9TH INTERNATIONAL CONGRESS OF PATHOPHYSIOLOGY
5TH CONGRESS OF PHYSIOLOGICAL SCIENCES OF SERBIA
WITH INTERNATIONAL PARTICIPATION

PROGRAM AND ABSTRACT BOOK

9TH INTERNATIONAL CONGRESS OF PATHOPHYSIOLOGY
5TH CONGRESS OF PHYSIOLOGICAL SCIENCES OF SERBIA
WITH INTERNATIONAL PARTICIPATION

Izdavač
Fakultet medicinskih nauka
Univerziteta u Kragujevcu

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9TH INTERNATIONAL CONGRESS OF PATHOPHYSIOLOGY

AND

5TH CONGRESS OF PHYSIOLOGICAL SCIENCES OF SERBIA

WITH INTERNATIONAL PARTICIPATION

JULY, 4th – 6th, 2023.

BELGRADE, SERBIA

FINAL PROGRAM AND ABSTRACT BOOK

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FOREWORD

Dear Colleagues,

The 9th International Congress of Pathophysiology and the 5th Congress of Physiological Sciences of Serbia with international participation will be jointly held from July 4th - 6th, 2023, in Belgrade, Serbia, at Hotel Crowne Plaza. For the last 22 years, the Serbian Physiological Society has organized a series of national and internationally recognized congresses and meetings in the fields of cardiovascular biology, risk factors, and health. These meetings were recognized internationally, attracted a worldwide scientific audience, and were supported by the Federation of European Physiological Societies (FEPS), the International Union of Physiological Sciences (IUPS), and the International Society for Pathophysiology (ISP). Such success in previous years directed us to plan and organize this event in order to improve the research background and make global research cooperation easier. Our wish is to have this meeting be traditional and progressive and to attract researchers from all over the world.

This scientific meeting will cover a very diverse range of topics such as metabolic disorders, COVID-19 and post-COVID disorders, cardiovascular diseases and novel therapeutic options, immunology and inflammation, nutrition and supplementation, health care, carcinogenesis and novel therapeutic options, oxidative stress, mitochondrial function, new molecular mechanisms in pathophysiology, skin diseases, regenerative mechanisms, neurological diseases, systemic inflammatory and autoimmune diseases, and many others.

The host city, Belgrade, stands as a captivating blend of rich history, vibrant culture, and modern allure. This magnificent metropolis offers much more than meets the eye. From ancient sacral and historical sites to contemporary galleries and museums, Belgrade seamlessly merges its storied past with its dynamic present. The city's heritage dates back centuries, making it a captivating destination for both scholars and enthusiasts alike. Moreover, Belgrade's charm extends beyond its historical significance. It serves as a thriving hub for cosmopolitan living and is adorned with a tapestry of modern establishments. As the capital city of Serbia, Belgrade offers a vibrant atmosphere that caters to all tastes and interests. Belgrade has evolved into a well-developed tourist destination, attracting visitors from both near and

far. Its welcoming spirit, coupled with a wide array of accommodation options and exceptional hospitality, ensures a memorable stay for every guest. We extend our heartfelt invitation to all attendees, welcoming you to experience the warmth and allure of Belgrade and central Serbia. Immerse yourself in the captivating history, indulge in the vibrant cultural scene, and embrace the energy and beauty of this remarkable host city. We are confident that your time in Belgrade will be nothing short of extraordinary.

On behalf of the Organizing & Program Committee

Vladimir Jakovljevic
Congress President

PROGRAM

Tuesday, July 4th, 2023

15.00 – 16.00 REGISTRATION/WELCOME COCTAIL

16.00 – 16.30 WELCOME MESSAGES

Jakovljevic Vladimir (Kragujevac, Serbia), Chair, Organizing Committee
Pechanova Olga (Bratislava, Slovak Republic), Chair, Organizing Committee
Bolevich Sergey (Moscow, Russian), Chair, Organizing Committee

16.30 – 19.00 KEYNOTE LECTURES
Chairs: Jakovljevic Vladimir (Kragujevac, Serbia), Pechanova Olga (Bratislava, Slovak Republic), Bolevich Sergey (Moscow, Russian)

Metabolic syndrome - targeted therapy versus natural polyphenolic substances
Pechanova O, Saman E, Barta A, Cebova M

Mechanisms for the loss of adrenergic support in heart failure
Dhalla NS

Noradrenergic control of astroglial vesicle dynamics and metabolic excitation: towards the noradrenergic hypothesis of cognitive decline
Kreft M, Fink K, Stenovec M, Vardjan N, Zorec R

Proteasomal degradation of TRAF2 mediates mitochondrial dysfunction in doxorubicin-cardiomyopathy
Dhingra R, Rabinovich-Nikitin I, Kirshenbaum LA

Experimental models of myocardial injury: is there possibility of cardioprotection induced by homocysteine-related vitamins?
Djuric DM, Jakovljevic Uzelac J, Mutavdzin Krneta S, Todorovic D, Stojanovic M, Stankovic S, Sobot T, Bajic Z, Stojiljkovic MP, Skrbic R

19.00 GROUP PHOTO/WELCOME RECEPTION

Wednesday, July 5th, 2023

Symposium 1: Extracellular Vesicles in Metabolic Diseases
9.00 – 10.30
Chairs: Bosch S (*Nantes, France*), Falcon-Perez JM (*Derio, Spain*)

Extracellular Vesicles in pancreatic beta-cell disease and therapy
Bosch S, de Beaurepaire L, Dauphin T, Bousquet C, Le Corre E, Salama A, Dubreil L, Pichon J, Jégou D, Dupont A, Pruvost Q, Lieubeau B, Haurogné K, Hervé J, Bach JM, Mosser M, Mignot G

Extracellular vesicles as biological vectors of atherosclerosis and nanobiomedicine approach to correct obesity via the hypothalamus
Andriantsitohaina R

High glucose and interleukin-1β synergize to activate NLRP3 activation and the release of extracellular vesicles in human aortic smooth muscle cells: prevention by IL-1R antagonists
San Hipólito-Luengo A, Valencia I, Vidal-Gómez X, Sánchez-Ferrer CF, Martínez C, Andriantsitohaina R, Peiro C

Extrahepatic systemic effects of hepatocyte-secreted extracellular vesicles
Falcon-Perez JM

Symposium 2: COVID-19 - Epidemiological Alert - Where are we now?
9.00 – 10.30
Chairs: Bugiardini R (*Bologna, Italy),* Buryachkovskaya L (*Moscow, Russia*)

COVID-19: different trajectories of death and treatments
Bugiardini R

Neutrophil extracellular traps in the pathogenesis of post-COVID syndrome
Kazimirskii AN, Kim AE, Salmasi JM, Poryadin GV, Panina MI, Larina VN, Stodelova EA, Stupin VA, Rogozhina LS

Platelets abnormalities in hospitalized patients with mild COVID-19 and their association with post-COVID conditions
Buryachkovskaya L Melkumyants A, Lomakin N, Zimchenok A

Similar changes of microcirculation in post-COVID19 syndrome and myalgic encephalomyelitis/chronic fatigue syndrome
Ryabkova V, Churilov LP

Coffe Break
10.30 – 10.45

Symposium 3: Advances in Cardiovascular Protection and Therapy
10.45 – 12.30
Chairs: Taskin Guven E (*Adiyaman, Turkey*), Tipparaju S (*Tampa, Florida, USA*)

Disulfide-Recovering HMGB1's Effect on Myocardial Ischemia/Reperfusion Injury
Taskin Guven E

Endocrine disruptors and their effects on human health: update on obesogens
Yilmaz B

Diabetic heart and strategies to mitigate injury
Tipparaju S

Interplay between clock genes and autophagy pathways in the heart
Rabinovich-Nikitin I

Mesenchymal stem cells in cardiovascular clinical trials: Advances and challenges
Dawn B

Melatonin against Doxorubicin-Myocardial Toxicity by HMGB1
Guven C

Symposium 4: Immunity and Inflammation – From Basic Research to Clinical Applications
10.45 – 12.30
Chairs: Buravkova L (*Moscow, Russia),* Sanchez-Ferrer CF *(Madrid, Spain)*

Senescence of multipotent mesenchymal stromal cells: the role of inflammation and oxidative stress
Buravkova L

Dipeptidyl peptidase-4 promotes human endothelial cell senescence and dysfunction by activation of PAR2-COX-2-TP axis and NLRP3 inflammasome
Valencia I, Vallejo S, Dongil P, San Hipólito A, Shamoon L, Carraro R, Erusalimsky JD, Romacho T, Peiró C, Sanchez-Ferrer CF

Th_{17}-lymphocytes and their cytokines in pathogenesis of autoimmune thyroiditis, accompanied by psychiatric disorders
Sobolevskaia PA, Gvozdeckii AN, Kudryavtsev IV, Chereshnev VA, Churilov LP

Correlations between the extracellular vesicles, inflammatory parameters and the parameters of the global hemostatic assays in patients with rheumatoid arthritis
Stojanovic A, Veselinovic M, Petrovic A, Jakovljevic V, Antovic A

Nonspecific neutrophil proteinases and their inhibitors: pathogenic role in the local and systemic inflammation development
Kubyshkin A, Fomochkina I

The role of inflammation and apoptosis in formation of multiple organ dysfunction syndrome at critical states
Nomerovskaya A, Kubyshkin A, Kharchenko V, Anisimova L, Fomochkina I, Golubinskaya E, Zyablitskaya E

Pause/Lunch
13.00 – 14.30

Symposium 5: Cardiovascular Disease: A Variety of Pathologies
14.30 – 16.00
Chairs: Lionetti V (*Pisa, Italy*), Pierce GN (*Winnipeg, Canada*)

MicroRNAs in the Heart-Brain Axis dysfunction: the strange case of miRNA29
Furini G, Baroni C, Perota A, D Aquaro G, Filomena Santarelli M, Dushpanova A, Terlizzi D, Cantile C, Galli C, Giorgetti A, Cattaneo A, Cellerino A, Lionetti V

The emerging role of Muscle enriched A-type Lamin Interacting Protein in cardio-protection
Burgon PG

High salt-induced human vascular remodeling and type 2 diabetes
Bkaily G, Jacques D

Bacteria and Cardiovascular Disease
Pierce GN

Symposium 6: Nutritional Strategies for Health Preservation
14.30 – 16.00
Chairs: Buttar HS (*Ottawa, Ontario, Canada*), Tyagi SC (*Louisville, USA*)

Healthful foods and lifestyle modifications are the best cost-effective strategies for the prevention of cardiovascular and cardiometabolic diseases
Buttar HS

Pathophysiological background for metabolic syndrome correction with grape polyphenols Iuliana Shramko I, Kubishkin A, Fomochkina I, Tarimov C, Nastoyashiy S

Sirt3 reverses renal dysfunction by trans-sulfuration pathway
Pushpakumar S, Kumar Juin S, Sen U, Tyagi SC

Pathogenetic substantiation of the nephroprotective action of polyphenols substances and ARBs in an experiment
Fomochkina I, Shevandova A, Kubyshkin A, Ametova L, Krupenko A, Gritsenko N, Pawar T

Coffe Break
16.00 – 16.15

Symposium 7: Cardiovascular Diseases - Unexpected Connections and Various Influences
16.15 – 18.00
Chairs: Turan B (*Ankara, Turkey*), Zivkovic V (*Kragujevac, Serbia*)

Antiphospholipid antibodies as independent predictors of cardiovascular failure: multidisciplinary approach is the key to the success
Stojanovich Lj, Djokovic A, Stanisavljevic N, Saponjski J

Redistribution of connexin 43 plays important role on long-QT characterized cardiac remodeling in insulin-resistant elderly mammalian heart
Turan B

Circulating vasopressin contributes to the development of hypertension via a switch in baroreflex inhibition to excitation
Brown CH

Opposite effects of meldonium in acute ischemia/reperfusion injury versus fecal- and LPS-induced sepsis in rat
Djurasevic S

The Effects of Different Exercise Types on a Rat Model of Myocardial Ishemia/ Reperfusion Injury
Zivkovic V, Glisic M, Nikolic Turnic T, Pindovic B, Nikolic M, Sretenovic J, Fisenko V, Bolevich S, Jakovljevic V

Symposium 8: Carcinogenesis and Metastasis
16.15 – 17.45
Chairs: Mogilenskikh A (*Yekaterinburg, Russia*),
Erdani Kreft M (*Ljubljana, Slovenia*)

Possibility of studying the receptor apparatus and proliferation in cell cultures obtained from luminal a subtype breast cancer tumors
Mogilenskikh A, Grebenyuk EV, Fadeev FA, Sazonov SV, Demidov SM

The role of angiogenic pathways in mechanisms of resistance in colorectal carcinoma
Seferov B, Golubinskaya E, Kalfa M, Fomochkina I, Kubyshkin V

Human amniotic membrane homogenate as a novel therapy for inhibiting bladder cancer cell migration and invasion
Janev A, Zeleznik Ramuta T, Dragin Jerman U, Obradovic H, Cemazar M, Erdani Kreft M

Analysis of co-expression of the cyclooxygenase (COX) gene in breast and colon tumors
Borisets A, Samburova NV

Teaching corner
17.45 – 18.15

Pathophysiology teaching strategy on a medical University
Litvitskiy PF

Application of "hallmarks" in pathophysiology
Grigoryan A, Vardresyan S, Petrosyan L, Ghazaryan D

18.00 – 20.00 POSTER SESSION I

Thursday, July 6th, 2023

Symposium 9: Redox Biology: Oxidative Stress in Pathophysiology
9.00 – 10.30
Chairs: Aburel OM (*Timisoara, Romania), Moskovtsev AA(Moscow, Russia)*

Methylene blue improves mitochondrial bioenergetics and mitigates oxidative stress: two birds with one stone
Aburel OM, Anechitei A, Danila MD, Sturza A, Borza C, Muntean DM

Variations of redox balance in different stages of childhood immune thrombocytopenic purpura
Medovic RH, Jakovljevic VLj, Medovic MV, Milosavljevic IM, Nikolic MR, Stojanovic AZ, Igrutinovic ZR, Srejovic IM

Small RNA during endoplasmic reticulum stress
Moskovtsev A, Zaichenko DM, Mesitov MV, Kubatiev AA

Oxidative stress in kidney tissue caused by methotrexate: effects of coenzyme Q10
Ilic S, Stojiljkovic N, Mitic N, Stojnev S, Ciric M, Stojiljkovic N

Symposium 10: Targeting Mitochondria: Past and Present
9.00 – 10.30
Chairs: Muntean D (*Timisoara, Romania*), Teixeira J (*Coimbra, Portugal*)

Monoamine oxidase is a novel target of SGLT2 inhibitors in the cardiovascular system
Muntean D, Hancu IM, Danila MD, Sturza A, Borza C

Repurposing monoamine oxidase inhibitors to reverse vascular hyperglycemic memory
Sturza A, Loredana IN, Soșdean R, Claudia B, Muntean DM

Mitochondria-targeted antioxidant based on hydroxycinnamic acid antiOxCIN4 improved liver steatosis in western diet-fed mice: the role of Nrf2-mediated cell signaling pathways
Teixeira J

Transcriptional regulation of mitochondrial stress responses
Trifunovic A

Coffe Break
10.30 – 10.45

Symposium 11: Novel Molecular Insights into Pathophysiological Cascades
10.45 – 12.30
Chairs: Todorovic Z (*Belgrade, Serbia*), Bozorgnia M (*Bratislava, Slovak Republic*)

High-fat diet exaggerates metabolic and reproductive features of estradiol valerate-induced pcos in rats
Joksimovic Jovic J, Rakic D, Pantovic S, Zivkovic V, Nikolic M, Sretenovic J, Nikolic M, Jovic N, Ristic N, Jakovljevic V

The effect of kynurenic acid on individual branches of the fibrotic cascade: an in vitro model of fibrosis
Bozorgnia M, Sykorova S, Vavrinec P, Vavrincova-Yaghi D

Effects of *galium verum* extract on cardiodynamic parameters and redox state of the isolated heart of psoriatic rats
Sretenovic J, Nikolic M, Jeremic N, Novakovic J, Bradic J, Joksimovic Jovic J, Savic M, Mihajlovic K, Nikolic M, Jakovljevic V

The effects of de novo lipid synthase inhibitors on cellular senescence
Todorovic Z

Structural changes of the corneal sub-basal nerve plexus and glycemic control in patients with type 1 diabetes mellitus
Bregovskaya A, Grineva EN, Gavrilova NY, Soprun LA, Lukashenko M, Churilov LP

Symposium 12: Cancer Initiation and Progression and Novel Therapeutic Options
10.45 – 12.30
Chairs: Kukreja RC (*Richmond, VA, USA*), Lukina SS (*Moscow, Russia*)

PDE5 Inhibition with Sildenafil Attenuates Cardiotoxicity and Improves Cancer Chemotherapy
Kukreja RC

Ovarian cancer metastasis: four hypermethylated long non-coding RNA genes in epigenetic regulation
Lukina S, Burdennyy AM, Pronina IV, Filippova EA, Loginov VI, Kazubskaya TP, Kushlinskii NE, Braga EA

Survivin conjugated gold nanoparticles enhance the potency of Abiraterone and Enzalutamide against prostate cancer cell lines via the increase in ROS formation and enhancement of apoptosis
Syed A, Baker A, Mohany M, Elgorban AM, Sajid Khan M, Al-Rejaie SS

The latent period of time between secretion of Ca^{2+} from ER-depo and activation of Ca^{2+} crac channels was observed in HL-60 cells
Astashkin E, Glezer M, Grachev S

Relationship between the expression of galectins-1,3 and the production of growth factors (VEGF and EGF) in patients with colorectal cancer, depending on the clinical and morphological characteristics of the tumor
Kurnosenko AV, Poletika VS, Reingardt GV, Vasileva OA, Kolobovnikova YV, Urazova OI

Pause/Lunch
13.00 – 14.15

Symposium 13: Pathophysiological aspects of skin disorders and regeneration
14.15 – 16.00
Chairs: Bradic J (*Kragujevac, Serbia*), Desyatova MA (*Yekaterinburg, Russian*)

Formulation and assessment of immortelle essential oil-based semi-solid preparations for wound healing
Bradic J, Andjic M, Petrovic A, Kocovic A, Tomovic M, Pecarski D, Jakovljevic V

Definition of FLG gene expression levels as a key biomarker in the pathogenesis of atopic dermatitis
Desyatova MA, Korotkov AV, Antonova SB, Makeev OG

Impact of siberian pine essential oil-containing ointment on wound healing in diabetic rats
Petrovic A, Bradic J, Andjic M, Nikolic M, Kocovic A, Tomovic M, Milojevic Samanovic A, Pecarski D, Jakovljevic V

Pathogenetic therapy of atopic dermatitis
Makeev OG, Korotkov AV, Desyatova MA, Bokovoj VD

Symposium 14: Pathophysiological Features of Neurological Disorders
14.15 – 16.00
Chairs: Stroev YI (*Saint Petersburg, Russia*),
Selakovic D (*Kragujevac, Serbia*)

The anxiety level alterations induced by orally administered fluorescent nanosized polystyrene particles in mice
Selakovic D, Rosic G

Magnesium deficiency in epilepsy – pathophysiological aspects
Stanojevic M, Parezanovic M, Spasic S, Lopicic S, Nedeljkov V, Jovanović Z, Vuckovic S

Role of endocrine factors in pathogenesis of autistic spectrum disorders
Sedelkova D, Stroev YI, Churilov LP

Antigen mimicry between human coronaviruses and candidate proteins involved in pathogenesis of small fiber neuropathy
Gavrilova NY, Normatov MG, Utekhin VJ, Churilov LP

Risk assessment of the development of neurodegenerative processes in cosmonauts
Korovin AE, Leontieva DO, Sobolevskaya PA, Tovpeko DV, Fedotkina TV, Churilov LP

Coffe Break
16.00 – 16.15

Symposium 15: COVID-19 - Learning from Experience
16.15 – 18.00
Chairs: Kolesnik S (*Moscow, Russia*),
Normatov MG (*Saint Petersburg, Russia*)

T-cell immune response in COVID-19 convalescents and following vaccination
Kolesnik S, Kudlay D, Gorodnova E, Krechetov S, Krechetova L, Vtorushina V, Inviyaeva E, Dolgushina N

Results of the study of gene polymorphism in children patients with COVID-19
Ageeva ES, Ablaeva RN, Rymarenko NV, Diadyura EN

Von Willebrand factor to ADAMTS-13 ratio as a prognostic factor of COVID-19 severity and thrombosis risk
Gorodnova E, Dolgushina N, Grachev S, Beznoshchenko O, Romanov A, Menzhinskaya I, Krechetova L, Ivanets T

Molecular mimicry of human coronavirus antigens and targets of autoimmune endocrine diseases: bioinformatic analysis and autopsy data
Normatov MG, Karev VY, Pakhomov VA, Utekhin VJ, Churilov LP

Pathomorphological changes of vasa vasorum in large arteries and their role in atherogenesis
Novitskaya TA, Fedotkina TV, Makarova YuA, Malakhova SA, Petrovskiy AN, Shapkina VA, Churilov LP

Symposium 16: Systemic Inflammatory and Autoimmune Disorders
16.15 – 18.00
Chairs: Zolotykh V*(St. Petersburg, Russia),*
Buravkov MS *(Moscow, Russian)*

Effects of silicone implants on immune system and autoimmune/autoinflammatory syndrome induced by adjuvants
Zolotykh V, Medvedeva KY, Utekhin KJ, Churilov LP, Yablonskiy PK

Markers of inflammation in gingival crevicular fluid in children with juvenile rheumatoid arthritis
Kozlitina J, Admakin O, Morozova N, Zakharova N, Morozova O

NK and T-cells in prion diseases
Bolevich S, Fokina M, Buravkov MS

The role of nonspecific inflammation and apoptosis in the pathogenesis of endometrial hyperplasia
Karapetyan O, Kubyshkin A, Kovalenko E, Ziablitskaya E, Golubinskaya E, Aliev L, Kubyshkin V, Podgorny G

Melissa officinalis as a nutritional strategy for cardioprotection in experimental autoimmune myocarditis
Milosavljevic I, Lazarevic N, Srejovic I, Zivkovic V, Novakovic J, Sretenovic J, Nikolic M, Jakovljevic V

18.00 – 20.00 POSTER SESSION II

CONCLUDING REMARKS & TAKE HOME MESSAGE

POSTER SESSION I
Chairs: Pierce GN (*Winnipeg, Canada*), Dawn B (*Las Vegas, USA*), Selakovic D (*Kragujevac, Serbia*), Bradic J (*Kragujevac, Serbia*)

PP01 Liraglutide pretreatment decreases oxidative stress and apoptosis in isoprenaline-induced myocardial injury in rats
Bajic Z, Sobot T, Uletilovic S, Mandic-Kovacevic N, Cvjetkovic T, Malicevic U, Vojinovic N, Jovicic S, Amidzic Lj, Djuric DM, Stojiljkovic MP, Skrbic R

PP02 Retinal, cardiovascular and renal markers in high risk of atherosclerosis
Barsukov AV, Korovin AE, Kulikov AN, Borisova EV, Churilov LP

PP03 Circulating endothelial cells in mild COVID-19 patients
Buryachkovskaya L, Melkumyants A, Lomakin N, Antonova O

PP04 Pathophysiological significance of blood cells in atherosclerosis: effects of simvastatin on hematological parameters in neonatal rats treated with mononatrium glutamate
Ciric M, Najman S, Pavlović V, Ilić S, Živković J, Mitić N,Trandafilović M, Janković-Veličković Lj, Ciric V

PP05 Development of a method based on hydrophilic chromatography mass spectrometry for the diagnosis of chronic kidney disease in children with vesicoureteral reflux
Danilova E, Eroshchenko N, Morozova O, Stavrianidi A, Nosyrev A

PP06 Glutathione system parameters and oxidative damage of DNA and proteins in girls and boys with constitutional obesity
Darenskaya M, Rychkova L, Kolesnikov S, Semenova N, Balshieva D, Nikitina O, Lesnaya A, Kolesnikova L

PP07 Cytokine profile and lipid peroxidation peculiarities in women with initial manifestations of pelvic venous insufficiency
Darenskaya M, Stupin D, Semendyaev A, Kolesnikov S, Kolesnikova L

PP08 Girls of two ethnic groups: the constitutional obesity most informative metabolic parameters
Darenskaya M, Rychkova L, Kolesnikov S, Semenova N, Nikitina O, Balshieva D, Yuzvak N, Rashidova M, Kolesnikova L

PP09 Curcumin attenuates oxidative stress in rats with CFA induced-rheumatoid arthritis: a pilot study
Djordjevic K, Pindovic B, Milojevic Samanovic A, Ilic I, Folic M, Zivkovic V, Bradic J, Jakovljevic V, Nikolic Turnic T

PP10 The potential cardioprotective effect of bile acids in isoprenaline-induced myocardial injury
Djukanovic Dj, Mihajlović D, Maksimovic Z, Uletilovic S, Mandic-Kovacevic N, Cvjetkovic T, Malicevic U, Gajic Bojic M, Vesic N, Milivojac T, Krivokuca A, Mikov M, Skrbic R

PP11 Molecular markers of bone metabolism in children with chronic kidney disease
Elovskaya A, Morozova N, Admakin O, Maslikova E, Potriasova A, Zakharova N, Morozova O

PP12 Expression of matrix metalloproteinases and their tissue inhibitor 1 in pancreatic tissues in rats against the background of acute experimental pancreatitis
Rogova LN, Ermak MV, Povetkina VN

PP13 The effects of creatine phosphate conditioning on heart function and redox balance during cardiac ischemia-reperfusion injury in rats
Gadzieva LA, Jakovljevic V, Bolevich SB, Bolevich SS, Kruglova MP, Saulin MP, Agabekyan AI, Yakhyev GK, Grachev SV

PP14 Methodological challenges in using human umbilical artery as a model for *in vitro* studies
Gajic Bojic M, Djukanovic Dj, Marinkovic S, Jovicic S, Stojiljkovic M, Savic MM, Skrbic R

PP15 Effects of supplementation of *Gallium Verum* extract on redox state in psoriatic rats
Goncakova IV, Jakovljevic V, Bolevich SB, Yavlieva KH, Kostich MZh, Silina EV, Bolevich SS, Kruglova MP, Sinelynikova TG

PP16 Prothrombotic state in COVID-19 pathophysiology
Gorodnova E, Grachev S, Dolgushina N, Beznoshchenko O, Romanov A, Menzhinskaya I, Krechetova L, Ivanets T

PP17 The effects of N-methyl-D-aspartate modulation of heart function and redox balance in a model of ischemia and reperfusion of isolated rat heart
Govorushkina NS, Bolevich SB, Fokina MA, Kolotilova ML, Bolevich SS, Silina EV, Agabekyan AI, Yakhyev GK, Saltikov AB, Jakovljevic V

PP18 The protective effects of glutathione against cumene hydroperoxide induced toxicity in leech retzius neurons
Jovanovic Z, Stanojevic M, Nesovic-Ostojic J, Todorovic J, Lopicic S, Spasic S, Nedeljkov V

PP19 Effects of forced running in mice with type II diabetes mellitus model
Kapilevich L, Zakharova A, Milovanova K, Orlova A, Kollantay O, Shuvalov I

PP20 Hybrid arterialization of the veins of the foot after multiple open and endovascular interventions by critical ischemia
Svetlikov AV, Gamzatov TK, Kebriakov AV, Gurevich VS

PP21 Phenotypic adaptation in conditions of chemical pollution of the environment
Enikeyev DA, Khisamov EN, Enikeyeva SA, Enikeyev OA, Fayurshin AZ

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PP31 Regional features of the epidemiology of cervical cancer (CC)
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PP32 Prototype of modular 3D bioprinter with cooling platform for gelatin-based bioinks
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PP33 Detecting the major mutation in hereditary breast cancer genes BRCA1/2, CHEK2 and PALB2 in Crimean patients
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PP34 Hereditary ovarian cancer genetics in crimean patients
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PP35 The Crimean population's leptin, *ADIPOQ*, *ADIPOR1* AND *ADIPOR2* genotypes association with the clinical manifestations of metabolic syndrome in comparison with the main European and Asian data
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POSTER SESSION II
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Nikolic M, Nedeljkovic N, Nikolic M, Jeremic N, Jakovljevic V

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ABSTRACTS

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METABOLIC SYNDROME - TARGETED THERAPY VERSUS NATURAL POLYPHENOLIC SUBSTANCES

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Metabolic syndrome is a cluster of conditions that occur together and increase the risk of cardiovascular disease, stroke, and type 2 diabetes. Statins are prescribed as the first-line pharmacological therapy for the reduction of cardiovascular risk. However, many patients show intolerance to these drugs, so routine treatment needs to be replaced by targeted therapy or appropriate alternatives. Therefore, we studied the effects of simvastatin-loaded polymeric nanoparticles and polyphenol-rich sources like Cornelian Cherries (CC) and *Lonicera caerulea* (LC) on the lipid profile and nitric oxide (NO)/reactive oxygen species (ROS) balance in adult male obese Zucker rats. The rats were divided into an untreated group, groups treated with empty nanoparticles, simvastatin-loaded nanoparticles (SIMV), or SIMV-Coenzyme Q-loaded nanoparticles (SIMV+CoQ) and groups treated with CC, or LC. After 6 weeks, the lipid profile in the plasma and the concentration of conjugated dienes (CD) in the liver were determined. Nitric oxide synthase (NOS) activity, Akt, endothelial NOS (eNOS), phosphorylated eNOS (p-eNOS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and nuclear factor kappaB (NF-kappaB) protein expressions were measured in the heart and aorta. SIMV and SIMV+CoQ10 treatments decreased plasma LDL levels, but only the combined SIMV+CoQ10 treatment increased NOS activity and expression of Akt, eNOS, and p-eNOS in both the heart and aorta. Both CC and LC treatments decreased plasma LDL levels and NOS activity, however without eNOS or Akt upregulation. Interestingly, CD levels, NADPH oxidase in the heart and NF-kappaB protein expressions in the aorta were decreased by all treatments. In conclusion, both simvastatin-loaded nanoparticles and polyphenol-rich sources used in the study decreased LDL levels and ROS production. Simvastatin-loaded nanoparticles did not affect NOS activity. Thus, we hypothesize that a decrease in oxidative stress is crucial for the reduction of LDL levels, while an increase in NOS activity has only additional beneficial effects.

Keywords: plasma LDL, reactive oxygen species, nitric oxide

MECHANISMS FOR THE LOSS OF ADRENERGIC SUPPORT IN HEART FAILURE

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Although there occurs a loss of adrenergic support for maintaining cardiac function in heart failure, the mechanisms for this defect are not fully understood. We have investigated alterations in the inotropic responses to isoproterenol in myocardial infarction induced heart failure in rats at different stages upon occluding the coronary artery. Furthermore, changes in different components of adrenergic signal transduction pathway including β_1-adrenergic receptors (β_1-AR), G-proteins and adenylyl cyclase were examined in the failing left ventricle. Depressions in the inotropic responses to isoproterenol were associated with reductions in the β_1-AR density at early, moderate and severe stages of heart failure. The activation of adenylyl cyclase and the increase in cardiac cyclic AMP content by isoproterenol were also decreased in the failing hearts. Gi-protein content, mRNA levels for Gi-protein as well as pertussis toxin (PT) catalyzed ADP-ribosylation and PT-induced activation of adenylyl cyclase were increased in heart failure. On the other hand, cholera toxin (CT) induced ADP-ribosylation and CT-induced activation of adenylyl cyclase were reduced in heart failure. The mRNA level for Gs-protein were increased in heart failure whereas Gs-protein content was depressed in failing hearts at severe stages only. It was also observed that alterations in β_1-AR mediated signal transduction pathway in heart failure were attenuated by treatment of heart failure animals with blockers of the renin-angiotensin system. These observations indicate that the depressed activation of adenylyl cyclase by isoproterenol may not only be due to decreased β_1-AR density but also due to increased Gi-protein content as well as decreased bioactivity of Gs-proteins in the failing hearts. In addition, various pathogenic factors such as oxidative stress, Ca^{2+}-handling abnormalities and metabolic defects appears to explain changes in the β_1-AR signal transduction as well as loss of adrenergic support in heart failure.

Keywords: β_1-adrenoceptors, adenylyl-cyclase, G-proteins

NORADRENERGIC CONTROL OF ASTROGLIAL VESICLE DYNAMICS AND METABOLIC EXCITATION: TOWARDS THE NORADRENERGIC HYPOTHESIS OF COGNITIVE DECLINE

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Astrocytes play a significant role in numerous processes, including brain energy metabolism. Their anatomical position between blood vessels and neurons positions them as effective mediators of glucose uptake from the bloodstream. Within astrocytes, glycogen serves as an energy buffer, bridging short-term energy requirements in the brain. The levels of glycogen reflect a dynamic equilibrium between its synthesis and glycogenolysis. While several factors, including hormones and neuropeptides such as insulin and adrenaline, likely modulate glycogen stores in astrocytes, detailed cellular-level mechanisms remain scarce. During cognitive efforts conducted by local neuronal networks, an additional 20% of energy is required, which is mediated by chemical messengers like noradrenaline (NA). NA plays a fundamental role in many functions, including attention, arousal, sleep/wakefulness, and consciousness as well as in learning and memory. Astrocytes may be involved in cognitive decline through the neurodegeneration of locus coeruleus (LC) neurons, which are the prime source of NA in the central nervous system. In our studies, we employ a glucose nanosensor based on Förster resonance energy transfer to monitor cytosolic glucose and lactate concentrations with high temporal resolution. Additionally, we utilized a cytochemical approach to assess glycogen stores in individual cells. Our findings indicate that upon stimulation by adrenaline or noradrenaline, the availability of cytosolic glucose and lactate promptly increases. We demonstrate that the uptake of D-glucose is crucial for the NA-induced rise in lactate concentration, which is dependent on glycogen degradation. This suggests that the majority, if not all, D-glucose molecules in NA-stimulated cells transit through the glycogen shunt during glycolysis. Moreover, under the defined transmembrane D-glucose gradient, the glycolytic intermediates were not only used to produce L-lactate, but also to significantly support oxidative phosphorylation, as demonstrated by an elevation in lactate concentration when the Krebs cycle was inhibited. Insulin enhances the process of glycogen formation. Alzheimer's disease is regarded as diabetes mellitus of the brain, or as type 3 diabetes mellitus, which implies metabolic impairment of neurons and glia. We also provided experimental evidence for the early onset of cell-autonomous astrocyte dysfunction in the context of familial AD that affects cell capability for targeted delivery and regulated vesicular secretion. We performed experiments on 3xTg-AD mouse model that mimics the histopathology of AD. In this animal model astrocytes express a single mutated gene, namely a mutant presenilin-1). Results obtained from these cells show that vesicle traffic, secretory discharge of a peptide gliotransmitter, and cytoplasmic Ca^{2+} homeostasis are all impaired, suggesting astroglia as a novel target for the development of pharmacologic manipulation in treating the early stages of AD.

Keywords: astrocytes, metabolism, noradrenaline, cognitive decline, insulin

PROTEASOMAL DEGRADATION OF TRAF2 MEDIATES MITOCHONDRIAL DYSFUNCTION IN DOXORUBICIN-CARDIOMYOPATHY

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Cytokines such as TNFα have been implicated in cardiac dysfunction and toxicity associated with doxorubicin (DOX). While TNFα can elicit different cellular responses including survival or death, the mechanisms underlying these divergent outcomes in the heart remains cryptic. The E3 ubiquitin ligase TRAF2 provides a critical signaling platform for K63 - linked polyubiquitination of RIPK1, crucial for NF-κB activation by TNFα and survival. Herein, we investigate TRAF2 signaling in the pathogenesis of DOX cardiotoxicity. Using a combination of *in vivo* (4 weekly injections of DOX (5mg/kg/week) in cardiac-myocyte restricted expression of AAV9-GFP and AAV9-TRAF2 mice (C57/BL6J), and in vitro approaches, we monitored TNFα levels, LDH, cardiac ultrastructure and function, mitochondrial bioenergetics and cardiac cell viability. In contrast to vehicle treated mice, ultrastructural defects including cytoplasmic swelling, mitochondrial perturbations, and elevated TNFα levels were observed in the hearts of mice treated with DOX. While investigating the involvement of TNFα in DOX cardiotoxicity, we discovered that in the absence of DOX, NF-κB was readily activated by TNFα. However, TNFα-mediated NF-κB activation was impaired in cardiac myocytes treated with DOX. This coincided with loss of K63- linked poly-ubiquitination of RIPK1, attributed to the proteasomal degradation of TRAF2. Further, TRAF2 protein abundance was markedly reduced in hearts of cancer patients treated with DOX. Impaired TRAF2 signaling resulted in mitochondrial perturbations, including disrupted bioenergetics, loss of membrane potential and permeability transition pore opening. We further established that the reciprocal actions of the ubiquitinating and de-ubiquitinating enzymes c-IAP1 and USP19 regulated the proteasomal degradation of TRAF2. An E3 ligase mutant of c-IAP1(c-IAP1 H588A) or gain of function of USP19, prevented proteasomal degradation of TRAF2 and DOX-induced cell death. Further, wild type TRAF2 but not a RING finger mutant defective for K63 linked polyubiquitination of RIPK1, restored NF-κB signaling and suppressed DOX-induced cardiac cell death. Finally, cardiomyocyte-restricted expression of TRAF2 (AAV9-TRAF2) *in vivo* protected against mitochondrial defects and cardiac dysfunction induced by DOX. Our findings reveal a novel signaling axis that functionally connects the cardiotoxic effects of DOX to proteasomal degradation of TRAF2. Disruption of the critical TRAF2 survival pathway by DOX, sensitizes cardiac myocytes to TNFα mediated necrotic cell death.

Keywords: doxorubicin, TRAF2, TNFα

EXPERIMENTAL MODELS OF MYOCARDIAL INJURY: IS THERE POSSIBILITY OF CARDIOPROTECTION INDUCED BY HOMOCYSTEINE-RELATED VITAMINS?

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During last time we established two different models of myocardial injury in rat (monocrotaline-induced heart failure and isoprenaline-induced myocardial injury/infarction) for the pre-clinical evaluation of the relevance of homocysteine-related vitamins (folic acid, vitamin B6) in cardioprotection. In both experimental models increased levels of high-sensitivity troponins were registered however in isoprenaline-induced myocardial infarction it was followed by an increased level of homocysteine. This led to an idea to examine possible cardioprotective effects of homocysteine-related vitamins taking into consideration their homocysteine-dependent or homocysteine-independent mechanisms. Four weeks treatment with folic acid reduced cardiomyocyte proliferation in the right ventricle (RV) wall even if did not significantly reduce hypertrophy of the RV wall, and affected different cardiometabolic and oxidative stress biomarkers (thiol groups, nitrotyrosine and reactive carbonyl group content, total GSH, SOD and GPx activities were affected in cardiac tissue) in monocrotaline-induced right heart failure. In addition, it was observed that co-application of folic acid and vitamin B6 did not attenuate hypertrophy of the RV wall but aggravated oxidative stress. Furthermore, it was examined the potential cardoprotective effects of seven-day pretreatment with folic acid on isoprenaline-induced myocardial injury. Folic acid pretreatment significantly decreased both homocysteine and high-sensitivity troponin levels. Moreover, folic acid moderately decreased the ROS levels (superoxide anion radical, hydrogen peroxide and thiobarbituric acid reactive substances), improved the antioxidative activities (catalase, superoxide dismutase, reduced glutathione), and significantly alleviated isoprenaline-reduced the nitrite level. Pretreatment with folic acid failed to prevent the ECG changes and histopathological abnormalities induced by isoprenaline. Taken together, it can be concluded that folic acid affected metabolism of the failing heart and modulated certain molecular mechanisms responsible for heart failure progression, and it could be cardioprotective supplement in myocardial injury in rat.

Keywords: folic acid, homocysteine, myocardial injury, rat, vitamin B6

EXTRACELLULAR VESICLES IN PANCREATIC BETA CELL DISEASE AND THERAPY

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The pancreatic beta cell is a central orchestrator at metabolic, nervous, endocrine and immune crossroads. Beta cell stress and dismiss are acknowledged to contribute to inflammation in beta cell diseases such as diabetes. As extracellular vesicles (EV) mirror epigenetic changes in their parental cell, they are important players in the local and systemic coordination of immune responses. The present work aims to characterize the beta cell vesiculome and identify its role in beta cell disease and potential opportunities for therapy. EV released by mouse insulinoma MIN6 (w/ or w/o exposure to stress) or human beta cells (untreated) were separated from culture supernatants by a method combining differential centrifugation, filtration and size-exclusion chromatography. EV were characterized for their morphology and cargo (tunable resistive pulse sensing, cryo-electron microscopy, western blot, real-time PCR, ELISA…) and assessed by flow cytometry for their aptitude to induce or modulate allogeneic immune responses *in vitro* (murine and human sEV) and in diabetes-prone NOD mice *in vivo* (murine sEV). Exposure to stress engenders an up to four-fold increase in the release of beta EV containing immune-active cargo (auto-antigens, microRNAs, cytokines, chemoattractants) promoting dendritic and macrophage cell activation. In contrast, EV derived from healthy beta cells present the ability to modulate T-cell proliferation and cytokine secretion in mixed lymphocytic reactions *in vitro* and to stimulate the secretion of immune-suppressive cytokines beta *in vivo*. In function of the parental cell's status, EV from pancreatic beta cells exert protective or maladaptive immune effects. EV constitute a new class of replication incompetent, filter sterilisable, off-the shelf drugs. In the future, the selection of EV from healthy beta cells with defined immune-regulatory signals might offer new leverage for beta cell disease immune therapy.

Keywords: beta cell disease, extracellular vesicles, immune homeostasis

EXTRACELLULAR VESICLES AS BIOLOGICAL VECTORS OF ATHEROSCLEROSIS AND NANOBIOMEDICINE APPROACH TO CORRECT OBESITY VIA THE HYPOTHALAMUS

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Obesity causes thousands of deaths per year worldwide. Indeed, abdominal obesity is frequently associated with insulin resistance, elevated blood pressure (BP), and dyslipidemia consisting in low levels of HDL-cholesterol and high levels of triglycerides; this cluster of events is denominated metabolic syndrome (MetS). MetS increased the risks of cardiovascular diseases and type 2 diabetes (T2D). Extracellular vesicles (EVs) are now considered veritable entities for diagnosis, prognosis, and therapeutics. Circulating EVs are increased in MetS patients and participated in atherosclerosis. On the other hand, EVs can be used as a "nanobiomedicine" approach to treat obesity through central regulation of energy balance. For the first point, we showed that large EVs (lEVs) overexpressing Rap1 are involved in atherosclerosis, accumulate in atherosclerotic plaques, and their circulating levels correlate with vascular risks. Indeed, proteomic analysis revealed that the small GTPase, Rap1 was overexpressed in lEVs from MetS *versus* those from nMetS patients. In addition, Rap1 was in GTP-associated active state, in lEVs. Rap1 lEV levels correlated with triglyceridemia, insulinemia, hs-CRP, TyG index and increased stenosis risk. lEVs promoted proliferation and migration of human aortic smooth muscle cells (SMC) , but, only those from MetS patients increased the expression of the pro-inflammatory molecules and the activation of ERK5 signaling pathway. Neutralization of Rap1 by specific antibody or pharmacological inhibition of Rap1 with GGTI-298, either partially or completely, prevented the effects of lEVs from MetS patients but not those from nMetS lEVs. In addition, HFD-fed $ApoE^{-/-}$ mice displayed an increased expression of Rap1 in both aortas and circulating lEVs. lEVs from HFD- fed $ApoE^{-/-}$ mice, but not those from mice fed with a standard diet, enhanced SMC proliferation. For the second point, we bring the first evidence of specific targeting of ventromedial hypothalamic (VMH) SF1 neurons by small EVs (sEVs) to treat obesity without altering food intake limiting inflammatory responses. We have demonstrated the effectiveness of this strategy using *iv* injections of sEVs loaded with a plasmid with a dominant negative (DN) of AMPKα1 under the SF1 promoter, which allows targeting of $SF1^{+}$ neurons at the VMH level. Administration of sEVs carrying SF1-AMPKα1- DN significantly decreased weight in mice fed a high-fat diet. This effect was not associated with changes in food intake, but involved activation of the sympathetic nervous system and increased brown adipose tissue thermogenesis. This approach opens a new avenue in the rational design of new treatment strategies for obesity and associated comorbidities and possibly other neurological diseases.

Keywords: extracellular vesicles, nanobiomedicine, atherosclerosis, metabolic syndrome

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HIGH GLUCOSE AND INTERLEUKIN-1B SYNERGIZE TO ACTIVATE NLRP3 ACTIVATION AND THE RELEASE OF EXTRACELLULAR VESICLES IN HUMAN AORTIC SMOOTH MUSCLE CELLS: PREVENTION BY IL-1R ANTAGONISTS

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Vascular complications are the major cause of morbidity and mortality in patients with diabetes melllitus (DM). Hyperglycemia but also inflammatory cytokines like interleukin (IL)-1β play a key role in such alterations. Understanding the pathophysiological mechanisms, including altered intercellular communication, by which both factors exacerbate vascular damage is essential to identify potential therapeutical targets. We investigated the reciprocal influence of IL-1β and high glucose on the activation of NLRP3 inflammasome, an innate immunity component linked to vascular disease and the production and cargo of extracellular vesicles (EVs) as key players in vascular intercellular communication. Human aortic smooth muscle cells (HASMC) were challenged for 18 h with IL-1β (10 ng/ml) and/or high glucose (22 mM) and used for protein determinations and EVs isolation. NLRP3, phospho-p65, ASC protein and caspase-1 were quantified by Western blot, while the formation of ASC-like specks, reflecting NLRP3 activation, was assessed by indirect immunofluorescence. In HASMC cultures, IL-1β favored NLRP3 inflammasome priming as shown by NF-κB (phospho-p65) activation and increased NLRP3 and pro-IL-1β levels. The cytokine equally promoted the NLRP3 inflammasome complex assembly, the activation of caspase-1 and the subsequent release of mature IL-1β in an auto-inflammatory loop. High glucose alone did not promote any of these effects, but it exacerbated the actions of IL-1β. High glucose also potentiated the release of HASMC-derived EVs induced by IL-1β and increased their cargo content in NLRP3 inflammasome components. The IL-1 receptor blocker anakinra (1mg/ml) blunted all these effects of IL-1β as well as their exacerbation by high glucose. Overall, high glucose exaggerates the priming and activation of the NLRP3 inflammasome by IL-1β and alters intercellular communication by modifying the number and content of EVs. By preventing such synergy, biological drugs blocking IL-1β receptors arise as potential pharmacological tools to attenuate vascular abnormalities associated with DM.

Keywords: interleukin-1β, hyperglycemia, NLRP3, extracellular vesicles

EXTRAHEPATIC SYSTEMIC EFFECTS OF HEPATOCYTE-SECRETED EXTRACELLULAR VESICLES

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Extracellular vesicles (EVs) constitute a novel biological entity to identify biomarkers, and active players in the development of liver diseases, and as entities for intercellular communication. Biodistribution analysis of EVs using non-lipophilic methods, single-vesicle global characterization by Raman-tweezers spectroscopy and omics technologies have been applied to characterize the distribution, content, and function of EVs secreted by hepatocytes in liver-associated diseases. Biodistribution analysis showed that hepatocytes-secreted EVs travel to different tissues and cross blood-brain barrier. Transcriptomics and proteomics of these EVs have provided several low invasive candidate biomarkers in serum and urine. The transcriptomic analysis of EVs and the cells that secrete those EVs made possible the identification of a sorting RNA signal that can incorporate the RNAs into the EVs to be exported out of the cells. Metabolomics has shown that hepatic EVs carry several active enzymes that are able to modify the serum metabolic composition what could have important implications for endothelial functioning. Biodistribution studies and the integration of several omics technologies in different experimental settings including the analysis of the cells, the EVs secreted by those cells, and the cells exposed to those EVs allow to dissect the EVs-mediated mechanisms underlying the development and progression of liver diseases and it provides novel therapeutics targets.

Keywords: exosomes, single-vesicle analysis, metabolic syndrome, extracellular vesicles, drug-induced liver injury

COVID-19: DIFFERENT TRAJECTORIES OF DEATH AND TREATMENTS

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Previous analyses on sex differences in case fatality rates at population-level data had limited adjustment for key patient clinical characteristics thought to be associated with COVID-19 outcomes. Neverthless the risk of specific organ dysfunctions and mortality in women and men is less known. This retrospective cross-sectional study included 17 hospitals within 5 European countries participating in the International Survey of Acute Coronavirus Syndromes (ISACS) COVID-19(NCT05188612). Participants were individuals hospitalized with positive SARS- CoV-2 from March 2020 to February 2022. Risk-adjusted ratios (RR) of in-hospital mortality, acute respiratory failure (ARF), acute heart failure(AHF), and acute kidney injury(AKI) were calculated for women versus men. Estimates were evaluated by inverse probability of weighting and logistic regression models. The overall care cohort included 4,499 patients with COVID-19 associated hospitalizations. Of these, 1,524(33.9%) were admitted to ICU, and 1,117(24.8%) died during hospitalization. Compared with men, women were less likely to be admitted to ICU (RR:0.80; 95%CI: 0.71-0.91). In general wards (GW) and ICU cohorts, the adjusted women- to-men RRs for in-hospital mortality were of 1.13(95%CI: 0.90-1.42) and 0.86 (95%CI: 0.70- 1.05; p interaction=0.04). Development of AHF, AKI and ARF was associated with increased mortality risk (ORs: 2.27; 95%CI; 1.73-2.98,3.85; 95%CI:3.21-4.63 and 3.95; 95%CI:3.04-5.14, respectively). The adjusted RRs for AKI and ARF were comparable among women and men regardless of intensity of care. By contrast, female sex was associated with higher odds for AHF in GW, but not in ICU (RRs:1.25; 95%CI0.94-1.67 versus 0.83; 95%CI:0.59-1.16, pinteraction=0.04). Women in GW were at increased risk of AHF and in-hospital mortality for COVID-19 compared with men. For patients receiving ICU care, fatal complications including AHF and mortality appeared to be independent of sex. Equitable access to COVID-19 ICU care is needed to minimize the unfavourable outcome of women presenting with COVID-19 related complications.

Keywords: COVID-19, acute heart failure, mortality

NEUTROPHIL EXTRACELLULAR TRAPS IN THE PATHOGENESIS OF POST-COVID SYNDROME

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Post-Covid Syndrome is a set of signs and symptoms that develop after a previous infection with COVID-19. By determining the content of neutrophil extracellular traps (NETs), it is possible to verify the inflammatory status of patients. In combination with other clinical and laboratory data, this makes it possible to predict the course of the disease and the development of complications, and to determine the mechanism for the development of the pathological process. The purpose of this study was to determine the involvement of NETs in the pathogenesis of post-COVID syndrome. The study included 47 people, aged 19 to 28 years, without established chronic diseases, BMI<25 kg/m^2. The isolation of neutrophils from the venous blood was performed using traditional isolation methods on a double ficoll density gradient. NETs were visualized and counted using fluorescence microscopy using fluorescent dye for dsDNA SYBR Green. Purine nitrogenous bases (PNB) were determined by the color reaction method based on their interaction with silver nitrate to form a colored compound. NETs in the form of thin single strands of DNA were found in patients with post-COVID syndrome. The size of DNA strands exceeds several tens of cell diameters (220-280 microns). Such filamentous structures do not form a net and are not capable of capturing pathogens with subsequent fiber retraction. The process of spontaneous enzymatic degradation of these DNA strands can cause the appearance of secondary alteration factors in the form of extracellular PNB. High concentrations of extracellular PNB in the blood of patients with post-COVID syndrome are also due to the long period of formation of NETs in the form of thin single strands of DNA for a long time (3 months or more). Our studies have shown that in patients with mild, moderate and severe forms of coronavirus infection, the concentration of extracellular PNB during the development of post-COVID syndrome was 22.89 ± 8.36, 23.27 ± 8.90 and 35.84 ± 19.25 mg/ml, respectively. A comparative study of extracellular PNB in patients with acute infectious pathology (appendicitis, cholecystitis) showed their presence in the blood, but their concentration was very low and ranges from 0.2 to 1.8 mg/ml. Extracellular PNB are toxic to the body and their effects are similar to some of the clinical manifestations of post-COVID syndrome. The results of our studies prove the important role of NETs in the development of post-COVID syndrome.

Keywords: post-COVID syndrome, neutrophil extracellular traps, inflammatory diseases, extracellular purine nitrogenous bases, pathogenesis

PLATELETS ABNORMALITIES IN HOSPITALIZED PATIENTS WITH MILD COVID-19 AND THEIR ASSOCIATION WITH POST-COVID CONDITIONS

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COVID-19 is associated with various hemostatic abnormalities. A large number of studies are devoted to plasma factors of coagulation and to prevention of venous thrombosis using anticoagulant therapy. Considerably less data has been collected about the role of platelets in this disease and the possible association of Post-COVID conditions with platelets activity. Thirty-one patients hospitalized with PCR-confirmed mild COVID-19 were included in a single-center retrospective observational study. During hospital stay, no patient was referred to ICU or demonstrated any symptoms of bleeding. The patients were hospitalized in 5.9±0.8 (1-11) days after manifestation of the first symptoms of the disease. More than half of the patients (58%) had comorbidities. The median age was 52.7±2.4 (27-74) years. Twenty COVID-19-negative persons constituted the control group. The scanning electron microscopy was utilized to examine the blood derived from the cubital vein at admission and discharge. The presence and severity of post-COVID symptoms were assessed using a telephone survey of the patients 18 months after discharge from the hospital. At admission, in 20 (64.5%) patients, most platelets were activated, but in 11 (35.5%) patients, they retained a native discoid form similar to that of healthy individuals. At discharge from the hospital, 24 (77.4%) patients had high platelet activation whereas number of patients without platelet activation decreased to 7 (22.6%). Such a change was associated with neither drug therapy used nor comorbidities or the clinical course of the disease. However, later hospitalization (5.2±0.6 vs 3.1±0.8 days since the onset of the disease) and longer duration of hospitalization (14.1±0.9 vs 10.6±0.4 days) were characteristic of the group with high platelet activation. During 2-6 months post-COVID symptoms retained in 19 (61.3%) patients. However, 17 of them had some symptoms even after 18 months. There occurrence of post-COVID symptoms did not depend on physical characteristics of the patients, their age, comorbidity, laboratory indices or the drug treatment. It is important that the majority of patients (up to 75%) with high platelet activation developed post-COVID conditions whilst neither of 7 patients who had at discharge the low number of activated platelets demonstrated post-COVID symptoms. Patients with COVID-19 differ in the degree of platelet activation. High platelet activation after recovery may be a predictor of the development of post-COVID complications. Patients with no platelet activation at discharge from the hospital seems to be protected from post-COVID complications.

Keywords: COVID-19, platelets, blood cells, post-COVID conditions

SIMILAR CHANGES OF MICROCIRCULATION IN POST-COVID19 AND MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROMES

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Post-COVID19 syndrome is defined by the World Health Organization as the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection. *The experience of patients with post-COVID19 syndrome has led to renewed interest in myalgic encephalomyelitis/chronic fatigue syndrome (MF/CFS),* wh*ich is known since the 19th century, because some symptom clusters of these conditions considerably overlap. There are still many unsolved questions, particularly those related to the pathogenesis of both entities. Increasing evidences witness for for the microvascular impairment common for ME/CFS and post-COVID19 syndrome. Post-occlusive (three-minute occlusion) reactive arterial hyperemia in the cutaneous microvasculature of the forearm was assessed with laser doppler flowmetry (LDF) in 27 patients suffered from post-COVID19 syndrome, 27 patients with ME/CFS not related to COVID-19 and in 31 healthy individuals. Patients with post-COVID19 sy*ndrome had significantly lower pre-occlusion steady-state perfusion, maximum post-occlusive perfusion and biological zero signal compared to the healthy controls. Patients with ME/CFS also presented with significantly decreased biological zero signal compared to the healthy controls. The results provide some indirect evidence for the capillary rarefication in post-COVID19 syndrome, which has been recently reported using other method of microcirculation assessment. During occlusion, biological zero signal obtained by LDF originates from Brownian motion of red blood cells in vessels and macromolecules in the interstitial space, and from the redistribution of blood between vessels, including reverse flow in the capillaries. To our knowledge, this is the first finding of decreased biological zero in post-COVID19 and ME/CFS. This phenomenon needs further investigation and may depend on alterations of both blood rheological properties and endothelial non-wettability. e.g. due to autoantibodies.

Keywords: microcirculation, COVID-19, myalgic encephalomyelitis/chronic fatigue syndrome, laser doppler flowmetry, long COVID

DISULFIDE-RECOVERING HMGB1'S EFFECT ON MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

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Myocardial ischemia-reperfusion injury (MIR) is a major health problem worldwide. Numerous studies have been evaluated to understand the MIR mechanism; however, significant problems still exist. Damage-associated molecular patterns (DAMPs), which are endogenous danger substances secreted by harmed or dying cells, including High Mobility Group Box-1 (HMGB1), These substances interact with pattern recognition receptors, such as Toll-Like Receptors (TLRs), to activate the innate immune system. HMGB1 is one of the non-histone proteins secreted passively by stressed or dying cells. Numerous pathologic circumstances lead to oxidative stress, which releases HMGB1. One of these pathological conditions is myocardial ischemia and reperfusion damage, which results from an excess production of radicals due to inadequate oxygen delivery. HMGB1 is secreted by tissues under various clinical circumstances. HMGB1 is secreted by cardiac cells under MIR circumstances. Toll-like receptors (TLR) 2 and TLR4 are directly activated by secreted HMGB1. HMGB1 may play a role in the pathophysiologic mechanisms behind MIR, according to some evidence, but more recent research suggests that it may also control how tissues repair following MIR. Its structure is the source of this disagreement. Because the protein's three cysteine residues, located at positions 23, 45, and 106, are vulnerable to redox-dependent alterations, there are three posttranslational redox modifications of it as a result. Cytein residues in HMGB1 can either be completely reduced, oxidized with a disulfide between 23 and 45 with 106 reduced, or terminally oxidized at all cyteines. So, they each serve as a chemokine, a cytokine, and a sign of inactivity. Therefore, HMGB1 undergoes oxidative alteration, which alters protein function by altering its external binding receptor. While the disulfide form of HMGB1 contributes to the release of cytokines by TLR4, the reduced form of HMGB1 exhibits chemotactic activity through CXCL12/CXCR4. The article addresses a new discovery that disulfide HMGB1 can promote myocardial repair following MIR, despite environmental redox conditions and by changing the TLR2/TLR4 axis.

Keywords: high mobility group box-1 protein, TLR2, myocardial ischemia–reperfusion injury

ENDOCRINE DISRUPTORS AND THEIR EFFECTS ON HUMAN HEALTH: UPDATE ON OBESOGENS

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Endocrine Disrupting Chemicals (EDCs) are a global problem for environmental and human health. They are defined as "an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action". It is estimated that there are more than 1000 chemicals with endocrine-acting properties. EDCs comprise pesticides, fungicides, industrial chemicals, plasticizers, nonylphenols, metals, pharmaceutical agents and phytoestrogens. Human exposure to EDCs mainly occurs by ingestion and to some extent by inhalation and dermal uptake. Most EDCs are lipophilic and bioaccumulate in the adipose tissue, thus they have a very long half-life in the body. It is difficult to assess the full impact of human exposure to EDCs because adverse effects develop latently and manifest at later ages, and in some people do not present. EDCs may interfere with synthesis, action and metabolism of sex steroid hormones that in turn cause developmental and fertility problems, infertility and hormone-sensitive cancers in women and men. Recently it has been suggested that some EDCs promote adipogenesis and cause obesity. These EDCs are called "obesogens". They may cause disturbance in energy homeostasis. Disruption of lipid homeostasis may involve multiple mechanisms: 1) increasing the number of adipocytes, 2) increasing the size of adipocytes, 3) altering endocrine regulation of adipose tissue development, 4) changing hypothalamic regulation of appetite, satiety and food preference, 5) affecting basal metabolic rate and 6) energy balance in favor of calorie storage and 7) altering insulin sensitivity in the liver, skeletal muscle, pancreas, gastrointestinal system and the brain. It has been shown that various EDCs alter adipogenesis through interfering with peroxisome proliferator-activated receptor-γ (PPAR-γ) function. We have recently shown that some obesogens such as tributyltin and p,p'-DDT may affect the feeding circuits in the hypothalamus. In this talk, potential EDCs, their obesogenic effects, epidemiological studies to analyze their effects on human health, analysis methods and recommendations for prevention will be reviewed.

Keywords: endocrine disrupting chemicals, adipogenesis, infertility

DIABETIC HEART AND STRATEGIES TO MITIGATE INJURY

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Diabetes and insulin resistance leads to cardiovascular complications and increased risk for death. Myocardial infarction is a leading cause for injury and sudden death. In diabetics the incidence of MI related death is high. We studied the diabetic heart to understand the pathophysiological changes and identify strategies for cardioprotection. Diabetic mice were utilized for testing the progression of disease and physiological and pathological changes. Treatment strategies were utilized for testing the small molecule that would mitigate the insulin resistance. Treatment strategies were evaluated for diabetes or diabetes along with Ischemia-reperfusion injury. Echocardiography and ECG changes show significant beneficial changes in the diabetic heart treated with small molecule compared with vehicle treated group. Biochemical evaluation showed increased activation of pAkt, p-eNOS and Beclin signaling with small molecule treatment. The NADH/NAD levels were significantly increased in diabetic heart and treatment with small molecule significantly decreased the NADH/NAD ratio. Overall, the present study shows that activation of NAD pathway allows for cardioprotection in the diabetic mouse models. Physiological and pharmacological evaluation indicated that the novel small molecule offers cardioprotective in diabetes.

Keywords: heart, diabetes, MI, ischemia, signaling

INTERPLAY BETWEEN CLOCK GENES AND AUTOPHAGY PATHWAYS IN THE HEART

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Virtually, all tissues of the body exhibit a circadian rhythm that follows a 24-hour cycle that coordinates the timing of several key biological and homeostatic processes. Circadian rhythms are essential for human health as they regulate many processes of the human body, including sleep-wake cycles, body temperature, hormone secretion, blood pressure, heart rate, and metabolic function. Our previous work has demonstrated that circadian clocks regulate an adaptive stress response critical for cardiac cell survival by transcriptionally coordinating autophagy and mitochondrial metabolism in cardiac myocytes. Autophagy is a highly conserved evolutionary process that regulates cell quality control through protein degradation, organelle turnover, and recycling of cellular components in response to nutrient cellular stress. Herein, we show that the circadian gene, Retinoic Acid-Related Orphan Receptors α (*RORα*) is cardioprotective through modulation of autophagy in cardiac myocytes. We show that *RORα* is downregulated during hypoxia, leading to increased death of cardiac cells and enhanced mitochondrial perturbations. We demonstrate that small molecules, such as Nobiletin can induce *RORα* induction and protect from cardiac cell death. Nobiletin is a polymethoxy flavonoid found in the peel of citrus fruits. We show that Nobiletin binds directly to *RORα* promoter, leading to activation of autophagic function, rescue of mitochondrial perturbations, and increased cell survival of cardiac myocytes during hypoxia. Interestingly, loss of *RORα* activity during hypoxia resulted in inhibition of cardiac protection by Nobiletin. Furthermore, inactivation of autophagy by *Atg7* knockdown also abrogated the cytoprotective effects of Nobiletin. Collectively, these results demonstrate that *RORα* regulates autophagy through activation with Nobiletin and interdictions that activate *RORα* may prove beneficial in reducing hypoxia- induced cardiac cell death.

Keywords: autophagy, circadian rhythm, nobiletin

MESENCHYMAL STEM CELLS IN CARDIOVASCULAR CLINICAL TRIALS: ADVANCES AND CHALLENGES

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Mesenchymal stem cells (MSCs) can be easily harvested from diverse adult tissues and expanded rapidly in culture. Because of their uniquely suitable biological properties, the reparative abilities of MSCs have been tested in numerous cardiovascular clinical trials over the past two decades. These trials enrolled relatively small number of patients in each and injected MSCs from different tissues in patients with acute myocardial infarction or heart failure. MSC therapy was found to be generally safe with variable improvements in cardiac structure and function or clinical outcome parameters during follow-up. In addition, pooled data from these clinical trials have been subjected to several meta-analyses over the recent years. The results from meta-analyses also broadly indicate that MSC therapy is safe and effective for several cardiovascular pathophysiological conditions. Nonetheless, a number of significant challenges remain to be overcome in order to realize the full potential of MSC therapy in clinical practice. The purpose of this talk is to present the current clinical evidence regarding the utility of adult MSCs in therapeutic cardiovascular repair, and to discuss emerging approaches to further enhance these outcomes.

Keywords: mesenchymal stem cell, cardiovascular, clinical trial

MELATONIN AGAINST DOXORUBICIN-MYOCARDIAL TOXICITY BY HMGB1

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Doxorubicin (DOX), an anticancer medication, is frequently used to treat certain solid cancers. DOX has toxic effects on numerous tissues, including the heart, pancreas, liver, kidney, and testis. However, the most concerning side effect of DOX is its cardiotoxicity, which can lead to heart failure. As the underlying mechanisms of DOX cardiotoxicity are not fully clarified, its undesired effects are still difficult to treat. Still, it is thought to involve the production of reactive oxygen species (ROS) and oxidative stress. ROS can damage the heart muscle cells, leading to decreased cardiac function and heart failure. Therefore, it is essential to closely monitor patients receiving DOX for any signs of cardiotoxicity and take preventative measures to reduce the risk of heart damage. It is well known that HMGB1 has recently been recognized as an essential damage-associated molecular model (DAMP). The TLR4 signaling pathway is a classical pathway involved in the induction of innate immune responses by identifying endogenous DAMPs. Many clinical and animal findings have revealed that endogenous HMGB1 released by injured tissues is heavily associated with activating the TLR4 signaling pathway. Melatonin is an antioxidant shown to protect against DOX-induced cardiotoxicity in animal studies. The effect of melatonin on doxorubicin heart toxicity is based on its antioxidant and anticancer effects. By reducing the production of ROS and oxidative stress, melatonin can help protect the heart muscle cells from damage and decrease the risk of heart failure. The protective effect of melatonin on the cardiotoxicity of DOX is not fully understood. This study will discuss melatonin triggers to secrete high mobility group box-1 protein (HMGB1), attenuating TLR2 but enhancing TLR4, eventually restoring mitochondria membrane potential and apoptotic cell death.

Keywords: doxorubicin toxicity, high mobility group box-1 protein, melatonin, TLR2

SENESCENCE OF MULTIPOTENT MESENCHYMAL STROMAL CELLS: THE ROLE OF INFLAMMATION AND OXIDATIVE STRESS

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Adult multipotent mesenchymal stromal cells (MSCs) are considered to be the integrative key of regeneration of damaged tissues. In this respect, age-related changes of MSCs are an important pathophysiological issue. Functional changes in MSCs during aging reflect the shifts of epigenome and proteome (some of them are reversible). Many cell properties (including differentiation) can be related to the tissue source, methods of isolation and cultivation, enhanced heterogeneity of MSCs. The intensive examination has identified that the cell senescent is accompanied by acquisition of specific secretory phenotype, which can influence survival, proliferation and differentiation of the nearby cells. Among others, this secretome can also have a carcinogenic effect. In the light of these data, the study of the involvement of proinflammatory mediators and the polarization of MSCs during aging is very important. The prolonged cultivation of cells until the replicative senescence, when the proliferative activity decreases (Hayflick limit) remains one of the most convenient approaches to the study of cell aging in vitro. It is also necessary to consider the influence of microenvironment factors (including oxygen level). Oxidative stress is known to can activate cell aging, so it is often applied as a model of "physiological stress" leading to division arrest or apoptosis. Nevertheless, the similarity between the mechanisms of replicative and stress-induced aging remains an open question, as well as their involvement in physiological processes. Cell senescent is accompanied by epigenetic changes and shifts in gene expression associated with age-related processes. The genes encoding proteins responsible for genome integrity and transcription is downregulated. Interestingly, different gene patterns are altered in various adult progenitor cells (e.g., MSCs vs HSCs) during aging. Obviously, cell senescent under changes in microenvironment factors (extracellular matrix, intercellular contacts, paracrine factors, oxygen level) must be taken into account when analyzing the physiological and pathological processes and developing the new cell technologies.

Keywords: MSCs, senescent, microenvironment, aging

DIPEPTIDYL PEPTIDASE-4 PROMOTES HUMAN ENDOTHELIAL CELL SENESCENCE AND DYSFUNCTION BY ACTIVATION OF PAR2-COX-2-TP AXIS AND NLRP3 INFLAMMASOME

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Obesity and type 2 diabetes mellitus (T2DM) are considered progeric conditions leading to premature vascular aging. Soluble dipeptidyl peptidase 4 (sDPP4) secretion from adipose tissue is enhanced in obesity and T2DM, where it was proven to mediate deleterious effects, albeit its contribution to vascular aging is unknown. We aimed to explore sDPP4 involvement in vascular aging by evaluating sDPP4-induced endothelial senescence and its mechanisms of action *in vitro* in human umbilical vein endothelial cells. As markers of cell senescence, we determined senescence-associated-β-galactosidase activity, DNA damage, senescence-associated secretory phenotype and pro-senescence markers expression. The priming and activation of nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing-3 (NLRP3) inflammasome upregulation was determined by Western blot and indirect immunofluorescence. We used human isolated mesenteric microvessels to study vascular function in reactivity experiments. This study demonstrates that, by a common signalling mechanism, sDPP4 triggers senescence in cultured human endothelial cells and endothelial dysfunction in isolated human resistance arteries. sDPP4 subsequently activates the metabotropic protease-activated receptor-2, cyclooxygenase 2 activity and the production of thromboxane A_2, which acts on thromboxane receptors in an autocrine or paracrine manner. This cascade in turn leads to the activation of NLRP3 inflammasome and the release of IL-1b. In the pathological context of human obesity, we explored some related parameters *in vitro* and *ex vivo*. Obese patients exhibited impaired microarterial functionality in comparison to control non-obese counterparts. Endothelial dysfunction in obese patients positively correlated with greater expression of DPP4, pro-senescent, and pro-inflammatory markers in the visceral AT nearby the resistance arteries. When DPP4 activity or sDPP4-induced pro-senescent signalling pathways were blocked, endothelial dysfunction was restored back to the levels of healthy subjects. These results reveal sDPP4 as a relevant mediator in early vascular aging by activating in the endothelium main pro-inflammatory mediators, such as the NLRP3 inflammasome complex. These effects might be blocked with pharmacological tools; antidiabetic DPP4 inhibitors, as well as NLRP3 inflammasome-targeted drugs, arise as potential therapeutic interventions for tackling the *inflammaging* scenario associated to cardiometabolic diseases.

Keywords: dipeptidylpeptidase-4, endothelium, senescence, obesity, inflammaging, NLRP3-inflammasome

TH_{17}-LYMPHOCYTES AND THEIR CYTOKINES IN PATHOGENESIS OF AUTOIMMUNE THYROIDITIS, ACCOMPANIED BY PSYCHIATRIC DISORDERS

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Autoimmune thyroiditis (AIT) alters thyroid by T-cells and autoantibodies. Th17 rather than Th1 lymphocytes predominate in AIT. It is often comorbid with psychiatric disorders (PD). In schizophrenia Th17 content increases and proinflammatory cytokines overproduced. 14 patients with AIT+PD, 46 - with AIT, and 20 healthy controls (HC) were involved. Lymphocyte subpopulations were detected by flow cytometry, serum levels of hormones and autoantibodies - by ELISA; cytokines - by multiplex technique. Within the general CCR6+Th17 pool, 4 main Th17 subpopulations were identified, distinct in patterns of chemokine receptors CXCR3 and CCR4 expression, and in functions. There were "classical" Th17 with CCR4+CXCR3, "double-positive" (DPTh17) CCR4+CXCR3+, "non–classical" CCR4-CXCR3+ (Th17.1), and "double-negative" CCR4–CXCR3– (DNTh17). The percentage of DPTh17 of the central memory (CM) Th17 in the AIT+PD was higher than in AIT ($p<0.001$). The total pool of effector memory (EM) Th17, and the percentage of DPTh17 in AIT+PD was higher than in AIT ($p<0.001$) or in HC ($p=0.025$); the percentage of Th17.1 in AIT+PD was lower than in AIT ($p=0.04$). The highest level of the CCL20 (main Th17 cytokine) was in AIT+PD, the lowest in HC. Significant inverse correlations existed between Th17 subpopulations and psychiatric symptoms: e.g. between the % of DPTh17 (%EMTh17) and autism or attention deficit, the level of Th17.1 (%EMTh17) and delirium, sleep disorder, or OCD. Direct correlation existed between DNTh17 (%EMTh17) and delusions, hallucinations, or OCD. Inverse correlation existed between DPTh17 (%CMTh17) and autism. Direct correlation was between Th17.1 (%CMTh17) and sleep disorder, but the level of "classic" Th17 (%CMTh17) correlated with that symptom inversely. An inverse correlation was between DNTh17 (%CMTh17) and anxiety, but the symptom directly correlated with delusions and hallucinations. In AIT+PD the features of lymphocyte spectrum and cytokine profile may promote migration of lymphocytes including Tregs to peripheral tissues and brain, chronic activation of Th17 and their mechanistic role in AIT-related PD.

Keywords: autoimmune thyroiditis, psychiatric disorders, lymphocytes, cytokines

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CORRELATIONS BETWEEN THE EXTRACELLULAR VESICLES, INFLAMMATORY PARAMETERS AND THE PARAMETERS OF THE GLOBAL HEMOSTATIC ASSAYS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The pathogenesis of rheumatoid arthritis (RA) is complex and comprises interactions between genetics, epigenetic modifications, and environmental factors, all of which contribute to the production of specific autoantibodies, systemic inflammatory response and joint destruction. Recently, the role of microparticles was implicated in the pathogenesis of RA. Microparticles, also called extracellular vesicles (EVs), are small membrane-coated vesicles 0.1–1.0 μm in diameter that are released from various cells during cell activation and apoptosis. EVs also have important procoagulant properties based on the availability of phosphatidylserine (PS) exposed on the surface after stimulation. The aim was to identify if there is any correlation of different EVs subpopulations in the plasma of RA patients with parameters of hemostatic potential and inflammation. Twenty women with established RA were included in the study (mean age 51.85 ± 9.43 years). The mean disease duration in patients was 13.0 ± 6.6 years, and disease activity was medium to high (DAS28 was 4.1 ± 1.2), at the moment of blood sampling. RA was diagnosed according to the classification criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010. The phosphatidylserine-positive (PS^+) EVs; platelet ($CD42a^+$), leucocyte ($CD45^+$), monocyte ($CD14^+$), and endothelial ($CD144^+$)-derived EVs as well as and EVs-expressing tissue factor ($CD142^+$), P-selectin ($CD62P^+$), and E-selectin ($CD62E^+$) were determined by flow cytometry analysis. Overall hemostasis potential (OHP) was assessed to follow the hemostatic disturbances, including the parameters for overall coagulation potential (OCP) and overall fibrinolytic potential (OFP) as well as clot lysis time (CLT). Total PS^+ EVs and $CD42a^+$ correlated with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), OCP, OHP, OFP and CLT. $CD45^+$ correlated with CLT. $CD14^+$ correlated with CRP, ESR, OFP and CLT. $CD62P^+$ corelated with CRP, ESR, OHP, OFP, CLT. $CD62E^+$ correlated with OFP and CLT. Levels of circulating EVs in patients with established RA are in correlation to inflammatory burden and coagulation activation. Larger studies are needed to confirm these preliminary findings.

Keywords: rheumatoid arthritis, extracellular vesicles, inflammation, coagulation

NONSPECIFIC NEUTROPHIL PROTEINASES AND THEIR INHIBITORS: PATHOGENIC ROLE IN THE LOCAL AND SYSTEMIC INFLAMMATION DEVELOPMENT

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Neutrophils take important part in nonspecific defense of organism by mechanism of phagocytosis. But also very important role of neutrophils in processes of exocytosis and formation of "extracellular traps". In all this mechanisms take part nonspecific proteinases of granulocytes, at first elastase, cathepsins and other trypsin-like proteinases. In experimental and clinical studies, the role of nonspecific neutrophil proteinases (elastase-like and trypsin-like activity) and their endogenous inhibitors (antitrypsin activity and acid-stable inhibitors) in the development of local inflammation in the lungs and systemic inflammation in critical conditions was studied. The results showed that the development of local inflammation leads for nonspecific reaction of proteinases and their inhibitors in the blood serum with increase of proteinases and their inhibitors levels. In the lungs investigation of the bronchoalveolar lavage fluid showed more specific changes, which are characterized by a phase reactions. In the acute period the changes can be characterized as compensatory, in chronic stage of inflammation, the level of proteinases increases with a decrease activity of local inhibitors. In critical conditions, both at the local and systemic levels, an imbalance develops in the proteinase-inhibitor system, which is characterized by a decrease activity of the antiproteinase potential and increase activity of proteinases in period within 24-48 hours of decompensated stage of shock. This studies made it possible to characterize the types of reactions of nonspecific proteinases and their inhibitors at the systemic and local levels. At the blood level, compensated and decompensated types of reactions were described. At the local level, 4 types of changes are described, which can be characterized as potentiated, compensated, destructive and decompensated. Depending on the types of reactions, it is possible to evaluate compensatory potential and decide on the use of proteinase inhibitors for therapeutic purposes. In addition, in critical conditions, the assessment of the proteinase-inhibitory balance made it possible to propose an original classification of shock. It is proposed to shock classification with primary and secondary formation of the systemic inflammatory response syndrome (SIRS). In the situations with the primary development of the SIRS, the accumulation of proteinases and cytokines had a primary nature due to damage to organs and tissues, in the secondary - development of the SIRS with activation and accumulation of proteinases and cytokines is a consequence of primary hemodynamic disorders. Various approaches to the implementation of therapeutic measures depending from the types of shock have been proposed.

Keywords: proteinases, proteinase inhibitors, inflammation, shock

THE ROLE OF INFLAMMATION AND APOPTOSIS IN FORMATION OF MULTIPLE ORGAN DYSFUNCTION SYNDROME AT CRITICAL STATES

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One of the most dangerous complications of critical states, which lead to the death of patients, is the multiple organ dysfunction syndrome (MODS). Development of organ injury may depend from activation of inflammatory damage and apoptosis of organ cells. But what factor may play more important role in organ injury is still unstudied. We studied the organs pathology development by using the model of reperfusion injury by applying the tourniquets on both posterior limbs of rats. Revasculization was provided 6 hours after applying the tourniquets. The activity of proteinase-inhibitory system components, concentration of cytokines IL-1β, IL-6 and TNF-α was determined. The morphological changes in the lungs, liver, kidneys, heart and intestine were studied using immunohistochemical (IHC) methods by detect total leukocyte antigen (CD 45), natural killer (CD 56), macrophage marker CD 68 and apoptotic markers bcl-2, CD95. Also caspase-3 expression was determinate by PCR. Results shown, that under reperfusion injury the activation of nonspecific proteinases occurs within 24 hours on the local (injured muscular tissue), systemic (blood serum) and organ levels (bronchoalveolar lavage, peritoneal fluid, tissues of kidney and liver). At the same time we have found more than tenfold grow of IL-1β and more intensive grow of IL-6 and TNF-α during 12-24 hours after the reperfusion. On microscopic level we have found the signs of inflammatory and necrosis changes in the target organs and ultrastructural cellular changes. IHC examination showed that in all organs activation of apoptosis was detected. Bcl2 marker decrease in all organs and FAS ligand (CD95) increase. But the level of changes in different organs varied. Thus, in the lungs, the decrease of Bcl2 and the increase of CD95 were more intensive compared to the kidneys and liver. Also, the increase in caspase-3 expression was more intensive in the lungs. This may indicate a greater sensitivity of the lungs to damage in critical conditions, which is manifested by earlier lung damage in critical conditions with the development of acute respiratory distress syndrome. The obtained results allow to conclude that excessive systemic activation of pro-inflammatory cytokines and nonspecific proteinases under decrease of inhibitor control may play an important role in activation of injury processes in the target organs by formation of numerous inflammatory foci and lose control of apoptosis. All these changes can leads to development of multiple organ dysfunction syndrome.

Keywords: inflammation, apoptosis, proteases, cytokines, caspase, organ dysfunction

MICRORNAS IN THE HEART-BRAIN AXIS DYSFUNCTION: THE STRANGE CASE OF MIRNA29

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The heart-brain axis (HBA) represents the set of circuits that control the bidirectional flow of information between the heart and brain, which can be altered during aging. Unfortunately, the lack of clinically relevant animal models hinders our understanding of the mechanisms and the development of therapies. Differential expression of microRNAs (miRNAs), a type of small noncoding RNAs, is being studied as a possible mediator of HBA dysfunction in the context of aging. The main objective of our research was to investigate the pathogenic role of miRNA-29b in HBA dysfunction, a new player in human aging, heart failure, and cognitive dysfunction. We generated thirteen male miRNA-29b knockout minipigs using the CRISPR-Cas9 strategy. Wild-type progeny (WT, six male minipigs) were used as controls. We then phenotypically characterized brain and heart function of sedated pigs at 4 months after birth using a multimodal approach based on magnetic resonance imaging (MRI, 1.5 Tesla) and 18F- fluorodeoxyglucose (18F-FDG) positron emission tomography (PET). Transgenic minipigs compared to WT animals of the same age showed a significant reduction of left ventricular (LV) ejection fraction due to enlargement of LV end-systolic and end- diastolic volume, while the heart rate was slightly reduced ($p<0.05$). The reduction in global brain 18F-FDG uptake, a hallmark of aging, was globally reduced ($p<0.05$) in fasted transgenic animals compared to WT minipigs of the same age. Our study demonstrates for the first time that knockout of miRNA-29b recapitulates functional features of the aged brain and heart in 4-month-old minipigs. Our transgenic pig model generated by targeting miRNA-29b provides the opportunity to better understand the mechanisms underlying aging-associated HBA dysfunction in a clinically relevant manner.

Keywords: heart-brain axis, miRNA-29b, aging

THE EMERGING ROLE OF MUSCLE ENRICHED A-TYPE LAMIN INTERACTING PROTEIN IN CARDIO-PROTECTION

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Cardiovascular disease is one of the leading causes of death in developed countries, putting a strain on medical resources. Despite advances in cardiovascular research and treatment, the mechanism(s) by which cardiomyocytes identify pathophysiological stressors and influence beneficial cardiac remodelling and adaptability remain unknown. According to extensive genetic studies, many genes and processes that regulate heart development in health and disease are poorly understood. The molecular basis of physiological (reversible) myocardial development and the early "stress responders" that initiate and aid cardiac adaptation is unknown. Muscle-enriched A-type lamin-interacting protein (MLIP), a newly discovered protein with unknown function, is required for cardiac adaptation to stress. Stress-induced hypertrophy and cardiac failure were accelerated in the hearts of MLIP-deficient mice. Transgenic and AAV-MLIP overexpression prevented pathologic remodelling while maintaining heart function. In an exome-wide array-based association study (EWAS), MLIP was one of eight loci independently linked to sporadic dilated cardiomyopathy. Several rare MLIP variants in children have recently been reported and linked to myopathy characterized by mild muscle weakness, exercise-induced muscle pain, variable susceptibility to rhabdomyolysis, and persistent basal elevated serum creatine kinase levels, as well as possible cardiac involvement with age. Collectively, evidence will be presented supporting the hypothesis that MLIP's cardio-protection results from directly activating pro-survival pathway(s) that induce favourable adult heart hypertrophy and adaptability. The overall objective of harnessing MLIP's potential therapeutic potential, thereby offering new hope for heart failure patients and shedding light on MLIP's vital role in preserving pro-survival pathways in healthy and ailing hearts.

Keywords: adaptation, pro-survival, cardiovascular disease

HIGH SALT-INDUCED HUMAN VASCULAR REMODELING AND TYPE 2 DIABETES

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Although insulin-induced cardiac hypertrophy is reported, very little information is available on the hypertrophic effect of insulin on human vascular smooth muscle (hVSMCs) and the regulation of hypertrophy and its associated increase in sodium and calcium homeostasis. In addition, diabetes patients regulate their diet to decrease the hyperglycemia associated with type 2 diabetes. However, they ignore their high consumption of sodium salt. Thus, high salt consumption may contribute to a higher increase in blood pressure associated with type 2 diabetes. Quantitative 3D imaging and ionic fluorescent probes were used in human vascular smooth muscle cells. Our results showed that hyperglycemia-induced remodeling of hVSMCs and high salt further aggravated remodeling by inducing a genomic memory for high salt. Taurine is a non-essential amino acid in low quantities in many foods, particularly seafood. It is also known to be an endogenous and exogenous powerful antioxidant. Our results showed that chronic treatment with taurine prevents the remodeling of hVSMCs induced by hyperglycemia associated with high salt-induced genomic memory of sodium. Hyperglycemia associated with hypernatremia induced a permanent remodeling of hVSMCs that could be prevented by taurine supplementation. This induced relaxation of arteries leading to a decrease in blood pressure. A better understanding of the effects of high Na^+ salt on the morphological remodeling of hVSMCs in type 2 diabetes may permit the development of new therapeutic interventions for treating salt-sensitive hypertension in type 2 diabetes and preventing associated cardiovascular diseases.

Keywords: salt-sensitive hypertension, type 2 diabetes, vascular remodeling

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BACTERIA AND CARDIOVASCULAR DISEASE

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Infectious disease and inflammation are thought to be involved in a variety of chronic diseases including heart disease. Chlamydia Pneumoniae is typically thought to be an infectious agent for lung disease. However, it has also been associated with atherosclerotic heart disease. The talk presented in this meeting will address the questions of a direct involvement of Chlamydia Pneumoniae in cardiovascular disease. How does a lung infection cause atherosclerosis? Can Chlamydial infection actually induce atherosclerosis directly? If so, what is its mechanism of action? What factors modulate the atherogenic response in the vasculature to this infectious agent?
Basic biochemical analyses will be used including histochemical tests, molecular biology and microbiological techniques. Data will be presented using both animal models of infection and cell culture approaches. Bacterial infections can induce significant changes in cardiac and vascular structure and function. Ultimately, we believe that bacterial infections like Chlamydia Pneumoniae can directly induce atherogenesis but the environment plays a critical role in modulating this response.

Keywords: Chlamydia Pneumoniae, cardiovascular disease

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HEALTHFUL FOODS AND LIFESTYLE MODIFICATIONS ARE THE BEST COST-EFFECTIVE STRATEGIES FOR THE PREVENTION OF CARDIOVASCULAR AND CARDIOMETABOLIC DISEASES

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Incidences of obesity, diabetes, cardiovascular and cardiometabolic diseases and healthcare costs are escalating globally. There is a great surge for finding cost-effective measures for the prevention of cardiovascular (CVD) and cardiometabolic diseases (CMD) associated with type 2 diabetes and obesity. *Hippocrates - Father of Medicine (ca.460-370 B.C.) - advocated the healing effects of foods: He said: "Leave your drugs in the chemist's pot if you can heal the patient with food"*. It is now well recognized that intake of healthful foods rich in flavonoids/polyphenols/carotenoids: namely fresh fruits and vegetables, and probiotics/prebiotics as well as lesser consumption of salt and sugar-loaded drinks, less saturated fat, smoking cessation, and moderate exercise (30 min/day), collectively help in the prevention of CVDs and CMDs. This holistic approach may be the most cost-effective method for health promotion and prevention of chronic diseases like cancer, diabetes, obesity, CVDs, CMDs, and neurodegenerative disorders. Overwhelming evidence suggests that Mediterranean-type diet consisting of whole grains, omega-3-fatty acid, poultry, nuts and seeds, olive oil, dairy products, less red meat, and moderate consumption of red wine are linked to the reduction of mortality and morbidity associated with CVDs and CMDs. Ingestion of functional foods, vitamins, minerals, and amino acids assist to improve overall health beyond basic nutritional functions. Emerging evidence suggests that dietary supplements containing flavonoids, carotenoids, and antioxidants modulate gene and protein expression and thereby modify endogenous metabolic pathways and homeostasis, and consequently reduce the risk of chronic diseases multifactorial in origin. The beneficial effects of plant-derived bioactive compounds and/or their metabolites are attributed to their combined anti-oxidant and anti-inflammation actions. Probiotics/prebiotics/symbiotics confer health benefits on the host through the promotion of healthy microbiota in the gut, improvement of gut endothelium integrity, and boosting the immune function in the body. Several well-designed studies have indicated that the incidence of nongenetic CVDs can be reduced by 75-80% by making lifestyle changes, eating healthful foods, and physical activity. The preventive strategies for CVDs and CMDs must be targeted at the primary health promotion level before some of the important underlying causes of these diseases seriously afflict an individual or a population at large. Such preventive approaches would not only help in reducing work-related absenteeism due to prolonged hospitalization, but would also decrease the costs of drugs and healthcare providers that impose high economic burdens on the healthcare systems of developed and developing countries. This communication will address the etiology and risks involved in the occurrence of CVDs and CMDs, and highlight the cost-effective non-pharmacological interventions for the prevention of these diseases.

Keywords: cardiometabolic diseases, diabetes mellitus, Mediterranean-diet, smoking cessation, exercise

PATHOPHYSIOLOGICAL BACKGROUND FOR METABOLIC SYNDROME CORRECTION WITH GRAPE POLYPHENOLS

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Metabolic syndrome (MS) is a complex of metabolic and organ disorders accompanied by changes in various cellular receptors' expression. The dynamics and effects of cellular receptors (PPARγ, GLUT4 and TLR4) in experimental MS and their correction by polyphenolic grape processing products (PGPP) were studied. In the group of modelled MS inflammatory response occurred - the rise of C-reactive protein (CRP) by 2 times ($p < 0.05$) and TLR 4 – by 5 times ($p < 0.05$), correlated positively together ($r=0.62$) were detected. The activation of lipid peroxidation (LP) proved by thiobarbituric acid products (TBA) increase by 1.43 times ($p <0.05$), decrease of peroxidase activity (PA) by 1.4 times ($p < 0.05$) and trypsin-like activity(TLA) by 2.2 times ($p < 0.05$) and a negative correlation between cholesterol concentration (Ch) and TLR 4 ($r= -0.6$). Also, decline in antioxidant protection (rise of catalase-like activity (CLA) by 5 times ($p < 0.05$) and ceruloplasmin (CP) by 2.2 times ($p < 0.05$)) was stated. Insulin resistance (IR) was confirmed by hyperglycemia, 2-time increase in GLUT 4 ($p < 0.05$) and a negative correlation between GLUT 4 and glucose ($r=-0.6$). IR resulted in an excessive synthesis of atherogenic lipids and intracellular accumulation of fats. This was confirmed by the rise of Ch and triglycerides' (TG) concentrations by 1.5 times ($p < 0.05$), reduction of high-density lipoproteins (HDL) by 1.9 times ($p < 0.05$), and enlargement of adipocytes by 2.1 times ($p < 0.05$), correlating positively with the concentration of Ch ($r =0.7$). Correction of MS by PGPP resulted in reduction of IR, and normalization in lipid metabolism. This was proved by serum glucose's two-times decline ($p <0.05$), normalization of adipocyte sizes, drop in TG, Ch and HDL concentrations ($p <0.05$) and a positive correlation between GLUT 4 and Ch ($r=0.7$). Inflammation inhibition confirmed by two- times drop of both TLR 4 and CRP ($p <0.05$). These tendencies combined with two- times increase in GLUT 4 and PPARy ($p <0.05$), a positive correlation between glucose and CRP ($r=0.7$), GLUT4 and CRP ($r=0.7$), CRP and HDL concentrations ($r=0.63$). An inflammatory oppression entailed the restoration of antioxidant systems and inhibition of LP manifested by a 2-times increase in CP ($p <0.05$), and negative correlation between PA and adipocyte size ($r=-0.6$). Thus, through the modulation of PPARs, TLR 4 and GLUT 4 receptors, PGPP caused the correction of IR in experimental MS.

Keywords: metabolic syndrome, molecular markers, receptors

SIRT3 REVERSES RENAL DYSFUNCTION BY TRANS-SULFURATION PATHWAY

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High protein diet is currently popular among weight watchers and people with type 2 diabetes. Although controversies exist regarding high protein diet (HPD) and renal health, the effect of long-term high protein intake on the kidney remains unclear. HPD is known to increase homocysteine levels (HHcy). Besides, dietary protein intake is strongly correlated with calcium and phosphorus intake that leads to hypercalciuria and hyperphosphatemia. Increased metabolite load leads to glomerular hyperfiltration to eliminate waste products. Overtime, chronic hyperfiltration can increase glomerular and tubular pressure causing damage. Further HPD induced HHcy can cause vascular atherosclerosis. Homocysteine metabolism yields methyl donors that are involved in DNA methylation reactions for several cellular functions. In pathological conditions, elevated homocysteine levels increase DNA methylation to suppresses gene expression in turn leading to renal injury. Defects in transsulfuration pathway increases homocysteine levels and disrupts redox homeostasis in the mitochondria to worsen the injury. There is strong evidence that HPD is linked to nephrotic syndrome, disturbances in the calcium and phosphorus metabolism leading to mitochondrial dysfunction. In this study, we hypothesize that defective epigenetic pathway creates HHcy, homocystinuria, dysregulates trans-sulphuration pathway, and mitochondrial bioenergetics, causing renal dysfunction. C57BL/6J mice (WT, 12-14 wks) were used in the study and fed normal diet (ND) or high protein diet (HPD) for 7 m. Honokiol (Hon), Sirt3 activator, was given at 0.2mg/Kg/d by i.p injections for 28 d. Capsaicin given at 0.3mg/Kg/d by i.p injections for 28 d. In HPD treated animals, plasma homocysteine, and phosphate levels were increased. HPD kidneys demonstrated epigenetic derangement (↑ DNMT, SAHH and AceCS1) and downregulated transsulfuration pathway (CBS/CSE/MPST enzymes), and was associated with mitochondrial stress, increased MMP-9 activity, and interstitial fibrosis. Honokiol (Sirt3 activator) treatment and TRPV1 agonism (Capsaicin) normalized homocysteine and phosphate levels, and the expression of VDAC1 and TRPV1 channels in the glomeruli and tubules. Sirt3 and Capsaicin attenuated mitochondrial stress, MMP-9 activity, and fibrosis in the kidney. HPD increased renal blood flow and caused hyperfiltration. Sirt3 treatment restored the altered renal blood flow and GFR in HPD kidneys. HPD causes hyperhomocysteinemia, disruption of transsulfuration pathway, defective TRPV1 and VDAC1 expression, mitochondrial and renal dysfunction. Sirt3 activation and TRPV1 agonism protects the mitochondria by modulating epigenetic mechanism and restoration of transsulfuration pathway thus kidney function.

Keywords: renal dysfunction, trans-sulfuration pathway, high protein diet

PATHOGENETIC SUBSTANTIATION OF THE NEPHROPROTECTIVE ACTION OF POLYPHENOLS SUBSTANCES AND ARBS IN AN EXPERIMENT

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Metabolic syndrome (MetS) is called the "pandemic of the XXI century" because there are more than 300 million people with this pathology in the world. One of the complications of MetS is the development of the chronic kidney disease (CKD). These worsen the prognosis of the underlying disease and the quality of patients of life. This study aim to study the mechanisms of development of MS and related nephropathies, the effectiveness of polyphenolic substances and angiotensin-II receptor antagonists for their adjustments in the experiment. In this study, 48 Wistar rats (age 2.5 months) of the SPF category were used. The animals were divided into 4 groups (n=12): 1) animals with MetS; 2) animals with MetS and Azilsartan's correction; 3) animals with MetS and correction by grape-derived stilbene concentrate ("GDSC"); 4) control group. The first 3 groups were fed for 24 weeks, with standard solid feed containing 60% fructose. The criteria of the International Diabetic Federation (FDA, 2005) were used for substantiate the development of MetS. The induced of MetS was accompanied by the development of classic signs: abdominal obesity, hyperglycemia, high lipid levels and hyperuricemia. The visceral fat mass was increase in the group with MetS by 2.5 times ($p<0.001$). The blood glucose level was 6.9 mmol/l in the MetS group which exceeded the indicator of the control group by almost 12.5 times. The use of " GDSC " led to a decrease blood glucose to 4.4 mmol/l, Azilsartan – to 4.8 mmol/l. Morphometric analysis showed that the glomerular area in rats of the MetS group decreased by 32.7%. The use of Azilsartan and "GDSC" increased the glomerular area by 15.5% and 7.1%. The significant decrease in the area of the urinary space in the renal body in the group with MetS was observed in comparison with the control group ($p<0.001$). The area of the urinary space increased in the group animals that took Azilsartan (by 24.7%) and "GDSC" (by 14.6%) compared with the experimental group. Glomerulosclerosis, arteriol hyalinosis and fibrosis are observed in the kidneys against the background of MetS. The current of the MetS was accompanied by the formation of all MetS main features. Hyperglycemia has affected various morphological structures kidneys this led to the development of nephropathy. "GDSC" and Azilsartan had a positive effect on the course of both MetS and nephroprotective effect.

Keywords: metabolic syndrome, chronic kidney disease, grape-derived stilbene concentrate, polyphenols

ANTIPHOSPHOLIPID ANTIBODIES AS INDEPENDENT PREDICTORS OF CARDIOVASCULAR FAILURE: MULTIDISCIPLINARY APPROACH IS THE KEY TO THE SUCCESS

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A major cause of morbidity and mortality in the context of the antiphospholipid syndrome (APS) is the occurrence of thrombotic events, including cardiac involvement.
On the other hand, accelerated atherosclerosis proven in APS, is accountable for the development of coronary and peripheral artery disease. This leads to a higher cardiovascular mortality rate in the population of patients with low incidence of the traditional atherosclerosis risk factors. The literature shows evidence of an association between APS and the occurrence of accelerated atherosclerosis. We investigated 585 patients: 404 pts with PAPS followed up for an average of 44.00±12.97y., and 148 pts with secondary APS (SAPS) in scope of SLE (47.74±14.84y). Antiphospholipid antibody (aPL) analysis included detection of aCL (IgG/IgM), ß$_2$GPI (IgG/IgM). Carotid ultrasound was performed, and the intima-media wall thickness (IMT) and presence of plaque was investigated in all patients and controls. Traditional vascular risk factors and APS-disease and treatment related factors were also analyzed. Presence of aCL IgG was more common (p=0.001) in SAPS, and LA in PAPS patients (p=0.002). More than one type of antibodies (category I) was present in 64.5%. Age was a significant risk factor for myocardial infarction (MI): 56.6 and 43.6 years, respectively (p=0.0001). Highly statistically significant difference was revealed considering presence of aβ$_2$GPI antibodies and carotid arteries plaque presence (p= 0.020), in pts with PAPS and aβ$_2$GPI (0.049), as well PAPS pts with smoking (p=0.008). PAPS and SLE patients did not differ among themselves regarding the occurrence of MI (p = 0,102), and UAP (p = 0.123) unstable angina pectoris (UAP), but presence more than 2 aPL was a significant risk factor for UA (p=0.017). Certain aPL types and levels are associated with distinct cardiovascular manifestation, suggesting their predictive role. In this subgroup of APS patients, a more aggressive approach towards prevention and control of standard atherosclerotic risk factors is crucial.

Keywords: antiphospholipid syndrome, antiphospholipid antibody, the intima-media wall thickness, myocardial infarction, unstable angina pectoris

REDISTRIBUTION OF CONNEXIN 43 PLAYS IMPORTANT ROLE ON LONG-QT CHARACTERIZED CARDIAC REMODELING IN INSULIN-RESISTANT ELDERLY MAMMALIAN HEART

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The regular heart rhythm and normal heart contractile activity can be maintained by a coordinated electrical activation in the heart. An electrical non-uniformity appears by the clustering of gap junction channels which contributes to remodeling processes in heart disease. Any change in gap junctions, therefore, can have an important impact on the modulation of arrhythmogenic mechanisms in the heart, via disruption of synchronous activation. The heart function insufficiency of elderly humans is usually characterized with a marked risk of ventricular arrhythmias. Also, there is a relationship between the complex spatial and temporal conduction properties of the heart with Connexins (Cxs), however, there is no exact mechanisms provided these relationships. So, we aimed to examine a correlation between long-QT and status of Cx43 phosphorylation and localization in elderly rats. The correlation between long-QT and connexin 43 (Cx43) status and localization in elderly rats was determined to demonstrate a correlation between insulin resistance (I-R), ischemia-reperfusion, aging, and heart dysfunction. Male Wistar rats are grouped as 24-month-old rats (Aged-group), those with metabolic syndrome (8 months old; MetS-group), or controls (8 months old; Con-group). Both experimental groups have long-QT and low heart rate. Immunohistochemical imaging and quantification showed marked decreases in Cx43 staining of intercalated disc with less localizations in the Aged-group and MetS-group. The lateralization of Cx43 on longitudinal cell membrane was significantly high in the MetS-group than in the Con-group with no significant change in the Aged-group. Its significant cytoplasmic internalization was higher in the Aged-group than in the MetS-group. There were marked decreases in phospho-Cx43 (pCx43) staining of intercalated disc with less localizations in both groups than in the Con-group. Furthermore, lateralization of pCx43 was significantly low in the Aged-group and MetS-group, whereas there were no significant changes in the cytoplasmic internalization of both groups compared with the Con-group. Furthermore, the ratio of pCx43 to Cx43 was significantly small in both groups. We determined increases in RhoA and endothelin-1 in both groups, further supporting decreases in pCx43. Our data indicate the important role of I-R on long-QT in aging heart through alterations in both Cx43 protein level and localizations, leading to an abnormal spreading of ventricular repolarization in I-R heart.

Keywords: hart function, ECG, insulin resistance, aging, metabolic syndrome

CIRCULATING VASOPRESSIN CONTRIBUTES TO THE DEVELOPMENT OF HYPERTENSION VIA A SWITCH IN BAROREFLEX INHIBITION TO EXCITATION

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The posterior pituitary hormone, vasopressin, is a potent vasoconstrictor. However, circulating vasopressin levels are paradoxically elevated in some hypertensive with but it is unknown what increases vasopressin levels in hypertension and whether circulating vasopressin contributes to the development of hypertension. Secretion of vasopressin is triggered by action potential firing in hypothalamic vasopressin neurons, which is normally inhibited by baroreflex activation of inhibitory GABAergic inputs to vasopressin neurons. Therefore, we hypothesised that blunted baroreflex inhibition of vasopressin neuron activity would increase blood pressure during the development of hypertension. Conscious transgenic Cyp1a1-Ren2 rats with inducible angiotensin-dependent hypertension were made hypertensive over seven days in the presence or absence of concurrent subcutaneous administration of the vasopressin V_1 receptor antagonist, dGly[Phaa1,d-tyr(et), Lys, Arg]vasopressin. Extracellular single-unit recordings of vasopressin neurons were made from urethane-anaesthetised rats and whole-cell patch-clamp recordings were made from vasopressin neurons in brain slices to determine whether hypertension affects vasopressin neuron activity and their GABAergic inputs. V_1 receptor antagonism did not affect blood pressure in non-hypertensive Cyp1a1-Ren2 rats but prevented the increase in blood pressure between days 3 and 7 of the induction of hypertension. Vasopressin neuron firing rate was higher in hypertensive rats than in non-hypertensive rats on day 7 and intravenous administration of the α_1-adrenoreceptor agonist, phenylephrine, caused baroreflex inhibition of vasopressin neurons in non-hypertensive rats, but not in hypertensive rats. In patch-clamp recordings, the $GABA_A$ receptor antagonist, bicuculline, excited vasopressin neurons from non-hypertensive rats and the $GABA_A$ receptor agonist, muscimol, excited vasopressin neurons from hypertensive rats. Taken together, our results suggest that increased vasopressin secretion contributes to the development of hypertension and is driven by reduced baroreflex inhibition of vasopressin neurons via a switch in GABA inhibition to excitation.

Keywords: hypothalamus, vasopressin, hypertension

OPPOSITE EFFECTS OF MELDONIUM IN ACUTE ISCHEMIA/REPERFUSION INJURY VERSUS FAECAL- AND LPS-INDUCED SEPSIS IN RAT

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Acute ischemia/reperfusion (I/R) is a clinical condition challenging to treat. Sepsis is a life-threatening condition, characterized by the exaggerated pro-inflammatory response and disturbed lipid metabolism leading to sequential organ failure. Meldonium is an anti-ischemic agent that shifts energy production from fatty acid oxidation to less oxygen-consuming glycolysis. Thus, we investigated the effects of meldonium pre-treatment on the acute I/R liver and kidney injury and faecal- and LPS-induced sepsis in male rats. Glucose, lactic acid, carnitine, FA, and glycerol were measured as markers of meldonium metabolic action. The extent of tissue injury was assessed by histology and NF-κB p65 activation and haptoglobin expression, with the Bax/Bcl-2 ratio and HMGB1 as the apoptotic and necrotic markers. The antioxidant defence was assessed by level of lipid peroxidation, Nrf2 activation and haem oxygenase 1 expression, SH groups and glutathione concentration, and activity of CuZnSOD, MnSOD, catalase, glutathione peroxidase, reductase and S-transferase. In liver and kidney meldonium reduces inflammation and injury and increases antioxidative defence. In contrast, in both model of sepsis meldonium increases mortality. Meldonium pre-treatment may represent a protective agent against I/R-induced injury in both the liver and the kidney, whit the potential clinical significance in surgical procedures. Paradoxically, but the same mechanism which makes meldonium protective in I/R also makes him detrimental in sepsis, underlining the importance of uninterrupted energy production in sepsis, and closely drawing attention to the possible harmful effects of lipid-mobilization impairment caused by certain therapeutics. This could lead to the much-needed revision of the existing guidelines in the clinical treatment of sepsis while paving the way for discovering new therapeutic approaches.

Keywords: ischemia/reperfusion, meldonium, LPS-induced sepsis

THE EFFECTS OF DIFFERENT EXERCISE TYPES ON A RAT MODEL OF MYOCARDIAL ISHEMIA/REPERFUSION INJURY

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The aim of present research was to examine the influence of different swimming and running protocols, as forms of physiological preconditioning on an isolated rat heart's ischemia/reperfusion injury. The study was conducted on male Wistar albino rats divided into groups following an aerobic or an anaerobic swimming and running protocol. Aerobic and anaerobic swimming trainings lasted 9 weeks while aerobic and anaerobic running trainings lasted 6 weeks. After the preconditioning protocols were completed, an *ex vivo* estimation of myocardial function was performed using the Langendorff technique. After a 30-minute global ischemia session, a 30-minute reperfusion period started. Different parameters for assessing myocardial function were measured with a sensor placed in the left heart ventricle: maximum and minimum rate of pressure development in the left ventricule (dP/dt max and dP/dt min), systolic and diastolic left ventricle pressure (SLVP and DLVP) and heart rate (HR). Coronary flow (CF) was measured flowmetrically. Our results show that anerobic running training decreased heart rate values while anerobic swimming training reduced coronary flow. These differences can be explained with the different physiological response of the heart to aerobic/anaerobic physical training. All data from this experimental study support many training effects: improved contractility, resting heart rate and increased physical work capacity and exercise tolerance. Anaerobic running physical training induces greater heart preconditioning to reperfusion injury in comparison to anaerobic swimming training. Physiological response to aerobic swimming physical training is considered more valuable for improving the exercise tolerance and coronary circulation.

Keywords: preconditioning, ischemia/reperfusion, swimming, running, rats

POSSIBILITY OF STUDYING THE RECEPTOR APPARATUS AND PROLIFERATION IN CELL CULTURES OBTAINED FROM LUMINAL A SUBTYPE BREAST CANCER TUMORS

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Breast cancer (BC) is a heterogeneous disease. There is evidence of changes in the cell receptor apparatus in the process of metastasis. The study of this process using primary cell cultures contributes to the expansion of knowledge about carcinogenesis. Immunohistochemical (IHC) analysis of the primary tumor was conducted in autostainer Dako (Denmark) using antibodies for Her2/neu (clone 4B5, Rabbit Monoclonal primary Antibody, Ventana, USA), Ki-67 protein (Clone SP6, Spring Bioscience, USA), estrogen receptor (clone 1D5, DAKO, Denmark) and progesterone receptor (clone PgR636, DAKO, Denmark). To obtain cell culture, tumor surgical specimens were crushed and cells were isolated by enzymatic dissociation. Cells were grown for 10 days, at which point they established a monolayer and were reseeded. Mammocult nutrient medium (STEMCELL, Canada) was used for cultivation. The fluorescence level of stained cells was assessed using a Navios 10 flow cytofluorimeter (Beckman Coulter, USA) and antibodies for vimentin (Alexa Fluor® 405 Anti-Vimentin antibody), pancytokeratin (Alexa Fluor® 488 Anti-pan Cytokeratin antibody)**,** estrogen receptor (Alexa Fluor® 647 Anti-Estrogen Receptor alpha antibody), Ki-67 proliferation marker (488 Anti-Ki67 antibody, SP6 Abcam, Canada). Cases were selected for IHC evaluation based on high expression of estrogen, progesterone, low Ki-67 cell proliferation index, absence of HER2 gene amplification, which is typical for Luminal A molecular biological BC subtype. At the first passage (P1), the cell culture showed high values of estrogen expression level and an increase in the Ki-67 cell proliferation index compared to the original tumor samples. Co-expression of estrogen receptor and Ki-67 proliferation marker averages 41.7% of cells. No cells stained only for vimentin were found. Most cells exhibited an epithelial phenotype, the rest expressed both vimentin and cytokeratin (about 7.5% of cells). At second passage (P2), there is a sharp decrease in the number of cells with co-expression of estrogen receptor and Ki-67. The population of cells with an epithelial phenotype is reduced in culture. Also, cells expressing only vimentin appear. In cultures of the Luminal A BC at 1st passage, the expression of estrogen receptors in tumor cells is reduced against the background of sufficiently high levels of proliferative processes. At 2nd passage, manifestations of epithelial-mesenchymal transition occur: appearance of cells expressing only vimentin, increase in the number of cells with keratin and vimentin co-expression, while there is a general decrease in the proliferative activity of cell culture.

Keywords: breast cancer, primary cell culture, ER receptors, proliferation, cytoskeleton

THE ROLE OF ANGIOGENIC PATHWAYS IN MECHANISMS OF RESISTANCE IN COLORECTAL CARCINOMA

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Colorectal carcinoma (CRC) is a heterogeneous group of oncological processes with specific molecular features, the most significant of which are mutations in the KRAS, NRAS, BRAF genes, HER2neu gene amplification, and microsatellite instability. In routine clinical practice, based on their presence / absence, not only the prognosis of the disease is determined, but also the predictive effect of therapy with targeted drugs and checkpoint inhibitors is assessed. Despite a wide range of CRC treatment methods, the disease often has a steadily progressive course with the development of resistance to various types of drug therapy and high mortality rates, which actualizes the details of the mechanisms of resistance to ongoing therapy courses. Indicators of local activity of neoangiogenesis (HIF1a, VEGF-A, Angiopoetin1, Angiopoetin2, Tie2, CD163) were studied on operation material in 152 patients with colorectal carcinoma, divided into groups depending on the degree of response to neoadjuvant therapy: TRG1-2 (n=30), TRG 3-4(n=90), TRG5(n=32). Morphometric analysis was carried out using ImageJ and ImageScope programs. The statistical significance of the differences was determined using the nonparametric Mann-Whitney U-test. As a result of our study of the microenvironment of colorectal carcinoma, the presence of 2 independent pathways of angiogenesis activation was established: 1. VEGF-dependent, mediated through the tissue hypoxia factor and correlated with the activity of tumor-associated macrophages; 2. Angiopoietin-dependent mediated by tumor cells and cells of the tumor microenvironment, primarily tumor-associated macrophages. In all patients with colorectal carcinoma, a high intensity of angiopoietin-dependent angiogenesis is determined, and therefore, the use of targeted therapy aimed at blocking Angiopoetin1 and Angiopoetin2 is pathogenetically justified. VEGF-A and Tie2 demonstrate heterogeneity of immunohistochemical reactions in the tumor bed in the form of overexpression ("hot tumors") in patients with TRG1-2, moderate activity in TRG3 and weak activity ("cold tumors" in TRG4-5). Before antiangiogenic therapy with VEGF blockers-dependent angiogenesis (bevacizumab, aflibercept and regorafenib), it is recommended to conduct an IHC study of surgical or biopsy material of the tumor in order to assess the VEGF-A and TIE2 status of the tumor to determine the predictive effect of targeted therapy. The optimal type of therapy for tumors with an intermediate and hot immunophenotype for VEGF expression is combined Bevacizumab or Aflibercept regimen with an isolated Angiopoetin2 blocker, trebananib, or a combined Angp2 and VEGF blocker, vanucizumab.

Keywords: colorectal cancer, angiogenesis, resistance

HUMAN AMNIOTIC MEMBRANE HOMOGENATE AS A NOVEL THERAPY FOR INHIBITING BLADDER CANCER CELL MIGRATION AND INVASION

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Bladder cancer is one of the ten most commonly diagnosed cancers in the world. At the time of diagnosis, approximately 25% of patients have muscle-invasive bladder cancer, which is characterised by a poor prognosis and often leads to the formation of metastases. Human amniotic membrane (hAM) is an extraembryonic membrane with anti-inflammatory, antifibrotic and antimicrobial properties. Despite the growing number of studies, the cell biological mechanisms associated with its anticancer activity are poorly understood. In the present study, we investigated the mechanisms of action of hAM homogenate on bladder cancer and normal urothelial cells. In the present study, we investigated the effect of hAM homogenate on the migration rate of normal porcine urothelial NPU cells, human papillary cancer urothelial RT4 cells and human muscle-invasive bladder cancer T24 cells, and on the invasion of the latter. Using time-lapse confocal microscopy, we examined the effect of hAM homogenate on the migration pattern of T24 cells stably transfected with enhanced green fluorescent protein (eGFP). We also measured the expression of small RhoGTPases involved in actin cytoskeleton remodelling. Focal adhesion kinase (FAK) expression and phosphorylation were assessed by Western blot analysis, while *protein tyrosine kinase 2* (*PTK2)* expression was quantified by RT-qPCR. Our study revealed that hAM homogenate impedes the migration rate of bladder cancer RT4 and T24 cells, but not of normal urothelial NPU cells. In addition to cell migration, hAM homogenate significantly decreased the invasion rate of muscle-invasive bladder cancer T24 cells. Moreover, hAM homogenate exerted its antimigratory effect by downregulating the expression of FAK and RhoGTPases, key proteins involved in actin cytoskeleton reorganisation. In conclusion, the results obtained support the potential use of hAM homogenate as a complementary agent in bladder cancer therapy.

Keywords: human amniotic membrane, bladder cancer, FAK, migration, invasion

ANALYSIS OF CO-EXPRESSION OF THE CYCLOOXYGENASE (*COX*) GENE IN BREAST AND COLON TUMORS

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It is recently discovered that *COX-2* (cyclooxygenase) is frequently expressed in cells, that are tend to transform into ontological cells or have already gone through this process. Besides most genes including *COX-2* conduct its effects in association with other genes, which could be detected by the analysis of gene co-expression. Co-expression may indicate the functional connectivity of genes, reflect their role in a particular process. To analyse the role of the *COX-2* and *COX-1* genes in breast cancer and colon cancer and determine its role in the pathogenesis of tumours. We analysed clusters of the *COX-2* and *COX-1* genes isolated from breast cancer and colon cancer using the methodology of a weighted gene co-expression network analysis (WGCNA). The distributions were tested for uniformity using Pearson's chi-squared test. The analysis of datasets is performed using STRING database. To characterize transcription factors and kinases interacting with genes that are co-expressed with *COX-1* and *COX-2*, we used TRRUST, enrich and X2K databases. Using *The human protein atlas* and *PubMed* we found out in which processes each of the regulating factors take part. It was found out that among the considered types of tumours, COX-1 plays an important role in oncogenesis in colon tumours (p-value 0,002), COX-2 - in breast tumours (p-value 0,062). Lysyl oxidase (LOX), Caveolin 1 (CAV1), C-C motif chemokine ligand 2 (CCL2), Tetraspanin-2 (TSPAN2), ADAM Metallopeptidase With Thrombospondin Type 1 Motif 2 (ADAMTS2) are proteins that interact with COX-1 and COX-2 in the corresponding types of tumours. Mitogen-activated protein kinase 3, 1 and 13 (MAPK3, MAPK1, MAPK14), Casein kinase 2 alpha 1 (CSNK2A1), RELA Proto-Oncogene (RELA), Homeodomain Interacting Protein Kinase 2 (HIPK2), cyclin-dependent kinase 2 (CDK2) are factors that potentially regulate the transcription and activity of module proteins containing COX-1 and COX-2. For the majority of certain TF (transcription factor) and kinases, a role in the oncogenesis of the tumours is shown, except for RELA. Detection of functional clusters could be possibly used to discover new drug targets and expanding the understanding of the pathological process underlying tumour formation. RELA is involved in many cellular processes, including cellular metabolism, chemotaxis and modulation of the immune response, therefore it serves as a potential target for the development of therapies for breast tumours.

Keywords: *COX-1 genes*, *COX-2 genes*, cyclooxygenase, colon tumours, breast tumours

PATHOPHYSIOLOGY - IS AN INTEGRATIONAL BASIS OF PHYSICIANS RATIONAL THINKING AND EFFICIENT ACTION

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The experience of medical training has shown that pathophysiology has on integration role in shaping in students the theoretical and methodological basis of rational physicians thinking and efficient practical action. This fact gave the reason to believe that a single course of pathophysiology for medical students is insufficient. Awareness of pathophysiological issues and an ability to carry out pathophysiological analysis specific clinical situations to an ever increasing extent is required at every successive stage of undergraduate studies and (evidenced by the results of a pool among medical graduates) in the process of postgraduate training of specialists. In the view of this fact the specialized course of clinical pathophysiology was introduced in the medical institutes of Russia. In some progressive institutes course of clinical pathophysiology was introduced at the undergraduate stage of and at the initial stage of postgraduate specialization of physicians (training of interns and residents). The suggested (3-stages) system of pathophysiology teaching will promote implementation of the two most important pedagogical principles: integration of various teaching levels, as well as continuity of the under and postgraduate stages of training schools of medicine. In the view of this fact pathophysiology in the medical institute is fundamental discipline not only in the general biological but in clinical physicians training.

Keywords: pathophysiology; integration basis; physicians training

APPLICATION OF "HALLMARKS" IN PATHOPHYSIOLOGY

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Over the last two decades, several papers have been published with "hallmarks" in the title, indicating the aim of identifying the most crucial pathogenetic mechanisms of specific pathologies. This "hallmarkisation" began with the seminal paper "Hallmarks of Cancer" in 2001 by Hanahan and Weinberg, which has become one of the most cited papers in the field. Since then, two more articles have been published, reflecting the contributions of different prominent scientists in the area. The number of cancer hallmarks has increased from 6 to 10 and has now arrived at 14. In 2013, Guido Kroemer and Carlos Lopez-Otin took the challenge and published the "Hallmarks of Aging," which was followed by an update in 2023. According to this authors an ideal hallmark should fulfill the following requisites: (1) it should be associated with the Disease; (2) its experimental or real-life perturbation should be vastly pathogenic; and (3) its experimental or medical maintenance or restoration should have a broad pro-health activity. Nevertheless, not all authors followed these rules. Recently, "Hallmarks of neurodegenerative diseases," "Hallmarks of environmental Insults," and "Hallmarks of Health" have been published. Paradoxically, the latter reminds a concise textbook of general pathophysiology. Even the mosaic theory of essential hypertension proposed by Irvine Page is a prototype of "the hallmarks of hypertension" later elaborated on by many other scientists. This trend shows a tremendous need for the generalization and conceptualization of the most common pathologies of contemporary human society. However, the hallmarks are usually considered as equally contributing causes or mechanisms of the pathologic process and tend to create a generalized, magnified point of view on etiology and pathogenesis. However, they fail to establish the proper causation of the disease in question. From pathophysiological point of view, it's important to delineate the hierarchy of the factors/mechanisms within. This uncertainty can be overcome by categorizing hallmarks into "initial," "promoting," "driving," and "manifesting." This will promote more pathophysiological thinking into the emerging hallmarks and serve as a basis for diagnosis and treatment.

Keywords: hallmarks, etiology, pathogenesis, health, aging

METHYLENE BLUE IMPROVES MITOCHONDRIAL BIOENERGETICS AND MITIGATES OXIDATIVE STRESS: TWO BIRDS WITH ONE STONE

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Abnormal energy metabolism and oxidative stress are central mechanisms in the pathophysiology of cardio-metabolic pathologies. Specifically, impaired mitochondrial bioenergetics with subsequent decrease in ATP synthesis and increase in reactive oxygen species (ROS) generation have been reported to occur both in animal models and humans. Alleviation of mitochondrial dysfunction and mitigation of oxidative stress in cardiovascular diseases and diabetes are unmet goals currently pursued by several research groups worldwide. Because mitochondrial function is dependent on redox components, drugs with redox activity have been increasingly used as mitochondrial modulators to enhance energy production and decrease oxidative stress. Methylene blue (MB), a tricyclic phenothiazine used for more than a century to treat a variety of disorders, has recently emerged as an important modulator of mitochondrial bioenergetics, which follows the pharmacological principle of hormesis, with the optimal effect at low doses. The present research was purported to: i) characterize the effects of MB on the bioenergetic profile of a cardiac cell line and ii) assess cardiac mitochondrial and endothelial dysfunction in the setting of experimental diabetes. The research methodology consisted in the *in vitro* evaluation of: i) mitochondrial bioenergetics using the extracellular flux analyzer and high-resolution respirometer ii) endothelial function of rat aortic rings in organ bath experiments using the myograph. H_2O_2 production and calcium retention capacity were also evaluated in rat heart mitochondria (RHM) by means of spectrofluorimetry. Incubation of a rat cardiomyoblasts (H9c2) with MB (0.1 μM) increased both the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), the two parameters of oxidative phosphorylation (OXPHOS) and glycolysis, respectively. OCR-linked parameters and ECAR were decreased in a dose-dependent manner when MB was administered in higher concentrations (1, 5, and 10 μM) and were not influenced by the lowest concentration tested (0.05 μM). We further demonstrated that in RHM isolated from diabetic and non-diabetic rat hearts, MB improved respiratory function and elicited a dichotomic, substrate-dependent effect on H_2O_2 production. Endothelial function and vascular oxidative stress were assessed in isolated aortas; *ex vivo* treatment with MB decreased oxidative stress and partially restored vascular reactivity in an endothelial-dependent manner. Methylene blue is a potential candidate for drug repurposing in cardio-metabolic diseases by alleviating both mitochondrial and endothelial dysfunction and mitigating the cardiovascular oxidative stress.

Keywords*:* methylene blue; mitochondria; bioenergetics; oxidative stress; cardio-metabolic pathologies

VARIATIONS OF REDOX BALANCE IN DIFFERENT STAGES OF CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA

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Some previous studies indicated the role of oxidative stress in pathogenesis of childhood idiopathic thrombocytopenic purpura (ITP) changes, but there is little data regarding changes in redox balance in different forms of the disease, and changes after therapeutic procedures. We aimed to investigate the values of pro-oxidants and antioxidative capacity in various forms of ITP before and after the applying therapy.The research included 102 children, classified into the following groups: 1) newly diagnosed ITP (ndITP), 2) persistent ITP (pITP), 3) chronic ITP (chITP), and 4) control groups - A) healthy control, and B) previously experienced ITP (peITP) – healthy children who had been suffering from ITP earlier. During the clinical assessment, a blood sample was taken from the patients, from which the value of pro-oxidants (index of lipid peroxidation measured as TBARS, nitrites (NO_2^-), as measurement of nitric oxide (NO) production, superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2)) and the capacity of antioxidant protection (activity of superoxide dismutase (SOD) and catalase (CAT), and quantity of reduced glutathione (GSH)) was determined spectrophotometrically. Our results demonstrated that values of pro-oxidants, especially reflected through the TBARS and O_2^-, were the highest in the ndITP and exacerbated chITP groups. Also, the activity of endogenous antioxidative defense system was the lowest in these groups. Intravenous immunoglobulins therapy in ndITP group exerted the most prominent effect on the redox balance. It can be concluded that severity and exacerbation of the ITP significantly correlates with redox status.

Keywords: childhood idiopathic thrombocytopenic purpura, corticosteroids, endogenous antioxidants, intravenous immunoglobulins, oxidative and nitrosative stress

SMALL RNA DURING ENDOPLASMIC RETICULUM STRESS

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We studied short RNAs in acute and chronic ER stress. We performed deep sequencing of small RNAs up to 35 nucleotides in T-lymphoblastoid Jurkat cells treated with dithiothreitol (DTT), which causes the accumulation of misfolded proteins in the ER lumen. Throughout the small RNA fraction, we noted the most significant changes in miRNA and tRNA-mapped reads. The relative quantity of tRNA-derived fragments was increased by 1.8-fold under ER stress. We found that tRNA isotypes with a high quantity of reads contained a large fraction of 32-nt reads among their sequenced fragments. The five tRNA isotypes that gave rise to almost 76% and 86% of all 32nt RNA fragments in ER-stressed and control cells, respectively, included glycine (Gly-tRNA^{GCC} and Gly-tRNA^{CCC}), glutamic acid (Glu-tRNA^{CTC}), aspartic acid (Asp-tRNA^{GTC}) and valine (Val-tRNA^{CAC}). The total number of reads of these tRNA species significantly increased in response to ER stress compared to other tRNA species. The characteristic patterns of tRNA-derived fragments resembled those generated by endoribonuclease A Angiogenin (*ANG*), which cleaves tRNA molecules within the anticodon loop. We observed increased expression of *Ang* mRNA in response to ER stress in Jurkat cells, whereas the expression of its inhibitor ribonuclease/angiogenin inhibitor 1 (*RNH1*) mRNA was significantly downregulated. At the global level, we observed a decrease in the quantity of miRNA reads compared to other small RNA classes in ER-stressed cells. Using RT-qPCR and ELISA in both tunicamycin- and DTT-treated Jurkat and EA. hy926 cells, we detected significant downregulation of important miRNA biogenesis factor *DICER1* at mRNA and protein levels. Acute ER stress in FRSN fibroblasts also reduced the expression levels of microRNA biogenesis components. Chronic ER stress led to the development of premature senescence phenotype in FRSN cells with high levels of beta-galactosidase activity and cells accumulation in the G1 and G2 phases of the cell cycle; morphological changes and several markers (*p21, MKI67, DNMT1*) were similar to replicative senescence. In fibroblasts subjected to chronic stress, the activity of numerous processes related to DNA synthesis and cell cycle regulation as well as RNA degradation, splicing, and mRNA processing was altered. Unlike acute ER stress, we did not observe a significant decrease in microRNA biogenesis components expression at the RNA level during chronic stress and replicative senescence. Analysis of small RNA fractions also showed that during chronic ER stress, the content of microRNA precursors and mature forms increased in cells, indicating no global decrease in microRNA abundance. Comparison of tRNA fragmentomes in replicatively senescent and chronically stressed cells revealed several common upregulated fragments e.g. Gly-tRNA^{CCC}. Thus, we present evidence that ER stress leads to small RNome remodeling, including significant upregulation of tiRNA, which we suggest as an important transcriptionally independent regulator of the ER stress response and cellular senescence. Downregulation of miRNA biogenesis was not observed in chronic ER stress and replicative senescence.

Keywords: cellular stress, ER stress, cellular senescence, small RNAs, tRNA-derived fragments

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OXIDATIVE STRESS IN KIDNEY TISSUE CAUSED BY METHOTREXATE. EFFECTS OF COENZYME Q10

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Methotrexate is an antimetabolic drug that is often used in the treatment of autoimmune diseases and malignant tumors. Its clinical application is limited due to a large number of adverse effects such as nephrotoxicity. Coenzyme Q10 is an endogenous, liposoluble substance that exhibits a strong antioxidant effect. The aim of our study was to investigate the possible protective effect of coenzyme Q10 on methotrexate -induced nephrotoxicity in rats. Experiments were performed on 24 Wistar rats divided into 4 groups of 6 animals. The first (C-group) group received normal saline (1 ml/kg), the second (Q-group) received coenzyme Q10 (10 mg/kg) for 8 days, the third (MTX-group) received methotrexate in a single dose (20 mg/kg) on the first day and the fourth (QMTX-group) group received simultaneously methotrexate and coenzyme Q10. Functional changes in kidney tissue were determined by biochemical analysis of blood, by determining the parameters of oxidative stress and histopathological analysis of kidney tissue. Administration of coenzyme Q10 for 8 days after methotrexate administration showed a significant protective effect by decreasing serum levels of creatinine and urea, elevating catalase activity and decreasing concentrations of AOPP and MDA in kidney tissue in QMTX group compared with MTX group of rats. In the same group, histological sections of kidney showed that coenzyme Q10 ameliorated degenerative changes of proximal tubules. There was focal degeneration of tubular cells without glomerular congestion, leukocyte infiltration or tubular dilatation. Coenzyme Q10 also reduced histopathological changes of glomeruli and glomerular basement membrane. The results suggest that coenzyme Q10 ameliorates oxidative stress and has the nephroprotective action which might be clinically useful. The results of our study also indicate that coenzyme Q10 can be used as a supporting agent in clinical practice in patients treated with methotrexate.

Keywords: nephrotoxicity, methotrexate, coenzyme Q10, oxidative stress

MONOAMINE OXIDASE IS A NOVEL TARGET OF SGLT2 INHIBITORS IN THE CARDIOVASCULAR SYSTEM

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The rise of cardiometabolic diseases in low and middle-income countries in the setting of "globesity" is responsible for the increased cardiovascular mortality. Chronic oxidative stress and low-grade inflammation are the two major pathomechanisms that underlie these pathologies, which promote each other in a vicious circle leading to disease progression and the occurrence of complications. Monoamine oxidase (MAO) with two isoforms, A and B, located at the outer mitochondrial membrane, have emerged in the past decades as important sources of oxidative stress in cardiovascular and adipose tissues. The sodium-glucose-cotransporter 2 inhibitors (SGLT-2i) are novel antidiabetic drugs which exert cardiovascular protection in the absence of diabetes via partially elucidated off-target effects. The aim of the present research was to investigate whether the empagliflozin can mitigate the MAO-related oxidative stress in human atrial and vascular tissues. Right atrial appendages and mammary arteries samples were harvested from overweight, non-diabetic patients undergoing elective open-heart surgery were acutely incubated *ex vivo* with empagliflozin in the presence or absence of angiotensin II (AII), lipopolysacharride (LPS) and glucose (GLUC) and used for the assessment of: MAO expression (qRT-PCR and immune-histochemistry), reactive oxygen species (ROS) production (dihydroethidium staining and ferrous oxidation xylenol orange), organ bath studies of the vascular samples (evaluation of the endothelial-dependent relaxation) and correlations with the echocardiographic parameters. Ex vivo incubation with AII, LPS and GLUC increased cardiac and vascular expression of MAO isoforms and the related oxidative stress. The effects were mitigated in the presence of acute exposure to empagliflozin. ROS production positively correlated with the size of the heart cavities and negatively with the left ventricular ejection fraction. Here we provide the evidence for a novel protective effect of empagliflozin by targeting MAO in the cardiovascular system in non-diabetic cardiac patients.

Keywords: MAO, SGLT2 inhibitors, cardiovascular protection

REPURPOSING MONOAMINE OXIDASE INHIBITORS TO REVERSE VASCULAR HYPERGLYCEMIC MEMORY

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Defined as the persistence of hyperglycemic stress after normoglycemia restoration, the "hyperglycemic memory" is a partially elucidated mechanism underlying cardiovascular complications in diabetic patients. Vascular oxidative stress has been reported to persist after glycemia restoration yet identification of the ROS-generating sources is still an unmet goal. Monoamine oxidases (MAOs, A and B), enzymes responsible for catecholamines metabolism with the constant generation of hydrogen peroxide, have been reported to be hyperexpressed and elicit endothelial dysfunction in metabolic diseases, particularly in the presence of inflammatory burden. The present study was purported to assess MAOs contribution to the vascular "hyperglycemic memory". Insulin (glargine)-treated mice with diabetic streptozotocin (STZ)-induced diabetes treated with MAO A (clorgyline) and B (selegiline) inhibitors were sacrified after 2 weeks and aortas were isolated and used for vasomotor studies (organ bath), oxidative stress (dihydroethidium staining and ferrous oxidation xylenol orange) and MAO expression (qRT-PCR and immune-histochemistry). MAOs expression and ROS production were increased in the diabetic aortas after 2 weeks of hyperglycemia together with impaired vascular relaxation. Partial glucose normalization with glargine failed to normalise ROS generation and vascular relaxation. *In vivo co*-administration of MAO inhibitors on the top of insulin additively reduce the oxidative stress and improved vascular relaxation in diabetic mice. In the murine model of STZ-induced diabetes, MAO inhibitors reversed the vascular "hyperglycemic memory", thus being viable candidates for drug repurposing as vasculo-protective agents in diabetes.

Keywords: MAO inhibitors, diabetes, hyperglycemic memory

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MITOCHONDRIA-TARGETED ANTIOXIDANT BASED ON HYDROXYCINNAMIC ACID ANTIOXCIN4 IMPROVED LIVER STEATOSIS IN WESTERN DIET-FED MICE: THE ROLE OF NRF2-MEDIATED CELL SIGNALLING PATHWAYS

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Mitochondria-targeted hydroxycinnamic acid derivative (AntiOxCIN4) can alter the cellular redox and energetic status and activate mitochondria-to-nucleus signaling pathways, such as Nrf2/Keap1 pathway. Understanding such mechanisms is crucial to potentiate the therapeutic application of mitochondriotropic antioxidants towards specific pathological conditions. Non-alcoholic steatohepatitis (NASH), the progressive form of non-alcoholic fatty liver disease (NAFLD), is a worldwide health problem with no effective treatment, offering a considerable market opportunity for developing novel drugs. Here, we investigated the potential effect of AntiOxCIN4 in the prevention of NAFLD development. Human hepatoma-derived cell line HepG2 was treated with the mitochondriotropic antioxidants based on hydroxycinnamic acids (AntiOxCIN4) for 48h in the presence and absence of fatty acid (FA) overload. Additionally, C57BL/6J mice daily supplemented with 2.5 mg AntiOxCIN4 were then fed with standard diet (SD) or Western diet (WD) (30% high-fat, 30% high-sucrose) for 16 weeks. After an initial decrease in oxygen consumption paralleled by a moderate increase in superoxide anion levels, AntiOxCIN4 led to a time-dependent Nrf2 translocation to the nucleus. AntiOxCIN4 treatment enhanced mitochondrial quality by triggering the clearance of defective organelles by autophagy and/or mitophagy, coupled with increased mitochondrial biogenesis. AntiOxCIN4 have the ability to maintain hepatocyte redox homeostasis, up-regulate cellular antioxidant defense system, regulate the electrophilic/nucleophilic tone, and protected HepG2 cells against the detrimental effects of FA overload. In a WD-fed mice model, AntiOxCIN4 decreased body weight gain and hepatic steatosis by decreasing LD number/size and its composition. These effects were correlated with increased cellular FAO activity. Although the cellular mechanisms behind NAFLD pathogenesis are still a focus of controversy, the multi-operational mechanism of action of AntiOxCIN4, make this molecule a valuable drug candidate in the context of metabolic-related hepatic disorders, such as NASH/NAFLD.

Keywords: mitochondria-targeted antioxidants, non-alcoholic fatty liver disease (NAFLD), Nrf2

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TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL STRESS RESPONSES

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When mitochondria experience stress or when dysfunction occurs, the organelle sends signals to the cell nucleus, which launches different types of adaptive cell responses. Understanding of the mitochondrial stress response in mammals remains incomplete. Numerus studies over last years showed that unlike in *C. elegans*, mammalian UPR^{mt} is not the primary response to mitochondrial dysfunction but rather part of more complex mitochondrial stress response sharing a signature of the integrated stress response (ISR), hence referred to as mitoISR. Our results have demonstrated that mitochondrial dysfunction is sensed independently of the respiratory chain deficiency, questioning current view on molecular mechanisms contributing to the development of mitochondrial diseases. We also showed that mitoISR is regulated by an intricate interplay between three transcription factors, CHOP, C/EBPβ, and ATF4. Contrary to its previously proposed role as a transcriptional activator of UPR^{mt}, we presented strong evidence that CHOP, through its interaction with C/EBPβ, attenuates prolonged ISR and mitochondrial cardiomyopathy by regulating the ATF4 levels. In parallel, we showed that mitokine FGF21, although highly upregulated is fully dispensable for the cell-autonomous and systemic responses to severe mitochondrial cardiomyopathy. In contrast, in the conditions of mild-to-moderate cardiac OXPHOS dysfunction, FGF21 regulates a portion of mitoISR, independent of ATF4. Collectively, our work highlights the complexity of mitochondrial stress responses by revealing the importance of the tissue specificity and dose dependency of mitoISR.

Keywords: mitochondria stress, transcription factor

HIGH-FAT DIET EXAGGERATES METABOLIC AND REPRODUCTIVE FEATURES OF ESTRADIOL VALERATE-INDUCED PCOS IN RATS

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Polycystic ovary syndrome (PCOS) is a common multifactorial endocrinopathy that affects women during their reproductive years and is frequently related to infertility and metabolic issues. The employing of animal models enables a better understanding of etiopathogenesis and an identification of the most effective therapeutic strategy. With the goal to examine PCOS-related changes with a specific emphasis on oxidative stress, we attempted to investigate the additive effect of estradiol-valerate (EV) and high-fat diet (HFD) in female rats. Three groups of animals: a control group (CTRL), an estradiol-valerate group (EV), and an estradiol-valerate group on HFD (EV+HFD) were used in the study. A single subcutaneous injection of long-acting EV was used to induce PCOS (in EV and EV+HFD group), while HFD was administered (in EV+HFD group) during the induction period of 60 days. Estrus cycle disruption, changes in anthropometric parameters, and hormonal abnormalities contribute to the obesity-type PCOS phenotype. Compared to EV protocol alone, glucose metabolism impaired after the addition of HFD to the EV treatment. The combination of the EV and HFD treatment resulted in more cystic follicles, according to histological examination. In the vast majority of the measured parameters, the cumulative effect of EV and HFD was clear. Our research clearly showed that PCOS in rats had both metabolic and reproductive characteristics; in the vast majority of the measured parameters, the cumulative effect of EV and HFD was clear. The development of PCOS-associated endocrine, reproductive, and metabolic features may be related to changes in oxidative stress markers.

Keywords: polycystic ovary syndrome, oxidative stress, rat, ovary

THE EFFECT OF KYNURENIC ACID ON INDIVIDUAL BRANCHES OF THE FIBROTIC CASCADE: AN IN VITRO MODEL OF FIBROSIS

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Fibrosis is a world-wide problem leading to increased morbidity and mortality in industrialized world. Currently no effective antifibrotic therapy is available, thus searching for a new antifibrotic agent is necessary. Indoleamine 2,3-dioxygenase (IDO) is a rate limiting enzyme of tryptophan (TRP) metabolism. IDO converts TRP to its metabolites kynurenine (KYN) and kynurenic acid (KYNA). Recently we showed that IDO has antifibrotic potential, thus we aimed to find out whether this can be held by IDO metabolites KYN or KYNA. In this study we used immortalized embryonic mouse fibroblasts (NIH/3T3) to examine the effects of KYN and KYNA on fibrosis in an in vitro model. We used recombinant Transforming Growth Factor β (TGF-β) to induce fibrosis in the cells, and then we treated them with KYN and KYNA to see how they affected the fibrotic cascade. We used western blot analysis to monitor an expression of fibrosis-related proteins, such as alpha-smooth muscle actin (αSMA), phosphorylated SMAD2 (pSMAD2), phosphorylated p38 (pp38), extracellular signal-regulated kinase (ERK), phosphorylated ERK (pERK), neural cadherin (N-cadherin), and epithelial cadherin (E-cadherin). Our findings suggested a potential route for the treatment of renal fibrosis by demonstrating how KYN and KYNA altered the TGF-mediated signaling cascade. In particular, it was discovered that KYN and mainly KYNA decreased the expression of αSMA, pSMAD2, pp38 and pERK. These alterations point to a reduction in fibrosis and an increase in epithelialization, both of which are desired effects for the therapy of renal fibrosis.Our research shows that KYN and KYNA can affect the fibrotic cascade and may be therapeutically useful in the treatment of renal fibrosis. However, more investigation is required to validate our results and establish the most effective dosages and methods of administration for KYNA as a potential fibrosis treatment.

Keywords: fibrosis, kynurenic acid, kynurenine, TGF-signaling

EFFECTS OF *GALIUM VERUM* EXTRACT ON CARDIODYNAMIC PARAMETERS AND REDOX STATE OF THE ISOLATED HEART OF PSORIATIC RATS

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Psoriasis represents a chronic inflammatory disease, which predominantly affects the skin, but recent findings show that it can affect various organ systems, including the heart. The aim of the study was to examine the effects of *Galium verum* extract administration on cardiodynamic parameters and redox state of the isolated heart of psoriatic rats.The study was conducted on 24 *Wistar albino* male rats, 10 weeks old, classified into 3 groups: control (CTRL), psoriasis (PSORI), and psoriasis with *Galium verum* extract (PSORI+GV). After induction of psoriasis, the *G.verum,* extract was administered daily by oral gavage in dose of 125mg/kg b.w. for 4 weeks. All isolated hearts were perfused according to modified Langendorff technique at gradually increased coronary perfusion pressures (40-120 cm H2O) and the following parameters were measured: dp/dt max/min, SLVP, DLVP, heart rate and coronary flow. Afterward, the isolated hearts were prepared for further histological analysis and stained with H/E. In collected samples of coronary venous effluent, the following parameters of the redox state were measured: index of lipid peroxidation (TBARS), nitrite (NO_2^-), hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-). The dp/dt max was increased in the PSORI+GV group compared to the CTRL group. The dp/dt min values significantly decreased in the PSORI group compared to the PSORI+GV and the control groups. SLVP was increased in the PSORI and PSORI+GV groups compared to the CTRL. DLVP values were significantly increased in the PSORI group while the values were decreased in PSORI+GV group compared to the CTRL groups. GV supplementation significantly decreases O_2 and H_2O_2 values at all pressures compared to the PSORI group. Nitrites were reduced at lower coronary perfusion pressures in the PSORI+GV group compared to the PSORI group. The diameter of cardiomyocytes was increased in the PSORI group by 45% and in the PSORI+GV group by 21% compared to the CTRL group. Supplementation of *Galium verum* extract has a positive effect on the cardiac function and redox status of the isolated heart of psoriatic rats.

Keywords**:** *Galium verum*, psoriasis, rat, heart, redox state

THE EFFECTS OF DE NOVO LIPID SYNTHASE INHIBITORS ON CELLULAR SENESCENCE

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Senescence and cellular aging are related but different concepts. Senescent cells accumulate during aging. With aging, certain diseases and conditions, such as cardiovascular diseases and malignant tumors, occur more often, and senescence plays a significant role in their pathogenesis. Factors that contribute to cellular senescence are DNA damage, telomere shortening, or oxidative stress. Senescent cells develop a senescence-associated secretory phenotype. They do not divide (permanent stop of division in the G1 or G2 phase) but are metabolically active (with increased glycolysis, redox homeostasis, and decreased beta-oxidation of fatty acids) and secrete proinflammatory molecules that damage the surrounding tissues. The de novo synthesis of lipids plays a significant role in the onset of senescence. Among the different signaling molecules that link senescence and lipid metabolism, p53 and mTOR have an essential place. Several classes of de novo lipid synthesis inhibitors, including statins, fatty acid synthase (FASN) inhibitors, and stearoyl-CoA desaturase (SCD) inhibitors, have been investigated for their potential to inhibit cellular senescence. However, it is difficult to determine which inhibitor is the most effective in inhibiting senescence, as their effectiveness can vary depending on the specific type of cell and the specific senescence-inducing stimulus. For example, atorvastatin reduced senescence in human endothelial cells induced by oxidative stress. FASN inhibitors, such as cerulenin and C75, reduced senescence in human fibroblasts induced by hydrogen peroxide. SCD inhibitors, such as A939572 and A939735, reduced senescence in human fibroblasts induced by ionizing radiation. Overall, while all these de novo lipid synthesis inhibitors have shown some anti-senescence effects in specific cell types and senescence-inducing stimuli, more research is needed to clarify which inhibitor is the most effective in inhibiting cellular senescence across a wide range of cell types and stimuli.

Keywords: cellular senescence, de novo lipid synthesis, fatty acid synthase inhibitors

STRUCTURAL CHANGES OF THE CORNEAL SUBBASAL NERVE PLEXUS AND GLYCEMIC CONTROL IN PATIENTS WITH TYPE 1 DIABETES MELLITUS

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Diabetic neuropathy (DN) is the most common complication of diabetes mellitus (DM). Corneal confocal microscopy (CCM) is a new noninvasive method that can help to study small nerve fibers in patients with DN. The study was aimed to evaluate corneal small nerve fibers in patients with type 1 DM and DN, and dynamics of the nerve fibers by CCM during of the DM treatment. 20 patients with type 1 dm and dn were included. Median age 29 years [18.0; 45.0], the median HbA1c 8.5% [7.0; 10]. Neuropathy was assessed by Neuropathy Total Symptom Score (NTSS-9), Neuropathy Disability Score (NDS), CCM, lower limb electroneuromyography. After adjustment of glycaemia to HbA1c<7%, all tests were repeated. After reaching HbA1c<7%, there was a clinically significant improvement in NTSS-9 scores by 66.3% ($p < 0.001$). There were increase in the number of nerve fibers (+16%), their thickness (+25.6%), the number of nerve branches (+32.4%) and nerve branch density – by 23.6% ($p<0.001$ for all parameters). The nerve fiber tortuosity decreased by 14.5%. Also, there was an increase of nerve conduction velocity of *n. tibialis* by 8.3% and *n. suralis* by 4.05% ($p<0.05$). Amplitudes of stimulation responses of these nerves increased by 9.9% and 8.5%, respectively ($p<0.05$). The results allow us to propose the use CCM for pathogenetic diagnosis of DN. Improved glycemic control has a positive effect on the structural characteristics of corneal nerves as an element of pathogenetic treatment.

Keywords: diabetes mellitus, diabetic neuropathy, corneal confocal microscopy, corneal nerves

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PDE5 INHIBITION WITH SILDENAFIL ATTENUATES CARDIOTOXICITY AND IMPROVES CANCER CHEMOTHERAPY

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Phosphodiesterase type 5 (PDE5) selectively hydrolyses the second messenger cGMP into 5'-GMP, thereby regulating its intracellular concentrations. Dysregulation of the cGMP-dependent pathway plays a significant role in cardiovascular (CV) and several other clinical disorders including Alzheimer Disease, heart failure, diabetes and metabolic diseases. The targeting of PDE5 with the potent inhibitors including sildenafil (Viagra) has been successfully used clinically for the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension. Several pioneering investigations primarily from my laboratory have described pleotropic effects of PDE5 inhibitors including protection against ischemia/reperfusion injury, ischemic cardiomyopathy, by limiting necrosis, apoptosis, fibrosis and preserving left ventricular (LV) function. Because PDE5 is highly expressed in prostate cancer (PCa), treatment of PCa cells with sildenafil in combination with the potent anti-cancer drugs such as doxorubicin (DOX) as well as Docetaxal-induced apoptosis, which was mediated by enhanced oxidative stress, nitric oxide generation, up-regulation of caspase-3 and caspase-9 activities, reduced expression of Bcl-xL, and phosphorylation of Bad. Furthermore, co-treatment with sildenafil and DOX or Docetaxal in mice bearing PCa xenografts resulted in significant inhibition of tumor growth as compared to individual drug treatment. Doppler echocardiography showed that sildenafil treatment also ameliorated DOX-induced LV dysfunction. These results provide provocative evidence that sildenafil is both a powerful sensitizer of DOX or Docetaxal-induced killing of PCa and a potent cardioprotective small molecule. The results suggest that modulation of cGMP could be a clinically translatable strategy in improving outcome of PCa patients receiving chemotherapy, considering that many PDE5 inhibitors are now approved for treatment of ED.

Keywords: PDE5, sildenafil, chemotherapy, cardiotoxicity

OVARIAN CANCER METASTASIS: FOUR HYPERMETHYLATED LONG NON-CODING RNA GENES IN EPIGENETIC REGULATION

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Ovarian cancer (OC) develops asymptomatically up to the terminal stages with metastasis, chemoresistance and poor prognosis, what ranks it first in mortality among onco-gynecological diseases. The study of long non-coding RNAs (lncRNAs) as biomarkers that involved in the regulatory mechanisms associated with the metastasis is a significant and promising field. The aim of our study was to assess the metastatic potential of lncRNA genes group based on the alterations in the level of expression and methylation and its contribution to epigenetic regulation of OC metastasis. The real-time RT-qPCR, methylation-specific qPCR, as well as the nonparametric Mann-Whitney criterion and the Spearman correlation criterion (RStudio) were applied. A set of 30 paired (tumor/norm) OC samples were tested. Our results determined statistically higher methylation level of HAND2-AS1, SEMA3B-AS1, ZNF667-AS1, KCNK15-AS1 genes in the tumor samples. We also revealed the metastatic potential of three (SEMA3B-AS1, ZNF667-AS1, KCNK15-AS1) genes that was higher in the samples with more severe stages and a larger tumor size, which indicates a connection with tumor progression ($p \leq 0.05$). We also found for these three genes a significant increase in methylation level with metastasis compared with samples without them ($p<0.001$). At the same time, a significant decrease of the expression level ($p \leq 0.05$) was found for the HAND2-AS1, KCNK15-AS1, SEMA3B-AS1 genes in tumor samples. These results suggested these three genes to function as suppressors in OC. We couldn't prove these findings for the ZNF667-AS1 gene as a decrease of its expression level was detected at trend ($p=0.08$). Moreover, for the lncRNA HAND2-AS1 a statistically significant decrease in expression level ($p<0.05$) was found in the samples of OC with later stages, metastasis and a more aggressive OC histological type, and for the ZNF667-AS1 – with a more aggressive OC histological type. We revealed a strong negative correlation between the levels of methylation and expression for 4 lncRNA genes: HAND2-AS1 ($r_s=-0.5$, $p=0.006$), SEMA3B-AS1 ($r_s=-0.63$, $p=0.0001$), ZNF667-AS1 ($r_s=-0.63$, $p=0.0003$), KCNK15-AS1 ($r_s=-0.46$, $p=0.01$). Thus, our results showed that hypermethylation of a number of lncRNA genes is involved in their expression regulation. Moreover, this epigenetic mechanism contributed to OC metastatic potential, advantage stages and aggressive histological type. The confirmation for application of our findings to predict metastasis in OC is needed. Using TCGA and GEO databases, three potentially interacting lncRNA-miRNA pairs were selected: HAND2-AS1/hsa-miR-135a, KCNK15-AS1/hsa-miR-484, KCNK15-AS1/hsa-miR-942; $Rs=-0.98$, $p<10^{-5}$.

Keywords: ovarian cancer, metastasis, lncRNA, epigenetic regulation

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SURVIVIN CONJUGATED GOLD NANOPARTICLES ENHANCE THE POTENCY OF ABIRATERONE AND ENZALUTAMIDE AGAINST PROSTATE CANCER CELL LINES *VIA* THE INCREASE IN ROS FORMATION AND ENHANCEMENT OF APOPTOSIS

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Despite being the foremost aggressive cancer around the globe; surgeries, chemotherapies, and other single or multiple drugs are not producing satisfactory results in the treatment of prostate cancer. However, targeted delivery with combinatorial drugs has emerged as an appropriate therapy with minimal side effects. In this study, a targeted nanomedicine with the combinatorial impact of abiraterone (Ab) and enzalutamide (Ez) bioconjugated with survivin antibody-loaded gold nanoparticles (AbEzSvGNPs) has been developed. Further, the AbEzSvGNPs were characterized by using different physical techniques, including UV-Vis spectroscopy, dynamic light Scattering, zeta potential, and transmission electron microscope. Interestingly, the combined effect of Ab and Ez in AbEzSvGNPs was greatly enhanced against DU145 (4.21μM) and PC3 (6.68μM) cells compared to the combined effect of the free pure drugs (Ab/Ez) (30.4μM, DU145; 21.8μM, PC3) and survivin loaded GNPs (100.58μM, DU145; 105.1μM, PC3). The cytotoxicity of AbEzSvGNPs against normal kidney cells (NRK) cell lines was reduced compared to the combination of pure drugs (Ab/Ez), affirming the safety of AbEzSvGNPs. Moreover, AbEzSvGNPs were found to increase ROS production in DU145 and PC3 cells as evidenced by increased DCFDHA fluorescence intensity compared to both the combination of pure drugs (Ab/Ez) and survivin-loaded GNPs. In addition, apoptosis was more pronounced in AbEzSvGNPs-treated DU145 and PC3 cell lines compared to either the pure drugs or survivin-loaded GNPs alone as shown by increased intensity of both DAPI and TUNNEL staining. Caspase-3 activity was significantly increased in the AbEzSvGNPs-treated DU145 and PC3 cells compared to the pure drugs (Ab/Ez) and survivin-loaded GNPs. Further, AbEzSvGNPs-treated DU145 and PC3 cells showed a significant reduction in mitochondrial membrane potential evidenced by reduced Mitotracker red stain intensity compared to the pure drug (Ab/Ez) and survivin-loaded GNPs. Taken together, AbEzSvGNPs potentiated the cytotoxicity of Ab and Ez against DU145 and PC3 cell lines through an increase in ROS formation, increased apoptosis, and reduction in mitochondrial membrane potential.

Keywords**:** GNPs, abiraterone, enzalutamide, survivin, prostate cancer

THE LATENT PERIOD OF TIME BETWEEN SECRETION OF CA^{2+} FROM ER-DEPO AND ACTIVATION OF CA2+ CRAC CHANNELS WAS OBSERVED IN HL-60 CELLS

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HL-60 human leukemic cells are widely employed model for study of Ca^{2+} signals which control of cell functional activity. HL-60 promyelocytes are pluripotent cells and can be differentiated into neutrophil-like or monocyte-like cells. Intracellular Ca^{2+} plays an important role in regulation of differentiation of HL-60 cells mediated by early response genes expressing. The Ca2+ content in the cells was determined by the Fluorescence of Fura-2AM on the Shimadzu-RF-510 fluorimeter with continuous mixing of the suspension using a magnetic mixer. Differentiation of HL-60 cells induced dibutiril-cAMP (db-cAMP). The Ca^{2+}-level in HL-60 cells ($[Ca2+]_i$) was changed by using the selective inhibitor of ER-depo Ca^{2+}ATPase-Tapsigargin (TG,1μM) and Formyl-peptide (FMLP, 2 μM) acting on the receptors of the plasma membrane. The undifferentiated (ud-HL-60) cells did not response to 0.1-5.0 μM FMLP, which in differentiated (d-HL-60) cells in Ca^{2+}-medium the optimal concentration (2 μM FMLP) caused the maximum increase $[Ca^{2+}]$i, then there was a decrease in $[Ca^{2+}]$i to the stationary level. TG in ud-HL-60 in Ca^{2+}-medium induced an increase of Ca^{2+}, then there was a latent period of time (lat-time), followed by a transient increase $[Ca2+]_i$. The duration of lat-time varied from 2 to 4 min, depending on the conditions. This time increased with a decrease in temperature. Ca^{2+} response after lat-time in the ud-HL-60 was suppressed by CRAC standart inhibitors and Trimetazidine (TMZ, 10 μM), which did not affect the ER-depo, but selectively blocked the CRAC channels. In the d-HL-60 cells in the Ca^{2+}-medium the latent period of time was not observed under the action of TG. It is assumed that the presence of a latent period of time between the Ca^{2+} release from intracellular ER-depo and activation of transport Ca^{2+} by CRAC channels of plasma membranes reflects the integrated, complex nature of the coupling of these two processes.

Keywords: HL-60 cells, Ca^{2+} release, ER-depo, CRAC channels, latent time

FORMULATION AND ASSESSMENT OF IMMORTELLE ESSENTIAL OIL-BASED SEMI-SOLID PREPARATIONS FOR WOUND HEALING

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Diabetes mellitus is associated with a high risk of developing serious micro- and macrovascular complications, while there is a great concern related to impaired wound healing, and development of chronic wounds and amputations. Therefore, the aim of this research was to prepare semi-solid formulations based on *Helichrysum italicum* essential oil (HIEO) and assess its efficacy in diabetic rats with induced incision. After creating full-thickness cutaneous wounds, forty-eight diabetic rats were divided into six groups: (1) negative control; (2) positive control; (3) ointment base; (4) gel base; (5) 0.5% HIEO ointment (6) 0.5% HIEO gel. Wound healing potential was assessed through following the healing time, percentage of wound contraction, and histological analysis. In order to reveal the mechanism of action, markers of redox status were examined in skin tissue as well. Our findings revealed a significant drop in the wound size after application of HIEO formulations compared with respective control groups. Formulations based on HIEO exerted benefits partially through a decrease in oxidative stress. Our study clearly suggest that HIEO based semi-solid formulations such as gel and ointment were able to accelerate the time for wound healing, while the gel exhibited superior wound repairing effect.

Keywords**:** diabetic wounds, *Helichrysum italicum* essential oil, semi-solid formulations, phytotherapy

DEFINITION OF *FLG* GENE EXPRESSION LEVELS AS A KEY BIOMARKER IN THE PATHOGENESIS OF ATOPIC DERMATITIS

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This article examines the expression of the *FLG* gene to determine whether it is a key biomarker of barrier function impairment in patients with previously established atopic dermatitis (ATD). In conclusion, the magnitude of filaggrin gene expression is not the only determinant for the development of AD. This article examines the expression of the *FLG* gene to determine whether it is a key biomarker of barrier function impairment in patients with previously established atopic dermatitis (ATD). We studied nucleated fractions of peripheral blood cells of six apparently healthy donors, as well as thirty-two patients of different sex and age (12-58 years) with AD of varying severity. To determine the expression level of the *FLG* gene, total RNA was isolated using Tri-Reagent (Sigma Aldrich, Germany) in a ratio of 1:4 according to the manufacturer's instructions. Changes in *FLG* gene expression accompany about 40% of cases of severe AD and up to 20% of moderate and mild AD. Previously it was shown that even 12 % of healthy European population might have a decreased level of expression of *FLG*. The results obtained indicate the presence of a synergistic effect of the interaction of several genes. *FLG* is just one gene out of more than seventy genes localized in epidermal differentiation complex. So we can consider it as a variable dynamic feature in the pathogenesis of the atopic dermatitis. Even in the absence of somatic mutations, the occurrence of AD is influenced by Th-2 cell cytokines, pH, and the presence of skin infections of various origins, which leads to changes in *FLG* expression. It is quite possible that the identification of the relationship between the state of the *FLG* gene in combination with other biomarkers (genes located in the region of chromosome 1q21 and epigenetic mechanisms) will clarify the pathogenesis of this multifactorial disease. Changes in *FLG* gene expression are not the only risk factor for the onset and development of AD. This implies the participation of additional biological mechanisms, including, among other things, epigenetic mechanisms.

Keywords: filaggrin gene (*FLG*), expression, polymerase chain reaction (PCR), epigenetics, atopic dermatitis

IMPACT OF SIBERIAN PINE ESSENTIAL OIL-CONTAINING OINTMENT ON WOUND HEALING IN DIABETIC RATS

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Siberian pine essential oil (SPEO) contains a high percentage of terpenes which exert anti-inflammatory, antioxidant and antimicrobial effects thus we assumed that it might be valuable agent in wound healing. Therefore, the aim of this investigation was to formulate topical ointment with SPEO, and to evaluate their wound healing potential. Phytochemical profile of SPEO and rheological characteristics of the formulation were analyzed. *Wistar albino* diabetic rats (n=32) with induced excision wounds were randomly divided into following groups: untreated group, topically treated with either a 1% silver sulfadiazine group, ointment base group or SPEO-containing ointment group. In order to determine wound healing activity, we measured percent of wound contraction, level of hydroxyproline and redox status parameters, and conducted histological analysis. Formulation containing SPEO was stable and safe for skin application. Three weeks of treatment lead to significant decrease in wound size and remarkably higher level of total hydroxyproline content in rats treated with SPEO ointment relative to all other groups. Level of hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-) and index of lipid peroxidation (TBARS) were decreased while activity of superoxide dismutase (SOD) and catalase (CAT) enzymes were enhanced after application of SPEO ointment. Additionally, results of histological analysis confirmed pro-healing effect of SEPO ointment. The present study revealed that SPEO ointment promote wound healing in excision wound model and might serve as a valuable approach for wound management.

Keywords**:** *Siberian pine*, essential oil, wound healing, ointment

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PATHOGENETIC THERAPY OF ATOPIC DERMATITIS

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Atopic dermatitis is a common pathology, accounting for 20-40% in the structure of skin diseases. For its treatment, systemic therapy drugs are currently used have side complications. A method of therapy for atopic dermatitis has been developed, based on the application of exosomes, including the transmembrane form of the Klotho protein, to the area of the skin defect. High efficiency of therapy has been achieved, with a reduction in the duration of treatment. The relevance of the development of treatment methods is due to the prevalence of this pathology (up to 40% of skin diseases), and the lack of safe and effective methods of therapy. The purpose of this study is to improve the quality of the treatment of atopic dermatitis. The goal is achieved by using exosomes containing the Klotho protein, obtained from genetically modified MMSCs by inducing overexpression of the transmembrane Klotho gene in the latter. Autologous material (10–30 mm^3) in the form of punch biopsy specimens was taken under aseptic conditions. Transfection of MMSCs was performed by liposomal transfection. The concentration of Klotho protein in cell lysates of the control group of cells was 74.22 ± 6.73 v and 93.03 ± 6.65 in the experimental group (pg of Klotho protein/μg of total protein). Cells were grown on serum-free StemPro-MSC. Subsequently, the supernatant was filtered through nitrocellulose filters (Sarsthted). To concentrate the exosome solution, the pellet was re-suspended to 1/10 of its original volume and centrifuged at 100,000 g for 120 minutes. Before use, a suspension of exosomes was prepared in saline, which was placed in a sterile syringe. A method for the treatment of atopic dermatitis has been created, based on the application of exosomes, including the transmembrane form of the Klotho protein, to the area of a skin defect.

Keywords: atopic dermatitis, klotho gene, MMSC transfection, exosomes

THE ANXIETY LEVEL ALTERATIONS INDUCED BY ORALLY ADMINISTERED FLUORESCENT NANOSIZED POLYSTYRENE PARTICLES IN MICE

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Whether originating from commercially manufactured or environmental degradation, nanoplastics (NPs) have an increasing contribution to environmental pollution. In this study, we analyzed the behavioral outcome of fluorescent polystyrene (PS) nanoparticles oral uptake in C57BL/6 mice (8-10 weeks old of both sexes), randomly divided into groups of 5-9 animals. PS nanoparticles were administered in a low (0.01 mg/day or 4.42 × 109 40 nm + 2.16 × 107 200 nm) and high (0.1 mg/day or 4.42 × 1010 40 nm + 2.16 × 108 200 nm) dose for five weeks. Behavioral testing for anxiety level estimation was performed in the open field (OF) and the elevated plus maze (EPM) test. Anxiogenic response to orally administered nanosized PS particles in female mice was observed in both OF test (the significant decrease in cumulative duration and frequency to the centre zone, with declined number of rearings) and EPM (the significant lowering of cumulative duration and frequency to open arms, with diminished exploratory activity and percentage of time moving) test. In contrast, nanosized PS particles intake in male mice resulted in anxiolytic effect. This was confirmed in the OF test (the significant increase in overall motor activity, including the parameters obtained in the center zone, and the number of rearings), as well as in the EPM test (the increase in all estimated parameters that counts for anxiolytic response). The analysis of the hippocampal tissue samples of female mice revealed the proinflammatory (the increase in NLRP3 and the decline in TGF-β) and proapoptotic (the increase in BAX and the decline in BCL-2 relative gene expression) effect of PS nanoparticles accompanied by the significant decline in both brain-derived neurotrophic factor (BDNF) and GABA-α5 subunit. The hippocampal tissue analyses in male mice showed an opposite response to the described treatment. At the same time, the confirmed accumulation of PS nanoparticles in testicular tissue was accompanied by a significant decrease in serum testosterone levels. Taken together, it seems that the observed impact of PS nanoparticles on anxiety levels was gender dependent. The basic difference underlying this phenomenon may be attributed to the disturbance of the neuro-endocrine axis in which physiologically higher testosterone levels in male mice contribute to the principal increased anxiety level that can be attenuated by lowered testosterone production following prolonged administration of nanosized plastic particles.

Keywords: polystyrene, nanoparticles, anxiety, hippocampus; mice

MAGNESIUM DEFICIENCY IN EPILEPSY – PATHOPHYSIOLOGICAL ASPECTS

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Magnesium has multiple functions in human body in health and disease. It is a bioessential mineral with multiple neuroactive effects (anaesthetic, analgetic, antimigrenous, antidepressant, anxiolytic, neuroprotective and anticonvulsive). Magnesium ion exerts an overall stabilizing effect on excitable membranes. Neuronal Mg^{2+} requirements are high and its level is higher in cerebro-spinal fluid (CSF) then in blood serum. Magnesium status is usually assessed by measuring the total level of Mg^{2+} in serum (tMg^{2+}). However, measuring ionized Mg^{2+} serum level (iMg^{2+}) is considered to be a more valid test to discover the disorders of magnesium homeostasis. Epilepsy is a common neurological disorder characterized by recurrent seizures (sudden interruptions in normal brain function during which large populations of neurons fire repetitively in high synchrony). The underlying mechanisms of epilepsy and seizures are not completely understood yet. Since the role of magnesium metabolism disorders in epilepsy is frequently neglected, a re-examination of literature on magnesium in preclinical and clinical studies of epilepsy was made. Magnesium deficiency is known to have epileptogenic effect. Hippocampal slices perfused with low Mg^{2+} artificial CSF readily generate epileptiform activity. Magnesium deprived diet induces seizures in experimental animals. In humans hypomagnesiaemia increases seizure susceptibility and frequency. In pharmacoresistant epilepsy (PhRE) hypomagnesemia increases the risk of sudden unexpected death from epilepsy (SUDEP). In epilepsy patients, both adults and children, iMg^{2+} levels are found to be low both in serum and CSF. In the majority of PhRE patients a low iMg^{2+} / tMg^{2+} ratio is found interictally. Magnesium exerts antiepileptic effect on experimental epilepsy in animal models both *in vivo* and *in vitro*. In humans, anticonvulsant magnesium effect is long known and clinically used to control several specific seizure types: in eclampsia, uremia, porphyria and hypomagnesemia. Magnesium infusion can also help control noneclamptic and refractory status epilepticus (SE). Magnesium penetration into the brain is normally prevented by the intact blood-brain barrier (BBB). However, in seizures the pathophysiologically increased BBB permeability permits for Mg^{2+} level in the brain to be restored upon infusion. It is considered that oral supplementation with magnesium has the potential to reduce seizure frequency. However, more studies are needed to adequately evaluate the promising benefit of magnesium supplementation as an adjunct therapy to improve the efficacy of epilepsy treatment, especially for PhRE cases.

Keywords: epilepsy, magnesium, pharmacoresistant epilepsy, magnesium deficiency

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ROLE OF ENDOCRINE FACTORS IN PATHOGENESIS OF AUTISTIC SPECTRUM DISORDERS

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The autism concept of was coined by Eugen Bleuler in 1908. Nowadays it is formally called autistic spectrum disorders (ASD) which encompasses several previous diagnoses, including Asperger syndrome, childhood disintegrative and pervasive developmental disorders. Etiology of ASD is unclear and apparently multi-factorial. Hormonal imbalance may play a role. Richard Asher reiterated the relationship between psychosis and hypothyroidism and introduced the term "myxedematous madness" (1949). Hypothyroidism is detected in 0.3-3% of patients in psychiatric hospitals. However, psychiatrists rarely examine hormone levels in patients with ASD. There is currently no definitely known impact of COVID-19 on individuals with autism. The study was aimed to check thyroid function in children and adolescents with ASD assessing the possibility of treating ASD by normalization of thyroid function. 36 children (average age: 6.7±0.3) and 53 adolescents (average age: 13.9±0.7) observed at Saint-Petersburg Regional Autism Center were examined. Their reason of seeing an endocrinologist was the ineffectiveness of psychotropic drugs. All had the serum concentrations of the following hormones checked: thyrotropin (TSH), free (F) thyroxine (T4) and triiodothyronine (T3), prolactin, cortisol, as well as levels of autoantibodies against thyroperoxidase (anti-TPO), TSH receptor (TSHrAb) and thyroglobulin (anti-Tg). 9 children (25%) had COVID-19. Control group consisted of 20 healthy children and adolescents. Of the 9 children who had COVID-19, one 6-year-old boy developed fulminant diabetes mellitus type 1, and another 3-year-old boy lost his speech skills. Other patients were reported to have experienced a mild or moderate course of the illness. The TSH level in ASD was 2.7±0.4 μIU/ml (maximally 8.44; in control group – 0.99±0.03 μIU/ml; $p<0.01$); FT4 – 14.2±1.1 pM/l (in control group – 22,55 ±1,59 pM/l, $p<0,001$). Other results showed no significant difference between the groups. The following were the findings: FT3 – 6.2±0.4 pM/l; anti-Tg - 20.14±13.9 IU/ml, anti-TPO - 7.5±1.2 IU/ml, TSHrAb - 0.69±0.1 IU/l, prolactin - 378.2 ±76.3 μIU/l, cortisol – 305.5±30.1 nM/l. All the patients were prescribed with levothyroxine: their sleep improved, it has been a noticeable improvement in patients' cognitive abilities – they were able to focus better, so their school performance improved as well. The symptoms of ASD may have dyshormonal origin, especially in case of delayed diagnosis of hypothyroidism. In children with ASD, thyroid status should be checked. Thyroid supplementation has been proven to be effective in amelioration of ASD cognitive dysfunction.

Keywords: autistic spectrum disorders, hypothyroidism, levothyroxine, COVID-19

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ANTIGEN MIMICRY BETWEEN HUMAN CORONAVIRUSES AND CANDIDATE PROTEINS INVOLVED IN PATHOGENESIS OF SMALL FIBER NEUROPATHY

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The infections, especially if caused by Herpesviridae and Coronaviruses, can cause persistent neuroimmunological disorders. Examples are some cases of chronic fatigue syndrome / myalgic encephalomyelitis, as well as post-COVID syndrome, with excessive systemic action of inflammatory mediators, activation of autoimmune reactions, damage to small nerve fibers, endothelial dysfunction, and dysautonomia. Among the modern concepts of autoimmunity (epitope spreading, cryptic epitopes, bystander activation), molecular mimicry also plays a part. It occurs when viral and human antigens share similar minimal epitopes (at least pentapeptides). We selected 6 human proteins – candidates, mentioned in literature as potential targets of autoimmunity in small fiber neuropathy, all expressed in fine nerve fibers, and 3 antigens from every one of 7 human Coronaviruses known to date: spike (S) protein, membrane (M) protein and nucleocapsid (N) protein. Amino acid sequences of all these proteins were obtained from the UniProt database. To determine the pentapeptides, we have created original computer program «Alignmentaj». Thanks to the PDB database and the PyMol program, we studied the location of similar pentapeptides in 3D structures of viral or human antigens. Pentapeptides of immunoreactive epitopes of human Coronaviruses were evaluated using the IEDB database. We found totally 30 pentapeptides shared between Coronavirus S-proteins and human small nerve fiber autoantigens, 5 shared pentapeptides in viral M-proteins and 15 pentapeptides – in viral N-proteins. Many of the shared pentapeptides found are located in the 3D surface structure of human or viral antigens. Almost all these pentapeptides located in the immunoreactive epitopes of human Coronaviruses. MERS-CoV proteins share similarities with the fibroblast growth factor receptor 3, a promising antigen for the autoimmune small fiber neuropathy and corneal pain, in the fibromyalgia-type post-COVID-19 syndrome. Peptide sharing is also typical for plexin-D1 and seasonal Coronaviruses, where the human protein is another promising target for the autoimmune aggression, especially in the face-related pain syndromes. Seasonal Coronaviruses share numerous amino acid sequences with the sodium channel protein type 9 subunit alfa, which dysfunction can cause neuropathic pain. The results obtained witness for probable autoimmune origine of many post-COVID and post-antiCOVID vaccination disorders.

Keywords: molecular mimicry, human coronaviruses, human autoimmunity, small fiber neuropathy

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RISK ASSESSMENT OF THE DEVELOPMENT OF NEURODEGENERATIVE PROCESSES IN COSMONAUTS

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The professional activity of cosmonauts impacted by a complex of extreme hazards: a redistribution of biological fluids, prolonged plethora in cerebral vessels, increasing intracranial pressure (alongside the flattening of the pituitary gland) - all can contribute to neuroendocrine damage, creating a risk of pre-clinical neurodegenerative processes. Of current interest are occupational specifics of space travelers' work in the context of risk factors of neurodegenerative processes and search of markers of their preclinical manifestations at different stages of their professional career. The study was aimed to evaluation of the of neurodegeneration risk in cosmonauts and its timely prevention. 7 male spaceflight veterans were involved (mean age 56.9±5.6 years, last flight more than 180 days ago), all clinically healthy. The comparison group consisted of 10 civil aviation pilots. All subjects signed voluntary informed consent to participate in the study. C-reactive protein, interleukin-6, interleukin-8, tumor necrosis factor-alpha (TNF-α), prolactin (by ELISA), and mitochondrial proteins parkin and prohibitin (by immunocytochemical analysis of buccal epithelium) were tested. Studies revealed the absence of correlations between the total number of days in space or the number of hours in open space - and most of the compared immunoendocrine parameters. However, a statistically significant direct correlation was established between the number of hours in outer space and the serum level of TNF-α with a very high reliability according to the Chaddock scale ($\rho = 0.991$; $p < 0.001$). Immunocytochemical analysis of the expression of mitochondrial proteins of the buccal epithelium showed an obvious trend towards a decrease in the level of expression of prohibitin and parkin proteins in the group of cosmonauts compared to the control group. The results witness for depressed mitochondrial functions or lack of protective mitochondrial proteins, which may be risky for the development of neurodegenerative processes. Cumulative Space Exposure Index (CSEI) integrating the spaceflightoccupational hazards was developed, tested, strictly correlated with above mentioned changes and introduced into practice. The results make pre-nosological diagnostics possible as a basis for the prevention and preventive therapy of neurodegenerative diseases. The introduction of CSEI in practice restrains the depreciation of the labor resource of space travelers, may be used in professional selection and will contribute to professional longevity of cosmonauts against the background of a shortage of such professionals.

Keywords: cosmonauts, cosmic radiation, hypergravity, neurodegeneration, microgravity

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T-CELL IMMUNE RESPONSE IN COVID-19 CONVALESCENTS AND FOLLOWING VACCINATION

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COVID-19 has been extensively studied, but much remains unknown regarding the pathogenetic features of the immune response. Increased T cells depletion and decreased functional diversity in COVID-19 predict severe disease. Despite the impaired response, COVID-19 convalescents developed coronavirus-specific memory T cells that remained detectable for 2 years post recovery. Thus, the T-cell immune response and its pathogenetic mechanisms in COVID-19 are far from being fully characterized, so their study is essential to better understand the immune response against the virus, and the long-term immunity that virus-specific T cells can provide. In addition, the role of vaccination plays in triggering specific T-cell responses against the virus should not be missed, since its analysis can help to determine the reliability and durability of protection. To evaluate the T-cell immune response by detecting IFN-γ-secreting T cells after stimulation with different SARS-CoV-2 antigens in COVID-19 convalescents and following vaccination with Gam-COVID-Vac vaccine. In addition, IgG humoral immunity was analyzed and compared with the specific T-cell response. The study included 102 blood samples and three study groups: COVID-19 convalescents (n=58), vaccinated individuals (n=26) and controls (n=18). The ELISPOT method (TigraTest SARS-CoV-2 reagent kit, JSC GENERIUM, Russia) was used to determine the number of Tcells responding with IFN-γ synthesis to stimulation by peptides containing epitopes of the S-protein or N-, M-, ORF3, and ORF7 proteins, using peripheral blood mononuclear cells. Determination of the IgG in serum was carried out by ELISA. T-cell immune responded to stimulation by both sets of peptides with increased IFN-γ production. COVID-19 convalescents and vaccinated individuals have more spots in the stimulation wells, compared to the controls ($p < 0.01$). The correlation between the studied parameters of T-cell immune response and humoral immunity in the groups was not found ($p>0.05$). So, the presence of T-cell immune response was confirmed approximately in 70% of convalescents and in 50% of vaccinated individuals. The presence of a humoral immune response was confirmed in more than 90%. Thus, the interlinked mechanism of the formation of specific receptors of naive T and B cells in the host results in a different level of T-cell and humoral antiviral immunity in COVID-19 convalescents and vaccinated individuals.

Keywords: COVID-19, T-cell immune response, Gam-COVID-Vac, ELISPOT

RESULTS OF THE STUDY OF GENE POLYMORPHISM IN CHILDREN PATIENTS WITH COVID-19

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Factors that may contribute to the manifestations of the disease are population density, sex and age differences. Infants and children develop a mild or asymptomatic of the disease. They have a unique immunophenotype and respond differently to SARS-CoV-2 infection. Interleukins play important role in the development of COVID-19. IL-2 controls the immune response, differentiation and survival of regulatory T cells. IL-6 induce an innate hyperinflammatory response in SARS-CoV-1. IL-4 induces differentiation of Th0 into Th2 cells and influences the outcome of SARS-CoV-1 infection. Genetic factors involved in the mechanism of immunity regulation have shown an association between the IL-6-174C variant and the severity of SARS-CoV-2. GA/AA genotypes (lower expression) of the IL-10 indicate a high susceptibility to COVID-19 damage. These genotypes do not allow IL-10 to control the immune response. IL4 gene polymorphism (risk allele: T) may also affect the outcome of SARS-CoV-1 infection. The aim was to analyze the frequency of occurrence of polymorphism of genes interleykines in children with COVID-19. 54 children (0-14 years old) with a COVID-19 infection were examined. Verification of the diagnosis was based on confirmation of the SARS-CoV-2 virus by RT-PCR and the presence of clinically symptoms and on x-ray. All patients signed an informed consent for the study. DNA was isolated from the whole blood of patients. To analyze polymorphisms of genes IL2 T-330G, IL4 **C**-589**T**, IL6 **C**-174G and IL10 G-1082**A** an allele-specific PCR with electrophoretic detection 3% agarose gel was used. The dominant genotype in patients with COVID-19 was the heterozygous TG genotype of the T-330G IL2 gene polymorphism (42%). Genotypes GG and TT of the polymorphism T-330G gene IL2 polymorphism was 32 and 26% respectively. Genotypes CC (48%) and CT (44%) polymorphism **C**-589**T** of gene IL4 was more often comparative genotype TT of IL4 **C**-589**T** (8%). Research of genotypes variants polymorphism of **C**-174G gene IL6 showed that CG – 56%, CC and GG – 24 and 20 % respectively. PCR analizes IL10 G-1082**A** determined that genotype GA was52%, AA – 32%, GG – 16%. Polymorphisms in immune-related genes to influence the outcome of COVID-19 disease. The results of the present study, despite their intrinsic limitations, showed that many different gene SNPs could be associated with a higher risk for COVID-19 infection. Understanding individual-specific polymorphisms may help better explain COVID-19 outcomes in genetic profiling to create personalized COVID-19 therapies.

Keywords: T-330G IL2, IL4 **C**-589**T**, IL6 **C**-174G, IL10 G-1082**A**, COVID-19, children

VON WILLEBRAND FACTOR TO ADAMTS-13 RATIO AS A PROGNOSTIC FACTOR OF COVID-19 SEVERITY AND THROMBOSIS RISK

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In a clinical picture closely resembling a secondary thrombotic microangiopathy COVID-19, which is often associated with a high prothrombotic risk, is suggested in some patients as an imbalance between von Willebrand factor (VWF) and ADAMTS13, or a high VWF to ADAMTS13 ratio. Further research is still needed to fully understand the mechanisms associated with the prothrombotic state in COVID-19. This includes studies on the way the VWF/ADAMTS13 axis imbalance is connected to the intermingled mechanisms of COVID-19 pathophysiology, such as immune dysregulation, complement overactivation, neutrophil extracellular traps, and autoantibodies, which may all join together to propagate COVID-19-associated coagulopathy. Since thrombosis is a hallmark of the evolving pathology, it is likely that the degree of impairment of the VWF/ADAMTS13 axis would progress concurrently with disease worsening. To study the association between the level and activity of ADAMTS-13 and the vWF blood level as a VWF to ADAMTS13 ratio in COVID-19 of varying severity and thrombosis risk. ADAMTS13 antigen and activity were evaluated in plasma with ELISA kits "TECHNOZYM ADAMTS-13 Antigen" and "TECHNOZYM ADAMTS-13 Activity". The vWF antigen and ristocetin-cofactor activity (vWF:RCo) assessment was performed using automatic coagulometer ACL TOP 700 (Instrumentation Laboratory, USA). Our observational prospective study included 141 patients with COVID-19. They were organized into three groups based on disease severity: mild (n=39), moderate (n=65) and severe COVID-19 (n=37). The vWF:RCo to ADAMTS-13:activity ratio in acute phase of COVID-19, vWF to ADAMTS-13 and vWF:RCo to ADAMTS-13:activity ratios in recovery period were significantly higher in patients with moderate and severe COVID-19. Using ROC analysis, we found the threshold values for these parameters to distinguish patients with mild COVID-19 from patients with moderate and severe COVID-19. In acute phase of COVID-19 the vWF:RCo to ADAMTS-13:activity ratio has a greater diagnostic value than the vWF to ADAMTS-13 ratio. The threshold levels for these indicators were 1.55 and 2.45, respectively. So, despite ongoing anticoagulant therapy, high levels of both ratios persisted over recovery period, which promoted prothrombotic activity of the vascular endothelium and pointed to thrombosis risk. Presence of high-risk inherited or acquired thrombophilia is also an additional risk factor for severe COVID-19. Thus, the vWF to ADAMTS-13 ratio can be used for assessing COVID-19 severity as well as the thrombosis risk.

Keywords: COVID-19 severity, von Willebrand factor (VWF) to ADAMTS-13 ratio, hypercoagulation, thrombosis risk

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MOLECULAR MIMICRY OF HUMAN CORONAVIRUS ANTIGENS AND TARGETS OF AUTOIMMUNE ENDOCRINE DISEASES: BIOINFORMATIC ANALYSIS AND AUTOPSY DATA

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Molecular mimicry between viral and host antigens can produce cross-reacting antibodies promoting autoimmunity. It plays a role in the etiology of autoimmune diseases provoked by exogenous pathogens. SARS-CoV-2 antigens can mimics human autoantigens as well. Similar minimal immune epitopes or pentapeptides can cause molecular mimicry. The molecular mimicry between human coronaviruses' antigens (spike, membrane and nucleoprotein) and marker targets of human autoimmune endocrine diseases (18 autoantigens of endocrinocytes) was subjected to bioinformatic analysis by original computer program «Alignmentaj». To study the location of pentapeptides in 3D-structures of human coronavirus antigens, we used the PyMol program and open database PDB. Epitope immunogenicity was assessed according with IEDB database. Pathohistological *post mortem* study of the endocrine glands from victims of severe COVID-19 was performed to check bioinformatics suggestions by wet lab data. 117 pentapeptides shared by human endocrinocyte autoantigens (in thyroid, Langerhans islets, adrenals and pituitary) with the coronavirus antigens were found, almost all of them located within immunodominant epitopes. The most pathogenic SARS-CoV-2 and SARS-CoV-1 displayed more shared sequences than did the less pathogenic seasonal coronaviruses. Spike protein was richest with shared pentapeptides. Only highly pathogenic coronaviruses shared epitopes with human adrenals. Less pathogenic seasonal ones manifested most mimicry with pancreatic islets. The pathohistological analysisrevealed in thyroid, adrenals and adenohypophysis (but not in neurohypophysis) of COVID-19 victims the abundant expression of SARS-CoV-2 and affluent mononuclear lymphoid infiltration, thus witnessing for immunopathological viral-induced inflammation, which is regarded as wet-lab confirmation of bioinformatics data. According the results of immunohistochemical tests for Caspase-9, the cell death in altered endocrine glands occurred mostly in non-apoptotic pathway. The results link the manifestations of antigenic mimicry with multiple cases of post-COVID-19 and post-anti-COVID vaccination autoimmune endocrine disorders described earlier, in particular, with autoimmune thyroiditis, adrenalitis, hypophysitis and exacerbations of type 1 diabetes mellitus. Autoimmune thyroid pathology was noted most often after COVID-19 in the clinic, and characteristically, most of the common pentapeptides found belong specifically to marker autoantigens of autoimmune thyropathies. The phenomenon of molecular mimicry cannot be blamed as their single prerequisite, but may serve as a tile in mosaic of autoimmunity. The facts revealed emphasize the need of endocrinological diagnostic alertness of a physician while observing patients with post-vaccination and post-COVID-19 health disorders.

Keywords: molecular mimicry, human coronaviruses, autoimmunity, endocrine diseases

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PATHOMORPHOLOGICAL CHANGES OF VASA VASORUM IN LARGE ARTERIES AND THEIR ROLE IN ATHEROGENESIS

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The role of *vasa vasorum* (VV) in the development of atherosclerosis is currently attracting much attention and is widely discussed, with new data strongly suggesting the potential of VV in atherogenesis and its complications. The VV network is considered as a possible venue for the inflammatory cells and atherogenic factors penetration, contributing to the plaque formation and progression. Therefore, we investigated the correlation between pathomorphological changes in VV of the large arteries and the condition of atherosclerotic plaques there. Autopsy material from 50 cases was examined, 6 of them perished from complications of atherosclerosis in post-COVID period. VV of large arteries were stained using histological (hematoxylin and eosin), histochemical (alcyan blue, picrofuchsin by van Gieson), and immunohistochemical (CD3, CD4, CD8, CD20, CD23, CD45 expression) methods. The atherosclerotic plaques of various types, both stable ones with a well-defined dense fibrous cover and a small atheromatous nucleus; and unstable ones, with sings oftearing, cracks and ruptures of connective tissue covers and greater amounts of extracellular lipids were revealed. In the area of unstable plaques, plenteous vascularization of the arterial wall was observed. In the areas of unstable atherosclerotic plaques, moderate thickening of the VV wall due to focal deposition of glycosaminoglycans, degenerative changes in the endothelium, focal inflammatory infiltrates, and a tendency to extravasation were revealed. Immunohistochemical examination revealed there pronounced positive expression of CD3 and CD45. In the areas of stable atherosclerotic plaque, noticeable expression of CD20, CD45 and weak expression of CD3 was noted. A correlation existed between the changes in the VV wall and the progression of plaques. The most pronounced changes of VV were noted in post-COVID cases. The VV changes most often occur in those arterial wall portions where exists marked chronic inflammatory cell infiltration with the presence of macrophages and CD3+ lymphocytes. There is a relationship between VV state and severity of plaque changes, potentially meaningful for the pathogenesis of atherosclerosis. Probably, COVID-19 can be regarded as a potential atherogenic factor.

Keywords: atherosclerosis, stable plaque, unstable plaque, *vasa vasorum*, COVID-19

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EFFECTS OF SILICONE IMPLANTS ON IMMUNE SYSTEM AND AUTOIMMUNE/AUTOINFLAMMATORY SYNDROME INDUCED BY ADJUVANTS

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The use of silicone implants is a topic of hot discussion. Breast implants are the most popular aesthetic surgeries (about 1.8 million operations per year globally). Their manufacturers sometimes do not indicate autoimmune diseases among contraindications. However, it is known that silicone may alter the immune system. The association between silicone implants and autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA) was reported by few authors, but rejected by others. Methods: Analysis of 119 aesthetic surgical interventions on the mammary gland was performed (breast augmentations, mastectomy without reconstruction, radical resection, sectoral resection, lifting, reduction, lipofilling without implants, breast reconstruction). The patients operated upon with silicone implants were compared with those operated without silicone. The following serum immune and endocrine parameters were measured before the operation and at control points (3, 6 and 12 months after surgery): autoantibodies against modified citrullinated vimentin (MCV-Ab), thyrotropin receptor (TSHR-Ab), thyroglobulin (TG-Ab), β2-glycoprotein 1 (Anti-β2-GP1), cardiolipin (ACLA) and annexin V (aAnV) - both IgG and IgM, IgG; concentrations of thyroid hormones, TSH, prolactin, estradiol and testosterone. All patients were interviewed in every control point with ASIA questionnaire for anamnestic factors and complaints matching the ASIA criteria. The significant progressing increase in concentrations of TSHR-Ab (but not other autoantibodies) was revealed after silicone implantation, interpreted as a result of silicone adjuvant action. In 78.1% of patients the level of TSHR-Ab increased more than 2 times for 12 months. Before breast surgery hyperprolactinemia was observed, later vanishing. Its prolonged cases requiring prolactostatics all belong to silicone patients. The incidence of ASIA - syndrome in patients who underwent breast surgery also increased. But it occurred both in silicone and non-silicone breast surgeries and hence was not explained solely by silicone action. Within 12 months after silicone mammoplasty patients with increase of TSHR-Ab nevertheless did not show any clinical and hormonal signs of overt thyroid disease, thus staying in pre-nosological state. Because silicone mammoplasty (unlike other breast surgery) increased of serum TSHR-Ab above the normal level, autoimmune thyroid disease is a contraindication to the silicone implantation. After silicone mammoplasty it is necessary to check the thyroid condition. Anamnestic ASIA incidence increases after various kinds of breast surgery. Hence, ASIA probably is not a disease, but pre-nosological entity resulted from multi-factorial hyperstimulation of the immune system.

Keywords: autoimmunity, adjuvant, mammoplasty, silicone implants, thyroid gland

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MARKERS OF INFLAMMATION IN GINGIVAL CREVICULAR FLUID IN CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

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Aim was to study the cytokine profile of the gingival crevicular fluid (GCF) in children with a juvenile rheumatoid arthritis (JRA). We examined 20 children with JRA and 10 patients without somatic pathology aged 6 to 16 years old. The condition of periodontal tissues was assessed by periodontal indices – gingival index GI (Loe, Silness, 1963) and gingival bleeding index GBI. Biomaterial sampling from the gingival sulcus was carried out using special endodontic absorbent paper points. Enzyme immunoassay for IL-18, IL-10, IL-1ß, IL-1RA, MCP-1, VEGFs in the GCF was performed using the test kits of Vector-Best LLC (Novosibirsk, Russia). Mean GI index in the JRA group was 0.31 ± 0.10 and in the control group – 0.20 ± 0.05 ($p < 0.05$), mean GBI index – 19.90 ± 3.14 and 10.80 ± 2.60 respectively ($p < 0.05$), which was accompanied by a more pronounced degree of inflammation of periodontal tissues. The GCF concentration of IL-18 in the JRA group was 6.70 (4.97–7.92) pg/ml, in the control group – 11.25 (8.70–13.10) pg/ml ($p < 0.05$), while the concentration of IL-1ß was 15.30 (13.79–17.18) pg/ml in the JRA group and 5.36 (5.32–5.54) pg/ml in the control group. The IL-10 concentration in the JRA group was 3.60 (2.89–4.45) pg/ml, which was comparable to the values of the control group. The concentration of IL-1RA was lower in the JRA group than in the control group: 3638.5 (2397.5–4133.5) pg/ml and 4951.0 (4303.0–5455.0) pg/ml respectively. The total GCF chemokine concentration for MCP-1 was determined at the level of 15.65 (14.15–17.39) pg/ml and 15.50 (12.80–21.20) pg/ml for the main and control groups and for VEGF – 49.60 (41.95–54.50) pg/ml in the JRA group and 12.00 (11.00–13.00) pg/ml in control group. In children with juvenile rheumatoid arthritis, an imbalance of pro- and anti-inflammatory cytokines in GCF plays a role in the development of gingivitis: an increased of IL-1ß concentration – a triggering factor of pro-inflammatory chains, a decreased concentration of IL-1RA – an anti-inflammatory cytokine, an increased concentration of VEGF – a marker of hypoxia.

Keywords: gingival fluid, cytokines, juvenile rheumatoid arthritis, markers of inflammation

NK- AND T-CELLS IN PRION DISEASES

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Prion diseases are the fatal neurodegenerative diseases, which occur deposition abnormal folded prion protein scrapie (PrP^{sc}). T-cell and NK-cell appears to be the protective cells in prion diseases. The aim of the work is to identify the role T and NK cells in prion diseases. Systematic literature review using PubMed. PrP^{sc}, simulating to the cellular prion protein, badly detected by immune system. For this reason prion-antibody low produced or not produced. Prion diseases are characterized by increasing level reactive oxygen species (ROS) and specify cytokines (pro-inflammatory – interleukin-1, TNF; anti-inflammatory – interleukin 4 and interleukin 10 and others). In prion associated inflammation is take activation the microglia and astroglia, which have a different subtypes and form various cells environment. This characteristic features the prion diseases have influence with function NK and T-cells. Activation microglia and astroglia leads to a dysfunction blood-brain barrier and an infiltration NK and T-cells in brain tissues. NK-cells is lymphocyte an innate immune system. They are activated in prion diseases via binding to the damage-associated molecular patterns. The pathogen-associated molecular patterns play the little part in activation NK-cells. NK-cells produced granzymes and perforines, absorbed the apoptotic bodies and eliminate the prions, but damage the neurons. Differentiation CD4+ T-cell depend on a cells environment. Complication ROS and interleukin 4 leads to formation Th2 and Treg cells. Since low level interleikin -2 number Th17 cells in brain tissues is small. Types and quantity cytokines by produced microglia change at different stages and in different brain regions, probably, in brain presents other T-cells. Treg produce interleukin-10, TGF-β. Interleukin-10 leads to formation anti-inflommatory subtype microglia and astroglia, that survival the neurons. Th2-cell play important the role in antibody production but their function in prion disease unclear. Th2 cells are able to suppress the Th1 response with interleukin-4. CD8+ T-cells eliminate prion protein though cell dependent cytotoxicity but damage neurons. The glia and T-cells have bilaterally bond. T and NK-cells have a protective function in prion disease but could damage neurons. The role Th1, Th9, Th17 and CD8+Tcells in prion diseases requires further research.

Keywords: neurodegeneration, prion diseases, T-cells

THE ROLE OF NONSPECIFIC INFLAMMATION AND APOPTOSIS IN THE PATHOGENESIS OF ENDOMETRIAL HYPERPLASIA

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Endometrial hyperplasia (EH) is the one of the most common gynecologic diseases and main ethiopathogenic factor of EH development is an imbalance of estrogens and progestogens. We propose that inflammation also plays a key role in the progression of endometrial hyperplasia. Endometrial scrapings were studied in 47 women of reproductive age, divided into 4 groups based on the morphological conclusion: control, simple endometrial hyperplasia without atypia, complex hyperplasia without atypia, adenocarcinoma. To study the parameters of local immunity, monoclonal antibodies (Dako, Denmark) were used to detect total leukocyte antigen (CD 45), natural killer population (CD 56) and macrophage marker CD 68. Also we used vascular endothelial growth factor A (VEGF-A), apoptotic markers bcl-2, cd95. Morphometric analysis was carried out using ImageJ and ImageScope programs. The statistical significance of the differences was determined using the nonparametric Mann-Whitney U-test. Results of the hyperplastic processes investigation, a gradual intensification of the expression of markers of cell populations of the immune response (total leukocyte antigen CD45, CD 56 NK cells, CD4 and CD8 T-lymphocytes) is noted, which indicates the presence of inflammatory reactions in the stroma of the endometrium against the background of hyperplasia of any form. There is an intensification of the production of vascular endothelial growth factor as the degree of cytological atypia in hyperplastic processes increases, with peak rates in cases of verified adenocarcinoma. The apoptotic markers Bcl-2 and CD-95 demonstrate a depression of the intensity of reactions in complex hyperplasia, especially atypical in comparison with the control parameters and in the group with simple glandular hyperplasia without atypia. We suggest that the initial responsibility for the development of simple endometrial hyperplasia belongs to systemic hyperestrogenemia and, in particular, local hyperestrogenia, but that the role of inflammatory processes increases in complex and atypical EH. Development of inflammatory changes in endometrial hyperplasia may be considered as a factor in the promotion and progression of pathology. This inflammatory process which follow EH, interpreted in our investigation such as "endometrial hyperplasia associated inflammation". Thus, cytological atypia in hyperplastic processes in the endometrium correlates with the intensity of inflammatory reactions, as well as the intensification of neoangiogenesis, which actualizes the modification of existing hormonal treatment protocols due to the introduction of anti-inflammatory therapy courses. VEGF-A may be a potential marker of endometrial malignant transformation, which requires further study.

Keywords: endometrial hyperplasia, inflammation, apoptosis

MELISSA OFFICINALIS AS A NUTRITIONAL STRATEGY FOR CARDIOPROTECTION IN EXPERIMENTAL AUTOIMMUNE MYOCARDITIS

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This study was aimed at investigating the potential of ethanolic *Melissa officinalis* extract (MOE) to prevent the development of myocarditis and its ability to ameliorate the severity of experimental autoimmune myocarditis (EAM) by investigating MOE effects on in vivo cardiac function, structure, morphology, and oxidative stress parameters. 50 male Dark Agouti rats were included in the study and randomly divided into the following groups: nontreated healthy rats (CTRL); nontreated rats with EAM (EAM); rats with EAM treated with either 50, 100, or 200 mg/kg of MOE for 3 weeks per os (MOE50, MOE100, and MOE200). Myocarditis was induced by immunization of the rats with porcine myocardial myosin (0.5 mg) emulsion on day 0. Cardiac function and dimensions of the left ventricle (LV) were assessed via echocardiography. Additionally, the blood pressure and heart rate were measured. On day 21, rats were sacrificed and the hearts were isolated for further histopathological analyses (H/E and Picrosirius red staining). The blood samples were collected to determine oxidative stress parameters. The EAM group characteristically showed greater LV wall thickness and lower ejection fraction (50.33 ± 7.94% vs. 84.81 ± 7.74%) and fractional shortening compared to CTRL ($p < 0.05$). MOE significantly improved echocardiographic parameters (EF in MOE200 81.44 ± 5.51%) and also reduced inflammatory infiltrate (by 88.46%; $p < 0.001$) and collagen content (by 76.39%; $p < 0.001$) in the heart tissues, especially in the MOE200 group compared to the EAM group. In addition, MOEs induced a significant decrease of prooxidants production ($O_2^{\cdot-}$, H_2O_2, and TBARS) and improved antioxidant defense system via increase in GSH, SOD, and CAT compared to EAM, with medium and high dose being more effective than low dose ($p < 0.05$). The present study suggests that ethanolic MOEs, especially in a 200 mg/kg dose, improve cardiac function and myocardial architecture, possibly via oxidative stress mitigation, thus preventing heart remodeling, development of dilated cardiomyopathy, and subsequent heart failure connected with EAM. MOEs might be considered as a potentially helpful adjuvant therapy in patients with autoimmune myocarditis.

Keywords: *Melissa officinalis*, myocarditis, oxidative stress, rat

LIRAGLUTIDE PRETREATMENT DECREASES OXIDATIVE STRESS AND APOPTOSIS IN ISOPRENALINE-INDUCED MYOCARDIAL INJURY IN RATS

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The potential cardioprotective role of liraglutide is based on multiple effects: reduction of insulin resistance and arterial blood pressure, body mass loss, improvement of lipid profile, and direct effect on the heart and vascular endothelium. The direct impact on the myocardium is reflected in the reduction of oxidative stress and anti-inflammatory effect. Pathophysiology of myocardial injury (MI) includes oxidative stress, which is associated with transcription factor nuclear factor kappa B (NF-κB) and apoptosis. The early phase of oxidative stress activates NF-κB, but long exposure to oxidative stress decreases NF-κB activity. The aim of the study was to investigate liraglutide's antioxidative and antiapoptotic effects in isoprenaline-induced MI. MI in Wistar albino rats was induced by isoprenaline in a dose of 85 mg/kg given on two consecutive days. The study included 4 groups of animals: control (C) – saline was administered for 10 days, and also on days 9 and 10; liraglutide (L) – rats were treated with liraglutide for 10 days + saline on days 9 and 10; isoprenaline (I) – saline was administered for 10 days + isoprenaline on days 9 and 10; liragutide+isoprenaline (LI) – rats were treated with liraglutide for 10 days + isoprenaline on days 9 and 10. After the sacrifice, blood samples were taken for assessment of oxidative stress markers, and hearts were isolated for histopathological analysis and evaluation of markers of apoptosis. Isoprenaline-induced MI was characterized by oxidative stress, proved by increased prooxidative markers, such as thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), and decreased nitrite (NO_2), catalase (CAT) and superoxide dismutase (SOD), and reduced glutathione (GSH). Liraglutide prevented oxidative stress by preventing TBARS and NO_2, and alleviating H_2O_2 and O_2^- changes. Liraglutide showed strong antioxidative potential in healthy rats and in rats with MI, proved by the significant increase in CAT, GSH and SOD. Histopathological analysis showed that liraglutide prevented severe myocardial damage. Liraglutide pretreatment also prevented rise of NF-κB, BAX, cleaved caspase 3, and reduction of BCL-2 in isoprenaline-induced MI. Liraglutide showed strong antioxidative and antiapoptotic effects against isoprenaline-induced MI in rats.

Keywords: isoprenaline-induced myocardial injury, liraglutide, oxidative stress, apoptosis

RETINAL, CARDIOVASCULAR AND RENAL MARKERS IN HIGH RISK OF ATHEROSCLEROSIS

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The state of the fundus in arterial hypertension is one of the indicators of the risk of atherosclerosis-associated diseases. The simultaneity of target organ damage accelerates the development of the cardiovascular continuum. The aim of the study was to establish the relationship between the diameters of retinal arterioles and venules, the area of the foveal avascular zone (FAZ), the subfoveal thickness of the choroid (STC) and parameters characterizing lipid metabolism, fibrosis, the state of the left heart and kidney function in middle-aged patients with uncomplicated essential hypertension. 115 people (86 men and 29 women, mean age 49.7±4.8 years) were examined. The main group - 70 patients with hypertension I-II degree. Control group - 45 normotensive persons. No patients with diabetes mellitus, impaired liver function, or clinically significant ophthalmic pathology were studied. The values of routine hemodynamic and biochemical parameters of blood; estimated risk of death from atherosclerosis-associated diseases over the next 10 years according to the SCORE scale; N-terminal propeptide III procollagen (PIIINP) blood; daily albuminuria; parameters of 24-hour ambulatory blood pressure monitoring; quantitative electrocardiographic (ECG) markers of left ventricular hypertrophy; echocardiography; fundus conditions. Based on the method of scanning laser ophthalmoscopy, the central retinal arterial (CRAE) and venous (CRVE) equivalents, arteriovenous ratio (AVR) were calculated. The FAZ area and STH were determined by optical coherence tomography**.** Patients with hypertension compared with normotensive individuals were characterized by: significantly lower CRAE values ($p=0.009$), significantly larger FAZ area ($p=0.019$), comparable values of CRVE, AVR, STH ($p>0.05$ for each indicator). According to the correlation analysis in the group of patients with hypertension, significant relationships were found: AVR and low-density lipoprotein cholesterol ($r=-0.3$; $p<0.05$); FAZ area and female gender ($r=0.42$; $p<0.05$); FAZ area and PIIINP content in blood ($r=0.3$; $p<0.05$); FAZ area and daily albuminuria ($r=0.37$; $p<0.05$); CRVE and R wave amplitude in lead aVL on ECG ($r=0.31$; $p<0.05$); CRAE and left atrial (LA) volume index ($r=-0.3$; $p<0.05$); STC and age ($r=-0.3$; $p=0.01$). In men, CRVE was associated with the Cornell voltage product ($r=0.3$; $p<0.05$). In women, age was negatively correlated with STC ($r=-0.54$; $p=0.01$), the SCORE index was inversely associated with STC ($r=-0.56$), ABC ($r=-0.53$; $p=0$ .01), CAES ($r=-0.3$; $p<0.05$). In hypertension, retinal microcirculation parameters are associated with indicators reflecting the state of other target organs – LA volume index, R wave amplitude in standard ECG lead aVL, daily albuminuria, and serum PIIINP concentration. Men tend to have a direct association of retinal venule diameter with a quantitative ECG marker of LVH, while women have an inverse association of STC, arteriole diameter with the SCORE index.

Keywords: retinal microcirculation, hypertension, foveal avascular zone, atherosclerosis

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CIRCULATING ENDOTHELIAL CELLS IN MILD COVID-19 PATIENTS

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Current coronavirus disease 2019 (COVID-19) pandemic reveals thrombotic, vascular, and endothelial dysfunctions at peak disease. Most COVID data are yielded from random clinical observations or autopsy of postmortem samples, while precise blood cellular data in survivors are insufficient. Samples of venous blood obtained from 31 hospitalized patients with a PCR-confirmed diagnosis of mild COVID-19 were analyzed using electronic microscopy and flow cytometry. We found that upon admission to the hospital, all patients have a very high number of circulating virus-damaged endothelial cells in their blood (404.6 ± 43.8 cells/ml, what is 40-100 times greater than in healthy individuals). For the first time, we showed the existence numerous holes on the membrane of circulating endothelial cells, comparable in diameter to the size of the capsid of the SARS CoV-2 virus. These data prove the fact that the virus penetrates into the endothelial cell, where it not only replicates, but also violate the fixation of endothelial cells in the vascular wall what leads to their exit into the blood flow. The consequence of such a damage is the denudation of blood vessels, which, as we have shown, leads, firstly, to the activation of platelets, which, interacting with erythrocytes and leukocytes, form microthrombi, and, secondly, to the formation of erythrocyte sludges, which, along with circulating endothelial cells, clog the microvascular bed, thus disrupting the supply of oxygen to tissues. We have also shown that such disorders persist in some patients even when they are discharged from the hospital, when standard laboratory parameters (especially, the markers of inflammation such as CRP, ESR, fibrinogen) are normalized. This means that after the treatment, the negative effects caused by viral damage to the endothelium are not eliminated, which can cause the so-called post-COVID syndrome, which affects people who have had COVID-19.

Keywords: COVID-19, circulating endothelial cells, electron microscopy, flow cytometry

PATHOPHYSIOLOGICAL SIGNIFICANCE OF BLOOD CELLS IN ATHEROSCLEROSIS: EFFECTS OF SIMVASTATIN ON HEMATOLOGICAL PARAMETERS IN NEONATAL RATS TREATED WITH MONONATRIUM GLUTAMATE

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The onset of atherosclerosis is primarily determined by the qualitative properties of the blood. In addition to lipidemia, blood cells too have a very important role in the initiation and progression of atherosclerosis. Among the drugs capable of keeping atherosclerosis in check, statins stand out as the drugs capable of reducing cholesterol levels in the plasma, but with numerous additional effects as well. Guided by these facts, we investigated the impact of simvastatin on the number of erythrocytes, leukocytes, platelets, amount of hemoglobin and hematocrit in rats, in the settings of general degeneration, aging, and metabolic disorders, such as these achieved in the monosodium glutamate (MSG) toxicity model. The experimental animals were treated with MSG at a dose of 4 mg/g bw at 48 hours, during the first 10 postpartum days, while the controls were treated with saline in a volume of 10 µl/g bw, following the same procedure. Two months after the onset of treatment, the first subgroup was treated with esophageal simvastatin intubation at a dose of 2 mg/100 g bw, once a day for 30 days, and the second subgroup with drinking water, 500 ml/100g bw, in the same manner. It was clear that there was a biological link between the hematological parameters, hypercholesterolemia and atherosclerosis, and we were able to show that simvastatin did not significantly affect the tested hematological parameters, but that there were gender differences in response to the studied agents.

Keywords: atherosclerosis, haematological parameters, simvastatin, monosodium glutamate

DEVELOPMENT OF A METHOD BASED ON HYDROPHILIC CHROMATOGRAPHY MASS SPECTROMETRY FOR THE DIAGNOSIS OF CHRONIC KIDNEY DISEASE IN CHILDREN WITH VESICOURETERAL REFLUX

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Being a children's most common congenital uropathy (CU), primary vesicoureteral reflux (VUR) has high probability of deterioration to the chronic kidney disease (CKD) and a subsequent renal failure. Despite that, efficient diagnostic methods for the early renal damage still do not exist. The development of an alternative diagnostic method based on the analysis of polar low molecular weight metabolites such as amino acids and their metabolites by a hydrophilic interaction liquid chromatography–mass spectrometry (HILIC-MS) is a promising approach for improving the chronic kidney disease diagnosis. The aim of this study was to evaluate the possibility of distinguishing Group of healthy children and Group of children with kidney damage with VUR by urine samples analysis using tandem targeted HILIC-MS method and to describe the main points in the development of a fast and robust metabolomic method based on HILIC chromatography combined with mass-spectrometry. The study involves 35 patients (average age 4.9±2.4 years), divided into 2 groups. First Group consist of 1 – 16 children with CU (grade II–V VUR), second Group of 19 patients without pathology of the urinary system. Analysis is performed on the mass-spectrometer SCIEX4500 coupled with Shimadzu LC20. Statistical data are processed by an unsupervised machine learning method (GraphPad Prism 8 and MetaboAnalyst 5.0). HILIC-based separation of low molecular weight metabolites such as amino acids and their metabolites method for was developed. The critical importance of the sample preparation and the matrix effect consideration in development of HILIC-MS method was shown. Metabolite analysis of urine samples was performed. It showed that the main difference between two groups was metabolites from kynurenine and amino acids metabolic pathways. These changes made it possible to distinguish samples of Group 1 from those of Group 2. Chromato-mass spectrometry is a modern approach to analysing urine samples that can be a step towards developing fast and non-invasive diagnostic techniques. The method demonstrates sensitivity and specificity. The low molecular weight metabolites such as amino acids and their metabolites in urine samples provide a possibility for differentiating groups of healthy patients and patients with VUR.

Keywords: metabolomic, biomarkers, hydrophilic interaction liquid chromatography, mass spectrometry, congenital uropathy

GLUTATHIONE SYSTEM PARAMETERS AND OXIDATIVE DAMAGE OF DNA AND PROTEINS IN GIRLS AND BOYS WITH CONSTITUTIONAL OBESITY

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Obesity is considered a serious burden for global health. Along with the increase in the prevalence of obesity in adults, there is an increase in this indicator among the child population. The main direction of research now is the identification of early modifiable risk factors that will improve the clinical assessment, treatment and prevention of the disease. More research is needed to study oxidative stress responses and antioxidant defense factors in obese children and adolescents. The aim was the analysis of the content of the thiol-disulfide system and the components of oxidative damage to DNA, proteins in adolescent girls and boys with constitutional obesity (CO). The study included 24 girls (mean age-14.00±1.97 years) and 23 adolescent boys (mean age-13.86±2.21 years) with an established diagnosis of grade 1 CO. The control groups consisted of 23 girls (mean age-14.00±1.26 years) and 20 boys (mean age-13.89±1.41 years), respectively. Height, body weight, waist circumference were measured, body mass index was calculated, and the stage of puberty according to Tanner was determined. Spectrophotometric, fluorometric and enzyme immunoassay methods of analysis were used. It was found that the level of DNA destruction index - 8-hydroxy-2'-deoxyguanosine showed increased values in the groups of girls ($p=0.019$) and boys ($p=0.007$) with obesity relative to the control groups. The indicator of protein oxidation - advanced oxidation protein products did not show significant differences between the groups ($p>0.05$). The activity of the thiol-disulfide system in adolescent patients with CO changed statistically significantly relative to the control parameters. Thus, there were reduced levels of GSH (boys ($p=0.015$), increased levels of GSSG (girls ($p=0.0003$), boys ($p=0.012$)) and GSH/GSSG ratio (girls ($p=0.0002$), boys ($p=0.004$)) in patients with CO compared with controls. The study showed a significant increase in the parameters of oxidative damage to DNA and an imbalance in the thiol-disulfide system in adolescents with CO, regardless of gender. In connection with these changes, in adolescents with CO, it is recommended to carry out corrective measures to stabilize the indicators, with the appointment of drugs with antioxidant properties.

Keywords: constitutional obesity, adolescents, 8-hydroxy-2'-deoxyguanosine, advanced oxidation protein products, thiol-disulfide system

CYTOKINE PROFILE AND LIPID PEROXIDATION PECULIARITIES IN WOMEN WITH INITIAL MANIFESTATIONS OF PELVIC VENOUS INSUFFICIENCY

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Women pelvic venous insufficiency (PVI) is widespread, is closely associated with the risk of reproductive disorders (in 15–25% of patients), and have the high rate of the disease recurrence after treatment. The factors involved in venous wall damage include atherogenic stimuli and chronic endotoxin aggression due to inflammatory processes. The changes in the initial stages of the disease are usually minor and selective. There is currently an urgent need to identify initial markers to develop preventive measures for correction of these changes. The aim of this study was to reveal the cytokine profile parameters' levels, as well as the peculiarities of lipid peroxidation (LPO) and antioxidant defense (AOD) reactions in women with initial manifestations of PVI. Thirty-nine female patients with PVI (mean age 37.4±9.1 years old) were the subjects of the study. The control group included 30 healthy women (mean age 33.5±6.3 years old). The diagnosis was verified by clinical and instrumental examination including ultrasound angioscanning of the pelvic veins and therapeutic and diagnostic laparoscopy, and it was finally confirmed histologically. Spectrophotometric, fluorimetric and enzyme immunoassay methods were used. Compared to the control group the cytokine profile of PVI patients characterized by increased concentration of proinflammatory interleukins (IL) (IL-6 and IL-8) and anti-inflammatory cytokines (IL-4 and IL-10) and higher values of IL-6/IL-10 ratio. The level of primary LPO products, conjugated dienes, was significantly increased with simultaneous final products - TBARs values decreasing in comparison to the control. The AOD system main enzyme, superoxide dismutase, activity was decreased, while the catalase activity increased. In patients with PVI, the glutathione reduced form concentration was lower than in the control group. The results of the study in women with PVI suggest negative changes in the cytokine profile and multidirectional changes in the indicators of the LPO system state in the initial stages of the disease. The control of these changes in patients with PVI should probably be an important component of preventive measures in the initial stages of the disease.

Keywords: cytokines, women, pelvic venous insufficiency, lipid peroxidation

GIRLS OF TWO ETHNIC GROUPS: THE CONSTITUTIONAL OBESITY MOST INFORMATIVE METABOLIC PARAMETERS

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besity constitutional form is leading among its various forms, and found mainly in girls. Lipid metabolism disorders, oxidative stress reactions activation, and capacity of the body's antioxidant system decrease often accompany this form of obesity. It is important to identify the most informative indicators describing the differences between groups of adolescents, taking into account the ethnic factor and elaborate targeted influence on metabolic parameters.

The aim of this research was to identify the most informative metabolic markers of constitutional obesity in Caucasians and Asian girls. 39 Caucasian and 44 adolescent Mongoloid girls with grade 1 constitutional obesity were examined. As a comparison, we used data from the same age and ethnicity practically healthy adolescent girls (control groups): 26 Caucasian and 59 Mongoloid girls. Spectrophotometric, fluorometric and statistical (discriminant analysis) methods were used. The results of this type analysis revealed the most informative metabolic parameters in Caucasian girls - glutathione-S-transferase, retinol, diene conjugates, triacylglycerol's, compounds with unsaturated double bonds; in Mongoloid girls - glutathione-S-transferase, very low density lipoprotein cholesterol, high density lipoprotein cholesterol, diene conjugates, oxidized glutathione, superoxide dismutase activity. The identification of the most informative indicators of lipid metabolism and lipid peroxidation-antioxidant defense processes makes it possible to build a mathematical model to substantiate pathogenetically the homeostasis correction methods in adolescents with exogenous constitutional obesity, depending on the ethnic factor.

Keywords: obesity, adolescents, metabolic markers, ethnos

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CURCUMIN ATTENUATES OXIDATIVE STRESS IN RATS WITH CFA INDUCED-RHEUMATOID ARTHRITIS: A PILOT STUDY

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Oxidative stress is defined as the persistent imbalance between the production of reactive oxygen species (ROS) and antioxidant defense culminating in irreversible cellular alterations. Curcumin (diferuloylmethane) is the active component derived from Curcuma longa. Curcumin is a potent scavenger of a variety of reactive oxygen species including superoxide anion radicals, hydroxyl radicals, and nitrogen dioxide radicals, and these protective effects are attributed to its antioxidant property. In this study, we analyzed whether curcumin modulates RA-induced oxidative stress and synovial hyperplasia and investigated the associated mechanism. Complete Freud's Adjuvant-induced arthritis (CFA) was developed in Wistar rats and used as a model resembling RA in humans. Rats were treated with curcumin (200 mg/kg three times per week *per os*) for 4 weeks. Effects of the treatment on redox status, local joint (paw thickness, articular score, X ray imaging), peripheral blood (inflammatory markers and oxidative stress), and synovial hyperplasia, in the pathogenesis of CFA were analyzed. Curcumin treatment inhibited the increased levels of pro-oxidative markers and improved antioxidant capacity in CFA rats. Our findings show that curcumin alleviates CFA-induced inflammation, synovial hyperplasia, and the other main features involved in the pathogenesis of CFA. These results provide evidence for the anti-arthritic properties of curcumin and promote its potential use for the treatment of RA.

Keywords: rheumatoid arthritis, curcumin, Complete Freud Adjuvant-induced arthritis, redox balance

THE POTENTIAL CARDIOPROTECTIVE EFFECT OF BILE ACIDS IN ISOPRENALINE-INDUCED MYOCARDIAL INJURY

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Cardioprotective effect of bile acids has already been described and it depends on how bile acids alter immune response and ROS activity. Oxidative stress takes an important place in pathophysiology of myocardial injury (MI) leading further to activation of several transcription factors and at the end to the apoptosis of the myocytes. The aim of this study was to investigate cardioprotective and antioxidative effects of ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) pretreatments in isoprenaline-induced MI. Healthy male Wistar albino rats were used in this study. UDCA (25 mg/kg) and CDCA (25 mg/kg) were dissolved in propylene glycol (PG) and administered by oral gavage, while myocardial injury was induced by subcutaneous administration of isoprenaline (I; 85 mg/kg) for two consecutive days. The animals were divided into 6 groups: control group (PG p.o. for 10 days and saline s.c. on day 9 and 10); I group (PG p.o. for 10 days and I s.c. on day 9 and 10); UDCA group (UDCA p.o. for 10 days and saline s.c. on day 9 and 10); UDCA+I group (UDCA p.o. for 10 days and I s.c. on day 9 and 10); CDCA group (CDCA p.o. for 10 days and saline s.c. on day 9 and 10) and CDCA+I group (CDCA p.o. for 10 days and I s.c. on day 9 and 10). The ROS activity was measured in the heart homogenate and the biochemical parameters were determined in the blood samples. Isoprenaline-induced MI was characterized by an increase of high sensitive troponin I (hsTnI) values in the I group compared to the control group. UDCA and CDCA pretreatment decrease the values of hsTnI in groups where isoprenaline was administered compared to the I group. Values of prooxidative marker thiobarbituric acid reactive substances (TBARS) were significantly lower in UDCA+I and CDCA+I compared to I group, while in the groups that did not receive isoprenaline its values were lower in UDCA compared to both control and CDCA groups. There is a trend in increasing the values of antioxidative markers CAT, SOD and GSH in groups that received UDCA and CDCA, without statistical significance except in GSH values in CDCA+I and CDCA groups. Pretreatment with UDCA or CDCA reduced isoprenaline-induced MI and have antioxidative potential to prevent oxidative stress on site.

Keywords: isoprenaline-induced myocardial injury, ursodeoxycholic acid, chenodeoxycholic acid, oxidative stress

MOLECULAR MARKERS OF BONE METABOLISM IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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Chronic kidney disease-mineral bone disorders (CKD-MBD) of dentoalveolar system (DAS) are observed in patients with end-stage CKD. Nowadays, more attention is paid to non-invasive diagnostics of oral pathology in children, namely, the use of saliva. Matrix metalloproteinase-8 (MMP-8) and osteoprotogerin (OPG) are considered the most significant markers of bone metabolism disorders in saliva. This study aimed to establish patterns of changes in salivary MMP-8 and OPG in 76 children aged 7 to 18 years with different severity of CKD. Groups of 19 people were formed: group 1 - children with CKD stage 1-2 receiving drug treatment; group 2 - children with end-stage CKD on hemodialysis; group 3 - children one year after kidney transplantation; group 4 (control) - children with minor surgical pathology without kidney disease. Periodontal status evaluated by SBI, PMA and Russell indices. Cone beam computed tomography (CBCT) measured in Hounsfield units (HU) was performed to determine jawbone density. Saliva was collected by absorption method in the morning before drug and food intake. MMP-8 and OPG measured by solid-phase ELISA using HumanTotal MMP-8 QuantikineELISAKit, R&D Systems and Osteoprotegerin, Biomerica (USA) reagent kits. The highest PMA, SBI and Russell indices was observed in group 2; group 1 values were close to the control; group 3 has a slight increase. When lower jaw density analyzing, its decrease in group 3 compared to the control and CKD stage 1-2 group. Bone density in the anterior part of upper jaw, was the same with both group 3 and 4, however, it was significantly lower in group 1. Bone density in the posterior part of upper jaw in all studied groups did not differ statistically. Salivary MMP-8 and OPG in children with different CKD stage was significantly higher compared to the control. The maximum values of MMP-8 were recorded in group 2. Higher salivary OPG was noted in group 1 and 3. The obtained results of salivary MMP-8 and OPG levels were compared with CBCT data and dental indices data. The data confirm the relationship between salivary MMP-8 and OPG levels and periodontal health in children, and also help to determine both hard and soft oral tissues damage severity in children with end-stage CKD. Thus, saliva makes DAS diseases diagnostics possible in children with CKD. Salivary markers measurement is non-invasive, so it can be widely used in clinical practice for the timely diagnosis of DAS changes.

Keywords: matrix metalloproteinase 8, osteoprotegerin, CKD-MBD, chronic kidney disease, dentoalveolar anomalies

EXPRESSION OF MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITOR 1 IN PANCREATIC TISSUES IN RATS AGAINST THE BACKGROUND OF ACUTE EXPERIMENTAL PANCREATITIS

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Acute pancreatitis (AP) is a polyethological and polypathogenetic inflammatory and destructive disease, which is based on necrosis and autolysis of the gland. The key pathogenetic event of destruction inside acinar cells is their calcium overload. However, the events occurring in the extracellular matrix have not been sufficiently studied. The purpose of this work was to study the activity of matrix metalloproteinases 1, 9, 19 (MMP1, MMP9, MMP19) and their tissue inhibitor (TIMP1) in gland tissues during remodeling of the extracellular matrix against the background of experimental AP in rats. The experiment was carried out on 20 rats of the "Wistar" line, divided into 2 groups - control and experimental. AP was modeled by mechanical traumatization of the gland. The animals were removed from the experiment after 7 days. Pancreatic tissues obtained during autopsy were examined by immunohistochemical method using monoclonal and polyclonal antibodies to MMP1, MMP9, MMP19) and TIMP1 in a 1:100 dilution. The evaluation of antibody expression was determined by calculating the relative area of the immunoreactive material using the Image Analysis module of the ZEN 1.1.2.0 program using an "Axio Lab. A1" microscope. MMP1 expression in the control group was 14.6±3.41% and increased to 25.15±8.46% in pancreatitis, $p < 0.001$. The study of MMP19 expression in pancreatic tissues also revealed an increase in the area of immunoreactive material from 13.58±3.77% in the control group to 21.77±7.64% in pancreatitis, $p=0.002$. There were no statistically significant differences in the determination of MMP9: in the control - 19.72% (12.1-24.47) and in pancreatitis - 19.32% (17.07-20.67), $p>0.1$. In the control, the expression area of TIMP1 was 14.22% (11.36-19.36), and in pancreatitis 20.02% (15.81-31.75), $p=0.0016$. Thus, against the background of an acute destructive-inflammatory process in the tissues of the gland, the number of cells expressing MMP1 produced by macrophages, endothelial cells and fibroblasts during inflammation increases, with a simultaneous increase in MMP19 also activated by macrophages, and the increase in activity of TIMP1 obviously acts as a protective-adaptive reaction limiting their excessive growth. The destructive-inflammatory process in pancreatic tissues in the acute phase is accompanied by an increase in MMP1, MMP19, TIMP1 with an unchanged level of MMP9, which is an important component of tissue remodeling in case of damage.

Keywords: acute pancreatitis, matrix metalloproteinases, inhibitor of matrix metalloproteinases

THE EFFECTS OF CREATINE PHOSPHATE CONDITIONING ON HEART FUNCTION AND REDOX BALANCE DURING CARDIAC ISCHEMIA-REPERFUSION INJURY IN RATS

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Despite the great scientific effort investigated into prevention and treatment of ischemic heart disease the morbidity and mortality remains high worldwide. The aim of this study was to assess the effects of creatine phosphate, applied during preconditioning, perconditioning, and postconditioning, on heart function and redox balance in a model ischemia and reperfusion injury using Langendorff technique of isolated rat heart. The isolated hearts were preconditioned, perconditioned and postconditioned with creatine phosphate (0.5 mmol/L, 5 min) and values of cardiodynamic parameters and oxidative stress biomarkers were compared to control group of hearts without conditioning. Creatine phosphate induced significant improvement of heart function and prevented increase of prooxidants. The most beneficial effects were recorded in the percondtitioned group of hearts, given that in this group cardiodynamic parameters were the most similar to the values before the induction of ischemia. Such results suggest that creatine phosphate has the most protective effects if it is applied during the ischemia itself. Altogether, results of this study indicate that creatine phosphate could be valuable tool for prevention of ischemic and reperfusion damage of myocardium in the future.

Keywords: creatine phosphate, heart conditioning, cardiodynamic parameters, oxidative stress, isolated rat heart

METHODOLOGICAL CHALLENGES IN USING HUMAN UMBILICAL ARTERY AS A MODEL FOR IN VITRO STUDIES

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Preparations obtained from umbilical cords serve as a tool for the important research fields, such as models for "organ bath" *in vitro* study of cardiovascular diseases, including preeclampsia. Considering that clinical studies on pregnant women are sporadic, and that the interpretation of results from animal models is insufficiently reliable, the experiments on isolated human umbilical arteries (HUA) have a special place in preeclampsia studies. The aim of the study was presentation of protocols that indicate potential outcomes in interpretion of the vascular effects of tested substances both, under physiological conditions and in a model of homocysteine-induced preeclampsia. The segments of umbilical cords (length 5–10 cm) were obtained from Clinics for Gynaecology and Obstetrics, University Clinical Centre of the Republic of Srpska. The HUAs were cleaned of Warthon's jelly and connective tissue and cut into rings (length 3-4 mm). The HUA rings were suspended between two wire hooks in organ bath chambers filled with 10 mL Krebs-bicarbonate solution, aerated with mixture of 95% oxygen/5% carbon dioxide. Equilibration time was 2h (with washout periods every 10 minutes) and resting tension 2.0 g. Serotonin, prostaglandin $F_{2\alpha}$ and phenylephrine were chosen for the vasoconstriction tests, in order to determine and compare their potencies and efficacies. Relaxing potential of HUA preparations was assessed using endothelium-dependent (acetylcholine) and endothelium-independent (minoxidil) vasodilators. Mechanism of minoxidil-induced vasodilatation in HUAs was determined using glibenclamide (K_{ATP} channel blocker), tetraethylammonium (BK_{Ca} and K_V blocker), and 4-aminopyridine (K_V blocker). Two different concentrations of homocysteine (100 and 300 μM) were incubated in medium for 2 h, in order to estimate the impact of homocysteine on both serotonin contraction and minoxidil relaxation. Serotonin exhibited the highest potency. Significant potentiation of the vasoconstrictor potenty and efficiency was observed when HUA rings were additionally incubated for 2 h compared to the controls. In half of the tested HUA preparations, phenylephrine caused significant vasoconstriction, while half of the HUA preparations had no response to phenylephrine at all. The largest number of HUA rings were non-responsive in the presence of acetylcholine, while a smaller number of preparations showed pronounced reactivity (some with significant relaxation, and others with biphasic activity). Homocysteine (300 μM) caused a significant left-shift of the serotonin curve and significantly down-shift of the minoxidil curve. The obtained results can be useful for planning the future *in vitro* studies of cardiovascular diseases, including preeclampsia.

Keywords: homocysteine-induced preeclampsia, human umbilical artery, *in vitro* studies

EFFECTS OF SUPPLEMENTATION OF GALLIUM VERUM EXTRACT ON REDOX STATE IN PSORIATIC RATS

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Psoriasis is a chronic disease mediated by immune mechanisms which affect the skin but also lead to systemic inflammation. However, there is lack of data in the literature regarding the systemic impact of psoriasis on the redox state. The aim of this study was to investigate the effects of *Gallium verum* extract on the system redox state in psoriatic rats. The study was conducted on 24 male *Wistar albino* rats, 10 weeks old, divided into 3 groups: control (CTRL), psoriasis (PSORI), and psoriasis with *Gallium verum* (PSORI+GV). For induction of psoriasis, 5% *imiquimod* cream was used. The animals were treated by daily application of the cream on their shaved back skin for 8 consecutive days. After induction, the animals from the PSORI+GV group received GV extract via nasogastric tube for 4 weeks. In the collected blood samples following redox state biomarkers were determined: index of lipid peroxidation (TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH). TBARS levels were increased in the PSORI group compared to the CTRL and PSORI+GV groups. The level of nitrites was significantly decreased in the PSORI group compared to the control and PSORI+GV groups. The O_2^- values were increased in the PSORI and PSORI+GV groups compared to CTRL. The level of H_2O_2 was increased in the PSORI and PSORI+GV groups compared to CTRL. GSH values as well as SOD values were significantly lower in the PSORI and PSORI+GV groups compared to the control values. Catalase activity was significantly increased in the PSORI and PSORI+GV groups compared to the control values. *Gallium verum* extract supplementation has a positive effect on the redox status in psoriatic rats.

Keywords: *Gallium verum*, psoriasis, rat, redox status

PROTHROMBOTIC STATE IN COVID-19 PATHOPHYSIOLOGY

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While COVID-19 was initially considered a respiratory illness, rapidly accumulating data suggests that it results in a unique, profoundly prothrombotic milieu leading to high thrombosis risk. Elevated D-dimer level has emerged as an independent risk factor for poor outcomes, including death. Several other laboratory markers and blood counts have also been associated with poor prognosis, possibly due to their connection to thrombosis. The meta-analysis indicated that the results obtained are very heterogeneous and controversial. The pathophysiology underlying the prothrombotic state in COVID-19 is poorly understood. Of concern is the observation that anticoagulation may be inadequate in COVID-19, highlighting the need for additional therapies. Studies investigating the pathophysiology of the COVID-19 associated prothrombotic state may provide valuable insights that can direct appropriate interventional strategies as well. The aim of this work was to study the hemostasis disorders and their prognostic significance in patients with varying severity of COVID-19, including patients at thrombosis risk. A study of 141 patients in both the acute phase and recovery period with varying degrees of COVID-19 severity evaluated a large list of plasma and platelet hemostasis parameters, serine proteases, markers of endothelial activation, ADAMTS-13, prevalence of polymorphisms in genes of the hemostasis system, complement, etc. COVID-19 patients in a prothrombotic state are at high thrombosis risk. Changes in plasma hemostasis in COVID-19 dependent on severity, both in acute phase and recovery period are: hyperfibrinogenemia; Prothrombin time and International Normalized Ratio elongation; decrease in prothrombin by Quick; Activated Partial Thromboplastin Time and thrombin time (Tt) elongation; increase in D-dimer, antigen of the Willebrand factor (vWF:Ag), ristocetin-cofactor activity of the Willebrand factor (vWF:RCo), plasminogen (PLG) and Plasminogen Activator Inhibitor; decreased factor XIII antigen. It is important to note that despite ongoing anticoagulant therapy in our patients, hypercoagulation persisted over recovery period, point to thrombosis risk. Presence of high-risk genetic or acquired thrombophilia is an additional risk factor for severe COVID-19. The following parameters: Tt, vWF:Ag, vWF:RCo, PLG, Protein S and Antithrombin III should be evaluated in progress over recovery period (min 1 month after disease), especially in patients who suffered from severe and moderate forms of COVID-19, to control and manage the prothrombotic state.

Keywords: COVID-19, prothrombotic state, hypercoagulation, thrombosis risk

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THE EFFECTS OF N-METHYL-D-ASPARTATE RECEPTORS ACTIVATORS AND INHIBITORS OF HEART FUNCTION AND REDOX BALANCE IN A MODEL OF ISCHEMIA AND REPERFUSION OF ISOLATED RAT HEART

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The ischemic heart disease remains one of the leading cause of death worldwide, whereby intensive scientific effort is invested in finding opportunities for better patient survival and monitoring of cardiac function. Beside the well-known role of N-methyl-D-aspartate receptors (NMDARs) in the brain physiology and pathophysiology, data from the increasing number of studies indicate possibility of their role in functioning of other organs and tissues. The aim of this study was to assess the effects of activation and inactivation of cardiac NMDARs during ischemia and reperfusion. Experimental protocol assumed the application of the NMDAR agonists glutamate (100 μmol/L) and (RS)-(Tetrazol-5-yl)glycine (5 μmol/L) and the NMDAR antagonists memantine (100 μmol/L) and MK-801 (30 μmol/L) before and after global heart ischemia using Langendorff technique of isolated rat heart. The ischemia lasted for 20 minutes, while subsequent reperfusion lasted for 30 minutes. In this study we used hearts of male Wistar albino rats. We measured cardiodynamic parameters and biomarkers of oxidative stress. MK-801, especially applied after ischemia, in the first five minutes of reperfusion exerted most pronounced antioxidative and cardioprotective effects. Taken all together, results from this study indicate that NMDARs could have role in managing of myocardial survival during ischemia and reperfusion.

Keywords: N-methyl-D-aspartate receptor, cardiodynamics, glutamate, isolated rat heart, oxidative stress

THE PROTECTIVE EFFECTS OF GLUTATHIONE AGAINST CUMENE HYDROPEROXIDE INDUCED TOXICITY IN LEECH RETZIUS NEURONS

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Glutathione (GSH), one of the most abundant low molecular weight non-protein thiols, plays pivotal roles in protecting cells against oxidative stress-induced cellular damage. Additionally, glutathione provides the cell with multiple defences not only against reactive oxygen species (ROS), but also against their toxic products. The present study was designed to examine the ability of GSH to detoxify exogenously applied cumene hydroperoxide (CHP). Experiments were performed on Retzius nerve cells of isolated segmental ganglia of the ventral nerve cord of the leech *Haemopis sanguisuga*. Membrane potentials were recorded in the conventional manner with glass microelectrodes filled with 3 M KCl. The outward potassium current was studied using the two-electrode voltage clamp (TEVC) method. One of the electrodes (the voltage-electrode) measures the membrane potential and connects to a feedback amplifier where this signal is compared with the desired (command) potential. Exposure of leech segmental ganglia to 1 mM CHP produced a depolarization of resting membrane potential of -51.84±3.52 mV to -44.6±3.11 mV (n=10; $p \leq 0.01$). Concomitantly with the depolarization, the action potentials broadened, and the amplitude decreased in a progressive way during the 20-min drug exposure. On average, the prolongation of action potentials amounted to 25.67±7.92 (n =16, $p \leq 0.01$). The application of CHP caused an initial excitation followed by depression of the spontaneous electrical activity. TEVC recordings showed that CHP induced significant changes in the outward potassium currents. At the test potential of +4 mV, the fast and late steady part of the potassium outward current dropped from 68 to 37 nA (46%) and from 36 to 22 nA (39%). GSH (0.2 mM) largely inhibited the effects of CHP on the action potential (action potential duration was extended from 9.22±1.14 ms to 12.45±1.56 ms ($p>0.05$). Also, GSH partially blocked the effect of CHP on the outward potassium currents. At the test potential of +17 mV, the fast and late steady part of the outward current dropped from 65 to 52 nA (20%) and from 46 to 39 nA (15%). These results suggest that the neurotoxic effect of CHP on the electrical properties of leech Retzius nerve cells was reduced in the presence of GSH. The protective effect of glutathione against cumene hydroperoxide-induced neurotoxicity may be due, at least in part, to its ability to scavenge ROS and to protect sulfhydryl groups on the ion transport proteins.

Keywords: neurotoxicity, cumene hydroperoxide, glutathione, Retzius cell, potassium channels

EFFECTS OF FORCED RUNNING IN MICE WITH TYPE II DIABETES MELLITUS MODEL

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The effect of forced running exercises on body weight and metabolic parameters in mice with type II diabetes mellitus was studied. To form a model of the disease, a high-fat diet was used, physical activity in the form of forced running was carried out for 4 weeks. The content of glucose and insulin was determined in plasma, glut-4, citrate synthase, OXPHOS and myokines in the muscle tissue was determined by Western blotting. We verified an experimental model of type 2 diabetes mellitus in mice based on a specially developed diet with an excess content of animal fats. The adequacy of the model is confirmed by the formation of hyperglycemia, decreased glucose tolerance and hyperinsulinemia. The resulting model may be particularly useful in the broad field of research on insulin resistance, diabetes and obesity, it will provide a better understanding of the pathogenesis. Forced physical activity in the form of daily treadmill running has been shown to have a number of pronounced effects on metabolism in mice with type II diabetes mellitus. This is manifested in a decrease in body weight, an increase in the rate of glucose uptake and an increase in insulin concentration. All of the above indicates the normalization of carbohydrate metabolism under the influence of regular physical activity and the involution of changes characteristic of type II diabetes. It is also important that physical effects are manifested differently in different age groups and depend on the time of day. In young animals, the effect of forced running on body weight is more pronounced, while in older animals, on the rate of glucose uptake. The use of activity during the period of the day in which the animals are passive (daylight hours), as well as shift training, have a greater effect. It has been proven that the mechanism of the revealed changes is associated with one of the main pathogenetic factors of diabetes - a fatty diet in mice is accompanied by a decrease in the content of GLUT-4 in muscle tissue, and forced physical activity, on the contrary, by its increase. The results obtained reveal a promising way of influencing metabolic processes both at the cellular and systemic levels, which is very important for finding new ways to correct metabolic disorders in type 2 diabetes mellitus.

Keywords: physical activity, myokines, diabetes

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HYBRID ARTERIALIZATION OF THE VEINS OF THE FOOT AFTER MULTIPLE OPEN AND ENDOVASCULAR INTERVENTIONS BY CRITICAL ISCHEMIA

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73-years old male with long history of tobacco smoking and chronic kidney disease admitted with rest pain and left foot trophic ulcer. Computed Tomography Angiography confirmed occlusion of the superficial femoral, fibular, and posterior tibial arteries of the corresponding leg. Patient was diagnosed with infrarenal abdominal aortic aneurysm (up to 4.4 cm in diameter) and both renal arteries were critically stenosed. Blood creatinine levels fluctuated from 150 mmol/l to 330 mmol/l within the last observational year. HBA1C on admission was 6.9%. Past history revealed myocardial infarction (2015) followed by aorto-coronary shunting in 2016. Consecutive 9 months (from 04.2017 to 01.2018) patient underwent multiple types of endovascular and hybrid interventions including femoro-popliteal vein bypass grafting followed by numerous bypass graft thrombectomies, plain and drug-coated balloon angioplasties, iliac arteries stenting and catheter-directed thrombolysis. However, bypass graft early reocclusions have been observed with inevitable progression of critical limb ischemia. An attempt to perform arterialization of the foot veins was regarded as a last chance to save the limb. Common femoral-medial marginal vein composite bypass grafting (politetrafluoroetileno + cephalic vein) has been successfully executed followed by great saphenous vein distal branches ligation and valvulotomy of medial marginal vein using a plain 3 mm balloon catheter. On control angio blood flow reached the metatarsal area. Ultrasound control on the 4-th day after procedure confirmed robust blood flow characteristics in medial marginal vein of the foot (peak systolic velocity — 79 cm/s, blood flow rate — 57 ml/min). Trophic ulcers of the foot showed good healing potential early following the procedure with gradual decrease in size and granulation tissue formation. On the 105th postoperative day, however, patient admitted urgently with an arterialized bypass graft thrombosis. Thrombectomy was attempted followed by medial marginal vein balloon angioplasty and regional thrombolysis. Control angiogram revealed small-sized outflow vessels with slow filling. Recurrent bypass graft thrombosis developed next day after surgery requiring mid-thigh amputation. Presented case demonstrates highly progressive disease burden in patient with peripheral arterial disease and chronic kidney disease with small vessel obliteration even after venous arterialization of the foot.

Keywords: peripheral arterial disease, critical limb ischemia, hybrid surgery, arterializations

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PHENOTYPIC ADAPTATION IN CONDITIONS OF CHEMICAL POLLUTION OF THE ENVIRONMENT

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The level of FRO in the blood system was determined together with hematological studies in the course of field experiments conducted in the conditions of intense chemical pollution at a distance of 3 km from the "Khimprom" chemical factory in Ufa, the Republic of Bashkortostan of the Russian Federation. The CL data of blood components were recorded at the baseline level and after 10, 30, 60, 90 days of the experiment. The obtained results were compared to the baseline data, which were taken as 100%. Summary indicators of luminol-initiated CL of ROS in the process of phagocytosis in animals at the initial level had the following numerical values. The light sum for 300 seconds averaged 28.5 x 10^2 counts. The average amplitude of the maximum luminescence was 23.2 counts/sec. After 10 days, the average values of the light sum for 300 seconds and the amplitude of the maximum luminescence slightly decreased and were equal to 22.2 x 10^2 counts (78.1%) and 17.0 counts/sec (73.4%), respectively. In the subsequent periods of the experiment, a further decrease in the numerical values of luminol-initiated CL of ROS in the process of blood cell phagocytosis was observed. After 30 days of the experiment, the light sum for 300 seconds and the amplitude of the maximum luminescence averaged 19.4 x 10^2 counts (68.2%) and 16.2 counts/sec (69.5%), respectively. After 60 days of experience, the average values of the light sum for 300 seconds were 20.0x10^2 counts (70%), the amplitude of the maximum luminescence was 15.3 counts/sec (66.1%). Indicators close to this level were observed after 90 days: the light sum for 300 seconds averaged 19.6 counts (68.9%), the amplitude of the maximum luminescence was 16.5 counts/sec (71.2%). Consequently, after 10 days of exposure to chemical pollution in animals, the phagocytic function of blood cells decreased, and with further continuation of field experiments, this process became more pronounced. Thus, the stay of animals in the zone of intense chemical pollution of the environment was accompanied by a decrease of luminol-initiated CL of ROS in the process of phagocytosis , both stimulated by cyolite and without stimulation. These indicators reflected the suppression of the phagocytic function of blood cells in the organism. This was observed in all periods of the experiment without noticeable signs of the formation of a long-term perfect adaptation on the part of the natural immune status of the organism as a functional system. The state of immunodeficiency in the organism observed in the process of field experiments indicates a high cost of adaptation. The immune system turns out to be a "defective" functional system in the conditions of an active adaptive redistribution of vital resources in favor of the perfection of energy supply mechanisms in a crisis situation.

Keywords: environmental pollution, blood, chemiluminescence, adaptation, environment

REACTIVE OXYGEN SPECIES OF BLOOD IN THE CONDITIONS OF CHEMICAL POLLUTION OF THE ENVIRONMENT

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The objective of the research was to study the state of free radical oxidation (FRO) in the blood in the conditions of exposure to chemical pollution. The use of the chemiluminescence (CL) method in the process of studying hematological parameters has expanded the possibilities to differentially assess the nature of the body's responses to the action of chemical environmental factors. In addition to other methods generally accepted in hematological practice, the study of the state of FRO was carried out in animals in various regions of the Republic of Bashkortostan of the Russian Federation, at a distance from the center of pollution (Ufa). Chinchilla rabbits were used in field experiments. An idea of the state of FRO in the blood system was compiled based on the intensity of CL of reactive oxygen species (ROS) in the process of phagocytosis of blood cells. The FRO study was conducted in Gorny village of Chishminsky district, "Tseh Keramiki" village of Blagoveshchensky district and in the cities of Belebey, Ufa, Ishimbay. The average values of the light sum for 300 seconds of luminol-initiated CL of ROS during phagocytosis in rabbits kept in Gorny village of Chishminsky district was 31, 10^2counts. A parallel study of CL of ROS in the process of phagocytosis of peripheral blood cells in rabbits kept in other districts of the Republic of Bashkortostan revealed significant discrepancies in comparison with data from Gorny village of Chishminsky district. For ease of comparison, the indicators of CL in the animals of Gorny village were taken as 100%. Thus, the data of CL of ROS in the process of phagocytosis of blood cells in rabbits kept in "Tseh Keramiki" village of Blagoveshchensky district were significantly lower than those in Gorny village of Chishminsky district. The average value of the light sum for 300 seconds of the luminol-initiated CL of ROS in the process of phagocytosis without stimulation was 23.1 x 10^2 counts, which accounted to 74.2% of the corresponding indicators of animals kept in Gorny village of Chishminsky district. The average amplitude of the maximum luminescence corresponded to 19.5 counts/sec. (72.8%). The study of CL of ROS phagocytosis of blood cells in rabbits kept in Belebey also revealed a decrease in the amplitude of maximum luminescence and light sum compared to those in animals in Gorny village of Chishminsky district. Thus, the light sum for 300 seconds of the luminol-initiated CL of ROS during phagocytosis without stimulation averaged 22.1 x 10^2 counts (71.1%), and the amplitude of the maximum luminescence was 18.8 counts/sec (69.9%). The same indicators of the phagocytosis process initiated by luminol CL of ROS and stimulated by cyolite were 28.4 x 10^2 pulses (79.2%) and 18.9 pulses/sec (78.1%), respectively. Consequently, there was also a decrease in phagocytic function in Belebey. In rabbits kept in Ufa, CL of ROS during the phagocytosis of blood cells revealed a further decrease in cellular immunity. Thus, the average value of the light sum for 300 seconds of the luminol-initiated CL of ROS of non-stimulated phagocytosis was 19.6 x 10^2 counts (62.9%), and the amplitude of the maximum luminiscence was 17.0 counts/sec (63.4%). The same parameters of cyolite-stimulated phagocytosis by luminol-initiated CL of ROS averaged 21.4 x 10^2 counts (59.8%) and 15.0 counts/sec (61.9%), respectively. Thus, the study of the condition of animals kept in the disricts of the Republic of Bashkortostan with varying degrees of chemical contamination by the method of initiated CL of ROS in the process of stimulated phagocytosis and without stimulation showed an ambiguous picture. The most favorable level of cellular immunity was observed in animals kept in Gorny village of Chishminsky district. A slightly depressed state was revealed in rabbits kept in "Tseh Keramiki" village of Blagoveshchensky district and in Belebey. Relatively lower indicators of phagocytic function of blood cells were observed in animals kept in Ufa and Ishimbay.

Keywords: environmental pollution, blood, chemiluminescence, environment

PEROXIDATION OF BLOOD LIPIDS IN DIFFERENT CONDITIONS OF CHEMICAL POLLUTION

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The objective of this study was to establish the nature of the body's reaction in response to chemical pollution of the environment in rural areas and in urban environments. In addition to the methods generally accepted in hematological practice, the state of free radical oxidation (FRO) was studied in Chinchilla rabbits kept under the influence of chemical environmental factors in various regions of the Republic of Bashkortostan of the Russian Federation. The observation was carried out in the process of field experiments. The idea of the state of free radical oxidation in the blood system in this study was compiled based on the intensity of lipid peroxidation (POL) in erythrocytes and blood serum. The FRO study was conducted in Gorny village of Chishminsky district, "Tseh Keramiki" village of Blagoveshchensky district and in the cities of Belebey, Ufa, Ishimbay. The study of erythrocyte lipids peroxidation chemiluminescence (CL) induced by hydrogen peroxide with iron sulfate in various regions of the Republic of Bashkortostan allowed us to assess the nature of the FRO process in red blood depending on the degree of chemical pollution of the environment. The light sum for 60 seconds of the induced CL of POL of peripheral blood erythrocytes in rabbits kept in Gorny village of Chishminsky district averaged 22.1 x 10^2 counts. The average amplitude of the maximum luminescence was 19.9 x 10 counts/sec. The indicators obtained from other regions were compared to the data on animals kept in Gorny village of Chishminsky district. Therefore, the above numerical values were taken as 100%. The average values of the CL light sum and the amplitude of the maximum luminescence in other regions of the Republic of Bashkortostan were more pronounced, and significantly exceeded 100%. These indicators in the village "Ceramics Workshop" of the Blagoveshchensk district were 27.7 x 10^2counts per 60 seconds (124.9%) and 25.4 x 10 counts /sec (128.1%), respectively. In Belebey, the summary indicators of induced CL of POL of erythrocytes significantly exceeded the previous ones. The average values of the light sum for 60 seconds were 28.5 x 10^2 counts (129.1%) and the amplitude of the maximum luminescence was 27.0 x 10 counts /sec (135.9%). In rabbits kept in the cities of Ufa and Ishimbay, the indicators of CL of POL of red blood cells were the most pronounced. Thus, in Ufa, the average value of the CL light sum for 60 seconds was 33.8 x 10^2 counts (153.1%), the amplitude of the maximum luminescence was 30.4 x 10 counts /sec (152.0%). In Ishimbay, the CL light sum and the amplitude of the maximum luminescence averaged 34.9 x 10^2 counts per 60 seconds (158.2%) and 32,1x10 counts /sec (161.3%). Consequently, the CL of POL of erythrocytes in rabbits that was induced by hydrogen peroxide with iron sulfate had the highest digital values in the cities of Ishimbay and Ufa, slightly less pronounced in "Tseh Keramiki" village and Belebey. Thus, the increase in CL of POL of the erythrocytes in rabbits kept in "Tseh Keramiki" village of Blagoveshchensky district, Belebey, and especially in the cities of Ufa and Ishimbay reflects the activation of FRO of red blood when staying in conditions of chemical pollution of the environment. In turn, an increase in FRO in erythrocytes is probably one of the links in the mechanism of hematological shifts observed in this case in the body of the animals, in particular, erythrocytopenia. Therefore, in the conditions of chemical pollution of the environment, there was an increase in the FRO of erythrocytes and blood serum components.

Keywords: environmental pollution, blood, chemiluminescence

PARTICIPATION OF NEUTROPHIL NETS IN THE ANTIVIRAL PROTECTION OF THE ORGANISM

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Neutrophils form neutrophil extracellular traps (NETs) as a defense mechanism against pathogens. However, the formation of neutrophil nets in viral diseases has not been practically studied. It is generally accepted that NETs are absent in the body of healthy people and are found only in inflammatory diseases. Neutrophil nets are described in some detail, but their presence in people resistant to viral infection is not known. There is no exact data on the presence of neutrophil nets in the peripheral blood of people who are in contact with infected persons, but who do not also become ill. This study aimed to comparite of NETs studies in healthy individuals during and outside the H1N1 influenza pandemic. The study included two groups of volunteers of 10 people aged 20-25 years. The inclusion criterion was the absence of acute diseases at the time of the study and the presence of chronic diseases in history. The exclusion criterion is acute infectious diseases within a month before the day of the study. Volunteers of the first group took part in the study in May-June 2022. During this period, a calm epidemic situation for influenza and COVID-19 was recorded. Representatives of this group were not in contact with people suffering from acute respiratory diseases. Volunteers from the second group took part in the study in December 2022. During this period, the H1N1 influenza epidemic threshold was exceeded in children and adults. Moreover, the volunteers of the second group were in contact with patients with H1N1 influenza, but they themselves did not get sick. The isolation of neutrophils from the venous blood of patients was performed using traditional isolation methods on a double ficoll density gradient. NETs were visualized and counted using fluorescence microscopy using a specific fluorescent dye for dsDNA SYBR Green. Neutrophils of volunteers from the first group, who had no contacts with sick people, were presented in the form of classical segmented granulocytes and did not spontaneously form neutrophil extracellular traps. Neutrophils of volunteers from the second group, who were not ill, but had contacts with sick people, spontaneously formed NETs in the form of neutrophil nets. The number of NETs was 8.58±0.51%, and their size was 39.68±3.52 μm. The neutrophils of this group spontaneously, without any additional stimulation, formed NETs in the form of neutrophil nets. The results of our studies indicate the protective function of NETs in antiviral immunity.

Keywords: neutrophil extracellular traps, healthy people, viral diseases, neutrophil networks, pathogenesis

EXPERIMENTAL MODELING OF THE CYTOKINE RESPONSE IN COVID-19 CONVALESCENTS AND IN VACCINATED INDIVIDUALS IN SPECIFIC AND NON-SPECIFIC STIMULATION WITH SARS-COV-2 ANTIGENTS

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In COVID-19, large numbers of innate and adaptive immune cells become activated and begin to produce pro-inflammatory cytokines. In most severe cases, hyper production leads to a cytokine storm with functional exhaustion of both innate and adaptive immune responses. The plasma cytokine levels analyzed in the majority of studies indicated the release of proinflammatory, mainly T-helper-1 (Th1) cytokines and T-helper-17 (Th17) cytokines in COVID-19. Cytokine production *in vitro* from peripheral blood mononuclear cells (PBMCs) is a good model to study of T-cell activation in COVID-19-convalescents and post vaccinated individuals, which can provide important data about of the pathogenesis of immune response in COVID-19. The aims of this work were to identify the cytokine-producing capacity of PBMC in COVID-19 convalescents and individuals after vaccination as well as in controls under specific and non-specific stimulation with SARS-CoV-2 antigens. We analyzed the secretion of selected Th1/Th2/CCC/GF cytokines by PBMCs. The study involved 13 COVID-19 convalescents, 14 vaccinated individuals and 10 controls. After stimulation of PBMCs *in vitro* the culture medium samples were analyzed using a multi-analyte flow assay kit for human cytokine 17-plex assay panel. SARS-CoV-2 peptide panels were used for specific stimulation: one set included peptides S-protein, and the another set included the peptides of proteins N, M, ORF3, ORF7. For non-specific stimulation Orthoclone (OKT-3) was used. In the absence of stimulation, was the systematic but unreliably reduced content of almost all cytokines in the culture medium in recovered and vaccinated individuals. In non-specific stimulation almost all cytokines were significantly increased for all individuals. At the same time, specific stimulation does not result in an increase in cytokines content in all samples of the culture medium. All instances of increased cytokine content after specific stimulation compared to non□stimulated PBMCs are unreliable. Thus, according to the data obtained, the cytokine response to specific stimulation of T-lymphocytes sensitized in COVID-19-convalescents and post vaccinated individuals is not accompanied by the accumulation of cytokines in the environment surrounding lymphocytes, which provides a balanced immune response without undesirable consequences, for example, in the form of a cytokine storm.

Keywords: COVID-19, cytokines, T-cells, *in vitro*, PBMCs

CHANGES IN THE SPECTRAL VALUE OF HEART RATE VARIABILITY IN MALE WISTAR RATS IN DIFFERENT MOTOR ACTIVITY

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The study of heart rate variability (HRV) in laboratory animals is an informative method for establishing homeostasis deviations under the influence of various stimuli. Changes in motor activity result in a new level of functioning, depending on the energy supply and body metabolic activity, however, the significance of these changes in the analysis of experimental data were not sufficiently investigated. The objective was to study HRV indicators in free-moving animals at rest and increased motor activity. The study was performed 16 male Wistar rats. HRV indicators were recorded using the Physiobelt 2.5.1 software and hardware complex (Neurobotics, Russia). HRV was analyzed by spectral indicators: the total power of the HRV spectrum (TR); the total power of the high-frequency (HF, ms^2, %), low-frequency (LF, ms^2, %), very low-frequency (VLF, ms^2, %) component of HRV; vagosympathetic interaction index (LF/HF); centralization index (IC). Physical activity was created using a 2-min treadmill run (Treadmill LE8710, Panlab, Spain) (speed – 15 m/min). The first recording was performed before exertion, the 2nd record – after the exertion. The third record was performed after 15-min rest. Statistical processing was carried out in Statistica 13.0 (StatSoft, Inc.). To compare the records we performed Student's paired t-test. $P<0.05$ was considered as significant. We found out the dynamics between the 1st and 2nd records: VLF (ms^2, %), HF, LF (%), LF/HF and IC. A decrease in LF/HF demonstrated an increasing role of the sympathetic nervous system (SNS). The spectral mechanisms dynamics proved the dominant role of humoral factors and sympathetic activation in the adaptation to motor activity. A characteristic sign of the onset of the recovery period was a significant change in VLF (ms^2, %), LF (%), LF/HF and IC between the 2nd and 3rd entries. The changes in the LF/HF value indicated an increase in the activity of the parasympathetic nervous system. A decrease in IC indicated the mechanisms of urgent humoral regulation shutdown and a decrease in the activity of the central mechanisms. The dynamics of the LF demonstrated inhibition of the SNS activity. Thus, the change in the motor activity of male Wistar rats was accompanied by the transition of the functional state of the organism to a new level of regulation. The obtained data can be used as prognostic indicators of the body fitness. Rest for 15 minutes after physical activity contributed to the return to the initial level of most of the studied HRV parameters.

Keywords: heart rate variability; Wistar rats; vegetative balance; motor activity

HISTOLOGICAL STUDIES OF THE RAT HEART DURING INTRAPERITONEAL INJECTION OF PERFLUOROCARBON EMULSION

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In this work we studied the effect of Russian blood substitute drug type "Perftoran" based on gas transport perfluorocarbon 20% emulsion of perfluorodecalin and perfluormethylcyclohexylpiperidine stabilized with 4% surfactant - proxanol-268 at an average emulsion particle size ("artificial red blood cells") 80-100 nanometers on rat heart at intraperitoneal administration in different doses: 8 ml/kg, 12 ml/kg, 16 ml/kg in 1 hour, 16 ml/kg in 10 hours, and 20 hours before removal of the heart for histology. An isolated cardiac specimen was embedded in paraffin and cut into 6-μm-thick sections. Sections were stained with a hematoxylin and eosin staining kit according to the manufacturer's instructions and photographed with a digital camera connected to a microscope. The degree of cardiac myocyte hypertrophy and myocyte cross-sectional area were determined on sections stained. Histological studies in the control group of rats showed abnormal arrangement of muscle fibers with the presence of wavy fibers, oval euchromatic nuclei, hypercellularity (hypereosinophilia) and the presence of transverse signs of ischemic changes. In rat heart tissues, after intraperitoneal injection of perfluorocarbon emulsion (PFE) at doses: 8 ml/kg, 12 ml/kg, 16 ml/kg in 1 hour, 16 ml/kg in 10 hours, and 20 hours before ischemia was created-Photo 1 (8 PFE, 12 PFE, 16 PFE, 10 PFE, 20 PFE), almost the same changes as in the control group were observed - regular arrangement of muscle fibers, hypercellularity (mainly around blood vessels), ischemic cell changes, euchromatic nuclei of muscle fibers oval and elongated shape. Only in the groups receiving the emulsion at doses of 16 mg/kg 10 hours and 20 hours before cardiac withdrawal was there a partial loss of transverse striation of individual cells (less pronounced signs of ischemic changes). Despite some minor changes, we can conclude that intraperitoneal injection of perfluorocarbon emulsion in different doses (8, 12, 16 ml/kg) with different time of emulsion circulation in the body (from 1, 10 to 20 hours before cardiac output) has shown that the studied preparation has no obvious effect on heart tissue after a single injection and can be used as a hemocorrector to compensate blood loss and increase body resistance to ischemia.

Keywords: blood substitution, perfluorocarbon, hemocorrector

THE EFFECTS OF PERFLUOROCARBON BASED BLOOD SUBSTITUTE PERFTORAN ON CARDYODINAMICS IN ANIMAL MODEL OF ISCHEMIA-REPERFUSION INJURY

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The main goal of this study was to investigate the cardioprotective properties in terms of effects on cardiodynamics of perfluorocarbon emulsion in *ex vivo*-induced ischemic-reperfusion injury of an isolated rat heart. The first part of the study aims to determine the dose of 10% perfluoroemulsion (PFT) that will show the best cardioprotective effect in rats on ex vivo-induced ischemic/reperfusion injury of an isolated rat heart. Depending on whether the animals received saline or PFT, the animals were divided into a control or experimental group, and depending on the application of a dose (8, 12, 16 ml/kg body weight) of saline or PFT. At a dose of 12 ml/kg, the maximum left ventricular pressure growth rate differed statistically significantly in the PFT group, ie there was an increase in this parameter at points R25 and R30, and the minimum left ventricular pressure growth rate in R15-R30 compared to saline-treated group. At a dose of 16 ml/kg, PFT also had a statistically significant effect on the change in cardiodynamic parameters in an isolated rat heart organ. Peftoran administered immediately before ischemia (1 hour) has less positive effects on myocardial function in a model of an isolated rat heart compared to earlier administration (10 and 20 hours). The effects of 10% peftoran solution are more pronounced if there is a longer period of time from application to ischemia, ie immediate application of peftoran before ischemia (1 hour) gave the weakest effects on the change of cardiodynamics of isolated rat heart.

Keywords: isolated rat heart, perfluoroemulsion, cardiodynamic parameters, *ex vivo*-induced ischemic-reperfusion injury

TRENDS IN THE EPIDEMIOLOGY OF GESTATIONAL DIABETES MELLITUS (GDM)

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In 2021, hyperglycemic conditions were observed in 16.7% of pregnancies worldwide. Hyperglycemia in pregnancy includes the following nosologically forms: pregestational diabetes, gestational diabetes mellitus (GDM) and diabetes in pregnancy. Hyperglycemia in pregnancy increases the risk of adverse outcomes for mother and fetus and risk of mother and child developing diabetes in the future. In different regions of the world, there is an upward trend in the hyperglycemia in pregnancy rate. GDM accounts for 80.3% of hyperglycemia in pregnancy. The frequency of GDM varies worldwide from 1% to 30%. The GDM rate is high in the Middle East (8.8-20.0%), Southeast Asia (9.6-18.3%), Western Pacific (4.5-20.3%). In the US, the GDM rate has gone up from 4.6 cases per 100 pregnancies in 2006 to 8.2 cases per 100 pregnancies in 2016. The GDM rate growth is noticeable among physically inactive low-income overweight women (body mass index 25-30). The prevalence of GDM in Europe is 10.9%. Among women who are overweight or obese, the prevalence of GDM is 2.29 and 6.79 times higher, respectively. The prevalence of GDM is 2.14 times higher in pregnant women aged >30 (compared with women aged 15-29). In Australia, over the 20 years from 2000 to 2021, the incidence of GDM more than tripled from 5.2% to 17.9%. The probability of GDM increases with age. In women aged 35-39, the frequency of GDM is 2.6 times higher than, in women aged 40–44, in 3.3 times higher, in women aged 45-49 it is 4 times higher than in women aged 15-19. The prevalence of diabetes in pregnant women in Russia has increased from 0.2% in 2005 to 7.8% in 2021. Over the past decades, there has been a noticeable increase in the rate of hyperglycemia in pregnancy, including GDM-related, in a number of countries. It is probably due to an increase in the age at which a woman becomes a mother, a growing number of overweight and obese women in the population.

Keywords: pregnancy, hyperglycemia in pregnancy, diabetes mellitus, gestational diabetes mellitus, epidemiology

THE PROMISE OF PERSONALIZED AND PRECISION MEDICINE (PPM) AS A UNIQUE HEALTHCARE MODEL - NO ONE IS BETTER PLACED THAN PATHOLOGISTS TO DRIVE THE PPM TOWARDS THE FUTURE

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To achieve the implementation of PPM concept, it is necessary to create a fundamentally new strategy based upon the recognition of biomarkers and thus the targets to secure the future of drug design and drug discovery. Along with the impact of genomics, pathology is the central specialty of PPM-related resources. It is pathology that provides the skills, infrastructure, and predictive and prognostic vision we need to lead the way in research-driven biobanking. The vast majority of tissue-based personalized and precision assays used in the clinic (including DNA sequencing, RNA and fusion protein identification by in situ hybridization, and immunohistochemistry to detect protein expression) can be performed on formal infixed, paraffin-embedded (FFPE) tissues. Translational applications and clinical trials depend on FFPE tissues, typically from a diagnostic procedure. Pathologists are reimbursed for interrupting clinical workflows for high-priority assessments (intraoperative consultations, cytologic adequacies). Personal pathology is an area of the clinical innovative tools focusing specifically on the biomarker-based OMICS-technologies.Integration of the concepts will provide a true challenge for the future, requiring collaboration between clinicians, physiologists, pathologists, biodesigners and bioengineers and remaining a real challenge to bioindustry. Pathology and Physiology are the central specialties of PPM. It is pathology that provides the skills, infrastructure, and scientific vision we need to lead the way in science-driven biobanking. In this sense, molecular diagnostics has a long tradition in pathology, especially in clinical one, where various OMICS-analyses of cancers are being incorporated into diagnostic. Although "the next-generation pathologists" have already been launched, further and continuous educational efforts must fully implement the paradigm shift into diagnostic molecular pathology practice and reinvent it as a leading diagnostic discipline in the PPM era. The combination of comprehensive biobanking and the next wave of theranostic pathology technologies provides a natural, externally visible infrastructure that now allows pathology as a discipline – to engage directly with the biotechnology and pharma sector. Pathology-driven biobanking is becoming both central to our core expertise and, even more importantly, a powerful enabler for many of the most promising growth areas of our discipline: PPM healthcare, clinical trials and drug development, theranostics. We stress that implementation of PPM thus requires a lot before the current model "physician-patient" could be gradually displaced by a new model "medical advisor-healthy person-at-risk". PPM will transform the way doctors practice and will shake up the entire pharmaceutical value chain.

Keywords: personalized and precision medicine

REGIONAL FEATURES OF THE EPIDEMIOLOGY OF CERVICAL CANCER (CC)

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Introduction. Cervical cancer (CC) ranks 4th among malignant neoplasms of women all over the world. In Russia, cervical cancer ranks 5th in structure of morbidity and 6th in structure of mortality from cancer among women. Up to 50% of cervical cancer are caused by human papillomavirus HPV 16 and 18. The aim of the work is to identify the correlation between the availability of HPV vaccination programs and the incidence of cervical cancer. Systematic literature review using PubMed, Scopus databases. Age-standardized rates (ASR) and the number of detected cases per 100,000 people were used as comparison criteria. The HPV vaccination program exists in European countries. In 2008, it was introduced in the UK. Mortality from cervical cancer decreased from 8.1/100,000 to 2.8/100,000, which is the good indicator among all countries in the Europe (-65.4%). Australia pioneered a fully funded national HPV vaccination program in 2007. Eight years later, the prevalence of HPV 16 and 18 decreased by 74.5% among women aged 13–24 years. The incidence of cervical cancer decreased from 17 to 10 cases per 100,000 women aged 20–69 years. Over 58% of all cases of cervical cancer in the world happen in Asian countries. The incidence of cervical cancer for 2020 in China is 10.7 cases per 100,000 women, in India 18.0/100,000, and Indonesia 24.4/100,000. The incidence in Russia increased from 39.4/100,000 cases to 50.5/100,000 from 2005 to 2020. Routine HPV vaccination reduces the incidence of HPV-associated pathology and cervical cancer. Screening and vaccination programs have an impact on reducing mortality from cervical cancer. It is difficult to make a definitive evaluation of vaccination programs, since all national programs are not very long in time.

Keywords: Cervical cancer, HPV vaccination

PROTOTYPE OF MODULAR 3D BIOPRINTER WITH COOLING PLATFORM FOR GELATIN-BASED BIOINKS

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Three-dimensional (3D) bioprinting is a novel promising technology of tissue constructs fabrication. It can be used in regenerative medicine, as example – corneal bioprinting. Besides 3D bioprinting can be used in organ-on-chip and similar microphysiological systems. In organ-on-chip systems bioprinting can be used for microfluidic chip fabrication as well as tissue equivalent fabrication technique. The example of bioprinting in organ-on-chip technology is the blinking-eye-on-chip for dry eye disease modelling. Gelatin as one of the highly used material for bioprinting shows low antigenicity, high biocompatibility, has a cell adherence sites and could be modified with a methacrylate group, creating the photocrosslinkable hydrogels used in extrusion bioprinting. The gelation of gelatins solution depends from temperature. The aim of this work was to develop the prototype of modular 3D bioprinter with cooling platform for gelatin-based bioinks. Designed bioprinter uses a Cartesian dimensional coordinate system. The printhead moves along the YZ-axes whereas the printing platform moves along the X-axis. The extrusion based technique was used for bioink dispensing. The syringe pomp was driven by stepper motor and placed on special rack. Printing table has mounted cooling system based on thermoelectric Peltier element. Excess heat from Peltier element is taken away by liquid cooling system. For bioprinting process glass slide or Petri dish 60 or 30 mm are fixed on cooling table. Preheated >35°C gelatin-based bioink must be loaded into syringe pump. When bioinks are dispensing onto cooled surface of Petri dish or glass slide, the gelatin in solution is starting to form gel. It makes able to print three-dimensional constructs. After printing, construct can be crosslinked and used in experiment. The designed system able to be used for tissue constructs bioprinting and for microfluidic chip fabrication in experiments with microphysiological organ-on-chip systems.

Keywords: bioprinting, gelatin, organ-on-chip, microphysiological systems, extrusion bioprinting

DETECTING THE MAJOR MUTATION IN HEREDITARY BREAST CANCER GENES BRCA1/2, CHEK2 AND PALB2 IN CRIMEAN PATIENTS

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Hereditary breast cancer – the most frequency oncopathology among women population. There are different sets of germline mutations and its frequencies in different geographical regions, that is valuable information for preventive medicine and target treatment. In this research we have detected the quality set and frequencies of mutations c.5266dupC, c.181T>G, c.5251C>T, c.5161C>T, 4035delA, c.1961delA, *c.4675G>A, c.68_69del, c.3700_3704del* in *BRCA1* gene, *c.3749dupA*, *c.961_962insAA* in *BRCA2* gene*, c.1100del, c.444+1G>A, c.893_897del, c.470T>C* in *CHEK2* gene*, c.1592delT* in *PALB2* gene. Aim of study to detect the quality set and frequencies of mutations in hereditary breast cancer candidate genes *BRCA1/2, CHEK2* and *PALB2* in slavic and crimeantatar populations in Crimea. Material for research is the whole blood of patients with hereditary breast cancer from Crimean Republican Oncological Clinical Dispensary named after V.M. Efetov. Methods for researching: to extract the DNA from the whole blood of patients on spin columns by using kit «Extract DNA Blood», Eurogen, Russia; to measure the concentration of DNA on fluorimeter with using kit «QuantiFluor® dsDNA System», Promega, USA; PCR in real time with detection of melting curves by using set «HRR-screening», Testgen, Russia. 90 sertified samples of DNA of patients with hereditary breast cancer were analized: 69 slavic patients and 21 crimeantatar patients. Patients of slavic population have mutations: in *CHEK2* gene *c.470T>C* with frequency 12%, in *BRCA1* gene*:* c.5266dupC – 4 %; c.1961delA – 3%; and four mutations: *c.4675G>A, c.3700_3704del,* c.5251C>T, c.181T>G with frequencies 1%. A patient of slavic population has once two mutations in *CHEK2* gene *c.470T>C* and *BRCA1* gene c.5266dupC. Crimeantatar population's patients of Republic of Crimea have mutations: in *CHEK2* gene *c.470T>C* with frequency 10%, in *BRCA1* gene c.1961delA – 5%. Patients with hereditary breast cancer have not mutations in *BRCA2* and *PALB2* genes. Mutation *c.470T>C* in *CHEK2* gene is the major mutation for slavic and crimeantatar populations in Republic of Crimea. Data for crimeantatar population of Republic of Crimea was obtained for the first time. Results of our research allow to doctors provide preventive and target therapy for patients with hereditary breast cancer.

Keywords: hereditary brest cancer, tumor suppressor genes, carcinogenesis, CHEK2

HEREDITARY OVARIAN CANCER GENETICS IN CRIMEAN PATIENTS

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Ovarian cancer (OC) is the most common cause of oncogynecological mortality. It is one of the most aggressive tumors due to heterogeneity of genetic variants and carcinogenesis pathways, late-stage cancer detection, rabid metastasis rate and frequent relapses. There're various risk factors of OC development including hereditary predisposition, menstrual and reproductive function abnormalities, hormonal contraceptives and hormone usage in post menopausal period, as well as lifestyle. The most common cause of hereditary OC is inactivation of tumor suppressor genes. Specific mutations detection can serve as the basis for early diagnosis, targeted therapy and disease prognosis. However, mutation variants and frequency vary according to ethnicity and region therefore it's important to study OC genetics typical for specific population. The aim of this work was to study qualitative and frequency spectrum of mutations responsible for OC development in Crimea. The study included 50 patients (42 belongs to Slavic ethnicity, 8 – to Crimean tatar group) with hereditary OC who received different types of treatment from February to November 2022, in presence of inclusion criteria: 1) age of manifestation up to 50 years; 2) family history; 3) bilateral OC and primary multiple cancer; 4) voluntary consent to participate in the study. To perform mutation detection we took blood samples from patients and extracted DNA out of them using spin columns and "ExtractDNA Blood" kit. Mutations were detected by real-time PCR using "HRR-screening' reagent kit, which includes primers for detecting 16 mutations in the *BRCA1,2*, *CHEK2*, and *PALB2* genes. In the dominant Slavic subgroup, 7 mutations were detected, 5 of which in *BRCA1:* c.5266dupC, c.181T>G, c.68_69del with 0.02 occurrence frequency each, c.4035delA with 0.04 frequency and 2 mutations in *CHEK2:* c.470T>C with 0.04 frequency. Mutations in tatars as well as *BRCA2* and *PALB2* mutations weren't detected as of now. This can be explained both by the small sample available and by the crucial role of different gene inactivation mechanisms. Obtained data shows significant differences between detected mutations and mutations considered the most common in Russian population. Thus, standard genetic panels for the diagnosis of OC and breast cancer includes *BRCA1* 5382InsC, 3819delGTAAA, 4153DelA, 85delAG, 3875delGTCT, 2080delA, 300T> G (Cys61Gly) and *BRCA2* 6174DelT mutations. Study results indicate a trend towards irrelevance of such panels for the Crimean population. Moreover, mutation detection failure in the presence of hereditary nature signs indicates the need to continue research to expand the data on molecular genetic basis of OC development.

Keywords: hereditary ovarian cancer, tumor suppressor genes, mutations, carcinogenesis, BRCA1

THE CRIMEAN POPULATION'S LEPTIN, *ADIPOQ*, *ADIPOR1* AND *ADIPOR2* GENOTYPES ASSOCIATION WITH THE CLINICAL MANIFESTATIONS OF METABOLIC SYNDROME IN COMPARISON WITH THE MAIN EUROPEAN AND ASIAN DATA

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The association of metabolic syndrome (MS) and type two diabetes mellitus (DM2) clinical symptoms with polymorphisms of *ADIPOQ* G (276)T (rs1501299), *ADIPOR2* (rs11061971 and rs16928751) and *LEP G*(-2548)A (rs7799039) genes was studied in the population of Crimea. It was found that in Crimean population the genotype GT of the *ADIPOQ* gene (rs2241766) was associated with high systolic blood pressure (SBP) and body mass index (BMI), contrasting with the European and Asian data. Genotype GG was associated with hyperglycemia and an increased glycated hemoglobin (HbA1c), as in the Russian populations studied earlier. In contrast to the data, the GG genotype was not associated with hypercholesterolemia (as in Asian populations) and an increased BMI (as in European and Russian studies). The genotype GT of *ADIPOQ* gene (rs1501299) in patients with MS was associated with high diastolic blood pressure (DBP), corresponding to European and Asian studies. The highest level of serum glucose associated with the GG genotype, which was not mentioned in the studied meta-analyses. The TT genotype of this polymorphism, similarly to Asian data, caused the highest HbA1c, cholesterol, and BMI. The genotype AA of *ADIPOR1* gene (rs2275737) and the TT genotype of the *ADIPOR1* (rs 2275738) in the Crimean population were associated with DM2 and MS. It was contrast to meta–analyses reviled CC genotype of *ADIPOR1* rs2275737 associated with the risk of DM2 in Latin American population, and AA and GG genotypes of *ADIPOR1* gene rs2275738 - in the Russian population. The genotype AA of *ADIPOR2* gene rs11061971 in the Crimean population associated with hyperglycemia, hypercholesterolemia and increased HbA1c. At the same time, the association of allele A and a decreased risk of DM2 were detected in the Russian population, while the T allele increased the specified risk. The genotype GG of *ADIPOR2* gene rs16928751 was associated with grade two obesity in the Crimean population. At the same time, the alleles +219T rs11061971 and +795A rs16928751 of the *ADIPOR2* gene were significantly associated with high SBP in DM2 patients in the Russian population.The analysis of *LEPG* gene (rs7799039) G (-2548) A polymorphism's genotypes revealed the GA genotype associated with hyperglycemia and hypercholesterolemia. This association is unique for the Crimean population, in contrast to both European and Asian studies. The role played by hereditary factors in MS and DM2 pathogenesis has not yet been fully established, despite the numerous meta-analyses.

Keywords: adiponectin, leptin, metabolic syndrome, polymorphism

EVALUATING THE CARDIOPROTECTIVE EFFECTS OF USNIC ACID AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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Usnic acid (UA) is a natural, dibenzofuranic secondary metabolite found in various lichens and has been widely studied for its biological activities. Its importance stems from its potential therapeutic uses, including antimicrobial, antitumor, and antioxidant effects. Although lichens containing UA are used in traditional medicine and numerous beneficial effects of usnic acid have already been confirmed, there is still insufficient data on its cardioprotective effects. The aim of this study was to evaluate the effect of UA on doxorubicin-induced cardiotoxicity in rats. UA was extracted from the acetonic extract of lichen *Xanthoparmelia stenophylla* (XSA) and identified by comparison with the standard. The study was conducted on 40 male *Wistar albino* rats. The UA was administered orally at a dose of 25 mg/kg for 28 days. After 28 days, doxorubicin was administered intraperitoneally at a cumulative dose of 15 mg/kg. Three days after doxorubicin administration, hearts were isolated and subjected to *ex vivo* examination on a Langendorff apparatus. Blood and coronary venous effluent samples were also collected in order to determine the markers of oxidative stress by spectrophotometric method. Administration of UA at a dose of 25 mg/kg for 28 days leads to the preservation of cardiac function in a model of doxorubicin-induced cardiotoxicity. Also, a reduction in cardiac oxidative stress can be observed in treated animals compared to the animals not treated with UA. Our results showed that UA exhibits cardioprotective and antioxidant activity, which indicates that UA can potentially be used as a cardioprotective agent.

Keywords: usnic acid, lichen, doxorubicin, oxidative stress, cardiotoxicity

WOUND HEALING EFFECTS OF AN OINTMENT CONTAINING *HELICHRYSUM ITALICUM* ESSENTIAL OIL

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Helichrysum italicum (H. italicum) is a typical Mediterranean plant belonging to the *Asteraceae* family and its anti-inflammatory, antioxidant, and antibacterial properties has been verified. *H. italicum* essential oil has been used traditionally for wound and burns treatment, but there is no scientific evidence that supports the traditional claim. The present study aimed to develop an ointment containing the *H. italicum* essential oil and investigate its wound healing effects on excision wounds in streptozotocin-induced diabetic rats. Thirty-two *Wistar albino* rats with the confirmed diabetes were used to evaluate *in vivo* wound healing effects of ointment. Wounds were created one week after confirmed diabetes. Firstly, animals were anesthetized, and the back of the rats was shaved and the open excision wounds of size 2 × 2 cm were created with scalpel and scissors. The animals were randomly divided into four groups: Group I was untreated. Group II was vehicle control (ointment base). Group III was 0.5% *H. Italicum* ointment. Group IV was standard (1% silver sulfadiazine ointment). The response to the treatment was assessed by macroscopic and biochemical analysis. Topical application of the *H. italicum* ointment showed the highest wound contraction with the highest content of hydroxyproline in comparison to the all examined groups. The *H. italicum* ointment showed significant wound contraction from day 7 to day 21 as compared to other groups. On the day 21, there was an average of 99.32% wound contraction in the *H. italicum* group, whereas the mean wound contraction in the untreated group and ointment base group was 71.36% and 81.26% respectively. Our findings revealed that the *H. italicum* ointment approach might serve as a promising and innovative tool for wound healing.

Keywords**:** *Helichrysum italicum*, essential oil, wound healing, ointment

EFFECTS OF ADAPTATION TO THE FACTORS OF SPACEFLIGHT И AIR FLIGHT ON THE DEVELOPMENT OF CARDIOVASCULAR DISEASES

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Cardiovascular pathology is a leading cause of death among the space travelers and pilots. During the flights the crew members are exposed to prolonged whole-body low-frequency vibration (LFV), launch acceleration, high noise levels, microgravity exposure and prolonged intensive stress. Their circadian rhythms are challenged. The aim of the study was to determine the contributions of various occupational pathogenic factors to the risk of cardiovascular diseases among these professionals. The study involved 10 civil pilots and 8 cosmonauts, who were tested by psychophysiologic scales (MMPI, Luscher's 8-color test, the Spielberg-Hanin scale for reactive and personal anxiety and SF-36 test) and functional instrumental tests for neural regulation of cardiovascular system (temperature sensitivity and galvanic skin test, diagnostics of orthostatic disorders on the stabilometric platform). In the study of correlations between the state of cardiovascular system and the data of instrumental studies, a high positive correlation ($\rho = 0.86$, $p < 0.05$) was revealed with the parameters of the frontal instability of the stance along the Y axis. But, in a psychophysiological study, all the subjects had a low and moderate indicators of reactive and personal anxiety, and there was no emphasis on hyperreactive personality types. There were no correlations of psychophysiological parameters with the state of the cardiovascular system ($\rho = 0.56$, $p > 0.05$). Perhaps, overload and vibration exposure as well as microgravity are the most significant factors in the development of cardiovascular pathology, but psycho-emotional stress, as a factor of pathogenic or sanogenetic adaptive cardiovascular changes in cosmonauts and looks much less insignificant.

Keywords: pilots, cosmonauts, atherosclerosis, arterial hypertension, occupational hazards

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THE EFFECT OF THE INFLAMMATORY PROCESS IN THE RESPIRATORY SYSTEM AND GASTROINTESTINAL TRACT ON THE SEVERITY OF GRANULOSA CELLS APOPTOSIS IN WOMEN WITH IMPAIRED REPRODUCTIVE FUNCTION

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Granulosa cells are a layer of somatic cells surrounding an oocyte during maturation and providing it with nutrition and protection. A number of studies have found that aberrant apoptosis in these cells can lead to disruption of the processes of oogenesis and, as a consequence, disruption of reproductive function in women. It is known that many factors can influence apoptosis, but the effect of extragenital inflammation on this process in granulosa cells has not yet been studied. This problem is now becoming more actual, taking into account the widespread of inflammatory diseases in the respiratory and digestive systems and the increasing number of women suffering from infertility.The purpose of the work: to assess the level of granulosa cell apoptosis and the outcomes of in vitro fertilization (IVF) in women with impaired reproductive function against the background of frequent diseases of inflammatory genesis in the respiratory and/or digestive systems. The study analyzed samples of granulosa cells of 60 women with a history of inflammatory pathology of the respiratory system (during the last 6 months) and/or digestive systems (during the last 6 months) who underwent treatment infertility by IVF methods in Clinic No.1 of the VSMU in the period from 2021 to 2022. Granulosa cell samples were collected from follicular fluid obtained during transvaginal puncture of preovulatory follicles. The number of cells with signs of apoptosis was assessed using a commercial flow cytometry kit "Dead Cell Apoptosis Kit with Annexin V FITC and PI" (Invitrogen, Thermo Fisher Scientific Inc.). The Fischer F–test method was used for statistical analysis. It was found that in women without extragenital pathology in the anamnesis (n=20) the number of fertilized egg cells is 11 [9-12] and the level of apoptosis of granulosa cells was 0,0088±0,0062%, which is significantly lower than in the group of women with inflammatory diseases of the digestive system in the anamnesis (n=20) – the number of fertilized eggs is 5,5 [4,0-6,75] and the level of apoptosis was 0,0140 ± 0,0099% (p=0.015) and a group of women with a history of inflammatory diseases of the respiratory system – the number of fertilized eggs was 3,0 [2,5-3,0] and the level of apoptosis was 0,0650 ± 0,0391% (p=0.033). The level of apoptosis of granulosa cells in women correlates with the presence of extragenital pathology of inflammatory genesis, which can potentially affect fertility indicators and requires further study.

Keywords: inflammation, granulosa cells, infertility, apoptosis

TOXIC EFFECTS OF CHRONIC ADMINISTRATION OF RUTHENIUM(II) COMPLEX VERSUS CISPLATIN ON SYSTEMIC REDOX STATUS, SERUM CARDIAC BIOMARKERS AND MYOCARDIAL HISTOPATHOLOGICAL CHANGES IN W. ALBINO RATS

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Ruthenium(II) complexes have been extensively investigated as potential metallopharmaceuticals due to their unique chemical and biological properties. These complexes can exhibit a range of biological activities such as anticancer, antimicrobial, and anti-inflammatory activities, with lower systemic toxicity than platinum(II) compounds. Therefore, the aim of our study was to assess the effects of [Ru(Cl-tpy)(bpy)Cl][Cl] and cisplatin (reference compound) on systemic redox status, cardiac injury markers and myocardial histopathological changes in rats. The levels of oxidative stress, serum cardiac biomarkers and histopathological changes were evaluated in 36 male Wistar albino rats (8 weeks old, body weight 190-250 g, n=12 animals per group) chronically (4 weeks) treated with [Ru(Cl-tpy)(bpy)Cl][Cl] (4 mg/kg/week), cisplatin (4 mg/kg/week) and saline (4 mL/kg/week). In collected blood samples, the following pro-oxidative parameters in plasma were measured spectrophotometrically: index of lipid peroxidation (TBARS), nitrite (NO_2^-), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), while antioxidative defense system in erythrocytes was estimated by determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activity, as well as reduced glutathione (GSH) and oxidized glutathione (GSSG) content and its ratio GSH/GSSG. The cardiac biomarkers lactate dehydrogenase (LDH), creatine kinase (CK), CK-MB and cardiotroponin (cTnT) were measured in serum samples spectrophotometrically. Histopathological changes were examined by hematoxylin/eosin method at magnification 400x. Our results showed that pro-oxidative markers (NO_2^-, O_2^-, H_2O_2) were significantly elevated in cisplatin group compared to control group, while levels of TBARS were increased in cisplatin group compared to ruthenium and control group. The concentrations of antioxidative markers were significantly decreased in ruthenium group compared to control group. The most pronounced effects on the levels of serum cardiac injury biomarkers (LDH, CK, CK-MB) were observed in cisplatin group compared to ruthenium and control group, while cTnT concentrations were highly affected by ruthenium administration. Cisplatin had the greatest potential for causing histopathological damage to heart tissue, followed by the ruthenium complex with bipyridine. These results may help in better understanding of effects of ruthenium(II) complexes on the systemic redox state and myocardial injury and indicates that investigation of ruthenium(II) complexes as potential metallopharmaceuticals is an exciting area of research with significant potential for the development of new drugs with possible less systemic toxicity.

Keywords: ruthenium(II) complex, cisplatin, toxicity, oxidative stress, cardiac injury markers

EXAMINING THE EFFECTS OF HBOT ON THE CARDIOVASCULAR SYSTEM AND OXIDATIVE STRESS IN INSULIN-TREATED AND NON-TREATED DIABETIC RATS

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The purpose of the present study was to verify the action of HBOT on the cardiovascular system and oxidative stress in streptozotocin-induced diabetic rats, insulin treated and non-treated. Diabetes was induced by intraperitoneal administration of 60 mg/kg streptozotocin to Wistar albino rats. 48 rats were devided into 4 groups: DM group (diabetes induced by streptozotocin (STZ) injection); DM + HBOT group (received both STZ injection and HBOT, exposed to 100% oxygen at 2.8 ATA (atmosphere absolute) for 1 h once daily, for 5 days (two weeks)); DM+INS group (neutral protamine hagedorn (NPH) insulin 5 U/day); DM+HBOT+INS (received both NPH insulin and HBO exposure for 2 weeks). The glycemic control, parameters of oxidative stress and cardiac function were evaluated. NPH insulin treatment significantly reduced blood glucose levels. NPH does not induce normoglicemia but significantly reduced hyperglicemia in treated groups. We can conclude that the lowest values of all determined pro-oxidative markers were recorded in the DM group, and that the application of insulin alone and in combination with HBOT led to a significant increase. Cardiac function was significantly improved by NPH insulin, and combination of NPH insulin and HBOT seems to be effective in restoring the cardiac function in diabetic animals.

Keywords: diabetes mellitus type 1, streptozotocin, hyperbaric oxygen therapy, neutral protamine hagedorn (NPH) insulin

LARGE IMMATURE CELLS IN ASSESSMENT OF COVID-19 PROGRESSION

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Coronavirus disease 2019 (COVID-19) strongly affects the immune and hematopoietic systems. An inadequate and exaggerated immuno-inflammatory response has a decisive role in the pathogenesis of severe COVID-19 and is reflected through reactive changes in the complete blood cell counts (CBC). We aimed to evaluate and compare the usefulness of initial CBC parameters in predicting COVID-19 progression. A total of 945 patients were enrolled during 2021. Inclusion criteria included confirmed diagnosis of COVID-19 and CBC analysis performed within 24h. COVID-19 progression was defined as a worsening of disease that required admission to an intensive care unit or a lethal outcome. The CBC parameters were tested by analysis of variance, receiver operating characteristic (ROC) curve analysis, and binomial logistic regressions. The disease progressed in 374 patients (39.6%), of whom 351 patients (37.2%) died. These patients had significantly higher absolute counts of leukocytes, neutrophils, eosinophils, large immature cells (LIC), red cell distribution width (RDW), and platelet distribution width (PDW), while decreased counts of platelets and monocytes, compared to milder disease. Lymphopenia (median 980±69/μl) was found in all of the patients at admission, but without significant association with the outcomes. Counts of atypical lymphocytes did not show marked differences. Among the CBC parameters, the ROC curve analysis provided LIC and RDW values as significant (p=0.000) in identifying COVID-19 progression (area under the curve (AUC) =0.605 and AUC=0.596, respectively). However, only LIC was significant in predicting progression of COVID-19 in logistic regression (odds ratio (OR)=1.934, p=0.035). The LIC population consists of various myeloid and lymphoid immature forms. Disease progression was accompanied with a rise in LIC counts of median 250±28/μl vs. 180±28/μl in other patients (p=0.000). The LIC count cut-off at ≥0.265 x10^9/L could predict disease progression (OR = 1.547, p=0.019). Patients with higher counts had a 54.7% greater likelihood of having COVID-19 progression. A strong influence of pro-inflammatory cytokines leads to enhanced generation and release of immature myeloid populations, which is observed in critically ill COVID-19 patients. In this regard, higher LIC, RDW and PDW levels were detected among severe COVID-19 patients in our study. An increase in LIC count was highly associated with worse outcomes, showed the best discriminatory ability, and represented an independent predictor of COVID-19 progression.

Keywords: lymphoid and myeloid progenitor cells, lymphopenia, leukocytes, plateletes, severe acute respiratory syndrome coronavirus 2

BENEFITIAL EFFECTS OF BILE ACIDS ON LPS INDUCED ACUTE LUNG INJURY IN RATS

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Lipopolysaccharide (LPS) from gram-negative bacteria contributes to general inflammation and development of multiple organ dysfunction syndrome (MODS). The lungs are considered to be the one of the most vulnerable organs in endotoxemia and the reactive oxygen species are thought to play a key role in the pathogenesis of lung damage. Bronchoalveolar lavage (BAL) is used as a dynamic tool for further biochemical analyses. A wide range of tests can be performed on BALF, including antioxidant enzymes activity and quantification of inflammatory mediators. The aim of the study was to examine the effects of UDCA (ursodeoxycholic acid) and CDCA (chenodoexycholic acid) pretreatment on oxidative stress parameters in the BAL fluid and plasma in rats with LPS induced endotoxemia. Male Wistar albino rats were used in this experiment. The endotoxemia was induced by administration of LPS (5,5 mg/kg bw) intraperitoneally. In order to alleviate the effects of LPS, the UDCA and CDCA (25mg/kg bw) was administrated by gavage as a pretreatment for 10 days. The animals were divided into 6 groups. Control group (propilene-glycol p.o. for 10 days and saline on day 10 ip; LPS group (propilene glycol p.o. for 10 days and LPS i.p on day 10); UDCA group (UDCA p.o. for 10 days and saline i.p. on day 10); UDCA+LPS group (UDCA p.o. for 10 days and LPS i.p. on day 10); CDCA group (CDCA p.o. for 10 days and saline i.p. on day 10); CDCA+LPS (CDCA p.o. for 10 days and LPS i.p. on day 10) .The markers of oxidative stress (antioxidative enzymes) in BAL fluid and plasma, and histological analyses of lungs were evaluated. Antioxidative enzymes, GSH, CAT and SOD, as markers of oxidative stress showed a significant increase in the LPS-treated group in the BAL ($p<0,001$, $p<0.01$) and plasma $p<0.001$, $p<0.001$ and $p<0.05$) in comparison to the control group, (Fig.1). UDCA and CDCA showed antioxidative effect in LPS induced endotoxemia. GSH and CAT, analyzed as antioxidative enzymes, showed a decrease in the UDCA pretreated group (BALF $p<0.01$ and $p<0.05$; plasma $p<0.01$ and $p<0.01$) and CDCA pretreated group (BALF $p<0.01$ and $p<0.05$; plasma $p<0.001$) in LPS induced endotoxemia (Fig.1). There was no significant difference in activity of SOD between groups measured in BAL and plasma. Pretreatment with UDCA and CDCA has protective effects against endotoxin-induced oxidative stress in lungs and plasma indicating that UDCA and CDCA are potent antioxidants. UDCA and CDCA reduce oxidative stress by raising antioxidative enzymatic protection (GSH and CAT). Histological evaluation also confirmed that UDCA and CDCA attenuated LPS-induced lung injury.

Keywords: LPS induced entotoxemia, ursodeoxicholic acid, chenodeoxicholic acid, oxidative stress

BIOCHEMICAL AND PATHOPHYSIOLOGICAL ANALYSES DETECT IMPAIRED CARDIAC FUNCTION IN HYPERTENSIVE RATS WITH PERIAPICAL LESIONS

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The study explored the possible association between experimentally induced periapical lesions (PA) and cardiac function in hypertensive rats. Forty-eight male normotensive Wistar Kyoto and spontaneously hypertensive (SHR) rats were randomly divided into four groups: control (C), normotensive with PA (PA), spontaneously hypertensive (SHR), and SHR with PA (SHR+PA). PA has been induced on the first right molar lower jaw by exposing the pulp to the oral environment for 4 weeks. Biomarkers of oxidative stress were determined from systemic redox state, carried out in blood samples collected from a jugular vein. The hemimandibles and cardiac tissue were analyzed pathohistologicaly. The dimensions of periapical periodontal ligament thickness and alveolar bone resorption, were significantly higher in the SHR+PA group ($p < 0.01$). The levels of systemic pro-oxidative markers showed that the levels of superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and the index of lipid peroxidation (TBARS) were significantly higher in the SHR + PA group than in C, PA, and SHR groups ($p < 0.01$), while the level of nitrites (NO_2^-) was significantly higher in the SHR + PA group than C and PA group. The activities of systemic antioxidative markers showed that the superoxide dismutase (SOD) and catalase (CAT) were significantly lower in the SHR + PA group than in C, PA, and SHR groups ($p < 0.01$), while the level of reduced glutathione (GSH) was not significantly different between all groups ($p > 0.05$). Histopathologically, the SHR+PA group showed significant changes in cardiac tissue including nucleus loss, cytoplasmic hypereosinophilia, and zonal necrosis of a large number of muscle cells with fiber fragmentation. PA was associated with increased values of prooxidative markers and decreased mobility of antioxidants, especially in the hypertensive state at the systemic levels. In addition, PA was linked with cardiac pathologic changes. Hypertension was correlated with increased periapical periodontal ligament thickness and alveolar bone resorption with normotensive conditions.

Keywords: periapical lesions, hypertension, oxidative stress

CHLORPYRIFOS INDUCED NEPHROTOXIC CHANGES IN WISTAR ALBINO RATS: A POSSIBLE PROTECTIVE ROLE OF *VISCUM ALBUM* L. LEAF EXTRACT

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Organophosphates belong to a wide group of compounds that act as cholinesterase inhibitors. Although the main target for organophosphates is the nervous system, many other organs are affected, and one of the suggested mechanisms is a generation of oxidative stress. The leaves of *Viscum album* L. possess therapeutic effects due to the presence of bioactive compounds. Therefore, this study was designed to determine the phytochemical profile of *Viscum album* extract (VAE) and to investigate the protective effects against nephrotoxicity induced by commonly used organophosphate, chlorpyrifos (CPF). The ultrasound extraction was performed and analysis of the polyphenolic profile showed that VAE is rich in flavonoids and hydroxycinnamic acids. This study was conducted on male rats as follows: group I was orally given a saline solution (NaCl) via gavage; group II was intragastrically administered with CPF (35 mg/kg); group III was supplemented with VAE in higher dose intraperitoneally (VAE1, 350 mg/kg); group IV was treated as group II and was supplemented with VAE1 (350 mg/kg), and group V was cotreated with CPF (as group II) and a lower dose of VAE (VAE2, 175 mg/kg). After 30 days of treatment, the animals were sacrificed, and serum and kidney tissue were collected for the analyses. The impaired kidney function in group II was reflected in increased biochemical parameters (urea and creatinine) and histological changes in the kidney. The occurrence of oxidative stress was confirmed by increased lipide peroxidation (LPO) and glutathione disulfide (GSSG) levels in kidney tissue. The measured activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) were significantly increased, while the activity of glutathione reductase (GR) was decreased. Animals supplemented with CPF and VAE1 or VAE2 showed stabilization of all measured parameters. The obtained results imply that phytoconstituents identified in investigated extract (such as rutin, kaempferol, quercetin, and apigenin) exhibit beneficial effects against kidney damage, and therefore VAE could be useful as therapeutic agent. However, future investigations are needed to clarify the exact mechanisms of VAE action.

Keywords: insecticides, oxidative stress, kidney damage, plant extract, flavonoids

SELECTED THIOUREA DERIVATIVES OF NAPROXEN AS POTENTIAL ANTI-INFLAMMATORY AGENTS: *IN VIVO*, *IN VITRO*, AND *IN SILICO* APPROACH

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The aim of the conducted study was to develop new potential dual COX-2 and 5-LOX inhibitors based on naproxen scaffold. We performed the evaluation of *in vivo* and *in vitro* anti-inflammatory activity of newly synthesized thiourea derivatives of naproxen containing *m*-anisidine and *N*-methyl tryptophan methyl ester in a side chain. An *in vivo* study was carried out using a carrageenan-induced paw edema model of acute inflammation. COX-2 and 5-LOX inhibitory potential of synthesized compounds was evaluated using fluorometric inhibitor screening kits. *In silico* study was performed in OEDocking 3.2.0.2 software with the FRED tool. Two investigated derivatives exhibited comparable anti-inflammatory activity to naproxen (56.32%) four hours after injection of carrageenan, with the percentage of inhibition being 54.01% (*m*-anisidine derivative) and 54.12% (*N*-methyl tryptophan methyl ester derivative). *In vitro* studies of COX-2 inhibition demonstrated that none of the tested compounds achieved 50% inhibition at concentrations below 100 μM, whereas the *m*-anisidine derivative accomplished comparable inhibition of 5-LOX (IC_{50} = 0.30 μM) to commercial 5-LOX inhibitor zileuton (IC_{50} = 0.36 μM). Inability of the tested compounds to form three hydrogen bonds with ARG120 and TYR355 could be a reason why these compounds showed weak COX-2 inhibition. The *m*-anisidine derivative formed a more stable complex with the 5-LOX enzyme (−8.39 kcal/mol), compared to *N*-methyl tryptophan methyl ester derivative (−7.98 kcal/mol), with the absence of the iron ion chelation in the active site in both cases. The significant *in vivo* anti-inflammatory activity of the *m*-anisidine derivative, together with the potent inhibition of 5-LOX, highlighted this compound as a promising anti-inflammatory agent.

Keywords: naproxen, thiourea, anti-inflammatory activity, COX-2 and 5-LOX, FRED

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INTERACTION OF SACUBITRIL AND VALSARTAN WITH MTORC1. *IN SILICO* IDENTIFICATION OF SIGNALING PATHWAYS INVOLVED IN PHARMACOLOGICAL PROMOTION OF BROWNING

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Rapamycin sensitive mTORC1 is dimeric complex composed of mTOR, RAPTOR, and mLST8 subunits. The mechanism of molecular recognition between sacubitril/valsartan and well-established upstream regulator of browning, mTORC-1, is still unknown. To identify potential signaling pathways involved in pharmacological promotion of browning, this *in silico* study initially examined the molecular docking of sacubitril active metabolite-sacubitrilat and valsartan into the different subunits of mTORC1. Rigid protein-flexible ligand docking protocol was executed using Lamarckian Genetic Algorithm with default settings in AutoDock 4.2 software. Within blind molecular docking studies, sacubitrilat and valsartan docked separately into the mTOR, RAPTOR, and mLST8 subunits of mTORC1. Considering the molecular docking of sacubitrilat into the mTORC1 subunits, the highest binding affinity was observed for mTOR subunit, with a free binding energy value of -37.15 kJ/mol. Sacubitrilat interacts with RAPTOR subunit with the slightly lower binding affinity in comparison to mTOR, with docking score (-35.90 kJ/mol), whereas the lowest binding affinity was observed for mLST8 subunit (-21.92 kJ/mol). On the other hand, valsartan exhibited the highest binding affinity towards mLST8 subunit, as evident from the value of free binding energy, -32.26 kJ/mol. During molecular fitting of valsartan into the mTOR subunit, comparable, but slightly higher free binding energy was noticed (-31.30 kJ/mol). The highest value of docking score was observed for RAPTOR subunit (-27.74 kJ/mol), due to the formation of relatively small number of non-covalent interactions (8). According to the high binding affinity of sacubitrilat and valsartan towards subunits mTOR and mLST8 observed in molecular docking study, we additionally performed the molecular dynamics (MD) simulations of these complexes throughout 25 ns, using Schrödinger Desmond 2020-4 software. The RMSD (Root Mean Square Deviation) plot of sacubitrilat-mTOR complex revealed that sacubitrilat showed the significant deviations in RMSD values at the beginning the simulation, and then complex became stabilized at 10 ns and remained stable till the end of simulation. On the other hand, RMSD diagrams of valsartan and mLST8 are mutual overlapped during entire simulation, indicating the formation of stable complex throughout time. Based on molecular docking and MD results, we can conclude that sacubitrilat and valsartan strongly interact with mTOR and mLST8 subunits of mTORC1, forming a stable complexes with mentioned subunits, which are maintained over time.

Keywords: mTORC1, sacubitrilat, valsartan, AutoDock, molecular dynamic

ALTERATION IN REDOX STATE FOLLOWING COMBINED SACUBITRIL AND VALSARTAN TREATMENT IN RATS WITH ISCHEMIA/REPERFUSION (I/R) INJURY

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Sacubitril (SAC) and valsartan (VAL) as inhibitors of neprylisine and angiotensin II receptor type 1 (AT1) has been considered as therapy with superior efficiency in reducing cardiovascular death. However, in ischemia/reperfusion (I/R) injury excessive reactive oxygen species are released which is the key factor in initiation of this pathophysiological condition. In this study, we aimed to examine the potential of combined SAC/VAL administration for 4 weeks to decrease oxidative stress in rats with metabolic syndrome exposed to I/R injury. This study enrolled forty *Wistar albino* male rats (8 weeks old; body weight, bw: 200 ± 20g) randomly divided into four equal groups depending on diet regime (standard or high fat diet) as follows: CTRL – healthy untreated rats; CTRL+SAC/VAL – healthy rats treated with SAC/VAL; MetS – rats with metabolic syndrome; MetS+SAC/VAL – rats with metabolic syndrome treated with SAC/VAL. The group of rats treated with SAC/VAL received the combination of these drugs in dose of 68 mg/kg/day by oral gastric gavage, while the animals from other two groups (CTRL and MetS) received the same volume of distillated water. After finishing the experimental protocol, rats were sacrificed and hearts were applied to Langendorff apparatus to induce *ex vivo* I/R injury (30'/60' I/R period). For the purpose of spectrophotometric determination of oxidative stress coronary venous effluent samples were used to detect the level of pro-oxidative molecules such as: superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitrites (NO_2^-) and index of lipid peroxidation measured as thiobarbituric acid-reactive substances (TBARS). The results of our study showed significant increase in all measured parameters in rats with MetS compared to healthy untreated group (CTRL). However, long term application of SAC/VAL for four weeks succeeded to decrease the level of pro-oxidative molecules ($O_2^{\cdot-}$, H_2O_2 and TBARS) in MetS group, managing to reach the values same as at stabilization period (S). Interestingly, treatment with SAC/VAL resulted in increase of NO_2^- in coronary venous samples during reperfusion period compared to untreated rats indicating vasodilatory potential of these drugs. In summary, combined administration of SAC/VAL offers great potential to save the hearts from I/R induced damage by decreasing the markers of oxidative stress but improving cardiac and vascular function mediated by increased nitric oxide bioavailability.

Keywords: sacubitril, valsartan, ischemia/reperfusion injury, oxidative stress, rat

MOLECULAR MIMICRY BETWEEN AUTOANTIGENS OF HUMAN ENDOTHELIOCYTES AND CORONAVIRUSES AND PROMOTION OF ATHEROGENESIS

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One of the known hypotheses of atherogenesis in humans is the viral one. We have examined atherogenesis in humans in terms of molecular mimicry. Molecular mimicry occurs when unrelated proteins have similar minimal epitopes (pentapeptides), this phenomenon may be the cause of autoimmune reactions in the human body. We performed an exhaustive bioinformatics search of all contiguous segments of human Coronaviruses with an exact identity to human protein pentapeptides. We selected 27 proteins known from literature as targets of autoimmune vasculitis, arthritis, aneurysms, key factors of lipid metabolism and atherogenesis and compared them with antigens of all 7 known human Coronaviruses (spike, membrane, evelope – proteins and nucleoproteins). Human and Coronavirus protein sequence files were downloaded from UniProt database. Only segments with a length of five amino acids were considered. To determine shared sequences, we created an original computer program called "Alignmentaj". The location of the found pentapeptides in the 3D structure of human Coronavirus antigens and autoantigens was studied using the PDB database and PyMol program. Further analysis was performed using the Immune Epitope Database, a database of experimentally validated epitopes and a tool to predict T cell and B cell epitopes. Analysis showed that there are has 142 shared pentapeptides. In addition, many pentapeptides are located on the surface in the 3D structure of human and Coronavirus antigens. Notably, a lot of shared peptides are part of immunogenic epitopes. The results obtained using the bioinformatics prediction tool for the phenomenon of molecular mimicry between autoantigens of atherogenesis and antigens of human Coronaviruseswitness for probability of autoimmune reaction against autoantigens involved in of atherogenesis. Coronaviruses may be ragarded as possible new atherogenic factor, which already has some wet lab autopsy confirmations.

Keywords: atherosclerosis, autoimmunity, vasculitis, molecular mimicry, human Coronaviruses

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MULTISENSORY STRIPPING VOLTAMMETRY FOR EARLY DIAGNOSIS OF KIDNEY DAMAGE IN CHILDREN WITH VESICOURETERAL REFLUX

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Primary vesicoureteral reflux (VUR) is children's most common congenital uropathy (CU). 25-60% of patients with VUR reach the terminal stage of chronic kidney disease. Today effective diagnostic methods for early renal damage do not exist. Multisensory stripping voltammetry is an electrochemical analysis method of non-volatile and volatile organic compounds (NOCs and VOCs) in urine samples. It could become a new approach to early diagnosis of kidney damage. To evaluate the possibility of separating healthy children and children with kidney damage with VUR using the analysis of the voltammograms of NOCs and VOCs in urine samples and compare them with the results of non-target mass spectrometry and proteomic analysis. This study involved 42 patients (average age 5.4±2.3 years), divided into 2 groups: group 1 – 24 children with CU (grade II–V VUR) and comparison group 2 – 18 patients with minor surgical pathology without pathology of the urinary system. Voltammetry was conducted on a «Microtox» device using planar electrodes and background electrolyte (BE). Statistical data was processed by factor analysis («Statistics 7» software package). Urine samples were additionally analysed by time-of-flight mass spectrometry using a «Reflectron» device. Urinary levels of MCP-1, IL-8, IL-18, VEGF and TGF-β1 were measured by solid-phase ELISA. Voltammetry signature of urine samples of VUR patients was determined. Its characteristic features are the presence of the small zinc current maximum, a slight cadmium current shift from -0,74V to -0,76V, and the absence of a lead current shift or a slight lead current shift from -0,54V to -0,56V. This method demonstrated 72,2% sensitivity and 77,8% specificity. The sensitivity was 96%, specificity – 56%. Normalised BE metal activity indexes were calculated, and factor analysis was performed. It showed that zinc and lead activity indexes are the most significant for discrimination between patients with VUR and the control group. During the time-of-flight mass spectrometry, composition changes in urine VOCs were detected. These changes made it possible to distinguish group 1 samples from comparison group 2. An increase in the concentration of inflammatory (MCP-1, IL-18, IL-8), angiogenesis (VEGF) and fibrosis (TGF-β1) markers was observed in the urine of children with VUR. Electrochemical sensors and biosensors can play a crucial role in developing cost-effective, portable and non-invasive diagnostic methods. The voltammograms of NOCs and VOCs analysis in urine samples helps to differentiate groups of healthy patients and patients with VUR. This method demonstrated high sensitivity and specificity.

Keywords: volatile organic compounds; stripping voltammetry; mass spectrometry; biomarkers; congenital uropathy

ASSOCIATION BETWEEN APICAL PERIODONTITIS AND CARDIAC FUNCTION IN DIABETIC RATS

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This study was designed to examine the possible link between apical periodontitis (AP) and cardiac function in type 2 diabetic rats. Adult male Wistar albino forty-eight rats were divided equally into healthy control (CTRL), healthy rats with apical periodontitis (AP), rats with diabetes mellitus type 2 (D), and D with AP (D + AP) groups. The pulp chambers of the mandibular first right molars were exposed to the oral environment for inducing AP. After sacrificing animals, cardiac function and the dimension of the left ventricle (LV) were evaluated via echocardiography. The following structural variables of the LV were measured: interventricular septal wall thickness at end-diastole (IVSd) and end-systole (IVSs), left ventricle (LV) internal dimension at end-diastole (LVIDd) and end-systole (LVIDs), LV posterior wall thickness at end-diastole (LVPWd) and ended-systoleVPWs), as well as fractional shortening (FS) percentage. The establishment of AP was verified and analyzed radiographically. The hemimandibles were analyzed pathohistologicaly. D + AP rats had significantly changed echocardiographic parameters (decreased diastolic and systolic interventricular septal wall thickness (IVS), as well as increased left ventricular internal diameter (LVIDd)) ($p < 0.05$) compared with CTRL, AP, and D groups. Similarly, percentages of fractional shortening and ejection fraction were decreased in rats with D+AP compared with CTRL, AP, and D groups ($p < 0.05$). The area of radiographic AP was significantly larger in the D + AP compared to the AP group ($p < 0.05$). The apical cementum resorption was significantly higher in the D + AP compared to the AP group ($p < 0.05$). In diabetic conditions, AP was linked with disturbed echocardiographic parameters. Diabetes type 2 was associated with increased radiographic AP area and apical cementum resorption compared with healthy conditions.

Keywords: apical periodontitis, diabetes, cardiac function, rat

EFFECTS OF STRAIN DIFFERENCES IN ALBINO OXFORD AND DARK AGOUTI RATS ON DEVELOPMENT OF OVARIECTOMY INDUCED ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurological disorder characterized by memory deterioration and loss of learning abilities. The main pathological features of AD are an extraneuronal accumulation of amyloid β (Aβ) and intraneuronal hyper-phosphorylated tau (p-tau) protein tangles. There is grooving a body of evidence that neuroinflammatory, genetic, hormonal and infectious factors and in particular oxidative stress may contribute to the development of neurodegeneration as seen in Alzheimer's disease (AD). The aim of this proposal is to define the differences in susceptibility and development of Alzheimer-like disease in different strains of rats which differ in their response to neuroinflammatory agents. Albino Oxford (AO) rats, unlike Dark Agouti (DA) rats are resistant to the induction of experimental autoimmune encephalomyelitis (EAE). The reasons for this difference appear to be related to different T lymphocyte responses and cytokines production. The AD was induced in adult female DA and AO rats by ovariectomy and D-galactose treatment lasting for 12 weeks (OVY/D-gal). Results of this study showed that expression of Aβ and p-tau were significantly higher in OVY/D-gal DA rats compared to OVY/D-gal rats both in hippocampus and prefrontal cortex. P-tau showed similar pattern of expression. AD rats which has Th1 immune response had much more pronounced expression of pathohistological hallmarks of AD. These results suggest that immune response has important role in development of AD. Further research will provide more information regarding the role of immune response and genetic background on the development of AD.

Keywords: Alzheimer's disease, amyloid β, hyper-phosphorylated tau protein, experimental model, ovariectomy

ASSOCIATION OF PLASMA GALECTINS-1,3 WITH FEATURES OF SUBPOPULATION COMPOSITION AND FUNCTIONAL ACTIVITY OF CIRCULATING T-LYMPHOCYTES IN COLON CANCER

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Dysregulation of antitumor immunity and the development of immunosuppression plays a central role in the pathogenesis of tumor growth. One of the mechanisms of tumor-induced immunosuppression may involve the production of galactose-binding proteins galectins. Galectins 1 and 3 are known to modulate cell-mediated immune response by regulating the differentiation and apoptosis of effector T-lymphocyte-helper (Th) types 1 and 17, as well as regulatory T-lymphocytes (Treg) with immunosuppressive properties. At the same time, the mechanisms of the influence of galectins 1 and 3 on the cells of the immune system in tumor diseases remain not fully elucidated. To evaluate the features of the subpopulation composition and cytokine-secretory activity of T-lymphocytes (Th1, Th17 and Treg) in relation to the concentration of galectin-1 and galectin-3 in the blood of patients with colon cancer. Total of 26 patients diagnosed with colon cancer were examined. The material of the study was whole peripheral blood, blood plasma and supernatants of suspension cultures of mononuclear leukocytes. Blood lymphocytes were typed by laser flow cytometry using monoclonal antibodies. The content of galectin-1 and galectin-3 (in blood plasma) and interferon (IFN) γ, interleukin (IL) 17A and transforming growth factor (TGF) β (in mononuclear leukocyte culture supernatants *in vitro*) was determined by enzyme immunoassay. Statistical analysis was done using the Statistica 10.0 software package. Differences were considered significant at $p<0.05$. In patients with colon cancer, a significant increase in the concentration of galectin-1 and galectin-3 in blood plasma was found, associated with a decrease in the content of $CD4^+T\text{-}bet^+$ Th1-lymphocytes, $CD4^+RORC2^+$ Th17-lymphocytes in the blood and hyposecretion of IL-17 by leukocytes *in vitro*. On the contrary, positive correlations were found between the concentration of galectins 1 and 3, the content of $CD4^+FoxP3^+$Treg cells in the blood, and the secretion of TGFβ by mononuclear leukocytes *in vitro*. In colon cancer, an increased level of galectins 1 and 3 in the blood is associated with a quantitative deficiency and inhibition of the secretory activity of effector T-lymphocytes, and, conversely, activation of the immunosuppressive functions of regulatory T-cells. The obtained results indicate a negative role of galectin-1 and galectin-3 in the mechanisms of regulation of the T-cell-dependent immune response in colon cancer.

Keywords: colorectal cancer; galectins; T-lymphocytes; cytokines; antitumor immunity

THE SPECIFIC METHOD OF ETIOPATHOGENETIC TREATHMENT OF PATIENTS WITH HPV-ASSOCIATED FORMS OF OROPHARYNGEAL PATHOLOGY

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Incidence of head and neck squamous cell carcinoma (HNSCC) has been increased recently and continues to rise among the population. Diagnosed HPV-positive (Human papilloma-virus) HNSCCs prevail over other types of cancer including cervical cancer. Etiology of HPV is confirmed by detecting in tumors and can explain the development of HNSCC in people who do not have traditional risk factors of that disease. The risk group with the development of HNSCC involved the patients that had associated with HPV chronic inflammatory forms of oropharyngeal pathology. Purpose: to determine markers of the malignant transformation of the oropharyngeal epithelium in HPV-associated chronic inflammatory forms of oropharyngeal pathology. Material and methods: 50 patients were involved in the research. The main group consisted of 25 patients with chronic inflammatory forms of oropharyngeal pathology; the control group consisted of 25 patients without oropharyngeal pathology. Real-time PCR (Polymerase chain reaction) diagnostics, HPV genotyping in oropharyngeal mucosa scrapings and cytological examination of oropharyngeal mucosa scrapings have been conducted in both groups. In the main group during the Real-time PCR diagnostics and HPV genotyping in oropharyngeal mucosa scrapings have been revealed HPV DNA (Deoxyribonucleic acid) samples of high (HPV 16, 18) and low (HPV 6, 11, 44) oncogenic risk. Based on results of cytological examination of oropharyngeal mucosa scrapings all the patients of the main group with high oncogenic risk HPV DNA samples had precancerous cell changes: dysplasia which is manifested in the form of dyskaryosis and polymorphism. Coylocytosis is also a sign of cell damage by HPV. The patients of the main group have been treated with Aminodihydrophthalazindione sodium according to the scheme. During the repeated diagnostics HPV 16, 18, signs of koilocytosis and epithelial dysplasia have been detected again in 3 patients (12%) of the main group and at the same time the signs of inflammatory changes of oropharyngeal mucosa have been preserved. In clinical practice Real-time PCR diagnostics and HPV genotyping are the methods of detecting markers of precancerous changes in HPV-associated chronic inflammatory forms of oropharyngeal pathology. Therapy with Aminodihydrophthalasindione sodium has demonstrated positive results in the treatment of HPV-associated chronic inflammatory forms of oropharyngeal pathology.

Keywords: human papilloma virus, chronic inflammatory forms of oropharyngeal pathology

EFFECTS OF GLP-1 AGONISTS ON REDOX STATUS IN RATS WITH METABOLIC SYNDROME

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Metabolic syndrome is a multifactorial disorder characterized by combination of insulin resistance, abdominal obesity, dyslipidemia and hypertension. These metabolic abnormalities have been shown to contribute to the development of oxidative stress in the body which can be further responsible for many complications. Accordingly, this study aimed to examine the potential effects of GLP-1 agonists on oxidative stress in rats with metabolic syndrome (MetS). We used 30 male, Wistar albino rats, 4 weeks old at the beginning of the protocol. To induce metabolic syndrome, rats were for 4 weeks fed with high fat diet (25% fat, 15% protein and 51% starch) and after 12h of hunger, we aplicated single injection of STZ intraperitoneal (0,25 mg/kg). 72 h after the administration of STZ, fasting blood glucose and blood pressure are measured. Rats with blood glucose level over 7 mmol/L, and blood pressure level over 130/90 mmHg were included in the study as rats with metabolic syndrome. After the induction, rats were divided into five groups. MetS – control group, fed with Hfd, rats treated with exenatide in dose 0.5μg/kg per day s.c.; rats treated with liraglutide s.c. (0.3mg/kg/day); rats treated with dulaglutide s.c.(0.3mg/kg/twice a week); rats treated with semaglutide s.c. (0.3mg/kg/day). After 6 weeks rats of treatment rats were sacrificed and blood was collected from jugular vein for evaluation of antioxidant and pro-oxidant parameters. The potentital of antioxidant system was determinated as a superoxide dismutase (SOD), level of reduced glutathione (GSH) and catalase (CAT). While as pro-oxidant parameters we measured the levels of superoxide anion radical (O_2^-), nitrites (NO_2^-), hydrogen peroxide (H_2O_2), and index of lipid peroxidation (measured as thiobarbituric acid reactive substances (TBARS)). The results of our research indicate that GLP-1 agonists had the ability to attenuate the oxidative stress present in metabolic syndrome. So GLP-1 agonists may have therapeutic potential for the treatment of metabolic syndrome and its associated complications, however further research is needed to fully understand the mechanisms underlying the beneficial effects of GLP-1 agonists and to optimize their clinical use in the treatment of metabolic syndrome.

Keywords: metabolic syndrome, GLP-1 agonists, oxidative stress

ANTIOXIDANT EFFECT OF DIFFERENT DIURETICS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is a leading cause of cardiovascular disease, and diuretics are one of the most commonly prescribed classes of drugs to treat hypertension. One of the reasons hypertension is so damaging is that it creates a pro-oxidant environment in the body, which can lead to oxidative stress and damage to cells, tissues, and organs. Reducing oxidative stress in hypertension may be an important therapeutic strategy for improving patient outcomes and preventing cardiovascular disease. Diuretics, in addition to lowering blood pressure by increasing the excretion of sodium and water from the body, also have antioxidant potential. The study's objective was to investigate the antioxidant effects of sub-chronic treatment of various diuretics in spontaneously hypertensive rats (SHR). The research included 40 spontaneously hypertensive male rats (*Wistar Kyoto* strain, body weight 250±30 g, 8 weeks old) grouped into 4 groups. The animals were treated for four weeks with 10 mg/kg of hydrochlorothiazide, indapamide or spironolactone *per os*. The 24 h after the last treatment, all rats were sacrificed and blood was sampled for spectrophotometric determination of markers of systemic oxidative stress. The oxidative status were estimated by determination of the following prooxidative marker from plasma: superoxide anion radical (O_2^-), nitrite (measured as NO), hydrogen peroxide (H_2O_2) and index of lipid peroxidation (measured as thiobarbituric acid reactive substances - TBARS). While from erythrocyte lysate following antioxidative parameters were determined: activity of superoxide dismutase (SOD), activity of catalase (CAT) and content of reduced glutathione (GSH). All three examined diuretics, hydrochlorothiazide, indapamide and spironolactone significantly reduced the production of prooxidants, which may play a role in reducing myocardial oxidative damage. On the other hand, all three investigated diuretics led to the depletion of reduced glutathione, while none of them significantly affected the activity of catalase and superoxide dismutase. Overall, the antihypertensive and antioxidant effects of diuretics make them an important tool in the treatment of hypertension and potentially other diseases.

Keywords: thiazide diuretics, thiazide-like diuretics, aldosterone blockers, antioxidant activity, spontaneously hypertensive rats

PATHOPHYSIOLOGY OF CONSTIPATION

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The Roman criteria of Constipation (Rome-IV, 2016) is “less than 3 defecation a week” (1-2 times/week). According to Robert Hegglin (1999), the diagnosis of “Constipation” should be said when “Absence of defecation within 24 hours” is established. From the standpoint of chronobiology defecation is the same circadian rhythm as the sleep-wake cycle. The definition of “Constipation” by R. Hegglin is more adequate than the Roman criteria. Therefore, new pathophysiological classification of Constipation can be proposed. First of all, from the standpoint of normal physiology, it should be stated that the physiological norm for daily nutrition is the daily rhythm of defecation. Normal defecation is a circadian rhythm with the frequency 7 times/week. The pathophysiological classification of Constipation contains three stages: First stage of Constipation (Mild) – with defecation frequency 5-6 times/week; Second stage (Moderate) – 3-4 times/week; Third stage (Severe) – 1-2 times/week. It was found (n=2501) that the first stage of Constipation occurs in almost 60% of cases, the second stage – occurs on average of 30%, and the third stage – about 10% of cases. Consequently, the new Pathophysiological classification of Constipation, taking into account a more complete range of defecation pathology, allows diagnosing 9 times more the earliest prenosological stages of pathology of defecation than the “Roman criteria of Constipation”. A new pathophysiological dependence of defecation frequency of on the timing of this act to the Morning acrophase of this rhythm has been revealed. The presence of Bowel Habit, timed to the Morning hours (from waking up to 12:00), is usually associated with the normal physiological frequency of defecation (7 times/week). The absence of Bowel Habit in the Morning is usually associated with the pathological frequency of the defecation rhythm (1-6 times/week). The propensity to take laxatives depends on the defecation frequency: at I stage of Constipation 13% of people took laxatives, at the II stage – 30%, and at the III stage – 64% of patients. The risk of Obesity in people with a regular rhythm of defecation was 7%, among patients with Constipation – 24%. The incidence of depression depends on the stage of Constipation: at the Mild stage, clinically pronounced depression occurred in 12% of patients, at the Moderate stage – in 17%, and at the Severe stage – in 43% of patients. Previously, it was found that Constipation increased the risk of Myocardial Infarction by 24%, and the risk of Stroke by 1.5 times. The new Pathophysiological classification of Constipation is aimed at identifying the earliest (prenosological) stages of this syndrome and can be used for early prevention of cardiovascular pathology.

Keywords: Constipation; frequency; defecation; laxative; depression

BOWEL HABIT AND IMMUNE SYSTEM

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The aim of the study was to compare the Immune System in individuals with Regular and Irregular Bowel Habits. The method of "Chronoenterography" was used, which allows to determine the frequency of defecation and its acrophase. A Regular Bowel Habit (RBH) was isolated at a stool frequency - 7 times/week but an Irregular Bowel Habit (IBH) – at a stool frequency 1-6 times/week. The clinical activity of the Immune System was determined by the method Kolbanov with 10 main symptoms: runny nose, cough, allergy, dry skin, asthenia, hyperdynamics, absent-mindedness, daytime drowsiness, insomnia, irritability. Falling asleep before or after 24:00 was taken into account. Satisfaction with nutrition and physical activity, the level of Quality of Life in 42 people aged 20-48. Regular Bowel Habit (RBH) was equally common for women and men, but Irregular Bowel Habit (IBH) was 2 times more common in women. IBH was 1.33 times more common than RBH. The morning phase of defecation in persons with RBH was 2.1 times more common than in persons with IBH. Nutritional satisfaction at RBH was 5.5 times more common than at IBH. In persons with RBH, falling asleep before 24:00 was 1.8 times more common than in persons with IBH. The high level of the Immune System at RBH was 1.3 times more common than at IBH. In persons with IBH, a reduced level of quality of life (in 29%) was 2.6 times more common than in persons with RBH (in 11%). 1. Irregular Bowel Habit is 33% more common than Regular one. 2. A regular Bowel Habit is 2.1 times more likely than an Irregular One to be associated with Morning acrophase of the circadian rhythm of defecation. 3. The high level of the Immune System in people with a Regular Bowel Habit was 1.3 times more common than in people with an Irregular Bowel Habit. 4. Irregular Bowel Habit not only reduces the Quality of Life by 2.6 times, but also reduces the activity of the Immune System by almost 30%.

Keywords: Bowel Habit, Immune System, Quality of Life, Regular, Irregular

COTREATMENT WITH PLACENTAL MSCS IN COMBINATION WITH FUCOXANTHIN LEADS TO REGRESSION OF LIVER FIBROSIS IN MICE

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The lack of effective non-surgical pathogenetic methods for the liver fibrosis treatment is a crucial health problem. At present, the transplantation of mesenchymal stromal cells (MSCs) holds great potential. However, the introduction of cell technologies into practical medicine is difficult due to their low survival rate and migration ability [1]. This leads to a decrease in the therapeutic potential of MSCs. Therefore, it seems promising to use combination therapy with antifibrotic drugs. Studies were carried out in 50 mice divided on nine groups: control, carbon tetrachloride (CCl_4), fucoxanthin (Fx), MSC, MSC + Fx. CCl_4 at a dose of 2 μl/g was injected intraperitoneally 2 times a week for 6 weeks. CCl_4 was diluted in peach oil. $1*10^6$ MSCs were injected into the tail vein once after modeling fibrosis. Fx was administered at a dose of 10 mg/kg daily for 5 weeks per os. Liver histopathology was evaluated by H&E and Sirius Red staining using the METAVIR scale. Immunohistochemical method was used to determine the number of CD45+ and α-SMA+ positive cells on 1 mm^2, as well as TIMP-1, MMP-9, MMP-13 and α-SMA+ positive areas. Using the enzyme immunoassay, COL1a1, TGF-β and HGF were determined in liver homogenate and IL–1ß, TNF–α were determined in blood serum. Serum ALT and AST activity, ALB, and total bilirubin levels were determined by biochemical assays. Fx, MSC and their combination reduced the severity of liver fibrosis according to the METAVIR scale. The α-SMA, TIMP-1 positive areas, as well as the number of CD45 and α-SMA positive cells was decreased. The MMP-9, MMP-13 positive areas was significantly increased. The levels of TGF-β, COL1A1, IL-1β, TNF-α and ALT, AST activity were also reduced. Fx, MSC and their combination increased HGF and ALB levels. The values of TGF-b, TIMP-1, IL-1b, albumin in the MSC + Fx group reached the level of the control group. In summary, we investigated that cotreatment had the greatest impact on liver fibrosis regression. The results demonstrated the effectiveness of the chosen direction. We hope that the data obtained will help accelerate the development and implementation of cellular technologies in clinical medicine.

Keywords: liver fibrosis, placental MSCs, fucoxanthin, TGF-b, cotreatment

COMPARATIVE ANALYSIS OF THE IMMUNOLOGICAL PROFILE AND PLATETET FUNCTION IN PATIENTS OPERATED ON FOR ABDOMINAL AORTIC ANEURYSMS

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In patients with abdominal aortic aneurysm (AAA), the total content, relative and absolute population composition of leukocytes, and the concentration of pro-inflammatory cytokines in the blood did not differ significantly from the reference values. There were two exceptions. The content of MMP-9 in the blood was increased by more than 3 times to 258+47 ng/ml (p=0.00002). These changes in the cellular composition and concentrations of serum metalloproteinase indicate the characteristic features of the pathological process in patients with AAA. In the group of patients after EVAR in the late postoperative period, a statistically significant (p=0.03) increase in the percentage of neutrophils and a decrease in the relative number of blood lymphocytes, mainly due to T- and B-lymphocytes, were noted. An increase in the concentration of the pro-inflammatory cytokine TNF-alpha (6.8+1.2 pg/ml vs. 1.0+0.2 pg/ml) was also found in the main group compared with patients who underwent open surgery (p=0, 01). There was an increase in the concentration of the pro-inflammatory cytokine - interleukin-6 (5.6±1.1 pg/ml versus 2.1±0.2 pg/ml) in the main group compared with patients who underwent open surgery (p=0, 02). When analyzing the functional activity of platelets in patients in the group with EVAR, a statistically significant (p=0.01) decrease in both spontaneous, without stimulation, and induced platelet activation was observed compared with patients in the comparison group. In patients of the main group, when measuring the functional activity of platelets, a significant decrease in the expression level of P-selectin (0.74 [0.40–3.90]%) on the surface of platelets was revealed compared with the group where open surgery was performed (17.40 [13.70–30.87]%, p=0.003). Statistically significant increase in the concentration of pro-inflammatory cytokines interleukin-6 (5.6+1.1 pg/ml versus 2.1+0.3 pg/ml) (p=0.02) and tumor necrosis factor (6.8+1.2 pg /ml versus 1.0+0.1 pg/ml) (p=0.01) confirms the presence of inflammation in the long-term period after EVAR, which is characteristic of aneurysmal disease.

Keywords: abdominal aortic aneurysm; endovascular abdominal aortic aneurysm repair; immunological profile; platetet function

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PEPTIDE HIS-PHE-ARG-TRP-PRO-GLY-PRO AMELIORATES GUT MUCOSAL MICROBIOTA COMPOSITION IN REPETITIVELY STRESSED RATS

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Repetitive stress (RS) could lead to the development of gut dysbiosis. This, in turn, might result in disruption of the functioning of the central nervous system. It is advisable to search for new approaches to solving this problem. As promising solution could be the use of an analogue of the adrenocorticotropic hormone (ACTH) – His-Phe-Arg-Trp-Pro-Gly-Pro (ACTH(6-9)-PGP), for which a wide range of neurotropic properties were already shown. Based on the above, the aim of the study was to study the state of the parietal microbiota of the colon when using ACTH(6-9)-PGP in conditions of repetitive stress. 55 male Wistar rats were used in the experiment. Experimental rats were divided into 5 groups (n = 11): 1 – control (non-stressed animals + saline administration), 2 – RS + saline; 3 – RS + ACTH(6-9)-PGP at dose of 500 μg/kg. RS was modeled by placing the rats in tight transparent plastic ventilated boxes for 2 hours for 14 consecutive days. To assess the mucosal microbiota, we performed assessment using the method of L.I. Kafarskaya and V.M. Korshunov. The middle part of the rat colon was homogenized and sown on different microbiological media. To identify the grown microorganisms, we performed matrix-assisted laser desorption/ionization mass-spectrometry. Statistical processing of the obtained data was carried out using R environment. The Kruskal–Wallis test with the Dunn post hoc test was used. P-value <0.05 was considered as significant. We found out that to a decrease in the number of obligate microorganisms: Lactobacillus spp., Bifidobacterium spp., Escherichia coli, Enterococcus spp. (1.3–2.3-fold, $p<0.01$). At the same time, an increase in the number of Proteus spp., Klebsiella spp., coagulase-negative Staphylococcus and Candida spp. (1.4–2.9-fold, $p<0.05$) was found. Moreover, under stress, Enterobacter spp., Citrobacter spp., Morganella spp., Acinetobacteria spp., and S. aureus were identified in the gut microflora which were not observed in the control animals. Against the background of administration of ACTH(6-9)-PGP at a dose of 500 μg/kg, we found an increase in the number of lactobacilli, bifidobacteria, E. coli, and enterococci (1.4–2.1-fold, $p<0.05$) against the background of a significant decrease in the number of Enterobacter spp., Proteus spp., Morganella spp., Acinetobacter spp., coagulase-negative Staphylococcus, Candida spp. (1.3–7.7-fold, $p<0.05$).
All things considered, daily iadministration of ACTH6-9-PGP at dose of 500 μg/kg prevented the development of the observed stress-induced shifts in the microbiocenosis of the mucin layer of the colon.

Keywords: His-Phe-Arg-Trp-Pro-Gly-Pro; gut mucosal microbiota; repetitive stress

FEATURES OF THE CARDIOVASCULAR ADAPTATION DISORDER IN YOUTH

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According to the World Health Organization, 17.9 million people died from cardiovascular disease (CVD) in the past year. To date, the most urgent problem is the increase in the number of CVD among young people. Assessment of the adaptive reserves of the cardiovascular system (CVS) and early detection of the risk of developing the disease is one of the effective strategies for personalized CVD prevention.The cohort study included 243 healthy volunteers (mean age 20±0.02 years). We formed the groups under the calculation of the index of functional changes (FCI) using indicators of sex, age, body weight, height, hemodynamic parameters according to the formula of R.M. Baevsky (1987). The first group (n=138) - FCI <2,6 - satisfactory CVS adaptation; the second group (n=59) - FCI =2,6-3,09 - adaptation tension; the third group (n=46) – FCI ≥3,10 unsatisfactory CVS adaptation. Research methods: questionnaire (identification of risk factors), anthropometric study, photoplethysmography (determination of vascular wall stiffness (VWS) and pulse wave velocity (PWV)), electrocardiography (ECG) (assessment of rhythmogram indicators at rest and tests with hyperventilation), statistical data processing StatTech v. 3.1.5. The gender factor was associated with hemodynamic parameters (blood pressure, pulse rate). The body mass index in the first group was 20,0±1.2, in the second – 22,7±1.8, in the third – 28,3±2,4 (p1.2-3<0.05). The study of VWS and PWV did not reveal any differences between the groups. Assessment of heart rate variability (HRV) indicators showed: RRNN in the first group was 812±20 ms, in the second - 713±21 ms, in the third - 689±27 ms ($p_{1-2,3}$<0,05). The SDNN indicator in the first group was higher relatively other groups data. The stress index in the first group was 99±15,9, in the second – 181,7±35,7, in the third - 219±46,5 ($p_{1-2,3}$<0,05). The index of sympathoadrenal tone in the first group was lower than the data of the second and third groups by 118,5 and 158,4% (p<0,05). The test with hyperventilation revealed a significant increase in HRV for most parameters in the first group of subjects. The analysis of the data from the second and third group did not reveal statistically significant changes. The most significant risk factors for reducing the adaptive reserves of the cardiovascular system in young people are male gender, increased body weight, impaired autonomic regulation of the heart rhythm (increased sympathoadrenal influences). Analysis of HRV is interesting as an important way to control the adaptive reserves of CVS in young people.

Keywords: cardiovascular system, adaptation, risk factors, heart rate variability

THE ROLE OF ITGB3, ITGA2, FGB GENE MUTATIONS IN THE PATHOGENESIS OF HEMOSTASIS DISORDERS IN PREGNANT WOMEN

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Coagulopathy in pregnant women increases the risk of developing uteroplacental blood flow disorders. Currently, the question of the role of genetic mutations in the pathogenesis of blood clotting disorders in pregnant women with preeclampsia (PE) remains open. A prospective study included 173 patients with singleton pregnancies (gestational age 22-41 weeks). Study groups: the first (n=63) - pregnant women with moderate PE; the second (n=58) - pregnant women with severe PE; control group (n=52) – patients with physiological pregnancy. The blood coagulation system was studied by hemostasiogram and thromboelastography (TEG) indices. The presence of mutant alleles of the platelet integrins ITGB3 (T1565C), FGB (G(-455)A) and fibrinogen FGB (G(-455)A) genes was determined by PCR diagnostics. With moderate PE, we noted an increase in the activity of the blood coagulation system relative to the control: shortening of the activated partial thrombin time by 17.52% ($p<0.05$), prothrombin time - by 17.65% ($p<0.05$), an increase in blood fibrinogen by 7.94% ($p<0.05$), decrease in antithrombin III by 11.10% ($p<0.05$), decrease in platelet count by 15.11% ($p<0.05$). TEG data: increase in α-angle by 25.26% ($p<0.05$), maximum amplitude - by 15.17% ($p<0.05$) and clot strength, and 30.72% ($p<0.05$); shortening of reaction time by 13.11 % ($p<0.05$) and clot formation time 31.79% ($p<0.05$), compared with the control group. In severe PE, similar changes in all of the above indicators were more pronounced. Assessment of platelet integrin and fibrinogen gene polymorphism in pregnant women with PE: the spread of the mutant allele C of the ITGB3 gene was observed in 17.3% (in the control group), 39.7% (in the first group) and 62.9% (in the second group) ($\chi2=22.8$, $p<0.001$); the T allele of the ITGA2 gene – in 9.6%, 31.0%, and 56.9% ($\chi2=27.9$, $p<0.001$) of cases at the same groups; allele A of the FGB gene – 7.7, 33.3 and 60.3% of cases at the same groups ($\chi2=33.6$, $p<0.001$). Homozygous variants of carriage of mutant alleles in the group of pregnant women with severe PE were significantly more common. PE is characterized by a significant increase in blood thrombotic potential, which corresponds to the severity of the condition. The above components of the pathogenesis of PE are associated with a high prevalence of mutant variants of platelet integrins and fibrinogen genes, which is an additional risk factor for adverse pregnancy outcomes.

Keywords: gene mutations, hemostasis, preeclampsia, pregnancy

THE EFFECT OF HYPERHOMOCYSTEINEMIA ON THE REDUCTION OF GINGIVAL BLOOD FLOW

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Hyperhomocysteinemia (HHcy), has already been associated with inflammation and bone resorption. As an independent risk factor for cardiovascular diseases, it is also a risk factor for periodontal disease (PD). The mechanism of HHcy influence on gingival blood flow and development of PD has not been fully investigated. The aim of this study was to examine the effects of HHcy on gingival blood flow in mice with congenital cystation β-synthetase deficiency and as well possible prevention and therapy of *Lactobacillus rhamnosus* (LGG) in these mice. This study included 24 mice, 12 wild-type mice (Wild type, WT, C57BL/6J) and 12 congenital β-synthetase deficiency mice ($CBS^{+/-}$ knock-out heterozygotes, B6.129P2□Cbstm1Unc/J 002853) which were classified into the following four groups: healthy mice (WT), $CBS^{+/-}$ mice ($CBS^{+/-}$), mice treated with probiotic LGG (LGG), $CBS^{+/-}$ mice treated with probiotic LGG ($CBS^{+/-}$ + LGG). LGG treatment involved quarterly oral administration, at a dose of 2.5×10^5 CFU per day. Animals were measured gingival blood flow by Laser Doppler and after sacrifice histomorphometry analyzes of periodontal tissue on histological preparation Hematoxylin and eosin (H&E) stained, zymographic analysis of matrix metalloproteinase activity (MMP-9), quantitative real-time chain polymerization reaction (TNF-α, IL-1β, IL-6, OPG, RANKL). The results of our study showed HHcy led to a decrease in gingival blood flow, thereby the distance between cementoenamel junction and the alveolar bone crest, epithelial downgrowth was increased by reduced number of fibroblasts. On the other hand, LGG has shown anti-inflammatory effects as cytokine levels were lower, periodontal tissue recovery improved and health of HHcy mice maintained. In summary, HHcy is a potential biomarker of PD and it can be helpful in elucidating mechanisms of pathogenesis and etiology of PD. Given its antimicrobial, anti-inflammatory and non-cariogenic properties, LGG could offer risk reduction, an inexpensive way to prevent and treat PD as well as an excellent option for achieving and maintaining periodontal health in individuals with elevated homocysteine levels.

Keywords: homocysteinemia, periodontal disease, *Lactobacillus rhamnosus*, laser Doppler

PLATELETS IN COVID-19, COMORBID WITH AUTOIMMUNE THYROIDITIS

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In patients with COVID-19, the amount of platelets (PLT) usually drops, which may have autoimmune reasons. Up to 97% of patients with autoimmune thyroiditis (AIT) also have anti-PLT autoantibodies leading to autoimmune thrombocytopenia. PLT parameters during AIT/COVID-19, comorbidity was unknown. The study was aimed at evaluation of PLT parameters in AIT comorbid with COVID-19. Catamnesis of 27 people with AIT who suffered from COVID-19-pneumonia was studied. Blood amount of PLT and following their parameters: mean platelet volume (MPV), platelet distribution width (PDW), and thrombocrit (PCT) were measured (flow cytometry, XN-9000, Sysmex, Japan). Before COVID-19, the PLT count was $262.3\pm15.6\times10^9$/l. In the initial period of COVID-19, it decreased to $214.7\pm12.7\times10^9$/l, in minimal cases – to 96×10^9/l. In acmatic phase of COVID-19 it increased significantly to 356.7±29.2 After COVID-19, PLT count fell down to $232.9\pm16.5\times10^9$l, which was lower than before the infection. In patients with COVID-19 without AIT, the number of platelets usually steadily decreases throughout the disease. The control group of COVID -19 patients (n=30), had platelet count of $213,9\pm 16,7\times10^9$/l in the acute phase. MPV before COVID-19 was 9.6±0.53 fL, At the height of COVID-19 MPV became 9.15±0.47fL, During the recovery period MPV increased to 10.51±0.38 fL. An increase in MPV may depend on higher share of young PLT due to compensatory stimulated thrombopoiesis. PDW before COVID-19 was 14.38±3.49%; At the peak of COVID-19 it was 14.69±1.0%, After the infection PDW moderately decreased to 13.5±0.86%. PDW characterizes the degree of platelet anisocytosis – with a lower value, platelets differ less in volume. Before COVID-19, thrombocrit was 0.25±0.07% At the height of COVID-19, thrombocrit was 0.21±0.02%. In the recovery period, thrombocrit grew up to 0.27 ± 0.02%. This gradual increase of thrombocrit can be explained with an increase in the count of platelets at the height of the disease, and an increase in their volume after the acute stage. At the beginning of COVID-19, the platelet count decreases. It remains unclear why in the acute stage of COVID-19 platelet counts in patients with AIT are increasing. This may be due to known hyperleptinemia in AIT, because leptin stimulates thrombopoiesis and positively correlates with PLT count. To prevent hemorrhage in patients with AIT during COVID-19, it is recommended to check not only PLT count, but also anti-PLT autoantibodies.

Keywords: autoimmmune thyroiditis, COVID-19, platelets

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URIC ACID AND ACUTE RENAL IMPAIRMENT IN PATIENTS ON ADMISSION FOR HOSPITAL TREATMENT

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The aim of this study was to evaluate value of uric acid as a risk factor for the development of acute kidney damage on admission for hospital treatment. We determined also, risk factors related to uric acid value, and its relationship with anemia. The study included 86 hospitalized patients with acute renal impairment who were divided according to the stage of renal impairment at admission to hospital treatment into three groups. The assessment of acute renal impairment and the classification of disease stages were based on the diagnostic stages by the K / DIGO group. All examined patients were over 18 years of age. In the first stage of the disease it was 12.79%, in the second stage it was 15.12%, and in the third 72.09% of patients. Results: uric acid values were the lowest in the first stage 551.00 ± 225.64μmol / L and highest in the third stage 670.02 ± 86.72μmol / L, but without statistical significance. The lowest percentage with renal function was 12.9% in patients with stage 3 acute renal impairment. There was a statistically significant difference with patients with stage 1 and stage 2 ($p < 0.017$) renal impairment. ROC curve for cut off value of uric acid 903,00μmol/L based on the Youden index method with sensitivity of 91.70% and predictive value of 17.00% as a marker of acute kidney damage in stage 2 disease is larger than in other stages acute kidney injury. Although there is a significant relationship with risk of uric acid with hemoglobin values according to the following results OR 1.036 (95% confidence interval CI: 1.003-1.071, $p= 0.030$) and hematocrit level based on predicted probability and OR 1.149 (95% confidence interval CI: 1.028-1.285, $p=0.015$). The study showed that high values of uric acid in patients with acute kidney damage at the beginning of hospital treatment did not show the same significance in different phases of kidney damage. We confirmed the link between anemia and uric acid levels.Ineffective renal uric acid clearance means progression of the disease course to the next stage, and over time as a sign of progression of the renal continuum to chronic damage.

Keywords: acute kidney injury, uric acid, risk factors, prognosis

THE ROLE OF OXIDATIVE STRESS AS A MECHANISM IN THE PATHOGENESIS OF HEART DAMAGE IN ACUTE KIDNEY DAMAGE

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Despite a large amount of research on synchronous and mutually induced kidney and heart damage, the basis of the disease is still not fully clarified. It is clear that healthy mitochondria are essential for normal kidney and heart function. Mitochondrial dysfunction occurs when the clearance or process of generation and fragmentation of mitochondria is disturbed. The kidney is the second organ after the heart in the number of mitochondria. Kidney tubules are rich in mitochondria due to the high energy requirements for absorption processes of large amounts of ultrafiltrate and dissolved substances. The place of action of oxidative stress is the influence on the balance in the production and breakdown of mitochondrial ROS. A more precise determination of the place and role of key factors that play a role in the onset of the disease is necessary for understanding the nature of the onset of the disease and the creation of therapy in the future. The review integrates results found in previously performed studies which have evaluated oxidative stress participation in renocardiac syndrome type 3.

Keywords: acute kidney damage, acute heart failure, cardio renal syndrome

CLINICAL AND PROGNOSTIC SIGNIFICANCE OF BAX EXPRESSION AND BCL2/BAX RATIO IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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The phenomenon of apoptotic resistance observed in malignant B lymphocytes in chronic lymphocytic leukemia (CLL) is attributed, at least in part, to inherent deficiencies in their apoptotic machinery. Numerous genetic modifications and anomalous regulation of apoptosis effectors, including the Bcl2 protein family, are encompassed within this phenomenon. The objective of this investigation was to examine the correlation between the expression of the pro-apoptotic Bax gene and the Bcl2/Bax ratio with the clinical characteristics of patients with CLL and with molecular prognostic markers, specifically the mutational status of rearranged immunoglobulin heavy variable (IGHV) genes and lipoprotein lipase (LPL) gene expression. The expression of Bax mRNA and Bcl2/Bax mRNA ratio was quantitatively analyzed in the peripheral blood mononuclear cells of 58 unselected CLL patients and 10 healthy controls using the reverse-transcriptase polymerase chain reaction. Our findings indicate a noteworthy upregulation of the Bax gene in CLL specimens relative to non-leukemic specimens ($p=0.003$). Additionally, we observed an increased Bcl2/Bax ratio ($p=<0.001$). The results indicate that the Bcl2/Bax ratio exhibited a statistically significant negative correlation with lymphocyte doubling time ($r=-0.307$; $p=0.0451$) in relation to prognostic markers. Additionally, high-level Bax expression was found to be associated with LPL-positive status ($p=0.035$). The expression of Bax and the ratio of Bcl2/Bax were comparatively elevated in patients with unmutated IGHV rearrangements in contrast to those with mutated IGHV rearrangements. However, this dissimilarity did not attain statistical significance. Based on our findings, it can be inferred that the dysregulated expression of Bcl2 and Bax, resulting in an increased Bcl2/Bax ratio in leukemic cells, plays a role in the development and clinical progression of CLL.

Keywords: Bax, Bcl2/Bax ratio, chronic lymphocytic leukemia, apoptosis

FORMULATION AND EXAMINATION OF *GALIUM VERUM* BASED ORAL GEL FOR APHTOUS STOMATITIS TREATMENT

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Aphthous stomatitis is a chronic inflammatory disease characterized by the presence of painful ulcerations located on oral mucosae. The available treatment is not sufficient to provide complete ulcer recovery without side effects although oral ulcers represent one of the most frequent oral mucosal disease. Therefore, the aim of this study was to prepare a mucoadhesive oral gel based on *Galium verum* (*G. verum*) ethanol extract (GVL gel) and detect its effects in the healing process of aphthous stomatitis in animal model. Rats with oral buccal ulcers were separated into the following groups: control group (untreated), gel base (ulcer was treated with the gel base, three times per day for 10 days), and GVL gel group (the ulcer was treated with GVL gel, three times per day for 10 days). Development and size of the aphthous lesion was measured every day until complete healing. At the day 0 and after 3, 6, and 10 day of treatment animals from each group were sacrificed and buccal tissue samples were collected for further analyses. Effects of oral gel on healing process were determined by clinical evaluation, as well as histopathological examinations. Our results for the first time suggest the potential of a mucoadhesive gel based on *G. verum* extract to accelerate oral ulcer healing which is reflected by a significant increase in percentage ulcer contraction throughout the duration of the treatment. Herbal extract such as *G. verum* significantly reduced healing time in the experimental group compared to control groups. This indicates that *G. verum* based oral gel might serve as effective and safe agent for the treatment of aphthous stomatitis, however future studies are required to reveal the overall potential of this formulation before human application.

Keywords: recurrent aphthous stomatitis, oral ulcer healing, Galium verum L., phytotherapy

CORRELATION OF THE MANDIBLE POSITION AND THE CARDIOVASCULAR STRESS REACTIVITY

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Dentists try to bring the occlusion of patients to the golden ratio and the central position of the lower jaw. It is known, that the position of the lower jaw is interconnected by the function of the cardiovascular system through the trigeminocardial reflex. We **aimed** to identify the correlation between the mandibular position and cardiovascular stress reactivity in patients with psychological stress (bruxers) and after physical stress (sit-ups). We have studied the mandibular position of 10 patients with bruxism and 10 nonbruxers 24-56 years old and 30 healthy students 20-23 years old with distal (n=10), medial (n=10) and central (n=10) mandibular position. All the students had their pulse and blood pressure measured before and 5 minutes after 30 active squats. Statistical analytics we made with Statistica SPP, using paired Student's test. We noticed the central mandibular position in 50% of bruxers and 10% of nonbruxers; distal mandible position 60% in the bruxers group and 10% in the group without bruxism. All the student groups had higher systolic blood pressure (5-15 units mmHg) after squats accept the group with central mandible position (10-15 units mmHg lower after the physical stress). All the student groups had higher pulse after active squats accept the group with central mandible position (5-12 beats per minute less after active squats). Student's t-test value 0.02. Differences are not statistically significant (p=0.980763). Number of degrees of freedom f=18. Critical value of Student's t-test = 2.101, at significance level $\alpha = 0.05$. In our study, we noticed, that the central mandibular position might correlate with better stress tolerance due to muscle activity. With this, we may suppose, that bruxism, as the mussel activity, is not a pathological process but it can be a stress-protective function. This stress-protective function should be maximally realized in patients with central mandible position. There are limitations in our study with a small sample group. Further studies are needed.

Keywords: mandible, bruxism, stress, blood, pressure

STUDY OF THE ASSOCIATION OF POLYMORPHISMS OF THE DNA REPAIR, CELL CYCLE AND TRANSPORT GENES WITH RESPONSE TO TREATMENT AND OBSTETRIC PARAMETERS IN ONCOLOGICAL DISEASES DURING PREGNANCY

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One on the one thousand pregnancies is complicated by cancer, and the issues of treatment tactics in such patients remain debatable. So work on the searching the markers for individualization of the treatment of pregnant women with cancer is extremely relevant. The study included 41 pregnant women with cancer treated with cytotoxic drugs. They consist of 34.1% breast cancer, 31.7% of cervical cancer, and 34.2% other cancers (intestinal, ovarian, lung, bone). Peripheral blood samples were taken from the patients before chemotherapy, and DNA was isolated. Polymorphisms of genes of DNA repair XRCC1 (rs25487), ERCC5 (rs17655), ERCC2 (rs13181), cell cycle control TP53 (rs1042522), CDKN1A (rs1801270), MDM2 (rs2279744), transport proteins ABCB1 (rs1045642, rs2032582), ABCC1 (rs4148353, rs35626, rs35625, rs35623, rs11866794) were analysed by real-time PCR with fluorescent allele-specific probes. The results were compared with the clinical response, the degree of pathological response of tumor, the percentile of the newborn (complex index of weight and length), and weight of the placenta using the logistic regression method. There was an association of allele C rs1042522 TP53 (p=0.04), allele A rs1801270 CDKN1A (p=0.03), allele A rs2032582 ABCB1 (p=0.05) with the achievement of complete clinical response, and allele G rs1045642 ABCB1 (p=0.04) with better clinical response. In the subgroup of breast cancer there was the relationship of clinical response with allele C rs1042522 TP53 (p=0.015), allele G rs2279744 MDM2 (p=0.015), and allele T rs35625 ABCC1 (p=0.005). In the subgroup of cervical cancer association of G rs35626 (p=0.018) and C rs35625 of ABCC1 (p=0.047) was observed. The data of pathological response was available for 25 patients. There was an association of allele G rs1045642 ABCB1 and the achievement of complete and grade 3 of pathological response (p=0.04). In the group of breast cancer there was the relationship of allele A rs1801270 CDKN1A (p=0.048). Changes in placental weight (less than 10 percentile) were found depending on the carriage of the allele G rs13181 ERCC2 (p=0.019), allele A rs2032582 ABCB1 (p=0.028), allele C rs35625 of ABCC1 (p=0.019), allele T rs35623 ABCC1 (p=0.013). An association of allele G rs35623 ABCC1 (p=0.028) with percentile of newborn was found. The work revealed the relationship of number of polymorphisms of studied genes with the clinical response, the degree of pathological response of the tumor, and changes in the placenta weight and the percentile of newborn. Thus, the study of gene polymorphisms is promising for the individualization of the treatment of pregnant women with cancer.

Keywords: pregnancy; cancer; chemotherapy; gene; polymorphism

ACTIVATION OF RECEPTOR EXPRESSION ON BLOOD CELLS BY VARIOUS PROINFLAMMATORY AGENTS

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Change of receptor expression on the surface of immune cells is one of manifestations of biological activity of proinflammatory agents, in particular endotoxins or allergens [Gomes at al., 2010]. We evaluated the involvement of the proinflammatory factors LPS *E. coli*, *Dermatophagoides pteronyssinus* Der p 2 (the house dust mite allergen [HDM]) or their combination in the activation of human whole blood monocytes and neutrophils by changing the expression levels of TLR4, CD14 and CD11b receptors. Der p 2 is a mimetic of the human protein MD-2, a TLR4 activation cofactor. Expression of the receptors was estimated with specific mAb by flow cytometry (EPICS XL-MCL, Beckman Coulter, USA).The expression of TLR4 and CD14 on monocytes decreased upon activation of blood cells by LPS *E. coli*, which may be associated with CD14-dependent endocytosis of these receptors [Tan et al., 2015]. The important role of the CD14+CD16+ monocyte subpopulation in the inflammatory response to endotoxins has been shown previously [Radzyukevich et al., 2021]. TLR4 expression on monocytes in response to Der p 2 allergen did not change remarkably. Allergen Der p 2 and its co-activation with LPS *E. coli* decreased the level of CD14 receptor expression on monocytes compared to controls. The expression of CD11b on monocytes showed no significant changes upon activation of blood cells with LPS *E. coli* and/or Der p 2. We found no effect of LPS *E. coli* on the level of TLR4 expression on the surface of neutrophils. The level of CD14 expression on neutrophils, unlike on monocytes, was insignificant and did not change considerably when activated by endotoxin or allergen. Activation of neutrophils by LPS *E. coli* markedly increased the expression of CD11b on the cell surface compared to the control, which agrees with the literature data [Kim et al., 2019]. Der p 2 protein caused a marked decrease in CD11b expression on neutrophils compared to the control. A decrease in the level of CD11b on neutrophils was also observed when cells were activated by a combination of Der p 2 allergen and LPS *E. coli*. These results reflect different functional activity of these cells depending on the inflammatory agent. Our studies of Der p 2 effect on TLR4 expression on innate immune cells showed an increase in the expression of this receptor on neutrophils. Allergens are known to trigger inadequate immune responses in direct interaction with TLR [Karp, 2010]. TLR4 expression controls neutrophil responses to LPS and allergens. When studying the involvement of neutrophils in the development of severe asthma, it was found that TLR4 stimulation of neutrophils by HDM regulates the apoptosis of these cells [Kim et al., 2015]. The results obtained in this study on receptor expression reflect the different specificity of monocyte and neutrophil activation mechanisms in the blood cell response to LPS, Der p 2 allergen and their combination, determining the specificity of the participation of these cells in the development of inflammatory responses.

Keywords: LPS *E. coli*, Der p 2, TLR4, CD14, CD11b