Session 2. Molecular Basis of Metabolic Diseases

Lectures

L2.1

New advanced molecular technologies for the diagnosis of metabolic diseases

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The recent advances in the genomic field and the development of new technologies for DNA testing started the revolution of the diagnostic laboratory. For the diagnosis of metabolic diseases, DNA-based diagnostics provide a sensitive alternative to protein-based diagnostics and the mutation detection is one of the most important areas of molecular diagnostics today and can be divided into two categories: a diagnostic mode, where specific tests are designed to detect known mutations and a scanning mode, where a stretch of DNA is searched for unknown mutations.

Advances in DNA analysis to develop methods, which are increasingly specific, sensitive, fast, simple, automatable, and cost-effective, are considered paramount. These demands are currently driving the rapid evolution of a diverse range of newer technologies.

Researchers have discovered hundreds of genes that harbour variations contributing to human illness, identified genetic variability in patients’ responses to dozens of treatments, and begun to target the molecular causes of some diseases. In addition, scientists are developing and using diagnostic tests based on genetics or other molecular mechanisms to better predict patients’ responses to targeted therapy.

For the future of genomics is demanding the rapid evolution of miniaturization (nanotechnology) and high-throughput genotyping technologies (next generation sequencing) toward increased speed and reduced cost. The speed, accuracy, efficiency, and cost-effectiveness of DNA sequencing have been improving continuously since the initial derivation of the technique in the mid-1970s. With the advent of massively parallel sequencing technologies, DNA sequencing costs have been dramatically reduced. The recent introduction of instruments capable of producing millions of DNA sequence reads in a single run is rapidly changing the landscape of genetics, providing the ability to answer questions with heretofore unimaginable speed.

In this arena Laboratory Medicine should play a major role.

L2.2

Molecular basis of chronic metabolic diseases: type 2 diabetes and osteoporosis

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Type 2 diabetes is a highly prevalent metabolic disease with strong comorbidity with obesity and cardiovascular diseases. A crosstalk between mitochondria and the insulin signaling cascade could be involved in the etiology of T2DM. Mitochondria play a key role in energy metabolism and ATP production in skeletal muscle, cardiac muscle, brain, liver. Mitochondrial dysfunction characterized by reduced ATP generation and mitochondrial number in skeletal muscle or reduced ATP generation and mitochondrial stimulus-secretion coupling in the pancreatic beta cell has been implicated in the pathology of metabolic disease associated with T2DM. The generation of reactive oxygen species from mitochondria and other cellular sources may interfere in insulin signaling in muscle, contributing to insulin resistance. Obesity and insulin resistance and T2DM are associated with a metabolically driven, low-grade, chronic inflammation. Several inflammatory cytokines as well as lipids and metabolic stress pathways can activate metabolic inflammation, which targets metabolically critical organs and tissues including adipocytes and macrophages to adversely affect systemic homeostasis. Fatty acid-binding proteins (FABPs) may play a role in metabolic inflammation and related diseases including obesity, diabetes, and atherosclerosis. The study performed in young women with overweight/obesity showed that adipocyte FABP was associated with atherogenic risk, inflammation and predicted obesity. Recent data suggest a role for mild zinc deficiency in low-grade systemic inflammation present in cardiometabolic diseases.

Bone remodeling requires an intimate cross-talk between osteoclasts and osteoblasts and is coordinated by regulatory proteins interacting through complex autocrine/paracrine mechanisms. Osteocytes, bone lining cells, resident osteal macrophages and vascular endothelial cells regulate bone remodeling through cell signalling networks of ligand-receptor complexes. T and B lymphocytes also mediate bone homeostasis via secreted and membrane-bound factors in the bone microenvironment. Recently discovered paracrine or coupling factors of importance are: RANKL, slerostin, osteoblast interleukin-33, EGFL6, osteoclast-derived semaphorin 4D, signals from T helper cells. Intracellular communication within the bone microenvironment is critical for the maintenance of normal bone integrity. Osteoporosis is caused by disruption of this multicellular communication.
L2.3

Cholinergic neurons - “locus minoris resistentiae” of the brain – metabolic backgrounds

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Pyruvate derived from glycolytic metabolism of glucose is a principal source of acetyl-CoA, which is generated by intramitochondrial pyruvate dehydrogenase complex (PDHC) in all types of brain cells. Cholinergic neurons like neurons of other transmitter systems cells, utilize acetyl-CoA for energy and N-acetyl-L-aspartic acid production in mitochondria and for diverse synthetic pathways in their extramitochondrial compartments. However, cholinergic neurons require some additional amounts of acetyl-CoA for acetylcholine (ACh) synthesis in the cytoplasmic compartment to maintain their neurotransmitter functions. Characteristic feature of several neurodegenerating diseases including Alzheimer’s disease and aluminum or thiamine diphosphate deficiency encephalopathy is the decrease of PDHC activity correlating with cholinergic markers deficits and losses in cognitive functions. Such conditions may generate acetyl-CoA deficits that were found to be deeper in cholinergic neurons than in the noncholinergic neuronal or glial cells, due to its additional consumption in the ACh synthesis. Therefore, any neuropathologic conditions are likely to be more harmful for the cholinergic neurons than for noncholinergic ones. This presentation demonstrates that common neurodegenerative signals such as excess of peroxynitrite, Zn, Al, vandyl anions, amyloid-β or thiamine diphosphate deficiency might induce more extensive dysfunction and structural impairment of cholinergic neurons than the noncholinergic ones, through the suppression their acetyl-CoA metabolism. The key targets for these neurotoxins were PDHC, ketoglutarate dehydrogenase complex and/or aconitate. Their inhibition or inactivation suppressed acetyl-CoA synthesis and/or its utilization in TCA cycle yielding depletion of intracellular ATP in the mitochondrial compartment. It caused inhibition of indirect and direct pathways of acetyl-CoA transport to cytoplasm. The shortages of acetyl-CoA in the latter compartment could cause multidirectional disturbances in ACh synthesis as well as its nonquantal and quantal release. Hence, cholinergic neurons survival correlated with intramitochondrial level of acetyl-CoA. On the other hand, intracellular ACh accumulation and its quantal release in different pathologic conditions correlated strongly with cytoplasmic levels of this metabolite. No such correlations were observed in noncholinergic neuronal cells, which appeared to be more resistant to neurodegeneration than the cholinergic ones. For this reason therapeutic strategies aiming to preserve proper rates of acetyl-CoA synthesis in the encephalopathic brains, should attenuate high susceptibility of cholinergic neurons to diverse neurodegenerative conditions.

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Oral presentations

O2.1

Adipocyte fatty acid binding protein and its association with atherogenic risk profile and insulin resistance in young overweight and obese women

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Aim: We evaluated the association of A-FABP with proatherogenic risk profile and insulin resistance (IR) in young non-diabetic overweight/obese women. Materials and methods: Serum A-FABP, hsCRP, adiponectin, glucose, insulin, lipids and apolipoproteins were measured in 104 women (20–45 yrs; BMI≥25 kg/m²) and age-matched healthy controls (n=76; BMI<25 kg/m²). All underwent blood pressure and anthropometric measurements. Results: A-FABP concentration was related with IR, anthropometric and atherogenic indices. A-FABP was an independent predictor of TG/HDL-C explaining 42% of its variation in overweight/obese women. At a cut-off 16 ng/mL A-FABP discriminated between controls and overweight/obese (AUC= 0.96) with high sensitivity and specificity. A-FABP predicted atherogenic risk with OR 11.2 (95% CI 3.7–34.2), 7.1 (1.9–27.2), 6.7 (2.6–17.2) for having elevated TG/HDL-C, apoB, CRP and IR with OR 5.6 (1.8–17.2). Conclusions: A-FABP seems to be a valuable predictor of atherogenic risk profile; if elevated contributes to cardiovascular disease beyond its effect on insulin resistance.
O2.2

Non-enzymatic modifications of cyclooxygenase-1 affect its bifunctional activity — implications for the diminished sensitivity of platelets to acetylsalicylic acid in diabetes

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Aim of the study: We tested whether the process of non-enzymatic glycosylation of cyclooxygenase-1 is able to effectively modulate the enzymatic activity of the enzyme and inhibit the subsequent non-enzymatic acetylation of the enzyme at these sites that are critical for the binding of the substrate.

Materials and methods: Isolated, purified ovine cyclooxygenase-1 enzyme was either incubated (at final concentrations given in parentheses) with glucose (300 mM), 1,6-bisphosphofructose (300 mM), methylglyoxal (100 mM) for 48h in 10°C or with acetylsalicylic acid (170 uM) for 24h in 10°C. Cyclooxygenase activity of COX-1 was monitored as oxygen consumption and measured with the use of high resolution respirometry. Activity of peroxidase domain of COX-1 and whole COX-1 activity were assessed with the use of fluorescent assays.

Results: We noticed decrease of peroxidase activity of COX-1 by 30% (p = 0.02) and PGE2 production by 47% (ns) for glucose, increase of cyclooxygenase activity of COX-1 by 69% (p = 0.001) and decreased prostaglandin E2 production by 80% (p = 0.002) for 1,6-bisphosphofructose and almost complete inhibition of enzyme activity (by 87% for peroxidase subunit, 46% for cyclooxygenase domain and 61% for a total enzyme activity) for methylglyoxal compared to the unmodified enzyme. In the case of incubation of COX-1 with acetylsalicylic acid peroxidase and cyclooxygenase activity of enzyme as well as PGE2 production was inhibited respectively by 87% (p < 0.0001), 88% (p = 0.0008), 92% (p = 0.0001). COX-1 peroxidase and cyclooxygenase activities were reduced after incubation with glucose by 57% (p = 0.006; by 12% (p = 0.03, respectively) and with methylglyoxal (by 78%, p = 0.0001; by 49%, p = 0.0001, respectively) while further acetylation with ASA was still susceptible to inhibition with ASA. Prior incubation with 1,6-bisphosphofructose increased cyclooxygenase activity of COX-1 (by 22%, p = 0.008), whereas the enzyme was still susceptible to inhibition with ASA (peroxidase by 62%, p = 0.0008; cyclooxygenase by 52%, p = 0.0001).

Conclusions: We showed that non-enzymatic modifications of COX-1 may change the activity of each of its functional domains in different way resulting in amended activity of whole enzyme. COX-1 modified with glucose and methylglyoxal was not further susceptible to inhibition with ASA. That may be one of the reasons of diminished sensitivity of platelets to acetylsalicylic acid in diabetes.

O2.3

Comparison the intensity of free radical processes in the liver and heart during chronic alcohol intoxication

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Chronic alcoholism is the cause of much somatic pathologies, the main of which are: liver cirrhosis, hepatitis and cardiomyopathy (Popova et al., 2008, Narod 2: 32–35). Currently the leading role assigned to the molecular mechanisms of alcoholism, including a free radical or lipid peroxidation (POL) (Pompella, 1997, Int J Nutr Metab 67: 289–297). Because of activation of lipid peroxidation is the destruction of structures of cells, tissues and organs through cytosis and exit of oxidase enzymes, decreased the activity of catalase (Zelickson et al., 2011, Biochim Biophys Acta 1807: 1573–1582).

The purpose of the study — to compare processes of free radical oxidation of lipids in the liver and heart in chronic alcohol intoxication (CAI).

Experimental rats were given 25% ethanol intragastrically (3.5 g/kg body weight), twice a day, during 7, 14, 21 and 28 days. Intact rats were given drinking water. In homogenized liver and heart tissues were determined level of thiobarbituric acid-reactive substances (TBARS), diene conjugates (DC) and catalase activity (Koroluk, 1988). Results. In liver homogenates noted the increase of DC on the 7th day CAI by 86% compared to control (p ≤ 0,05), with subsequent normalization. Increased activity of catalase is observed on the 14th day CAI by 85% (p ≤ 0,05). Increasing the concentration of TBARS did not happen. Thus, in the liver by the end of the first week of CAI activated POL processes, which further terms reduced over time by increasing the activity of catalase, which may indicate the induction of adaptive mechanisms. There is a time delay catalase response to the intensification of the POL processes, which is probably due to increased formation of acetaldehyde in the first week of CAI. In the heart there is also a pronounced oxidative stress within 2 weeks of CAI: the level of DC on the 14th day CAI increases by 67% and the concentration of TBARS — by 81% in comparison with the control.

In contrast to the liver, in the heart at high intensity POL during CAI not marked activation of catalase. This may be due to a violation of the membranes of cardiomyocytes and with the development tolerance of membranes to the presence of ethanol. Thus, activation of lipid peroxidation and their inhibition in the liver and the heart at the CAI is performed by different mechanisms.
O2.4

Protective effects of amino acids on the heart in alcohol treated rats

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Background: Binge drinking can be a cause of dilated cardiomyopathy, referred to as alcoholic cardiomyopathy (ACM). A number of mechanisms including oxidative damage, deposition of triglycerides, altered fatty acid extraction and impaired protein synthesis have been proposed for the development of ACM. Nonetheless, the exact pathogenesis of ACM is incompletely understood. It is generally accepted that amino acids are the powerful natural means of metabolic therapy of alcoholism.

Aims: This study was designed to evaluate the preventive effects of amino acids on ethanol-induced cardiomyopathy.

Methods: We investigated the effect of intragastrical administration of L-arginine (500 mg/kg) + L-glutamine + (500 mg/kg) + succinate (50 mg/kg) on myocardial tissue of male Wistar rats under interrupted alcohol intoxication (8 mg/kg/day (7 days of alcohol intoxication and 7 days of abstinence) during 56 days).

Results: It was shown that interrupted alcohol intoxication produces a number of histological abnormalities of the contractile elements including myocytes loss, disruptions in the myofibrillary structure and inflammatory infiltrates. Mixture of L-arginine, L-glutamine and succinate, when co-administered with alcohol completely prevents alcohol-induced histological changes in myocardial tissue.

Conclusions: These findings demonstrate that histological abnormalities in myocardial tissue caused by interrupted alcohol intoxication can be prevented by administration of L-arginine + L-glutamine + succinate mixture. The results of this study suggest that this mixture is a promising therapeutic agent to prevent ethanol-induced adverse effects on myocardial tissue. Further study is needed to determine the utility of L-arginine + L-glutamine + succinate mixture as a potential drug for treatment of ACM.

O2.5

Effect of long-term impaired carbohydrate metabolism on mitochondrial respiratory parameters and cyclooxygenases activity in blood platelets

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Introduction: In a diabetes-related states of chronic hyperglycemia, the prolonged exposure of blood platelets to high concentrations of glucose may impair functioning of platelet mitochondria, but also affect other platelet oxygen-consuming systems, like cyclooxygenases. The principal aim of this work was to assess the effects of chronic hyperglycemia on oxygen consumption by platelet mitochondria and prostaglandin H₂ synthases (COX).

Materials and methods: Diabetes mellitus was induced in Sprague Dawley rats by injection of streptozotocin. Platelet mitochondrial respiratory capacity and platelet COX activity were monitored as oxygen consumption and measured with use of high resolution respirometry. In addition, COX activity was assessed with the use of peroxidase fluorescent assay and plasma thromboxane B₂ (TXB₂) with immunoenzymatic assay.

Results: In streptozotocin-diabetic rats blood platelet mitochondria demonstrated significantly elevated routine respiration and maximal respiratory state, compared non-diabetic animals. However, we did not observe changes in Leak respiration or Leak Control Ratio. We also revealed significantly elevated COX-related arachidonic acid metabolism in diabetic platelets, both when using polarographic and peroxidase assay, as well as elevated concentrations of serum TXB₂.

Conclusions: Our results indicate that although long-term diabetes can result in altered respiration of platelet mitochondria, it does not lead to a decreased control ratio and has no effect on respiratory complexes. In addition, increased arachidonic acid metabolism related to platelet COX activity, observed in diabetic subjects, may significantly contribute to elevated thromboxane generation in diabetes. However, the molecular mechanism(s) underlying these changes remain elusive.
Posters

P2.1

Reduced bone formation and elevated bone resorption markers in children with cystic fibrosis

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Introduction: Scientific studies show decreased bone mineral density and increased fracture frequency in most adult patients with cystic fibrosis (CF). It is mainly caused by imbalance between bone formation and bone resorption processes. This problem has not been sufficiently resolved in children and adolescents with CF. The aim of this study was to explain whether bone abnormalities in CF patients relate rather to bone formation than bone resorption process. We assessed biochemical bone formation markers: procollagen type I carboxyterminal propeptide (PICP) and total osteocalcin (OC) with its carboxylated (cOC) and undercarboxylated (ucOC) forms as well as resorption markers: C-terminal cross-linked telopeptide of type I collagen (CTX) and receptor activator of nuclear factor κB ligand (RANKL).

Patients and methods. We examined 40 children aged 5–10 years with diagnosed CF treated at the Department of Pediatrics of the Institute of Mother and Child in Warsaw. All patients had exocrine pancreatic insufficiency, were clinically stable, and received standard multivitamin supplementation. The control group consisted of 40 healthy subjects in the same age, who did not suffer from bone metabolism diseases. Bone metabolism markers were determined in serum of patients and healthy subjects using immunoenzymatic methods with specific monoclonal antibodies.

Results. Mean serum concentration of calcium, phosphate and 25-hydroxyvitamin D in both groups of children were within the reference ranges. In patients with CF, we observed a decrease in PICP concentration (median values: 218 ± 241 ng/ml, p<0.05) and a similar level of total OC (p<0.05) and higher of ucOC (p<0.05) in CF patients than he control. The serum levels of bone resorption markers were elevated in patients affected by cystic fibrosis. Mean CTX concentration was slightly higher (by about 15%) but RANKL level was about 2-fold higher (p<0.05) in CF children compared with the healthy ones.

Conclusions. Our results indicate that reduced PICP and carboxylated osteocalcin concentrations may lead to abnormal bone formation in patients with cystic fibrosis. Decreased bone formation together with increased bone resorption markers indicates an imbalance in bone turnover in pubertal period. That is why patients affected by cystic fibrosis may be at risk of osteopenia and osteoporosis in later life.

P2.2

Autophagy regulation in podocytes by insulin-dependent ROS production

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Autophagy constitutes an important intracellular defense mechanism which is responsible for turnover of damaged or non-functional cellular constituents and provides cells with energy and essential compounds under unfavourable environmental conditions. It is especially important for non-proliferating cells, eg podocytes, which are thought to be the most vulnerable part of glomerular filtration barrier. Altered morphology and reduced number of podocyte cells are found in type 2 diabetes, and these are the earliest pathological signs of diabetic nephropathy. We presume that alteration of podocyte autophagy may occur due to insulin resistance development or changes in insulin concentration in extracellular fluid, what leads to podocyte injury and improper functioning.

All experiments were conducted on primary culture of rat podocytes. Podocytes were transfected LC3 plasmid (expressing RFP-GFP-LC3 fusion protein). Insulin effects on autophagy were investigated in untransfected podocytes cultured for 60 minutes, 3 or 5 days in medium supplemented with 300 nM insulin. In order to determine the role of reactive oxygen species (ROS) in insulin-dependent autophagy regulation podocytes were incubated with both insulin (300 nM) and apocynin (100 μM) – inhibitor of NAD(P)H oxidase subunit NOX4. The expression level of proteins involved in autophagy (LC3, beclin1, AMP-activated protein kinase (AMPK), phosphoinositide 3-kinase (PI3K) class III) was analysed by Western blot. Changes in AMPK activity were determined by immunoblotting against AMPKa P-Thr172. Autophagy in transfected podocytes was detected based on presence of autophagosomes and autophagolysosomes. The increases of LC3-I and LC3-II expression were observed in untransfected podocytes after 60 min incubation with insulin and these effects were abrogated by apocynin. After 5 days incubation the amount of LC3-I and LC3-II was comparable to control. Insulin decreased P-AMPK/AMPK ratio (by 40%) after 60 min of incubation. However after 3 days of incubation P-AMPK/AMPK ratio amounted to 128% of control, whereas in the presence of apocynin it was reduced by 35% (p<0.05 vs control). The expression of beclin1 showed slightly upward tendency dependent on time of incubation with insulin. The effect of insulin on PI3K class III expression in podocytes was not observed.

The results obtained up till now demonstrate that insulin may affect autophagy through ROS-dependent mechanism in podocytes and this may implicate the importance of this process in pathogenesis of diabetic nephrphathy.

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Amino acids can prevent ethanol-induced oxidative stress in the liver

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Background: Alcoholic liver disease is one of the most serious consequences of chronic alcohol abuse. Oxidative stress via generation of reactive oxygen species is suggested to be the major mechanism of alcohol-induced liver injury. Aims: This study tested the hypothesis that ethanol-induced oxidative stress in the liver can be prevented by the administration of amino acids mixture in the experimental model of alcoholism. Methods: We investigated the effect of intragastrical administration of L-arginine (300 mg/kg) + L-glutamine (300 mg/kg) + succinate (50 mg/kg) on oxidative stress and antioxidant status in the liver of male Wistar rats under chronic alcohol intoxication (8 mg/kg/day during 60 days). Results: The results revealed that chronic alcohol treatment caused a significant increase in the level of lipid peroxidation indicated by increase in the level of thiobarbituric acid (TBARS), levels of superoxide dismutase (SOD)/glutathione peroxidase (GSH-Px) activities and decrease in the activity of glutathionereductase (GSH-Red). Supplementation of L-arginine + L-glutamine + succinate mixture significantly lowered the activities of SOD and GSH-Px, decreased the level of lipid peroxidation, and enhanced the antioxidant status. Conclusions: This study demonstrated that ethanol-induced liver damage is associated with oxidative stress and co-administration of L-arginine + L-glutamine + succinate mixture may attenuate this damage by decreasing oxidative stress in the experimental model of alcoholism.

Cytokines secretion by Peripheral Blood Mononuclear Cells (PBMCs) in children diagnosed with Autism Spectrum Disorders (ASDs)

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Autism Spectrum Disorders (ASDs) are defined as a group of developmental disabilities characterised by impairment in social interactions and communication, and repetitive, stereotypical patterns of behaviour. ASDs, according to The Autism and Developmental Disabilities Monitoring (ADDN) Network, affect about 1 in 88 children in US (data for 2008).

Immediate cause of Autism Spectrum Disorders remains unknown, although some common factors are observed. In majority of patients are registered abnormalities in brain morphology and function, mutations and other genetic aberrations (fragile chromosome X syndrome, Rett’s syndrome, SNPs, etc.). List of possible causes of ASDs consist: parental (maternal diabetes) and prenatal factors, birth defects, enviromental exposures, immune dysfunctions and autoimmune diseases, among others.

According to one of theories disruptions in opioid system function may be possible cause of so called autistic behaviour. Elevated levels of both endogenous and exogenous (β-casomorphins; BCMs) opioid peptides were reported in sera of ASDs patients. B-casomorphin-7 and -5 were also detected in urine of autistic children in opposite to healthy controls where no BCMs were present.

Although there is no specific diet for ASDs patients, they are often recommended to avoid milk products and products with high sugar content. Milk and milk products are major source of allergens especially in diet of children and were confirmed to be external source of β-casomorphins. Casomorphins may cause immunological response independently of milk allergens.

The aim of our research was to observe in simplified in vitro model, immunological response of ASDs patients to diet containing milk as an external source of β-casomorphin-7. Our experiment consisted isolation of PBMCs (Peripheral Blood Mononuclear Cells) from blood of patients diagnosed with ASDs. PBMCs were cultured in vitro for 5 days in presence of selected substances: non-hydrolysed milk (MNH), hydrolysed milk (MH) and bovine β-casomorphin-7 (BCM-7) in three concentrations: 1 µg/mL, 1 ng/mL and 1 pg/mL. We examined secretion of three cytokines: interleukin-4 (IL-4), interleukin-10 (IL-10) and interferon-gamma (IFN-γ) in post culture media via use of commercially available kits.

Although our primary results show that immunological response differs among ADSs patients, levels of IL-4 were decreased in majority of cases. The problem of immunological response of ADSs patients to milk and its products as a potential source of opioid peptides needs further investigation.
ROS dependent pro-apoptotic effects of visfatin in human colorectal carcinoma HCT-116 cell line: an in vitro study

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Background: Obesity and related metabolic abnormalities, including adipokine dysbalance, are risk factors for colorectal carcinoma. Visfatin, an adipokine that is highly expressed in visceral fat, is suggested to play a role in the progression of human malignancies. Oxidative stress basically defines a condition in which cellular redox state is shifted toward prooxidative environment; cellular biomolecules undergo severe oxidative damage, ultimately compromising cells viability. Oxidative stress may cause cellular apoptosis via both the mitochondria-dependent and mitochondria-independent pathways. Aims: Aims of the study were to examine the effects of visfatin on reactive oxygen species production and hydrogen peroxide level in human colorectal carcinoma HCT-116 cell line (by flow cytometry analysis), as well as visfatin’s effects on HCT-116 cells viability/cytotoxicity using MTS assay. Results: We demonstrated that visfatin increased intracellular ROS level in HCT-116 cells, by elevating the superoxide and hydrogen peroxide levels. Our results showed that visfatin significantly inhibited cells growth in a dose-dependent manner according to the MTS assay and induced morphological changes consistent with apoptosis, confirmed by DAPI staining and fluorescent microscopy analysis. Addition of visfatin to HCT-116 cells, resulted in the increase of apoptosis frequency measured by flow cytometry Annexin-V staining assay. Simultaneously we also observed a decrease in cell viability caused by the visfatin treatment; in these conditions, the clonogenic ability of the cells was also significantly decreased. Cell cycle analysis revealed that visfatin increased the proportion of these cells in G0/G1 and also decreased the percentage of cells in S and G2/M phases. Conclusions: The visceral obesity results in adipokine accumulation and play a crucial role in local metabolic pathways regulation. Visfatin stimulate colorectal cancer cells to produce ROS and to activate apoptosis pathway. Results suggested that mitochondrial oxidative stress caused by hormonal exposure is responsible not only for keeping cells in quiescent state (G0 phase) and prevent cancer proliferation but also induced cell death by apoptosis pathway.

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Looking for a cure to diabetes — the LPC-based modulators of GPR119

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Diabetes mellitus is one of the most common metabolic diseases nowadays and it is associated with elevated blood sugar level. The most often it results from insulin deficiency due to destruction of the cells in pancreas (type 1 diabetes, T1D) or insulin resistance and relative deficiency resulting from reduced number of insulin receptors, disruption of cellular signaling pathways or both (type 2 diabetes, T2D). T2D counts for over 90% of all cases of the disease and affects over 340 million people worldwide. This also entails development of other disorders, like heart diseases, strokes, obesity, diabetic retinopathy, kidney failure or even poor blood circulation in limbs. Due to high epidemiology rates of the disease and related severe effects, there exists a great need to search for a successful method of treatment. A great part of research, especially connected to T2D, is focused on understanding of cellular signaling connected with regulation of insulin release from beta pancreatic cells. The idea connected with “adorption” of orphan receptors involved in those pathways by specific ligands became a basis for screening of potential drugs. A G protein-coupled receptor present in the membrane of beta pancreatic cells, GPR119, is being a very often research target nowadays, as it was proven to be involved in modulation of functions of pancreatic cells, blood glucose level, body weight and other related aspects. Moreover, recent reports have shown that lysophosphatidylcholine (LPC) is one of the probable activating ligands of the receptor found to induce insulin secretion from beta pancreatic cells, activation of glucose uptake and effective decrease of blood sugar level (murine model of T1D and T2D). However, the molecular mechanisms behind these observations have not been understood yet. Concluding, the nature of endogenous ligands to GPR119, as well their potential physiological role in direct regulation of insulin secretion, requires an explanation.

The following study is devoted to preliminary in vitro investigation of novel analogues of LPC, possessing probable prolonged activity, in terms of regulation of the GPR119-related cellular signaling and their hypothetic therapeutic properties. The presented results concern LPC analogues originally synthesized at the Institute of Technical Biochemistry, Lodz University of Technology, Poland.

References:

Cystic fibrosis (CF) is characterized by impaired secretion of exocrine glands and affects many organs, especially the digestive and respiratory systems. The glands produce thick mucus, which provides an ideal environment for continuous development of respiratory infections, especially by Pseudomonas aeruginosa and Staphylococcus aureus, leading to progressive lung damage and respiratory failure, and ultimately to death [1]. The cause of the disease is the presence of mutations in the gene responsible for the synthesis of CF transmembrane conductance regulator protein (CFTR) [2].

Oxidative stress is defined as an imbalance between oxidant and antioxidant processes in favor of the former, what leads to excessive production of reactive oxygen species (ROS) and, consequently, damage to biomolecules. Imbalance in oxidative processes and antioxidant underlies many diseases, including CF. Severe oxidative and nitrosative stress and inefficient removal ROS increases pathological inflammation in patients with CF [3]. The presence of the defective CFTR contributes to the imbalance in the processes of the epithelial cells and extracellular fluids, and excessive synthesis of ROS [4].

This study was aimed at characterization of the relationship between inflammatory and oxidative stress components in exhaled breath and blood in patients with respiratory diseases and control subjects. We evaluated the oxidative stress markers in blood (the total antioxidant capacity (TAC), carbonyl and sulfhydryl group content) and in the exhaled air (superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase, as well as plasma level of advanced oxidation protein products (AOPPs) and advanced glycation end products (AGEs). In addition, concentration of nitric oxide (NO) was measured in the exhaled air from the lower and upper respiratory tract of pediatric patients with cystic fibrosis.

We observed elevated levels of carbonyl groups content, AGEs and AOPP level in plasma of CF patients compared to control plasma. In addition, we have shown that the level of antioxidant enzymes in the blood (catalase, glutathione S-transferase and superoxide dismutase) is also increased. We noted increased levels of Amadori products in CF patients. The concentration of nitric oxide in the airways did not differ between patients with CF and healthy children.

References:

Heavy metal ions are environmentally persistent toxins. There are three main reasons for their high toxicity in living organisms: 1) high affinity for thiol-, histydyl-, amino- and carboxyl groups of amino acid residues in proteins, 2) effect on the antioxidant potential of cells, 3) the modification of the activity of enzymes. The aim of the study was to determine changes in activity and expression of γ-cystathionase (CST) and 3-mercaptoppyruvate sulfurtransferase (MST), enzymes participating in the non-oxidative metabolism of cysteine, in liver and kidney of frog Pelophylax ridibundus and Xenopus tropicalis exposed to lead (28 mg/l) and mercury (1,353 mg/l) ions during 10 days. Liver and kidneys are characterized by high activity of sulfurtransferases as compared to brain or muscles. In kidney, in response to lead ions an increased activity of rhodanase, CTH and MST was detected. In contrast, exposure to copper ions leading to reduced activity of CTH and MST and consequently had the effect of lowering the level of sulfane sulfur. The expression of CTH gene was comparable to the control group while the expression of MST gene increased in animals exposed to lead ions. Mercury ions, had no effect of the expression of MST. In liver, after exposition to lead and mercury ions the expression of CTH was not changed in comparison with the control group. The expression of MST was lower after exposition to mercury ions. Further studies will be conducted to clarify the mechanism of reduced activity and effects of heavy metals on the expression of the investigated enzymes.

Mutations in the gene encoding human mitochondrial polymerase γ

Human mitochondrial DNA (mtDNA) is a circular double-stranded molecule approximately 16 500 base pairs in size and encodes 13 polypeptides that are subunits of respiratory chain complexes, 2 ribosomal RNAs and 22 types of transfer RNAs. Almost all of the proteins necessary for mitochondrial function and mtDNA maintenance are nuclear-encoded and transported into the mitochondria (Sharer, 2005).

Mitochondrial polymerase γ is a heterotrimer and consists of a catalytic subunit (POLG) and 2 accessory subunits (POLG2). The catalytic subunit is divided into 3 domains: an exonuclease proofreading domain and a polymerase domain separated by a linker region which plays a role in DNA binding and processivity through its contact with accessory subunits (Hudson & Chinnery, 2006; Szczepanowska & Foury, 2010). DNA replication errors are caused by the selection and insertion of an incorrect nucleotide, failure to proofread the misincorporated nucleotide, and subsequent extension of the mispaired 3’ terminus (Graziwecz et al., 2004). Additionally POLG pathogenic mutations can cause mtDNA replication stalling. Little is known about the fate of stalled mtDNA replication forks, but they are suggested to lead to double-strand breaks, whose repair processes can in turn result in mtDNA rearrangements (Kirshnan et al., 2008).

In our group we perform molecular diagnosis of mitochondrial syndromes such as chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome and Pearson syndrome. This is a group of multiorgan disorders affecting especially post-mitotic tissues with a high energy requirement, in particular skeletal muscle and nerves and may be caused by the accumulation of multiple mtDNA deletions. We present results of POLG sequence analysis in patients with confirmed multiple mtDNA deletions or strong clinical indications of a depletion syndrome.

Acknowledgements

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References:

P2.11

The neuroprotective effects of deferoxamine in newborn rats exposed to anoxia

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Iron, released from heme and ferritin and deposited in the brain, contributes to neurodegeneration in asphyxiated newborn. It is known that hypothermia provides neuroprotection, then newborn mammals, showing spontaneously reduced body temperature, might avoid the iron-mediated neurotoxicity. Moreover, iron chelation with deferoxamine reduces the hypoxia/ischemia-induced brain lesions. Its action mechanism is based mainly on inhibiting the Fenton reaction. Therefore, we decided to verify the role of iron during the hyperthermia-induced oxidative stress on the base of evaluation of oxidative stress extent at control and anoxic animals administered with deferoxamine (DFO).

Two days old newborn rats were exposed to anoxia in 100% nitrogen atmosphere. Their rectal temperature was kept at 33°C (typical of rat neonates), or elevated to the level typical of febrile (39°C) adults. Control rats were exposed to atmospheric air in the respective thermal conditions. Saline solution or DFO were injected subcutaneously twice: immediately after exposure to anoxia or normoxia in the hyperthermic conditions and 24 h later. After two weeks the level of lipid peroxidation products and activities of antioxidant enzymes were determined in brain homogenates. Febrile body temperature, which amplifies cerebral hyperferremia, induces oxidative changes in the brain. On the other hand, the protection against the brain harmful oxidative processes can be achieved by both the reduced physiological neonatal body temperature and postasphyxic DFO administration.

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P2.12

To die or not to die, that is the question — analysis of cell death pathways in Leber’s hereditary optic neuropathy

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Leber’s hereditary optic neuropathy (LHON) was the first human pathology to be associated with a mitochondrial DNA point mutation. The clinical phenotype of LHON is the degeneration of retinal ganglion cells (RGCs) and a progressive degeneration of the optic nerve. In contrast to the pleiotropic phenotypes observed in other mitochondrial diseases, in most patients affected with LHON the only symptom is vision loss. LHON has a markedly reduced penetrance with a clear gender bias. Approximately 50% of men and approximately 10% of women harbor one of the three primary pathogenic mutations developing visual failure. 95% patients with LHON carry one of three mutations in mtDNA — 11778/ND4, 3460/ND1 or 14484/ND6. The remaining cases are due to rare mutations in different genes encoding subunits of complex I of the respiratory chain. The pathogenic processes leading to optic nerve atrophy are largely unknown. One of the most common hypotheses which explain the retinal ganglion cell death is that mtDNA mutations increase the level of apoptosis in this tissue. Moreover, other types of cell death could be also involved in retinal ganglion cell degeneration in LHON, for example autophagy. In this study we present the results of apoptosis and autophagy analysis in different cells lines derived from patients with LHON diagnosed in Poland.
P2.13

Neurochemical laterality in alcohol intoxication

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Aim of this study was investigate particularity of amino acids and neurotransmitters pool, their derivatives and same enzymes of its metabolism in symmetric parts in of the brain in experimental alcohol intoxication. Way for calculation of laterality index (Li) was propose for determination scale of laterality of biochemical compounds in symmetric anatomical structures. Experiments demonstrates, that during acute alcohol intoxication (AAI) was observed dose dependents effects. Maximum inversion of Li noted in rats with AAI in dose 1 g/kg b.w., exposition 1h. Intermittent alcohol administration (4 day’s alcohol+3 day’s withdraw-al) maximum transform amino acids and biogenic amines pool. In the same time changes of Li was observed in case of 2 cycle of relapse. Li of activity of alanine aminotransferase, aspartate aminotransferase and gammaglutamyltranspeptidase are stable in all case of alcoholisation.

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P2.14

Impact of the joint introduction of lead acetate and ethanol on the levels of neurotransmitters in the midbrain of rats

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Ethanol and lead can increase the effects of each other at the joint admission, is particularly strong in this case chang-es the activity of enzymes sensitive to lead (Sajitha GR et al., 2010, Indian J Clin Biochem 25: 280–288). Their joint introduction activates production of free radicals. In the liver and kidneys is reduced level of GSH and protein-free SH groups, as well as the level of vitamin E and C. There was a decrease in activity ALAD in the blood and increased levels of ALA in the urine. The above changes were the sum of independent effects of ethanol and lead (Jurczuk M et al., 2007, Food Chem Toxicol 45: 1478–1486).

To study the effects of a coadministration of lead and ethanol on the level of biogenic amines and neuroactive amino acids in the rat striatum and midbrain, we performed an experiment in which animals one time intraperitoneally were injected lead acetate (150 mg/kg) then for 10 days was administered ethanol (4.5 g/kg). The second group of animals received ethanol without prior introduction of lead. After 10 days animals were decapitated, in the striatum and midbrain by HPLC were determined levels of biogenic amines and neuroactive amino acids such as aspartate, glutamate, glycine, taurine and GABA.

On a midbrain after ethanol administration significantly in-creased the level of metabolite of dopamine (DA) - homovanillic acid (HVA) so that the index of turnover of dopamine (DA/HVA) decreased, prior single injection of lead acetate prevented these changes and caused the decrease of the content level of norepinephrine. At separate introduction of ethanol, there was a decrease in the level of 5-hydroxytryptophan, 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT). This increases the ratio 5-HT/5-HIAA, indicating weakening of 5-HT turnover, can be caused by decrease in level tryptophan. Joint effects of ethanol and lead in midbrain serotonergic system showed the same changes as at introduction of one ethanol.

Significant changes in levels of neurotransmitter amino acids both at separate and at joint introduction of ethanol and lead did not happen.

Conclusions. The intake of lead acetate and subsequent subchronic administration of ethanol by the animals leads to additional changes in the levels of metabolites and precursors of biogenic amines (NE, Trp). Moreover, has an impact on ethanol-induced changes in the midbrain, due to the normalization of dopamine turnover, which are increased when, administered ethanol.
Lead is a cumulative poison that accumulates in the tissues, primarily the bone marrow, spleen, and liver. Lead cations cause negatively effects on the cells metabolism, matrix synthesis and energy balance. The toxicity of lead compounds is determine by ability to interfere in the divalent cations metabolism, which resulting in numerous biochemical effects. The lead cations of, along with other heavy metals react with SH-groups of different macromolecules, enzymes and structural proteins, as well as some peptides and amino acids in the first place. Violation of intracellular Ca2+ homeostasis, due to Pb2+ competition for Ca2+-channel plasma and reticulum membranes. Chronic lead poisoning has a negative impact, especially on the central nervous system cells and the immune system, thereby affecting the metabolism of the whole body.

The aim of the study was to study the effect of lead poisoning on the exchange of amino acids in the spleen, one of the most important organs of the immune system, and also lymphocytes separated from the spleen. During 30 days female rats weighting 140–160 g were administrated intragastrically the lead acetate at a dose of 30 mg/kg. Determination of free amino acids in perchlorate extracts of spleen and dialyse lymphocytes performed by the reversed HPLC, using chromatographic system Agilent 1100. Chronic intake of the lead decreased the total content of proteinogenic amino acids (from 25764±476 to 22233±1194 nmol/g) in the spleen. Reduces of asparaginic acid (14.8%), asparagine (17.7%), glutamine (25.6%), threonine (34.6%) and phosphoethanolamine (20.1%) was observe.

In the lymphocytes isolated from the spleen, no significant changes in the structure of amino acid fund was not observed, however, the total amount of BCAA (leucine, isoleucine, valine) from 2.1±0.24 to 2.8±0.18 nmol/10^6 cells increased significantly. Thus, the observed changes in the spleen tissue are different from those in lymphocytes and it is probably due to diverse cellular composition of tissue, which includes not only the cells of the immune system, but red blood cells, as well as stromal proteins and fibroblasts. Changes in the spleen take place in the pool of proteinogenic amino acids, and membrane phospholipids, which indirectly indicates a possible change in the functioning of the spleen as an immun organ.

**P2.16**

The selected parameters of hemostasis in children with hypersensitivity type I

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Hypersensitivity type I is a condition of the immune system, in which the antigens recognized by IgE, are bound to receptors on the mast cells and basophiles and consequently cause clinical symptoms known as allergies.

The von Willebrand factor (vWF) is a protein synthesized in endothelial cells and megakaryocytes. The main function of vWF is binding clotting factor VIII, and binding to the platelet glycoproteins and subendothelial matrix during the primary homeostasis. The elevated concentration of vWF is observed in the course of physical and mental effort, in times of stress, depression, inflammation, cancer and atherosclerosis, but there are no unambiguous reports in the literature about the correlation between vWF and hypersensitivity type I in children.

In the experiment were analyzed following parameters of haemostasis: concentration of von Willebrand factor (vWF:Ag), activity of von Willebrand factor (vWF:CBA) and concentration of factor VIII. The concentration of C-reactive protein (CRP) was also determined. The citrate plasma was collected from 20 children with allergy and 20 healthy children (control group). As the standard was used pooled citrate plasma. The concentration of all these parameters was determined using ELISA. Differences between groups were analyzed with t-test. The mean concentration of vWF in children with an allergy was 206.3%, which was twice as high as in the plasma of healthy children, which was 104.4% (the norm: 50–150%). The mean activity of vWF in children with an allergy was 214.7%, whereas in the control group — 99.1% (the norm 50–150%). The differences between both parameters were statistically significant. The concentration of factor VIII in children with an allergy was determined at the level of 73.9% and at 68.5% in healthy (the norm 50–220%), and the differences were not statistically significant. CRP levels in children with an allergy was 448.1 ng/ml and in the control group — 61.26 ng/ml (the norm < 5000 ng/ml).

The studies show that in children with type I hypersensitivity content of vWF was high and its activity was abnormally elevated. It is connected with the stimulation of endothelial cells in the inflammation. However, there was no increase in levels of CRP — observed level was normal in the children, similarly like coagulation factor VIII. While CRP is considered a marker of inflammation, in this case its the level of vWF that shows high activity of the immune system. For this reason VWF is often classified as an acute phase protein and a marker of inflammation.
Multiple sclerosis (MS) is a demyelinating immune-mediated disease of the central nervous system, in which neurons, as well as oligodendrocytes and microglia are included in the demyelinating process. In this autoimmune disease, the oxidative stress plays an important role due to moderate of leukocyte migration, condisc of oligodendrocyte damage and axonal injury and what’s more the activation of haemostasis. In MS a physiological process that ensures the liquidity circulating and the efficient inhibition of bleeding after cessation of the continuity of the walls of blood vessels — haemostasis are disturbed. Understanding the causes of haemostasis disturbances and mechanisms of blood platelet hyperactivity may bring benefits in prophylaxis and treatment MS and variety of autoimmune neurological diseases, which are the highest risk of death.

Blood platelets are multiresponding cells, with respect to the different number of physiological compounds, including collagen, ADP and thrombin. Activation of blood platelets, induced by various agonists, manifested as their secretion, aggregation and activation in inflammatory process and cancers.

In the present study we focus our attention on the first step of platelet activation — the platelet adhesion in vitro to collagen and fibrinogen. During blood platelet activation stimulated by an agonist, a signal is transmitted into the platelets that produce special biochemical mediators, including reactive oxygen/nitrogen species (ROS/RNS). ROS/RNS may act as signaling molecules — second messengers — and may control platelet functions. The present work was also designed to study the production of $O_2^{-}$ in blood platelets in vitro. Adhesion of blood platelets to fibrinogen and collagen type I was determined both, in resting blood platelets and in thrombin-stimulated platelets. Generation of $O_2^{-}$ in resting platelets was measured by cytochrome c reduction.

The recent studies have shown that the platelet adhesion to collagen and fibrinogen was significantly higher in MS patients than in the healthy people. Moreover, we observed that stronger generation of $O_2^{-}$ takes place in blood platelets obtained from MS patients. Our study confirm that MS is responsible for generation of oxidative stress mediators in blood platelets and supports the importance of free radicals in platelet functions, including adhesion process.
Cardiovascular risk factors in inflammatory processes of the lung

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Cardiovascular risk factor, such as hypercholesterolaemia, smoking, diabetes mellitus, inflammatory mediators or cytokines, factors related to coagulation and fibrinolysis and hyperhomocysteinaemia, are closely related to the development of atherosclerosis. Several experimental and clinical studies support the notion that oxidative stress plays a significant role in this process. Over the past decades, some novel markers of cardiovascular risk have been identified. There are a lot of experimental data on the effect of inflammation on atherogenesis. However, the exact mechanisms of this process are unknown. De Chiara et al. showed that total cardiovascular risk factors burden is associated with increased total cystein levels. They are reports of increased total cystein levels in patients with vascular diseases, and that cystein is a stronger marker of endothelial dysfunction [1]. It is well-known that homocysteine and cysteine react via redox pathways in the extracellular space, and their reduced, oxidized, and protein-bound forms participate in the dynamic system known as redox thiol state. They play an important role in the connection between environmental influences and progression of detrimental changes associated with the presence of cardiovascular risk factors.

Cystein effects endothelium-dependent contraction by generating O₂−, which rapidly inactivates the endothelium-derived relaxing factor — nitric oxide (NO). Furthermore, in the experimental setting, auto-oxidation of homocysteine was found to be dramatically accelerated by the presence of either cystein or cystine [1].

We carried out measurement of cysteine and homocysteine in the blood plasma (using the HPLC) in patients with inflammatory processes in the lungs (pneumonia and tuberculosis). It was found that a statistically higher level of cysteine in both groups of patients (561.7±177.5 mmol/L (pneumonia), 514.1±117.5 mmol/L (tuberculosis), against — 418.3±95.3 mmol/L in the control group, p<0.0001 and p 0.001, respectively). The obtained data may suggest that the substantial inflammatory processes may potentiate the process of atherogenesis is not only due to the pro-inflammatory factors [2], but also due to the activation of prooxidant processes in the formation of which sulfur containing amino acids play a key role.

References:

Hyperhomocysteinemia — an important factor in the formation of metabolic syndrome and hypertension

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The role of hyperhomocysteinemia in the pathogenesis of metabolic syndrome is not fully established. Pravenec M et al. have shown that if the spontaneously hypertensive rat fed a folate-deficient diet observed increase in plasma total homocysteine concentration and impaired glucose tolerance. That is developing metabolic syndrome and also increased systolic blood pressure. In addition, the low-folate diet was accompanied by significantly reduced activity of antioxidant enzymes and increased concentrations of lipoperoxidation products in liver, renal cortex, and heart [1]. These work demonstrate that the the spontaneously hypertensive rat is susceptible to the adverse metabolic and hemodynamic effects of low dietary intake of folate (B9). It is known the main manifestation of a lack of folate is hyperhomocysteinemias.

The main objective of our study was to determine the level of homocysteine in patients with arterial hypertension and correction capabilities of its level with the additional intake of vitamins.

Patients (~30 years old) with hypertension has been found elevated levels of total homocysteine — 9.79±0.35 (7.7 – 12.5) mmol/L, in the control group — 6.83±1.81 mmol/L (p 0.05). Supplementing with vitamin B9 and B12 within 30 days at a dose equivalent to the daily needs of significantly reduced hyperhomocysteinemia in patients (7.13±0.29 mmol/L, p<0.05). In addition there was a decrease levels of cholesterol, triacylglycerols, LDL, glucose and increase HDL in blood, but these changes were not statistically significant.

These findings demonstrate that, taking vitamins involved in the catabolism of homocysteine, is essential for prevention and treatment of patients with hypertension and metabolic syndrome. Because folate and cyanocobalamin deficiency can promote hyperhomocysteinemias, oxidative stress and multiple features of the metabolic syndrome that are associated with increased risk for diabetes, hypertention and cardiovascular disease.

Reference:
Anti-heat shock proteins antibodies as a risk factor of ischemic stroke
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Ischemic stroke is the second leading cause of death worldwide. One of the important mechanisms involved in the development of vascular lesions that leads to ischemic stroke is the immune response to heat shock proteins (HSP). This proteins form a ubiquitous, abundant and highly conserved group. HSPs serve as molecular chaperones, aiding in protein folding. They are mostly known as stress proteins because they are overexpressed under various physical, chemical, and biological stresses. Large amounts of HSPs are found accompanying atherosclerotic plaque. Evolutionary conservation has resulted in a high degree of sequence homology between microbial and human HSPs. This can lead to cross-response to bacterial proteins after infection, consequently triggering an autoimmune response towards human HSPs. We determined the antibody levels against bacterial HSPs in blood plasma from patients in acute phase of stroke and from a control group using ELISA technique. The antibody levels were also correlated with several stroke risk factors. Statistical analysis was performed using Statistica software package, version 10 (Statsoft Inc). The data was analyzed with one-way ANOVA, Chi², Pearson's correlation coefficient and multiple regression analysis. In all tests, the value of 0.05 was assigned a significance level (p<0.05). The group of stroke patients had elevated level of anti-hsp antibodies. The difference was statistically significant to the control group. We also observed correlation between some stroke risk factors (previous stroke, hyperthyroidism) and elevated levels of antibodies.

Our results provide strong evidence that level of the anti-HSP antibodies is linked with ischemic stroke. We consider this an argument, that autoimmunity could underlie in the formation of atherosclerosis plaque and lead to stroke.

Cytoprotection effect of oregonindiarlyheptanoid with a course administration of cyclophosphamide
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Diarylheptanoids do not only increase the effect of cyclophosphamide against lymphoma cells, but also, inhibit lipid peroxidation. Oregin administration in vivo increases the speed of activation and cytotoxic activity of NK cells, which are proved the main link of antitumor immunity. This is one of the mechanisms of anti-tumor effect of oregin. In addition, diarylheptanoids including oregin have antioxidant activity.

The aim of the study was to investigate the protective effect of oregin on amino acid-protein metabolism with a course administration of cyclophosphamide. Animals were injected cyclophosphamide intraperitoneally at a total dose of 160 mg/kg, oregin — intragastrically throughout the experiment at a dose of 5 mg/kg. The animals were decapitated 24 h after the last injection of the drugs. Free amino acids in plasma were determined by HPLC the reversed method.

The total number amino acid derivatives, the ratio of non-essential/essential amino acids decreased in blood plasma, but the total number BCAA and the ratio of proteinogenic/amino acid derivatives increase. Citrulline, arginine, taurine, β-alanine, tyrosine concentrations in plasma reduce, but the levels of α-amino butyric acid (1.7 times), leucine (1.6 times), lysine (1.9 times), histidine (2.2 times) and threonine (2 times) increase.

With the co-administration oregin and cyclophosphamide the total number of amino acids and their derivatives, the amount of proteinogenic amino acids, total number nonessential amino acids and the ratio of proteinogenic/derivatives of amino acids in the blood plasma of rats increased. The levels of serine (1.3 times), glutamine (1.4-fold), histidine (2.4 times), fosfoetanolamina (1.7 times), threonine (2.0 times) α-amino butyric acid (1.7 times), lysine (1.9 times) in the blood plasma increased, but the concentration of cysteic acid (50.6%), citrulline (30.7%), arginine (27.1%), β-aminobutyric acid (58.2%) reduce compare to the control group.

Thus, co-administration of cytostatic and oregin reduces the degree of amino acid imbalance in the blood plasma, which is effected in the correction of metabolic disorders caused by the administration of cyclophosphamide.
Mitochondrial nucleotide metabolism — molecular analysis of ANTI and RRMB genes

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The maintenance of a mitochondrial DNA (mtDNA) copy number and integrity is essential for oxidative phosphorylation and is controlled by a complex homeostatic network. All known components of this network are nuclear-encoded and are involved in mtDNA replication and repair or in a mitochondrial nucleotide metabolism, providing a balanced nucleotide pool for mtDNA synthesis which is independent from the cell cycle (mtDNA is continuously turned over) [1].

MtDNA replication rate, processivity and fidelity are highly dependent on mitochondrial nucleotide pool availability. The mitochondrial nucleotide metabolism is based on a nucleotide salvage pathway and transport of nucleotides and their precursors from the cytosolic pool. De novo synthesis takes place only in the cytosol — it is performed by ribonucleotide reductase (encoded by the RRMB gene) which is responsible for mass nucleotide production during the S phase of the cell cycle [2]. Cytosolic and mitochondrial nucleotide pools are separated by the inner mitochondrial membrane but nucleotide exchange is enabled by the activity of specific mitochondrial protein carriers. One of the most abundant components of the inner mitochondrial membrane are proteins of the adenine nucleotide translocator family. One of these proteins is isoform 1 (encoded by the ANTI gene), which is specific for skeletal muscle and heart [3]. Mutations of these genes cause mitochondrial genome destabilization. They result in general decrease of mtDNA copy number (depletion) or qualitative sequence changes, e.g. accumulation of multiple deletions of mitochondrial DNA. Insufficient ATP production, caused by mtDNA defects, is a characteristic feature of mitochondrial DNA. Mutations of these genes cause mitochondrial genome destabilization. They result in general decrease of mtDNA copy number (depletion) or qualitative sequence changes, e.g. accumulation of multiple deletions of mitochondrial DNA. Insufficient ATP production, caused by mtDNA defects, is a characteristic feature of mitochondrial DNA. Mutations of these genes cause mitochondrial genome destabilization. They result in general decrease of mtDNA copy number (depletion) or qualitative sequence changes, e.g. accumulation of multiple deletions of mitochondrial DNA. Insufficient ATP production, caused by mtDNA defects, is a characteristic feature of mitochondrial DNA. Mutations of these genes cause mitochondrial genome destabilization. They result in general decrease of mtDNA copy number (depletion) or qualitative sequence changes, e.g. accumulation of multiple deletions of mitochondrial DNA. Insufficient ATP production, caused by mtDNA defects, is a characteristic feature of

References:

Analysis of concentration of sphingosine-1-phosphate in patients with chronic kidney disease, depending on the renal replacement therapy

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Introduction: Chronic kidney disease (CKD) is a progressive loss of kidney function, resulting from damage or reduction of the number of active nephrons, destroyed by disease processes in the renal parenchyma. CKD requires the implementation of renal replacement therapy (peritoneal dialysis, hemodialysis, renal transplantation). Sphingolipids play an important role in the development, physiology and pathogenesis of many kidney diseases. Sphingosine-1-phosphate (S1P) influences the processes involved in cell survival, migration and inflammation, so it can be assumed that it is closely involved in the process of CKD. The role of S1P in the pathophysiology of renal function has not yet been fully elucidated. It is known that expression of S1P1 receptor in the kidney plays an important role in maintaining the integrity of the endothelial cells and lymphocytes circulation. Fingolimod — one of the immunosuppressive drugs used after transplant, is S1P receptors agonist. Sphingolipids can also be potential indicators of kidney damage or kidney graft function. Recent reports indicate that bioactive lipids play a key role in the regeneration of damaged or transplanted kidney.

Aim of the study: Comparison and analysis of concentrations of basic biochemical parameters and sphingosine-1-phosphate in patients with chronic kidney disease, depending on the renal replacement therapy.

Materials and Methods: The study group consisted of patients of Department of Nephrology, Transplantology and Internal Diseases, PUM, with CKD in stage IV and V — hemodialysed (30), on peritoneal dialysis (30) and during the conservative treatment (30). Peripheral blood samples were collected from patients from all groups. Biochemical analyses of the samples were carried out using spectrophotometric methods and the concentration of S1P was measured using RP-HPLC.

Results: Statistically significant differences were observed in the mean concentrations of studied biochemical parameters between patients on hemodialysis (HD) before and after intervention. Most parameters, such as cholesterol, LDL, phosphorus, Ca x P, achieved a higher average concentration in HD patients group after intervention, while the remaining parameters, i.e. creatinine, uric acid, and TAG — in HD patients before intervention. The highest average concentration of S1P was obtained in patients on peritoneal dialysis (DO) (83.83±18.99 mg/dL), while the lowest in the group of treated conservatively (58.06±20.38 mg/dL). The average concentration of S1P in HD patients before (71.52±19.86 mg/dL) and after (77.83±26.48 mg/dL) treatment was similar. Statistically significant differences were found between patients from DO group and conservatively treated (p=0.0002), and between HD after intervention and conservatively treated (p=0.003).

Applications: 1) Haemodialysis significantly affects the concentration of all lipid and biochemical parameters, what is associated with impaired homeostasis due to intervention.
2) Higher concentrations of S1P in HD and DO patients, compared to patients treated conservatively, indicate the activation of cellular sources of this lipid as a result of activation of coagulation system and increased oxidative stress.

**P2.25**

**Correction of amino acid imbalance in liver lymphocytes caused by alcohol intoxication with aminozol "tritarg"**

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The primary effect of alcohol on the cell membrane is proved to be alcohol-membranotoxicity action revealed in the membrane fluidization. Effect of ethanol on the membrane is determined by its interaction with membrane structures and it is not specific. Oxidative stress develops in the metabolism of alcohol also causes damage to biological membranes and subsequent death of cell. The consequence of these toxic and metabolic effects of ethanol is the change in the functioning of key transport and metabolic pathways, including those which insure the need of the cells in amino acids. The most active amino acid metabolism occurs in the cells of the immune system, where amino acids are used for with biosynthetic (synthesis of mediators) and energy (glutamine) aims. A number of amino acids - taurine, arginine, tryptophan, have immunomodulatory properties. The effect of zinc supplementation of on the immune system has been proved.

The aim of this study was to study the influence of minizol "tritarg" (arginine, taurine, tryptophan and zinc aspartate) on the metabolism of free amino acids in the liver lymphocytes of rats after a course of ethanol administration.

Investigations were carried out on rats, the first 7 days the experiment rats were treated intragastrically to ethanol - 7.5 g/kg, and during 6 days to ethanol 5 g/kg every day and "tritarg" — 350 mg/kg. Determination of free amino acids in liver lymphocytes dialysates perchlorate extracts was performed reversed HPLC.

The administration of ethanol reduces in liver lymphocytes the total number nonessential (from 63.2±14.06 to 32.5±4.51 nmol/10⁶cells) and essential amino acids (from 29.2±5.88 to 15.1±1.64 nmol/10⁶cells), sulfur-containing amino acids (from 20.1±4.77 to 8.6±1.33 nmol/10⁶cells), the number of BCAA (from 11.7±2.28 to 5.1±0.62 nmol/10⁶cells). Co-administration of ethanol and minizol normalizes total amino acid content in lymphocytes, thus the number of essential and nonessential amino acids and BCAA increases. Thus, the administration of "tritarg" has a protective effect against alcohol intoxication on the metabolism of amino acids in the lymphocytes are isolated from the liver, which is likely to have significance for the development of the inflammatory process in the tissue induced by ethanol.
The aim of this study was to elucidate ability to correct the disturbance of free amino acids pool induced by ethanol intoxication by administration of the solution, containing branched-chain amino acids (BCAAs) and taurine. Chronic ethanol intoxication (ChAI) was simulated by substituting the drinking water with ethanol solution (20%) during 14 weeks, average daily ethanol consumption registered — 8 g/kg of body weight. During last 7 days of alcoholization one of the groups of rats received solution of BCAAs and taurine (500 mg/kg) intragastrically. The levels of amino acids and their derivatives were assayed using HPLC with fluorescence detection after derivatization with o-phtalaldehyde and FMOC-chloride. ChAI lasting 14 weeks decreased levels of glycine, methionine, histidine and 3-methylhistidine and increased levels of tyrosine and glutamate in blood plasma. The ratio of the total levels of BCAAs to aromatic amino acids (AAAs) was also reduced by ChAI. Administration of BCAAs and taurine solution decreased levels of aspartate, proline and tryptophan, increased levels of serine, histidine, citrulline, tyrosine, α-aminobutyrate, ethanolamine, leucine, sulfur-containing compounds (methionine, cystathionine, cystine and taurine). Levels of glutamate, histidine and 3-methylhistidine, changed by ChAI, normalized after administration of composition. Rise of arginine concentration may be also considered as beneficial effect of the composition. Among the indices of amino acids pool the normalisation of BCAA/AAA levels worth to notice, as well as increased portion of essential amino acids in total pool of proteionogenic amino acids. Discriminant analysis revealed that tyrosine, glutamine, cystathionine, ethanolamine and threonine was most significant (F > 4) meaning that its levels specifically determine similarity between the action of the opiates and symptoms of the ASDs. It has been proven that children who suffer from autism have increased the levels of opioid peptides, which are occurred in the central nervous system in humans. It was found that appearance of β-casomorphin-7 in the diet is leads to development of many diseases and psychological disorders. In significant number of people who suffer from autism is application of milk-free and gluten-free diet lessens clinical symptoms of ASDs. All experiments were approved by the Bioethics Committee. For the research we used the serum and urine of the children with diagnosed ASD. The samples were taken from Regional Children's Specialized Hospital, Olsztyn, Poland. The aim of this study was to demonstrate the content of β-casomorphin-7 in the serum and urine of children, who suffer from autism. Samples of serum and urine where tested for the presence of β-casomorphin-7 by ELISA test. We observed elevated levels of β-casomorphin-7 in serum in children with diagnosed ASD. We also demonstrated presence of β-casomorphin-7 in urine of children with ASDs. Opiate dependence and autism disorders in children should be confirmed by further studies.
**P2.28**

**Blood ALDH1 and GST activity in diabetes type 2 and its correlation with glycated hemoglobin**

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There is an increasing evidence that oxidative stress (OS) plays a major role in the pathogenesis of diabetes mellitus (DM) and the development of its complications [1]. The results of OS in the blood of DM patients are observed not only in serum but also in erythrocytes where the activity of enzymes involved in the antioxidant defense mechanism is altered. As one of the consequences of OS is increased lipid peroxidation (LP), the aim of our studies was to check, how the activity of two enzymes involved in the detoxification of aldehydes formed during LP [2], glutathione S-transferase (GST) and aldehyde dehydrogenase 1 (ALDH 1) is changed in patients suffering from DM. GST and ALDH1A1 activities were determined in whole blood samples from DM type 2 patients (n=64) and healthy controls (n=60) using spectrophotometer (for GST activity) and fluorometer (for ALDH1 activity). Additionally HbA1c, serum glucose level and CBC were measured. GST and ALDH1A1 activities were found to be significantly increased in diabetics when compared with healthy control (p<0.05). Moreover, the enzymes activities were higher in uncontrolled (HbA1c≤7.5%, n=47; p<9.0%, n=12). The increase of ALDH1A1 and GST activity in DM seems to be associated with severity of the disease and might be a compensatory effect against oxidative stress.

**References:**


**P2.29**

**Aminotransferases and amylases activity in children with atopic dermatitis**

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For the majority of patients with atopic dermatitis (AD) disorders of the digestive system are typical, which not only support the allergic inflammation in the skin, but can also be caused by it. Therefore, patients with AD need a timely complex examination of the functional state of the gastrointestinal tract (GIT), followed by a rational therapy. Aim: assessment of alanine aminotransferase (AlAT), aspartate aminotransferase (AsAT) and amylase in children with atopic dermatitis.

Material and methods: The analysis of transaminases and amylase in 90 patients in the allergological department of the children's hospital in Grodno in age from 1 month to 17 years. The level of the enzyme was determined by kinetic methods using reagent kits "Analysis Plus" on a biochemical analyzer BS-200. For the normal levels in children taking the following values: AlAT 0–40 U/L, AsAT 0–40 U/L, amylase 0–80 U/L. Statistical analysis of the results using a non-parametric U Test Mann-Whitney. Results: Associated pathology in 46 (51.1%) of the patients (I group) was presented by digestive diseases, 30 (33.3%) — other allergic diseases (allergic rhinitis, bronchial asthma) — II group and in 14 (15.6%) occurred concomitant allergic and digestive pathology (III group). Biochemical Analysis of the data showed that the activity of the enzymes (U/L) in all patients is within the reference values. Statistically significant differences between the groups were revealed representative only amylase level (p I–III<0.05), whose activity was higher in patients with concomitant disorders (48.52±21.36) as compared with isolated disease of the digestive system (33.40±18.89) and other allergic diseases (37.50±13.33). AlAT level was higher in group I (31.37±15.66) compared to II (25.97±10.05) and III (23.53±9.74) groups. AsAT activity was 37.50±13.33 in group I; 34.00±9.74 — in II and 32.21±8.27 — in groups III. The results of these studies allowed us to formulate the following conclusions: 1. In majority of the patients (67%) with atopic dermatitis digestive organs involved in a pathological process. 2. Transaminases and amylase in the study groups was within the reference values. 3. The presence of other allergic diseases in patients with AD and diseases of the gastrointestinal tract leads to increase activity of amylase.
### P2.30

**Influence of paroxetine on levels of tryptophan and its metabolites in plasma and hypothalamus of rats with chronic tryptophan depletion**

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The purpose of our work was to estimate the levels of Tryptophan (Trp) and its metabolites in plasma and brain of rats depleted with Trp, and effect of administration of Paroxetine on the levels of Trp and metabolites. 40 male rats of heterogeneous stock were used. 20 rats received a full-fledged diet within 4 weeks, 20 were subjected to low tryptophan diet. For next 2 weeks both groups were divided into 2 subgroup which were administered intragastrically with Paroxetine 5 mg/kg daily and with water, respectively. Concentrations of Trp, 5-hydroxytryptophan (5-HTP), serotonin (5-HT), 5-hydroxyindolacetic acid (5-HIAA) were determined in plasma and hypothalamus by HPLC with fluorescence detection. Assessment of the significance of interactions were made by ANOVA (LSD-test). We found that Paroxetine treatment in a dose of 5 mg/kg to a full-fledged diet reduced plasma concentration of 5-HIAA, with the Trp, 5-HTP, 5-HT levels being intact. The concentrations of Trp, 5-HTP, 5-HIAA in the hypothalamus increased. The increase of the 5-HTP level could be explained by activation of Trp hydroxylation, and that of 5-HIAA — by increase of a portion of the whole 5-HT available for brain monoamine oxidase (MAO). The concentration of 5-HT remained unchanged. Paroxetine addition in the situation of chronic Trp depletion reduced plasma concentration 5-HIAA, 5-HT, and also level 5-HIAA in hypothalamus that suggest the exhaustion of compensation of Trp metabolism at chronic Trp deficiency leading to decrease of 5-HT production.

### P2.31

**Molecular mechanisms of blood platelet disorders in autoimmune diseases**

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Blood platelets are the main mediators in the initial stages of the blood coagulation process resulting in the arrest of bleeding sites of vascular injury. Besides their well-recognized role in vascular homocostasis, sample data are emerging on the many immunoregulating functions of platelets, indicating crosstalk between the coagulation and inflammation system. Because of extremely high numbers of platelets and their ability to release inflammatory intermediaries, they are preferably positioned to play a sentry role and to provide early signals to immune cells. During autoimmune diseases, platelets are chronically exposed to potent stimuli, resulting in enhanced platelet activation. Blood platelets are multisresponding cells, with respect to the different number of physiological compounds. Activation of platelets induced by various agonists, manifested as their adhesion, secretion and aggregation plays a very important role in the pathogenesis of cardiovascular diseases (CVD). Mounting evidence indicates that CVD events are a main cause of excessive mortality of autoimmune disorders. The underlying mechanism is unknown, but might involve blood platelets and endothelial dysfunction secondary to inflammatory disease activity.

The main aim of the present study was to assess the platelet reactivity to different physiological agonists manifested by platelet aggregation. The purpose of the presented work was to evaluate and compare platelet activation induced by ADP, collagen and arachidonic acid *in vitro* between healthy people and patients suffering from Multiple Sclerosis (MS) and Hashimoto disease. The platelet aggregation induced by ADP — 10 μM, collagen — 2 μg/ml or arachidonic acid — 160 μg/ml, respectively was measured by turbidimetric method using a dual-channel Chrono-log optical Lumi-aggregometer (Chronolog, USA). In our study we have used three different platelets agonists affect different ways of signaling pathways and our results confirmed that platelet obtained from MS or Hashimoto were more sensitive to all tested platelet agonists than platelets from healthy individuals. Increased platelet aggregation in autoimmune diseases clearly pointing to their role in CVD disorders. These insights could provide us with new therapeutic perspectives for not only the increased thrombotic risk observed in many autoimmune diseases, but also for various disease-specific events triggered by platelet activation.
P2.32

Connection between chitotriosidase activity and ischemia-modified albumin (IMA) and markers of inflammation in diabetic chronic hepatitis C patients

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The enzyme chitotriosidase (ChT) is one of the most abundant and indicative proteins secreted by activated macrophages. Serum ChT activity is significantly increased in patients with established atherosclerosis in relation to the severity of the lesion, suggesting a possible role for ChT as a marker of atherosclerosis. Inflammation and oxidative stress have been reported in patients with chronic hepatitis C (CHC) infection, but their influence on chitotriosidase levels and diabetes prevalence remains unknown.

Seventy-four CHC patients, 40 with diabetes, and 45 healthy controls were enrolled in the study. ChT activity was measured by the fluorescence method. Concentrations of oxidative stress markers [ischemia-modified albumin (IMA), advanced oxidation protein products (AOPPs) and Nε-(carboxymethyl)lysine-advanced glycation end products (CML-AGEs)], pro-inflammatory cytokines [interleukin-6 (IL-6) and tumor necrosis factor alfa (TNF-α)], and high-sensitivity C-reactive protein (hsCRP) were assessed.

CHC patients with diabetes showed increased chitotriosidase activity in serum as compared to healthy controls. Moreover, compared with the controls, the CHC patients with diabetes showed a significant increase in plasma concentrations of IMA, TNF-α, and hsCRP (P<0.01). The values of ChT, IMA, AOPPs and TNF-α were more elevated in patients with diabetes than without diabetes (P<0.05). The positive relationships were found between ChT and presence of diabetes (P<0.05), and TNF-α levels (P<0.001). CML-AGEs did not show any significant correlation with ChT, markers of inflammation and presence of diabetes.

In conclusion, we have documented significant elevation in serum activity of chitotriosidase and levels of IMA in CHC patients. In addition, circulating ChT was associated with inflammation markers and diabetes prevalence. This observation suggests a relationship between chitotriosidase and inflammation in CHC patients with diabetes, which may represent one of the mechanisms involved in the accelerated atherosclerosis in this population.

P2.33

Chitotriosidase, chronic hepatitis C and diabetes: a novel triad

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CHC patients with diabetes showed increased chitotriosidase activity in serum as compared to healthy controls. Moreover, compared with the controls, the CHC patients with diabetes showed a significant increase in plasma concentrations of AOPPs, TNF-α, and hsCRP (P<0.01). The values of ChT and TNF-α were more elevated in patients with diabetes than without diabetes (P<0.05). The positive relationships were found between ChT and presence of diabetes (P<0.05), and TNF-α levels (P<0.001). CML-AGEs did not show any significant correlation with ChT, markers of inflammation and presence of diabetes.

In conclusion, we have documented significant elevation in serum activity of chitotriosidase and levels of AOPPs in CHC patients. In addition, circulating ChT was associated with inflammation markers and diabetes prevalence. This observation suggests a relationship between chitotriosidase and inflammation in CHC patients with diabetes, which may represent one of the mechanisms involved in the accelerated atherosclerosis in this population.
Correlation between polymorphism of μ-opioid receptor (A118G) and its gene expression in children with food allergy

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with heterogeneous clinical phenotypes reflecting exposure to environmental factors and a personal genotype. It has been reported, that occurrences of food allergy to cow’s milk proteins may lead to atopy. Additionally, in the majority of patients an IgE-dependent reaction is observed, which is connected with consumption of food allergens. Milk proteins are not only particularly strong allergens, but also source of bioactive peptides including β-casomorphin-7 (BCM7). These opioid peptides are released from the β-casein fractions of milk and may permeate through human intestinal barriers. BCM7 may exert on nervous, digestive, and immune functions via μ-opioid receptors (MORs). These systems are most important in terms of allergies. It has been shown that polymorphism in the μ-opioid receptor gene (A118G) may affect on the bond strength of the various opioids.

The aim of this study was to determine the frequency of allele A and G at position 118 of the gene μ-opioid receptor (A118G) and examined the effect of the polymorphism on MOR gene expression in mononuclear cells isolated from peripheral blood of healthy children (control) and children with food allergy with coexisting atopic dermatitis.

Participation in the study involved 100 people aged from 5 to 18 years. DNA was isolated from blood or epithelium obtained from patients. Afterwards, PCR-RFLP reaction was performed to evaluate polymorphism A118G of the μ-opioid receptor. Subsequently, 20 healthy children and 30 children with atopic disease were selected and blood samples were collected to the study of MOR gene expression. Peripheral blood mononuclear cells (PBMCs) were isolated and incubated with the BCM7 solution and peptide extracts of cows’ milk. The expression of MOR gene was determined with real-time PCR.

The obtained results allowed to demonstrate that the gene polymorphism in μ-opioid receptor may influence susceptibility to food allergy in correlation with the consumption of milk.