O2.1
The prion protein: from monomers to infectious fibrils
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The molecular basis of the prion model and the mechanistic models of the prion amplification are summarized. The infection is a conversion of the host encoded prion protein PrPC from its cellular isoform PrPC into a pathological and infectious isoform PrPSc. The conversion process was investigated by in vitro studies on recombinant PrP. Dimeric and oligomeric PrP conformations were identified as intermediate states. In particular, a monomer-dimer equilibrium of partially denatured PrP was described as the essential step preceding fibrillization. During the last years fibrils could be formed from recombinant PrP, and indeed these fibrils exhibited infectivity; by these experiments the prion model can be regarded today as experimentally proven. Since in vivo PrPC is presented on the outer surface of the cell membrane and has to be detached from the membrane during the conversion process, the interaction of PrPC with raft like lipid membranes was studied by quantitative methods. PrPC can form oligomers on the surface of the membrane. The thermodynamic and kinetic parameters are the basis of the so-called two-phase-model which takes into account the role of the cell membrane in the conversion process of PrPC into PrPSc.

O2.2
Molecular genetics of Alzheimer's disease and frontotemporal dementias — the most common forms of amyloido- and tauopathies
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Aggregates of dysfunctional proteins and peptides in or between brain neurons are key neuropathological features of dementias and are believed to directly cause or substantially contribute to the development of these diseases. Fundamental parts of the mechanisms underlying the dysregulation of proteins in Alzheimer’s disease, frontotemporal dementia and other dementing disorders are now well characterized, mainly due to the discovery of genes causing dominantly inherited disease forms. Alzheimer’s disease (AD), the most common form of dementia, is characterized by two types of brain lesions, referred to as senile plaques and neurofibrillary tangles. A series of endoproteolytic cleavages of amyloid precursor protein (APP) controlled by α-, β-, and γ-secretases leads to a formation of non-amyloidogenic (α-secretase pathway) and amyloidogenic (β-secretase pathway) products which are essential for neurodegeneration. According to “the amyloid hypothesis”, the accumulation of amyloid β (Aβ) peptides in the brain is a primary event in the pathogenesis of AD. One of the strong pieces of evidence supporting this hypothesis was the identification of pathogenic mutations within APP, Presenilin 1 and Presenilin 2 genes responsible for familial autosomal dominant cases of AD. The mutations affect APP processing causing overproduction of Aβ42 alloform. Over 85% of patients can be considered as sporadic late onset AD with more complex mode of inheritance and a multifactorial etiology. Aβ aggregation in sporadic AD may be induced by yet unknown post-translational modifications of Aβ and/or by altered mechanism of Aβ clearance from the cell. Biochemical studies confirm that changes in cholesterol metabolism in neurons may underlie the pathological processes in AD. The APOE*4 allele of Apolipoprotein E gene is a major risk factor in sporadic late onset AD. Frontotemporal dementia (FTD) is characterized by atrophy of the frontal and temporal lobes of cortex and an abundant pathology of tau protein. Tau promotes assembly of microtubules and is involved in neurite outgrowth, axonal transport and cell polarity. In human brain, three isoforms of 3R-tau type and three isoforms of 4R-tau type are produced in ratio 1:1 by alternative splicing of the Microtubule Associated Protein Tau (MAPT) gene. So far, over 30 pathogenic mutations within the MAPT gene have been found in families with autosomal dominantly inherited forms of FTD with parkinsonism.
linked to chromosome 17 (FTDP-17). Most of the mutations change the biochemical properties of the protein and/or alter the regulation of exon 10 alternative splicing leading to disturbances in 4R-tau isoforms production. Moreover, a small subset of ubiquitin-positive FTDP-17 cases can be explained by mutations in the Progranulin (PGRN) gene. A variety of pathogenic consequences of the MAPT and PGRN gene defects could explain the wide range of clinical features observed in different families with FTDP-17.

O2.3

Cellular senescence as a new approach in anti-cancer therapy

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Cellular senescence is a physiological program of terminal growth arrest that can be triggered by alteration of telomeres or by different forms of stress. Senescent cells can not divide even upon mitogen stimulation, but they remain metabolically active and show characteristic changes in morphology such as enlarged and flatten cell shape and increased granularity. Based on the in vitro and in vivo experiments, induction of cellular senescence was shown to play an important role in suppressing tumorigenesis in mouse and human. During the process of carcinogenesis only the cells that are able to bypass cellular senescence acquire fully transformed phenotype. It has now become apparent however, that tumor cells can be forced to undergo senescence by genetic manipulations or by treatment with chemotherapeutic drugs, radiation or differentiating agents. Since cellular senescence leads to inhibition of cancer cells proliferation, it seems to be an attractive approach to anti-cancer therapy. Based on our own experiments we were able to show that treatment of HCT116 human colon cancer cell line with low dose of doxorubicin leads to induction of cellular senescence characterized by morphological and biochemical changes typical for this process like enlargement of cells, induction of p53 and p21 proteins and increased activity of SA-β-galactosidase. Moreover, DNA content analysis revealed that drug-induced senescence of colon cancer cells was correlated with polyploidisation. Although the majority of polyploid cells acquired senescent phenotype few days after doxorubicin treatment, we simultaneously observed rare events of aberrant mitotic cell division of some polyploid cells. The process of defective mitosis gave rise to aneuploid progeny as confirmed by chromosomal spreads analysis. What seems to be particularly important, those aneuploid cells gain the ability to proliferate leading to overgrowth of the population of undividing, senescent cell. Thus we can conclude that induction of HCT116 colon cancer cell senescence is correlated with induction of genetic instability, which can contribute to tumor development thus questioning cancer cell senescence as a promising anti-cancer therapy.
Biochemical bone turnover markers and vitamin D levels in prepubertal vegetarian children

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In general, most children on well-planned vegetarian diets can achieve normal growth and development. However, elimination of animal products from the diet decreases the intake of some essential nutrients, such as calcium and vitamin D and may influence bone metabolism. This is especially important in childhood and adolescence, when growth and bone turnover are most intensive. Biochemical bone metabolism markers which showing global skeletal activity have lately been developed and validated for the assessment the dynamics of bone formation and resorption processes. Among them, products of the osteoblast activity (osteocalcin — OC, bone alkaline phosphatase — BALP), which are markers of bone formation, and products of osteoclast activity (collagen type I C-terminal telopeptide — CTX) markers of bone resorption are considered to be clinically useful. The aim of this study was to investigate the serum concentrations of biochemical bone turnover markers and vitamin D in prepubertal vegetarian children. We examined 50 children on vegetarian and 50 on omnivorous diets aged 2–10 years. In group of vegetarian children there were 28 lacto-ovo-vegetarians, 4 lacto-vegetarians, 5 ovo-vegetarians and 13 vegans. Dietary constituents were analysed using a local nutritional program. Serum bone formation and resorption marker concentrations were determined by specific enzyme immunoassays (ELISA) and 25-hydroxyvitamin D by chemiluminescence method (CLIA). Average daily energetic value and the percentage of energy from protein, fat and carbohydrates in diets were similar in both groups of children and were within the recommended range. We showed about two-fold lower daily intake of calcium and vitamin D in vegetarian diet than in the omnivorous one. Concentration of calcium and phosphate in serum all tested children were within physiological range, but 25-hydroxyvitamin D level in vegetarian children was nearly 2-fold lower compared with omnivores. In vegetarians, as compared to those in non-vegetarians, mean serum concentrations of OC, BALP and CTX were significantly lower by about 20%, 10% and 15%, respectively. Examined vegetarian children were on different kinds of diet, but mean values of bone markers for the vegans and lacto-ovo-vegetarians were not significantly different. Our preliminary results suggest that an inadequate dietary intake of calcium and vitamin D may impair bone turnover rate in children on vegetarian diets. Thus biochemical markers which reflect bone formation and resorption processes should be controlled in order to prevent abnormalities in bone metabolism in these populations.

MALDI tissue profiling/imaging — a new approach for biomarker discovery

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In the field of biomarker discovery, researchers are screening for different classes of molecules. Some groups are employing a proteomic approach with classical separation techniques like 2D-PAGE or 2D chromatographic approaches to investigate peptide or protein levels. Other screening methods can also be utilised, evaluating the metabolite or lipid components. Frequently the difficulty in biomarker discovery is in the sample reproducibility and sample treatment over the whole process, ensuring that detected differences are valid. Also, significant differences must clearly be identified and correlated to a disease or sample type. Fast, simple and reproducible methods are therefore essential when attempting this kind of screening approach. In recent years, MALDI tissue profiling and imaging has come in to focus and gained increasing popularity. The scientist can section the crude tissue sample as a starting material. Instead of intensive sample treatments and fractionation, the analysis can be performed directly from the tissue section after applying the appropriate MALDI matrix. This presentation will explain different techniques developed by Shimadzu and collaborators and shows examples of direct tissue analysis by MALDI mass spectrometry. We will include analysis of various cancer sections and tumour margin zones. These have shown some promising results which will give this field more momentum for the future.
Identification of ecto-nucleotidases in human cerebrospinal fluid

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In central nervous system, ecto-purine and ecto-pyrimidine nucleotides play a role in neurotransmission and neuromodulation of neurons and glia cells. The source of ecto-purines in the CNS is exocytosis from all type of brain cells, endothelial and blood cells. Ecto-purines cooperate with two classes receptors: P1-adenosine receptors and P2-nucleotide receptors. Concentration of ecto-nucleotides and ecto-nucleosides is regulated by soluble exo-enzymes and ecto-enzymes bound to cell membrane. These enzymes are involved in termination of nucleotide signal and may produce other messengers (ADP and adenosine). Previous research showed presence nucleotides and nucleosides in cerebrospinal fluid (CSF) and nucleotide P2X receptor on the surfaces of the cerebrospinal fluid contacting neurons. Also, the activity of ENTPDases was found in the rat CSF, and activity of ecto-adenosine deaminase in human CSF. Additionally, our unpublished results show, that there are number of nuclotidases bound to cell membrane of ependyma. The aim of this research was to identify the enzymes metabolizing purines and pyrimidines in the cerebrospinal fluid. Using the immunodetection and catalytic study of CSF of patients with neurological diseases we detected an activity of soluble adenyate kinase and enzymes which belong to NTPDases and NPPases. Both these hydrolyses require an alkaline pH, while the optimal pH for adenyate kinase is 6.5. NPPases of CSF prefer ATP as a substrate and hydrolyze it to AMP and pyrophosphate. Soluble forms of NPPases are activated by magnesium and strongly inhibited by Ap5A, which is also inhibitor of adenylate kinase. Soluble NTPDases from CSF hydrolyze pyrophosphate bonds of diphosphonucleotides (ADP and GDP). Their catalytic activity requires presence of calcium ions and is partially inhibited by suramine. In CSF we also found a new type of nucleotidase. That enzyme hydrolyzes ATP to ADP, requires either calcium or magnesium ions for activity, is insensitive to Ap5A and has an optimum pH 8.5. Maximal activity of this enzyme was found in CSF of patients with a stroke. Presence of phosphate groups in a products of the above enzyme excludes the participations of NPPases in this reaction. Therefore, it is an NTPDases-like soluble enzyme which prefers triphosphonucleotides as a substrate. Our earlier investigations have showed that CSF of patients with various CNS disorders differ in composition and quantity of purines and pyrimidines. These results suggest that above differences may result from change of exo-nucleotidases activity.

Serum interleukin-12 and interleukin-18 concentrations in patients with oesophageal, gastric and colorectal cancer

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Introduction: Interleukin-12 (IL-12) and interleukin-18 (IL-18) play important role as immunomodulatory factors in cancer pathogenesis. The prognosis of patients with digestive tract cancers, especially oesophageal and colorectal cancer is poor. New biomarkers estimated in serum could be very helpful in diagnosis and monitoring of the cancer progression. The aim of this study was determination of IL-12 and IL-18 concentrations in patients with oesophageal, gastric and colorectal cancer, and determine, whether serum IL-12 and IL-18 can be use as diagnostic markers in these cancers.

Material and methods: Peripheral blood was obtained before treatment from: 34 patients with oesophageal cancer, 28 patients with gastric cancer and 17 patients with colorectal cancer. Control group included 30 healthy individuals. The serum IL-12 and IL-18 concentrations were determined by immunoenzymatic ELISA test. Data were analysed using Mann-Whitney U test.

Results: Medians of serum IL-12 and IL-18 concentrations were significantly higher in patients with oesophageal cancer than in the healthy subjects ($P<0.001$ and $P=0.022$ respectively). Serum IL-12 and IL-18 medians were significantly higher in patients with gastric cancers in comparison with control group ($P<0.001$ and $P=0.0027$ respectively). Differences between concentrations of IL-12 and IL-18 in patients with colorectal cancers were also statistically significant ($P=0.002$ and $P=0.047$ respectively). Conclusions. Concentrations of serum IL-12 and IL-18 are significantly higher in patients with oesophageal, gastric and colorectal cancer than in the healthy subjects. We suggest that IL-12 and IL-18 can be used as markers in diagnosis and monitoring progression of cancer in oesophageal, gastric and colorectal.
O2.8

Gelatinases MMP-2 and MMP-9 from umbilical cord blood in preeclampsia

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Our previous studies have demonstrated that preeclampsia causes a decrease in MMP-1, MMP-3, MMP-2 and MMP-9 content from the umbilical cord artery (UCA). These enzymes and their inhibitors (TIMPs) participate in remodelling of UCA extracellular matrix. They may be involved in the preeclampsia-associated increase in the collagen content in the UCA wall and other fetal arterial vessels. MMP-2, MMP-9 and their inhibitors (TIMP-1 and TIMP-2) play an important role in vascular remodelling and are implicated in pathological fibrosis. Alterations in these enzymes activity may contribute to the pathophysiology of preeclampsia. We have hypothesised that these enzymes and their inhibitors could be present in umbilical cord blood, and that the concentration of these proteins could have effects on both vascular remodelling and vascular reactivity. The effect of preeclampsia on the content and activity of metalloproteinases in umbilical cord blood serum and plasma was elevated by immunoenzymatic methods and zymographic technique. A high amount of MMP-2 (gelatinase A), MMP-9 (gelatinase B), TIMP-1 and TIMP-2 was detected in all investigated fluids. Preeclampsia is associated with a distinct increase in MMP-9 content in umbilical cord blood plasma. The amount of TIMPs is so high enough to inhibit the activity of MMPs. It can be concluded from zymography that MMP-2 (gelatinase A) is present as proenzymatic form in both control and preeclamptic umbilical cord blood. Control umbilical cord plasma does not demonstrate activity of MMP-9 only, even after activation with APMA. High content of investigated metalloproteinases and their inhibitors in the umbilical cord blood may be a result of releasing those proteins from vessel wall cells. It can be concluded that an increase in MMP-9 concentration in umbilical blood, in turn, can alter vascular function in newborns delivered by mothers with preeclampsia.

O2.9

Chronic kidney denervation induces hypersensitivity to agonist of P2X receptors and increases expression of P2X1 receptor mRNA

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Background and purpose: The kidney receives very dense innervations of adrenergic nerves affecting renal haemodynamics and tubular function. ATP has been established as a cotransmitter and as neuromodulator molecule in sympathetic nerves. This purinergic signaling involves P2 receptors, which are structurally and functionally divided into ion channels (P2X receptors) and metabotropic receptors (P2Y1,2,4,6,11-14). The adrenergic and purinergic transmission may affect each other which, in turn, may modify renal function. The aim of the present study was to investigate whether renal denervation modulates the results of activation of P2X receptors.

Experimental approach: The clearance experiments in anaesthetized Wistar rats were performed 7 days after bilateral denervation of the kidneys. The changes in glomerular filtration rate (GFR), renal plasma flow (RPF), sodium and water excretion in urine were investigated during systemic infusion (i.v. 2 µmol/kg + 20 nmol/kg per min) of β,g-methylene ATP (β,g-meATP), the poorly hydrolysable ATP analogue — P2X receptors agonist with greatest potency at P2X1 receptor. For the purpose of qualifications of the possible changes in the level of P2X1 receptor in denervated kidney we examined, by RT-PCR, the mRNA level for P2X1.

Key results: The infusion of β,g-meATP increased RPF and fractional sodium excretion from proximal and distal nephron segments without significant changes in fractional water excretion from distal nephron segments in control group (sham-operated, n=5). The chronic renal denervation (n=6) produced several times greater β,g-meATP-induced diuresis and natriuresis than in control group. The mean arterial pressure and GFR remained unchanged in both group during β,g-meATP infusion. The RT-PCR experiments showed increased expression of P2X1 receptor with relation to reference gene b-actin (0.24±0.02 vs. 0.08±0.04).

Conclusions and implications: Our results indicate that renal chronic denervation leads to hypersensitivity to β,g-meATP, which may be due to, at least in part, increased expression of P2X1 receptors.

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O2.10

Does VEGF165 and sFLT gene transfer prevent the radiation-induced lung fibrosis?

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Ionizing irradiation is associated with an acute inflammatory reaction leading to pulmonary fibrosis, a significant medical problem. The radiosensitivity of the lung limits the dose of radiation, which can be delivered to tumors in the thoracic region. The interaction between VEGF proinflammatory cytokine, the main mitogen for endothelial cells, and its soluble receptor sFLT, specific VEGF inhibitor, may play an important role in lung microvascular restoration during post-radiation inflammatory and fibrotic phases. We examined therapeutic nonviral, intramuscular gene transfer of human VEGF165 and sFLT1, in radiation-induced late pulmonary fibrosis in experimental animals. To investigate its antifibrotic activity we used pVEGF165 and psFLT1 expression vector, constructed in our laboratory. Therapeutic plasmids were previously tested in vitro and in vivo for its ability to cell transfection, gene expression and angiogenic and antiangiogenic potency. C57Bl/6J mice, well documented animal model for thoracic irradiation, were exposed to single dose 15 Gy and randomly divided into three groups, which received intramuscular injection of plasmids as follows (1) control-“empty” plasmid, (2) plasmid encoding VEGF165 and (3) plasmid encoding sFLT. In this study DNA-plasmid was complexed with PEI (Polyethylenimine, 25 kDa) and served in 30 µg per mouse. To enhance the transfection efficiency the plasmid injections were repeated in two weeks intervals, for three months. Plasmid injections in group of animals receiving pVEGF were started two weeks later, because of possibility of its action as vascular permeability factor (VPF) in early post-radiation phase. For histopathologic changes examination some mice were euthanized at 1 or 2 months after irradiation. Remaining twenty four animals were observed, weighted and its survival time was noted. In conclusion, our data demonstrate that sFLT1 derived from plasmid transfection may inhibit endogenous VEGF and increase its survival time was noted. In conclusion, our data demonstrate that sFLT1 derived from plasmid transfection may inhibit endogenous VEGF and increase.

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O2.11

The amino-terminal pro-brain natriuretic peptide in patients treated by maintenance haemodialysis

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The brain natriuretic peptide (BNP) is synthesized and released by cardiomyocytes in response to increased transmural wall stress. It is produced by ventricular myocytes as circulating propeptide (proBNP) and further processed to N-terminal proBNP (NT-proBNP). The increase plasma NT-proBNP concentration may be useful indicator for asymptomatic left ventricular dysfunction. This phenomenon is very important particularly in patients with end-stage renal disease, because heart failure is a leading cause of their death.

The aim of this study was assessing the NT-proBNP plasma concentration and finding the influencing factors on its plasma concentration in patients treated with maintenance haemodialysis (mHD).

Studies were taken on 66 mHD patients (mean duration of HD 47.35±37.12 months), 20 of them suffered from coronary artery disease. HD procedures were performed 3 times a week for 4 hours with polysulphone capillary dialyzers with a regimen of i.v. iron (112.52±15.25 mg per week) and i.v. rhHuEPO (5392.85±3234.35 IU per week). Blood samples were drawn from fasting patients at the start of the second HD of the week. Reference values were obtained from 20 healthy volunteers (HV).

NT-proBNP and soluble transferrin receptor (sTfR) concentrations were assessed by ELISA method. The concentrations of selected hematological variables (RBC, HCT, HGB), total cholesterol, LDL-cholesterol, triglycerides (TG) and creatinine were evaluated by the routine laboratory tests.

In mHD patients plasma concentration of NT-proBNP (152.2±68.6 fmol/ml) and sTfR (1.54±0.84 mg/ml) were significantly higher (P<0.05) in comparison to HV: NT-proBNP (35.6±21.3 fmol/ml) and sTfR (0.71±0.84 mg/ml). The values of RBC, HCT, HGB in serum were significantly (P<0.0001) lower in mHD than in HV group. Total cholesterol, LDL-cholesterol and TG were slightly (with no statistical significance (P=0.07)) decreased in mHD. The positive correlations between NT-proBNP and the presence of the coronary artery disease (r=0.19, P=0.01), duration of HD treatment (r=0.31, P<0.0001) and creatinine serum concentration (r=0.31, P<0.0001) were observed. Plasma concentration of NT-proBNP inversely correlated with total cholesterol (r=-0.55, P<0.0001), LDL cholesterol (r=-0.5, P<0.0001), RBC (r=-0.36, P=0.006) and HGB (r=0.041, P=-0.274). Between NT-proBNP and sTfR, TG no significant correlations were revealed.

Studied patients, especially those with coronary artery disease showed the propensity to the high plasma NT-proBNP concentration. The concentration of this parameter may be influenced by the duration of HD treatment.
and serum creatinine concentration. It is probably because it is cleared from plasma by renal secretion. The inverse correlations between NT-proBNP, selected haematological and lipids parameters suggest that anaemia and malnutrition, atherosclerosis and inflammation syndrome may be another factor influencing NT-proBNP.

**O2.12**

**Cell surface-bound of cathepsin B and leukocyte elastase expression on polymorphonuclear leukocytes in type 2 diabetes**

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Cathepsin B (CB) and leukocyte elastase (LE) are proteolytic enzymes widely distributed among various cells and tissues of the human organism. They are also contained within the azurophilic granules of polymorphonuclear leukocytes (PMN). These proteases play the essential role both in physiological and pathological processes. First of all CB participates in the turnover of proteins, while LE mainly in the non-specific host defence by degradation of engulfed microorganisms or cell debris. The pathological role of LE is mainly manifested in pathogenesis and development of diseases connected with inflammation, and CB — with the carcinoma progression. However both proteases participate in the diabetes development and formation of vascular late complications. The excessive rise in activity of these enzymes, their massive extracellularly release and translocation into cell surface are mainly observed in pathological states. Specific forms of proteases, which are expressed on the cell surface, show some different proprieties, but the most important is increased resistance to the action of endogenous inhibitors. This situation is characteristic for inflammatory diseases, e.g. diabetes, when PMNs are overactivated by higher level of glycaemia. There are informations about membrane expression on PMN such enzymes as LE, cathepsin G and proteinase 3, however not about cathepsin B. The activity of cell surface-bound CB was mainly studied in homogenates or sections of different tissues, but not in PMN's fractions. Our previously investigations have shown enlarged activity and concentration, both CB and LE in type 2 diabetic patients, in the comparison with healthy persons, particularly in PMN. Now we estimated expression of membrane-bound cathepsin B (MBC) and leukocyte elastase (MBL) in the PMN's fraction. We developed a modified method for membrane-bound forms of these proteases on the ground of activity measured by fluorimetric assays. PMNs were isolated from the blood, and then was fixed with the solution of PBS containing 3% paraformaldehyde and 1% glutaraldehyde. We also measured total activity of CB and LE in cellular lises, obtained by homogenisation, as well as in plasma of the same patients. We compared these results with the control group. We noticed the essential increase, both the activity of cell surface-bound CB and LE, and the total activity of these proteases in cell's lysates and plasma of diabetic patients compared to the control group. Moreover, the percentage participation of MBC and MBL in the total activity of these enzymes was larger in diabetics in comparison with healthy subjects, what confirmed PMNs stimulation.
MBC and MBL showed also various degree of sensitivity to their specific endogenous inhibitors, while “typical” forms of CB and LE were almost completely inhibited.

O2.13

Plasma homocysteine and birth defects

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Background: Homocysteine is a sulfur-containing, non-proteinogenic amino acid biosynthesized from methionine that takes a key place between the folate and activated methyl cycles. This amino acid is involved in cysteine biosynthetic pathway (transsulfuration), remethylation in methionine synthesis, transmethylation of DNA, proteins and lipids, biosynthesis of small hormonal and neuronal signaling molecules. Hyperhomocysteinemia has been associated with both folate and cobalamin deficiencies, and also with pregnancy complications (pre-eclampsia, spontaneous abortion, intrauterine growth restriction, birth defects), mental disorders, psoriasis and some tumors. Hyperhomocysteinemia is associated with the occurrence of congenital defects including failure of neural tube closure, conotruncal anomalies in the heart, and orofacial clefts. Congenital anomalies have become more important causes of infant morbidity and mortality since the prevalence rates of infectious diseases and nutritional problems during the childhood have decreased over the last decades.

Objective: To measure the concentration of plasma total homocysteine in pregnant women of birth defects child. We aimed to estimate association between increased plasma homocysteine level and different kinds of congenital anomalies.

Methods: Blood samples were collected into tubes containing heparin, immediately chilled in the ice and centrifuged. Samples prepared at the day of analyses. Total homocysteine was determined by high-performance liquid chromatography (HPLC) with fluorescence detection in both pregnant women of birth defects child (n = 102) and pregnant women with unaffected offspring (n = 52).

Results: Plasma homocysteine was significantly higher in pregnant women with affected offspring compared with control group (P<0.05). It was revealed in the cases of hydrocephalus, neural tube defects, Down’s syndrome, nuchal cystic hygroma and diaphragmal hernia. We found that homocysteine plasma concentration was higher in the cases of skeletal dysplasia, teratomas, kidney and lung polycystosis compared to control group but it was not significant. We failed to confirm role of homocysteine in genesis congenital heart defects and orofacial clefts.

Conclusion: Our data confirm role of homocysteine in genesis of some birth defects. Several hypotheses can be proposed to explain biological mechanisms through which elevated homocysteine level could cause birth defects. Homocysteine itself is embryo-toxic during the process of embryogenesis. Decreased levels of methionine due to a disturbed remethylation of homocysteine to methionine will result in decreased levels of S-adenosylmethionine and disturb embryogenesis by an inadequate gene methylation. We consider that it is necessary use estimation of plasma homocysteine in prenatal diagnostic.
Interactions between PPARα and MTHFR genes polymorphisms and conventional risk factors of coronary artery disease in determining the risk of the disease

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Background: Progression of atherosclerosis, the main reason of cardiovascular diseases, depends on multiple genetic and environmental factors. Genetic susceptibility to CAD may be determined by specific polymorphic variants of genes encoding isoforms involved in processes important in the pathogenesis of atherosclerosis. Participation of single polymorphic variants is relatively small, however its significance may increase in the presence of specific genetic or environmental background. Peroxisome proliferator-activated receptor alpha (PPARα) is a ligand-activated transcription factor which regulates the expression of genes involved in lipid metabolism, monocyte recruitment and adhesion, foam cell formation. It has also anti-inflammatory and antioxidant properties. Therefore it is thought to regulate proatherosclerotic processes in the vessel wall. Methylene tetrahydrofolate reductase (MTHFR) is an enzyme which plays an important role in homocysteine (Hcy) metabolism. The 677C>T polymorphism of MTHFR gene results in a decrease of the enzyme activity that leads to mild hyperhomocysteinemia. Elevated plasma level of homocysteine has been recognized as an independent risk factor of cardiovascular disease.

Aim: The aim of the study was an evaluation a possible association between G>C intron 7 polymorphism of PPARα gene or 677C>T polymorphism of MTHFR gene and CAD, as well as analysis of interactions between polymorphic variants and conventional risk factors of CAD in determining the risk of the disease.

Methods: We studied 339 white Caucasians, including: 177 patients with angiographically confirmed CAD and 162 blood donors without history of CAD. Polymorphisms were genotyped using RFLP-PCR (Restriction Fragments Lenght Polymorphism-Polymerase Chain Reaction) method. Data were analyzed using STATISTICA 6.0 and EpiInfo-6 (WHO) software.

Results: We did not observed the differences in the distribution of genotypes and alleles of MTHFR and PPARα genes. There were only tendency to higher prevalence of T and C alleles, respectively in CAD group. However we noticed significantly higher frequency of carriers of both “proatherosclerotic” variants in CAD group (19.2%) than in controls (8%) (P=0.004, OR=2.73). The PPARα gene polymorphism shows also cumulative and synergic effect on CAD with elevated level of triacylglycerols (P=0.0004, OR=4.23, SIM=1.57) and overweight or obesity (P=0.0001, OR=3.62, SIM=2.8). The strongest influence on CAD was observed for carrier-state of PPARα C allele with carrier-state of MTHFR T allele and overweight or obesity (P=0.0002, OR=7.49, SIM=2.78).

Conclusions: The PPARα gene G>C polymorphism in intron 7 together with MTHFR gene 677C>T polymorphism may be regarded as a risk factor of premature CAD, especially in the presence of specific conventional risk factors.
**P2.2**

**Total antioxidant status in cystic fibrosis infants on complete formula containing vitamins A and E**

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In cystic fibrosis (CF), an imbalance between the production of reactive oxygen species and their inactivation by protective systems has been suggested. Digestive malabsorption in CF patients, especially those with exocrine pancreatic insufficiency, may lead to malnutrition and spread of oxidative conditions generated by chronic pulmonary infections. Because biological symptoms of malnutrition (such as low blood level of vitamin E) may occur in the few weeks after birth it is important to preserve normal nutritional status of CF patients. Therefore routine multivitamin supplementation should be supported by diet with adequate quantity of antioxidants. The aim of the study was to assess total antioxidant status in cystic fibrosis infants fed a complete formula containing vitamins A and E compared to healthy controls. 21 cystic fibrosis children with exocrine pancreatic insufficiency aged 3–15 months taking Milupa Cystilac Formula (vitamin A – 141 mg/100 ml; vitamin E – 3.7 mg/100 ml) were enrolled in the 6-month long study. Total antioxidant status (TAS) was measured as Trolox-equivalent antioxidant capacity and vitamin A (retinol) and E (α-tocopherol) concentrations were determined by the method of high-pressure liquid chromatography (HPLC). We have found that in plasma of CF patients before nourishing with complete formula mean concentration of TAS was significantly lower than in healthy children (1.16 ± 0.08 mmol/l vs 1.30 ± 0.04 mmol/l; P < 0.0001). Plasma levels of retinol were in the controls within the normal range (0.8–2.8 µmol/l). In CF children vitamin A concentration below the normal limit was shown in 5 patients. Plasma levels of α-tocopherol were lower in group of CF infants (11.56±4.35 µmol/l) than in healthy ones (18.41±3.10 µmol/l). The differences were statistically significant (P<0.0001). In CF children TAS level elevated following treatment (pre 1.16±0.04 range 1.03–1.31 mmol/l; post 1.23±0.07 range 1.15–1.40 mmol/l; P<0.01). Similar increases in the plasma concentration of retinol (pre 1.13±0.39 µmol/l; post 1.40±0.36 µmol/l; P<0.05) and α-tocopherol (pre 11.56±5.35 µmol/l; post 17.85±4.09 µmol/l; P<0.0001) was observed. After six months intake of diet based on specific nutritional needs of infants with cystic fibrosis their total antioxidant status improved by about 12% and was in the similar range as in control subjects (1.22–1.40 mmol/l). Our results suggested that studied diet is effective in normalising level of total antioxidant status and plasma vitamin A and E concentrations and could be associated with clinical benefit to CF patients.

**P2.3**

**Clinical value of IL-8 in prognosis of complication in acute pancreatitis**

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**Introduction:** Acute pancreatitis is an inflammatory process of a gland and the adjoining or distant organs as well. The direct mechanism, which triggers the development of the disease, is still unknown and therefore any causal treatment is impossible. If the inflammatory process of pancreatitis is reversible it depends on the form of a pathological appearance of the parenchyma. Severe pancreatitis is connected with high mortality rate. The studies of inflammatory markers both in mild and severe pancreatitis show that this proinflammatory cytokine can be helpful in differential diagnosis.

**Aim:** The aim of this paper is to follow through the dynamics of IL-8 in patients with mild and severe pancreatitis. The profile of parameter might give certain information to forecast the course of the acute pancreatitis and therapeutic and prognostic instructions.

**Material and methods:** The studied group consisted of 35 patients, F-15, M-20, aged 21 to 87 years (average age 54 ± 33 years). Cytokine was determined by immunoenzymatic tests (ELISA), according to producer’s instructions. The sensitivity of tests was 10 pg/ml. In all patients apart from routine tests, the concentration of IL-8 in the blood serum on I, III, VII, XIV day of hospitalization was determined. The dynamics of proinflammatory cytokines IL-8 was estimated and the obtained results underwent statistical analysis. Patients were divided into 2 groups: with mild, and severe pancreatitis. In the group with mild form consisted of 20 patients, the symptoms were poorly expressed and they required only pharmacological therapy. In severe pancreatitis as a result of enzymatic toxemia it came to the loss of fluids and proteins from vascular bed, multi organ failure and extensive necrotic changes in pancreas; predominated signs: strong pains of the abdomen, vomiting, flatulence of the abdomen, fever. In this group, consisted of 15 patients, surgical procedures were applied.

**Results:** Biochemical analysis showed that in patients with mild pancreatitis the average level of IL-8 were: on first day 4.76 pg/ml, S.D. ± 2.42; on third day 4.92 pg/ml, S.D. ± 3.51; on seventh day 5.44 pg/ml, S.D. ± 4.61; on fourteenth day 5.12 pg/ml, S.D. ± 4.43. Determined levels of IL-8 in this group were about the norm. The average levels of IL-8 in patients with severe pancreatitis were: on the first day the average level of IL-8 was 15.81 pg/ml, S.D. ± 12.87. On next days the values increased and theirs level was constant till the fourteenth day (3rd day 18.71 pg/ml, S.D. ± 13.90, 7th day 18.61 pg/ml, S.D. ± 13.86, 14th day 18.83 pg/ml, S.D. ± 13.65). The diagnostic value of IL-8 in the inflammation of the pancreas shows that this chemokine reflects the seriousness of the course of inflammation.
and confirms the facts in literature that it is perceived as a “lethal cytokine”.

**Conclusions:** The profile of concentrations of IL-8 remaining on higher level can suggest that this chemokine reflects the severity of the course of inflammation.

**P2.4**

**The new perspectives of the angiotensin II action as a factor modulating the proliferation of the cellular lines of the breast tumor**

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Breast cancer is a major cause of morbidity and mortality among women worldwide especially in middle age and growth factors has been found to play an important role in the etiology and progression of this disease. Several proteins of the Renin–Angiotensin–Aldosterone system (RAS) have been implicated in the processes of growth promotion or inhibition and are found present both in normal and cancerous breast tissues. Breast epithelial cells express components of the renin angiotensin system and studies suggest that these may be altered in disease progression. Angiotensin II (AngII), a peptide of the renin-angiotensin system, can not only regulate the water-electrolytic balance and control the arterial blood pressure but it can also influence the cellular proliferation. It is known, that this peptide influences not only the hyperplasia of the smooth muscle of the blood vessels but also the cells of the anterior pituitary lobe and the cells of the adrenal gland and how the latest research has shown, it influences also the proliferation of some tumor cellular lines. The aim of the study was to find out if the AngII can modulate the cell growth of two cellular lines of the breast tumor MCF7 (hormon-dependent line) and MDA-MB-231 (hormon-independent line). The influence of the peptide on the cell proliferation was assessed using standard MTT assays and changes of the tyrosine kinases activity (enzymes which are directly engaged in the cell proliferation). The influence of the 17-β-estradiol on the activity of the AngII has also been estimated. AngII $10^{-9}$M, $10^{-10}$M and $10^{-11}$M has strongly slowed down the activity of the tyrosine kinases and cell proliferation in the MDA-MB-231 line. AngII alone in all concentration has very weakly influence on the activity of the tyrosine kinases in the MCF7, but in the presence of estradiol in the concentration $10^{-8}$ this peptide strongly inhibited examined enzyme. The results show that AngII can be the next factor, which modulates the proliferation of the cellular lines of the breast tumor.
Adrenergic receptors are protein G-coupled receptors for catecholamines. Several groups of adrenergic receptors were discovered and cloned by now (α1, α2, β1, β2). β2 adrenergic receptors (ADRβ2) are considered to contribute (after stimulation) to pathogenesis of congestive heart failure and hypertension. The ADRβ2 intronless gene is located on human chromosome 5q31-32 and an extracellular amino terminus, three extracellular and three intracellular loops, with a small fourth loop before the palmitoylation site. β2-adrenergic receptors are cell surface receptors which activate adenyl cyclase by coupling to guanine nucleotide binding proteins (G proteins). These receptors mediate vasodilation in response to adrenergic agonists in vascular smooth muscle and together with β1 take part in chronotropic and inotropic responses in healthy heart. β2 adrenergic receptors are also present on adrenergic nerve terminals in the heart, where they facilitate norepinephrine release. ADRβ2 gene is highly polymorphic. Several polymorphisms within it have previously been examined for the relationship with cardiovascular disease but the results are conflicting. We examined the A46G polymorphism of ADRβ2 which results in amino acid substitution (Gly for Arg at amino acid position 16) within the coding region. According to the literature the replacement of the arginin in codon 16 with glycine (Arg16Gly) makes the protein more susceptible to down-regulation in response to β2 agonists, may lead to decreased agonist-mediated in vitro vasodilatation and may modulate the cardiovascular risk. The aim of our study was to evaluate the influence of Arg16Gly polymorphism in an adrenergic receptor on the risk of MI among Polish, young (<45 years) population. One hundred ninety five patients with documented myocardial infarction (MI) (age below 45 years) not suffering from diabetes mellitus or hypertension were included into our study group. The control group contained 130 healthy voluntaries. Genotype GG was predominant in studied group of patients in comparison to control group (40% vs. 34%, P<0.05). Moreover, the wild type genotype AA was less frequent in patients suffering from MI compared to healthy voluntaries (14% vs. 18%). The polymorphism A46G (Arg16Gly) seems to play a role in enhancing the risk of premature MI and could be considered as a potential genetic risk factor in studied group of patients. 

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P2.7

Noninvasive evaluation of liver function of nursing mothers

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Scientific literature has the proofs of the use of breast milk for evaluation of vitamin and hormonal status of nursing mothers. Though in this biological liquid it was not possible to determine the levels of activity of hepatocyte enzymes, by means of which it is a possible to evaluate the functional condition of the liver in the organism of a nursing mother.

48 healthy puerperas and 83 having preeclampsia of different degrees of severity during pregnancy were examined to evaluate the activity of enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDG) in blood serum and breast milk) on the 3rd day of post natal period using automatic biochemical analyzer “Architect C8000” by ABBOTT (USA).

The activity of enzymes, digitally determined in breast milk is the same to the activity of enzymes in blood serum. It was revealed the direct correlation dependence between studied indices (for ALT correlation coefficient made up r = +0.85; for AST r = +0.87; for LDG r = +0.810).

It was discovered the increase of activity of all examined enzymes according to the level of preeclampsia severity: the activity of ALT made up more then 50.0 Unit/l, AST – more then 47.5 Un/l, LDG – more then 316 Un/l and correlated with clinical presentations of liver lesion (pain in right hypochondrium and epigastric area) that puerperas suffered from preeclampsia had.

Thus noninvasive method of evaluation of liver function of nursing mothers in post natal period is effective, informative and safe in comparison to the traditional one.

The use of breast milk for noninvasive evaluation of liver function by means of determination of activity of hepatocyte enzymes creates the possibility for manifold research, preservation of peripheral veins for subsequent intravenous introduction of medical products, save the time of laboratory assistants and hospital nurses while receiving biological samples, prevent infectious complications and appearance of stress reaction while receiving biological samples.

P2.8

Cofilin and gelsolin in wound healing fibroblasts

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Myofibroblasts (or smooth-muscle like cells) are now well known key-players of many physiological and pathological processes such as wound healing, hypertrophic scars formation, fibromatoses, stromal reaction, morphogenesis and differentiation. These cells differentiate from fibroblasts under TGF-β stimulation and are characterized by the expression of α-SMA (smooth muscle actin) what allows them to generate a tensile force required for wound contraction. Our studies were focused on the role of specific actin binding proteins and on their localization within migrating wound healing fibroblast. We have also paid attention to the changes in the organization of actin cytoskeleton and TGF-β induced myofibroblasts α-SMA expression. Specific monoclonal antibodies against cofilin, gelsolin and actin were used to visualize proteins in fluorescent confocal microscope. Filamentous actin was stained with rhodamine-conjugated phalloidin. We have observed pivotal changes in the localization of cofilin and gelsolin in fibroblasts migrating in the wound healing assay as well as in TGF-β induced myofibroblasts. Our results underline a crucial role of these protein during dynamic changes of actin cytoskeleton in the processes of wound healing and others where a myofibroblast is involved.
Correction of metabolic disturbances in rats with experimental alimentary non-alcoholic fatty liver

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**Background and aim:** Non-alcoholic steatohepatitis (NASH) represents an advanced stage of fatty liver disease developed in the absence of alcohol administration. It has been clinically demonstrated possibility of its progression to advanced fibrosis and even cirrhosis. Among the factors initiating this pathology are obesity, insulin resistance, hyperlipidemia. Therefore, we used for experimental NASH therapy hypolipidemic, insulin sensitizing and membrane stabilizing agents (bezafibrate, metformin, betaine and tauroursodeoxycholic acid (TUDCA)).

**Methods:** Adult male Wistar rats (n = 10/group) were treated for 10 wks with high-fat methionine-choline deficient diet (MCDD) only, or MCDD diet with either metformin (50 mg/kg b.w.), bezafibrate (100 mg/kg), betaine (100 mg/kg) or TUDCA (80 mg/kg) during last 4 wks. 10 rats served as healthy controls. The severity of liver damage was evaluated histologically. Serum and liver lipids and lipoproteins content, serum insulin, glucose, bilirubin, TNF-α, leptin and adiponectin levels, serum marker enzymes activities were measured.

**Results:** MCDD-fed group had enhanced liver relative mass and severe hepatic steatohepatitis of score stage 3: almost 75% of the parenchyma developed steatosis. Hepatocyte ballooning and inflammatory scoring stage 1 were observed. Serum marker enzymes, AIAT, AsAT, alkaline phosphatase, were dramatically activated as compared to healthy control rats. Concentration of hepatic triglycerides, cholesterol and phospholipids were increased, while the serum lipids content was decreased. We noted significant rise of the serum proinflammatory cytokine TNF-α, increase in the serum leptin concentration and blood glucose content, whereas serum insulin and adiponectin levels were decreased. The administration of all studied agents caused significant beneficial changes in most of the histological and biochemical parameters measured. All compounds normalized serum marker enzyme activities. The treatment with either TUDCA or metformine was decreased blood glucose concentration and normalized serum insulin level. A normalization of the serum lipid and lipoprotein parameters was mostly seen in the TUDCA-, betaine- and metformine-treated groups. All compounds significantly decreased liver lipids (triglycerides, cholesterol, phospholipids) content. All the compounds normalized serum adiponectin level, whereas concentration of TNF-α and leptine were decrease only in TUDCA- and betaine-treated groups.

**Conclusions:** 1. The treatment with TUDCA, betaine and metformine and, in the less extent, bezafibrate had a significant hepatoprotective effect in rats with experimental NASH. 2. The clinical implication of this finding is promising for the treatment NASH in the clinics.

The role of theophylline and N-acetylcysteine in ketogenesis examined in vitro

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**Theophylline** and **N-acetylcysteine** (NAC) are preparations most often prescribed for protracted diseases of the respiratory system, such as bronchial asthma or chronic bronchitis. Written sources discussing theophylline and NAC do not contain any information on whether these preparations affect one of the most essential processes — ketogenesis — and if they do, in what way it happens. There is also no information on whether the preparations are hepatotoxic, despite the fact that it is recommended to minimize the dosage of both the chemical compounds in cases of any liver dysfunction.

Biotransformation of theophylline and acetyl cysteine takes place in the liver, accompanied by P-450 cytochrome isoenzymes. This may become a reason for hepatocyte metabolism disruption. Consequently, it can not be ruled out that these preparations disturb oxidative homeostasis in the liver, including ketogenesis.

The aim of this work was to assess metabolytic competence of isolated rat hepatocytes incubated in the presence of theophylline and/or N-acetylcysteine. The competence was measured by the intensity of ketogenesis.

The test was carried out using hepatocytes isolated by enzymatic method employing collagenase IV obtained from the livers of Wistar breed rats. The hepatocyte suspensions were incubated on “Hepatocyte Medium” base with the addition of theophylline and NAC. They were placed in Type BB 16 Heraeus incubator at the temperature of 37°C in CO2 atmosphere.

After 30 and 120 minute incubation period the concentrations of ketone bodies — acetoacetate and β-hydroxybutyrate were measured by means of tests produced by Wako GmbH Neuus, Germany. Also, the ratio between mole concentrations of acetoacetate and β-hydroxybutyrate (KBR) was calculated.

The observed changes in concentrations of ketone bodies indicate the interference of both the preparations in the course of ketogenesis and, by that, in the physiological balance between the reduced and the oxidated form of nicotinamideadenine dinucleotide. In consequence, the presence of N-acetylcysteine in the hepatocyte base resulted in the rise in KBR value, regardless of the incubation time. On the other hand, theophylline, which, as the time of incubation passed and the doses increased, caused lowering of acetoacetate concentration and increase in the concentration of β-hydroxybutyrate. This resulted in lowering of KBR value.
P2.11

The influence of doxorubicin on the activity of lactase dehydrogenase of squamous cell carcinoma cells AT478 in vitro

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An catalytic enzyme in the last part of glikolisis is LDH, lactase dehydrogenase [EC 1.1.1.27]. LDH is cytoplasmatic enzyme, marker of many chronic illuess: cardiopathy, hepatopathy, and new growth. The influence of doxorubicin on activity of lactase dehydrogenase, LDH, of squamous cell carcinoma (SCC) cells of the line AT478 from mous C3H was studies. The squamous cell carcinoma (SCC) is a special line of cell culture that has the ability for megacoloneies creation growing in continous contract with ground and other cells. Our studies were carried out on six groups: 3 study groups, exposition for doxorubicine 24 h, and 3 study groups, exposition for doxorubicine 72 h. In 2 control groups (24 and 72 h) the cells grew with out any influence of doxorubicine. The influence of doxorubicine on activity of lactase dehydrogenase, LDH, in cells medium was investigated. Our results suggest that doxorubicine, in used doses and time periods, influences the biochemical metabolism of squamous cell carcinoma in vitro.

P2.12

Gelatinase A activity in Dupuytren's disease

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Purpose: Dupuytren's disease is a connective tissue disorder viewed as a progressive pathological process involving multiple molecular events that lead ultimately to considerable changes in cell phenotype and function and to the deposition of excess matrix proteins in the extracellular space of the palmar aponeurosis, resulting in a flexion deformity of the fingers and loss of hand function. It is suggested, that the fibrosis of the palmar fascia may be the result of disturbed physiological balance between MMPs and their endogenous antagonists. Gelatinase A (MMP-2) is a member of the matrix metalloproteinase (MMPs) family of proteolytic enzymes that contribute to remodeling the extracellular matrix by degrading its components. The aim of this study was to investigate the activation level of MMP-2, in palmar fascia with Dupuytren's contracture in relation to the clinical stages of disease progression.

Methods: The level of relative MMP-2 activation, was investigated with use of the substrate gel sodium dodecyl sulfate-polyacrylamide gel electrophoresis zymography in 53 pathological tissues representing four clinical stages of disease progression and 12 fragments of normal palmar fascia obtained from patients surgically treated for carpal tunnel syndrome. The intensity of each sample band was determined densitometrically using software (ImageJ) and expressed as a ratio of the percentage of active to latent MMP-2 (activation ratio). The results were analyzed statistically with the nonparametric Mann-Whitney U test and the Kruskal-Wallis test for comparing the 4 populations of patients with different disease stages. A p value of less than 0.05 was considered statistically significant. Results: We found that the level of MMP-2 activation was significantly elevated in the palmar fascias with Dupuytren's contracture compared with normal tissues. We did not find statistically significant differences between groups with different stages of the disease progression. Conclusion: The differences in MMP-2 activation between contractured and normal fascia suggest a participation of this enzyme in the promotion of Dupuytren's disease.
P2.13

The association of C34T polymorphism of the AMPD1 gene with features of metabolic syndrome in patients with coronary artery disease without heart failure

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Background: The common C34T polymorphism in the AMP deaminase-1 (AMPD1) gene results in premature stop codon, and thus, in an inactive enzyme. Reduced activity of AMP deaminase may increase tissue concentrations of AMP and adenosine, a potent cardioprotective agent. There were reports of association of T34 allele with prolonged survival of patients with coronary artery disease (CAD) or heart failure (HF). It was also reported that variation in AMPD1 gene is associated with insulin clearance and may participate in syndromes of insulin resistance.

The aim of the study was to investigate the associations between AMPD1 genotype and features of metabolic syndrome in patients with CAD without HF.

Methods: 79 CAD patients were genotyped by PCR-RFLP. The PCR product (11 bp) of wild-type C34 allele was cleaved by TaqI into 8 bp and 21 bp fragments. The mutated T34 allele was identified by lack of TaqI restriction site. CAD was diagnosed on the basis of coronary angiography and patients with HF evidenced by clinical symptoms, echocardiography or high brain natriuretic peptide (BNP) plasma concentration were excluded from the study group.

Results: AMPD1 C34T genotype frequencies were consistent with Hardy-Weinberg equilibrium: 68 (70%) CC, 23 (24%) CT and 6 (6%) TT. There were no significant differences between T allele carriers (CT+TT, n=2) and non-carriers (CC) regarding patients’ age, gender, CAD duration, ejection fraction and BNP concentration.

T34 carriers had significantly lower body mass index (26.5±2.7 vs. 28.8±4.7 kg/m², P=0.026), waist circumference (89.5±8.5 vs. 98.2±11.3 cm, P=0.00013) and waist-to-hip ratio (0.92±0.08 vs. 0.97±0.08, P=0.0050). The prevalence of obesity (BMI≥30 kg/m²) was also lower in T34 carriers than in CC homozygotes (14% vs. 37%, P=0.029). Fasting serum glucose concentrations were not significantly different between the genotype groups (101.3±8.7 for CT+TT and 108.0±19.0 mg/dL for CC, P=0.35), but the prevalence of diabetes and fasting serum glucose ≥126 mg/dL was significantly lower in T34 carriers (0% vs. 16% and 0% vs. 15%, respectively, both P=0.030).

Conclusions: Carriage of AMPD1 T34 mutated allele in CAD patients without HF is associated with lower prevalence of two features of metabolic syndrome: diabetes and obesity. The possible mechanisms of the association, including hypothetical changes in AMP-activated protein kinase activity, need further investigation.

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Synergistic and cumulative effects between polymorphisms of PAI-1 and IL-6 genes and smoking in determining coronary artery disease

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Background: Progression of atherosclerosis is the main cause of coronary artery disease (CAD). It results from the interaction between multiple genetic and non-genetic factors combinations. Genetic predisposition to the disease may be co-created by functional polymorphic genes, encoding variants of proteins with altered biological activity that are involved in the processes important for the pathogenesis of atherosclerosis. Polymorphic variants of genes encoding markers of proinflammatory state, such as interleukin-6 (IL-6) and prothrombotic state, such as plasminogen activator inhibitor-1 (PAI-1) may determine a susceptibility to the disease.

Aim: The aim of the study was to assess a relationships between single genetic polymorphisms of IL-6 and PAI-1 and effects between polymorphic variants of these genes and cigarette smoking and CAD.

Material and Methods: The study was performed on 394 white Caucasians, including: 191 patients with angiographically documented CAD (aged 43.8±6.1, 64 females and 127 males) and 203 blood donors (aged 35.3±10.5, 49 females and 154 males) with no signs of CAD in the interview. Genetic polymorphisms analysis was performed using PCR-RFLP method. Data were analyzed using STATISTICA 6.0 and EPI-INFO (WHO) software.

Results: The genotypes frequencies were in agreement with Hardy-Weinberg equilibrium. We observed higher frequency of 5G allele of –674–675insG polymorphism of PAI-1 gene in CAD patients than in controls (47.6% vs 40.4%) (P=0.04, OR=1.34 95%CI 1.00–1.80). 5G allele carriers (4G5G+5G5G genotypes) were more frequent in CAD group (74.3%) compared to controls (66.0%) (P=0.048, OR=1.77 95%CI 1.00–3.14). In CAD female subgroup carriers of 5G allele were significantly more frequent than in female blood donors (78.1% vs. 57.1%) (P=0.031, OR=5.51 95%CI 1.13–26.78). We did not observed correlation between IL-6 –174G>C polymorphism and CAD. The frequency of IL-6 C allele was significantly higher only in male CAD subgroup (51.2%) compared to male blood donors (42.2%) (P=0.035, OR=1.44 95%CI 1.01–2.05). Contemporaneous carrier-state of PAI-1 5G allele and IL-6 C allele did not differentiate analyzed groups. In spite of rather weak association between analyzed polymorphic variants and the disease we found cumulative effects between specific genotype patterns and smoking in determining CAD, especially for PAI-1 (4G5G+5G5G), IL-6 (CC) and smoking (P=0.0001, OR=11.02 95%CI 2.36–70.99). There was also observed quite strong synergistic effect between homozygous CC of IL-6 gene and carriers of 5G allele of PAI-1 gene and smoking (synergy index SIM=3.71). The SIM value indicates that effect of both genetic and non-genetic factors on CAD is almost four-fold stronger than the effects of these factors considering separately.

Conclusion: The present study indicates that IL-6 –174G>C polymorphism and PAI-1 –674–675insG polymorphism have cumulative and synergistic effects together with smoking in determining the risk of CAD.
**P2.15**

**Analysis of sites susceptible to N-homocysteinylisation in human blood proteins**

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Clinical studies have shown that elevated plasma total Hcy is associated with an increased risk of the development of vascular and neurological diseases. An underlying mechanism of Hcy pathogenicity may involve Hcy incorporation into protein, which occurs in the human body. The pathway involves metabolic conversion of Hcy to Hcy-thiolactone catalyzed by methionyl-tRNA synthetase during protein biosynthesis. Hcy-thiolactone is chemically reactive and forms adducts with protein, N-Hcy-protein, in which the carboxyl group of Hcy is linked by an amide bond to ε-amino group of a protein lysine residue. This reaction impairs protein structure and function. Major pathophysiological consequences of protein N-homocysteinylate include induction of anti-N-Hcy-protein auto-antibodies and thrombogenesis in humans, which contribute to atherosclerosis in humans. The bulk of Hcy circulating in human blood is N-linked to hemoglobin and albumin. We have previously reported that Lys525 is a predominant site of N-homocysteinylation in human serum albumin in vitro and in vivo. In the present work we analyzed native and Hcy-thiolactone-modified human serum proteins by proteomic approaches. Protein samples were digested with trypsin; resulting peptides were purified by HPLC and subjected to MALDI-TOF mass spectrometric analyses. We found that Lys4, Lys12, Lys137, Lys159, and Lys99 are sites for the modification of human serum albumin by Hcy-thiolactone. Two sites in hemoglobin susceptible to the modification of by Hcy-thiolactone, βLys18 and αLys16, were also identified. Furthermore, we have found that a peptide containing N-Hcy-Lys525 is easily detected in MALDI-TOF mass spectrum of a tryptic digest of human serum proteins. Based on this finding, we have developed a method that allows direct monitoring of N-Hcy-albumin in human serum.

**P2.16**

**Correlation of hormonal and antioxidant homeostasis in the late stages of gestosis with the evidence of endogenous intoxication**

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Recently the literary data been indicative on level changing of liposoluble vitamins, hormones and in pregnant with gestosis the activation of processes of peroxide oxidation of substrata of protein, carbohydrate and lipid metabolism that is accompanied by the accumulation of free radical metabolites which play the leading role in origin of systemic hypoperfusion and hipoxia and cause the development of this complication of pregnancy.

The objective of our study were to investigate vitamino-hormonal status indexes in comparison with the the evidence of endotoxins in women in III trimester of pregnancy concerning normal and gestosis of different severity.

In accordance with the objective of study 83 women with gestosis in the different degrees of severity and 48 pregnant women who haven’t complication of gestosis were observed. 1-3 groups were formed according to severity of gestosis.

To estimate the activity of antioxidant system in blood plasma it was determined liposoluble antioxidants (A, E) by flowmeter; malon dialdehyd (MDA) spectrophotometrically. The content of estradiol, cortisol was determined by the radioimmunological methods. Level an endotoxicosis was estimated by calculation of leukocytic intoxication index (LII).

The intensity of systemic oxidant stress in the late stages of gestosis registered by dynamics of plasmic level of three interactive indexes (vitamins A, E and MDA), as well as hormones was changed depending on degrees of severity in the late stages of gestosis. Pregnant women with gestosis developed increasing of MDA in contract to normal pregnancy. Only womans of second group was showed its increasing in 33%. In the first and second group of woman the content of vitamin A, E and cortisol was higher than in control, and the content of estradiol was low in comparison with physiologically running pregnancy. In the third group the content retinol and cortisol was higher than in control and the content of estradiol was lower in 56%. On the grounds of obtained findings we can suppose that the redundant content in of lipid peroxides lead to increasing outlet of tocoferol and retinoids into blood from tissues. Tissue deficiency in antioxidants accelerates formation of peroxides and contributes to desorganization of cell metabolism and main cell functions. A vicious circle has been locked, which was supported by hormonal disbalance. In calculation of LII it was revealed it was 1.0 0.1 units in healthy women and in gestosis of light, mild and severe degrees it was in average about 1.2±0.1 (P<0.05); 1.70,2 (P<0.05); 2.6±0.3 (P<0.001) units accordingly. This is due to reducing of eosinophil number (some women had aneosinopenia) and increasing an amount
of stab and nucleosegmental neutrophils in women with gestosis. Thus, obtained results help to consider exchange indexes of vitamins, hormons in blood plasma, accounting indexes of endogenous intoxication as additive criteria, characterizing degree of the evidence in gestosis.

P2.17

Dentition status in twin youngsters with regard to composition of the diet and components of the polymetabolic syndrome

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The present study was undertaken on assumption that dentition status as a manifestation of lifestyle and certain hereditary predispositions may take part in modifying risk factors of CVD in youngsters from families with cardiovascular diseases and may adversely affect their state of health in future.

We enrolled 15 pairs of twins with a CVD family history. After oral health investigation the DMFT index assessing dental status was calculated. Body mass index and ratio of waist and hip, as overweight or obesity and adiposity markers, were calculated. The following laboratory tests in blood serum were done: hs-CRP, fibrinogen, lipids, apolipoproteins and glucose concentrations. Calcium, magnesium and fluoride contents were determined in enamel of teeth. Information about alcohol using, cigarette smoking, physical activity and a single 24-h dietary recall was collected. Calculated DMFT index values were classified from 1 to 29. Rising value of DMFT was connected with worse dental health status in twins. The DMFT histogram was bimodal for values < 15 and >15. The latter distribution was observed in boys only. The significant positive correlations between DMFT index values and serum LDL-C (r = 0.903), Apo B (r = 0.954) and glucose (r = 0.975) contents were shown in boys and young men with DMFT levels over 15. The correlations between DMFT index values and intake of: amount of hard fat in diet (r = 0.54) and alcohol volume (r = 0.41) and cigarette smoking (r = 0.39) were shown in whole group of twins. The significant negative correlations between DMFT values and ApoA1 (r = –0.39), intake of amount of soft fat (r = –0.58), dairy-products intake (r = –0.39), sweets intake and the content of Mg2+ in tooth enamel (r = –0.52) were observed in this group. The significant and negative correlations were shown between content of fluorides in tooth enamel and HDL-C (r = –0.52) and ApoA1 (r = –0.63) concentrations in whole group of twins. Multivariate regression analyses revealed that DMFT values were affected by daily consumption of calories and hard fats through the influence exerted on the amount of body water and fat mass, as well as by the content of bioelements (F+, Mg2+) in enamel as determined by composition of the diet. An effect of the concentration of acute phase protein in serum was noted.
Deterioration of dentition status may coincide with elevated concentrations of risk factors of atherosclerosis. Dental care and prevention of periodontal disease should constitute an important element of primary prevention of CVD in offspring at high familial risk of CVD.

P2.18

De Ritis ratio after binge drinking

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Background: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) participate in gluconeogenesis by catalysing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid respectively. Both aminotransferases are sensitive indicators of liver-cell injury and both are released into the blood in increasing amounts when the liver cell membrane is damaged. AST present in cytosol and mitochondria isoenzymes of many organs, is not specific for the liver. ALT is more specific to the liver cytosol. Although, ALT is more specific to the liver injury, its activity may be normal even in severe alcoholic intoxication or liver disease. In addiction, the established normal range of ALT and AST, varies widely among laboratories. The evaluation of aminotransferase activities in serum is improved by the ratio of AST: ALT (De Ritis ratio). A ratio of AST to ALT greater than 2 is characteristic to alcoholic hepatitis, however, in many forms of acute and chronic liver injury or steatosis, the ratio is less than or equal to 1. There are no studies on the effect of single occasion drinking of high dose of ethanol, on AST/ALT ratio in serum.

Aim: The purpose of this study was to determine the Ritis ratio in blood serum during 2–5 day period, after single dose ethanol intoxication.

Materials and Methods: Serum of eight healthy (22–31 years old) binge drinkers (>5 standard drinks in a row, at one sitting) was collected before and 2 and 5 days after ingestion of ethanol (2.0±0.38 g/kg; mean±S.D.). The activity of AST and ALT (IU/l) were determined by using routine methods (with bioMerieux reagents, France). The results were processed with statistical program Statistica 6 (Statsoft). P<0.05 was considered statistically significant.

Results and Conclusion: The De Ritis ratio decreased significantly during 2–5 day period after ethanol consumption (P<0.05). Because high De Ritis ratio is specific for severe alcohol liver injury and increases as damage progresses, the decrease of AST/ALT ratio seems to be connected with the recovery period, after ethanol intoxication.
P2.19

Some biochemical studies on the saliva and dental plaque of the 10–13 years old children and their caries' level as well as everyday diet

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Background: The solubilization of both enamel of teeth and dentine due to the acids being made with the oral bacteria from carbohydrates is the main etiological concept of caries. However, one has to consider that the bacteria involved need not only sugars to live. So the other constituents of diet as proteins, lipids and so on might affect both the growth of bacteria and the salivary composition. The latter contains many proteins playing cariostatic as well as cariogenic properties. Also the sugar contents of saliva and its saturation with calcium phosphate(s) as well as the salivary pH are very important for caries growth. So are the activities of proteolytic enzymes which might play the contradictory effects in this case.

Aims: 1. The evaluation of total proteolytic activity in the pH 5 and 7 both in saliva and dental plaque taken from the mouth of 10–13 years old children with the different level of caries, different everyday diet and oral hygiene being established. 2. The determination of total sugar, inorganic phosphate, protein and pH of both saliva and dental plaque of this group of children.

Methods: 1. Proteolytic activity was assayed with bovine serum albumin (BSA) as a substrate with our own method based on UV absorbance of acid-soluble products of BSA hydrolysis. 2. Inorganic phosphate (P) was assayed with our own modification of Delsal-Manhouri method. 3. Total sugar was evaluated with colorimetric red-ox method with 2,4-dinitrosalicylate. Besides, the caries level was examined and relevant coefficient(s) of caries were calculated for 285 children 10–13 years old. They were inquired with our own questionnaire on the oral hygiene habits and care as well as everyday diet. The results were analyzed with the statistical methods. The results: Some significant dependencies, differences and correlations were between (1) proteolytic activity of dental plaque and saliva (2) P and pH of saliva (3) total sugar, pH and PI therein. What is more, some of above mentioned data correlate somehow both with some measures (coefficients) of caries growth as well as some elements of everyday diet e.g. eating the meat products. The caries and diet correlate each other in some points. So they do with the height and weight of this group of children.

P2.20

Homocysteine, its thiolactone and the activity of homocysteine thiolactonase in the saliva of 10–13 years old children and their caries’ level and everyday diet

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Background: There just a few papers on thiol compounds including homocysteine (Hcy) in the human saliva. No one paper deals with the active derivative of Hcy i.e. homocysteine thiolactone (T-Hcy) therein. T-Hcy is being formed from Hcy within the cells, human and bacterial with ATP and methionyl-r RNA and its synthetase. T-Hcy quite easily passes through the cell membranes in both directions. T-Hcy might be split only outside the cells with Ca2+ dependent enzyme: homocysteine thiolactone hydrolase (thiolactonase). Neither this enzyme nor T-Hcy itself within the saliva have been studied till now. They might serve as the additional cariogenic-cariostatic system in the oral cavity.

Aims: (1) to evaluate free Hcy (sum of S-S and SH forms) and T-Hcy in the saliva taken out from the mouth of 10–13 years old children with different level of caries and with different everyday diet being evaluated; (2) to determine the activity of Hcy thiolactonase in the same saliva samples.

Methods: (1) The free Hcy contents have been estimated, after deproteinization and S-S reduction, with the Abbot enzyme immunoassay colorimetric kit; (2) The same method has been applied for T-Hcy but this time after previous alkaline hydrolysis of T-Hcy to Hcy. (3) T-Hcy thiolactonase has been assayed with our own spectrophotometric method based on the decrease of A280 due to Ca2+ dependent enzymatic hydrolysis of T-Hcy. 285 children 10–13 years old were studied. These studies and their analysis is described in the accompanying communication (B. Piskorz et al.). Results: We have found both homocysteine thiolactone and its thiolactonase activity within saliva of studied children. Some significant correlations of the above mentioned data with the coefficients of caries growth and some features of the everyday diet of children have been found. So, possibly thiolactonase might serve as the defence against caries, whereas T-Hcy might be cariogenic to some extent.