

Pteridines
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Abstracts

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TD-GC-MS Analysis of Volatile Metabolites Released by *Candida albicans*

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The aim of this project is to enlarge the basic knowledge about volatile metabolites in a wide range of microorganisms to identify biomarkers in human breath. Nosocomial infections are abundantly found in patients in intensive care units (ICU) and significantly impair diagnosis. Hence, methods for the fast and easy monitoring of infections would be elemental to improve diagnosis. In this project the release of volatile organic compounds from *Candida albicans* was investigated.

Candida albicans was inoculated in tryptic soy broth and cultivated under strictly controlled ventilation. For controls and after 1.5, 3, 4.5, 6, 7.5, 26 and 28 hours 200 ml of headspace air was sampled and preconcentrated by adsorption on solid sorbents and then analyzed by gas chromatography mass spectrometry. Proliferation of cells was monitored by plating of cells and measurements of optical density.

The results show the release of numerous compounds. Released VOCs comprise several classes of compounds like aldehydes (e.g. acetaldehyde), ketones (e.g. 2,3-butanedione), alcohols (e.g.

ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol), esters (e.g. ethyl formate, ethyl acetate) and pyrazines (e.g. 2,5-dimethylpyrazine).

This work is an important step to get more knowledge about VOCs released from fungi and the obtained results are giving a detailed overview. The big variety of released compounds give evidence that specific biomarkers for diseases can be found which has to be confirmed by breath gas analysis.

Relationships between CSF Cytokines and CSF Neopterin in Neurological and Psychiatric Disorders

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Recent analysis of CSF with modern methods established in clinical neurology showed in more than 40% of therapy-resistant schizophrenic and affective disorders CSF pathology, demonstrating low level neuroinflammation or blood-CSF barrier dysfunction, compatible with the mild encephalitis hypothesis (1). Over this, neopterin was increased in more than 30% of patients with in CSF but not in blood. To better understand the

relationship between CSF neopterin increase and an underlying pathogenic process, which seemed to represent low level neuroinflammation, is of interest. Therefore, various inflammatory and non-inflammatory neurological disorders were compared to affective and schizophrenic disorders. Respective CSF neopterin increases were further analyzed regarding associations with CSF cytokines increases. In psychiatric disorders the cytokine increases are overall small moderate, generally considerably less than in inflammatory neurological disorders, nevertheless share some characteristics with findings in inflammatory neurological disorders. The relationship between neopterin increases in acute inflammatory neurological disorders or systemic inflammatory disorders, to the psychiatric disorders especially within intrathecal spaces will be discussed in more detail.

References:

- 1 Bechter K, et al. Cerebrospinal fluid analysis in affective and schizophrenic spectrum disorders. Identification of subgroups with immune responses and blood-CSF barrier dysfunction. *J Psych Res* 2010;44:321-30.

Neuronal Nitric Oxide Synthase Knock-out Attenuates Ischemia Reperfusion Injury

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Donor- pretreatment with tetrahydrobiopterin was shown to attenuate Ischemia-reperfusion-injury (IRI)-related graft pancreatitis in a murine pancreas transplantation model. Since the under-

lying mechanism of H₄B-mediated action is still unclear, it was aim of this study to investigate the role of neuronal nitric oxide synthase (NOS) cofactor activity of tetrahydrobiopterin regarding its protective effects on ischemia reperfusion injury using an nNOS^{-/-} model.

In a heterotopic pancreas transplantation model male syngeneic C57Bl6 mice (wild types and nNOS^{-/-}) were used as donor-recipient pairs. Donors were either pretreated with 50mg/kg bw tetrahydrobiopterin or untreated. Non-transplanted animals served as controls. Pancreatic grafts were exposed to 16h prolonged cold ischemia time to induce pancreatitis. Following 4h reperfusion, microcirculation was analyzed by intravital fluorescence microscopy. Parenchymal damage and peroxynitrite-formation were histopathologically and immunohistochemically evaluated, respectively. Intra-graft tetrahydrobiopterin levels were determined by HPLC. Finally, all groups were tested for recipient survival.

Compared to non-transplanted controls, prolonged cold ischemia time resulted in significantly impaired microcirculation in untreated wild-type grafts ($p < 0.01$). Only in untreated nNOS^{-/-} grafts microcirculatory deficits were absent. While tetrahydrobiopterin pretreatment preserved blood flow in wild type grafts, it did not further increase microcirculation in nNOS^{-/-} grafts. Tetrahydrobiopterin significantly reduced parenchymal damage and nitrotyrosine formation in wild types ($p < 0.05$). nNOS^{-/-} grafts were less prone to develop IRI-associated parenchymal damage ($p = 0.07$) and showed no differences between treated and untreated grafts. Significantly prolonged recipient survival was only observed in animals receiving nNOS^{-/-} pancreatic grafts and in grafts treated with tetrahydrobiopterin, independent of their genotype (all $p < 0.01$).

nNOS^{-/-} of the donor attenuated IRI-induced lethal graft pancreatitis in this model. These data suggest a crucial involvement of the neuronal NOS isoform in the pathogenesis of IRI following pancreas transplantation.

Genetic Variation in the ABCA1-gene Influences the Protective Effect of Adiponectin on Carotid Intima Media Thickness

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The ATP-binding cassette transporter A1 (ABCA1) facilitates amongst others the initial step of the reverse cholesterol transport by transporting cholesterol from macrophages to lipid-poor HDL precursors. Variants in the ABCA1-gene have been associated with a lower carotid intima media thickness (IMT) (1). Adiponectin levels are correlated negatively with carotid IMT and positively with HDL-C (2). Recent *in vitro* data suggested an influence of adiponectin on ABCA1-mediated cholesterol efflux from macrophages (3-5). Our objective was to investigate the R219K polymorphism of the ABCA1 gene influences the atheroprotective effect of adiponectin.

680 men randomly were selected from the SAPHIR-population. The R219K-variant was determined by RFLP-analysis, carotid IMT by high-resolution B-mode ultrasound and plasma adiponectin levels by ELISA. In carriers of the K-variant (n = 334) adiponectin shows a negative correlation with carotid IMT ($r = -0.137$, $p = 0.013$), whereas in wild-type subjects (n = 346) homozygous for the R allele no correlation can be detected ($r = -0.044$, $p = 0.42$). In carriers this atheroprotective effect persisted after adjustment for age, blood pressure, LDL-cholesterol, C-reactive protein and insulin resistance (standardized Beta = -0.129 , $p = 0.009$). Plasma adiponectin levels correlated positively with HDL-C in the whole study population ($r = 0.337$, $p < 0.001$), in wild-type subjects ($r = 0.312$, $p < 0.001$) and in carriers of the K-variant ($r = 0.387$, $p < 0.001$). However, adjustment for HDL-cholesterol abrogates the correlation of IMT and adiponectin in the whole population, wild-type subjects and carriers of the K-variant.

In conclusion, variation in the ABCA1-gene influences the atheroprotective effect of adiponectin, suggesting that adiponectin influences carotid IMT at least partly via ABCA1.

- 1 Sandhofer A, et al. The influence of two variants in the adenosine triphosphate-binding cassette transporter 1 gene on plasma lipids and carotid atherosclerosis. *Metabolism* 2008;57:1398-1404.
- 2 Iglseider B, et al. Plasma adiponectin levels and sonographic phenotypes of subclinical carotid artery atherosclerosis: data from the SAPHIR Study. *Stroke* 2005;36:2577-2582.
- 3 Matsuura F, et al. Adiponectin accelerates reverse cholesterol transport by increasing high density lipoprotein assembly in the liver. *Biochem Biophys Res Commun* 2007; 358:1091-1095.
- 4 Tian L, Luo N, Klein RL, Chung BH, Garvey WT, Fu Y. Adiponectin reduces lipid accumulation in macrophage foam cells. *Atherosclerosis* 2009;202:152-161.
- 5 Tsubakio-Yamamoto K, et al. Adiponectin prevents atherosclerosis by increasing cholesterol efflux from macrophages. *Biochem Biophys Res Commun* 2008;375:390-394.

Use of *in vivo* Microdialysis to Study Phenylalanine/Tyrosine Levels in PKU Patients

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Phenylketonuria (PKU) is an autosomal recessive metabolic genetic disorder characterized by an error in the genetic code for phenylalanine hydroxylase (PAH), leading to misfolding of the protein and subsequent loss of function. This enzyme is necessary to metabolize the amino acid phenylalanine (phe) to the amino acid tyrosine (tyr). In clinical routine practice single blood sampling and measurement of phe and tyr are performed. This leaves questions about changes due

to fluctuating phe values, phe-uptake by food, circadian changes and behavioural alterations. Aim of our study is to introduce continuous sampling by *in vivo* microdialysis and to study changes of phe and tyr in clinical practice to avoid repeated blood sampling.

In 10 adult PKU patients we will compare intravenous blood sampling of phe and tyr levels with the respective values of abdominal microdialysis, measured by MS/MS analysis. Supplied food is controlled for all amino acid content, especially phe and tyr. Measurements will be repeated for up to 24 hours to control for circadian effects.

In a healthy volunteer we were able to control the setup and to study intravenous serum, intravenous microdialysis and abdominal microdialysis values for phe and tyr. In the microdialysis samples about 60 to 80% of the serum values could be found, keeping in mind, that this is a comparison of a state value (serum) and continuously collected value (microdialysis). Only small increases of phe and tyr values were observed after food intake. A peak for both phe and tyr values could be observed at about 10 pm in the evening.

In vivo microdialysis might become a useful tool to study individual reactions of phe and tyr levels on different stimuli especially in children.

Effects of Antipsychotics on the Expression of Hydrogensulfide Producing Enzymes in Human Cell Lines

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Hydrogensulfide is a gaseous endogenous modulator (H₂S) with physiological functions such as neuromodulation or even neuroprotection. H₂S is synthesized mainly by the two enzymes cystathionin- γ -lyase and cystathionin- β -synthetase.

Some studies show an interaction of H₂S with glutamatergic neurotransmission, either on the glutamatergic transmitter release or on the NMDA receptor. Thus, our hypothesis was that H₂S might be involved in the pathophysiology of schizophrenia. So the aim of our study was to show that antipsychotics affect the synthesis of H₂S on the level of gene expression of the two enzymes Cystathionin- γ -lyase and Cystathionin- β -synthetase.

Human monocytic U-397 cells and human neuroblastoma cells SH-SY5Y cells were incubated with the antipsychotics Haloperidol, Clozapin, Olanzapin and Risperidon respectively (0.3, 3, 30 and 300 μ M). Genexpression of Cystathionin- γ -lyase and cystathionin- β -synthetase were studied using RT-PCR. Either haloperidol or the atypical antipsychotics Clozapin, Olanzapin and Risperdal lead to significant reductions of both enzymes in both cell lines.

Our results demonstrate that antipsychotics affect the expression of both H₂S synthesizing enzymes. This confirms our hypothesis that H₂S might be involved in the pathophysiology of schizophrenia.

***Helicobacter pylori* Seropositivity and Serum Kynurenine-Tryptophan Ratio in Gastric Cancer**

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Although *H. pylori* has been recognized as a Class I human carcinogen, the bacterium does not induce carcinogenesis by itself. The present scientific consensus is that the bacterial oncogenic role is mediated by the chronic active inflammation. Chronic gastritis is a common consequence among the *Helicobacter pylori* (*H. pylori*) infected individuals. However, the molecular basis of evading mechanisms of *H. pylori* from host immune system is lacking. Thus the aim of our study was to determine the effect of serum

indoleamine 2,3-dioxygenase (IDO) activity, neopterin, nitric oxide, urinary neopterin and biopterin levels which may sustain the persistence of *H. pylori* seropositivity in gastric cancer. Ninety five adults with gastric cancer and 108 cancer free patients with extra-digestive diseases were involved in the study. Each group was assigned into two subgroups according to *H. pylori* IgG seropositivity. Exposure to *H. pylori* was determined by serum IgG test (ELISA). Serum tryptophan, kynurenine, neopterin and nitrite concentrations were measured. Serum IDO activity was calculated by kynurenine to tryptophan ratio. The frequencies of increased serum IDO activities of *H. pylori* seronegative and seropositive colorectal cancer groups were estimated by comparing with the average amount of serum IDO activity of *H. pylori* seronegative tumor-free patients. Urinary neopterin and in order to estimate tetrahydrobiopterin, urinary biopterin and creatinine levels were assayed. Serum tryptophan levels of both *H. pylori* seronegative and seropositive gastric cancer patients were significantly decreased. However, only *H. pylori* seropositive cancer patients demonstrated significant rise in kynurenine-tryptophan ratio. The elevation in urinary biopterin concentrations, as well as the decrease in serum nitrite levels of *H. pylori* seropositive cancer cases might be attributed to the excessive reactive oxygen radical production. Thus it can be stated that in gastric cancer, the persistency of *H. pylori* seropositivity might enhance the immune tolerance due to the significantly higher kynurenine-tryptophan ratio and reduced nitric oxide.

Phenylalanine Hydroxylase

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Th1-type cytokine interferon- γ (IFN- γ) strongly induces the key enzyme for biosynthesis of pteridines, GTP-cyclohydrolase I (GTP-CH, EC 3.5.4.16) in various cells and cell lines. In turn, most human cells and cells from other species

form 5,6,7,8-tetrahydrobiopterin (BH₄), but high output neopterin production is observed in human monocyte-derived macrophages and dendritic cells. BH₄ is the necessary cofactor and hydrogen donor of several monooxygenases including phenylalanine 4-hydroxylase (PAH; EC 1.14.16.1).

Recent studies indicate that the turn-over of the essential amino acid phenylalanine (Phe) is disturbed during inflammatory diseases (1), e.g., increased blood concentrations of Phe have been described in patients with HIV-1 infection, trauma, sepsis, burn, and malignancy, but also with older age.

The reason for the increase of Phe and of Phe/Tyr is still unexplained, Phe/Tyr being a useful measure of PAH activity, better than Phe alone. Increased Phe concentrations as well as an increased Phe and Phe/Tyr in such patients indicate an impaired activity of phenylalanine 4-hydroxylase (PAH; EC 1.14.16.1), which could contribute to the development of neuropsychiatric symptoms because Tyr, the product of PAH, is an important precursor amino acid for the biosynthesis of neurotransmitters like L-DOPA (L-3,4-dihydroxyphenylalanine) and catecholamine hormones dopamine, epinephrine and norepinephrine in the adrenal medulla. Thus, impaired dopaminergic, adrenergic and noradrenergic neurotransmission might develop when Phe concentrations and Phe/Tyr are increased. Indeed, in the elderly, higher Phe and Phe to tyrosine (Tyr) ratio (Phe/Tyr) correlated with neurovegetative symptoms, including sleep disturbance, digestive symptoms, fatigue, sickness, and motor symptoms and with increased inflammation (2). The spectrum of symptoms differed considerably from that which is observed to be related with the enhanced tryptophan degradation during clinical states of immune activation and inflammation which is most probably due to accelerated IDO activity.

Oxidative metabolites induced by IFN- γ may reduce availability of BH₄ which is extremely sensitive to oxidation (1). When BH₄ is destroyed, the conversion rate of Phe to Tyr by PAH becomes impaired and should be reflected by an increased Phe/Tyr. Thus, oxidative stress developing during diseases which are associated with increased production of IFN- γ and neopterin

could be involved in the increase of serum Phe concentrations and Phe/Tyr. Alternatively or in addition, ROS may interfere with redox-sensitive structural elements of proteins such as sulfhydryl residues and thereby influence their tertiary structures (3). This may impair binding of the substrate and/or the cofactor to enzyme PAH (4).

In conclusion, the determination of Phe/Tyr seems to well reflect PAH activity and may allow conclusion about the availability of BH₄ in clinical conditions. Data indicate that in inflammatory diseases BH₄ may become subnormal, and as a consequence not only PAH but also the other BH₄-dependent enzymes namely tyrosine- and tryptophan-hydroxylase as well as nitric oxide synthases and glycerylether monooxygenase may become impaired.

Is Decline of Exhaled Isoprene in Cancer Patients a Consequence of Immune Activation?

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Human breath contains a variety of endogenous volatile organic compounds (VOCs). The origin and pathophysiological importance of these VOCs is poorly investigated. The observation that dogs seem to possess the ability to sniff out early stage colorectal cancer together with other observations initiated studies to examine VOCs in exhaled breath as non-invasive screening for cancer development (1). Breath isoprene (2-methylbuta-1,3-diene; mass: 68D) levels have been reported to be altered in a number of clinical conditions and during efforts (2). Isoprene concentration in exhaled breath therefore might be of diagnostic potential (3). Isoprene represents a precursor molecule of isoprenoids (steroids, ter-

pens), and available data suggests that isoprene is related to cholesterol biosynthesis. However, the physiological meaning of isoprene changes has not been established and isoprene testing has not yet reached the level of routine clinical methods. Nevertheless, lower concentrations of isoprene, acetone (both $p < 0.01$) and of methanol ($p = 0.01$) have been reported recently in lung cancer patients as compared to healthy volunteers (4).

Utilizing proton-transfer-mass spectroscopy (PTR-MS), a new technology for the online detection of VOC patterns, we analyzed VOCs in breath samples in Tedlar bags collected from 78 lung cancer patients (23 females, 56 males; 13 stage I, 4 stage II, 25 stage III and 28 stage IV, 8 patients with missing stage information; 8 grade I, 18 grade II, 30 grade III, 12 grade IV, 10 with missing grade information. In addition, concentrations of immune activation marker neopterin (ELISA, BRAHMS, Hennigsdorf, Germany), lipid parameters (routine enzymology) and C-reactive protein (CRP) were measured.

Isoprene concentrations ranged from 19.4 to 105 [ppb] (mean \pm S.D.: 48.5 ± 16.7 [ppb]) and was lower than in controls. Isoprene concentrations correlated significantly with total cholesterol ($r_s = 0.281$, $p < 0.01$), LDL cholesterol ($r_s = 0.236$, $p < 0.05$) and triglycerides ($r_s = 0.164$, $p < 0.01$) but not with HDL cholesterol ($r_s = 0.048$) and CRP ($r_s = -0.115$, both not significant). There was no relationship with staging, grading or age, but the correlation between isoprene and neopterin was significant ($r_s = -0.215$, $p < 0.05$), and neopterin correlated with total cholesterol ($r_s = -0.343$, $p = 0.001$), HDL cholesterol ($r_s = -0.273$, $p = 0.01$), LDL cholesterol ($r_s = -0.236$, $p < 0.05$) and CRP ($r_s = 0.230$, $p < 0.05$) but not with triglycerides ($r_s = 0.035$, $p > 0.05$).

Our investigation confirms the decreased isoprene exhalation in lung cancer patients, and we found several significant associations of isoprene with parameters of lipid metabolism indicating a link between the decline of isoprene and of cholesterol concentrations in lung cancer patients. Moreover, a significant but rather weak inverse correlation was observed between breath isoprene and serum neopterin concentrations, which were also related to lipid metabolic abnormalities. Similar relationships between elevated neopterin

and decreased lipids concentrations have been reported earlier in patients with HIV-1 infection (5). All together data suggest that immune activation, oxidative stress and oxidation could play a role in the decline of isoprene and probably as a consequence also of lipid metabolic changes. With regards to dogs sniffing cancer it has to be considered that these animals not necessarily need to detect an increased concentration of a compound in breath, it could also relate to the decrease of a specific compound's concentration which can be detected by the dog.

- 1 Rieder J, et al. Analysis of volatile organic compounds: possible applications in metabolic disorders and cancer screening. *Wien Klin Wochenschr* 2001;113:181-5.
- 2 King J, et al. Physiological modeling of isoprene dynamics in exhaled breath. *J Theor Biol* 2010;267:626 - 37.
- 3 Salerno-Kennedy R & Cashman KD. Potential applications of breath isoprene as a biomarker in modern medicine: a concise overview. *Wien Klin Wochenschr* 2005;117:180-6.
- 4 Bajtarevic A, et al. Noninvasive detection of lung cancer by analysis of exhaled breath. *BMC Cancer* 2009;9:348.
- 5 Zangerle R, et al. Decreased plasma concentrations of HDL cholesterol in HIV-infected individuals are associated with immune activation. *J Acquir Immune Defic Syndr* 1994;7:1149-56.

Side Chain Oxidation Modulates Local Dynamics of Phenylalanine Hydroxylase

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The homotetrameric monooxygenase phenylalanine hydroxylase (PheH, EC number 1.14.16.1) catalyzes the hydroxylation of phenyl-

alanine to tyrosine, which is achieved by molecular oxygen and the reductive co-factor tetrahydrobiopterin (BH₄) at the non-heme iron containing active site. Dysfunction of PheH, which catalyzes the committed step of phenylalanine degradation, causes phenylketonuria, a well-examined genetic disease leading to mental retardation. Besides the catalytic domain PheH comprises a tetramerization region and an autoregulatory domain physically blocking the active site in the deactivated state. PheH is further regulated by the cofactor BH₄, phosphorylation of Ser16 in the autoregulatory domain and allosteric binding of phenylalanine. Recent studies demonstrate immune activation and inflammation to increase the ratio of phenylalanine to tyrosine in blood, which indicates an inhibition of PheH. As immune activation of macrophages is paralleled by the release of toxic reactive oxygen species, oxidative stress is discussed as chemical background for PheH inactivation (1). Further modulation of PheH activity has been reported upon reaction with disulfide reagents (2).

To investigate the impact of oxidative stress on PheH dynamics at atomic level, molecular dynamics simulations of the catalytic domain were applied. Inspection of X-ray structures of the catalytic domain revealed one apparent oxidizable site at two cysteine residues (Cys203, Cys334) distant from the active center. Mutations of both cysteine residues were shown to influence PheH activity though their distance to the active site (3, 4). Comparative molecular dynamics (MD) simulations were performed to analyze conformational differences of native PheH and PheH containing a hypothetical disulfide bond between these residues.

Starting from the X-ray structure of human PheH with bound co-factor (PDB code: 1J8U) (5) MD simulations of 100 ns were carried out for each of the systems using the AMBER forcefield ff99SB (6). Analyses of MD trajectories revealed a modulation of local dynamics in a loop region near the active site of PheH. The increased flexibility of the region in the oxidized state is accompanied by a motion of the loop towards the active site which possibly reduces PheH activity analogue to the autoregulatory sequence by physical blocking access to the catalytic center.

A molecular dynamics study of PheH was presented, demonstrating a potential molecular mechanism of PheH downregulation upon oxidative stress by modulation of local dynamics of a loop region near the active site.

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- 1 Ploder M, et al. Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations. *Amino Acids* 2008;35:303-7.
- 2 Koizumi S, et al. Sulfhydryl modification and activation of phenylalanine hydroxylase by dinitrophenyl alkyl disulfide. *Biochemistry* 1987;26:6461-5.
- 3 Guldberg P, et al. A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. *Am J Hum Genet* 1998;63:71-9.
- 4 Eisensmith RC, et al. Molecular basis of phenylketonuria and a correlation between genotype and phenotype in a heterogeneous Southeastern US population. *Pediatrics* 1996;97:512-6.
- 5 Andersen OA, et al. High resolution crystal structures of the catalytic domain of human phenylalanine hydroxylase in its catalytically active Fe(II) form and binary complex with tetrahydrobiopterin. *J Mol Biol* 2001;314:279-91.
- 6 Hornak V, et al. Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins* 2006;65:712-25.

Inhibition of Oxidised Low Density Lipoprotein Initiated Oxidative Stress by 7,8-dihydroneopterin

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Advanced atherosclerotic plaques are characterised by the formation of significant regions of necrotic cells. The death of the macrophage cells within these necrotic zones appears to be driven in part by the cytotoxic effects of oxidised low density lipoprotein (oxLDL). OxLDL is relatively inert chemically but when given to macrophage cells it triggers the generation of elevated levels of intracellular oxidants. This oxidant generation causes cell necrosis through the loss of cellular GSH, key metabolic enzymes and the ability of the cells to generate ATP. *In vitro* 7,8-dihydroneopterin is a potent antioxidant which inhibits oxLDL induced macrophage death. Previous studies suggested that 7,8-dihydroneopterin protects the cells by scavenging intracellular oxidants but the finding that 7,8-dihydroneopterin also decreases oxLDL uptake and CD36 receptor levels suggests that the protection may be more complex (1).

Using human monocyte derived macrophages we have examined the separate effects of oxidant scavenging and reduced oxLDL uptake by 7,8-dihydroneopterin. Measurement of dihydroethidium fluorescence showed OxLDL induced a large intracellular radical flux within 3 hrs of addition to the HMDM cells. 7,8-Dihydroneopterin addition reduced the oxLDL induced radical production by over 90% after 3 hours. HPLC analysis of cell lysates showed 7,8-dihydroneopterin was being rapidly taken up by the cells and significant levels of neopterin were generated during the oxLDL induced radical flux. This supports the hypothesis that 7,8-dihydroneopterin was scavenging intracellular radicals generated in response to the presence of oxLDL. One of the main sterol oxidation products of LDL oxidation is 7-ketocholesterol. Using the 7-ketocholesterol as a marker of oxLDL uptake, 7,8-dihydroneopterin was only found to reduce oxLDL uptake in the macrophages at the 12 hour time point but not before. Western blot analysis showed that CD36 was down regulated over the 12 hours of incubation but appeared to be too slow to account for the protection of cell viability observed with the 7,8-dihydroneopterin. To directly test the protective effect macrophages

were preincubated with with 7,8-dihydro-neopterin for 24 hours to down regulate the CD36. The removal of the 7,8-dihydro-neopterin and the addition of oxLDL resulted in the same level of cell viability loss as observed with the cells not preincubated with 7,8-dihydro-neopterin. These these results clearly show 7,8-dihydro-neopterin can act as a intracellular radical scavenger in macrophage cells exposed to oxLDL and CD36 down regulation has little protective effect.

- 1 Giese SP, et al. Oxidant production, oxLDL uptake, and CD36 levels in human monocyte-derived macrophages are downregulated by the macrophage-generated antioxidant 7,8-dihydro-neopterin. *Antioxid Redox Signal* 2010;13):1525-34.

Immunomodulation of Operating Room Personnel: Evaluation of Neopterin Levels and Tryptophan Degradation

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Various chemicals are found in many workplaces and require careful attention to avoid overt acute poisoning as well as the chronic exposure-related adverse health effects. Operating room personnel such as surgeons, anaesthesiologists, technicians and nurses are exposed to a variety of noxious agents in their workplace. The possible contaminants in air of the operation rooms may produce oxidative stress, sensitization, inflammation, and modulation of the immune system. Nowadays, particular attention is paid to non-invasive early markers of immune modulation such as neopterin, an unconjugated pteridine, and kynurenine, the main degradation product of tryptophan. The present study was undertaken to eval-

uate immunomodulatory effects of anaesthetics in operating room workers by measuring neopterin levels and the kynurenine to tryptophan ratio (kyn/trp) as an estimation of tryptophan degradation. Additionally, the results of all measured parameters in samples obtained from two different hospitals were compared. Two operation room personnel groups from two different hospitals in Ankara (n = 20, each) volunteered for this study. The unexposed group as a control was 30 healthy subjects not working in operating room. Serum tryptophan and kynurenine levels were determined by high-performance liquid chromatography (HPLC) while serum neopterin levels were measured by enzyme-linked immunosorbent assay (ELISA) (BRAHMS, Hennigsdorf, Germany). Serum neopterin levels were 5.36 ± 0.94 nmol/L (mean \pm SD) in controls and 4.97 ± 0.99 nmol/L in exposed group. Kyn/trp were 28.69 ± 5.01 μ mol/mmol and 25.51 ± 3.88 μ mol/mmol in control and exposed groups, respectively. Both neopterin and kyn/trp levels were statistically lower in exposed group ($p < 0.05$, both). No significant differences were observed between the operating room personnel of two hospitals ($p > 0.05$). The data obtained in this study may point out the immunosuppressant effects of anaesthetics. Future analysis with increased number of subjects and additional parameters supporting immunomodulatory effects should be performed.

- 1 Gruber G, et al. Neopterin as a marker of immunostimulation: an investigation in anaesthetic workplaces. *Anaesthesia* 2002;57:747-50.
- 2 Estelberger W, et al. Determination of renal clearance of neopterin by a pharmacokinetic approach. *FEBS Lett* 1993;329:13-6.
- 3 Fuchs D, et al. The role of neopterin as a monitor of cellular immune activation in transplantation, inflammatory, infectious, and malignant diseases. *Crit Rev Clin Lab Sci* 1992;29:307-41.

Spontaneous and Stimulated Neopterin Production and Tryptophan Degradation in Freshly Isolated PBMC from Healthy Donors

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Human peripheral blood mononuclear cells (PBMC) proliferation assays are widely used to characterize functional immune status of healthy individuals or patients with HIV-1 infection and cancer, respectively. During the past few years we have applied a PBMC assay to test for a potential immunomodulatory effect of compounds, drugs and plant extracts using unstimulated cell preparations and cells stimulated with mitogens phytohaemagglutinin (PHA) and/or concanavalin A (Con A) (1,2). The stimulation degree of PBMC was determined by the measurement of neopterin production and tryptophan degradation, both biochemical pathways being efficiently induced by T-cell-derived cytokine interferon- γ . In this study we investigated possible associations between these read-outs in unstimulated and stimulated PBMC and the demographics of donors as well as haemoglobin concentrations and liver enzyme glutamic-pyruvic transaminase (GPT) activity.

Freshly isolated PBMC from whole blood of 173 healthy blood donors (78 females, 85 males, aged mean \pm S.D.: 42.2 \pm 13.8 years) collected during 2006-2009 were used for proliferation assays. Cells were isolated by standard separation techniques using density gradient centrifugation. For stimulation of PBMC 10 μ g/ml PHA or Con A were added. Neopterin concentration was measured by ELISA (BRAHMS, Hennigsdorf, Germany). Tryptophan and kynurenine concentrations were determined by HPLC via fluorescence and UV absorption detection as described earlier (3).

Plasma neopterin concentrations of blood donations were 5.5 \pm 1.42 nmol/L, haemoglobin was 152 \pm 17.1 g/dl and GPT was 29.4 \pm 15.5 (U/L). Neopterin concentrations were positively correlated with donor age ($r_s = 0.360$, $p < 0.001$),

and there existed a good correlation between neopterin production and tryptophan degradation in the blood donors ($r_s = 0.304$, $p < 0.001$). Looking at the *in vitro* data, also in the supernatants of cultured unstimulated PBMCs a significant correlation between neopterin production and tryptophan degradation expressed as the kynurenine to tryptophan ratio (kyn/trp) was evident ($r_s = 0.555$, $p < 0.001$). Also the decline of tryptophan ($r_s = -0.709$) and the increase of kynurenine concentrations ($r_s = 0.655$) correlated with neopterin concentrations (both $p < 0.001$). After stimulation of PBMCs with either mitogen, significant correlations were observed between neopterin production (inverse), tryptophan degradation (positive) and kynurenine production (positive, all $p < 0.001$). No different effects were observed when the two mitogens were compared. There were also no significant differences between males and females in any of the measurements performed (neopterin, kyn/trp, tryptophan, kynurenine) of donors, cultured unstimulated PBMCs and stimulated PBMCs, respectively. Only neopterin production upon PHA stimulation was significantly higher in males than in females ($U = 2.75$, $p < 0.01$) in absolute values, but this significant difference was lost when concentrations were expressed in percent of baseline concentrations.

In conclusion, an association between age and neopterin concentrations in blood donors could be found, and there was good correlation between neopterin concentrations and kyn/trp in blood donors. Both these findings agree well with existing literature. Also the spontaneous neopterin production and tryptophan degradation correlated strongly in resting PBMC. The neopterin and kyn/trp response was similar in PBMC stimulated with either mitogen and no obvious influence of sex on results of mitogen-stimulated PBMC proliferation was observed.

- 1 Schroecksnadel K, et al. Antioxidants down-regulate Th1-type immune response *in vitro*. *Drug Metabol Lett* 2007;1:166-71.
- 2 Jenny M, et al. *In vitro* testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors. *Inflamm Res*

2011;60:127-35.

- 3 Widner B, et al. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. Clin Chem 1997;43:2424-6.

Potential Role of Biogenic Amines in Prenatal Depression and Stress

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Prenatal depression occurs in up to 20% of pregnancies and is associated with many unfavorable pregnancy outcomes. The causes of prenatal depression vary, and include poverty, stress, and preexisting depression, among other etiologies. We have recently found a correlation between prenatal depression and *Toxoplasma gondii* IgG titers.

This is a report of 370 *T. gondii* negative women between 16 and 25 weeks of pregnancy. *T. gondii* positive women are excluded because of the potential immune activation in seropositive immunocompetent individuals, and as immune activation results in activation of indoleamine 2,3-dioxygenase (IDO).

We measured plasma precursor amino acids of biogenic amines (HPLC) associated with mood and found that women with scores indicating clinical prenatal depression (n = 20) had significantly lower tryptophan (p < 0.02; Mann Whitney U-test) and phenylalanine (p < 0.01) levels, a lower phenylalanine /tyrosine ratio (p < 0.01, and higher nitrite concentrations (p = 0.05). Both tyrosine and phenylalanine were significantly inversely correlated with perceived stress, as measured by Cohen's Perceived Stress Scale. Other correlates of depression in both *T.gondii* positive and negative pregnant women included poverty, perceived

stress scores, and number of children.

A subset of the sample (n = 120) had plasma cytokines, interferon- γ , tumor necrosis factor- α , and interleukin-10, measured by multiplex technology. The only correlates of amino acids were an inverse relationship between interferon- γ and the nitrite concentrations (r = -0.20, p < 0.03) and a nearly significant relationship between tryptophan and tumor necrosis factor- α (r = 0.18, p = 0.059).

These data implicate complex neuroimmune pathways in prenatal depression and stress. Depletion of monoamines could result from poor diet or changes in metabolic pathways. Essential amino acid involved in serotonergic, adrenergic, and nitric oxide pathways appear to play some role in prenatal depression and perceived stress and may potentially interact with neuroimmune pathways to affect cellular immunity. Whether the unique physiological features of pregnancy are important in these relationships remains to be studied.

Wittgenstein, Gödel and the Vienna Circle

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Around 1918 philosophers and scientists met in Viennese coffeehouses to discuss developments in logic and philosophy of science. This led to the formation of the Vienna Circle ("Wiener Kreis") around the German physicist/philosopher Moritz Schlick. I discuss the Circle's dream to create an algorithmic unified science ("Einheitswissenschaft") based only on empirical observations, free of metaphysical concepts and constructed using formal logical methods developed by Bertrand Russell. These methods were brought to Vienna by Ludwig Wittgenstein (LW), the author of the *Tractatus Logico-Philosophicus* (TLP) and a "student" of Russell. Realizing the misinterpretation of the TLP by the members of the Circle, LW's sympathy for its program faded quickly and permanently. The logician and lifelong Platonist Kurt Gödel participated occasionally in the

Circle's meetings and was also an early, but restrained critic of the Circle's logical positivism.

The work of the Circle continued and in 1929 its members produced a manifesto ("Die Wissenschaftliche Weltauffassung") in honor of Schlick. I describe some of the tenets of this pamphlet (e.g. the Verification Principle) and point out implications of the Circle's ideas for currently used statistical approaches. A description of the Diaspora of the Circle's members to the US and the UK after the assassination of Schlick on the steps of the University of Vienna in 1936 concludes the presentation.

Impact of Lunar Phases on Neopterin Dynamics in a Healthy Woman

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Due to the moon's gravitational forces on the tides, its influence on human physiology, behavior and health has often been discussed. However, evidence is inconsistent. This integrative single-case study on a healthy 25-year old woman investigated the impact of lunar phases on cellular immune activity using time-series analysis. For this purpose, the proband collected her entire urine in 12-hour intervals for a period of 63 days for the determination of urinary neopterin. Observation time lasted from December 16th, 1999 to February 16th, 2000. Information on lunar phases was gathered from the Institute of Astrophysics, University Innsbruck, Austria. Lunar phases were parameterized using a cosine function which defined full moon as value 1 and new moon as 0. Two full moons occurred during the observational period, the first on December 22nd and the second on January 21st (lunar cycle length = 30 lags). Unadjusted cross-correlational analyses revealed that there was a significant lunar influence on the proband's immune system, particularly during the night where the human immune system seemed to be synchronized to this cosine curve. Strongest neopterin increases were

found 6 days after full moon (lag6: $r = .482$; $p < 0.05$) whereas strongest decreases occurred 6 days after new moon (lag21: $r = -.513$; $p < 0.05$). This investigation showed that adequate research designs are needed to study the highly dynamic nature of the lunar-immune interaction. Though, the current results are encouraging further integrative single-case studies must follow for generalization.

Neopterin During Targeted Therapy for Metastatic Renal Cell Carcinoma

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Recently, targeted agents, including bevacizumab, sorafenib, sunitinib, pazopanib, temsirolimus and everolimus, have shown significant activity in patients with metastatic RCC. Unfortunately, there are currently no biomarkers that could predict response to targeted agents in this disease. In earlier studies, it was demonstrated that increased urinary neopterin concentrations predict poor prognosis in patients with metastatic renal cell carcinoma (RCC). Urinary and serum neopterin concentrations were followed in metastatic RCC patients treated with bevacizumab/interferon-alpha combination, sunitinib, sorafenib or everolimus. In patients treated with bevacizumab-based combinations, increased neopterin concentrations were observed after interferon-alpha administration, but the administration of mammalian target of rapamycin inhibitors did not lead to significant changes of neopterin concentrations. Initial urinary neopterin

concentrations above 214 $\mu\text{mol/mol}$ creatinine were associated with poor prognosis. In conclusion, neopterin is a significant prognostic indicator in patients with metastatic RCC treated with targeted therapy.

Rapid Accurate Testing Systems for Markers of Inflammation

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Excellent point-of-care systems can be made for large molecular weight analytes. The home pregnancy test show clearly what can be achieved. However, the performance of classical immunoassay is often found challenging when applied to small molecular weight analytes, for example, biomarkers of inflammation such as pteridines. The problem is simply size. Large analytes can support the simultaneous binding of both capture and detector antibodies, allowing typical excess-reagent sandwich immunoassays to be formatted in which increasing analyte concentration provides an increase of observable signal over a very low zero background. Small molecules are simply not large enough to support such simultaneous binding. Alternative, inferior, 'competitive-format' systems in which analyte is cast into a competitive role with analyte-analogue for capture antibody sites have therefore had to be used. In effect, such systems measure how much analyte is not present. This causes major problems in terms of precision and sensitivity where, classically, increasing concentration of analyte reduces the signal produced by the system - making devices often difficult to read and assess. What is required are robust generic systems in which there is no observable signal in the absence of analyte but even low level samples give an obvious, quantitatively measurable, signal over this zero background. This need has led to our developing the Selective and Apposition systems. In these a secondary antibody against a pri-

mary anti-analyte antibody is raised that can: (i) still bind the primary antibody when the primary antibody has itself bound to the small molecular weight analyte but (ii) not bind the primary antibody in the absence of analyte - when the primary antibody has been free to bind a specific blocking molecule into its antigen binding site. Secondary antibody binding therefore becomes quantitatively analyte-dependent. Among the various areas to which these systems have now been successfully applied include those of rapid quantitative drug, pesticides and cortisol detection. Other markers of inflammatory disease, such as neopterin, are the focus of our present work which will be described.

Cell-based Assays and Gene Expression Profiling in Human Hepatoma HepG2 Cells Exposed to the Polyherbal Formula PADMA 28 Reveal Direct as Well as Indirect Antioxidant Capacities by Transactivation of Cytoprotective Genes.

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The polyherbal preparation PADMA 28 is derived from an ancient Tibetan formula, which contains twenty different powdered plants including natural camphor and calcium sulphate and was traditionally applied for the treatment of diseases associated with inflammation. To date, a number of studies have been conducted on the effects of PADMA 28 on biochemical pathways associated with inflammation. A significant suppressive effect of PADMA 28 on neopterin production and tryptophan degradation was also described in stimulated and unstimulated human peripheral blood mononuclear cells (1). PADMA 28 also showed promising results in experimental models of atherosclerosis and in patients suffering from chronic hepatitis B infections. In 1977, PADMA 28 has been approved by the Swiss drug authorities (Swissmedic) as a Tibetan medicine

for the symptomatic treatment of circulatory disorders, including those of peripheral arterial occlusive disease. In addition to direct radical absorbance capacities of an antioxidant, a potential antioxidant for the treatment of oxidative stress-related ailments should provide also further properties such as the interference with enzymes involved in cytoprotective mechanisms. To gain insight into the transcriptional changes influenced by PADMA 28 in human liver-derived HepG2 cells, we applied gene expression profiling using Affymetrix U133 Plus 2.0 DNA microarrays complemented with pathway- and network-oriented analysis. The obtained results were validated using reporter gene analysis and quantitative real-time PCR. Additionally, cell-free and cell-based assays were used to evaluate the antioxidant capacity of PADMA 28. In this study, we could show that PADMA 28 exhibits potent direct radical scavenging effects by a remarkable high oxygen radical absorbance capacity and a potential to decrease peroxy-radical-induced formation of intracellular reactive oxygen species in HepG2 cells, as well as indirect antioxidant capacities by transactivation of cytoprotective genes. Integrated transcriptome analysis revealed a major influence of PADMA 28 on pathways associated with phase I and II drug metabolism. Although extrapolation of these findings obtained in HepG2 cells to the *in vivo* situation is challenging, the presented findings provide further evidence for prospective beneficial effects of PADMA 28 during treatment of oxidative stress-related pathologies.

- 1 Neurauter G, et al. PADMA 28 modulates interferon-gamma-induced tryptophan degradation and neopterin production in human PBMC *in vitro*. *Int Immunopharmacol* 2004;4:833-9.

Effective Induction of Indoleamine 2,3-dioxygenase Competence Without Neopterin Release in Human Monocyte Derived Dendritic Cells After Activation With Prostaglandin E2

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Indoleamine 2,3-dioxygenase (IDO), the rate limiting enzyme for tryptophan catabolism, is generally regarded a feedback mechanism of immune activation. Neopterin, a low molecular mass compound synthesized from guanosine-triphosphate, has been shown to correlate with immune activation and IDO activity under several pathogenic conditions (1-3). In our recent studies we showed that human monocyte-derived dendritic cells (DCs) stimulated with lipopolysaccharide (LPS) and interferon- γ (IFN- γ) displayed sustained IDO competence. These IDO-competent DCs inhibited allogeneic T-cell responses and supported the induction of suppressive T cells under specific culture conditions *in vitro*, pointing at the tolerogenic potential of IDO (4). In these studies, neopterin release was closely related to IDO competence.

Prostaglandin E2 (PGE2) has recently been described to induce IDO competence in human DCs (5, 6). Yet, the immunomodulatory potential of such stimulated DCs was controversially discussed (7). We hypothesized that - similar to IFN- γ -stimulated DCs - PGE2-stimulated IDO-competent human DCs would exert their tolerogenic potential under certain culture conditions.

We show that PGE2 like IFN- γ can induce sustained IDO competence, i.e. IDO activity even after removal of the IDO-stimulating agent. However, only DCs stimulated with IFN- γ concomitantly released neopterin (average 95nmol/L) while the production of neopterin was low in DCs stimulated with PGE2 (average 3nmol/L).

When testing the stimulatory capacity of differently stimulated DCs we found that PGE2-stimulated IDO-competent DCs potently suppressed allogeneic T-cell responses at a high DC:T-cell ratio, similar to our former observations with IFN- γ -stimulated IDO-competent DCs. Suppression could be reversed by addition of the IDO inhibitor MTHF. Additionally, we analysed neopterin release in cell cultures of differently stimulated IDO-competent DCs that were either

re-cultured alone or in the presence of allogeneic T cells for different time periods. Strikingly, even though we did not find considerable differences in proliferation of allogeneic T cells after co-culture with either IFN- γ -stimulated or PGE2-stimulated IDO-competent DCs independent of DC:T-cell ratio, high neopterin release (range 45 to 85nmol/L) was restricted to co-cultures with IFN- γ -stimulated DCs. Neopterin release remained low (average 10nmol/L) in co-cultures with PGE2-stimulated DCs.

In a next step we tested whether suppression of T-cell responses by IDO-competent DCs was sustained upon re-stimulation. Surprisingly, a re-stimulation of the inhibited T cells with IDO negative stimulators resulted in enhanced proliferation. Additionally, activated CD25⁺ T cells, sorted after co-culture and re-cultivated, showed enhanced proliferative capacity upon a second stimulus and even without stimulation, in contrast to sorted CD25⁻ T cells. These data provide evidence that IDO-mediated tolerance induction in allogeneic T cells functions in a reversible manner.

Finally, we examined whether PGE2-stimulated IDO-competent DCs would support the formation of regulatory T cells. We recently showed that IFN- γ -stimulated IDO-competent DCs supported the induction of regulatory activity in allogeneic T cells only under specific culture conditions (re-supplementation with fresh medium during the culture period). Applying these conditions on co-cultures of PGE2-stimulated DCs and allogeneic T cells resulted in a CD25⁺ T cell population that was able to potently suppress proliferation of fresh T cells upon polyclonal stimulation.

Together, our observations suggest that DC-stimulating molecules, such as IFN- γ or PGE2, which are believed to be associated with promoting inflammatory immune responses, would concomitantly induce counter-regulatory (anti-inflammatory) pathways, such as IDO. Thus, IDO induction may constitute a feedback mechanism helping to limit excessive immune responses. However, as shown for PGE2, IDO induction is not necessarily related to neopterin release.

1 Murr C, et al. Immune reaction links disease progression in cancer patients with depression.

Med Hypotheses 2000;55:137-40

- 2 Huang A, et al. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. *Brit J Cancer* 2002;86:1691-6
- 3 Capuron L, et al. Interferon-alpha-induced changes in tryptophan metabolism. Relationship to depression and paroxetine treatment. *Biol Psychiatry* 2003;54: 906-14
- 4 Jürgens B, et al. Interferon-gamma triggered indoleamine 2,3-dioxygenase competence in human monocyte-derived dendritic cells induces regulatory activity in allogeneic T cells. *Blood* 2009;114: 3235-43
- 5 Braun D, et al. A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation. *Blood* 2005;106:2375-81.
- 6 von Bergwelt-Baildon MS, et al. CD25 and indoleamine 2,3-dioxygenase are up-regulated by prostaglandin E2 and expressed by tumor-associated dendritic cells *in vivo*: additional mechanisms of T-cell inhibition. *Blood* 2006;108:228-37.
- 7 Krause P, et al. Prostaglandin E2 is a key factor for monocyte-derived dendritic cell maturation: enhanced T cell stimulatory capacity despite IDO. *J Leukoc Biol* 2007;82:1106-14

Urinary Neopterin and the Risk of Atherosclerosis in Patients with Breast Carcinoma

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With improved survival of many types of cancer, long-term sequelae of the disease or therapy have become an important issue. An increased incidence of complications of atherosclerosis has been noted in cancer survivors. The aim of the present study was to investigate relation between intima-media thickness (IMT) and laboratory parameters of atherosclerosis risk in patients with breast carcinoma. One-hundred and ninety-two female patients with histologically verified breast carcinoma were included in the present study. IMT was assessed with sonography. Retinol, alpha-tocopherol, glycosylated hemoglobin and urinary neopterin were measured by high performance liquid chromatography. C-reactive protein (CRP), lipoprotein (a), cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, homocysteine and urinary albumin were determined immunochemically. Peripheral blood cell count, D-dimers, fibrinogen, glucose, magnesium, creatinine, uric acid, albumin and urinary N-acetyl-beta-D-glucosaminidase (NAG) were determined by routine methods. Differences between groups of patients were analyzed by the Mann-Whitney U test. Correlations were analyzed using Spearman's rank correlation coefficient. Patients with metastatic disease had significantly higher fibrinogen, CRP, urinary neopterin and mean IMT, and significantly lower serum albumin. Significant correlations were observed between CRP, urinary neopterin, mean IMT and other parameters of cardiovascular risk. In conclusion, systemic inflammatory response in patients with advanced breast cancer is associated with increased risk of atherosclerosis.

Modulation of Alkylglycerol Monooxygenase Activity in RAW264.7 Macrophage Cell Lines

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The tetrahydrobiopterin dependent enzyme alkylglycerol monooxygenase is so far the only enzyme known able to cleave the ether bond of ether lipids. Though already described for the first time in 1964, the enzyme has been resistant to sequence assignment for decades. Recently we were finally able to show that TMEM195 is the gene coding for alkylglycerol monooxygenase. Many properties of alkylglycerol monooxygenase are so far not described and especially its physiological function remains unknown. In this work we want to use the obtained sequence information to clarify the physiological role of alkylglycerol monooxygenase by studying its regulation in the mouse macrophage-like RAW264.7 cell line:

In order to answer the question if TMEM195 is the only gene responsible for ether lipid cleavage, we introduced three different shRNA-constructs targeting the TMEM195 mRNA into RAW264.7 cells by lentiviral transduction. In these cell lines, knockdown can be induced with doxycyclin via a tet-repressor. An shRNA construct targeting luciferase served as control. Alkylglycerol monooxygenase activity in cell pellets of all three knockdown lines were strongly reduced, while there was no effect on the luciferase control. These data we can confirm that TMEM195 is the major gene responsible for alkylglycerol cleavage.

The influence of intracellular tetrahydrobiopterin levels on ether lipid degradation was measured by inhibition of tetrahydrobiopterin synthesis with a specific GTP-CH1 inhibitor (DAHP). A fluorescent labelled alkylglycerol was used to measure the capability of RAW264.7 cells to cleave ether lipids. Addition of 5 mM DAHP strongly reduced tetrahydrobiopterin to levels below the detection limit and reduced alkylglycerol monooxygenase activity 8-fold. Treatment with sepiapterin recovered intracellular tetrahydrobiopterin concentrations as well as alkylglycerol monooxygenase activity. These results show that alkylglycerol monooxygenase activity in living cells is directly dependent on intracellular BH₄ concentrations.

Dynamic Profiles of Volatile Organic Compounds (VOCs) in Exhaled Breath - a Non-Invasive Window to the Body

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Breath gas analysis is based on the compelling concept that the exhaled breath levels of endogenously produced volatile organic compounds (VOCs) can provide a direct, non-invasive window to the blood and hence, by inference, to the body. In this sense, breath VOCs are regarded as a comprehensive repository of valuable physiological and clinical information, that might be exploited in such diverse areas as diagnostics, therapeutic monitoring or general dynamic assessments of metabolic function, pharmacodynamics (e.g., in drug testing) and environmental exposure (e.g., in occupational health).

The success of using breath VOC concentration profiles for tracking physiological and metabolic processes will mainly hinge on the availability of valid mechanistic descriptions for the observable exhalation kinetics of the trace gas under scrutiny. However, quantitative approaches remain challenging due to the multifaceted impact of medical parameters (such as cardiac output or breathing patterns) as well as due to the sparse and often conflicting data regarding potential biochemical sources or sinks of such substances in the human body.

Within this framework, we focus on real-time measurements of VOCs during distinct physiological states, e.g., rest, exercise, and sleep. An experimental setup combining breath-by-breath analyses using proton transfer reaction mass spectrometry (PTR-MS) with data reflecting the

behavior of major hemodynamic and respiratory variables is presented. Furthermore, a methodology for complementing continuous VOC profiles obtained by PTR-MS with simultaneous SPME/GC-MS measurements is outlined. These investigations allow for the identification of several species revealing characteristic rest-to-work transitions in response to variations in ventilation or perfusion, such as isoprene, methyl acetate, butane, DMS, acetone, and 2-pentanone.

Building on these experimental findings, the second part of this contribution is devoted to a thorough study of the physiological flow of acetone and isoprene, which rank among the most notable compounds studied in the context of breath gas analysis. In particular, based on its end-tidal breath concentration dynamics during exercise, various lines of supportive evidence for an extrahepatic tissue source of isoprene are presented. The results discussed are a first step towards employing breath gas analysis as a tool for quantitatively examining general exhalation, storage, transport, and biotransformation processes associated with volatile organic compounds *in vivo*.

Fatigue is Related with Immune-Mediated Tryptophan Degradation in Patients with Lung Cancer

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Fatigue and decreased quality of life are frequently encountered in patients suffering from cancer. Immune activation and immune-mediated degradation of the essential amino acid tryptophan might be involved in the development of these common complications of tumor disease.

Fifty patients with lung cancer answered ques-

tionnaires, namely the fatigue subscale (FACT-F1) of FACT-G and the anaemia subscale (FACT-An) of FACT-G, and the MAC (Mental adjustment to cancer) questionnaire to assess their fatigue and coping capacity. Furthermore patients scored their quality of life and fatigue feeling on a scale ranging from 1-5 (1 = best score; 5 = worst score), and the physical performance status of patients was assessed by ECOG scores. Neopterin, C-reactive protein as well as haemoglobin concentrations and tryptophan degradation were determined in the blood of patients and were correlated with scores of the performed tests.

Patients with lung cancer suffered from an increased fatigue feeling, half of the patients reported about moderate to very severe fatigue. Markers of inflammation and immune activation were elevated in patients, and also enhanced immune-mediated tryptophan degradation was found. Fatigue scores were associated significantly with concentrations of inflammatory markers, immune-mediated tryptophan degradation and haemoglobin concentrations of patients. Immune-mediated tryptophan degradation was enhanced in patients with an impaired physical performance, patients who reported about a worse quality of life had lower tryptophan concentrations. Neither immune activation nor tryptophan catabolism was related with coping strategies of patients (i.e. MAC scores). Higher CRP and haemoglobin values, increased tryptophan degradation and lower fatigue scores were predictive for the survival of patients (10 patients died within 3 months of follow-up).

Immune-mediated tryptophan degradation might play a role in the development of fatigue and an impaired quality of life. However, coping strategies of patients do not seem to be influenced by immune activation or tryptophan catabolism.

On the Association between Vitamin D and Neopterin Concentrations in Patients with Dementia

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In patients with Alzheimer's disease (AD), pathologically low 25-hydroxyvitamin D concentrations have been described (1), and lower vitamin D levels were associated with a worse cognitive test performance. E.g., correlations were found between vitamin D concentrations and mini-mental-state examination (MMSE) scores, as well as low levels of vitamin D were associated with substantial cognitive decline in the elderly population (2-4). However, in healthy adults without dementia no such relationship was found (4). Still there is also some hope for new preventive strategies for cognitive impairment using vitamin D supplements. Vitamin D deficiency is common in a wide range of inflammatory diseases, and earlier a significant relationship between lower vitamin D and higher neopterin concentrations was observed in patients with HIV infection (5) and in cardiovascular diseases (6). Data suggest a link between the chronic inflammatory status of patients and the increased risk of developing vitamin D deficiency.

In this study, in 43 patients with AD (26 female, 17 male; age: 81.7 ± 10.5 years), concentrations of vitamin D3 (colecalciferol) were measured by RIA and were compared to cognitive test results (MMSE, and clock drawing test), and serum concentrations of neopterin (ELISA; BRAHMS, Hennigsdorf, Berlin), and tryptophan and kynurenine (HPLC) were measured, the kynurenine to tryptophan ratio (kyn/trp) was calculated.

In patients with AD, vitamin D concentrations were mean \pm S.D.: 12.1 ± 8.5 ng/ml, 21 patients presented with <10 ng/L vitamin D. Vitamin D concentrations tended to be lower in patients with older age ($r_s = -0.173$, not significant), there was however no association vitamin D levels with the cognitive decline according to MMSE and clock test results correlated positively (!) ($r_s = 0.267$, $p < 0.05$). Older age was associated with higher neopterin concentrations ($r_s = 0.596$, $p < 0.001$) and kyn/trp ($r_s = 0.373$, $p < 0.01$), both findings confirming the known association between ageing and immune activation and inflammation also

in patients with AD. Higher neopterin concentrations were associated with lower tryptophan levels ($r_s = -0.288$, $p < 0.05$) and higher kynurenine concentrations ($r_s = 0.415$, $p < 0.01$) and a higher tryptophan degradation rate as indicated by kyn/trp ($r_s = 0.659$, $p < 0.001$). There was only a weak tendency of a correlation between higher neopterin and lower vitamin D levels ($r_s = -0.172$, not significant). Neopterin concentrations correlated with MMSE ($r_s = -0.320$, $p < 0.05$) and the clock test ($r_s = -0.270$, $p < 0.05$). AD patients with MMSE < 15 were older ($U = 2.45$, $p < 0.05$), had higher neopterin concentrations ($U = 2.27$, $p < 0.05$) and a trend to higher kyn/trp ($U = 1.82$, $p < 0.06$) as compared with those with better cognitive performance. There were neither such relationships to the clock-test results nor between any of these parameters and vitamin D concentrations.

Our results add to the discussion that the association found between hypovitaminosis D and cognitive impairment is inconclusive when a more homogenous group of patients is studied. Still our data confirm that patients with AD frequently present with subnormal vitamin D levels. However, no clear relationship existed between vitamin D concentrations and cognitive test performances. Also no significant correlation was found between increased immune activation and the high rate of hypovitaminosis D, albeit some coincidence of such abnormalities was observed in the whole group of patients in this study. Still our study fails to show any statistically significant relationship between higher neopterin and lower vitamin D concentrations in AD patients. Inflammation and immune activation were confirmed to relate to the cognitive decline of patients, but any possible role of vitamin D supplementation in prophylaxis and prevention of AD remains unclear. More prospective trials with long follow up periods are still needed.

- 1 Sato Y, et al. High prevalence of vitamin D deficiency and reduced bone mass in elderly women with Alzheimer's disease. *Bone* 1998;23:555-557.
- 2 Oudshoorn C, et al. Higher serum vitamin D3 levels are associated with better cognitive test performance in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord*

2008;25:539-543.

- 3 Annweiler C, et al. Association of vitamin D deficiency with cognitive impairment in older women: cross-sectional study. *Neurology* 2010;74:27-32.
- 4 Przybelski RJ & Binkley NC. Is vitamin D important for preserving cognition? A positive correlation of serum 25-hydroxyvitamin D concentration with cognitive function. *Arch Biochem Biophys* 2007;460:202-205.
- 5 Haug C, Müller F, Aukrust P, Frøland SS. Subnormal serum concentration of 1,25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. *J Infect Dis* 1994;169:889-893.
- 6 Murr C, et al. Inflammation and immune activation may underlie vitamin D deficiency. The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Pteridines* (in press).

Impact of Early Morning Bright Light Exposure on Neopterin, Melatonin and Tryptophan Metabolites

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The daily pattern of consolidated sleep and wake is strongly influenced by the timing of exposure to light and darkness. Workplace concentration and output depends on a variety of endogenous and exogenous factors, some exhibiting circadian variations. Melatonin is a key hormone for the regulation of the circadian rhythm in humans showing an acrophase in the early morning hours and declining to low levels during daytime. It is well established that a significant numbers of employees suffer from early morning lassitude at their workplaces. Bright light is known

to suppress melatonin formation. Therefore we hypothesize that early morning full spectrum bright light (5.000 lux) stimulates alertness and concentration due to a reduction in melatonin concentration. Moreover, we hypothesize that bright light exposure suppresses the melatonin precursor tryptophan and also neopterin formation.

A prospective cross-over study was performed, 31 healthy volunteers (mean age and SD 33.1 ± 7.3 years; 14 males, 17 females) participated. Urine samples of melatonin (measured as aMT6-s) and neopterin (neopterin/creatinine-ratio; measured by HPLC) were collected at 6:00, 7:05, 9:20 and 11:30 a.m.. Tryptophan was analysed by HPLC from serum collected at 7:10, 9:30 and 11:40 a.m.. Subjects were exposed to full spectrum light (5.000 lux, 30 min; light cabin produced by Bartenbach LichtLabor, Aldrans) and with one week interruption to normal light (400 lux, 30 min) at 7:45 a.m.. Thereafter a computerized test to analyse selective attention and concentration ability (DAUF, testform S3 Vienna test system) was performed. For statistical analysis MANOVA for repeated measures was applied.

The participants showed more adequate choice reactions and shorter reaction times under standard light (400 lux) as compared to intensive bright light (5.000 lux). aMT6-s decreased between 6:00 and 11:30 a.m. under both experimental light conditions with no significant difference between 400 lux and 5.000 lux. There were no changes in serum tryptophan and urinary neopterin within and between both light designs.

The initial hypothesis that early morning bright light exposure (5.000 lux) improves concentration has to be rejected. It may be possible that light intensity was too high and exposure time in the light cabin was too long causing overfatigue of the visual system. Since the morning reduction in melatonin was independent of light intensity an early morning bright light exposure has no additional effect on the circadian rhythm of this hormone. Early morning bright light does not influence tryptophan and melatonin concentrations.

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Biomarker for Detection of Viral Infection-Neopterin in Combination with C-Reactive Protein

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A misuse of antibiotics has been observed in many countries and is resulting in the development of multiple resistant pathogens. This study has investigated the value of the CRP to neopterin (C/N) ratio to differentiate bacterial from viral aetiology in patients. A neopterin immunotest was developed as a rapid screening for patients with viral infection.

Serum samples were collected from respiratory tract infection (RTI) patients at Emergency Department. The CRP and neopterin concentrations determined by ELISA and C/N ratio were calculated. A semi-quantitative immunotest called InfectCheck NeoPT was developed to test with the serum samples and results were compared to a commercial neopterin ELISA.

A total of 561 patients were recruited to the study. Viral and/or bacterial aetiological associations were detected in 35% of patients. The median of C/N ratio was 13-times higher in bacterial infections than viral infections (15.2 vs 1.2 mg/nmol; $P < 0.0001$), and 51 times higher than those in healthy subjects (15.2 vs 0.3 mg/nmol; $P < 0.0001$). Sensitivity analysis achieved a good sensitivity (95%) and specificity (94%) for ruling in/out bacterial/viral infection using a C/N ratio > 3 as the cutoff.

A total of 217 samples were measured by the developed immunotest. Results showed 78.8% agreement with the neopterin ELISA.

C/N ratios showed a good potential in differen-

tiating bacterial from viral causes. The developed neopterin immunotest had a good agreement (78.8%) with ELISA. Such immunotest has a great potential in screening for any unknown viral infection. With the combination of CRP and neopterin, the differentiating power could be enhanced and allow a more appropriate treatment.

Donor Preconditioning with Tetrahydrobiopterin Protects Murine Aortic Allografts from Chronic Allograft Vasculopathy

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Chronic allograft rejection characterized by chronic allograft vasculopathy is still a major obstacle to long term graft survival and is thought to be strongly associated with ischemia reperfusion injury. Previously, the essential cofactor for nitric oxide synthases tetrahydrobiopterin was shown to significantly reduce ischemia reperfusion injury in a murine pancreas transplantation model. Herein we analyzed whether attenuation of ischemia reperfusion injury by tetrahydrobiopterin pretreatment of the donor may also attenuate chronic allograft vasculopathy.

A fully MHC mismatched (BALB/c to C57BL/6) mouse cervical aortic transplantation model was used. Grafts were subjected to 24h cold ischemia time. Donor animals received either tetrahydrobiopterin (50mg/kg b.w.) or saline. Grafts without cold ischemia time and syngeneic donor-recipient pairs (C57BL/6 to C57BL/6) served as controls. Ten hours following graft reperfusion glutathione tissue levels and CD-31 immunohistochemistry were analyzed to assess ischemia reperfusion injury associated damage. Four weeks following reperfusion inti-

mal hyperplasia, an indicator of chronic allograft vasculopathy, was quantified by histopathology (H.E. staining) and immunohistochemistry (alpha-smooth muscle actin, α -SMA).

Prolonged cold ischemia time resulted in a significant reduction of glutathione tissue levels ($p < 0.05$). In contrast, tetrahydrobiopterin treatment restored glutathione tissue levels ($p < 0.05$). Additionally, reduced CD-31 expression following cold ischemia time was attenuated by tetrahydrobiopterin ($p < 0.05$). Four weeks after transplantation pretreatment with tetrahydrobiopterin resulted in attenuation of the prominent intimal hyperplasia observed in untreated grafts ($p < 0.001$), which was similar to syngeneic controls and grafts without cold ischemia time (not significant). Treatment with tetrahydrobiopterin elicited a significant reduction of α -SMA positive cells within the intima ($p < 0.05$).

These data show that the degree of ischemia reperfusion injury strongly correlates with chronic allograft vasculopathy development. Donor preconditioning with tetrahydrobiopterin may therefore represent a promising strategy to prevent chronic allograft vasculopathy.

Urinary Neopterin in Patients with Metastatic Colon Cancer Treated with Patupilone

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Despite increased efficacy of regimens combining cytotoxic drugs and targeted agents, most patients with metastatic colorectal cancer will ultimately die of the disease. Only a limited number of drugs have reproducible activity in this

tumor, and new active agents are urgently needed. Promising results in the therapy of metastatic colorectal cancer have been reported for patupilone, an epothilone analogue. Similarly to taxanes, the cytotoxic mechanism of epothilones involves the stabilization of microtubules. Increased serum or urinary concentrations of neopterin have been described in patients with tumors of different primary locations, including colorectal cancer. Neopterin concentrations were reported to increase during the administration of cytotoxic agents, including taxane-based chemotherapy. We have studied serum neopterin in patients with colorectal cancer before and during the therapy with patupilone. Urinary neopterin was determined by high performance liquid chromatography. Increased urinary neopterin concentrations were observed at baseline in the majority of the patients. In most patients, neopterin concentrations further increased during the therapy, and a significant increase of urinary neopterin was observed in patients with normal baseline neopterin concentrations. A trend of decreased survival was observed for patients with high initial neopterin concentration. In conclusion, urinary neopterin is increased in colorectal cancer patients presenting for second or higher line of treatment. An increase of urinary neopterin during patupilone therapy suggests an activation of immune response by this agent.

Development of Calcium Pterins as Anti-Tumor and Anti-Viral Agents

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Pterin, an immuno-modulator present in the

blood and tissues of mammals, is excreted in the urine of cancer patients in elevated amounts relative to normal persons. When combined with calcium, pterin demonstrates both anti-tumorigenic and anti-viral (hepatitis B; HBV) properties in mouse models. In the tumor studies, the following cytokines were analyzed by stepwise regression analysis: IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, IFN- γ , and TNF- α . Calcium pterins were found to inhibit MDA-MB-231 human breast cancer xenographs in nude mice in a manner correlated with increased plasma IL-10 and decreased IFN- γ in the case of CaPterin; and increased plasma IL-12 and IL-4 concentrations with dipterinyl calcium pentahydrate (DCP). Both forms of calcium pterin decrease plasma IL-6 levels and inhibit indoleamine 2,3-dioxygenase (IDO), an immunoinhibitory enzyme, in human peripheral blood mononuclear cells (PBMCs).

DCP has also been shown to significantly reduce, in a dose-response manner, log liver HBV DNA as measured by PCR in female HBV mice. In this study, a larger number of serum cytokines and chemokines (IL-1, IL-1, IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-12, MCP-1, TNF- α , MIP-1, GM-CSF, RANTES), along with the IDO metabolites tryptophan (Trp) and kynurenine (Kyn), were followed. Three significant changes in these biomarkers were found to be caused by DCP: 1) decreased MCP-1; 2) decreased Kyn/Trp (an estimation of IDO activity); and 3) increased GM-CSF.

Immuno-modulation via the IDO or tryptophan 2,3-dioxygenase (TDO) pathways, along with blood cytokine and chemokine changes are proposed to play roles in the anti-tumor and anti-viral mechanisms of calcium pterins.

Zinc and Neopterin in Patients with and without Angiographic Coronary Artery Disease The Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

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Zinc is important for the function of numerous biological processes including enzymatic reactions, regulation of gene expressions and maintenance of membrane structure and function. Zinc deficiency is common among the elderly and has been associated with oxidative stress, immune dysfunction and cardiovascular disease. In earlier studies we have observed significant relationship between decreased serum zinc and higher concentrations of immune activation marker neopterin (1) in patients with chronic diseases like cancer, HIV infection but also after allograft transplantation (2-4). Moreover, results showed that the decline of serum zinc was related to an enhanced renal clearance of zinc most probably due to reduced tubular reabsorption of the metal ion. Recently, an inverse association between serum zinc concentrations and adverse outcomes of patients referred to coronary angiography was reported within the LUDwigshafen RISK and Cardiovascular Health (LURIC) study (5).

In order to further examine a possible association between serum zinc and immune activation, in a cross-sectional approach we investigated 2048 patients within the LURIC cohort of whom paired zinc, neopterin and C-reactive protein (CRP) concentrations were available. To test for kidney function concentrations of creatinine and cystatin C were included in this analysis.

Zinc concentrations were not significantly different in patients with coronary artery disease (mean \pm SD: 87.1 \pm 15.4 μ g/dL) and controls (86.7 \pm 14.5 μ g/dL; Welch's t test: p = n.s.). In contrast, patients with coronary artery disease had higher neopterin (mean \pm SD: 8.6 \pm 7.4 nmol/L), CRP (mean \pm SD: 9.7 \pm 19.6 mg/L), creatinine (87.8 \pm 34.3 μ mol/L) and cystatin C (1.0 \pm 0.4 mg/L) concentrations compared to controls (neopterin: 7.5 \pm 4.8 nmol/L; Welch's t test: p =

0.0005; CRP: 5.5 \pm 10.0 mg/L; creatinine: 80.6 \pm 24.3 μ mol/L, cystatin C: 0.9 \pm 0.3 mg/L; Welch's t test for all: p < 0.0001). There was a significant inverse correlation between zinc concentrations and neopterin (Spearman's rank correlation: r_s = -0.222; p < 0.0001), CRP (r_s = -0.166; p < 0.0001) and cystatin C (r_s = -0.163; p < 0.0001) levels and age (r_s = -0.214; p < 0.0001), whereas no significant association could be found between zinc and creatinine concentrations (r_s = -0.028; p = n.s.).

Data do not show an association of coronary artery disease with zinc concentrations, but there was a highly significant association between lower zinc and higher neopterin or CRP concentrations. Data imply that renal wasting of zinc could play a role for development of zinc deficiency also in patients at risk for atherosclerosis and may relate to immune activation and oxidative stress. This hypothesis is not contradicted by the inverse correlation between zinc and cystatin C concentrations in our study, as renal elimination of cystatin C being solely influenced by glomerular filtration, but not by tubular function. In contrast, serum creatinine inaccurately estimates true glomerular filtration rate because of tubular secretion of creatinine.

- 1 Murr C, Widner et al. Neopterin as a marker for immune system activation; *Curr Drug Metab* 2002;3:175-187.
- 2 Melichar B, et al. Association between increased urinary zinc and neopterin concentrations in women with gynaecological cancer. *Tumor Diagn Ther* 1993;14:110-112.
- 3 Melichar B, et al. Are disturbances of zinc metabolism in human immunodeficiency virus type 1 (HIV-1) infection caused by immune activation? *Pteridines* 1993;4:195-199.
- 4 Melichar B, Aichberger et al. Immune activation and enhanced urinary zinc concentrations in allograft recipients. *Presse Med* 1994;23:702-706.
- 5 Pilz S, et al. Low serum zinc concentrations predict mortality in patients referred to coronary angiography. *Br J Nutr.* 2009;101:1534-1540.

Inflammation and Immune Activation May Underlie Vitamin D Deficiency. The Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

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Maintenance of serum calcium and phosphorus concentrations and interaction with bone mineralization and neuromuscular function are the major classical effects of 1,25-dihydroxyvitamin D (1,25OH₂-vitamin D, calcitriol) the active form of vitamin D (1). The major biologically inert precursor of 1,25OH₂-vitamin D is vitamin D₃ (cholecalciferol), which can be provided by ingestion of food or can be formed when 7-dehydrocholesterol in the skin is exposed to solar ultraviolet B. This vitamin D precursor is converted to 25-hydroxyvitamin D (25OH-vitamin D, calcidiol) when it enters the liver. It represents the major circulating form of vitamin D. Additional hydroxylation in the kidneys leads to the biologically active 1,25OH₂-vitamin D. Blood concentrations of vitamin D were found to be subnormal in patients with cancers, autoimmune syndromes, cardiovascular diseases, diabetes, and infections and also in the healthy elderly (1). It is believed that supplementation with vitamin-D might help to reduce the risk of acquiring such disorders. In patients referred to coronary angiography within the Ludwigshafen Risk and Cardiovascular Health (LURIC) study it was shown that both, lower serum 25OH-vitamin D and 1,25OH₂-vitamin D concentrations, are independently related to an upregulated circulating renin-angiotensin system, thereby being responsible for high blood pressure (2). Worse outcome to

be linked with subnormal vitamin D levels could also be shown in a subgroup of patients with chronic kidney disease (3). Aside from low vitamin D concentrations, markers of inflammation and immune activation are associated with adverse outcomes in patients with cardiovascular risk (4) but also in other clinical conditions like HIV infection (5) and cancer (6). To further study this relationship we investigated whether low vitamin D levels are related to immune activation and coronary artery disease (CAD).

Serum concentrations of 25OH-vitamin D and 1,25OH₂-vitamin D and the immune activation markers neopterin and C-reactive protein (CRP) were measured in 2015 patients derived from the LURIC-study, which comprises a cohort of patients referred for coronary angiography. Serum concentrations of 25OH-vitamin D and 1,25OH₂-vitamin D did not differ between patients with CAD and controls (25OH-vitamin D in patients, mean \pm SD: 17.4 \pm 9.4 vs. controls: 18.4 \pm 11.7 μ g/L; 1,25OH₂-vitamin D in patients: 34.4 \pm 13.3 ng/L vs. controls: 35.3 \pm 12.7 ng/L; Welch's t test: $p = \text{n.s.}$), whereas CAD patients had higher neopterin (8.6 \pm 7.4 nmol/L) and CRP (9.7 \pm 19.6 mg/L) concentrations compared to controls (neopterin: 7.5 \pm 4.8 nmol/L; CRP: 5.4 \pm 10.0 mg/L; both $p < 0.001$). There was an inverse correlation between serum 25OH-vitamin D or 1,25OH₂-vitamin D concentrations and serum neopterin (Spearman's rank correlation: 25OH-vitamin D: $r_s = -0.183$; 1,25-dihydroxyvitamin D: $r_s = -0.230$) and CRP (25OH-vitamin D: $r_s = -0.142$; 1,25OH₂-vitamin D: $r_s = -0.130$; all $p < 0.0001$) concentrations.

Our analysis shows an inverse relationship between neopterin and vitamin D concentrations, and this result may indicate that low vitamin D levels in patients are related to increased inflammatory processes. Interestingly a similar association was reported earlier in patients with HIV infection (7). The available data may indicate that the subnormal vitamin D concentrations in patients develop because of the inflammation background which could contribute to an increased demand of vitamin D when its biosynthesis is hampered and/or its degradation is accelerated. Further studies should clarify whether inflammation related processes like oxidative

stress would contribute to vitamin D deficiency by inhibition of the formation or induce the degradation of the active vitamin D metabolites.

- 1 Zhang R, Naughton DP. Vitamin D in health and disease: current perspectives. *Nutr J* 2010;9:65.
- 2 Tomaschitz A, et al. Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Clin Chim Acta* 2010;411:1354-1360.
- 3 Pilz S, et al. Vitamin D status and mortality in chronic kidney disease. *Nephrol Dial Transplant* 2011 (in press).
- 4 Grammer TB, et al. Neopterin as a predictor of total and cardiovascular mortality in individuals undergoing angiography (The Ludwigshafen Risk and Cardiovascular Health Study). *Clin Chem* 2009;55:115-146.
- 5 Murr C, et al. Neopterin as a marker for immune system activation. *Curr Drug Metabol* 2002;3:175-187.
- 6 Sucher R, et al. Neopterin, a prognostic marker in human malignancies. *Cancer Lett* 2010;287:13-22.
- 7 Haug C, et al. Subnormal serum concentration of 1,25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. *J Infect Dis* 1994;169:889-93.

The Role of iNOS in Systemic Iron Homeostasis and *Salmonella* Infection

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Iron homeostasis and nitric oxide (NO) biology are closely connected to each other since the transcription of inducible NO synthase (iNOS) is controlled by iron while the post-transcriptional control of iron homeostasis via iron regulatory proteins (IRPs) is affected by NO since this labile molecule stimulates the binding affinity of IRPs to its target iron responsive elements (IREs) which links maintenance of iron homeostasis to optimal formation of NO for host defence.

We studied the systemic effects of NO on body iron homeostasis in iNOS^{+/+} and iNOS^{-/-} mice. iNOS disruption led to significant accumulation of iron in liver, spleen and peritoneal macrophages. Iron deposition in the spleen of iNOS^{-/-} mice occurred mainly in macrophages and was paralleled by a significantly decreased ferroportin-1 (Fpn-1) mRNA expression in these cells. The massive iron overload was paralleled by increased hepcidin-1 mRNA expression in the liver of iNOS^{-/-} mice. The cause-effect relationship between NO and Fpn-1 expression was underscored by the observation that the pharmacological NO donor Nor-5 increased Fpn-1 expression in peritoneal macrophages. In addition, peritoneal macrophages from iNOS^{-/-} mice showed reduced TNF and IL-12p35 expression following infection with the intracellular pathogen *Salmonella Typhimurium*. While *Salmonella*-infected iNOS^{-/-} macrophages displayed increased bacterial load, the iron chelator desferasirox abrogated the differences observed between iNOS^{-/-} and iNOS^{+/-} macrophages and restored TNF and IL-12p35 production in iNOS^{-/-} cells.

Our results demonstrate that NO is a central regulator of body iron homeostasis and that its reduction results in an increased iron accumulation in macrophages which can be traced back to down-regulation of Fpn-1 expression, most likely due to a transcriptional mechanism. The accumulation of iron in iNOS^{-/-} macrophages reduces the expression of M1-type innate host response mechanisms which may partly underlie the impaired immune response of iNOS^{-/-} mice.

Inflammatory Markers and Neurohormones in Sepsis their Levels and Correlation with Mortality

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Results of laboratory diagnostic parameters are an important source of information in intensive care unit (ICU) patients. They are important and sometimes fundamental in the monitoring, diagnosing and at the medication of ICU patients. Laboratory examinations and imaging methods provide approximately 60% of the available information for the physician, and this percentage is even higher in the ICU. In fact, a wide range of parameters is applied and quality of laboratory tests regarding specificity, sensitivity, determination speed and also availability (point of care testing) is constantly improving. The development of new parameters includes natriuretic peptides, hsTnT, TnI, but also "neurohormones" adrenomedulin (ADM), arginine vasopressin (AVP, ADH; a key regulator of water balance, plays an important role in hypothalamus-hypophysis-adrenal/suprarenal gland axis and reflects individual stress reactions of the organism; however unstable in plasma and serum) and copeptin (CT-pro AVP, peptid, 39 AA, releases from AVP-aeqimolar, stable in plasma, serum, which can provide indirect information on AVP concentration.

In clinical studies copeptin turned out as a prognostic marker in septic patients, lung infections, pneumonias, myocardial diseases and in ischemic vascular brain attack. The physiologic a pathophysiologic role of vasoactive peptide ADM is not clear till now. ADM was isolated from feochromocytoma (adrenal medulla) and is not stable in plasma or serum, but MR-proADM released from ADM is stable, and the measurement MR-proADM provides information about ADM concentration, and ADM is a prognostic marker in sepsis.

The aim of this retrospective pilot study was to

examine copeptin and adrenomedulin concentrations in plasma samples of septic and non-septic patients from ICU stored at -35°C , to test for the utility of these parameters in reality, to compare results with data in the literature and to markers of the inflammatory response namely procalcitonin, C-reactive protein and macrophage product neopterin (all tests from BRAHMS, Hennigsdorf, Germany).

In 14 ICU patients (5 male, 9 female, aged 33-81 years, mean: 58) at the University Hospital of Bratislava collected from September 2010 - February 2011, 8 with sepsis, 6 without, parameters Copeptin, MR-proADM, procalcitonin (PCT), C-reactive protein (CRP) and neopterin were measured by ELISA (BRAHMS, Hennigsdorf, Germany). All tests used fulfill laboratory diagnostic criteria, they are simply and robust, are easily available, are free for the daily use in clinical laboratory and in clinical praxis. They fulfill all criteria such as availability, quality control and reproducibility. All tests except neopterin are set on the automatic analyzer, which is its only limitation. Although the number of patients in both groups are very low, especially the results of neopterin measurements revealed good association with the outcome of patients. It has to be achieved that neopterin measurements will be available on great automated analyzers. Those companies who will take the chance will be the winners.

***Toxoplasma gondii*, Suicidality and Suicide: an Immune Mediation?**

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Yearly suicide leads to premature death of more than 1 million people worldwide. The most important risk factors for suicide are history of attempting suicide and recurrent depression. Previously, immune activation has been linked to

suicidal behavior and mood disorders. We hypothesized that chronic latent infection with *Toxoplasma gondii*, a widespread neurotropic parasite infecting one third of the world population, through immune activation, would be associated with suicide attempts. We are presenting converging data connecting a history of suicide attempts with titers of *T. gondii* IgG antibodies in cross-sectional models in patients with mood disorders in Maryland (1), psychiatric inpatients in Turkey (2), schizophrenics in Germany (Okusaga et al in preparation), and predictive associations in cohorts of mothers in Denmark (Pedersen et al in preparation). We have also found an ecological association between national *T. gondii* seropositivity rates in women at reproductive years and completed suicide rates in postmenopausal age groups (3). *T. gondii* may also be involved in exacerbation of mood disorders, personality disorders and personality changes. Limitations of these studies will be presented and discussed. Potential mechanisms include misdirected immune responses involved in containing *T. gondii*, immune evasion by the parasite to avoid elimination, auto-antibodies, and downstream production of kynurenine and its metabolites. Other mechanisms could consist of reactivation of the latent infection, and intracellular deprivation of cholesterol. Confirming this relationship and understanding its mechanisms may lead to novel therapeutic and prophylactic targets in suicide prevention.

- 1 Arling TA, et al. *Toxoplasma gondii* antibody titers and history of suicide attempts in patients with recurrent mood disorders. *J Nerv Ment Dis* 2009;197:905-8
- 2 Yagmur, F., et al. May *Toxoplasma gondii* increase suicide attempt-preliminary results in Turkish subjects? *Forensic Sci Int* 2010;199:15-17
- 3 Ling et al.. *Toxoplasma gondii* Seropositivity and Suicide Rates in Women. *J Nerv Ment Dis* (in press)

Modulation of Intracellular Signaling Pathways and ROS Production by the Pteridines BH₄ and ABH₄ During Cellular Stress

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The pteridines tetrahydrobiopterin (BH₄), an essential cofactor of several enzyme systems, including nitric oxide synthases (NOS), and its structural analogue and competitive inhibitor 4-aminotetrahydrobiopterin (ABH₄) have previously been shown to attenuate ischemia-reperfusion injury (IRI) and to improve organ survival and performance during solid organ transplantation. Moreover, pro-apoptotic effects have been described. The underlying mechanisms are poorly defined and may be independent of NOS as demonstrated previously. Intracellular signaling pathways and mitochondrial ROS are vital for controlling cellular responses to stress as it occurs during tumor growth as well as during transplantation in form of IRI.

BH₄ and ABH₄ effects were analyzed under two conditions of cellular stress: stress caused by hypoxia and reoxygenation (HR) as an *in vitro* model for ischemia and reperfusion (IR) and the transformation with the RAF oncogene. Tumors are characterized by the use of alternative pathways for energy production and by regulating the generation of signaling molecules like reactive oxygen species (ROS). Cell systems studied included: cardiomyocytes (HL-1), endothelial cells (HUVEC), hematopoietic cells (32D) and fibroblasts (NIH3T3). Intracellular signaling was monitored in cell lysates using phosphorylation-specific antibodies. Mitochondrial ROS levels were determined by imaging of cells pre-loaded with MitoTracker Red CM-H2XRos. Viability was assessed through trypan blue and propidium-iodide staining.

The continuous presence of BH₄ and ABH₄ blocked cell proliferation in various cell models, while only ABH₄ caused significant cell death. Short time pre-treatment with ABH₄ but not BH₄ inhibited two major mitogenic and anti-apoptotic pathways leading to the activation of ERK and Akt. Furthermore, prolonged incubation with either pteridine resulted in a concentration-dependent activation of ERK, JNK and p38 with more potent effects in the case of BH₄.

During hypoxia and reoxygenation a pronounced activation of these signaling kinases was observed at early reperfusion in HUVECs and HL-1 cardiomyocytes as described previously. This HR-associated activation of ERK, JNK and p38 was further augmented by BH₄ and to a lesser extent by ABH₄ in the case of p38 and JNK, whereas ERK was not affected. Finally, the presence of BH₄ and ABH₄ significantly reduced both the HR-induced increase in mitochondrial ROS production as well as basal ROS levels in HL-1 cells and HUVECs.

The pteridines BH₄ and ABH₄ influence intracellular signaling in distinct and complex ways, which can lead to anti-oncogenic and pro-apoptotic effects in transformed cells. A protective effect of BH₄ and ABH₄ during ischemia-reperfusion may be linked to the antioxidant capacity of these compounds.

Chronic Immune Stimulation to Cause Moderate Impairment of Phenylalanine 4-hydroxylase

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Phenylalanine (4)-hydroxylase (PAH, E.C. 1.14.16.1) converts phenylalanine (Phe) to tyrosine (Tyr). Activity of PAH is reduced in 'classical' phenylketonuria (PKU), whereas in 'atypical' PKU biosynthesis of the cofactor 5,6,7,8-tetrahy-

drobiopterin (BH₄) is disturbed. Additionally, in BH₄-responsive PKU supraphysiological doses of BH₄ can decrease Phe concentrations. In contrast, depletion of BH₄ pool may cause an increase of Phe concentrations. As BH₄ is easily oxidized, situations with elevated production of reactive oxygen species (ROS) can lead to deficiency of this essential cofactor and as a consequence to reduced PAH activity. In fact, it was shown that patients with inflammatory and infectious diseases (with supposed high ROS load) may present with elevated plasma Phe concentrations. Additionally, Phe to Tyr ratio (Phe/Tyr) was elevated in these situations. BH₄ is not only cofactor for PAH, but also for enzymes involved in central metabolic pathways e.g. synthesis of neurotransmitters. Therefore, elevated Phe/Tyr may additionally be an easy available indicator for the disturbance of BH₄-dependent pathways and should decrease with substitution of BH₄.

High Fat Diet Causes Iron Deficiency via Hepcidin-Independent Reduction of Duodenal Iron Absorption

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Obesity is often associated with iron deficiency, however, the underlying mechanisms are not fully understood. Hepcidin is a key regulator of iron metabolism and has been suggested to be responsible for obesity driven iron deficiency. Herein, we used an animal model of diet-induced obesity to study high fat diet induced changes in iron homeostasis and the expression of iron metabolism genes in various tissues. C57BL/6 mice were fed a standard (SD) or high fat diet (HFD) for eight weeks, and in addition half of the mice were supplemented with high dietary iron

(Fe⁺) for the last two weeks. Hepatic and splenic iron contents were significantly lower in HFD fed than in SD fed mice supplemented with iron ($p < 0.001$). While neither hepatic and adipose tissue nor serum hepcidin concentrations differed significantly between SD and HFD fed mice, dietary iron supplementation resulted in increased hepcidin mRNA and protein expression in SD as compared to HFD mice ($p < 0.001$ and $p < 0.05$). This was mirrored by a significant reduction of duodenal iron absorption in HFD fed mice. Accordingly, the mRNA expression of the duodenal iron transporters Dmt1 ($p < 0.01$) and Tfri ($p < 0.05$) were higher in HFD fed mice indicating enterocyte iron deficiency, whereas the mRNA levels of the duodenal iron oxidoreductases Dcytb and hephaestin were lower in HFD fed mice. In parallel, the expression of markers of inflammation and adipocytokines in the liver did not vary as a function of dietary fat content. Our study suggests that HFD results in iron deficiency on the basis of impaired iron absorption which is neither due to increased hepcidin expression nor to inflammation. In contrast, discordant expression of duodenal oxidoreductases indicates impaired iron transfer across enterocyte membranes.

PBMC Assay to Guide Separation of Immunomodulatory Compounds from *Horminum* Extracts

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During recent decades clinical conditions associated with chronic inflammation became more and more recognized. Among them the age-associated cardiovascular and neurodegenerative disorders represent a major challenge for health care systems. To combat these clinical disorders, the development of new and more effective anti-inflammatory drugs is of utmost importance. In

the cell culture model of peripheral blood mononuclear cells (PBMC), the measurement of neopterin formation and tryptophan degradation has been shown to be a useful screening method for the testing of anti-inflammatory capacities of drugs, chemicals and plant extracts (1). The catalyzing enzymes GTP-cyclohydrolase I (GCH) and indoleamine-2,3-dioxygenase (IDO) are both induced by pro-inflammatory cytokine interferon- γ during Th1-type immune response.

Earlier diterpene derivatives (phytocannabinoids) have been demonstrated to modulate the mitogen-induced immune responses in PBMC resulting in changes of the tryptophan metabolism and neopterin formation (2). The plant family of *Lamiaceae* is rich in such diterpene compounds and in this study the PBMC assay was chosen to guide isolation and characterization of active plant compounds of *Horminum* (*H.*) *pyrenaicum* L. (syn. Dragon's mouth), the only representative of the monophyletic genus *Horminum*. Dichloromethane (DCM) extract of the roots of the plant was chosen for isolation. The pharmacological potential of extract fractions from whole DMC extract of *H. pyrenaicum* on Th1-type immune response was evaluated and the results were used to guide the further separation by monitoring the tryptophan catabolism and neopterin formation.

Investigation of the DCM extract of the root material was carried out by means of silica gel and Sephadex LH 20 column chromatography as well as high-speed countercurrent chromatography. Semi-preparative HPLC and LC-SPE-NMR enabled the identification of four abietane-diterpene quinone derivatives, two nor-abietane diterpene quinones and two abeo 18 (4 \rightarrow 3) abietane diterpene quinones. The compounds were identified by mass spectrometry and 1- and 2-D NMR spectroscopy as horminone, 7-O-acetylhorminone, inuroyleanol and its 15,16-dehydro-derivative. Additionally, the nor-abietanes agastatinone and 3-deoxyagastatinone and the abeo 18 (4 \rightarrow 3) abietanes agastol and its 15,16-dehydro-derivative were identified.

Whole DCM extracts and most extract fractions dose-dependently down-regulated neopterin formation and also tryptophan degradation. Upon further enrichment and identification of com-

pounds the diterpene horminone and a mixture of 15,16-dehydroinuroyleanol and 15,16-dehydroagastol were found to again dose-dependently down-regulated the immunobiological effects. In contrast, the diterpenes 7-O-acetylhorminone, 3-deoxyagastaquinone and agastaquinone had only minor influence on neopterin formation. Inuroyleanol and agastol showed no capacity to inhibit Th1-type immune response in stimulated PBMC at all.

The results of this study emphasize that PBMC represent a suitable and reliable assay to guide the separation of immunomodulatory compounds and could help to save time and resources for the development of future drugs.

- 1 Jenny M, et al. *In vitro* testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors. *Inflamm Res* 2011;60:127-35.
- 2 Jenny M, et al. Δ 9-Tetrahydrocannabinol and cannabidiol modulate mitogen-induced tryptophan degradation and neopterin formation in peripheral blood mononuclear cells *in vitro*. *J Neuroimmunol* 2009;207:75-82.

Identification of a Glutamate Residue in Alkylglycerol Monooxygenase Crucial for Tetrahydrobiopterin Binding

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In 1964, an enzyme was first described which cleaves ether lipids in a tetrahydrobiopterin-dependent manner. In contrast to other enzymes depending on this cofactor no major progress in characterising this enzyme named alkylglycerol monooxygenase was achieved in the following 46 years due to unsuccessful attempts to purify the

protein and assign a sequence. Very recently, we were able to identify the gene coding for alkylglycerol monooxygenase by pattern-based bioinformatics combined with standard molecular biology techniques. This now enables us to investigate the enzymatic properties in more detail. In earlier studies we could already show that alkylglycerol monooxygenase resembles the aromatic amino acid hydroxylases with respect to cofactor handling and dependency on non-heme iron. Sequence homology now reveals that this enzyme belongs to the family of fatty acid hydroxylases which all have the eight conserved histidines comprising diiron motif in common. None of these membrane-bound proteins has ever been successfully purified to homogeneity, most probably because of the intrinsic instability following solubilisation protocols.

To identify residues in alkylglycerol monooxygenase which are crucial for its enzymatic activity, we exchanged 10 residues in the coding sequence of alkylglycerol monooxygenase which are conserved throughout the whole fatty acid hydroxylase family by site-directed mutagenesis and heterologously expressed mutant and wild-type 6xmyc tagged constructs in Chinese hamster ovary cells which were analysed after 48 hours for their ability to cleave the fluorescent substrate 1-O-pyrenedecylglycerol in a tetrahydrobiopterin-dependent way. Protein expression was verified by immunoblotting.

Activity analyses showed that 9 mutants displayed significantly reduced etherlipid activity, one of these mutants was not detectable on the protein level and was therefore excluded from further analysis. We then also analysed 6 of these mutants (E137A, E152A, D153A, H189A, E203A, E212A) for their KM values for tetrahydrobiopterin and the fluorescent substrate. While no changes were detected in the KM values for substrate binding the KM for tetrahydrobiopterin was increased 18-fold in mutant glutamate 137 to alanine.

A similar analysis has identified a glutamate residue critical for binding of the cofactor also in phenylalanine hydroxylase. Despite a lacking overall sequence homology between this enzyme and alkylglycerol monooxygenase, this mechanistic feature seems to be conserved along with the

dependence of catalysis on histidine-bound iron.

Serum Neopterin in not Increased in Obese Juveniles

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Inflammation and immune activation are closely associated with the pathogenesis of cardiovascular disease (1-3), and concentrations of Th1-type immune activation markers like neopterin (4) significantly predict outcome in adults. We investigated serum neopterin concentrations (ELISA, BRAHMS, Hennigsdorf, Germany) as well as tryptophan metabolism by HPLC in 356 overweight and obese (aged 11.3 ± 2.97 years; mean \pm S.D.), otherwise healthy children and 32 non-obese controls.

Body mass index (BMI) differed significantly between obese and non-obese probands (28 ± 5.64 vs. 18 ± 2.19 kg/m², $U = 9.24$, $p < 0.0001$, Mann Whitney U-test). Neopterin concentrations were similar in both groups, although rather low as compared to reported adult data.

Obesity in juveniles is not associated with increased neopterin concentrations suggesting that obesity at least in the early course of the disease does not lead directly to Th1-type immune activation and associated cardiovascular disease. Only in the later course a switch to Th-1 type immune activation and associated cardiovascular diseases may take place. Chronic infections or other cofactors might be important to trigger cytokine production and elevated neopterin concentrations at the later stage.

1 Tatzber F, et al. Elevated serum neopterin levels in atherosclerosis. *Atherosclerosis*

1991;89:203-208.

- 2 Huber C, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. *J Exp Med* 1984;160:310-316.
- 3 Mangge H, et al. Low grade inflammation in juvenile obesity and type 1 diabetes associated with early signs of atherosclerosis. *Exp Clin Endocrinol Diabetes* 2004;112:378-382.
- 4 Weiss G, et al. Modulation of neopterin formation and tryptophan degradation by Th1- and Th2-derived cytokines in human monocytic cells. *Clin Exp Immunol* 1999;116:435-440.

Tryptophan Metabolism and its Association with Ghrelin and Obestatin in Obese Children

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Childhood obesity is not only associated with increased risk of metabolic diseases, but also with a range of behavioral/psychological disorders; these are linked to abnormalities in the serotonergic system. In adults, disturbances in tryptophan metabolism have been suggested to contribute to the development/maintaining of the metabolic syndrome. Ghrelin and obestatin, which are involved in the regulation of energy homeostasis and appetite, have been suggested to be involved in mood regulation and the pathogenesis of depression (1-3).

357 overweight and obese children (body mass index (BMI) $>P90$ or $P97$, mean \pm SD: 28 ± 5.64 kg/m²), 33 normal weight controls (BMI $<P90$, 18 ± 2.19 kg/m²); aged 11.3 ± 3 years, respectively. Concentrations of tryptophan and kynurenine (HPLC), neopterin, ghrelin, obestatin (RIA, Phoenix Europe), fasting insulin (ELISA,

BRAHMS Hennigsdorf, Germany), plasma glucose (Glucose oxidase method, Beckman Instruments, Fullerton, CA, USA), cholesterol and triglycerides (enzymatic) and liver transaminases (colorimetric, Boehringer Mannheim GmbH, Mannheim, Germany) were measured.

BMI differed significantly between obese and non obese probands (28 ± 5.64 vs. 18 ± 2.19 SD kg/m² U = 9.24, p <0000.1, Mann Whitney U-test). Tryptophan concentrations were significantly lower in the obese (80.7 ± 12.7 μmol/L) vs. non-obese subjects (74.7 ± 18.8 μmol/L; U = 2.384, p = 0.017), while there was no difference in kynurenine concentrations and kynurenine to tryptophan-ratio (Kyn/Trp). Tryptophan levels positively correlated with fasting insulin ($r_s = 0.20$, p <0.001), serum triglycerides ($r_s = 0.23$, p <0.001), HOMA-IR ($r_s = 0.21$, p <0.001) and GPT and GGT ($r_s = 0.09$ and 0.12 , p <0.05), negatively with ghrelin ($r_s = -0.14$, p <0.05). Kyn/Trp negatively correlated with obestatin ($r_s = -0.23$, p <0.001) within the obese group only.

Obese children and adolescents show lower levels of peripheral tryptophan suggesting increased degradation as compared to normal weight controls. In contrast to adult data, no difference in levels of kynurenine and peripheral tryptophan degradation (Kyn/Trp) were observed. Data suggests no role of indoleamine 2,3 dioxygenase (IDO). However, liver-based tryptophan 2,3-dioxygenase (TDO) might be important as a result of fatty liver or induced by relative hypercortisolism, but also enhanced activity of tryptophan-(5)-hydroxylase cannot be excluded to contribute to the decline of tryptophan levels especially when kynurenine concentrations remained unchanged. Orexogenic ghrelin and its anorexiogenic counterpart obestatin might be involved in mood regulation in obese children.

- 1 Rofey DL, Kolko RP et al. A longitudinal study of childhood depression and anxiety in relation to weight gain. *Child Psychiatry Hum Dev* 2009;40:517-26.
- 2 Oxenkrug GF. Tryptophan kynurenine metabolism as a common mediator of genetic and environmental impacts in major depressive disorder: the serotonin hypothesis revisited 40

years later. *Isr J Psychiatry Relat Sci* 2010;47:56-63.

- 3 Kluge M, et al. Effects of ghrelin on psychopathology, sleep and secretion of cortisol and growth hormone in patients with major depression. *J Psychiatr Res* 2011;45:421-6.

Sequence Identification of Alkylglycerol Monooxygenase by Pattern-Based Bioinformatics

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In addition to aromatic amino acid hydroxylases (phenylalanine, tyrosine and two tryptophan hydroxylases) and nitric oxide synthases (neuronal, endothelial and inducible isoform) alkylglycerol monooxygenase (glycerylether monooxygenase, E.C. 1.14.16.5) is known to require tetrahydrobiopterin. The enzyme has so far resisted all attempts to purify it to homogeneity because it rapidly lost activity. A screening of pools of expression libraries was also unsuccessful. From the virtually complete databases of human and mouse protein encoding genes we selected candidates for alkylglycerol monooxygenase based on four strategies. Two candidate genes (FAM43A and TMEM79) came from proteomic comparison of partially purified fractions from rat liver microsomes with and without enzymatic activity. Three candidate genes were selected from prediction of structural similarities to

phenylalanine hydroxylase (CDC132, C11ORF2 and MOXD1). One candidate gene (C17ORF28) came from an attempt to generate a unified tetrahydrobiopterin binding sequence for all tetrahydrobiopterin-dependent enzymes with characterised sequence, i.e. aromatic amino acid hydroxylases and nitric oxide synthases. Further candidates were chosen by browsing protein family (PFAM) motifs. These are amino acid motifs characteristic for a certain class of proteins with related function. More than 10.000 such families have been defined. One PFAM motif, the fatty acid hydroxylase motif, attracted our attention. This family of proteins includes lipid desaturases and hydroxylases carrying out reactions with similarity to the alkylglycerol monooxygenase reaction. From the six human proteins with the fatty acid hydroxylase motif we selected three, SC4MOL, C5ORF4 and TMEM195. TMEM195 was chosen due to the presence of a PFAM motif with unknown function. We then transfected the ten selected candidate genes to Chinese hamster ovary cells and monitored tetrahydrobiopterin-dependent alkylglycerol monooxygenase activity. We found that TMEM195, a predicted membrane protein with unknown function containing the fatty acid hydroxylase motif, encoded for alkylglycerol monooxygenase. TMEM195 has no sequence homology with aromatic amino acid hydroxylases or nitric oxide synthases. Thus alkylglycerol monooxygenase forms an additional, third class of tetrahydrobiopterin-dependent enzymes.

Neopterin Correlates with Visfatin Levels in a Cohort of Obese and non Obese Children and Adolescents

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Low grade inflammation has been associated with increasing body mass index (BMI) in the adult population. In childhood, however, this has been diversely discussed. Visfatin, originally known as pre B colony enhancing factor, expressed in peripheral blood neutrophils upon inflammatory stimulation, has been recently rediscovered as powerful adipokine through its insulin mimetic capacity expressed by the lowering of plasma glucose levels (1, 2). Visfatin furthermore regulates metabolism in adipose tissue by transfer of biochemical signals to organs of metabolic importance such as brain, liver and the activated immune system. In light of the rising prevalence of paediatric obesity it is necessary to unravel its association with immunologic processes in search of therapeutic intervention capabilities.

In a cross sectional approach, serum visfatin and neopterin concentrations were examined in 357 otherwise healthy obese (body mass index, BMI >25 kg/m² and 33 normal weighted (BMI 19-25 kg/m²) children and adolescents. Their age varied from 11.3 ± 2.97 years, female to male distribution was 2:1. Visfatin and neopterin were measured by enzyme linked immunosorbent assay (Phoenix peptides, Karlsruhe, Germany and BRAHMS, Hennigsdorf, Germany). We furthermore tested fasting triglyceride levels enzymatically (CHOD-PAP and GPO-PAP calorimetric methods, Boehringer Mannheim GmbH, Mannheim, Germany). Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA, USA). Plasma insulin concentrations were measured by chemiluminescence microparticle method (ELISA Mercodia, Uppsala Sweden). Insulin resistance was assessed by homeostatic model assessment-insulin resistance (HOMA-IR). Differences in distribution of laboratory variables among patients groups were statistically analyzed by non-parametric Mann-Whitney test, correlation analyses were done by Spearman's rank test.

BMI differed significantly (obese mean ± SD 28: ± 5.64 vs. non-obese: 18.2 ± 2.19 kg/m², U = 9.24, p <0.00001). Visfatin was significantly higher in the obese group (11.67 ± 18.07 vs. 8.82 ± 3.59 ng/ml, p <0.02) and so were fasting glu-

cose levels (88.8 ± 9.49 vs. 75.6 ± 7.61 mmol/L, $p < 0.0001$), insulin (16.8 ± 14.37 vs. 5.58 ± 2.85 U/ml, $p < 0.0001$) and the HOMA-IR index (3.52 ± 3.39 vs. 1.01 ± 0.18 , $p < 0.0001$). Visfatin levels correlated with neopterin in the obese ($r_s = 0.166$, $p = 0.005$), but there was only a trend for such an association in the control group ($r_s = -0.140$, $p = 0.07$). In the obese group no associations were observed between visfatin levels and fasting glucose ($r_s = 0.045$), insulin ($r_s = -0.073$) and HOMA-IR indices ($r_s = -0.074$, all $p > 0.05$), whereas they were significant in the non-obese controls (glucose: $r_s = 0.343$, insulin: $r_s = 0.347$, HOMA-IR: $r_s = 0.391$, all $p < 0.03$). Only in the obese but not in the non-obese controls, there existed inverse correlations between neopterin concentrations and body size ($r_s = -0.128$, $p < 0.01$) and weight ($r_s = -0.117$, $p < 0.02$).

Neopterin concentrations and other markers of immune activation were found to be increased in obese adults (3) but this was not the case not in children and juveniles (4). In agreement with these latter findings, we found an inverse correlation between serum neopterin and body size and weight indicating weight gain to be associated with decreasing neopterin levels in obese children/juveniles. In them also a positive association was found between neopterin and visfatin, an adipokine and pre B colony stimulating factor dedicated previously as of the Th1 immunity. In our subjects, the well known insulin-mimetic feature of visfatin was exclusively found in the normal weight children, but not in the obese.

- 1 Fukuhara A, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005;307:426-30.
- 2 Dedoussis GV, et al. Visfatin: The link between inflammation and childhood obesity. *Diabetes Care* 2009;32(6):e71.
- 3 Thewissen MM, et al. Abdominal Fat mass is associated with adaptive immune activation: the CODAM study. *Obesity* 2011 (in press)
- 4 Mangge H, et al. Serum neopterin is not increased in obese juveniles. *J Obes* 2011;946795.

Serum Neopterin and Citrulline in Patients with Rectal Carcinoma Treated with Chemoradiation

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Adjuvant or neoadjuvant chemoradiation may be considered the standard of care of patients with stage II or III rectal carcinoma. Chemoradiation regimens used are associated with significant toxicity, and gastrointestinal toxicity is the most important of these side effects. Despite the fact that gastrointestinal toxicity is one of the most common side effects of anticancer therapy, the diagnosis and assessment of this toxicity still depends mostly on anamnestic data. Serum citrulline represents a potential indicator of gut function. In earlier studies, a correlation was observed between the laboratory parameters indicating gut integrity, and neopterin concentrations. In the present study, serum neopterin and citrulline was investigated in patients with rectal carcinoma during chemoradiation. A decrease of serum citrulline was accompanied by increased neopterin concentrations. A rise of serum neopterin could be observed in patients who had complicated course. These preliminary data indicate an activation of systemic immunity as well as impairment of the gut function, reflected in decreased citrulline concentrations, during chemoradiation for rectal carcinoma.

Influence of Interferon- α Therapy in Patients with HCV Infection on Neopterin Production, Tryptophan Degradation and PAH Activity

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One important side-effect of the therapy with cytokines like interferon- α (IFN- α) is the development of mood changes and depression. In such patients cytokine-induced biochemical changes seem to play a major role, thereby indoleamine 2,3-dioxygenase (IDO) is in the focus of intense research. But also phenylalanine (Phe) metabolism appears to be influenced by immune activation and inflammation, e.g., the Phe to tyrosine ratio (Phe/Tyr), an index of phenylalanine hydroxylase (PAH) activity was found increased in patients suffering from infections, cancer and after trauma and to correlate with immune activation markers such as neopterin.

To investigate whether changes of Phe metabolism might occur under treatment with IFN- α we investigated Phe and tryptophan metabolism in sera of 25 patients with established hepatitis C virus (HCV) infection (aged mean \pm S.D. 44.5 ± 11.0 years; CD28B polymorphism: 8 C/C, 12 C/T, 5 T/T) who were treated with IFN- α . HCV load was determined by polymerase chain reaction before treatment and d after 1 months of

therapy. Concentrations of neopterin (ELISA; BRAHMS, Hennigsdorf) and of Phe, Tyr were measured at baseline, after 1 month and after 6 months. Statistical comparisons were made using non-parametric tests because some of the data sets did not show normal distribution: Wilcoxon paired rank test for group comparisons and Spearman rank correlation to test for associations between variables.

Under therapy, not only neopterin concentrations increased but also Phe concentrations and Phe/Tyr increased (both $p < 0.05$; paired test). Moreover, there existed a significant correlation between Phe/Tyr and neopterin concentrations ($r_s = 0.216$, $p < 0.05$).

The finding that IFN- α therapy significantly influenced PAH activity supports the concept that immune activation in addition to IDO activity may modulate dopaminergic neurotransmitter biochemistry. However, any association between impaired PAH activity and adrenergic and/or noradrenergic neurotransmission as well as related side-effects of IFN- α therapy still has to be demonstrated.