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Abstracts

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Influence on neopterin and cytokine release by the duration of cold ischemia in ortothopic liver transplantation

Bengtsson A, Tomasdottir H, Ronholm E, Haeger M

Department of Anesthesiology and Intensive Care, Sahlgrenska University Hospital, Gothenburg, Sweden

The aim of the study was to determine if the duration of the cold ischemia influences activation of complement, the release of neopterin and interleukin-6 and 8 in orthotopic liver transplantation.
Eighteen consecutive patients undergoing orthotopic liver transplantation were studies and they were divided into two groups; Group 1) Duration of cold ischemia longer than 12 hours (n=11) and Group 2) Duration cold ischemia shorter than 12 hours (n=7). Blood samples for complement activation (C3a and SC5b-9), neopterin and interleukin-(IL)-6 and IL-8 determinations were drawn preoperatively, 1min before perfusion of the grafted liver and 120 minutes after the start of perfusion of the grafted liver.
Preoperatively there were no significant differences regarding complement variables, neopterin and IL-6 and IL-8 between the two studied groups.
Increased concentrations of C3a, SC5b-9, neopterin, IL-6 and IL-8 were found 120 minutes after revascularization of the transplanted liver in both groups. In patients receiving a liver with a cold ischemic time longer than 12 hours (Group 1) the concentration of neopterin and Il-8 were higher ($p<0.05$) compared to what was found in patients receiving a liver with a cold ischemia time shorter than 12 hours (Group 2). Group 1 required a higher volume of erythrocyte concentrate and fresh frozen plasma during the operation ($p<0.05$). They also got more albumin and buffer ($p<0.05$).
This study indicates that plasma levels of IL-8 is influenced by the duration of the cold ischemic time. It is of importance to avoid a longer duration of cold ischemia than 12 hours in orthotopic liver

Inhibition of tetrahydrobiopterin synthesis promotes apoptosis in the death of human U251 astrocytoma induced by lipopolysaccharide and interferon

Bezin L, Anastasiadis PZ, Foster JA, Gordon LJ, Corcoran GB, Levine RA

Gossett Neurology Laboratories, Henry Ford Hospital, Detroit Veterans Administration Medical Center; Department of Pharmaceutical Sciences and Department of Psychiatry, Wayne State University, Detroit, MI, USA; and Laboratory of Molecular Neuropharmacology, CNRS-UMR5542, Lyon, France

Malignant gliomas account for the majority of primary brain tumors and are difficult to eradicate. While gamma interferon (IFN-γ) is cytotoxic to glioma in animal models, clinical trials have yielded poor therapeutic results. In accordance with these previous reports, IFN-γ alone did not cause death of cultured human U251 astrocytoma in our experiments. However, lipopolysaccharide (LPS) plus IFN-γ caused significant cell death by apoptosis, which was not dependent on nitric oxide metabolism. In numerous cell lines, LPS plus IFN-γ induces the content of 6R-5,6,7,8-tetrahydrobiopterin (BH4) and the expression of GTP cyclohydrolase I (GCH1), the ratelimiting enzyme in BH4 biosynthesis. We speculated that BH4 metabolism plays a role in the survival status of U251 cells. GCH1-mRNA, GCH1 avctivity and intracellular BH4 content increased linearly during the course of LPD plus IFN-γ induced ed cell death. Inhibition of BH4 synthesis prior to and during treatment accelerated the appearance of apoptosis. Raising BH4 content prior to and during treatment delayed the onset of apoptosis, while total death remained constant. Our data indicate that reducing BH4 biosynthesis during the induction of glioma cell death

may be a useful adjunct therapy to promote apoptosis. The potential for modulating the route of glioma cell death by altering BH4 metabolism merits further investigations to elucidate the underlying mechanisms.

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Platelet serotonin levels in children with dihydropteridine reductase defect and 6-pyruvoyl-tetrahydropterin synthase defect

Earl JW, Alexander I, Dossitor D, Coakley J

Biochemistry, Gene Therapy and Psychiatry Departments, New Children's Hospital, Sydney, Australia.

New-born infants unable to synthesize or regenerate tetrahydrobiopterin, the essential cofactor for production of serotonin and dopamine, have almost undetectable levels of 5-hydroxyindole acetic acid (5-HIAA) in urine and require treatment with L-DOPA and 5-hydroxytryptophan (5-HTP). We previously showed that such infants, prior to treatment with 5-HTP, have extremely low cerebrospinal fluid 5-HIAA levels and have difficulty in sleeping, this being the main observable symptom of serotonin deficiency at this early age. Older patients with dihydropteridine reductase (DHPR) defect, being treated with L-DOPA and folinic acid and no longer taking 5-HTP, have detectable levels of 5-HIAA in urine and do not exhibit symptoms of serotonin deficiency such as disturbed sleep, movement disorders or psychological problems.

To further investigate this phenomenon, platelet serotonin was measured in two patients with DHPR defect and in an infant with 6-pyruvoyl-tetrahydropterin synthase (PTPS) defect and the results were compared with patients who may have other disturbances in serotonin metabolism/physiolo-gy. Platelet serotonin was extremely low in the infant with PTPS defect; 0.3 ng serotonin /10^9 platelets but higher in a 12 year old boy with DHPR defect; 49ng/109 and also in his 19 year old sister 48ng/109 with DHPR defect, both untreated with 5-HTP. Dietary sources of serotonin and low level synthesis based upon folinic acid may account for the presence of serotonin in platelets and the lack of serotonin deficiency symptoms in these older patients. These patients have no evidence of psychiatric or behavioral disorders.

By comparison, a 15yr old girl with episodic rage attacks had platelet serotonin of 46ng/109. One 12 yr old boy with attention-deficit / hyperactivity disorder (ADHD) with pronounced aggression, initially had a platelet serotonin of 224ng/109 which dropped to 8.3ng/109 after 4 months therapy with sertraline, a selective serotonin reuptake inhibitor. In a 10 year old boy with ADHD and Tourette's disease, platelet serotonin dropped from 264 ng/109 to 43ng/109 after 4 days on sertraline.

There is still much to understand about the interrelationships between serotonin metabolism/physiology and symptomatology in pteridine defect diseases where there is a true limitation in serotonin availability and in psychiatric and behavioral diseases, which are treated with drugs to alter serotonin availability.

Progressive apoptotic death of dopamine neurons in substantia nigra following striatal excitotoxic damage: a new model of Parkinson's disease

Foster JA, Groc L, Bezin L, Christopherson PL, Levine RA

Gossett Neurology Laboratories, Henry Ford Hospital, Detroit Veterans Administration Medical Center; Department of Pharmaceutical Sciences and Department of Psychiatry, Wayne State University, Detroit, MI, USA; and Laboratory of Molecular Neuropharmacology, CNRS-UMR5542, Lyon, France

Parkinson's disease is characterized by progressive death of dopamine neurons in the pars compacta of the substantia nigra, which can occur by apoptosis. Our experiments investigate nigrostriatal cell death in the short- (1, 2, 3 weeks) and long-term (5 months) following excitotoxic striatal lesion with kainic acid. Striatal excitotoxic lesion destroys neurons originating in the striatum and initially spares the striatal terminals of substantia nigra dopamine neurons; these cells subsequently die in the long-term by an unknown mechanism. While the striatal excitotoxin-induced death pattern is distinct from Parkinson's disease, this lesion can be used to examine the mechanisms of progressive death of nigral neurons. Striatal kainic acid induces short-term activities of striatal tyrosine hydroxylase and GTP cyclohydrolase, the initial enzyme in BH4 biosynthesis. We hypothesize that catecholamine or BH4 metabolism contributes to apoptotic death of nigrostriatal dopamine neurons during loss of support from striatal target cells (i.e. non-dopamine neurons). Kainic acid (2(g) or vehicle was unilaterally injected into the striatum of male Sprague Dawley rats (225-275g) under stereotaxic control. In situ end-labeling of tissue sections revealed apoptotic nigral neurons as early as 1 week postlesion. Furthermore, a 25-30% decrease in tyrosine hydro-xylase-positive dopamine neurons was observed in the ipsilateral substantia nigra (compared to contralateral substantia nigra) 5 months post-lesion. These data suggest that removal of striatal target cells of nigrostriatal dopamine neurons by kainic acid causes pro-

gressive death of nigral dopamine neurons by apoptosis. Since the production of nitric oxide has been linked to excitotoxicity, we are also investigating the role of nitric oxide in dopamine neuronal loss. Experiments are underway to determine if apoptotic death of nigrostriatal dopamine neurons following target cell loss can be prevented by selective inhibition of catecholamine, BH4, or nitric oxide metabolism. These studies will enable us to under-cover the mechanisms behind premature nerve cell death that occurs in many neurological disorders as well as in normal aging. The results obtained will allow for the development of rational and effective interventions in the process of premature nerve cell death.

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Inhibition of neuronal nitric oxide synthase by 4-amino pteridine derivatives: structure activity relationship of antagonists of (6R)-5,6,7,8-tetrahydrobiopterin cofactor

Fröhlich L, Kotsonis P, Traub H, Taghavi-Moghadam S, Al-Masoudi N, Hofmann H, Strobel H, Matter H, Pfleiderer W, Schmidt HHHW

Department of Pharmacology and Toxicology, University of Würzburg; Hoechst Marion Roussel, Frankfurt; Faculty of Chemistry, University of Konstanz, Konstanz, Germany

The family of nitric oxide synthase (NOS) catalyze the conversion of L-arginine to L-citrulline and nitric oxide (NO). Interestingly, NOS can be maximally activated by the ubiquitous cofactor, (6R)-5,6,7,8-tetrahydrobiopterin (BH4) [1,2]. Antagonists of BH4 may be of therapeutic importance to inhibit pathologically high NO-formation. Indeed, the 4-amino substituted analogue of BH4 was reported to be a potent NOS inhibitor [3,4]. Therefore we developed a series of novel 4-amino pteridine derivatives, antipterins, to pharmacologically target the neuronal isoform (NOS-1).

In order to functionally characterize the pterin/antipterin interaction and to establish a structure activity relationship (SAR), we systemically altered the functionalities in 2,4,5,6 and 7-position of the pteridine nucleus. Varying the substitution pattern in the 2, 5 and 7-position had no significant inhibition effect on enzyme activity. In contrast, bulky substituents in 6-position such as phenyl markedly increased the inhibitory potency of the reduced 4-amino-5,6,7,8-tetrahydropteridines, possibly as a consequence of hydrophobic interactions within NOS-1. However, this was not the case for the aromatic 4-amino-pteridines. Interestingly, chemical modification of the 4-amino function by dialkyl/arylation together with 6-arylation of the aromatic 2,4-diaminopteridine re-sulted in potent and efficacious inhibitors of NOS-I, suggesting possible hydrophilic interactions with hydrogen donating groups within NOS-1. Thus che-mical alterations at these positions of the endogenous BH4 cofactor represents a novel class of pterin based antagonists that are potent and efficacious inhibitors of NOS functions, as their IC50 values on NOS are in the very low micromolar range.

Interestingly, we found that the inhibitory effect of selected compounds on human and NOS I-III isoforms activity was slightly lower than was the case for native porcine NOS I. Thus there is great potential for isoenzyme-selective inhibitors of NOSs by following the antipterin approach. The present SAR agrees a) with the recently published crystal structure of the oxygenase domain of inducible NOS isoform (NOS-II) [5] b) with comparative molecular field ana-lysis (CoMFA) of a data-set of selected NOS-I inhibitors, which resulted in a statistically significant and predictive 3D-QSAR model of the pterin binding site interactions (unpublished results). Further optimization should be possible when the full length structure of NOS becomes available.

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Inverse relationship between alpha tocopherol and neopterin concentrations in cerebrospinal fluid of patients dementia

Fuchs D, Leblhuber W, Widner B, Walli F, Sattler W

Institute of Medical Chemistry and Biochemistry, University of Innsbruck, and Ludwig Boltzmann institute for AIDS Research, Innsbruck; Department of Gerontology, Geriatric Clinic Wagner-Jauregg, Linz; and Institute of Medical Biochemistry, University of Graz, Graz, Austria

Oxidative damage is considered as an important aspect in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease. However, *in vivo* measurements of oxidative stress are only limited available and very often suffer from low sensitivity. Concentrations of alpha-tocopherol (vitamin-E) were found significantly decreased in the cerebrospinal fluid (CSF) of patients with Alzheimer's disease, and deficiency of alpha-tocopherol in the brain might be a consequence of increased oxidative stress.

We compared CSF concentrations of alpha-tocopherol and neopterin in 12 patients with dementias (5 Alz-

heimer's disease, 2 Huntington's disease, 4 incipient dementias, 1 vascular dementia; mean age 72.9 years). Alpha tocopherol was measured by high pressure liquid chromatography with fluorescence detection, and neopterin concentrations were determined by ELISA (BRAHMS; Berlin, Germany). Compared to healthy controls, patients had significantly increased CSF neopterin and low alpha- tocopherol concentrations, and there existed an inverse correlation between CSF neopterin and alpha-tocopherol concentrations.

Neopterin is produced from monocytic cells upon stimulation with interferon-γ. A significant association exists between the amount of neopterin released by activated macrophages and their capacity to release reactive oxygen species, and increased neopterin concentrations in body fluids therefore not only reflect immune activation but also can be regarded as an indicator of oxidative stress. The finding of lowest alpha-tocopherol in patients with highest neopterin concentrations indicates that chronic immune activation and oxidative stress seem to contribute to depletion of alpha-tocopherol and decreased concentrations of the antioxidant in patients are unlikely to be just a result of reduced dietary intake.

Role of Tyr171 and ser158 in the catalytic reaction of sepiapterin reductase as evidenced by site-directed mutagenesis

Fujimoto K, Inaba A, Tomomura A, Ichinose H, Nagatsu T, Katoh S

Department of Biochemistry, Meikai University, School of Dentistry, Japan; Institute for Comprehensive Medical Science, School of Medicine, Fujita Health University, Japan

Sepiapterin reductase (SPR) is essential for BH4 biosynthesis by catalyzing the last step of the synthesis, and is important for controlling the level of BH4 in tissues. SPR, identified as a member of the NADP(H)-preferring short-chain dehydrogenase / reductase (SDR) family, has a molecular subunit size of about 20-30 kD and NADPH-preferring activity for the reduction of various carbonyl compounds. The catalytic site of SDR family enzymes consists of serine, tyrosine, and lysine (the Ser-Tyr-Lys triad). Recently Auerbach et al. determined the crystal structure of mouse SPR. They demonstrated that the Ser-158-Tyr171 triad in the SPR subunit was located near the carbonyl groups of sepiapterin. To prove if these amino acids of SPR act for catalyzing the substrate, we cloned SPR and prepared mutants of it by the methods of constructed deletion and site-directed mutagenesis.

SPR consists of 262 (rat) or 261 (human and mouse) amino acids, as deduced from the respective cDNAs. Clones encoding rat SPR (rSPR) and human SPR (hSPR) were amplified by PCR. Amplified rSPR and hSPR cDNA were ligated into expression vectors, and expressed recombinant SPRs were purified by ammonium sulfate fractionation and affinity chromatography. The purified fusion SPRs showed the values of Km and Vmax for sepiaterin and NADPH similar to those of native SPR.

An N-terminal truncation mutant, rSPR13-262 showed similar Kms and about 10% of the kcat for sepiapterin and NADPH, compared with the wild-type value. Mutants of rSPR29-262, rSPR34-262, and hSPR34-261 did not show any activity. C-terminal truncation mutants of rSPR1-227 and hSPR1-227 showed no activities, either. These results suggest that the important regions are located between residues 1-12 for supporting the appropriate conformation of the catalytic domain, and in 228 - 262 for binding the pterin substrate. However, the distance between the A-X-L-L-S sequence, previously reported as a putative pterin binding site, and the pteridine substrate, was too far as judged from crystal analysis, so this sequence might preferably function as a component in the region for binding coenzyme rather than substrate.

Site-directed mutants of rSPRS158D, rsSPRY171 V, and rSPRK1751 had similar Kms for sepiapterin but showed low activity (kcat/Km values) of about 25% of that of the wild type. Since the two amino acids Tyr and Ser were located within a similar distance to the carbonyl group of sepiapterin in the crystal structure of mouse SPR, the double point mutant rSPRY171V&S158D was cloned and found to be totally inactive. These results show that Ser158, Tyr171, and Lys175 contributed to the catalytic activity and the especially both Tyr 171 and Ser 158 are necessary for the most effective proton transfer to the carbonyl functional groups of the substrate.

Patients suffering from BH4 deficiency have most frequently a 6-pyruvoyl-tetrahydropterin synthase deficiency or GTP cylohydrolase I deficiency. But no inherited abnormality of SPR has been reported, which suggests than SPR is essential for organisms to live. This study, however, shows an additional reason for undetectable deficiency of SPR because the natural rate of simultaneous mutation at Tyr171 and Ser158 of SPR may be infinitesimally low.

Potential Involvement of Oxidative Stress in the Cell Death of Developing Nigral Neurons in vivo

Groc L, Bezin L, Foster JA, Weissmann D, Levine RA

Gossett Neurology Laboratories, Henry Ford Hospital, Detroit Veterans Administration Medical Center; Department of Pharmaceutical Sciences and Department of Psychiatry, Wayne State University,

Detroit, MI; and Laboratory of Molecular Neuropharmacology, CNRS-UMR5542, Lyon, France

Naturally occurring cell death via apoptosis has been reported in the developing substantia nigra, culminating during the perinatal period. The cellular pathway(s) mediating the developmental apoptosis in nigral neurons is still unknown. Deprenyl, a monoamine oxidase (MAO)-B inhibitor, has therapeutic benefits in Parkinson's disease, which is characterized by death of dopamine neurons in the substantia nigra. Deprenyl also has numerous antioxidant properties, which may mediate its protective effects in catecholaminergic neurons during neurotoxic-induced apoptosis. The present study investigated: 1) whether daily treatment with deprenyl (0.1mg/kg) from embryonic day (ED) 18 to postnatal day (PND) 42 could prevent apoptosis of dopamin-ergic neurons in the substantia nigra, and 2) whether antioxidant enzyme activities are modified during the development of the substantia nigra in both control and deprenyl-treated rats. Dopamine neurons were counted on sections from the substantia nigra following immunohisto-chemical detection of tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis. Total neurons were counted following Nissl-staining on another set of nigral sections from each brain. The ability of high doses of deprenyl (1, 10 mg/kg) to pre-vent nigral neuronal apoptosis was determined by counting the number of apoptotic cells during the main apoptotic period (PND3). Activities of the anti-oxidant enzymes, superoxide dismutase (SOD), cata-lase, and glutathione peroxydase (GPx) were measu-red in substantia nigra and striatum in control and treated rats.

Deprenyl treatment did not alter the number of dopamine neurons or total neurons in the pars compacta and the pars reticulata of the substantia nigra. These results exclude the ability of low and high doses of deprenyl to rescue neurons from cell death in the developing substantia nigra. It is possible that the mechanism of deprenyl's protective action in adults was not yet mature during the peak of apoptosis in the developing substantia nigra. It is also possible that multiple pathways can lead to neuronal apoptosis within the substantia nigra, some of which are not sensitive to deprenyl treatment. We are currently identifying the pathways mediating naturally occurring apoptosis of dopaminergic neurons during development. These studies will reveal apoptotic pathways that may be inappropriately activated in neurological diseases, particularly Parkinson's disease.

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The importance of neopterin, C-reactive protein and serum-transferrin receptor for peripartal assessment of iron status

Guntermann H, Perewusnyk G, Breymann C, Huch R, Huch A

Department of Obstetrics, University Hospital of Zurich

Upon delivery, a "physiological" inflammatory process begins which lasts for several days.

It is know that the value of ferritin as a parameter to assess iron status is lost in the presence of inflammation. This means that in cases with postpartum elevated C-reactive protein (CRP) values, ferritin can not be used to determine the iron status. In contrast, serum transferrin receptors (s-TfR) are not affected by inflammatory processes, meaning that when there are indications of inflammation, s-TfR rather than ferritin should be used.

To determine whether an activation of the cellular immune response can be shown by peripartal neopterin levels and whether s-TfR be used as a reliable parameter in the first 48 hours post partum to assess iron status.

In a prospective study with 65 normal singleton pregnancies, a differential blood count and iron status as well as neopterin and CRP levels were determined. The first measurement was taken within 48h prior to the second within 48h after delivery.

The average CRP was 9.93 mg/l antepartum, compared with a significantly elevated level post partum of 51.69 mg/l. IL-6 and leukocytes, usually already elevated prior to delivery, increased significantly post partum (II-6 : $p = 0.0002$; WBC : $p = 0.0016$). Post partum a just barely significant in-crease in neopterin was found compared to pre-partum, when it was also, on average, above the normal range (prepartum: 9.91 nmol/l vs. postpartum: 10.62 nmol/l). Neither prior to nor after delivery was any correlation found between neopterin and hemato-crit, or between neopterin and IL-6. Post partum the average s-TfR concen-tration was significantly lower than before delivery ($p<0.0001$). For anemic women (Hb<11.0 g/dl) an inverse relationship was found between the estimated blood loss and the postpartum s-TfR values.

Increased peripartal neopterin values indicate an activation of the cellular immune response. It was shown that within the first 48 h post partum, s-TfR can be used as a diagnostically reliable parameter for the evaluation of iron status. This might be a consequence of blood loss during delivery, which leads to hamodilution in the subsequent hours.

Demonstration of monoamine-releasing action of 6R-L-erythro-tetrahydrobiopterin in RBL2H3 and PC-12 cells

Hasegawa H, Nakazawa N, Yamamoto K, Nakanishi N, Oguro K

Department of Biosciences, Teikyo University of Science and Technology, Uenohara, Yamanashi ; and Department of Biochemistry, Meikai University School of Dentistry, Sakado, Saitama, Japan

6R-L-erythro-tetrahydrobiopterin(6R-BH4) stimulates serotonin release from RBL2H3 cells and from PC-12 cells which has been loaded with 5HT. RBL2H3 cells, an established cell line retaining many characteristics of mast cells, synthesize serotonin as well as BH4. The cellular serotonin is released by stimulation such as an antigen/IgE, reflecting its mast cell nature. The release can also be triggered by high KCl (+30 mM KCl) up to 2.3 % of the cellular serotonin or by appro-priate calcium ionophores such as A23187 (30 nM) up to 9.1 % within 1 hour. On exposure to 6R-BH4, the cells also secreted serotonin in a concentration de-pendent manner. The half maximal effect of 6R-BH4 was observed at about 0.1 nM and the maximum re-lease reached about 10% of the cellular serotonin. With sepiapterin, minimal release was observed under similar experimental conditions at higher concentrations than 1 μM. Since the effective concentration of this pterin was much higher than that of 6R-BH4, the mechanism of stimulation might be different. Sepiapterin was not effective in the presence of methotrexate (50% inhibition was caused by 1 μM), suggesting that sepipapterin worked after conversion to BH4 in which dihydrofolate reductase was involved. Sepiapterin administration causes intracellular BH4 concentration rise, with 10 μM sepipaterin, for examples, cellular BH4 content reached about 20 fold within 1 hour (in the absence of methotrexate). The increased cellular BH4 decays at a half life of about 2 hours, suggesting that the accumulated BH4 either leaks out or decomposes in the cell. The possible leak of BH4 after sepiapterin administration was accounted for the observed serotonin release. 6S-BH4, the diastereomer of 6R-BH4, did not cause significant release of serotonin at doses up to 100 μM. Furthermore, 6S-BH4 inhibited serotonin release which was caused by 6R-BH4 simultaneously administered. The serotonin-releasing response of PC-12 which was established from a rat pheochromo-cytoma cell line was essentially the same with RBL2H3 cells. This suggests that the observed cellular response to low concentration of 6R-BH4 might be common in a wide range of monoaminergic endocrine or paracrine cells and presumably in neurons as well. In 1990, Miwa and his coworkers observed dopamine release in rat CNS caused by infusion of 6R-L-erythro-tetrahydrobiopterin (6R-BH4) using the brain in vivo microdialysis technique (1). Liang and Kaufman confirmed dopamine release by in vitro superfusion of rat striatal slices (2). Present work may serve as analytical system to elucidate cellular mechanism of the mono-amine releasing action of 6R-BH4.

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Pteridines enhance degradation of the porphyrin ring system in heme proteins

Horejsi R, Jung C, Reibnegger G

Medical Chemical Institute and Pregl Laboratory, University Graz; Max-Delbrück-Center for Molecular Medicine, Berlin-Buch; Germany

The effects of 11 pteridine derivatives with different structures (tetrahydrobiopterin, tetrahydroneopterin, tetrahydropterin, dihydrobiopterin, dihydroneopterin, dihydroxanthopterin, biopterin, neopterin, monapterin, xanthopterin and pterin) on the heme proteins cytochrome c, hemoglobin and myoglobin were investigated using electronic absorption spectroscopy. The heme proteins were incubated with 10μmol of the pteridine over a period of 24 hours and the UV-VIS spectra were scanned between 200 and 700 nm.

The most distinctive effects were caused by the tetrahydropterins, followed by the dihydrocompounds. A high ability to reduce the cytochrome c immediately after addition of the tetrahydropterin derivatives was observed. The transfer from the ferric to the ferrous form was performed between seconds and very similar spectra were measured over a period of 24 hours. As we suggest that tetrahydro-compounds dissociate into xanthopterin, and this dissociation can be seen within a few minutes, a more drastically degradation of the heme could be prevented. The dihydrocompounds show this reducing effect slower and not in that extent: after 24 hours about 70 % are reduced. Cytochrome c is the only hemeprotein that is reduced by aromatic pterins to a degree of 40 percent after 24 hours in this in vitro assay.

It is known from earlier experiments that aromatic pteridine derivatives enhance radical-induced effects. This reducing capacity of aromatic pteridine derivatives could be possibly attributed to an activated oxygen species generated in that air-saturated atmosphere. Hemoglobin and myoglobin interact in a distinctly different way with the pteridines. Tetrahydrocompounds and dihydropteridines cause the cleavage of the porphyrin as it was observed in a decrease of the Soret-band and synchronously an increase of the amplitude CO-difference spectrum. The range of degradation is between 10 and 40%. Aromatic pterins

seem to show no or nearly no interaction with the heme proteins, this percentage of porphyrin degradation occurs also in buffer in an air saturated atmosphere without addition of pterins.
These findings might get a certain importance in patients with an activated immune system (higher neopterin levels) corresponding to higher dihydroneopterin levels in the same ratio.
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An evaluation of low-cost progression markers in HIV-1 seropositive Zambians.

Hosp M, Lisse IM, Fuchs D, Quigley M, Mwinga AM, Godfrey-Faussett P, Porter JDH, Luo N, Pobee J, Wachter H, McAdam KPWJ

Department of Medicine, University Teaching Hospital, Lusaka, Zambia; Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK; Department of Pathology, Hvidovre Hospital, Copenhagen, Denmark; Institute of Medical Chemistry and Biochemistry, University of Innsbruck, and Ludwig Boltzmann Institute for AIDS-Research, Innsbruck, Austria

In this study, we compared T subset counts obtained by the immunoalkaline phosphatase (IA) method on blood slides with results from FACScount analysis. In addition was assessed the correlation of CD4 counts with neopterin, beta-2-microglobulin (B2M), total lymphocyte count and hemoglobin, and we investigated correlations between progression markers and clinical parameters in HIV-1 seropositive Zambians.
Of 147 HIV-1 seropositive patients presenting to an outpatient clinic in Lusaka between February and April 1995, blood was taken for CD4 counts, serum progression markers and full blood count. A detailed clinical history and medical examination was taken at that point, and the patients were seen 3-monthly over the following 12 months. Further CD4 counts were taken after 6 and 12 months.
CD4 counts obtained by the immunoalkaline phosphatase method were consistently higher than CD4 counts from FACScount analysis (mean difference 149/µl), but were highly correlated ($r = 0.89$, $p<0.001$). Neopterin, B2M, lymphocyte count, and hemoglobin showed a strong correlation with CD4 count. Of the serum progression markers, neopterin was more sensitive than B2M to detect HIV related symptoms and to predict weight loss and death in the follow-up period. A lymphocyte count of less than 2000/µL at baseline would identify 69% of patients with a CD4 count below 200/µL, with a specificity of 60%. Lymphocyte count and hemoglobin were significantly lower in patients falling sick or dying in the follow-up period. Hemoglobin also correlated with diarrhoea, weight loss, and oral candidiasis. When stratified by sex, these associated were very strong in males, but weaker in females.
Determination of CD4 counts on slides is a valuable and cheap alternative to flow cytometry based methods. Neopterin and B2M correlate well with CD4 counts; neopterin appears to be more sensitive for the clinical evaluation of the patients in this study. Hemoglobin might only be useful as a progression marker in male individuals. More studies are needed to assess the power of total lymphocyte counts to predict CD4 counts in African patients.

Structure of the human sepiapterin reductase gene

Ichinose H, Ohye T, Suzuki T, Inagaki H, Katoh S, Nagatsu T

Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan; and Department of Biochemistry, School of Dentistry, Meikai University, Sakado, Sitama, Japan

Sepiapterin reductase (SPR) catalyzes the final step of the biosynthetic pathway of tetrahydrobiopterin, which is an essential cofactor for aromatic amino acid hydroxylases and nitric oxide synthases. To aid the analysis of any possible human disease caused by mutations in SPR, we have cloned and characterized the human SPR gene.
We screened a human genomic library constructed in FixII, and isolated seven strongly hybridized clones (hg SPR 2-8) using the cloned human SPR cDNA (1) as a probe. Structural analysis of these clones revealed that the entire human SPR gene is approximately 4kb in length and consists of three exons. A 3.4kb EcoRI genomic fragment containing exon 1 was subcloned, and the region 300 bp immediately upstream of the 5' end of exon 1 was sequenced. No TATA-like sequence and no CAAT-box motifs were found in the upstream vicinity of the transcription start site, consistent with features of a housekeeping gene. Recognition site for the DNA-binding factor Sp 1 was found in the promoter region. The direct R-banding fluorescence in situ hybridization method was performed for chromosomal localization of the human SPR gene with an EcoRI digested genomic DNA fragment (3.4 kb in length) containing exons 1 and 2 used as a probe. Of the 100 R-banded (pro)metaphase plates examined, 14%exhibited complete twin spots on both homologues, 59% gave incomplete single and/or twin spots on either or both homologues. 59% gave incomplete single and/or twin spots on either of both homologues, and no spots were detectable in the others (27%). The signals were localized to p13 bands of chromosome 2. No twin spots was observed on other chromosomes.

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Decreased levels of 6-tetrahydrobiopterin in psoriatic epidermis

Kaczorowska AK, Beazley WD, Weller R, Schallreuter KU

Clinical and Experimental Dermatology, Department of Biomedical Sciences, University of Bradford; and University Department of Dermatology, Edinburgh, UK

Psoriasis is a common chronic skin disease of unknown pathogenesis. The major manifestations of the disease are inflammatory reactions in the skin and hyperproliferation and aberrant differentiation of epidermal keratinocytes. The switch between the processes of human keratinocyte proliferation and diffe-rentiation is governed by the level of intracelluar calcium ions which may be regulated by catecholamines. Catecholamine production in cells is regulated by the essential cofactor - (6R) L-erythro 5,6,7,8-tetrahydrobiopterin (6-BH4) - required for the hydroxylation of phenylalanine and tyrosine, the two initial steps in catecholamine de novo synthesis. 6-BH4 is also an essential cofactor for nitric oxide synthase and increased levels of the inducible form of this enzyme have been reported in psoriasis.
The aim of this study was to investigate 6-BH4 levels in epidermal suction blisters obtained from patients with psoriasis and their possible link with abnormal proliferation and differentiation of keratinocytes in psoriatic epidermis.
Total biopterin levels and the specific activity of the rate-limiting enzyme for *de novo* synthesis of 6-BH4, GTP-cyclohydrolase I (GTP-CH-I) were determined in epidermal cell extracts from lesional (n=4) and non-lesional (n=10) psoriatic skin and skin from healthy donors (n=10) using reverse-phase HPLC. 6-Biopterin levels were significantly reduced in both lesional and non-lesional psoriatic samples compared to healthy controls. However, GTP-CH I activity measured under substrate saturating conditions remained within the normal range.
We postulate that the low levels of 6-biopterin may be due to the activity of soluble guanylate cyclase which converts GTP, the 6-BH4 precursor in the de novo biosynthetic pathway, to cGMP. The levels of cGMP and the activity of soluble guanylate cyclase have been shown to be increased in psoriatic cells. Our results suggest that the reduced level of 6-BH4 may lead to perturbed catecholamine biosynthesis and subsequently give rise to the abnormal differentiation pattern seen in psoriasis. This hypothesis can be supported by decreased 2-adrenoreceptor densities reported previously in psoriatic keratinocytes.

Novel regulatory mechanisms of tetrahydrobiopterin in NO Synthase function

Kotsonis P, Fröhlich LG. Shutenko ZV, Horejsi R, Oettl K, Ghisla S, Köster SH, Pfleiderer W, Schmidt HHHW

Department of Pharmacology and Toxicology, Julius-Maximilians University, Würzburg, Germany; Institute of Medical Chemistry and Pregl-Laboratory University, Graz, Austria; and Faculty of Chemistry and Faculty of Biology, University of Konstanz, Konstanz, Germany

The family of NO synthases are homodimeric enzymes which catalyze an NADPH-dependent conversion of L-arginine to L-citrulline and the signaling molecule, nitric oxide (NO). Interestingly, all NOS require the ubiquitous cofactor, (6R)-5,6,7,8-tetrahydrobiopterin (BH4), for maximal activity [1,2] although the underlying mechanism(s) of activation is not fully understood. This may involve allosteric, structural and redox effects within the catalytic center. To further elucidate the function of BH4 in purified neuronal NOS (NOS-I), we developed pterin-based inhibitors termed anti-pterins [3].
In the present study we examined the effect of BH4 and anti-pterins on enzyme activity, quaternary structure and spectroscopic heme absorption properties.
Initial investigations using purified neuronal NOS (NOS-I) revealed a lack of intrinsic 4a-pterincarbinolamine dehydratase activity arguing against any classical BH4 redox cycling in NOS analogous to the aromatic amino acid hydroxylases. Interestingly, in the absence of either L-arginine or BH4, NOS-I quaternary structure was perturbed resulting in a loss of active, dimeric enzyme and the appearance of inactive monomers. This latter phenomenon was related to the uncoupling of reductive oxygen activation from L-arginine oxidation in the active center and the subsequent formation of superoxide (O_2^-) and hydro-gen peroxide (H_2O_2) since it was inhibited by either superoxide dismutase, catalase or N-nitro-L-arginine. The stabilizing effect of BH4 was also mimicked by the antipterin 2-amino-4,6-dioxo3,4,5,6,8,8a,9,10-octa-hydroaxazolo [1,2f]-pteridine (PHS-32), which also displaced both endogenous BH4 and exogenous [3H]BH4 in a competitive manner. In contrast, PHS-32 did not mimic the allosteric effect of BH4 on an L-arginine-dependent absorbance change of the NOS-1 heme Soret band. Taken together, these findings reveal a protective and stabilizing effect of BH4 and L-arginine on enzyme structure during catalysis. We suggest that BH4 increases the affinity of NOS-I for substrate, promotes coupled L-arginine turnover and, thereby, suppresses the formation of autodamaging uncoupled catalysis products. Interestingly, exogenous BH4 may play an

additional role within the catalytic center by directly "scavenging" excessively generated $O_2^{-\cdot}$. Consistent with this, mass spectroscopy measurements revealed that NO is capable of oxidizing BH4 to biopterin and a novel reaction product that is yet to be characterized.

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Reconstitution of a metabolic pathway with triple-cistronic IRES-containing retroviral vectors for correction of tetrahydrobiopterin deficiency

Laufs S, Kim SH, Kim S, Blau N, Thöny B

Division of Clinical Chemistry and Biochemistry, Department of Pediatrics, University of Zurich, Zurich, Switzerland; Institute for Molecular Biology and Genetics, Seoul National University, Seoul, South-Korea

Tetrahydrobiopterin (BH4) is an essential cofactor for catecholamine and serotonin neurotransmitter biosynthesis. BH4 biosynthesis is carried out in a three-enzyme pathway involving GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS) and sepiapterin reductase (SR). Treatment of genetic defects leading to BH4 deficiency requires neurotransmitter replacement since synthetic cofactor does not efficiently penetrate the blood-brain barrier. Autologous fibroblasts transplanted into the brain as depository cells for drug delivery might offer an alternative. However, normal fibroblasts do not express GTPCH, and fibroblasts from PTPS patients lack two biosynthetic enzymes for BH4 production. Here, we engineered primary fibroblasts by the use of triple-cistronic, retroviral vectors for cofactor production. Constitutive SR activity in these cells enabled BH4 biosynthesis by transducting GTPCH and PTPS cDNAs together with a selective marker coupled in a single transcript with two IRES-elements in tandem. Upon reaching a critical threshold concentration (<400 pmol/mg protein) of intracellular BH4, the fibroblasts efficiently released cofactor even under non-dividing conditions. The use of triple-cistronic vectors for single transduction to reconstitute metabolic pathways or to treat multi-genetic diseases may be useful for engineering, for instance, depository cells for various organs, including the nervous system.

Melanocyte tyrosine hydroxylase could supply the substrate, L-DOPA, fortyrosinase for melanogenesis

Marles LK, Schallreuter KU

Clinical and Experimental Dermatology, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire, UK

Tyrosine hydroxylase (TH: EC 1.14.16.2) is a pterin dependent enzyme catalysing the rate limiting step in the biosynthesis of the catecholamines. TH is present within the central nervous system and localised areas of the brain. More recently, TH has been shown to exist in keratinocyte of the human epidermis and catecholamine biosynthetic properties of these cells have been demonstrated. Human epidermal melanocytes hold a unique position in the pigmentation process. These cells possess all enzymes necessary for de novo synthesis/recycling/regulation of (6R)-L-erythro 5,6,7,8 tetrahydrobiopterin (6BH4). Therefore, it was of interest to determine whether melanocytes also express TH. PCR studies involving total melanocyte cDNA template followed by se-quence analysis were able to confirm the expression of TH mRNA in vitro. Isoform specific priming demonstrated the expression of hTH-1. At the present time, it remains unclear whether hTH2, hTH3 and hTH4 are also expressed.

Immunohistochemical studies utilising monoclonal mouse anti-human tyrosine hydroxylase antibody were unable to detect TH within melanocytes of hu-man full thickness skin. However, TH was observed to be localized in granules within melanocytes when the same method was applied directly to cells cultured.

The lack of in vitro or in vivo evidence for catecholamine synthesis in melanocytes suggests a novel activity for TH within the melanocyte. We therefore propose that TH could be coupled with tyrosinase (1.14.18.1), the key enzyme for melanogenesis, by supplying the substrate activator L-DOPA for enzyme induction. TH activity is reliant on the presence of the essential cofactor (6R)-L-erythro-5,6,7,8-tetrahydrobioterin (6BH4). In contrast the 'dopa oxidase' activity of tyrosinase is neither dependent on, nor inhibited by 6BH4. In this context, it is noteworthy that hydroxylation of tyrosine by tyrosinase is allosterically inhibited by 6BH4. These properties allowed the development of an assay, using purified bovine TH and unknown tyrosinase, which demonstrates the coupling of TH and tyrosinase in vitro by following the formation of dopachrome at 475nm. The reaction is pH dependent.

Neopterin concentrations in cerebrospinal fluid compared to serum in neurodegenerative diseases

Leblhuber W, Widner B, Walli F, Fuchs D.

Department of Gerontology, Geriatric Clinic Wagner-Jauregg, Linz; Institute of Medical Chemistry and Biochemistry, University of Innsbruck, and Ludwig Boltzmann Institute for AIDS Research, Innsbruck;

Department of Internal Medicine, University of Graz, Graz; Austria

Within a study of 71 patients with neurodegenerative diseases, of 29 patients (12 Alzheimer's disease according to NINCDS/ADRDA criteria, 17 Huntington's disease proven genetically or by autopsy) parallel serum and cerebrospinal fluid (CSF) specimens were available. In these specimens neopterin concentrations weres measured by ELISA (BRAHMS Diagnostica, Berlin Germany). Compared to healthy controls of similar age, significantly increased neopterin concentrations have been observed in serum and CSF in both groups of patients. Alzheimer's disease patients had higher neopterin concentrations than patients with Huntington's disease. Interestingly, neopterin concentrations were significantly higher in serum than in CSF in both groups, and there existed a significant correlation between serum and CSF neopterin concentrations in either the whole group of patients or in both sub-groups. The data further confirm and extend the observations made earlier that significant immune activation is going on in patients with Huntington's disease and Alzheimer's disease. Although there is a relationship between older age and higher neopterin concentrations, there is evidence for an additional impact of neurodegenerative disorders to further stimulate neopterin production in serum and CSF of patients. The higher concentrations of neopterin in serum than in CSF may indicate a higher degree of immune activation systemically rather than intrathecally. However, higher neopterin concentrations would also result when the population of cells capable of neopterin production namely monocytes / macrophages is greater in the peripheral blood than in the brain even when the concentration of cytokines present in the circulation is lower in blood than that in brain. Further studies are necessary to clarify this issue.

The influence of D-neopterin and dihydrobiopterin on endothelial cells *in vitro*

Martinsons A, Rudzite V, Bratslavska O

Latvia Medical Academy, Latvia Institute of Cardiology, and Latvia Institute of Microbiology, Riga, Latvia

We have examined the effect of neopterin and dihydrobiopterin on endothelial cells of lungs in vitro (CRA cultur). After the adhesion of the cells we incubated the culture with neopterin (5, 15, 30 nmol/L) or dihydrobiopterin (5, 15, 30 nmol/L) for 48 hours. We examined the total DNA synthesis (by adding 3H thymidine 2 μCi/ml from the 47th till the 48th hour of our experiment; registration the 3H thymidine incorporation in Packard chamber). We examined 5000 cells by light microscope and determined the mitotic index, the amount of pathological mitosis, apoptosis and necrosis.
We have observed that neopterin 30 nmol/L and dihydroneopterin 15 and 30 nmol/L induce pathologi-cal mitosis of endothelial cells of lungs. The incorporation of 3H thymidine (the total DNA synthesis) increases by incubation of the endothelial cells with neopterin (30nmol/L) and dihydrobiopterin (15nmol/L). The incubation of endothelial cells with dihydroneopterin 15 and 30 nmol/L leads also to necrosis of these cells.
These data allow us to suggest that neopterin and dihydropterin promote endothelial cell damage through the pathological mitosis, but dihydroneopterin can also be added to the factor, which leads to the necrosis of endothelial cells.

Regulation of the biosynthesis of tetrahydrobiopterin

Milstien S

Laboratory of Cellular and Molecular Regulation, NIMH, NIH, Bethesda, MD, USA

De novo synthesis of tetrahydrobiopterin is regulated mainly by the activity of the first enzyme of the biosynthetic pathway, GTP cyclohydrolase I (GTPCH). GTPCH has long been known to be feedback inhibited by the end product of the pathway, tetrahydrobiopterin, but until the purification and cloning of a tightly GTPCH-associated regulatory protein, this was considered to be an inherent property of GTPCH. In the liver, the GTPCH feedback regulatory protein (GFRP) forms a complex with GTPCH and confers a unique type of endproduct feedback sensitivity: to inhibition by tetrahydrobiopterin which can be overcome specifically by L-phenylalanine. GFRP is expressed in many other tissues besides liver, including the brain, and thus tetrahydrobiopterin synthesis in these tissues may also be regulated in the same manner. Quantitative determination of GFRP mRNA expression in various regions of the rat brain was carried out by ribonuclease protection assays and compared with GTPCH mRNA expression. Although aminergic neurons expressed both GFRP and GTPCH mRNAs, the ratio of GFRP mRNA to GTPCH mRNA was much greater in serotonergic neurons than in dopaminergic neurons, suggesting that GFRP may regulate tetrahydrobiopterin levels, and in turn, serotonin synthesis in serotonin neurons, by a feedback regulatory mechanism whose setpoint is determined by the relative concentrations of tetrahydrobiopterin and phenylalanine.
Tetrahydrobiopterin is also absolutely required by all three isoforms of nitric oxide synthase and it may regulate production of the important free radical gas, nitric oxide (NO). Availability of intracellular tetra-

hydrobiopterin likely plays a role in controlling NO-dependent vascular tone under normal and pathological conditions. Furthermore, overproduction of NO as a result of immune stimulation is thought to contribute to septic shock and may cause neuronal cell death. We have shown that cytokines increase NO production by stimulating parallel expression of the inducible form of NO synthase and GTPCH expression and tetrahydrobiopterin synthesis in glial cells. These cytokine-mediated stimulations are significantly higher in glial cells and hepatocytes from insulin receptor knockout mice, models for type II diabetes, compared to cells from wild type or heterozygote mice. Furthermore, insulin blocks cytokine-mediated increases in NO and tetrahydro biopterin in cells from normal animals, suggesting that neuropathology of type II diabetes might result from overproduction of NO as a result of defective insulin signaling.

FT-Raman spectroscopic investigations on the oxidation of 6(R)-L-erythro 5,6,7,8 tetrahydro biopterin by hydrogen peroxide and UVB light

Moore J, Beazley WD, Wood JM, Schallreuter KU

Clinical and Experimental Dermatology, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorks, UK

Previous work on the oxidation of 6(R)-L-erythro 5,6,7,8 tetrahydrobiopterin (6BH4) has resulted in conflicting hypotheses and different oxidation mechanisms including cleavage of the dihydroxy propyl side chain yielding 7,8-dihydroxanthopterin or the full oxidation of the tetrahydro pyrazine ring resulting in 6-biopterin [1,2]. In both cases the oxidation products were investigated using spectrophotometric techniques leading to ambiguous interpretation of the results. As Fourier Transform Raman spectroscopy (FT-Raman) yields unique information regarding the vibrational behavior of the fused heterocyclic pterin ring system, it is possible to determine the structure of different reaction products. Additionally, this method can directly follow molecular interactions leading to a greater understanding of the possible 6BH4 oxidation mechanism [3].

The resulting oxidation products were further analysed by high performance liquid chromatography. The oxidation of 6BH4 by hydrogen peroxide was followed 1) at different pHs in the absence of buffers and 2) after different doses of UVB radiation. Preliminary findings show at pH 0.45 that the hydrogen peroxide generated reaction products are 6-biopterin and 6-neopterin. A possible explanation for the lack of side chain cleavage may result from the absence of an acid base reagent (i.e a buffering agent) which could facilitate the side chain cleavage reaction.

Using isotopic exchange, we were able study the change in oxidation products because the N2' amino, N3, N5, N8, C1' and C2' hydroxy protons rapidly exchange with deuterium in solution. Since N5 is the main site for protonation, replacing this proton with a deuterium atom resulted in the formation of 6 biopterin and 6 hydroxy methyl pterin [4]. The formation of 6-hydroxy methyl pterin may follow a similar mechanism to the periodate oxidation/borohy-dride reduction reaction with deuterated 6BH4 acting as a reducing agent.

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Structural implications of cofactor binding on phenylalanine hydroxylase – effects of 6 and 7 tetrahydrobiopterin on the secondary structure of monomeric, dimeric and tetrameric phenylalanine hydroxylase

Moore J, Wood JM, Schallreuter KU
Clinical and Experimental Dermatology, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorks, UK

The tetrahydropterin dependent amino acid hydroxylase, phenylalanine hydroxylase [PAH: EC 1.14.16.1], catalyses the hydroxylation of the essential amino acid L-phenylalanine to L-tyrosine in the presence of molecular O_2. 7(R)-L-erythro-5,6,7,8 tetrahydrobiopterin (7BH4) is non-enzymatically formed when the natural cofactor of PAH, 6(R)-L-erythro-5,6,7,8 tetrahydrobiopterin (6BH4) is recycled. It has been shown that 7BH4 can act as a poor substrate and as a potent inhibitor of PAH [1]

Previously, Resonance Raman spectroscopic studies on tyrosine hydroxylase have only investigated the resonance-enhanced vibrations of bound catecholate and phenolate complexes and they have not revealed any structural changes in the enzyme [2]. Employing a modified PAH assay, the influence of different cofactors can be followed accurately, excluding catalytic recycling of the cofactor 6BH4. Using activation / inhibition plots of PAH versus cofactor concentration we were able to produce cofactor – enzyme complexes that correspond with the known active structures of this enzyme.

We present for the first time the FT-Raman spectra of phenylalanine hydroxylase with bound 6BH4 and the bound structural isomer 7BH4. In addition the coordination and oxidation state of the iron centre has been probed with regard to alteration of ligand interactions and the production of active monomers

and dimers as well as the catalytically inactive tetramers.

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Non-invasive immunologic monitoring in heart-, kidney-, and combined kidney-pancreas transplantation

Müller TF, Neumann MC, Vogl M, Grebe SO, Radsak K, Reibnegger G, Müller MM, Lange H

Department of Nephrology, Institute of Virology, Philipps-University, Marburg, Germany; Institute of Laboratory Diagnostics, Kaiser-Franz-Josef-Hospital, Vienna; and Institute of Medical Chemistry, Karl-Franzens-University, Graz, Austria

Infections and rejections are the principal complications in organ transplantation. They require opposite therapies. Thus, an early and reliable differential diagnosis, ideally by non-invasive parameters, is needed. In kidney transplantation (KTx) non-invasive markers of organ function (creatinine, cystatin C) as well as immunologic parameters (serum amyloid A SAA, serum and urine neopterin S-/U-NEOP) are available. In contrast, the main diagnostic tool in heart transplantation (HTx) is still the endomyocardial biopsy. In combined kidney-pancreas transplantation (KPTx) renal markers are used together with amylase excretion via bladder drainage of the pancreas graft.

Do non-invasive immunologic parameters detect the host's immune response in HTx and KPTx as well? Is a routine non-invasive immunologic monitoring possible independent of the type of organ transplanted? In addition to organ-specific markers like creatinine clearance, urine amylase concentrations, and biopsies the macrophage product S-NEOP (nmol/l, RIA; BRAHMS (Berlin) and the acute phase reactant SAA (mg/dl, nephelometry, Dade-Behring (Marburg) were measured daily in 10 patients with KPTx and 29 patients with KTx during their stay on ward. In 13 HTx patients the parameters were determined 2 to 3 times during the stay on ward and on each visit to the outpatient clinics. Periods of acute rejection (REJ), bacterial and viral infection (BACT-INF, VIR-INF) as well as stable graft function (STABLE) were diagnosed clinically and retrospectively. The parameter behavior was analyzed using mean and median values and calculating Kruskal-Wallis and Brown-Mood tests. Individual cut-off values for the parameter increases during the periods of stable graft function and rejection were defined to estimate sensitivity and specificity.

	HTx			KTx		
	n	mean (SD)	median	n	mean (SD)	median
SAA [mg/dl]						
REJ	16	15.6 (11.6)	12.7	66	9.5 (8.7)	6.1
STABLE	71	7.2 (7.1)	5.0	285	7.0 (6.7)	5.6
BACT-INF	33	14.5 (21.8)	8.0		-	
VIR-INF	8	8.6 (6.3)	5.2	82	8.7 (13.0)	5.8
S-NEOP (nmol/l)						
REJ	19	54 (45)	38.0	66	100 (115)	43.0
STABLE	134	19 (18)	14.0	284	54 (62)	35.0
BACT-INF	62	115 (79)	88.0		-	
VIR-INF	13	48 (27)	44.0	77	68 (89)	35.0

	PKTx		
	n	mean (SD)	median
SAA [mg/dl]			
REJ	21	15.6 (10.4)	14.1
STABLE	24	4.3 (3.3)	3.2
BACT-INF	20	21.5 (17.4)	12.5
VIR-INF	18	14.0 (15.6)	6.8
S-NEOP (nmol/l)			
REJ	22	105 (96)	43.0
STABLE	24	31 (23)	22.0
BACT-INF	21	94 (79)	50.0
VIR-INF	18	199 (107)	173.0

The table gives the number of measurements (n), mean values with standard deviations (SD), and median values of SAA and S-NEOP during the different clinical situations. S-NEOP and SAA ahowed lowest values during stable graft function. Both, SAA and S-NEOP were markedly elevated during rejections and infections. The highest SAA values were seen during rejections and infections in PKTx and during rejections in HTx, the maximum s-NEOP levels during viral infections in KPTx. The diagnostic accuracy of SAA and S-NEOP as rejection markers was 82% and 78% respectively in cases of KTx, 84% and 75% in HTx, and 94 and 71% in PKTx.

We conclude that S-NEOP and SAA reflect the immune response in HTx and combined PKTx. Non-invasive immunologic monitoring is possible not only in KTx but also in HTx and PKTx and may help to reduce the number of biopsies.

Urinary neopterin concentrations in females with breast cancer

Murr C, Bergant A, Widschwendter M, Heim K, Schröcksnadel H, Fuchs D

Institut of Medical Chemistry and Biochemistry and Department of Gynecology and Obstetrics, University of Innsbruck, Innsbruck, Austria

In malignant tumor diseases the frequency of neopterin elevation in serum or urine of patients varies from about 90% in hematological neoplasias to about 20% in tumors like breast cancer. In various studies, higher neopterin concentrations in serum or urine were significantly associated with more rapid disease

progression and death, the prognostic value usually being jointly predictive to stage of the tumor. This was shown in hematological neoplasias, in carcinoma of the uterine cervix or of the ovaries, in colon carcinoma, lung cancer, prostate cancer, hepatocellular cancer and squamous cell carcinoma of the oral cavity. So far, female breast cancer was only poorly examined concerning the predictive power of neopterin.
Therefore, in a retrospective study, 129 females with breast cancer at the moment of diagnosis were investigated. Tumor histology and routine laboratory parameters were concomitantly examined. Urinary neopterin and creatinine concentrations were determined by an automated high-performance liquid chromatography technique. The thereby resulting urinary neopterin/creatinine ratios were referred to the individual sex and age dependent reference values generated earlier.
Patients were followed up for up to 13 years, and the ability of all variables to predict fatal outcome was assessed by calculating product limit estimates and multivariate Cox's proportional hazards model. Urinary neopterin values which were elevated in 18% of the patients, did not correlate with tumor size or lymph node status. Neopterin levels were influenced by the presence of distant metastases ($p=0.04$) and by tumor differentiation ($p=0.01$). In univariate analysis, the presence of distant metastases ($p<0.001$), neopterin ($p<0.001$), tumor size ($p=0.001$) and lymph node status ($p=0.002$) were significant predictors of survival. 17/23 patients with elevated, but only 39/106 with normal urinary neopterin levels died from malignancy during the observation period. By multivariate analysis, a combination of the variables presence of distant metastases ($p<0.001$), neopterin ($p=0.03$) and lymph node status ($p=0.01$) was found to jointly predict survival.
Urinary neopterin concentrations provide valuable prognostic information in females with breast cancer. Neopterin is not a tumor marker in the usual sense of the word. The predictive power of elevated neopterin in patients with malignancies might be explained by a chronic immune stimulation, which is unable to eliminate the stimulating agent, namely the tumor. As neopterin production by monocytes/macrophages being paralleled by the production of reactive oxygen species, neopterin may also be regarded as an indicator for oxidative stress caused by an activated immune system. Such reactive oxygen species may promote tumor growth and contribute to the development of neoplastic transformation and thereby for poor patients' outcome.

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Formation of oxygen radicals in solutions of 7-8-dihydroneopterin

Oettl K, Wirleitner B, Baier-Bitterlich G, Fuchs D, Grammer T, Reibnegger G

Medical Chemical Institute and Pregl Laboratory, Graz; Institute for Medical Chemistry and Biochemistry, Innsbruck, Austria

Neopterin and 7,8-dihydroneopterin, two compounds which are secreted by activated macrophages, have been shown to interfere with radicals generated by cellular and different chemical systems. Reduced pterins were reported to scavenge, whereas aromatic neopterin was reported to enhance radical mediated reactions. However, recently it was found that high concentrations of 7,8-dihydroneopterin promote luminol dependent chemiluminescence and T-cell apoptosis, suggesting an enhancement of free radical formation. In addition, using ESR spectroscopy and spin trapping hydroxyl radicals were detected in solutions of 7,8-dihydroneopterin.
In our study aromatic hydroxylation was used for detection of hydroxyl radicals. It was shown that in solutions of 7,8-dihydroneopterin hydroxyl radicals were formed in the absence of any radical source. The presence of EDTA chelated iron enhanced, and addition of deferoxamine inhibited hydroxyl radical formation. While addition of iron accelerated the hydroxylation reaction, 7,8-dihydroneopterin was responsible for the degree of hydroxylation without altering the kinetics. In the presence of superoxide dismutase or catalase as well as by helium purging hydroxylation was inhibited.
Our data suggest that in solutions of 7,8-dihydroneopterin superoxide radicals are generated which are converted to hydroxyl radicals by Fenton or Haber-Weiss type reactions. The detailed role of 7,8-dihydroneopterin in the processes of radical generation is currently under investigation.
This work was financially supported by the Austrian „Fonds zur Förderung der wissenschaftlichen Forschung", project P12366CHE.

Iron-dependent Changes in Cellular Energy Metabolism: Influence on Citric Acid Cycle and Oxidative Phosphorylation.

Oexle H, Dlaska M, Gnaiger E, Weiss G

Department of Internal Medicine and Department of Transplant Surgery, D.Swarovski Research Laboratory, University Hospital Innsbruck, Innsbruck, Austria

Iron modulates the expression of the crucial citric acid cycle enzyme aconitase via a translational me-

chanism involving iron regulatory proteins. Thus, the present study was undertaken to investigate the consequences of iron perturbation on citric acid cycle activity, oxidative phosphorylation and mitochondrial respiration in the human cell line K-562. In agreement with previous data iron increases the activity of mitochondrial aconitase while it is reduced upon addition of the iron chelator desferrioxamine (DFO). Interestingly, iron also positively affects three other citric acid cycle enzymes, namely citrate synthase, isocitric dehydrogenase, and succinate dehydrogenase, while DFO decreases the activity of these enzymes. Consequently, iron supplementation results in increased formation of reducing equivalents (NADH) by the citric acid cycle, and thus in increased mitochondrial oxygen consumption and ATP formation via oxidative phosphorylation as shown herein. This in turn leads to down-regulation of glucose utilization. In contrast, all these metabolic pathways are reduced upon iron depletion, and thus glycolysis and lactate formation are significantly increased in order to compensate the decrease in ATP production via oxidative phosphorylation in the presence of DFO. Our results point to a complex interaction between iron homeostasis, oxygen supply and cellular energy metabolism in human cells.

Induction of neopterin and nitrite formation in monocytoid cell lines by products from gram-positive bacteria

Peterbauer A, Werner ER, Werner-Felmayer G

Institute for Medical Chemistry and Biochemistry, University of Innsbruck, Innsbruck, Austria

In previous work, we have shown that bacterial compounds such as lipopolysaccharide from the outer membrane of gram-negative cell walls and partial structures of these as well as compounds and exotoxins from gram-positive bacteria can cause immune stimulation in monocytoid cell lines. Addition of interferon-γ (IFN-γ) strongly augments the effect of these bacterial compounds (1). This causes induction of guanosine-5´-triphosphat cyclohydrolase I (GTP-CH I), resulting in increased tetrahydrobiopterin levels, and of nitric oxide synthase (iNOS). Due to species-specific differences in this metabolism (discussed in 2) this induction leads to release of neopterin in human monocytoid cell lines and of nitrite formed from NO in rodent monocytoid cells.
Based on these observations, we have established an *in vitro* assay for the detection of bacterial pyrogens which should replace the currently used rabbit pyrogenicity test. This test uses IFN-γ-treated monocytoid cell lines which are cultured in presence of different immunostimulatory bacterial compounds. As a read-out, neopterin is quantified in supernatants from THP-1 cells by ELISA, and nitrite is quantified in supernatants from RAW 264.7 cells by a photometric assay using the Griess reaction.
The only accepted alternative *in vitro* method for pyrogene detection is the limulus amoebocyte lysate (LAL) assay. As this test only detects pyrogens from gram-negative bacteria, an additional test system for sensitive identification of gram-positive compounds is needed as well. Here, we show that the test based on using IFN-γ-treated monocytoid cell lines could meet these requirements. As is shown, a variety of defined compounds from gram-positive and mycobacteria as well as crude supernatants of gram-positive bacterial cultures (especially *Staphylococcus aureus* cultures from patient isolates) could be detected by the cell lines.
Further characterization of the supernatant of such a *S.aureus* culture indicated that the substances responsible for the immune stimulation are heat-resistant lipoproteins from below 6.5 kD to above 66 kD. The strongest immunostimulatory activity can be identified at 65 kD. Our results are in accordance with previous findings obtained with phenol extracts from *S.aureus* suggesting that immune stimulation is mediated by a monomer <15 kD non-covalently bound to the peptidoglycan layer of gram-positive bacteria which forms aggregates or micelles of high molecular weight. These immune stimulating complexes may contaminate all compounds purified from gram-positive bacteria and therefore be exclusively responsible for the immune stimulation caused by components from gram-positive bacteria (3).

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The electronic structure of tetrahydropteridines

Reibnegger G, Werner ER

Medical Chemical Institute and Pregl Laboratory, University, Graz; Institute of Medical Chemistry and Biochemistry, University, Innsbruck, Austria

Recently, the 4-amino analogue of tetrahydro biopterin was found to be a strong inhibitor of nitric oxide synthase while being bound to the enzyme in a manner similar to the natural cofactor tetrahydrobiopterin. We were interested in the electronic properties of these compounds and studied therefore the following model tetrahydropteridine structures: tetrahydrolumazine, tetrahydropterin, 4-amino-analogue of tetrahydropterin and the N5-methyl-tetrahydropterin.
The quantum chemical software program package GAUSSIAN 94 (Gaussian Inc., Carnegie, PA, USA)

was used with basis set 6-31G** after geometry optimization with basis set 3-21G*. Computed were the total electron density, molecular orbitals, orbital densities, and the electrostatic potential of the molecules. We used a UNIX based version of this software package running on a Silicon Graphics Workstation Indigo 2 (Silicon Graphics, Vienna, Austria). The properties calculated were visualized using program AVS EXPRESS (Advanced Visual Systems Inc., Waltham, MA, USA) running on a personal computer.
The *ab initio* quantum chemical computations reveal a dramatic difference in distribution of electronic charge and all the molecular properties derived thereof, between a) the lumazine system, b) the normal pterin system, and c) the 4-amino analogue. In particular, electronic properties between tetrahydropterin and its N5-methy-derivative are negligible.
Our results are compatible with recent speculations that the striking differences between the effects of the tetrahydropterin structure and its 4-amino analogue on enzymatic activity may be due to electronic interaction between the pyrimidine moiety of the pterin ring system and the heme group.

Neopterin-induced expression of intercellular adhesion molecule-1 in type II-like alveolar epithelial cells

Rieder J, Seibel M, Smolny M, Schobersberger W, Fuchs D, Grote J, Hoffmann G

Department of Anaesthesiology and General Intensive Care Medicine, and Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Innsbruck, Austria; and Department of Physiology, University of Bonn, Bonn, Germany

Production and release of proinflammatory mediators like tumor necrosis factor-α and neopterin are common events following the activation of the cellular immune system. Concerning inflammatory disorders of the lung, high serum neopterin levels correlate well with the severity of diseases such as sepsis, sarcoidosis, and tuberculosis. These situations are often associated with an increased expression of adhesion molecules in type II alveolar cells. In our study, we investigated the potential of neopterin to stimulate ICAM-1 expression in type II pneumocytes. Detection of ICAM-1 gene expression by reverse transcriptase-polymerase chain reaction revealed a dose-dependent effect of neopterin with maximum impact following 12h incubations. Data were averaging 2.3 + 0.21 amol ICAM-1 cDNA/μg total RNA following 1μM neopterin, 5.5 ± 1.2 amol ICAM-1 cDNA/μg total RNA following 10 μM neo-pterin, 10.0 ± 1.8 amol ICAM-1 cDNA/μg total RNA following 100 μM neopterin, and 11.5 ± 2.2 amol ICAM-1 cDNA/μg total RNA following 1000 μM neopterin, respectively. Comparable results were obtained when ICAM-1 protein synthesis was measured by cell-based ELISA. From these data we conclude that neopterin stimulates ICAM-1 production in type II alveolar epithelial cells. In vivo, induction of ICAM-1 by the stable pteridine compound neopterin might contribute to the retention of the inflammatory response, thus promoting cytotoxic cell host defense mechanisms that lead to damage of the airway epithelium.

Are dendritic cells a relevant source for neopterin in humans?

Romani N, Widner B, Reider D, Ebner E, Wirleitner B, Fuchs D

Department of Dermatology, and Institute for Medical Chemistry and Biochemistry, University of Innsbruck; and Ludwig Boltzmann Institute of AIDS Research, Innsbruck, Austria

In vitro, monocytes/macrophages isolated from peripheral blood and stimulated with interferon-γ after 24 hours are most potent producers of neopterin derivatives. Also the human myelomonocytic cell line THP-1 and very few other human monocytic cell lines were found to be capable of producing high amounts of neopterin upon stimulation with interferon-γ. However, it is not yet clear which cells out from the monocyte/macrophage lineage are exactly the source of increased neopterin production during states of immune activation *in vivo*.
As monocytes/macrophages, dendritic cells are highly specialized antigen presenting cells. They are uniquely capable of activating resting, naive T cells. Ontogenetically, the major part of dendritic cells belongs to the myeloid lineage. In contrast to monocytes / macrophages, nothing has been reported so far about neopterin and dendritic cells. We therefore wondered, whether dendritic cells can elaborate this substance.
Dendritic cells were generated from monocytes of human blood by culture in the presence of GM-CSF and IL-4 for 7 days followed by an additional 3 days in the presence of monocyte-conditioned medium as a maturation stimulus. This yielded mature dendritic cells (CD83+, 86+, 115-). Parallel cultures in the absence of the maturation stimulus left the cells in an immature state (CD83-, 86-,115+). These two populations were further cultured at a density of 1x10E6/ml without or with an additional maturation stimulus; CD40-ligand expressing transfectant cells (kind gift of Dr.R.A.Kroczek, Berlin). Neopterin was determined in 48hr supernatants by ELISA (BRAHMS Diagnostica, Berlin, Germany).
Unstimulated dendritic cells secreted on average 8 nmol/l of neopterin (range 2.0-23.0 nmol/l; n=18). There was no difference between immature and mature

populations. In response to CD40 ligation immature dendritic cells doubled their output of neopterin (16.8 nmol/l; range 5.8-27.0 nmol/l; n=9; $p<0.01$ by Student's t-test for paired samples). The same treatment did not induce a significant rise in neopterin in populations of mature dendritic cells. A slight but insignificant increase was noted in response to bacteria (SACS) in either population.
We conclude that dendritic cells constitute a substantial source of neopterin. This production can be augmented by stimuli that also lead to maturation of the cells. A functional role for neopterin in dendritic cells remains to be elucidated.

Same-day mutation analysis of the genes involved in the synthesis and regeneration of tetrahydrobiopterin, BH4

Romstad A, Guldberg P, Güttler F

The John F. Kennedy Institute, Glostrup, Denmark

Tetrahydro biopterin (BH4) is the obligatory cofactor of the three aromatic hydroxylases, phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH). PAH catalyses the conversion of phenylalanine to tyrosine. TH converts tyrosine to dopa and TPH is responsible for the conversion of tryptophan to 5-hydroxytryptophan, which is subsequently decarboxylated to serotonin. Lack of BH4 influences the activity of these enzymes and thus the levels of neurotransmitters required for normal brain development and function.
The synthesis of BH4 is catalyzed by three enzymes, GTP cyclohydrolase I (GCH1), 6-pyruvoyl tetra-hydropterin synthase (PTPS), and sepiapterin reduc-tase (SR). Through the activity of for example PAH, BH4 is oxidized to quinonoid BH2 (qBH2) and recyc-led to BH4 by pterin-4α-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR). Deficiency of BH4 can be caused by defects in either the synthesis or the recycling of BH4. The enzymes involved in disease are mainly GCH1, PTPS and DHPR.
We have developed a simple method that scans all coding sequences and splice sites of these three genes (*GCH1*, *PTS* and *DHPR*) at the same time and allows fast detection of mutations. We have also included the 5' and 3' untranslated regions of the *GCH1* gene, in which mutations have recently been reported. The method combines PCR with denaturing gradient gel electrophoresis (DGGE) and can be completed within one day. We have applied the method to the detection of mutations in all three genes in patients clinically diagnosed as BH4-deficient.

Similarity between the action of pteridines and kynurenines on lipid metabolism

Rudzite V, Jurika E, Widner B, Fuchs D

Latvia Institute of Cardiology, Riga, Latvia; and Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Innsbruck, Austria

The activation of immune system due to viral and bacterial infection is followed by increased formation of neopterin and kynurenine in the organism. The results of our previous investigations have shown a similitude in the action of these metabolites on lipid metabolism: neopterin and kynurenine induced an increase of saturated and a decrease of polyunsaturated fatty acids incorporation into phospholipids as well as an elevation of cholesterol concentration in samples used for phospholipid biosynthesis in vitro. An inverse relationship we observed after addition of 5,6,7,8-tetrahydrobiopterin, its precursors as well as after addition of 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid to samples used for phospholipid biosynthesis in vitro: these metabolites induced a decrease of saturated and an increase of polyunsaturated fatty acid incorporation into phospholipids as well as lowered the cholesterol concentration in samples. The aim of this study was to continue these observations by examining the effect of anthra-nilic, kynurenic, xanthurenic, picolinic and nicotinic acids on lipid metabolism using the same method as in our previous investigations. We have found that the addition of anthranilic, kynurenic, xanthurenic and picolinic acids to incubation medium for phospholipid biosynthesis in vitro is followed by an increase of saturated and a decrease of polyunsaturated fatty acids incorporation into phospholipids as well as by elevated concentration of cholesterol in samples. So, the above mentioned metabolites of kynurenine showed a similar to kynurenine and neopterin action on lipid metabolism. After nicotinic acid addition to incubation medium for phospholipid biosynthesis in vitro we observed a decrease of saturated and an increase of unsaturated fatty acids incorporation into phospholipids, as well as a decrease of cholesterol concentrations in samples. Therefore nicotinic acid had a similar effect as 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid on lipid metabolism as well as the similar influence to tetrahydrobiopterin and its precursors.

Serum neopterin in rheumatoid arthritis: correlation with clinical activity and response to therapy

Samsonov M, Nassonov E, Chichasova N, Nikipho-rova E, Fuchs D

Moscow Medical Academy, Moscow, Russia; and Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Innsbruck, Austria.

To assess whether circulating concentrations of neopterin reflect clinical activity and whether changes of neopterin are dependent on the effect of treatment in patients with rheumatoid arthritis (RA).
Neopterin cocnetrations were assessed in 30 control subjects and serially for up to 48 weeks in 67 RA patients, by radioimunoassay (BRAHMS, Berlin, Germany). Concentrations of C-reactive protein were also measured by ELISA.
Concentrations of neopterin in RA patients (10.5 ± 6.1 nmol/L) were significantly higher than in controls (6.4 ± 2.2 nmol/L) ($p < 0.0001$). There were a significant correlations between neopterin concentrations and clinical and laboratory indices of RA activity.

Measures of activity	r s	p
Swollen joint count	0.409	< 0.001
Tender joint count	0.301	< 0.01
Total articular count	0.251	= 0.05
Pain (VAS)	0.271	= 0.05
Index Lee	0.362	< 0.002
Activity index	0.374	< 0.002
Severity index	0.441	< 0.0001
ESR	0.458	< 0.0001
CRB	0.335	< 0.001

Disease-modifying anti-rheumatic drugs (DMARDs) such as low dose oral methotrexate (MT) significantly reduced serum neopterin concentrations only in patients with improvement of indices of disease activity ($p < 0.01$)

Changes of body weight in patients treated with antiretroviral therapy

Sarcletti M, Petter A, Shetty-Lee A, Fuchs D, Zangerle R

HIV Unit, Department of Dermatology and Venereology, and Institute of Medical Chemistry and Biochemistry, University of Innsbruck; and Ludwig-Boltzmann Institute of AIDS Research, Innsbruck, Austria

Progressive body mass depletion is a major characteristic of chronic human immunodeficiency virus (HIV) infection. The magnitude of body cell mass depletion was found to determine the timing of death from wasting in acquired immunodeficiency syndrome (AIDS). Weight loss in HIV infection is multifactorial, and alterations of food intake, absorption and metabolism may vary among patients and at different times during the disease course. Urinary neopterin was found to be a good predictor of unintentional weight loss of >10% of body weight before the occurrence of AIDS-defining infections. Neopterin is a sensitive marker for endogenous formation of interferon gamma which may be an important mediator of HIV associated weight loss. Highly active antiretroviral treatment is associated with a decrease in immune activation, including decreased levels of neopterin. Is the decrease in immune activation in antiretroviral treated patients associated with an increase in body weight? To answer this question we evaluated patients during the first six months of antiretroviral therapy for the change in urinary neopterin levels, CD4+ T lymphocytes, HIV-1 RNA levels and body weight.
The study population comprised 61 individuals with a mean age of 36.6 ± 6.6 years, 22 were female, 14 were injecting drug users, 17 were homosexual men, 18 were heterosexual contacts and one man was a recipient of blood products. Twenty-two of the patients had AIDS, 25 were in CDC stage B and 14 were asymptomatic. Thirty-nine patients were treated with inhibitors of reverse transcriptase (at least two nucleoside analogues) and 22 received antiretroviral combination therapy including protease inhibitors.
The median body weight increased significantly from 66 kg at initiation of therapy to 70 kg six months later and CD4+ T lymphocytes increased from 166/µL to 239/µL (for both, $p<0.0001$). Median HIV-1 RNA levels decreased from 5.20 log10 copies/mL to 2.58 log10 copies/mL and urinary neopterin levels decreased from 598 µmol/mol creatinine to 216 µmol/mol creatinine (for both, $p<0.0001$). A strong correlation was found between the change in body weight and the change in urinary neopterin levels (r_S = -0.56, $p<0.001$). The change in HIV-1 RNA levels correlated only weakly with the change in body weight (r_s = -0.36, $p = 0.01$) and no correlation was found for the absolute change in CD4+ T lymphocytes. However, the percentage of the change of the CD4+ T lymphocytes from baseline correlated with the change in body weight ($r_s = 0.44$, $p = 0.001$). Although correlations among pathologies cannot be considered to be equivalent to causation, our data support the concept that chronic immune activation and concomitant release of inhibitory cytokines (e.g. interferon-γ and TNF-α are involved in the pathogenesis of HIV-associated weight loss.

Biopterin in human semen

Sawada Y, Shinataku H, Asada M, Bungo K, Fujita K, Murata R, Isshiki G

Department of Pediatrics, Osaka City General Hospital; Department of Pediatrics, Osaka City

University; and Department of Gynecology, Osaka City General Hospital, Japan

Pteridines including neopterin and biopterin are contained in various body fluids such as urine, blood, cerebrospinal fluid, saliva and breast milk. We measured pteridines in human semen for the first time. Semens were collected from nine patients with consent who consulted the department of gynecology in order to examine the cause of sterility. Concentration of neopterin and biopterin were analyzed by the modified method described by Fukushima and Nixon using HPLC on an ODS column with fluorimetric detection.

Number	Age [Y]	Total neopterin [nM]	Total biopterin [nM]	Neopterin/ biopterin
1	36	6589	14033	0.47
2	36	2330	9053	0.26
3	37	1294	5560	0.23
4	37	934	2376	0.39
5	37	642	961	0.67
6	38	676	6559	0.10
7	38	1578	9001	0.18
8	39	865	6628	0.13
9	41	485	2019	0.24

Number	Sperms [x10^6/µl]	Motility [%]	Biopterin/ protein [nmol/g]
1	89	56	471
2	73	50	271
3	40	40	129
4	43	40	63
5	35	36	22
6	87	56	116
7	35	30	213
8	55	42	201
9	55	45	47

According to our results, concentration of biopterin varies among individuals. But human semen seems to have the highest concentration of biopterin in all body fluids. Of course, there remains a question of whether biopterin found in semen is tetrahydrobiopterin or not. But if human semen contains a large amount of tetrahydrobiopterin, what does it mean?
We also measured NO2/NO3 in semen (data not shown). We are to discuss the relation between NO and biopterin in semen.

The control of 6-tetrahydrobiopterin in melanogenesis by α-MSH

Schallreuter KU, Wood JM, Tobin DJ

Clinical and Experimental Dermatology, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire, UK

Human skin color is a combination of constitutive pigment and the induction of facultative *de novo* tanning. The process of skin pigmentation takes place in epidermal melanocytes. Recently it has been shown that both melanocytes as well as keratinocytes under *in vitro* conditions express mRNA's and enzyme activities for an autocrine *de novo* synthesis/recycling/regulation of (6R)-L-erythro5,6,7,8 tetrahydrobiopterin (6BH4) (1,2).
Furthermore, it was demonstrated that tyrosinase is subject to allosteric inhibition by 6BH4 via a regulatory binding domain on the enzyme (3). We were able to show that L-tyrosine is formed from L-phenylalanine in the cytosol of human melanocytes. Studies on L-phenylalanine to L-tyrosine clearly proved a significantly higher production of L-tyrosine via phenylalanine hydroxylase (PAH) compared to L- tyrosine uptake alone. The tyrosinase/6BH4 inhibitor complex can be fully reactivated by 1:1 binding of 6BH4 to alphamelanocyte stimulating hormone (α-MSH). Therefore, we were interested whether α-MSH could affect PAH activity. Also, it has been shown earlier, that 6BH4 controls PAH in two ways: in the presence of high 6BH4 levels it forms inactive tetramers and it acts as cofactor/electron donor on the catalytic site of the enzyme. As a dimer, α-MSH can activate PAH by removal of 6BH4 from inactive tetramers to form active dimers (4). Melanogenesis takes place in the melanosomes. It is here, where α-MSH, 6BH4 and tyrosinase are located. Using [^{14}C] labelled phenylalanine we could demonstrate the incorporation of [^{14}C] formed L-tyrosine in these organelles by following melanin formation. In conclusion, α-MSH/6BH4 can control the substrate supply and the first step in melanogenesis via tyrosinase.

Correlations between neopterin levels and *Chlamydia pneumoniae* antibodies among blood donors

Schennach H, Mayersbach P, Gerald Hessenberger G, Schönitzer D

Central Institute for Blood Transfusion and Immunology, University Clinics; and Institute of Biostatistics and Documentation, Univesrsty of Innsbruck, Innsbruck, Austria

In the beginning of this century Sir W. Osler assumed an infectious background for cardiovascular diseases. This theory got lost in the following decades because a lot of non-infectious risk factors were documented (cholesterol, smoking, hypertension, ...). But in the last years the "response to injury model" stated that endothel-lesions might have an infectious cause and could lead to atheroma formation.
Intracellular bacterias as Chlamydia, Rickettsia are now discussed as pathogens. Genus Chlamydia

includes four species: *C.trachomatis, C.psittaci, C.pneumoniae, C.pecorum*. C. are obligate intracellular bacteria, host cells are macrophages, endothelial cells, and a variety of other cells. *C.pneumoniae* causes a flu-like illness, sometimes pneumonia, but also chronic lung processes have been associated with C. (sarcoidosis, COPD, asthma).
The epidemiology depends on the region, northern countries have more widespread epidemics. The antibody prevalence is about 50% in middle-aged adults (males > females) and people can have two or three *C.pneumoniae* infections during their lifetime.
The purpose of this study was to investigate whether there is an association between elevated neopterin levels and the presence of antibodies against Chlamydia. 368 blood donors (normal/elevated neopterin: 195/173) were tested for IgG, IgA and IgM antibodies against Chlamydia (MEDAC, Hamburg). We analyzed distribution of the antibodies and correlations between neopterin elevated donations and antibodies. IgG antibodies: positive:187 (50.8%), negative:158 (42.9%), unclear result: 23 (6.3%), IgA antibodies: positive: 123 (33.4%), negative: 217 (59.0%), unclear result: 28 (7.6%), IgM antibodies: positive: 22 (6.0%), negative: 333 (90.5%) unclear result: 13 (7.6%)
Neopterin normal group: IgG positives: 96 (53%), IgA positives: 50 (27.5%), IgM positives: 15 (7.9%),
Neopterin elevated group: IgG positives: 91 (55.5%), IgA positives: 73 (46.2%), IgM positives: 7 (4.2%)
Fisher's exact test showed no correlation between neopterin elevated specimens and IgG and IgM antibodies but with IgA-antibodies ($p < 0.001$).
Helsinki Heart-Study: Chronic *C.pneumoniae* shows a higher IgA-titer as an independent risk-factor for coronary heart disease. There are also hints that these antibodies are associated with arteriosclerotic changes. We found in our study a discrete correlation between higher neopterin levels and IgA antibodies. This leads to some questions: Are there another proofs for correlations between neopterin and chronic chlamydial infections with regard to coronary heart disease or atherosclerotic changes? And what is the role of neopterin, is it an enhancer, a diagnostic factor? It seems to be interesting to look for answers.

Neopterin in the screening for viral infections of blood donations

Schennach H, Schönitzer D, Hessenberger G, Fuchs D

Central Institute for Blood Transfusion and Immunology, University Clinics; Institute of Biostatistics and Documentation, and Institute of Medical Chemistry and Biochemistry, University of Innsbruck, and Ludwig Boltzmann Institute for AIDS Research, Innsbruck; Austria

In 1986 neopterin was introduced in the Austrian Tyrol as an additional test for the screening of blood donations. Since 1994 neopterin has been mandatory for all Austrian blood banks as non-specific indicator of immune activation to prevent viral transmission by blood donations. Four studies from our Institute were examined for the positive/negative predictive value of neopterin in correlation to transfusion transmitted infections:
Neopterin screening and acute cytomegalovirus infections in blood donors, raised prevalence of IgM antibodies to Epstein-Barr virus and parvovirus B19 in blood donations with elevated neopterin concentration, association between chronic Hepatitis C virus infection and increased neopterin concentrations in blood donations, human Parvovirus B19 detection in blood donations: correlations with neopterin levels. We wanted to illuminate the value of neopterin screening with regard to the prevention of transmission of viral infections via blood donations by analyzing the predictive value of neopterin.
Neopterin screening and acute cytomegalovirus infections in blood donors: 2855 blood donors were screened for CMV-IgM antibodies: Positive predictive value (PPV): 5.26, negative predictive value (NPV): 99.7
Raised prevalence of IgM antibodies to Epstein-Barr virus (EBV) and parvovirus B19 in blood donations with elevated neopterin concentration: EBV: 1486 blood donors were tested for EBV IgM antibodies. PPV: 5.5, NPV: 98.
Parvovirus B19: 1522 blood donors were tested for IgM antibodies: PPV: 6.9, NPV: 97.8.
Association between chronic Hepatitis C virus infection and increased neopterin concentrations in blood donations (Schennach et al.): 54402 blood donations were tested for HCV antibodies and if positive PCR was performed: PPV: 0.16, NPV: 99.97 (with regard to PCR results).
Human Parvovirus B19 detection in blood donations: correlations with neopterin levels (Schennach et al.): 675 blood donors were screened for parvovirus B19 DNA. PPV 3.4, NPV: 98.6.
In all studies a significantly high negative predictive value for neopterin is found, i.e., the probability to transfuse infectious blood which is neopterin negative is very low. The value of an unspecific marker for viral infections as neopterin is not the "specific" detection of a viral agent (PPV) but is the "non detection" of the viral agent in the neopterin negative donor population (NPV).

Dominant negative allele (N47D) in a compound heterozygote for a variant of 6-pyruvoyl-tetrahydropterin synthase deficiency causing transient hyperphenylalaninemia

Scherer-Oppliger T, Matasovic A, Laufs S, Levy HL,

Quackenbush EJ, Thöny B, Blau N

Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland; Division of Genetics, Children's Hospital and Harvard Medical School, Boston, USA

Mutations in the 6-pyruvoyl-tetrahydropterin synthase (PTPS) gene result in persistent hyperphenyl alaninemia and severe catecholamine and serotonin deficiencies. We investigated at the DNA level a family with a PTPS-deficient child presenting with an unusual form of transient hyperphenylalaninemia. The patient exhibited compound heterozygosity for the PTPS-mutant alleles N47D and D116G. Transfection studies with single PTPS alleles in COS-1 cells showed that the N47D allele was inactive while D116G had around 66% of the wild-type activity. Upon co-transfection of two PTPS alleles into COS-1 cells, the N47D allele had a dominant negative effect on both the wild-type PTPS and the D116G mutant with relative reduction to about 20% of control values. Whereas the mother and the father had reduced enzyme activity in red blood cells (34.7 % and 51.7 %, respectively) and skin fibroblasts (2.8 % and 15.4 %, respectively), the clinically normal patient had in these cells activities at the detection limits, although PTPS-cross-reactive material was present in the fibroblasts. The specifically low PTPS activity in the mother's cells corroborated the evidence of a dominant negative effect of the maternal N47D allele on wild-type PTPS.

Serine 19 of human 6-pyruvoyl-tetrahydropterin synthase is a substrate for cGMP-protein kinase II-dependent phosphorylation

Scherer-Opplinger T, Leimbacher W, Pfeifer A, Ruth P, Blau N, Thöny B

Division of Clinical Chemistry and Biochemistry, Department of Pediatrics, University of Zurich, Zurich, Switzerland; Institute for Pharmacology and Toxicology, Technical University, Munich, Federal Republic of Germany

6-Pyruvoyl-tetrahydropterin synthase (PTPS) participates in tetrahydrobiopterin-cofactor biosynthesis. We previously identified in a PTPS-deficient patient an inactive PTPS-allele with an Arg 16 to Cys codon mutation. Arg 16 is located in the protein-surface exposed phosphorylation motif R16RIS19, with the Ser19 as the putative phosphorylation site for serine/threonine protein kinases. Purification of recombinant PTPS-S19A from bacterial cells resulted in an active enzyme (k_{cat}/K_M= 6.4 x10^3 M^{-1} s^{-1}) which was similar to wild-type PTPS (k_{cat}/K_M =4.1 x $10^3 M^{-1}s^{-1}$). In assays with purified enzymes, wildtype but not PTPS-S19A was a specific substrate for the cGMP-dependent protein kinase (cGK) type I and II. Upon expression in COS-1 cells, PTPS-S19A was stable, but not phosphorylated and had an activity reduced to ca.33%. Extracts from several human cell lines including brain contained a kinase that bound to and phosphorylated immobilized wild-type, but not mutant PTPS. Addition of cGMP stimulated phosphotransferase activity 2-fold. Extracts from transfected COS-1 cells over-expressing cGKII stimulated Ser19-phosphorylation over 100-fold, but only 4-fold from cGKI over-expressing cells. Moreover, fibroblast extracts from mice lacking cGKII exhibited significantly reduced kinase activity for PTPS. These results suggest that Ser 19 of human PTPS is a substrate for CGKII phosphorylation in vivo.

Neopterin and serum TfR in pregnant women with iron deficiency anemia

Schneider J, Breymann C, Perewusnyk G, Huch R, Huch A

Department of Obstetrics, University Hospital of Zurich, Zurich, Switzerland

Neopterin is released from macrophages when cellular immune resistance is activated by cytokines. It is considered an indirect marker for iron metabolism disorders. The serum transferrin receptor (s-TfR) is a new parameter which is purported to detect iron deficiency. The combination of both neopterin and s-TfR might result in improved diagnosis of the iron status, respectively, of anemia, and thus also influence the therapy strategy. This study was performed to determine (1) whether elevated neopterin levels in pregnant women with iron deficiency anemia are an indication of insufficient iron mobilization / erythropoiesis, and (2) how reliably the s-TfR detect iron deficiency anemia in pregnancy.

In a prospective, randomised, on-going study, pregnant women (n= 15 to date) in the 15th-39th gestational week with iron deficiency anemia (ferritin < 15µg/L, CRP ≤ 10 mg/L), were treated during a minimum of 2 weeks as follows: 2x/week 200mg iron sucrose i.v. or 200mg iron sucrose plus 300 IE rhEPO/kg BW i.v.. Treatment was discontinued when hemoglobin ≥ 11g/dL or after 4 weeks. Blood count, erythrocyte and reticulocyte differentials, s-TfR and neopterin levels were determined 2x/week and iron status. CRP and EPO concentrations 1x/week.

In the group as a whole, there was a poor correlation between neopterin and Hb (r^2=0.18, p=0.12), CRP (r^2=0.01, p=0.69) and also endogenous EPO (eEPO : r^2 = 0.08, p=0.31). The correlation between neopterin and Hct was just barely significant (r^2=0.31, p=0.03). The correlation of s-TfR to ferritin was not significant (r^2=0.10, p=0.25) but to Hb (r^2=0.37, p=0.02) and eEPO (r^2=0.52, p=0.003) it was

significant. When the entire group was stratified according to s-TfR ≥ or < 8.5mg/L, the s-TfR < 8.5 mg /L subgroup (n=8) was found to have diminished neopterin levels (<10 nmol/L). In the s-TfR > 8.5 mg/L subgroup (n=7), three women had neopterin levels < and four > 10 nmol/L. Three of these four had eEPO levels < 70 U/L.
Discussion: These data indicate that ferritin probably is not a reliable parameter for diagnosis of iron deficiency in pregnancy. However, even though there does seem to be a better association between s-TfR and other anemia parameters (hemoglobin, eEPO), this trend and the role of neopterin as indicators of iron status during pregnancy are not yet convincing. With our continuing investigations, we hope to obtain more conclusive results.

Negative as well as positive emotions interfere with urine cortisol, sTNF-R and neopterin concentrations in a patient with systemic lupus erythematosus

Schubert C, Lampe A, Geser W, Widner B, König P, Fuchs D, Schüßler G

Department for Medical Psychology and Psychotherapy, Department of Internal Medicine, and Institute of medical Chemistry and Biochemistry, University of Innsbruck, Innsbruck, Austria

A variable's dynamics - whether it be a biochemical or psychological variable - consist of characteristic longitudinal patterns of interdependent time-points. A time-series has a unique structure. When we average time-series data from different individuals this uniqueness is lost. In order to be able to analyze the dynamic interdependence of emotional and biological factors it is therefore necessary to consider individuals on a single basis. Using an integrative single-case approach our objective is to investigate whether daily psychosocial stressors and associated emotions interfere with the dynamics of various biochemical parameters in patients with systemic lupus erythematosus (SLE).
Patients under study collect their urine at home, for a period of at least 50 days, on a daily basis, divided into day and night urine. Additionally, patients fill out questionnaires twice a day to determine their emotional state, life style and disease activity. Each week, patients were examined clinically and interviewed to identify the past week's stressors using the Incidents and Hassles Inventory (IHI, Brown and Harris). Statistical analysis of the serial data is performed using time-series analysis according to Box and Jenkins.
Using this integrative single-case design we have been able to show in a 52 year old woman with SLE causal links between psychosocial encounters, emotions and both stress hormone levels (urine cortisol) and immunological parameters (urine neopterin, soluble tumor necrosis factor receptor I). Negative incidents as well as negative emotions led to a suppression of cortisol and an increase in immune parameters. Similarly, positive incidents as well as positive emotions such as lowered irritation and elevated mood corresponded to an increase in cortisol and a decrease in immune parameters.

The molybdopterin cofactor: biosynthesis and deficiencies in humans

Schwarz G, Stallmeyer B, Reiss J, Mendel RR

Department of Botany, Technical University Braunschweig; Department of Human Genetics, University of Göttingen, Germany

The molybdenum cofactor (Moco) forms the catalytically active center of all molybdoenzymes except nitrogenase. It consists of a molybdenum group covalently bound to the unique pterin compound molybdopterin (MPT), a tetrahydro-pyranopterin. The cofactor is highly conserved in archaebacteria, eubacteria, and eukaryotes. Molybdoenzymes are essential for such diverse metabolic processes like sulfur detoxification and purine catabolism in mammals, nitrate assimilation in autotrophs, and phytohormone synthesis in plants. Human Moco-deficiency is characterized by the combined loss of function of three molybdoenzymes namely sulfite oxidase, xanthine oxidase and aldehyde oxidase. The clinical signs of Moco deficiency are attributed to the accumulation of toxic metabolites due to the reduced activity of these enzymes, mainly sulfite oxidase. Interestingly, Moco-deficient patients show severe neurological abnormalities such as microcephaly, increased muscle tone, rigid posturing, myoclonus, abnormal movements and refractory seizures. Affected patients die early postnatally since no therapy is available yet.
The biosynthesis of Moco requires the multi-step synthesis of the MPT moiety followed by the subsequent transfer of molybdenum. At least six gene products involved in Moco-biosynthesis have been identified in E.coli and plants. In humans, Moco is synthesized in three steps: In the first step GTP is converted to Precursor Z, a 6-alylpterin with a four carbon side chain and a terminal cyclic phosphate. This precursor could already be a pyranopterin like the mature Moco. In E.coli and plants, this reaction is catalyzed by two separately expressed proteins, but in humans the corresponding two gene products are expressed by on bicistronic mRNA (mocs1). For humans, this rare phenomenon is described for the first time. Surprisingly, the same situation is found for the second step of Moco-biosynthesis, where Precursor Z

is converted to MPT through insertion of a dithiolene group catalyzed by the heterodimeric enzyme MPT-synthase. The two enzyme subunits are expressed as bicistronic mRNA (mocs2) with overlapping reading frames. MPT-synthase is recharged with sulfur by the sulfurylase Mocs3. Results from our work suggest that the last step of Moco synthesis, the transfer of Mo to MPT, is carried out within one complex reaction step of converting Precursor Z directly to Moco by the MPT synthase.
We will present evidences that the neuroreceptor-anchor protein gephyrin binds with high affinity MPT and in addition to clustering neuroreceptors - is also involved in Moco-biosynthesis in mammals. Further we will discuss ways for a therapy of human Moco-deficiecy by protein-bound Precursor Z and MPT/Moco.

Apoptosis in neurodegeneration, a role of pteridines ?

Spöttl N, Wirleitner B, Böck G, Widner B, Mayr C, Fuchs D, Baier-Bitterlich G

Institute for Medical Chemistry and Biochemistry; and Institute for Experimental Pathology, University of Innsbruck; and Ludwig Boltzmann Institute of AIDS-Research, Innsbruck, Austria

Apoptosis plays an important role in neurodegeneration, although the mechanisms and mediators in the brain are still largely unknown. Neurodegenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease are characterized by a slow, progressive loss of specific subsets of neurons. It has been hypothesised that oxidative stress and damage by free radicals play an important part in these diseases.
Data in literature show that activated microglia may produce several neurotoxins, such as reactive oxygen intermediates, nitric oxide, glutamate, cytokines or proteases which potentially contribute to neuronal cell death. Antioxidants such as cysteine and glutathione and reactive-oxygen scavenger enzymes, manganese superoxide dismutase and catalase, may counteract this process. It has also been shown that microglia cells (U138 MG) that were stimulated with interferon-γ produced increased amounts of neopterin and biopterin.
Microglial cell activation has been suggested to contribute to apoptosis in neurological disorders, and recently, neopterin and 7,8-dihydroneopterin were found to interfere with redox-sensitive intracellular signalling pathways, and data implied a potential role of neopterin-derivatives in oxidative stress-mediated apoptosis.
We have studied the impact of various conjugated and unconjugated pteridines on the apoptosis of neuronal NT2 cells. We observed that the aromatic pteridines biopterin and neopterin did not induce apoptosis, whereas reduced forms like 7,8-dihydroneopterin, 7,8-dihydrobiopterin, 5,6,7,8-tetrahydrobiopterin and 7,8-dihydrofolic acid turned out to be potential mediators of apoptosis of neuronal cells. Antioxidants like pyrrolidine-dithiocarbamate well inhibited this type of apoptosis, whereas the effect of catalase and N-acetylcysteine was less significant. We also succeeded in differentiating neuronal precursor NT2 cells which led to a population of approximately 40% neurons and 60% astrocyte-like cells. When exposed these cells to various pteridines we observed similar effects as described for the precursor cells. These results imply a possible role for pteridines in the pathology of neurodegenerative diseases.

Laser capture microdissection (LCM) - a new and unique tool for rapid "specific cell" molecular and biochemical diagnostics

Travis JC

ARCTURUS ENGINEERING, INC., Mountain View, CA, USA

Molecular analysis of populations of pure cells in their native tissue environment will be an important component of the next generation of medical genetics. Unfortunately, tissues are complicated three-dimensional structures, composed of a large number of different types of interacting cell populations where the cell subpopulation of interest might constitute a tiny fraction of the total tissue volume. In breast cancer for example, to analyze the genetic changes in the premalignant cells or the malignant cells, these subpopulations are frequently located in microscopic regions occupying less than 5% of the tissue volume. Analysis of extract of this complex tissue using sophisticated microarray hybridization technology may, therefore, be severely impaired if the integrity of the analyzed cell population is compromised. Cell cultures may not accurately represent the molecular events taking place in the actual tissue they were derived from as the gene-expression pattern of the cultured cells is influenced by the culture environment and can be quite different from the genes expressed in the native tissue state. Cellular heterogeneity has thus been a significant barrier to the molecular analysis and biochemical expression of normal and diseased tissue.
In normally developing and diseased tissue progression, analysis of critical gene expression and protein patterns requires the cell capture and extraction of a microscopic subpopulation of homogeneous cells from their complex tissue environment. This "specific cell" sampling from heterogeneous tissue must fully preserve the cell integrity for subsequent quantitative analysis by PCR, reverse transcriptase-PCR, or enzymatic (protein)

function. Laser capture microdissection (LCM) has been developed to provide a fast, dependable and robust method for capturing and preserving specific cells from tissue for subsequent molecular and biochemical analytical procedures.

In LCM, a thin, transparent thermoplastic film is placed on the surface of a tissue specimen or cytological smear on a glass slide. The tissue region of interest is visualized with a standard inverted microscope. An infrared laser coupled to the microscope is pulsed through the film, causing the specific cell(s) underneath it to adhere to it, with its morphology intact. The film (attached to a "cap") is lifted off the slide by a transport arm and placed onto a 0.5 ml microfuge tube that contains extraction buffer for molecular or other analysis.

LCM has been used successfully prior to "gene-chip" methodologies for microarrays used in DNA and RNA studies in cancer, Alzheimer's disease, fetal cell analysis in maternal serum for prenatal diagnosis, lung sputum analysis and infectious disease. In colon cancer for example, DNA extraction and subsequent PCR analysis on LCM specimens demonstrated loss of heterozygosity (LOH) of microsatellite markers near specific loci, which may have prognostic significance.

The significance of LCM for studying the molecular and/or biochemical regulation of cell-specific cytokine-pteridine interaction remains to be investigated, but is suggestive.

Structure, genomic localization and recombinant expression of the mouse 6-pyruvoyl-tetrahydropterin synthase gene

Turri MO, Ilg E, Thöny B, Blau N

Division of Clinical Chemistry and Biochemistry, and Division of Oncology, Department of Pediatrics, University of Zurich, Zurich, Switzerland

The 6-pyruvoyl-tetrahydropterin synthase (PTPS) is the second enzyme in the biosynthetic pathway from GTP to tetrahydrobiopterin (BH4). BH4 is an essential cofactor of NO synthase and aromatic amino acid hydroxylases, the latter being responsible for hepatic phenylalanine degradation and monoamine neurotransmitter biosynthesis. BH4 deficiency due to autosomal recessive mutations in the human gene for PTPS leads to a broad range of phenotypes ranging from mild hyperphenylalaninemia to high phenylalanine levels concomitant with neurotransmitter depletion. An animal model to study PTPS deficiency is thus desired to investigate the molecular basis of the disease and its variability. Here, we report on the isolation and recombinant expression of the mouse PTPS gene, Pts. It was located on chromosome 9C-D, and contained six exons with an open reading frame of 144 codons. The derived protein monomer has a molecular mass of 16,187 Da and 93% identity to its human and rat counterparts, respectively. The mouse PTPS was expressed in bacterial cells and purified to homogeneity. The kinetic properties of the recombinant protein, apparent Km of ca.10μM and kcat of 0.27 s-1, were similar to the native mouse enzyme in liver and brain extracts, and to the corresponding human and rat PTPS.

Neopterin in saliva of patients with squamous cell carcinomas and inflammatory diseases of the oral cavity

Vrecko K, Städtler P, Schultes G, Reibnegger G

Medical Chemical Institute and Pregl Laboratory; Department of Conservative Dentistry; and Department of Oral Surgery, University Dental Hospital, University of Graz, Graz, Austria

Berchtold et al. (1) and Murr et al. (2) investigated urinary neopterin in patients suffering from squamous cell carcinomas of the oral cavity. They found significantly increased urinary neopterin levels in patients with squamous cell carcinomas compared to healthy controls. In univariate and multivariate analyses neopterin was an independently significant predictor of survival.

We were interested in local immune activation in patients with squamous cell carcinomas compared with inflammatory diseases of the oral cavity and in the correlation between local neopterin concentration in saliva and urinary neopterin. We investigated 79 patients (17 healthy subjects, 8 patients with fractures, 7 with fibromas and cysts, 19 with inflammatory diseases and 28 with squameous cell carcinomas of the oral cavity).

Urinary neopterin concentrations of healthy subjects and patients with fractures and fibromas were in the normal range. The highest neopterin values were found in patients with carcinomas (135.9 μmol/mol creatinine, SD=72.6). The differences of the distributions of urinary neopterin between the different patient-groups are significant (Kruskal-Wasllis test: H=11.53;P=0.021). Salivary neopterin behaved similarly; the highest concentrations were found in patients with inflammatory diseases of the oral cavity (11.6 nmol/l, SD=10.41) and in patients with squamous cell carcinomas (18.0 nmol/l, SD=25.0). The differences between the patient groups is also significant (H=13.40, P=0.0095). Using Mann-Whitney U-test for comparing salivary and urinary neopterin between healthy controls and patients with fracturesor with fibromas, we found no significant difference. However, in patients with inflammatory diseases salivary neopterin was much more significantly increased than in urine (urine: U=106, P=0.079; saliva: U=78, P=0.008). In patients with

malignancies, neopterin in both fluids was significantly different (urine: U=119, P=0.0053; saliva: U=120, P=0.0057). Salivary neopterin concentrations showed a moderate however significant positive correlation with urinary neopterin among all studied subjects (Spearman's rank correlation coefficient rs=0.316, P=0.0046).
As a logical next step we plan to follow up neopterin concentrations in saliva and urine in patients with squamous cell carcinomas of the oral cavity in order to determine how neopterin in saliva compares with urinary neopterin as a predictor of patient's survival.

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Interactions of cellular immune effector function, iron metabolism and disease activity in patients with chronic hepatitis C virus (HCV) infection

Weiss G, Umlauft F, Urbanek M, Herold M, Gordeuk VR

Department of Internal Medicine, University Hospital, A-6020 Innsbruck, Austria; and Divison of Hematology/Oncology, George Washington University, 20037 Washington, DC, USA

We studied the functional interactions of macrophage activity, T-helper cell type 1 (Th-1)/Th-2 responses, and iron status in 55 patients with hepatitis C virus (HCV) related liver disease and 28 control patients with non-infectious liver disease. Serum concentrations of soluble tumor necrosis factor receptor type II (sTNF-R75), a macrophage activation marker, were higher in cirrhotic than in non-cirrhotic patients (p=0.0005) regardless of the HCV status, while levels of neopterin (Th-1 marker) and IL-4 and IL-10 (Th-2 markers) did not differ significantly.
STNF-R75 levels and transferrin saturation (TfS) correlated positively with levels of AST (p<0.001 for sTNF-R75 and p=0.028 for TfS) and ALT (p=0.003 for sTNFrec75 and p=0.039 for TfS) in HCV positive patients. Increased serum ferritin levels and TfS correlated significantly with both, more advanced liver disease and a predominant Th-2 pattern in HCV patients.
Our data suggest an association between macrophage activation and hepatic dysfunction in HCV and control patients, and that iron status may affect the clinical course of HCV infection by modulating Th-1/Th-2 responses *in vivo*.

Expression of GTP-cyclohydrolase I feedback regulatory protein (GFRP) in human myelomonocytic (THP-1) cells

Werner ER, Werner-Felmayer G

Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Innsbruck, Austria

Using primers from the published sequence of rat GFRP (1), we cloned human GFRP from THP-1 cells. The sequence we found exactly matched the sequence of human GFRP meanwhile published in genbank (U78190). We inserted the reading frame of human GFRP into a pET21a vector and expressed the protein in *Escherichia coli (E.coli)*. To test for GFRP activity, we also expressed human GTP cyclohydrolase I in *E.coli* using a pET16b expression system, and purified the enzyme over DEAE- and hydroxylapatite columns. Similar to the findings with rat GFRP, human GFRP enhanced the feedback inhibition of tetrahydrobiopterin on GTP cyclohydrolase I activity and was required for efficient inhibition of the enzyme by 2,4-damino 6-hydroxy pyrimidine (DAHP). L-phenylalanine, but not L-tyrosine, L-tryptophan or L-arginine, stimulated human recombinant GTP cyclohydrolase I in presence of GFRP.
Northern blot analysis showed that GFRP is constitutively expressed in THP-1 cells. The expression of GFRP is not altered by interferon-gamma. Bacterial lipopolysaccharide (LPS), however, drastically downregulates GFRP mRNA levels. To test for GFRP activity in intact cells, we measured neopterin formation by interferon-gamma and interferon-gamma plus LPS treated THP-1 cells in presence of increasing amounts of sepiapterin. Consistent with the results from Northern blot analysis, neopterin formation by THP-1 cells was increased by L-phenylalanine when the cells had been treated with interferon-gamma as single stimulus. In cells treated with interferon-gamma plus LPS, however, no effect of L-phenylalanine on neopterin formation could be detected. Our results provide evidence for downregulation of GFRP by LPS in human myelomonocytic THP-1 cells, thus rendering pteridine synthesis independent of L-phenylalanine concentrations.

(1) Milstien S, et al. Purification and cloning of the GTP cyclohydrolase I feedback regulatory protein, GFRP. J Biol Chem 1996;271:19743-19751

New alternatively spliced GTP cyclohydrolase I mRNAs cloned from human myelomonocytic (THP-1) cells

Werner-Felmayer G, Strohmaier W, Heufler-Tiefenthaler C, Werner ER

Institute of Medical Chemistry and Biochemistry, and Department of Dermatology, University of Innsbruck, Innsbruck; Ludwig Boltzmann Institute of Experimental and Clinical Traumatology, Vienna, Austria

GTP cyclohydrolase I cDNA sequences have been cloned from different sources including *Escherichia coli (E.coli)* (1), rat (2) and human liver (3), as well as human pheochromocytoma cells (4). Further, two splicing variants were reported to occur in human liver (3). In order to investigate whether treatment with interferon-γ (IFN-γ) which is known to strongly increase GTP cyclohydrolase I activity and mRNA expression in human cells (5), leads to induction of an isoform of GTP cyclohydrolase I, we searched a cDNA library from IFN-γ-treated THP-1 human monocytoid cells using a 500 bp probe generated by PCR with primers specific for GTP cyclohydrolase I sequences conserved in different species. Two clones were isolated which contained a GTP cyclohydrolase I encoding reading frame. CH21-8, a full-length mRNA, contained the reading frame for the 27.9 kDa GTP cyclohydrolase I reported for human liver (3). The mRNA sequence is identical to the one isolated from human pheochromocytoma cells (4). CH21-3 encodes for a not yet described 25.7 kDa GTP cyclohydrolase I protein containing a different C-terminal sequence. Sequence analysis showed that alternative splicing occurs within exon 6 (6). Functional expression in *E.coli* showed that only clone CH21-8 yielded an active protein whereas the protein encoded by CH21-3 was rapidly degraded. In Western blot analysis from THP-1 cell extracts, only the 27.9 kDa protein was detectable although the 25.7 kDa protein is stained by the antibody as well. These data suggest that the shorter protein may not be active in vivo. It remains to be seen, however, whether this protein can interact with the full-length enzyme. By using various primers located within exon 5 (sense) and at different positions within exon 6 (anti-sense), we then checked whether we could identify CH21-8, CH21-3 and the two splicing variants described by Togari et al. (4) in THP-1 cells. All these GTP cyclohydrolase I mRNA species were detectable by PCR. An additional sequence was identified which is also alternatively spliced within exon 6 but splicing does not affect the reading frame. In summary our data rule out the possibility of a cytokine-induced isoform of GTP cyclohydrolase I in human THP-1 cells but indicate that various alternatively spliced mRNAs, i.e. those identified already in human liver (4) and two additional ones, do occur. Interestingly, all these variants are spliced within exon 6. The functional role of these mRNA species remains to be determined.

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Procalcitonin and neopterin in infectious diseases

Wiedermann FJ, Rieder J, Fries D, Fuchs D, Schobersberger W

Department of Anesthesiology and Intensive Care Medicine; and Institute of Medical Chemistry and Biochemistry, Leopold-Franzens-University of Innsbruck; and Ludwig Boltzmann Institute for AIDS Research, Innsbruck, Austria

Procalitonin (PCT) is a polypeptide consisting of 116 amino acids. It is the precursor protein of the hormone calcitonin which consists of 32 amino acids. Active calcitonin is produced from procalcitonin by means of specific proteolytic enzymes in the C-cells of the thyroid gland. During severe infections (bacterial, parasitic and fungal) with systemic manifestations, PCT levels may rise over 100 ng/ml. After endotoxin injection, PCT begins to increase at 3-4 h in blood of healthy volunteers and then rises rapidly to reach a plateau at 6 h, remaining elevated at least 24 h. Thyroid is not the sole tissue involved in the secretion. The liver or neuroendocrine cells in the lung are possible sites of extrathyroidal PCT production in septic patients. It was demonstrated that PCT m-RNA is expressed by human mononuclear cells (MNC) and modulated by lipopolysaccharide (LPS) and sepsis related cytokines. However, in a whole blood model, LPS stimulation on blood samples from healthy volunteers showed no production of PCT.

PCT in healthy humans are < 0.1ng/ml. During severe generalized bacterial infections with systemic manifestations, PCT levels may rise over 100 ng/ml. In contrast, an inflammatory response to viraemia only leads to a small elevation in PCT levels. In children with meningitis initial C-reactive protein, cerebrospinal fluid (CSF) proteins, and white blood cell count in CSF were not sufficiently discriminative to distinguish between bacterial and viral meningitis. PCT was discriminative in all cases. PCT seems to be a specific marker of bacterial sepsis in HIV-infected patients, as the baseline plasma level of PCT was low (0.5 ng/ml ± 0.37), even in the latest stages of the HIV disease. In HIV-infected patients with parasitic, viral, fungal, mycobacterial infections, and bacterial pneumonia or other localized bacterial infections, the levels of PCT were lower than 2.1 ng/ml. Significantly increased neopterin concentrations have been described in patients suffering from various virus infections (e.g. measles, rubella, CMV, EBV, varicella, hepatitis, HIV). Acute localized bacterial infections are usually not associated with increased

neopterin concentrations, except infections with intracellular bacteria, e.g. pulmonary tuberculosis, where neopterin concentrations correlate to the extent and the activity of the disease. There are conflicting results regarding the laboratory diagnostic role of PCT in fungal infections. In severe malaria increased PCT plasma concentrations have been reported. In patients with suspected malaria the high specificity and negative predictive value suggest that PCT may serve as rapid exclusion test for acute malaria. Very high neopterin concentrations have been described in patients with malaria due to *Plasmodium falciparum* and *vivax*. When PCT is compared with other routinely measured parameters for inflammation / infection such as the white blood cell count, platelet count, or C-reactive protein, PCT seems to be more sensitive and specific in the monitoring of septic patients. Elevated PCT levels dropped significantly within 48 h in the case of successful surgical treatment of the septic focus. Persistence of elevated or increasing PCT levels were related to failure to eliminate the septic focus and were associated with a higher mortality. During the early course of acute respiratory distress syndrome (ARDS) PCT and neopterin were able to discriminate ARDS of bacterial etiology and ARDS of non-infectious (toxic) etiology. Sepsis and septic shock are associated with markedly elevated concentrations of neopterin, they correlate with scores for severity of sepsis like APACHE II and strongly predict progression to multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF). A clinical study showed that increase in plasma PCT was closely related with biliary pancreatitis. All patients with toxic (e.g. alcohol abuse) or idiopathic pancreatitis had normal PCT levels despite excessive increases in acute-phase proteins such as C-reactive protein and IL-6. In the same study neopterin concentrations were also higher at onset in patients with biliary versus toxic pancreatitis. Neopterin concentrations were significantly associated with the course of pancreatitis and were predictive for the development MOF. PCT values for patients with acute graft rejection after renal transplantation did not differ significantly from those of healthy transplant recipients. In contrast, PCT was clearly elevated during invasive bacterial infection or partial graft necrosis. After liver transplantation an increase in plasma PCT was observed (up to 40 ng/ml) without any clinical or laboratory signs of systemic infections. A continuous twofold daily reduction of this value down to 0.5 ng/ml was associated with a complication-free clinical course. PCT was not helpful to distinguish acute viral infection and acute graft rejection, however in both cases there was an elevation of neopterin. In patients with systemic lupus erythematosus (SLE) and with systemic anti-neutrophil cytoplasmatic antibody (ANCA)-associated vasculitis but without systemic infection, serum PCT levels were within the normal range (i.e., < 0.5 ng/ml), whereas the values for neopterin, IL-6, and C-reactive protein were elevated in patients with active underlying disease. Systemic bacterial infections were associated with PCT levels that were markedly elevated. During the postoperative and post-traumatic systemic inflammatory response syndrome (SIRS) PCT may be elevated during the first five days dependent on the type of operation and the severity of trauma. In the case of a slow decrease or even a further increase in PCT levels a septic complication is most likely.

Prognostic value of urinary neopterin in lung cancer

Widner B, Prommegger R, Unger A, Murr C, Salzer GM, Fuchs D

Institute of Medical Chemistry and Biochemistry; and Division of Thoracical Surgery, Department of Surgery, University of Innsbruck, Innsbruck, Austria

In malignant diseases, neopterin measurement were found to provide diagnostic information regarding survival prognosis.[1] Higher neopterin levels were significantly associated with worse prognosis in many types of tumors and the frequency of elevated neopterin concentrations in tumor patients depends on the tissue affected by malignant outgrowth. On this basis, we examined urinary neopterin at the time of lung cancer diagnosis to substantiate its predictive value for survival.

From 110 lung cancer patients (103 non small cell lung cancer, 7 small cell lung cancer) preoperative urine concentrations of neopterin per creatinine were measured. Neopterin levels were then correlated to postoperative survival. For discrimination between high and low survival probability, the invariable 4th quartile limit of the neopterin distribution from the patients' collective being 212 µmol/mol creatinine was applied. Subjects presenting with increased neopterin concentrations due to infections were excluded from the study. Infections were diagnosed on the basis of clinical observation and routine laboratory parameters. Clinical staging after mediastinoscopy and health status allowed in all patients surgical intervention with lung resection. After surgery, tumor histology and staging according to the TNM classification was evaluated.

The median age at diagnosis was 64 years. Median follow-up of patients was 17.4 months. After the end of study 27 patients (24.5%) have died of tumor progression, 16 of these patients (57%) had preoperative neopterin levels in urine > 212 µmol/mol creatinine. No relation of tumor histology and neopterin could be determined. Pathological stage I of lung cancer were found in 66 patients. Of these patients, 11 presented with neopterin > 212 and nine

died of tumor progression (82%) in the observed period. From the other subgroub with neopterin < 212, only 11 died on their tumor (20%). Kaplan Meier survival statistics showed a significantly worse prognosis of survival ($p < 0.0001$) for patients whose neopterin level were > 212 compared to those with lower levels. Univariate analysis according to the generalized savage test revealed high prognostic values regarding tumor death for dichotomized neopterin with the cut off value 212, for infiltration stage T and for lymph node status N. Thereby values indicating higher tumor progression significantly contribute to lower survival probability. No predictive information was obtained from variables implying metastases or grading (tumor differentiation stage), respectively. Multivariate Cox regression analysis yielded an approach for survival probability including T and the neopterin criterion as significantly influencing parameters with coefficients b = 0.91 ($p = 0.0011$) and 1.1 ($p = 0.0089$), respectively. From these data neopterin presents with high predictive value beside pathological staging.

Preoperative urine neopterin is a sound prognostic parameter for survival in patients with lung cancer. Since neopterin elevation has its origin in the cellular immune response, aggressive tumors with worse tumor biology could be closely associated with high neopterin levels and with lower survival probability. From this view point, neopterin measurement in lung cancer diagnosis represents an additional laboratory parameter independent from pathological staging. In patients with high risk for lung resection due to reduced respiratory capacity, high neopterin values at diagnosis may support the physicans decision making in surgery. Further studies should be undertaken to examine whether neopterin could be useful to influence the therapeutical approach in lung cancer.

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ESR detection of oxygen radicals in aqueous solutions of 7,8-dihydroneopterin

Wirleitner B, Czaputa R, Öttl K, Reibnegger G, Fuchs D, Baier-Bitterlich G
Institute for Medical Chemistry and Biochemistry, University of Innsbruck, and Ludwig Boltzmann Institute of AIDS-Research, Innsbruck; Institute for Experimental Physics; and Institute of Medical Chemical and Pregl Laboratory, University of Graz, Graz, Austria

Pteridine derivatives are capable of modulating the action of free radicals and both pro-oxidant and antioxidant properties have been described [1-4], e. g. whereas neopterin and high doses of 7,8-dihydroneopterin enhanced hydrogen peroxide-induced luminol chemiluminescence, low concentrations of 7,8-dihydroneopterin acted as scavenger in this assay.

To further test the impact of 7,8-dihydroneopterin on the production of oxygen radicals, we intended to determine whether itself catalyses the production of oxygen free radicals. Electron spin resonance (ESR) spectroscopy was employed in combination with dimethyl pyrroline N-oxide (DMPO) spin trapping as a specific and sensitive tool to investigate the ability of 7,8-dihydroneopterin to generate free radicals. We show that 7,8-dihydroneopterin in aqueous solutions leads to formation of DMPO-OH spin adducts, suggesting a generation of hydroxyl radicals. Addition of superoxide dismutase (SOD) completely blocked DMPO-OH formation by 7,8-dihydroneopterin, indicating the involvement of superoxide anion in the generation of hydroxyl radicals. DMPO-OH production in the presence of 7,8-dihydroneopterin was dependent on the concentration of chelated iron in the solution.

Upon these results, we suggest the formation of superoxide anion, further reacting to form hydrogen peroxide via the Fenton and Haber-Weiss reaction in the presence of 7,8-dihydroneopterin in aqueous solution.

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Impact of 7,8-dihydroneopterin on oxidative stress and FasL expression *in vitro* and *in vivo*

Wirleitner B, Kowald E, Spöttl N, Widner B, Mayr C, Sepp N, Fuchs D, Baier-Bitterlich G

Institute for Medical Chemistry and Biochemistry, Department of Dermatology, University of Innsbruck and Ludwig Boltzmann Institute of AIDS-Research, Innsbruck, Austria

7,8-Dihydroneopterin has been reported to be involved in redox-sensitive processes in different cells. Using human Jurkat cells, increased concentrations of 7,8-dihydroneopterin were shown to induce apoptosis by disturbance of the redox-balance and to trans-activate HIV-1 and HTLV-1 promoters [1, 2].

Activation-induced cell death in T-lymphocytes requires the inducible expression of Fas-ligand (FasL), triggering apoptosis in Fas receptor bearing cells. In recent studies, involvement of redox processes in the regulation of FasL expression was discussed [3-5]. In line with these data, we tested the role of 7,8-dihydro-neopterin on the expression of FasL in Jurkat TAg cells. In promoter reporter assays and western blot analysis, we found that 7,8-dihydroneopterin is

capable to enhance FasL expression. This effect was blocked by antioxidants superoxide dismutase (SOD), catalase and PDTC. As 7,8-dihydroneopterin was reported to have an impact on NF-kB and AP-1 (1), two redox-sensitive transcription factors also present in the FasL-promoter region, their involvement in 7,8-dihydroneopterin-mediated induction of FasL promoter was assayed. Whereas activation of AP-1 binding sites seemed to be necessary for spontaneous as well as pteridine-mediated FasL expression, binding of NF-κB was only crucial in 7,8-dihydroneopterin induced FasL promoter activation. Following these results, a correlation between production of neopterin-derivatives and expression of FasL was tested in sera of 43 patients with systemic lupus erythematosus (SLE). Thereby, a strong correlation between neopterin as well as soluble FasL concentrations was detected. Our results indicate a direct role of 7,8-dihydroneopterin in oxygen radical mediated processes, thereby possibly actively contributing to the pathogenesis of various diseases involving a disturbed redox balance e. g. SLE and AIDS.

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PCD/DCoH in control of the transcription of the tyrosinase gene in human melanocytes

Wood JM, Ashraf W, Hibberts NA, Wei YW, Gibbons NJP, Lindsey NJ, Schallreuter KU

Clinical and Experimental Dermatology, Department of Biomedical Sciences, University of Bradford; West Yorkshire, UK

Human melanocytes cultured under *in vitro* conditions in serum free MCDB 153 medium express all of the mRNA's and enzymes for the *de novo* synthesis, recycling and regulation of 6[R]-L-erythro 5,6,7,8-tetrahydrobiopterin (6BH4). Furthermore, melanocytes synthesize a pool of L-tyrosine (10^{-4} M) via an extremely active phenylalanine hydroxylase (PAH)/pterin 4a carbinolamine dehydratase (PCD) system. This pool of tyrosine controls *de novo* melanogenesis through the activity of tyrosinase, the specific enzyme that regulates pigmentation in melanocytes. Therefore, as a tetramer, PCD functions as the rate-limiting step for the recycling of 6BH4 and plays a key role in L-tyrosine synthesis in the cytosol compartment of melanocytes. The dissociation of PCD from a tetramer to its dimeric form DCoH provides a catalyst for the dimerization of the transcription factor hepatocyte nuclear factor α1 (HNF-1) in the nucleus. The human tyrosinase gene promotoer contains a single 16 base binding domain for the HNF-1 homodimer, suggesting a possible role for DCoH/HNF-1 in the transcription of the tyrosinase gene. A 439 bp fragment of the human tyrosinase promotor containing the DCoH/HNF-1 homodimer consensus sequence has been isolated and confirmed by DNA sequence analysis from 3 healthy individuals. All DNA sequences were identical. Specific binding of DCoH/HNF-1 complex to the tyrosinase promoter was confirmed by gel shift analysis. Our results suggest a dual fuinction for PCD(DCoH in controlling the supply of L-tyrosine for melanogenesis, as well as the transcription of the tyrosinase gene.

Similar DCoH/HNF-1 binding domains have been found in the dopa decarboxylase and phenylethanolamine-N-methyl transferase (PNMT) gene promoters. Therefore, it is possible that PCD/DCoH controls the tyrosine supply and the transcription of two important genes in catecholamine biosynthesis also.

Changes of hemoglobin levels in patients treated with antiretroviral therapy

Zangerle R, Sarcletti M, Petter A, Shetty-Lee A, Fuchs D

HIV Unit, Department of Dermatology and Venereology, and Institute of Medical Chemistry and Biochemistry, University of Innsbruck; and Ludwig-Boltzmann Institute of AIDS Research, Innsbruck, Austria

Anemia is a frequent complication in the course of human immunodeficiency virus (HIV) infection. Lower hemoglobin levels were found to be significantly associated with accelerated progression to AIDS and death. In vitro, HIV is able to infect erythroid progenitor cells. In humans with HIV infection and in monkeys with experimental SIV infection virus could not be detected in isolated progenitor cells. In HIV-1 infected patients lower levels of hemoglobin are associated with increased levels of serum and urinary neopterin. Highly active antiretroviral treatment is associated with a decrease in immune activation, including decreased levels of neopterin. Is the decrease in immune activation in antiretroviral treated patients associated with an increase in hemoglobin levels? To answer this question we evaluated patients during the first six months of antiretroviral therapy for the change in urinary neopterin levels, CD4+ T lymphocytes, HIV-1 RNA levels and hemoglobin levels.

The study population comprised 61 individuals with a mean age of 36.6 ± 6.6 years, 22 were female, 14 were

injecting drug users, 17 were homosexual men, 18 were heterosexual contacts and one man was a recipient of blood products. Twenty-two of the patients had AIDS, 25 were in CDC stage B and 14 were asymptomatic. Thirty-nine patients were treated with inhibitors of reverse transcriptase (at least two nucleoside analogues) and 22 received antiretroviral combination therapy including protease inhibitors.
The median hemoglobin levels increased weakly from 13,4 g/dl at initiation of therapy to 14,0 g/dl six months later ($p = 0.001$) and CD4+ T lymphocytes increased from 166/μL to 239/μL ($p<0.0001$). Median HIV-1 RNA levels decreased from 5.20 log10 copies/mL to 2.58 log10 copies/mL and urinary neopterin levels decreased from 598 μmol/mol creatinine to 216 μmol/mol creatinine (for both, $p<0.0001$). A strong correlation was found between the change in hemoglobin levels and the change in urinary neopterin levels ($r_S = -0.53$, $p<0.001$). The change in HIV-1 RNA levels correlated only very weakly with the change in body weight ($r_S = -0.30$, $p = 0.04$) and no correlation was found for the absolute change in CD4+ T lymphocytes and . and the change in body weight. However, the percentage of the change of the CD4+ T lymphocytes from baseline correlated with the change in body weight ($r_S = 0.43$, $p = 0.002$). Although the pathogenesis of HIV-associated anemia is still far from being understood our data findings indicate that there is no direct role of HIV in the development of anemia. The data of our study are consistent with the view that HIV may induce chronic immune stimulation in patients which in turn is responsible for inhibition of erythropoiesis.

Ranopterins - amphibia skin pteridines displaying hematopoietic, immunomodulating and macrophageal proliferating biological activities

Zvetkova E

Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Ranopterins are biologically active substances isolated from the dermal chromatophores (cyanophores, xanthophores, erythrophores) of amphibia skin (1, 2). Our ultrastructural investigations (2, 3) on the pteridine-containing chromatophores in Rana ridibunda and Rana agilis skin demonstrated the existence of specific differentiations - pterinosomes in their cytoplasms, consisting of different number of electron-dense elementary lamellae. Pteridine fractions neopterin and pterin-6-carboxylic acid, isolated from the chromatophores of Rana ridibunda skin were tested in vitro and in vivo - in different experimental models. They display immunomo-dulating, macrophage-proliferating and haematopoetic activities (4 - 10). The in vivo administered ranopterin - neopterin enhances NOS macrophageal activity (4) as well as the macrophageal and spleen T-lymphocyte proliferation and T-cell responses to the action of specific mitogens (5, 6). The same pteridine fraction has an immunomodulating and tumour suppressive effect when applied in animals with experimentally induced tumours before tumour implantation (Zvetkova et al., Pteridines, In press). The pteridine fraction pterin-6 carboxylic acid displays MG-CSA in vitro (in mouse bone marrow agar cultures) influencing proliferation and differentiation of bone marrow early progenitors from the macrophage and granulocyte series (7, 8). Neopterin has in vitro stimulating effects on the proliferation and differentiation of mouse bone marrow lymphocytes and stromal cells from the macrophageal and fibroblast-like series (9, 10) - probably through the nuclear transcription factor κB (11).

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