Free Rad. Res., Vol. 29, pp. 331–338
Reprints available directly from the publisher
Photocopying permitted by license only

Inhibition of Xanthine Oxidase by Pterins

IRENE WEDE[a], ZEYNEP Z. ALTINDAG[b], BERNHARD WIDNER[a], HELMUT WACHTER[a] and DIETMAR FUCHS[a,*]

[a]*Institute for Medical Chemistry and Biochemistry, University of Innsbruck and Ludwig Boltzmann Institute of AIDS-Research, Fritz Pregl Strasse 3, A-6020 Innsbruck, Austria;* [b]*Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey*

Accepted by Prof. M. Grisham

(Received 15 February 1998; In revised form 10 June 1998)

The effect of a panel of pterins on xanthine oxidase was investigated by measuring formation of urate from xanthine as well as formazan production from nitroblue tetrazolium. The pterin derivatives, depending on their chemical structure, decreased urate as well as formazan generation: 200 μM neopterin and biopterin suppressed urate formation (90% from baseline) and formazan production (80% from baseline) as well. Their reduced forms, 7,8-dihydroneopterin and 5,6,7,8-tetrahydrobiopterin, showed a lesser but still strongly diminishing influence (40% from baseline). Another oxidized pterin namely leukopterin showed only a weak inhibitory effect. Xanthopterin, a known substrate of xanthine oxidase, had a strong effect on urate formation (80% inhibition), but a lesser effect on formazan production (30% reduction). When iron-(III)–EDTA complex was added to the reaction mixture all the effects were more pronounced. Superoxide dismutase, which removes superoxide anion by dismutation into oxygen, decreased formazan production in addition to pterin derivatives and had a small but enhancing effect on urate formation. Also the reductant N-acetylcysteine had an additive effect to pterins to diminish formazan production in a dose-dependent way. The results of our study suggest that depending on their chemical structure pterins reduce superoxide anion generation by xanthine oxidase.

Keywords: Pteridine,, xanthine oxidase, urate, formazan, superoxide

INTRODUCTION

Human macrophages stimulated with interferon-γ produce and release neopterin in large amounts.[1] The determination of urinary and serum neopterin concentrations is widely used for monitoring cellular immune activation status in diseases like viral infection, autoimmune disorders and malignancies (reviewed in refs. 2–5). So far, there is no clear biological function for the production of neopterin derivatives by the activated human macrophages. In an earlier study an association was described between the amount of neopterin produced by stimulated macrophages and the capacity of the same cells to produce reactive oxygen species,[6] and recently it was found that pteridine derivatives are able to interfere with processes mediated by reactive oxygen and chloride metabolites.[7] Measuring luminol chemiluminescence a number of pteridines scavenged hydrogen peroxide-derived reactive oxygen intermediates.[8–10] In contrast, at more stringent environmental

*Corresponding author. Tel.: ++43 512 507 3519. Fax: ++43 512 507 2865. E-mail: Dietmar.Fuchs@uibk.ac.at.

conditions, namely at neutral or slightly alkaline pH and in the presence of iron-(III)–EDTA, neopterin and other non-reduced pterins like biopterin were found to enhance chemiluminescence.[9,11] Similar effects of pterins have been also found investigating chloramine-T-induced chemiluminescence.[9,12] Also in cell culture systems neopterin enhances effects mediated by reactive oxygen metabolites, e.g., exogenous neopterin suppresses production of erythropoietin in the hepatocytoma cell line Hep-G2,[13] and it also induces nitric oxide synthase gene expression in rat vascular smooth cells.[14] From these data it is concluded that neopterin may amplify effects of reactive oxygen metabolites also in biological systems and probably in general. In contrast to this, Kojima *et al.*[15] found that 7,8-dihydroneopterin acts as an antioxidant in the hypoxanthine/xanthine oxidase (XOD) system and in rat macrophages stimulated with phorbol ester. Furthermore neopterin was found to suppress the generation of superoxide anion in macrophages due to its inhibitory influence on plasma-bound NADPH oxidase.[16] These observations seem to contrast the enhancing effect of neopterin on reactive oxygen metabolites in the studies mentioned earlier. To gain some more insight into the interaction of pterins with enzyme reactions we investigated the influence of a panel of reduced and non-reduced pteridines on XOD in more detail.

MATERIALS AND METHODS

Reagents

Pterins (5,6,7,8-tetrahydrobiopterin, 7,8-dihydroneopterin, L-erythro-biopterin, D-erythro-neopterin, xanthopterin and leukopterin) were

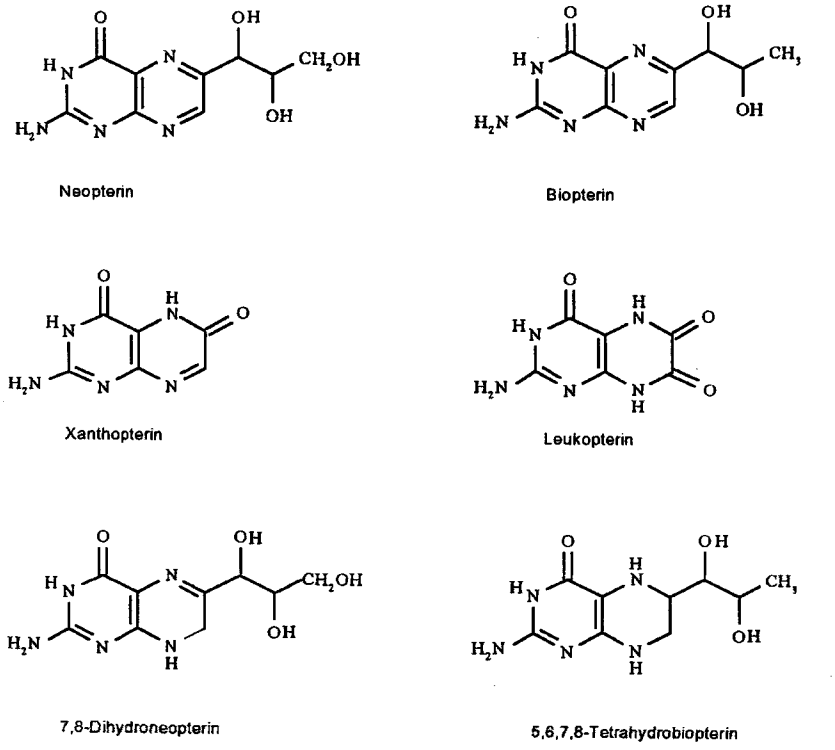

FIGURE 1 Chemical formulas of different oxidized and reduced pterins.

purchased from Dr. B. Schircks Laboratories (Jona, Switzerland). The chemical structures of these pterin derivatives are shown in Figure 1. Xanthine, XOD (from buttermilk), nitroblue tetrazolium (NBT), superoxide dismutase (SOD) and N-acetylcysteine (NAC) were from Sigma Chemical Co. (St. Louis, MO, USA). Iron-(III)-chloride, ethylenediamine tetraacetic acid disodium salt (EDTA), potassium hydroxide, potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate ($K_2HPO_4 \times 3H_2O$) were obtained from Merck (Darmstadt, Germany, analytical grade).

Preparation of buffer solution: KH_2PO_4 and $K_2HPO_4 \times 3H_2O$ were dissolved in distilled water reaching a final concentration of 13 mM. Solutions with pH-values 5.0–9.5 were prepared by different mixtures of these stock solutions according to Sørensen.

XOD Activity Measured by Urate Formation

Effects of pterins on XOD activity were measured by spectrophotometric detection of urate formation.[17] Reaction mixtures contained 340 μM xanthine diluted in 13 mM potassium phosphate buffer (pH 5.5–9.5). Different pterins (final concentrations: 10, 25, 50, 100 and 200 μM), as well as 5–500 μM iron-(III)-chloride in 10 μM–1 mM EDTA or 1.25–10 U/mL SOD or 0.15–300 μM NAC buffered in pH 7.5 were added to the test system. After addition of 0.052 U/mL XOD and incubation for 10 min at 25°C, absorbance at 290 nm wavelength was measured (U 2000 double beam spectrophotometer; Hitachi Ltd., Tokyo, Japan).

Superoxide Radical-generation by XOD Measured by Reduction of Nitroblue Tetrazolium (NBT)

Reaction mixtures contained 170 μM xanthine and 320 μM NBT diluted in 13 mM phosphate buffer (pH 5.0–9.0). The effects of different pterins were investigated applying concentrations from 10–200 μM. The influence of ferric ion, SOD, and NAC was detected by supplementation of the reaction mixture with 1–100 μM iron-(III)-chloride in 2–200 μM EDTA or 1.25–10 U/mL SOD or 0.15–300 μM NAC. The reaction was started by adding 0.052 U/mL XOD. After incubation for 10 min at 25°C, NBT reduction was detected by measuring absorbance at 560 nm (U 2000 double beam spectrophotometer; Hitachi Ltd., Tokyo, Japan).[18] Solutions of pteridine derivatives (10–200 μM) were used as blank controls. By this procedure absorbance of the compounds at 290 nm was taken into consideration.

RESULTS

Assessment of Assay Effects

Generation of superoxide, measured by xanthine oxidation, increased linearly according to the concentrations of xanthine, and a strong linear correlation between XOD concentration and the formation of urate was observed. The time course showed that accumulation of urate reached a plateau after approximately 600 s and increased with higher pH. Urate formation was highest at slightly alkaline pH 7.5. Formazan production also increased linearly in dependence on xanthine and NBT concentrations. Formazan levels reached a plateau after 600 s, and the reaction was pH-dependent, the maximum formazan production was observed at pH 8.0.

Effects of Different Pterins on Urate Formation

Compared to the control experiments, urate formation was diminished by supplementation with pterins in a concentration-dependent manner (Figure 2). Neopterin and biopterin strongly suppressed the production of urate at 50–200 μM. The neopterin-induced inhibition ranged

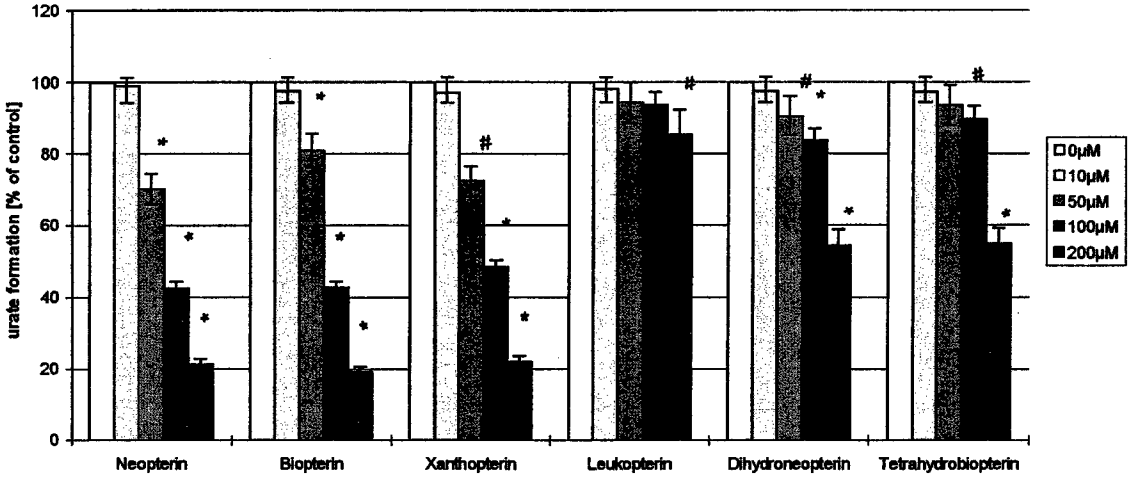

FIGURE 2 Concentration-dependent influence of pterins on urate production (mean values and standard deviations are shown; $^{\#}p < 0.05$, $^{*}p < 0.01$).

from 29.8% to 78.8%. Using biopterin a 19.1–80% decrease from baseline was observed. Also xanthopterin in dependence on the concentration diminished the urate formation. In contrast, leukopterin was less effective to suppress urate formation, but at a concentration of 200 µM leukopterin still a significant reduction (−14.6%) was caused. The reduced pteridines, 7,8-dihydroneopterin and 5,6,7,8-tetrahydrobiopterin, also induced a concentration-dependent suppression of urate production (16.3% and 10.2% at 100 µM). HPLC measurements[4] were performed before and after incubation of specimens, and it was apparent that the concentrations of pteridine derivatives were unaltered upon incubation with XOD.

Effects of Pterins on Formazan Production

The generation of formazan was diminished by different pterins (Figure 3). Supplementation with neopterin resulted in a decline of formazan production (41.4–88.2% with 50–200 µM neopterin), and also biopterin diminished formazan production (57.4–90.9% reduction with 50–200 µM biopterin). The effect of xanthopterin on formazan production (200 µM xanthopterin inducing a 37% reduction) was less marked compared to the more dramatic influence on urate formation. As with urate formation leukopterin had very low activity on formazan production. The di- and tetrahydroforms of neopterin and biopterin also diminished the formazan production, at the highest concentration of 7,8-dihydroneopterin and 5,6,7,8-tetrahydrobiopterin, the statistically significant suppression reached 22.5% and 53.7%. When pteridine derivatives were incubated with NBT alone, neither any production of formazan nor any change of pteridine concentrations was seen.

Stability of Pterins in the Presence of XOD

In order to evaluate eventual chemical modification of the pterins when exposed to XOD we examined absorbance spectra in a time-dependent fashion during 12 min (U 2000 double beam spectrophotometer). No change of absorbance spectra was observed with most pterins, except xanthopterin which was chemically modified upon incubation with the enzyme as reflected by a decline of UV-absorbance around 350–420 nm, the characteristic absorbance of

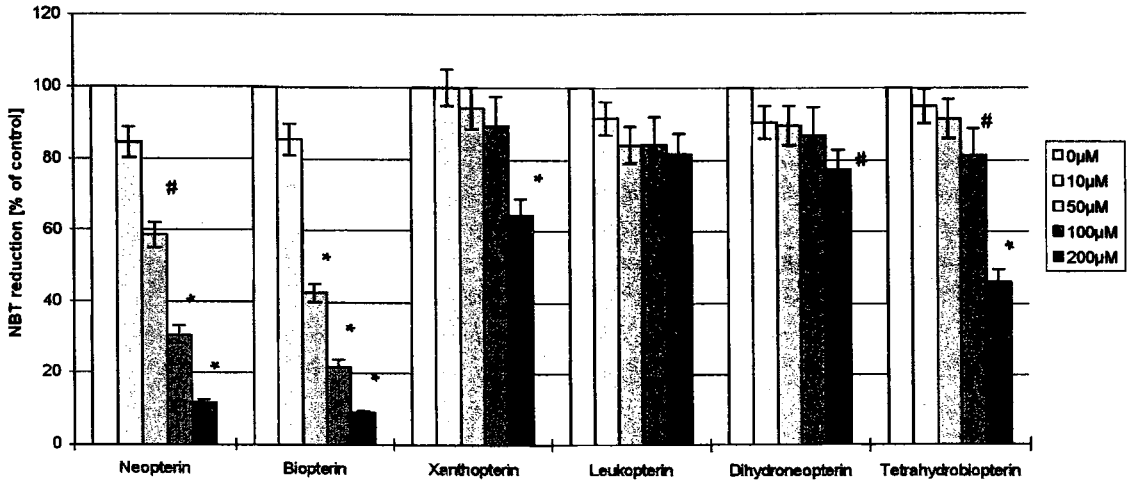

FIGURE 3 Concentration-dependent influence of pterins on formazan production (mean values and standard deviations are shown; $^{\#}p < 0.05$, $^{*}p < 0.01$).

TABLE I Influence of neopterin and 7,8-dihydroneopterin on the formazan production after incubation of xanthine with xanthine oxidase in the presence of iron-(III)–EDTA, SOD and NAC (% of control ± SEM)

	Control	Fe(III)–EDTA (5/10 μM)	NAC (300 μM)	SOD (1.25 U/mL)
Neopterin (μM)				
0	100	72.1	70.9	44.1
50	53.6 ± 3.3	28.8 ± 2.2	19.9 ± 1.4	7.9 ± 0.4
200	16.9 ± 1.7	6.5 ± 1	3.5 ± 0.7	1.3 ± 0.2
Dihydroneopterin (μM)				
0	100	72.1	70.9	44.1
50	90.6 ± 4.7	58.3 ± 3.6	50.3 ± 3.5	25.1 ± 1.8
200	46.1 ± 1.3	31.7 ± 3.6	29.1 ± 3.5	15.8 ± 2.2

xanthopterin, and a concomitant appearence of UV-absorbance of 300–360 nm wavelength. These results were strengthened by monitoring the concentrations of various pterins by fluorescence detection (emission: 440 nm, excitation: 350 nm) after high-performance liquid chromatography:[4] no decline of concentrations of pterins during XOD reaction could be detected, except xanthopterin (data not shown).

Effects of Ferric Ion, EDTA, SOD and NAC

Addition of iron-(III)–EDTA to the reaction mixture in the concentration range of 1–500 μM decreased formation of urate and of formazan in a strong dose-dependent way. When neopterin or 7,8-dihydroneopterin in different concentrations (50–200 μM) was added to the reaction mixture which contained 5 μM iron-(III)–EDTA, formazan production was still more reduced compared to the iron–EDTA supplemented control. Neopterin had a two- to four-fold higher scavenging activity on formazan production than 7,8-dihydroneopterin. The scavenging effects of the pterins were concentration-dependent (Table I). The same was true for urate formation (data not shown in detail).

SOD supplementation reduced formazan formation, but in contrast urate generation was increased with high concentrations of 10 U/ml SOD (data not shown). Low concentrations of

SOD had no effect on urate formation. Supplementation of pterins to the reaction mixture containing 1.25 U/mL SOD caused an additive decrease of formazan formation with neopterin and 7,8-dihydroneopterin (Table I).

Addition of NAC in the concentration range of 0.15–300 μM had no influence on urate formation, and formazan production was slightly decreased and this was true even at the lowest concentration of 0.15 μM. When NAC was added, the scavenging activities of pterins were additive (Table I).

DISCUSSION

Superoxide anion as well as hydrogen peroxide are generated by XOD with xanthine.[19] The catalytic activity of XOD can be measured by urate formation or by formazan production with the NBT reduction method.[18,20] By measuring production of uric acid from xanthine, indirectly the generation of superoxide anion is monitored. By this way, the influence of supplemented substances on XOD can be indirectly determined by monitoring urate production.[19] In an alternative way, spectrophotometric monitoring of formazan production from NBT allows to estimate potential additional effects of supplemented compounds on superoxide anion stability and content plus effects on XOD. At pH 7.5 the XOD reaction was optimal, at lower pH XOD is denaturated, and it is inactived at higher pH. Also the rate constant for spontaneous dismutation of superoxide anion decreases at alkaline pH.[20]

Various pterins depending on their chemical structure (Figure 1) decrease formazan and urate production (Figures 2 and 3). Different interactions may be possible:

(1) Certain pterins themselves can be a substrate of XOD, being hydroxylated during the enzymatic reaction. According to Rembold[21,22] XOD may attack the ring system in positions 2, 4, and 7 of non-hydrogenated pterins in a nucleophilic fashion. It has been shown by Forrest *et al.*[23] and Rembold[21] that the double bonds of the pyrazino moiety in unsubstituted pterins such as 7,8-dihydropterin or isoxanthopterin are readily susceptible to nucleophilic attack. In our study, xanthopterin was diminished during the reaction and a shift of UV-absorption spectra was observed. In agreement with earlier studies, xanthopterin is converted to leukopterin by XOD.[21–23] By this reaction, xanthopterin may hinder urate generation in a competitive way. There was only a small effect of xanthopterin on formazan production, which shows that XOD reaction and thus superoxide anion production takes place in the presence of xanthopterin. However, spectrophotometric and fluorescence measurements showed that no chemical modification of all the other investigated pterins, neopterin, biopterin, leukopterin and the reduced forms 7,8-dihydroneopterin and 5,6,7,8-tetrahydrobiopterin occurred in our experiments. In agreement, all pteridine derivatives which strongly diminished urate production also reduced formazan production (Figures 2 and 3). If pteridines were a substrate of XOD, one would expect that production of superoxide anion[24] and thus formazan from NBT should be unaltered.

(2) Steric factors and or electronic distributions of pterins could be important for the inhibition of XOD. Having a similar chemical structure to xanthine and also to the molybdenum cofactor, these pterins may occupy the active center of the enzyme or may engage the binding site of the cofactor without being a substrate for the enzyme. Neopterin and biopterin possess an almost identical side chain with several hydroxyl groups at position 6 as compared with the cofactor, and these compounds had a strong diminishing effect on urate and formazan production. Absence of such a side chain at position 6 in case of leukopterin resulted in a drastically smaller scavenging effect on urate and formazan production than with the other oxidized pterins. This leads to the suggestion that the presence of the

propyl group in position 6 is important for strong interaction of pterins with XOD. Reduced pterins, carrying additional hydrogens, e.g., 7,8-dihydroneopterin and 5,6,7,8-tetrahydrobiopterin, also decreased urate and formazan generation but less dramatically than neopterin and biopterin. 5,6,7,8,-Tetrahydrobiopterin may interfere with the redox-recycling of the molybdenum cofactor and consequently lower urate and formazan production. Such a reaction is not possible for the 7,8-dihydro-forms and the oxidized pterins, neopterin and biopterin. Schindelin *et al.*[25] discussed that although pterins in the coenzyme do not directly participate in the actual electron transfer, their oxidation state-dependent interaction with the molybdenum could influence the electronic properties of the metal, thereby inhibition of XOD would be possible.

(3) Several oxidized pterins and especially those with the proper side chain at position 6, e.g., neopterin, are known to enhance effects of various reactive species[12] and may thus be capable of reacting with reactive oxygen intermediates and therefore may also influence the reduction of NBT. The diminished formazan production was not necessarily only a result of a lower content of superoxide anion due to an inhibitory influence of pterins on XOD, but also a direct interaction of pterins with superoxide anion to catalyze the production of, e.g., $OH^{\bullet}$ seems possible. Indeed, the effect of neopterin and biopterin on formazan production was even stronger than that on urate formation. This observation would well agree with the assumption that the oxidized pteridines, neopterin and biopterin, additionally diminished the production of formazan from NBT possibly by catalyzing the conversion of superoxide anion to other reactive species whereas reduced pteridines, dihydroneopterin and tetrahydrobiopterin, have no such effect.

In the presence of iron, urate and formazan production were reduced and the influence of pterins was even more expressed. Superoxide anion reduces ferric into ferrous ion, which has strong reducing activity on hydrogen peroxide-forming hydroxyl radical (Fenton reaction). The catalytic activity of ferric ion seems to be required for the effects of pterins on XOD, and chemical reduction of active agents by iron-(III) may scavenge superoxide-dependent formazan production[26] (Table I). Fe could also interact with formazan radicals and also $OH^{\bullet}$ could simply inactivate XOD. SOD and NAC diminished formazan but not urate production, the effects of pteridine derivatives were in addition. SOD eliminates superoxide anion by dismutation and NAC as well as cysteine (data not shown) inhibit formazan production by reducing oxygen radicals through oxidation of their free sulfhydryl groups which may explain the scavenged rate of formazan production (Table I). NAC can also directly interact with formazan. High concentrations of SOD even increased urate production. SOD may prevent oxidative inactivation of XOD by its own toxic products.[18] This observation may also serve as an explanation for the drastic inhibitory effects of neopterin and biopterin on urate formation. The oxidized pterins could inhibit XOD by enhancing the inactivation of the enzyme by its oxidative products. This explanation would be in line with the known enhancing potential of these compounds on reactive oxygen metabolites.

We conclude, oxidized and reduced forms of pterins may inhibit conversion of xanthine by XOD in different ways. Substrates of XOD like xanthopterin may competively bind in the active center. Reduced pterins like 7,8-dihydroneopterin may interfere with the pterin residue in the molybdenum cofactor. In contrast, oxidized pterins may interact with the superoxide produced, which may enhance the feedback inhibition of XOD and also directly reduce the amount of reactive metabolites for NBT reduction.

Acknowledgements

This work was financially supported by the Austrian Funds "Zur Förderung der

wissenschaftlichen Forschung", project 10776-MED. The support by a grant of the Austrian Ministry of Science and Transport to Z.Z.A. is gratefully acknowledged.

References

[1] C. Huber, J.R. Batchelor, D. Fuchs, A. Hausen, A. Lang, D. Niederwieser, G. Reibnegger, P. Swetly, J. Troppmair and H. Wachter (1984) Immune response-associated production of neopterin: Release from macrophages primarily under control of interferon gamma. *Journal of Experimental Medicine*, **160**, 310–316.

[2] D. Fuchs, A. Hausen, G. Reibenegger, E.R. Werner, M.P. Dierich and H. Wachter (1988) Neopterin as a marker for activated cell-mediated immunity. *Immunology Today*, **9**, 150–155.

[3] H. Wachter, D. Fuchs, A. Hausen, G. Reibenegger and E.R. Werner (1989) Neopterin as a marker for activation of cellular immunity. Immunologic basis and clinical applications. *Advances in Clinical Chemistry*, **24**, 81–141.

[4] D. Fuchs, G. Weiss, G. Reibenegger and H. Wachter (1992) The role of neopterin as a monitor of cellular immune activation in transplantation, inflammatory, infectious, and malignant diseases. *Critical Reviews in Clinical Laboratory Sciences*, **29**, 307–341.

[5] H. Wachter, D. Fuchs, A. Hausen, G. Reibenegger, G. Weiss, E.R. Werner and G. Werner-Felmayer (1992) *Neopterin: Biochemistry – Methods – Clinical Application*, DeGruyter, Berlin/New York.

[6] C.F. Nathan (1986) Peroxide and pteridine: A hypothesis on the regulation of macrophage antimicrobial activity by interferon-γ. In: *Interferon*, Vol. 7, pp. 125–143, I. Gresser and J. Vileck (eds.). Academic Press, New York.

[7] D. Fuchs, G. Baier-Bitterlich, I. Wede and H. Wachter (1997) Reactive oxygen and apoptosis. In: *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. J. Scandalios (ed.). Cold Spring Harbor Laboratory Press, New York, pp. 139–167.

[8] S.J.R. Heales, J.A. Blair, C. Meinschad and I. Ziegler (1988) Inhibition of monocyte luminol-dependent chemiluminescence by tetrahydrobiopterin, and the free radical oxidation of tetrahydrobiopterin, dihydrobiopterin and dihydroneopterin. *Cell Biochemical Function*, **6**, 191–195.

[9] G. Weiss, D. Fuchs, A. Hausen, G. Reibenegger, E.R. Werner, G. Werner-Felmayer, E. Semenitz, M.P. Dierich and H. Wachter (1993) Neopterin modulates toxicity mediated by reactive oxygen and chloride species. *FEBS Letters*, **321**, 89–92.

[10] R. Shen (1994) Inhibition of luminol-enhanced chemiluminescence by reduced pterins. *Archives of Biochemistry and Biophysics*, **310**, 60–63.

[11] C. Murr, D. Fuchs, W. Gossler, A. Hausen, G. Reibnegger, E.R. Werner, G. Werner-Felmayer, H. Esterbauer and H. Wachter (1994) Enhancement of hydrogen peroxide-induced luminol-dependent chemiluminescence by neopterin depends on the presence of iron chelator complexes. *FEBS Letters*, **338**, 223–226.

[12] G. Reibnegger, D. Fuchs, C. Murr, M.P. Dierich, W. Pfleiderer and H. Wachter (1995) Effects of pteridines on luminol–dependent chemiluminescence induced by chloramine-T. *Free Radicals in Biology and Medicine*, **18**, 515–523.

[13] W. Schobersberger, G. Hoffmann and J. Fandrey (1996) Nitric oxide donors suppress erythropoietin production *in vitro*. *Pflügers Archives of Physiology*, **432**, 980–985.

[14] W. Schobersberger, G. Hoffmann, J. Grote, H. Wachter and D. Fuchs (1995) Induction of inducible nitric oxide synthase expression by neopterin in vascular smooth muscle cells. *FEBS Letters*, **377**, 461–464.

[15] S. Kojima, T. Icho, Y. Kajiwara and K. Kubota (1992) Neopterin as an endogenous antioxidant. *FEBS Letters*, **304**, 163–166.

[16] S. Kojima, T. Nomura, T. Icho, Y. Kajiwara, K. Kitabatake and K. Kubota (1993) Inhibitory effect of neopterin on NADPH-dependent superoxide-generating oxidase of rat peritoneal macrophages. *FEBS Letters*, **329**, 125–128.

[17] S. Stirpe and E. Della Corte (1969) The regulation of rat liver xanthine oxidase. *Journal of Biological Chemistry*, **244**, 3855–3859.

[18] J. Robak and R.J. Gryglewski (1988) Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*, **37**, 837–841.

[19] L.S. Terada, J.A. Leff and J.E. Repine (1990) Measurement of xanthine oxidase in biological tissues. *Methods in Enzymology*, **186**, 651–656.

[20] S.J. Klebanoff (1992) Oxygen metabolites from phagocytes. In: *Inflammation: Basic Principles and Clinical Correlates*. J.I. Gallin and I.M. Goldstein (eds.). Raven Press, New York, pp. 541–565.

[21] H. Rembold (1970) *Catabolismus of Unconjugated Pteridines*. International Academic Printing, Tokyo, pp. 163–178.

[22] H. Rembold and W. Gutensohn (1968) 6-Hydroxylation of the pteridine ring by xanthine oxidase. *Biochemical Biophysical Research Communications*, **31**, 837–842.

[23] H.S. Forrest, G. Glassman and H.K. Mitchell (1956) Conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in D. Melanogaster. *Science*, **124**, 725–728.

[24] K. Oettl, S. Dikalov, H.J. Freisleben, W. Mlekusch and G. Reibnegger (1997) Spin trapping study of antioxidant properties of neopterin and 7,8-dihydroneopterin. *Biochemical and Biophysical Research Communications*, **234**, 774–778.

[25] H. Schindelin, C. Kisker, J. Hilton, K.V. Rajagopalan and D.C. Rees (1996) Crystal structure of DMSO reductase: Redox-linked changes in molybdopterin coordination. *Science*, **272**, 1615–1620.

[26] K. Fukuzawa, K. Soumi, M. Lemura, S. Goto and A. Tokumura (1995) Dynamics of xanthine oxidase and Fe^{3+}–ADP-dependent lipid peroxidation in negatively charged phospholipid vesicles. *Archives in Biochemistry and Biophysics*, **316**, 83–91.