Pteridines

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Neopterin: a Mediator of the Cellular Immune System*

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Medical Informatics and Technology, Austria*Dedicated to Prof. Dr. Dr. h. c. Helmut Wachter on the occasion of his 75th birthday

Abstract

In this review, we will summarize our investigations on the potential biochemical, physiological, and pathophysiological effects of neopterin in the course of infection and inflammation. In a number of *in vitro*-studies we could show that the pteridine compound neopterin manifested distinct biochemical properties via interfering with cellular redox mechanisms. Among the observed actions of neopterin are the stimulation of inducible nitric oxide synthase gene expression and nitric oxide release, the activation of the transcription factor nuclear factor- κ B, the induction of apoptotic cell death, the inhibition of erythropoietin synthesis and release, and the stimulation of tumor necrosis factor- α generation in different cell culture as well as organic models. By these means, neopterin may represent a part of the host-defence reactions. In addition, an excessive or continuous release of neopterin due to a chronically activated cellular immune system may contribute to the deleterious events commonly observed in progressive infectious or inflammatory diseases. Our data are supported by the findings of a strong correlation between raised levels of neopterin and the severity and the outcome of these disorders.

Key words: neopterin, infection, inflammation, oxidative stress

Introduction

Since its isolation in 1967 by Sakurai and Goto (1), neopterin has been shown to be a useful indicator for an activated cellular immune system in humans. Studies by Wachter and co-workers reported increased levels of neopterin in serum, urine, synovial and cerebrospinal fluid or saliva in diseases characterized by a stimulation of the cell-mediated immune response. Thus, high neopterin concentrations are a reliable indicator of the host-defence reaction in infectious or malignant diseases. Furthermore, neopterin levels are augmented during graft rejection with or without infectious complications. Due to its quality as a disease marker, neopterin measurements have become a significant tool for blood and organ donor screenings (2-6).

Our specific interest in neopterin focused on its potential biochemical and physiological (or pathophysiological) effects in the course of infectious diseases. Early studies suggested the promotion of oxidative stress to be a key principle of neopterin's mode of action. In cell-free solutions, neopterin enhanced chlo-

ramine T- and hydrogen peroxide-mediated chemiluminescence. Since these effects depended on the presence of transition metals, it was hypothesized that neopterin stimulates the production of hydroxyl radicals (7-9). In our own studies, we investigated the potential effects of neopterin in various cell culture systems and organic models. The results with respect to their clinical implications will be summarized in this review.

Biochemical effects of neopterin

Reactive nitrogen species derived from nitric oxide are produced in excessive amounts in a number of infectious diseases, e.g. sepsis and septic shock. Due to its vasodilatory effects, nitric oxide (NO) is discussed to be a paramount mediator of the detrimental hypotensive reactions in the course of endotoxemia. Raised levels of serum neopterin have been reported in sepsis and there was a correlation between the concentration of this pteridine compound and the severity as well as

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the outcome of the disease (10). In our studies using vascular smooth muscle as a cellular model, we observed a neopterin-initiated expression of inducible nitric oxide synthase (iNOS) (Fig. 1) and a subsequent

Figure 1. Qualitative analyses of inducible nitric oxide synthase (iNOS) gene expression in vascular smooth muscle cells following a 6-h incubation with neopterin (10 μ M), a combination of tumor necrosis factor- α (500 U/ml) and interferon- γ (100 U/ml) (C), and a mixture of neopterin plus cytokines (N+C). U represents unstimulated controls.

increase of NO generation which was most likely triggered via an activation of the transcription factor nuclear factor- κ B (NF- κ B) by neopterin. NF- κ B activation may provide a link between neopterin, oxidative stress, and iNOS gene expression. Moreover, neopterin-induced NO synthesis may explain the observations made in endotoxemic animals and humans, where elevations of neopterin concentrations were accompanied by an increase in nitrite/nitrate levels (11,12). Pro-inflammatory cytokines, especially tumor necrosis factor- α (TNF- α) have been reported to be involved in the pathogenesis of sepsis and septic shock as well (13,14). Considering synthesis and secretion, similarities between neopterin and TNF- α were shown. For example, neopterin was found to amplify the secretion of TNF- α from activated peripheral blood mononuclear cells (15). We could identify neopterin to be a potent stimulus of TNF- α gene expression (Fig. 2) as well as TNF- α protein synthesis

Figure 2. Qualitative analyses of tumor necrosis factor- α (TNF- α) gene expression in vascular smooth muscle cells following a 3-h incubation with neopterin at increasing concentrations: 1 μ M (N1), 10 μ M (N10), 100 μ M (N100), and 1000 μ M (N1000). U represents unstimulated controls.

in vascular smooth muscle cells (16). Via the affection of septic mediators like NO or TNF- α , the synthesis of neopterin may contribute to the deleterious events in the course of bacterial infections (Fig. 3). This hypothesis is further supported by the observation of disturbed coronary flow and cardiac contractility following neopterin perfusion in the isolated rat heart (17).

In addition to raising NO levels, neopterin was found to induce apoptotic death in vascular smooth muscle cells (18). With respect to intracellular signal transduction mechanisms, there might be a connection between neopterin-induced nitric oxide generation and neopterin-mediated programmed cell death. In fact, the simultaneous incubation of smooth muscle cells with neopterin plus an inhibitor of the iNOS enzyme resulted in a loss of neopterin's impact on apoptosis in our studies (18). In blood vessels, the combination of raised levels of neopterin, apoptotic cell death, and nitric oxide generation is found in the process of atherosclerosis (19-21). Although the exact role of apoptosis in atherogenesis is not clear to date, stimulation of iNOS gene expression by neopterin might represent an important step towards programmed cell death under these conditions. In the early phase of atherosclerosis, this might trigger a beneficial limitation of plaque growth while in the later steps, apoptosis may contribute to a destabilization of massive plaques and may thus promote a pro-thrombotic state.

The concept of neopterin acting as a pro-apoptotic mediator could be confirmed in studies using the alveolar epithelial type II-like cell line L2. Single application of neopterin increased the rate of apoptosis in these cells. Furthermore, additive effects of neopterin and the pro-inflammatory cytokines TNF- α and interferon- γ (IFN- γ) could be observed (Fig. 4). In contrast to vascular smooth muscle cells, neopterin-induced programmed L2-cell death was not affected by iNOS-inhibition or the application of NO-donors. This suggests that apoptosis in L2 cells triggered by neopterin does not involve the production of NO (22). Within the pulmonary system, apoptosis is supposed to be a significant event in the pathogenesis and the course of inflammatory diseases of the lung, e.g. sarcoidosis, tuberculosis, and lung cancer (23). A combined action of neopterin and other pro-inflammatory mediators like TNF- α might contribute to the deterioration of pulmonary functions under these conditions. This hypothesis is further supported by the observations of an increased expression and release of intercellular adhesion molecule-1 (ICAM-1) in L2 cells following incubation with neopterin (24). ICAM-1 mediates the migration and activation of leukocytes. The accumulation of macrophages, neutrophils, or lymphocytes as well as their secretory products at the site of infection

Figure 3. Potential role of neopterin in the pathogenesis of sepsis and septic shock. Bacterial infections lead to the activation of TH1-type lymphocytes. The ensuing release of interferon- γ (IFN- γ) activates monocytes/macrophages to produce and release neopterin. As a single stimulus or in addition to cytokines like tumor necrosis factor- α (TNF- α), neopterin triggers pro-inflammatory events. Among those, inducible nitric oxide synthase (iNOS) gene expression and the subsequent release of nitric oxide in excessive amounts favours the pathophysiological hypotensive reaction characteristic for septic shock.

is part of the unspecific host-defence reaction. However, a continuous stimulation of these events may result in damage of the airway epithelium. Although neopterin and ICAM-1 synthesis are not generally connected in infectious diseases, a significant correlation between both compounds could be described in the broncho-alveolar lavage fluid in patients with sarcoidosis (25). Taken together, these data as well as our own *in vitro*-experiments point towards a role of neopterin in the regulation of ICAM-1 synthesis within the alveolar epithelium.

Chronically increased activities of the cellular immune system like sepsis and others described in this review are regularly associated with anaemia (26,27). A major trigger for anaemia under these conditions is the suppression of erythropoietin (EPO) production by pro-inflammatory mediators, e.g. TNF- α , interleukin-1 β , (IL-1 β), or bacterial lipopolysaccharides (28-30). Various data provided evidence that neopterin might be involved in the onset of anaemia, too. Thus, the

highest concentrations of neopterin in cancer patients are reported in tumors associated with the most pronounced degree of anaemia (31), and inverse correlations have been observed between serum concentrations of neopterin and iron as well as transferrin (32). Indeed, neopterin proved to suppress hypoxia-induced EPO generation in the human hepatoma cell line HepG₂ (33). These observations could be confirmed in a subsequent study using isolated, hypoxically perfused rat kidney. Addition of neopterin into the perfusate strongly reduced hypoxia-stimulated EPO release (34). So far, no involvement of oxidative stress in these processes could be established, since the anti-oxidative compound N-acetylcysteine did not change the effects of neopterin on hypoxia-triggered EPO synthesis in our organ model (35). In addition, changes in intracellular calcium concentrations [Ca²⁺]_i cannot serve as an explanation for the observed influence of neopterin on EPO generation, since verapamil did not affect the suppressed EPO-release following neopterin

Figure 4. Potential role of neopterin in the activation of programmed cell death (apoptosis). Pro-apoptotic signals trigger the release of cytochrome C from mitochondria. Subsequently, a cascade of enzymes (caspases) is activated which finally leads to the characteristic events of apoptosis, e.g. DNA fragmentation (which can be visualized via agarose gel electrophoresis of isolated cellular DNA, left insert) or blebbing of cells (which can be visualized via fluorescence microscopy, right insert). Most likely, neopterin acts via oxidative stress pathways to initiate apoptosis, while cytokines like tumor necrosis factor- α (TNF- α) require specific cell death receptors and different intracellular signalling mechanisms to activate mitochondria. This allows for additive or synergistic effects of both compounds with respect to activation of programmed cell death.

treatment.

However, this last observation does not exclude a potential interaction between neopterin and shifts in intracellular calcium. In a human myelomonocytic cell line, neopterin was found to increase $[Ca^{2+}]_i$ (36). With respect to monocytic cells, elevation of $[Ca^{2+}]_i$ contributes to the activation of NADPH oxidase and may thus promote the production of reactive oxygen species. In contrast to these findings, we found that neopterin inhibited Ca^{2+} transients triggered by ATP in L2 cells, suggesting that neopterin interferes with $[Ca^{2+}]_i$ in a cell-type specific manner (37). Considering the importance of an increase in cytoplasmic Ca^{2+} for surfactant release by alveolar type II epithelial cells, disturbances of cellular calcium homeostasis by neopterin may contribute to the pulmonary dysfunctions in the course of inflammatory processes.

Conclusions

The above-mentioned findings provide evidence

that neopterin acts as a mediator of host-defence reactions most likely via interfering with cellular redox systems. Although the exact role and the importance of neopterin in the course of infection and inflammation still remains unclear, the pteridine compound appears to be a part of the extracellular arsenal of IFN- γ -stimulated macrophages. Following an ineffective acute immune response, the chronic activation of neopterin synthesis and release may contribute to various adverse effects observed under these conditions. The findings summarized in this review may shed further insight into the complex mechanisms of a chronically activated cellular immune system and may provide an explanation for the commonly observed correlations between raised levels of neopterin and the severity of infectious diseases.

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