Review

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Immune activation and inflammation increase the plasma phenylalanine-to-tyrosine ratio

Abstract: The proinflammatory cytokine interferon (IFN) γ activates GTP-cyclohydrolase I. In turn, the production of neopterin in human monocytic cells and of 5,6,7,8-tetrahydrobiopterin (BH₄) in other human cells and cells of other species is markedly upregulated. BH₄ is cofactor for the biosynthesis of the neurotransmitters 5-hydroxytryptamine (serotonin) and the catecholamines dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline). The finding of increased neopterin concentrations in patients with viral infections, autoimmune syndromes, malignant tumors, and during treatment with specific cytokines corresponds well with its immunobiological background. However, there is no clear information about BH₄ concentrations in these patients. Furthermore, higher blood phenylalanine (Phe)-to-tyrosine (Tyr) ratios have been described in patients with ovarian cancer, after multiple trauma and with sepsis, in patients with HIV-1 infection, in elderly individuals, and in patients with HCV infection under IFN-α therapy. Recent studies already showed that the alterations of Phe metabolism are associated with mood changes and depression. Results point to an impaired hydroxylation of Phe when the enzyme phenylalanine 4-hydroxylase (PAH) is less efficient. As the decrease of PAH activity might result from a diminished availability of BH₄, the determination of the Phe/Tyr ratio may serve as an indirect measure of BH₄ availability.

Keywords: immune activation; inflammation; interferon γ; oxidative stress; phenylalanine; phenylalanine 4-hydroxylase (PAH); tetrahydrobiopterin (BH₄).

Introduction

Catecholamines dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline) are important neurotransmitters that are crucially related to neurobehavioral aspects. On the one hand, hormones such as adrenaline or noradrenaline facilitate immediate physical reactions associated with a preparation for violent muscular action, the so-called fight-or-flight response [1]. On the other hand, alterations of the neuroadrenergic pathway participate in the pathophysiology of various neuropsychiatric symptoms [2–4].

Catecholamines derive from the amino acids phenylalanine (Phe) and tyrosine (Tyr) and are synthesized by the adrenal gland, the central nervous system, and brain cells. The pteridine derivative 5,6,7,8-tetrahydrobiopterin (BH₄) is cofactor of the two aromatic amino acid monooxygenases in this biosynthetic cascade, namely phenylalanine 4-hydroxylase (EC 1.14.16.1; PAH) and tyrosine 5-hydroxylase (EC 1.14.16.2) [5]. The initial step in the production of cofactor BH₄ is achieved by enzyme GTP-cyclohydrolase I (GCH, EC 3.5.4.16). Stimulation of GCH by the Th1-type cytokine interferon (IFN) γ and some other proinflammatory stimuli such as lipopolysaccharide (LPS) and cytokine tumor necrosis factor α (TNF-α) leads to the production of neopterin at the expense of BH₄ in human macrophages [5]. Therefore, inflammation and immune activation affect BH₄ availability, and as a consequence, the biosynthesis of the above-mentioned monoamine neurotransmitters is altered. This is also true for neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) and nitric oxide (NO), which are also formed by BH₄-dependent enzymes tryptophan 5-hydroxylase (EC 1.14.16.4) and NO synthases (EC 1.14.13.39) [5].

Genetic abnormality of the PAH gene is the most relevant cause of a dysfunction of PAH, and in the case of homozygosity, phenylketonuria develops (PKU) [6]. PKU is characterized by hyperphenylalaninemia and a dramatic increase of the Phe/Tyr ratio in the blood. Phe/Tyr is considered to allow an estimate of PAH activity [7], although
Pteridines and Phe metabolism during immune response

IFN-γ centrally influences the biochemistry of the pteridine derivatives BH₄ and neopterin in humans [5]. The effect of IFN-γ to stimulate GCH can be further upregulated by other proinflammatory stimuli such as LPS and TNF-α, which itself is only a very weak inducer of GCH [9]. IFN-γ is produced and released during the cellular immune response, which is mediated by type 1 T-helper cells. Because human monocyte derived-macrophages and dendritic cells are deficient in 6-pyruvoyl-tetrahydropterin synthase (PTPS; EC 4.2.3.12), which is responsible for the conversion of intermediate 7,8-dihydroneopterin triphosphate to sepiapterin and BH₄ [5], human cells of the monocyte-macrophage lineage produce neopterin and 7,8-dihydroneopterin at the expense of BH₄. This biochemical peculiarity explains why these cells are defective in producing NO in high concentrations, whereas other human cells such as endothelial cells are capable of doing so, and this is even more the case for nonhuman/primate cells [10]. At the same time, the concentrations of neopterin derivatives in human body fluids are much higher than in those from other species.

In clinical conditions that are associated with immune activation, neopterin concentrations in the blood, urine, cerebrospinal fluid, or other body fluids are often increased and are of laboratory diagnostic value, e.g., to detect blood donations contaminated with infectious agents or to predict outcome of patients with HIV-1 infections, cardiovascular disease, or malignant tumor disease [11, 12], and neopterin determinations were also found helpful to judge treatment efficacy in patients with pulmonary tuberculosis, rheumatoid arthritis [11], and multiple sclerosis receiving IFN-β therapy [13].

Interestingly, increased serum Phe concentrations have already been reported, e.g., by Roth and colleagues, in the 1980s when amino acid profiles were examined in patients with HIV infection [14], cancer [15], after trauma, and with sepsis [16, 17]. This kind of moderate hyperphenylalaninemia was observed in treatment-naïve patients with HIV infection throughout all stages [14]. Also, in burn patients, not only higher levels of Phe but also higher Phe/Tyr values have been described, and the increases were found to correlate with the clinical course and predict nonsurvival [18]. In summary, it seemed that the pattern of diseases, which were found to be associated with moderate hyperphenylalaninemia, greatly overlapped with those found independently with elevated neopterin, representing a sign of immune activation.

Significant correlations between Phe metabolism and concentrations of immune activation marker neopterin were reported from patients after trauma [19], with ovarian cancer [20], with HIV-1 infection [21], with coronary artery disease [22], as well as in the healthy elderly [23], and neopterin concentrations followed the course of other immune activation markers such as interleukin 6, soluble interleukin 2 receptor α, and the 75-kDa TNF receptor [24].

Thus, the results indicate that processes taking place during immune activation are responsible for the development of moderately increased serum Phe concentrations,
which are most probably due to a reduced conversion rate of Phe to Tyr by PAH [23]. In accordance, children with PKU who were treated with BH₄ were described to require increased dosage during episodes of infections [8].

Because IFN-γ is the most relevant trigger for high output of ROS [25], it was assumed that the lability of the PAH cofactor BH₄ could be the reason for the impaired enzyme function when BH₄ will undergo oxidation in a situation of an overwhelming ROS production [26] that wipes out antioxidant compounds and defense systems. The correlation found between the concentrations of the oxidative stress marker isoprostane-8 and Phe underscores this assumption [20]. However, ROS and the oxidizing milieu in the neighborhood of activated macrophages can also impair proper enzyme function when tertiary structures of enzymes are influenced. Such scenario was recently demonstrated to utilize model calculations of the interaction of PAH protein with its substrate Phe [27].

**Disturbed Phe metabolism and the development of neuropsychiatric symptoms**

Chronic inflammatory diseases such as infections, autoimmune syndromes, or cancer are often accompanied by fatigue, mood changes, and depression especially in the later stage of disease. The precise biochemical background of these symptoms is still unresolved, but the disturbed metabolism of biogenic amines is highly discussed [3, 4, 28]. In patients with psychiatric disorders including major depression [29] and schizophrenia [30, 31], BH₄ deficiency was described to relate to the development of neuropsychiatric symptoms. Associations between neuropsychiatric scores and changes of Phe metabolism were also observed in elderly individuals [23]. Further support of the view that cytokine-induced alterations of pteridine metabolism is involved in neuropsychiatric abnormalities derives from observations made in patients under treatment with cytokines such as IFNs and TNF-α [32]. Disturbed conversion of Phe to Tyr was documented in patients treated with malignant melanoma [33] or HCV infection [34] under IFN-α therapy. Moreover, increased blood Phe/Tyr correlated with fatigue scores and with lower dopamine levels in the cerebrospinal fluid [35].

Results confirm the assumption that the impaired conversion of Phe to Tyr in the liver and the concomitantly decreased Tyr availability affect the transport of the amino acid into the brain and its further conversion to its downstream metabolites L-DOPA, dopamine, epinephrine, and norepinephrine [3]. Owing to its background, Phe/Tyr could serve as a convenient surrogate indicator of BH₄ availability because in situations when BH₄ production is disturbed, Phe/Tyr concentrations raise. Thus, an increased Phe/Tyr can serve as an indicator of impaired BH₄ availability, which could represent a reliable alternative to the direct measurement of BH₄ in the blood of patients [36], but although with good feasibility, preanalytical requirements are complex and thus not easily applicable in clinical studies.

**Therapeutic considerations**

Increased Phe/Tyr concentrations should improve upon administration of BH₄ [8, 37], and it is also well established that antioxidants such as vitamin C stabilize BH₄ and prolong its lifespan in enzyme reactions [38]. In vitro studies also show that immunosuppressants [39] or other anti-inflammatory drugs such as aspirin or salicylic acid and antioxidant compounds such as vitamins C and E or the stilbene resveratrol [40] are able to attenuate the inflammatory response and suppress cytokine-induced production of ROS and neopterin. If these in vitro findings hold true in vivo, one might expect that a diet rich in antioxidants and/or supplemented antioxidants could contribute to some amelioration of lowered mood and depressive disorders.

**Conclusion**

An association between moderate hyperphenylalaninemia and neuropsychiatric symptoms has been observed in the elderly and in patients with HCV infection under treatment with IFN-α/ribavirin. The measurement of Phe/Tyr might allow a conclusion whether adrenergic or serotonergic treatment is preferable for the individual patient with neuropsychiatric abnormalities. It should also be possible to improve abnormalities of Phe metabolism to a certain extent by reduction of inflammation. A threshold of Phe concentrations and/or Phe/Tyr needs to be defined at which such intervention strategies should be considered. The determination of the Phe/Tyr may serve as an indirect measure of BH₄ availability.
References


