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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 (BH_4) in other human cells and cells of other species is markedly upregulated. BH_4 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 BH_4 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 BH_4, 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 (BH_4).

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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 (BH_4) 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 BH_4 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 BH_4 in human macrophages [5]. Therefore, inflammation and immune activation affect BH_4 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 BH_4-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

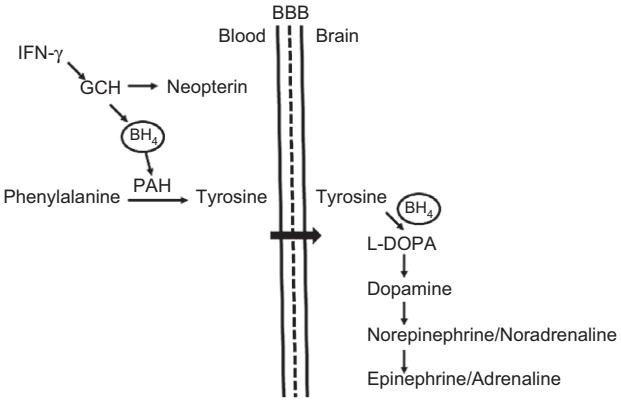

Figure 1 The proinflammatory cytokine IFN-γ is preferentially released during the process of Th1-type immune activation. The cytokine induces the expression of enzyme GCH, which, in most human cells and cells from other species, results in the formation of cofactor BH_4. Only human and primate monocyte-derived macrophages and dendritic cells produce neopterin instead of BH_4. In the liver and kidneys, the enzyme PAH converts Phe to Tyr, requiring BH_4 as a cofactor. Tyr deficiency may cause an insufficient production of biogenic amine neurotransmitters noradrenaline (norepinephrine) and adrenaline (epinephrine), which can underlie the development of mood changes and fatigue in patients with inflammatory conditions.

this approach is limited by the fact that the product of PAH, namely Tyr, is again a substrate for further BH_4-dependent enzyme reaction [2, 3] (Figure 1). Alternatively, Phe conversion can also be impaired when endogenous production of BH_4 is insufficient. The most dramatic form of BH_4 deficiency presents as atypical PKU, which develops due to genetic defects among the biosynthetic enzyme machinery for BH_4 production [6]. Atypical PKU can be successfully treated with BH_4 supplementation [8]. However, aside from classical PKU, genetically normal or heterozygous individuals may develop moderate hyperphenylalaninemia, and it seems that immune activation and oxidative stress can play a role [3].

Pteridines and Phe metabolism during immune response

IFN-γ centrally influences the biochemistry of the pteridine derivatives BH_4 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-pyruvoyltetrahydropterin synthase (PTPS; EC 4.2.3.12), which is responsible for the conversion of intermediate 7,8-dihydroneopterin triphosphate to sepiapterin and BH_4 [5], human cells of the monocyte-macrophage lineage produce neopterin and 7,8-dihydroneopterin at the expense of BH_4. 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 BH_4 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 BH_4 could be the reason for the impaired enzyme function when BH_4 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], BH_4 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 BH_4 availability because in situations when BH_4 production is disturbed, Phe/Tyr concentrations raise. Thus, an increased Phe/Tyr can serve as an indicator of impaired BH_4 availability, which could represent a reliable alternative to the direct measurement of BH_4 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 BH_4 [8, 37], and it is also well established that antioxidants such as vitamin C stabilize BH_4 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 neurospsychiatric 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 BH_4 availability.

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References

1. Cannon WB. Bodily changes in pain, hunger, fear and rage: an account of recent researches into the function of emotional excitement. New York: Appleton-Century-Crofts, 1915.
2. Shintaku H. Disorders of tetrahydrobiopterin metabolism and their treatment. Curr Drug Metab 2002;3:123–31.
3. Neurauter G, Schröcksnadel K, Scholl-Bürgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, et al. Chronic immune stimulation correlates with reduced phenylalanine turn-over. Curr Drug Metabol 2008;9:622–7.
4. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 2012;37:137–62.
5. Werner-Felmayer G, Golderer G, Werner ER. Tetrahydrobiopterin biosynthesis utilization and pharmacological effects. Curr Drug Metab 2002;3:159–73.
6. Opladen T, Hoffmann GF, Blau N. An international survey of patients with tetrahydrobiopterin deficiencies presenting with hyperphenylalaninaemia. J Inherit Metab Dis 2012;35:963–73.
7. Andersson DN, Wilkinson AM, Abou-Saleh MT, Blair JA. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand 1994;90:10–3.
8. Trefz FK, Scheible D, Frauendienst-Egger G, Korall H, Blau N. Long-term treatment of patients with mild and classical PKU by BH4. Mol Gen Metab 2005;86:S75–80.
9. Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Wachter H. Tumour necrosis factor-alpha and lipopolysaccharide enhance interferon-induced tryptophan degradation and pteridine synthesis in human cells. Biol Chem Hoppe Seyler 1989;370:1063–9.
10. Sperner-Unterweger B, Kohl C, Fuchs D. Immune changes and catecholamines: is there a role in depression? Prog Neuropsychopharmacol Biol Psychiatry (in press).
11. Widner B, Murr C, Wirleitner B, Mayr C, Spöttl N, Baier-Bitterlich G, et al. The importance of neopterin as a laboratory dianostic marker of immune activation. Pteridines 1999;10:101–1.
12. Fuchs D, Avanzas P, Arroyo-Espliguero R, Jenny M, Consuegra Sanchez L, Kaski JC. The role of neopterin in atherogenesis and cardiovascular risk stratification. Curr Med Chem 2009;16:4644–53.
13. Hu X, Miller L, Richman S, Hitchman S, Glick G, Liu S, et al. A novel PEGylated interferon beta-1a for multiple sclerosis: safety, pharmacology, and biology. J Clin Pharmacol 2012;52:798–808.
14. Ollenschläger G, Jansen S, Schindler J, Rasokat H, Schrappe-Bächer M, Roth E. Plasma amino acid pattern of patients with HIV infection. Clin Chem 1988;34:1787–9.
15. Watanabe A, Higashi T, Sakata T, Nagashima H. Serum amino acid levels in patients with hepatocellular carcinoma. Cancer 1984;54:1875–82.
16. Chang XJ, Yang CC, Hsu WS, Xu WZ, Shih TS. Serum and erythrocyte amino acid pattern: studies on major burn cases. Burns Incl Therm Inj 1983;9:240–8.
17. Rath T, Roth E, Keidl R, Meissl G. Phenylalanine: total amino acid ratio in 45 burn patients. Scand J Plast Reconstr Surg Hand Surg 1987;21:297–300.
18. Roth E. Veränderungen im Aminosäuren- und Proteinstoffwechsel bei chirurgischen Patienten. In: Ahnefeld FW, Hartig W, Holm E, editors. Klinische Ernährung, vol 26. Munich: Zuckschwerdt-Verlag, 1987:38.
19. Ploder M, Neurauter G, Spittler A, Schroecksnadel K, Roth E, Fuchs D. Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations. Amino Acids 2008;35:303–7.
20. Neurauter G, Grahmann AV, Klieber M, Zeimet A, Ledochowski M, Sperner-Unterweger B, et al. Serum phenylalanine concentrations in patients with ovarian carcinoma correlate with concentrations of immune activation markers and of isoprostane-8. Cancer Lett 2008;272:141–7.
21. Zangerle R, Kurz K, Neurauter G, Kitchen M, Sarcletti M, Fuchs D. Increased blood phenylalanine to tyrosine ratio in HIV-1 infection and correction following effective antiretroviral therapy. Brain Behav Immun 2010;24:403–8.
22. Mangge H, Schnedl WJ, Schroecksnadel S, Geisler S, Murr C, Fuchs D. Immune activation and inflammation in patients with cardiovascular disease are associated with elevated phenylalanine to tyrosine ratios. Pteridines 2013;24:51–5.
23. Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low grade immune activation in the elderly is associated with increased tryptophan catabolism and altered phenylalanine turnover: role in neuropsychiatric symptomatology. Biol Psychiatry 2011;70:175–82.
24. Sperner-Unterweger B, Neurauter G, Klieber M, Kurz K, Meraner V, Zeimet A, et al. Enhanced tryptophan degradation in patients with ovarian carcinoma correlates with several serum soluble immune activation markers. Immunobiology 2011;216:296–301.
25. Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med 1983;158:670–89.
26. Fuchs D, Jaeger M, Widner B, Wirleitner B, Artner-Dworzak E, Leblhuber F. Is hyperhomocysteinemia due to oxidative depletion of folate rather than insufficient dietary intake. Clin Chem Lab Med 2001;39:691–4.
27. Fuchs JE, Huber RG, von Grafenstein S, Wallnoefer HG, Spitzer GM, Fuchs D, et al. Dynamic regulation of phenylalanine hydroxylase by simulated redox manipulation. PLoS One 2012;7:e53005.
28. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46–56.
29. Hashimoto R, Mizutani M, Ohta T, Nakazawa K, Nagatsu T. Changes in plasma tetrahydrobiopterin levels of depressives in depressive and remission phases: reconfirmed by measurement with an internal standard. Neuropsychobiology 1994;29:57–60.
30. Richardson MA, Read LL, Taylor Clelland CL, Reilly MA, Chao HM, Guynn RW, et al. Evidence for a tetrahydrobiopterin deficit in schizophrenia. Neuropsychobiology 2005;52:190–201.
31. Richardson MA, Read LL, Reilly MA, Clelland JD, Clelland CL. Analysis of plasma biopterin levels in psychiatric disorders

suggests a common BH4 deficit in schizophrenia and schizoaffective disorder. Neurochem Res 2007;32:107–13.
32. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 2006;27:24–31.
33. Van Gool AR, van Ojik HH, Kruit WH, Bannink M, Mulder PG, Eggermont AM, et al. Pegylated interferon-alpha2b treatment in melanoma patients: influence on amino acids, 5-hydroxyindolacetic acid and pteridine plasma concentrations. Anticancer Drugs 2004;15:587–91.
34. Zoller H, Schloegl A, Schroecksnadel S, Vogel W, Fuchs D. Influence of interferon-α therapy on phenylalanine hydroxylase activity in patients with HCV infection. J Interferon Cytokine Res 2012;32:216–20.
35. Felger JC, Li L, Marvar PJ, Harrison DG, Raison CL, Miller AH. Tyrosine metabolism during interferon-alpha administration: association with fatigue and CSF dopamine concentrations. Brain Behav Immun 2012; doi: 10.1016/j.bbi.2012.10.010 [Epub ahead of print].
36. Biondi R, Ambrosio G, De Pascali F, Tritto I, Capodicasa E, Druhan LJ, et al. HPLC analysis of tetrahydrobiopterin and its pteridine derivatives using sequential electrochemical and fluorimetric detection: application to tetrahydrobiopterin autoxidation and chemical oxidation. Arch Biochem Biophys 2012;520:7–16.
37. Muntau A, Röschinger W, Habich M, Demmelmair H, Hoffmann B, Sommerhoff CP, et al. Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. N Engl J Med 2002;347:2122–32.
38. Heller R, Unbehaun A, Schellenberg B, Mayer B, Werner-Felmayer G, Werner ER. L-Ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. J Biol Chem 2001;276:40–7.
39. Schroecksnadel S, Sucher R, Kurz K, Fuchs D, Brandacher G. Influence of immunosuppressive agents on tryptophan degradation and neopterin production in human peripheral blood mononuclear cells. Transpl Immunol 2011;25:119–23.
40. Jenny M, Klieber M, Zaknun D, Schroecksnadel S, Kurz K, Ledochowski M, et al. In vitro testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors. Inflamm Res 2011;60:127–35.