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Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations

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Summary. Increased blood concentrations of phenylalanine in patients with trauma and sepsis are common but unexplained. We examined the potential relationship between serum concentrations of phenylalanine and the immune activation marker neopterin in 84 specimens of 18 patients (14 males and 4 females) post-trauma during 12–14 days of follow up. Compared to healthy controls, average phenylalanine and neopterin concentrations were elevated in patients, and there existed a positive correlation between concentrations of the two analytes ($r_s = 0.375$, $p < 0.001$). No such association existed between neopterin and tyrosine concentrations ($r_s = -0.018$), but neopterin concentrations correlated to the phenylalanine to tyrosine ratio ($r_s = 0.328$, $p = 0.001$).

Increased phenylalanine implies insufficient conversion by phenylalanine (4)-hydroxylase (PAH). Oxidative stress due to immune activation and inflammation may destroy cofactor 5,6,7,8-tetrahydrobiopterin and impair PAH activity. This assumption is further supported by the correlation found between higher neopterin concentrations and higher phenylalanine to tyrosine ratio, which estimates efficacy of PAH.

Keywords: Phenylalanine – Neopterin – Immune activation – Oxidative stress – Trauma

Introduction

The aromatic amino acid phenylalanine is essential for humans and is substrate for phenylalanine (4)-hydroxylase (PAH). PAH produces tyrosine, another important amino acid, which is precursor for the biosynthesis of DOPA and catecholamines dopamine, epinephrine, and norepinephrine (Fig. 1) (Shintaku, 2002). Increased blood levels of phenylalanine were reported in patients with HIV infection, with cancer and with trauma, sepsis, and after burns (Watanabe et al., 1984; Roth et al., 1985; Rath et al., 1987; Ollenschläger et al., 1988), in burned patients, the increase of phenylalanine was found to correlate with clinical course and to be predictive for non-survival (Chang et al., 1983; Rath et al., 1987). All the above-mentioned clinical conditions are known to be linked with inflammation and immune activation and with increased concentrations of immune activation marker neopterin, e.g., increased neopterin concentrations predict poor prognosis in patients with HIV infection, in patients with various types of malignancy and in patients after multiple trauma (Strohmaier et al., 1987; Fahey et al., 1990; Murr et al., 2002; Melichar et al., 2006).

Increased serum phenylalanine concentrations in patients are probably due to a reduced conversion rate of phenylalanine by PAH (Shintaku, 2002). This should be reflected by an increase of the phenylalanine to tyrosine ratio (phe/tyr), which can serve as an estimate of PAH activity (Anderson et al., 1994). Indeed, not only higher phenylalanine but also higher phe/tyr has been described in patients after burns to be associated with reduced survival (Chang et al., 1983; Rath et al., 1987).

In our study, the concentrations of phenylalanine and tyrosine were examined in patients post-trauma and with sepsis. Phe/tyr was calculated, and results were compared to neopterin concentrations.

Materials and methods

Patients

Eighteen patients (14 males, 4 females; aged mean ± S.D.: 45 ± 19 years, range: 20–77 years, 15 post-trauma and 3 with sepsis; apache score: 18.9 ± 6.75 range: 8–34; ISS: 39 ± 13.1, range: 18–57) were included in this study (Table 1). Samples were collected during 12–14 days of follow up every third day. In total every patient contributed 3–5 samples to the total number of 84 sera analyzed over the whole period of follow-up. All patients received standard parenteral nutrition (1700 kcal/d, 100 g amino acids/d).

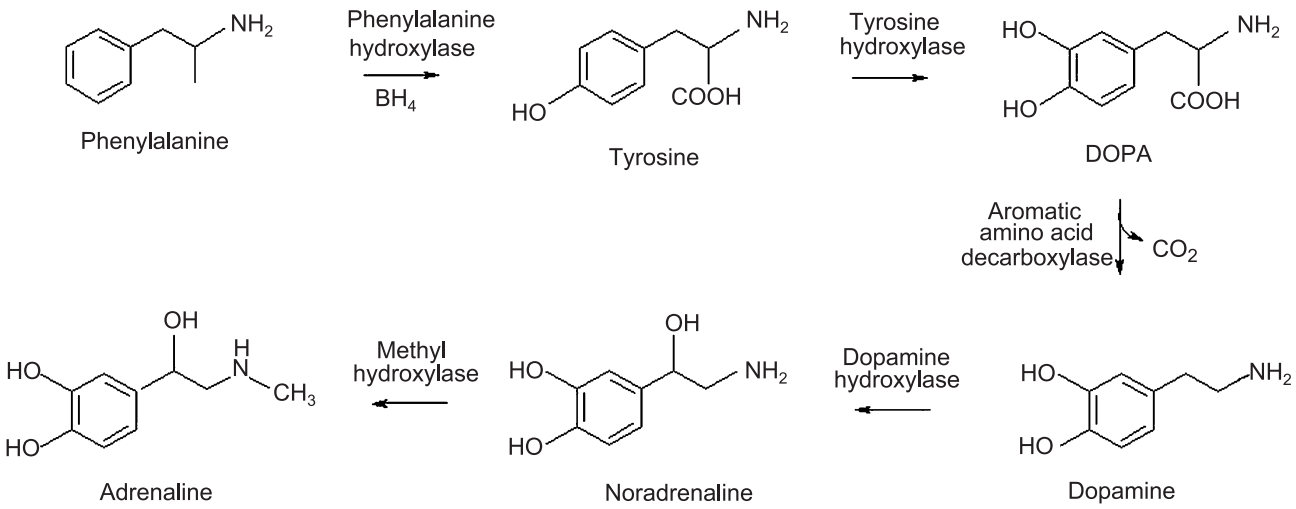

Fig. 1. Biosynthesis of tyrosine from phenylalanine by phenylalanine (5)-hydroxylase requires cofactor 5,6,7,8-tetrahydrobiopterin (BH4). Product tyrosine is further converted by tyrosine hydroxylase to DOPA, this second enzymatic step again requires cofactor BH4. Within both enzymatic reactions, BH4 is oxidized to quinonoid dihydrobiopterin and recycled (= reduced) by dihydropteridine reductase employing NADPH as hydrogen donor. Upon further conversion of DOPA, biogenic amines adrenaline and noradrenaline are produced

Table 1. Demographic data of patients

Number	18
Age (mean ± S.D.)	45 ± 19
Gender (female/male)	4/14 (22.2%/77.8%)
Diagnosis (trauma/sepsis)	15/3 (83.3%/16.7%)
Days at ICU (mean ± S.D.)	26.4 ± 19.6
Inotrop (yes/no)	17/1 (94.4%/5.6%)
Comorbidity (yes/no)	3/15 (83.3%/16.7%)
Apache II score (mean ± S.D.)	18.9 ± 6.75
ISS (mean ± S.D.)	39.0 ± 13.1
Survival (yes/no)	11/7 (61.1%/38.9%)

Assays

Concentrations of both amino acids phenylalanine and tyrosine were determined by HPLC (Ollenschläger et al., 1988). The phenylalanine to tyrosine ratio was calculated. Neopterin was measured by ELISA (BRAHMS, Hennigsdorf/Berlin, Germany) according to the manufacturer's instructions with a detection limit of 2 nM.

The observed values of all study parameters had no influence whatsoever on the course of the therapy. The protocol was approved by the local ethics committee, and written consent was granted by the next of kin.

Statistical evaluation

Because some of the parameters in this study did not show normal distribution, non-parametric Mann–Whitney *U*-test was applied for comparison of grouped data and Spearman's rank correlation coefficients were calculated. *p*-values <0.05 were considered to indicate significant differences.

Results

Absolute concentrations of phenylalanine and tyrosine were increased in the patients after trauma compared to the normal range in healthy controls (Table 2). Neopterin concentrations were increased in patients, and 67/84 (79.8%) measurements revealed concentrations above 8.7 nM, which represents the 95th percentile of healthy controls (Murr et al., 2002). Throughout follow-up, phenylalanine concentrations increased compared to day 1 ($p < 0.05$ from days 4–11; see Fig. 2).

Phenylalanine concentrations correlated with the Apache score at day 1 ($r_s = 0.494$, $p < 0.05$) but not thereafter. Tyrosine concentrations did not correlate with Apache scores throughout the study, whereas the associa-

Table 2. Concentrations of phenylalanine and tyrosine, the phenylalanine to tyrosine ratio (Phe/tyr) and neopterin concentrations (mean ± S.D., and range in brackets) in patients post-trauma (18 patients, 84 measurements) compared to the normal range in healthy controls

	Patients	Healthy controls
Phenylalanine [μM]	78.9 ± 20.5 (39.0–141)*	59.0 ± 15.0 (Roth, 1985)
Tyrosine [μM]	72.7 ± 34.3 (34.0–224)*	51.7 ± 11.3 (Roth, 1985)
Phe/tyr [μmol/mmol]	1.21 ± 0.39 (0.25–2.27)	1.16 ± 0.24 (Roth, 1985)
Neopterin [nM]	30.3 ± 36.4 (4.0–168)*	5.3 ± 2.7 (Murr, 2002)

*$p < 0.01$ compared to controls

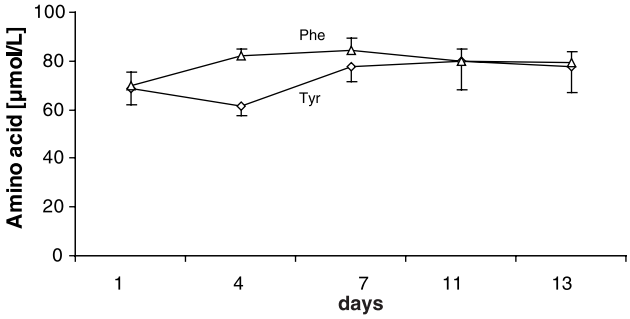

Fig. 2. Concentrations (mean ± S.E.M.) of serum phenylalanine (Phe, triangles) and tyrosine (Tyr, diamonds) in 18 patients during follow-up for 2 weeks (phenylalanine concentrations on days 4–11 were significantly higher compared to day 1; all $p < 0.05$, changes of tyrosine were not significant)

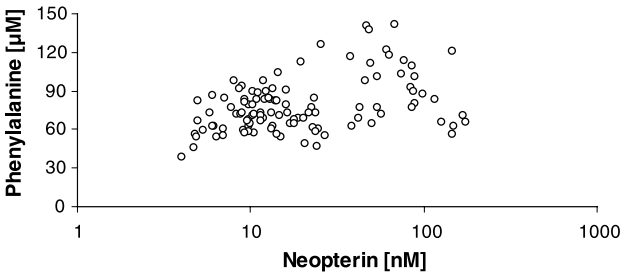

Fig. 3. Correlation between serum neopterin and phenylalanine concentrations ($r_s = 0.374$, $p < 0.001$) in 18 patients during follow-up for 2 weeks (84 specimens; note: graph shows log-scale of neopterin concentrations)

tion between Apache scores and neopterin concentrations became significant after day 4 (day 4: $r_s = 0.378$, $p = 0.068$; day 7: $r_s = 0.594$, $p < 0.01$; day 11: $r_s = 0.541$, $p < 0.05$; day 14: $r_s = 0.527$, $p < 0.05$). In the total set of measurements ($n = 84$), phenylalanine concentrations were higher in non-survivors (87.2 ± 23.1 mmol/L) compared to survivors (73.8 ± 17.0 µmol/L; $U = 2.55$, $p = 0.01$), and significantly higher phenylalanine concentrations were observed in non-survivors compared to survivors on day 1 ($p < 0.05$), later on the differences in individual days did not reach the level of statistical significance. No differences existed for tyrosine concentrations or for phe/tyr.

Significant correlation existed between phenylalanine concentrations and levels of neopterin when all results were considered ($r_s = 0.374$, $p < 0.001$; Fig. 2). Although phenylalanine and tyrosine concentrations were correlated ($r_s = 0.435$, $p < 0.001$), there was no correlation between neopterin and tyrosine ($r_s = -0.018$, n.s.). However, phe/tyr was found to correlate with neopterin concentrations as well ($r_s = 0.328$, $p = 0.001$). When comparing results of measurements on day 1 only, phenylalanine ($r_s = 0.618$) and phe/tyr ($r_s = 0.631$, both $p < 0.01$) correlated with neopterin concentrations.

Discussion

Our study confirms increased serum phenylalanine and neopterin concentrations in patients post trauma or with sepsis. A positive correlation between the serum concentrations of these two parameters was observed. There was no such association of neopterin concentrations with tyrosine levels, but a positive correlation existed between phe/tyr and neopterin concentrations.

Neopterin is produced and released in large amounts from human monocyte-derived macrophages and dendritic cells upon stimulation with Th1-type cytokine interferon-γ (Huber et al., 1984; Wirleitner et al., 2002), and thus, increased neopterin concentrations in patients indicate an activated cellular immune system. Increased neopterin concentrations are well documented during virus infections, in malignant tumor diseases and autoimmune syndromes, as well as after multiple trauma. Thereby, neopterin concentrations follow the extent, the activity and thus, the clinical course of the disease, and higher neopterin levels were found to predict disease progression in patients (Strohmaier et al., 1987; Fahey et al., 1990; Murr et al., 2002; Melichar et al., 2006). The association found between increase of phenylalanine and neopterin concentrations in our study together with the findings of increased phenylalanine levels in diseases (Watanabe et al., 1984; Roth et al., 1985; Rath et al., 1987; Ollenschläger et al., 1988), which are known to be associated with increased neopterin levels, suggests a more general relationship between immune system activation and the accumulation of phenylalanine (Widner et al., 2001). However, further studies are required to demonstrate such a general association.

In macrophages stimulated by interferon-γ, neopterin formation is paralleled by the release of toxic reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) or hypochlorous acid (HOCl) (Nathan, 1986). Neopterin itself was found to enhance oxidizing capacity of ROS (Hoffmann et al., 2003; Cirillo et al., 2007), and higher neopterin concentrations are thus often associated with, e.g., increased protein oxidation products (Witko-Sarsat et al., 1996) and the depletion of antioxidants and development of oxidative stress (Murr et al., 1999, 2007). The chronic immune system activation appears to be a major cause of the loss of antioxidants and the development of oxidative stress, e.g., subnormal concentrations of antioxidants, like vitamin C and E, have been observed in diseases associated with immune activation and inflam-

mation (Halliwell, 1991; Galli et al., 2005), and it could also lead to an impaired availability of BH_4 in patients post trauma and with sepsis.

Increase of essential amino acid phenylalanine is likely a result from impaired conversion of phenylalanine to tyrosine by PAH (Ponzone et al., 1993; Anderson et al., 1994; Shintaku, 2002). Phe/tyr allows to estimate the activity of PAH (Anderson et al., 1994; Hoekstra et al., 2002). Accordingly, the correlation between phe/tyr and neopterin concentrations suggests that impaired PAH activity in our patients is related to immune activation. An impaired PAH activity could be due to insufficient supply with cofactor 5,6,7,8-tetrahydrobiopterin (BH_4) which is required as a hydrogen donator within the enzymatic hydroxylation reaction (Fig. 1). BH_4 is very sensitive to oxidative stress, and its reaction with oxygene is irreversible because the side-chain of the pteridine structure is eliminated (Connor et al., 1979). One might speculate that in the situation of oxidative stress, depletion of BH_4 will occur, and in turn, BH_4-dependent enzymes like PAH may suffer from insufficient cofactor supply (Widner et al., 2001). In case of PAH deficiency, concentration of phenylalanine is supposed to increase. Notably, PAH-product tyrosine itself is substrate for a subsequent enzymatic reaction: tyrosine hydroxylase forms DOPA and represents another BH_4-dependent enzyme (Fig. 1). This fact may explain why also tyrosine concentrations were increased in our patients compared to control values. As a sum effect, BH_4 deficiency results in impaired production of DOPA and neurotransmitters like dopamine, epinephrine and norepinephrine (Shintaku, 2002).

Antioxidant vitamins like vitamin C (ascorbic acid), vitamin E (α-tocopherol) and carotenoides are known to stabilize BH_4 (Martinez-Cruz et al., 2002; Kuzkaya et al., 2003; Heller et al., 2004). It would be interesting to know whether supplementation with antioxidant vitamins is able to normalize the elevated phenylalanine concentrations in patients post trauma.

In conclusion, our study demonstrates an association between increased phenylalanine and neopterin concentrations post trauma. Although a correlation found does not necessarily reflect a cause-effect relationship, our data could be explained by a decline of PAH activity in patients as a consequence of oxidative stress.

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