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Immune activation and inflammation in patients with cardiovascular disease are associated with elevated phenylalanine-to-tyrosine ratios

Abstract: Higher serum neopterin concentrations and kynurenine-to-tryptophan (Kyn/Trp) ratios are associated with increased mortality in patients with coronary artery disease (CAD). Preferentially, Th1-type cytokine interferon-γ stimulates tryptophan breakdown and neopterin production by GTP cyclohydrolase I (GCH-I) in parallel in monocyte-derived macrophages and dendritic cells. In other cells, activation of GCH-I leads to the formation of 5,6,7,8-tetrahydrobiopterin (BH4), the necessary cofactor of amino acid hydroxylases such as phenylalanine 4-hydroxylase (PAH) and nitric oxide synthases. In 31 CAD patients (70.3±9.9 years; 21 males, 10 females), we determined serum concentrations of phenylalanine, tyrosine, and Kyn/Trp by HPLC, neopterin by ELISA, and nitrite by the colorimetric Griess assay. The phenylalanine-to-tyrosine ratio (Phe/Tyr) served as an estimate of PAH enzyme activity. Elevated Phe/Tyr concentrations were detected in a subgroup of CAD patients and correlated with Kyn/Trp ($r=0.396$, $p<0.05$) and neopterin ($r=0.354$, $p<0.05$) and inversely with nitrite ($r=-0.371$, $p<0.05$) concentrations. Higher Phe/Tyr in patients is associated with immune activation and indicates subnormal PAH activity that might be involved in the precipitation of neuropsychiatric symptoms in CAD patients.

Keywords: coronary artery disease; neopterin; phenylalanine; phenylalanine hydroxylase; tetrahydrobiopterin.

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Introduction

The development and progression of coronary artery disease (CAD) is closely associated with signs of immune activation and inflammation. Thereby, activated macrophages and the proinflammatory cytokine interferon-γ (IFN-γ) appear to play a major role [1]. In monocyte-derived macrophages and dendritic cells, IFN-γ triggers GTP cyclohydrolase I, the key enzyme for the biosynthesis of pteridine derivatives such as neopterin and 5,6,7,8-tetrahydrobioptein (BH4) [2, 3]. Accordingly, an elevated neopterin concentration is a sensitive indicator of Th1-type immune response. In parallel to GTP cyclohydrolase I, IFN-γ also stimulates tryptophan breakdown rate by the enzyme indoleamine 2,3-dioxygenase [3, 4]. The close association found between elevated serum neopterin concentrations and a higher tryptophan breakdown index, the kynurenine-to-tryptophan ratio (Kyn/Trp), in CAD patients underlines this relationship [5]. Moreover, a strong prognostic value for cardiovascular and total mortality is well documented for neopterin and Kyn/Trp concentrations [6–10].

It has been indicated that inflammation and immune activation also disturb the conversion of phenylalanine to tyrosine: in patients suffering from clinical conditions, which is in agreement with immune activation and inflammation such as sepsis, cancer, or HIV-1 infection, but also in the healthy elderly, increased phenylalanine and an increased phenylalanine-to-tyrosine (Phe/Tyr) concentrations have been described correlating with markers of immune activation such as neopterin [11–14].

In this pilot study, we determined serum concentrations of phenylalanine and tyrosine as well as the Phe/Tyr ratios in CAD patients and compared results to concentrations of neopterin, Kyn/Trp, and nitrite.

Patients and methods

Thirty-one patients (21 males, 10 females), aged 70.0±11.9 years (males, 67.7±13.2 years; females, 74.9±6.7 years), with coronary heart

disease confirmed by coronary angiography were included in this study. Patients were recruited from the Practice for General Internal Medicine (Dr. W.J. Schnedl, Bruck/Mur, Austria). Ten patients (4 females, 6 males) had coronary artery stenosis with no intervention, 13 patients (3 females, 10 males) were after stent implantation, and eight patients (3 females, 5 males) were after coronary artery bypass graft surgery. Patients received several medications, mainly statins, antihypertensive and antidiabetic drugs according to current treatment guidelines for coronary heart disease [15]. Sixteen patients (52%) presented with a body mass index (BMI) >30 kg/m^2, and 15 patients (48%) had a systolic blood pressure >135 mm Hg. Twenty patients suffered from diabetes mellitus type 2 with blood fasting glucose concentrations >100 mg/dL.

Concentrations of phenylalanine, tyrosine, tryptophan, and kynurenine were assayed by HPLC as described earlier [16, 17]. Phe/Tyr was calculated to estimate activity of enzyme phenylalanine hydroxylase (PAH) [11, 18, 19], and Kyn/Trp indicated the rate of tryptophan breakdown [5]. Neopterin concentrations were quantified by commercially available ELISA (BRAHMS-Thermo Fisher, Hennigsdorf, Germany). Nitrite concentrations were determined colorimetrically utilizing the Griess reaction [20]. Other standard laboratory markers such as total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, as well as triglycerides, glucose, and urate concentrations were measured by routine laboratory diagnostic methods.

The study was in accordance with the Helsinki declaration, and all individuals included gave informed consent that results of additional measurements were solely used for scientific purposes in a blinded manner. All laboratory measurements were performed in a blinded manner without knowledge about the diagnoses and disease status of patients.

Concentrations of analytes were given as mean values±SD. For comparison of mean values of grouped data, Student's test was applied, and associations between parameters were tested by linear correlation analysis. The Statistical Package for the Social Sciences (PASW Statistics 18, Chicago, IL, USA) was used. A p-value <0.05 was considered to indicate statistically significant differences and correlations.

Results

Concentrations of markers are shown in Table 1. There was no significant influence of gender. Twenty patients (47%) presented with phenylalanine concentrations above the upper limit of normal in middle-aged healthy individuals which is 75 µmol/L [21], 12 patients (28%) had Phe/Tyr >1.15, the cut-off value previously considered to be useful [22]. Phe/Tyr correlated with age (r=0.391), with neopterin concentrations (r=0.354) and with Kyn/Trp (r=0.396, all p<0.05; Figure 1). Further, neopterin concentrations correlated with Kyn/Trp (r=0.614, p<0.001; Figure 2) and inversely with nitrite concentrations (r=−0.371, p<0.05).

When only the subgroup of males (n=21) was considered, the associations between marker concentrations were similar: Phe/Tyr correlated with age (r=0.426,

Table 1 Results of laboratory measurements in 31 patients suffering from cardiovascular disease and split into two groups by gender (mean values±SD are shown; all differences between males and females are non-significant).

	All (n=31)	Females (n=10)	Males (n=21)
Age, years	70±11.9	74.9±6.7	67.7±13.2
Tyrosine, µmol/L	69.6±13.4	75.2±10.4	66.9±14.0
Phenylalanine, µmol/L	76.9±15.2	84.4±16.9	73.3±13.3
Phe/Tyr, µmol/µmol	1.12±0.23	1.13±0.19	1.12±0.25
Nitrite, µmol/L	14.1±13.5	14.7±9.77	13.8±15.2
Neopterin, nmol/L	10.1±7.21	8.42±4.90	10.9±8.01
Tryptophan, µmol/L	57.4±10.2	53.5±7.99	59.3±10.8
Kynurenine, µmol/L	3.25±1.05	3.49±1.51	3.13±0.75
Kyn/Trp, mmol/mol	58.5±22.8	65.9±31.1	55.0±17.6
Total cholesterol, mg/dL	169±41.2	179±45.6	164±39.2
HDL-cholesterol, mg/dL	49.7±14.7	53.5±19.6	47.8±11.8
LDL-cholesterol, mg/dL	93.0±35.4	93.6±36.0	92.8±36.0
Triglycerides, mg/dL	132±55.0	160±66.5	118±44.4
Urate, mg/dL	6.38±1.40	6.21±1.46	6.46±1.40
Glucose, mg/dL	118±35.1	134±51.5	111±22.1
Body mass index, kg/m^2	30.1±4.36	31.1±4.84	29.7±4.15
Blood pressure systolic, mm Hg	137±19.8	135±22.9	137±18.7
Blood pressure diastolic, mm Hg	78.2±10.8	79.5±12.4	77.6±10.2

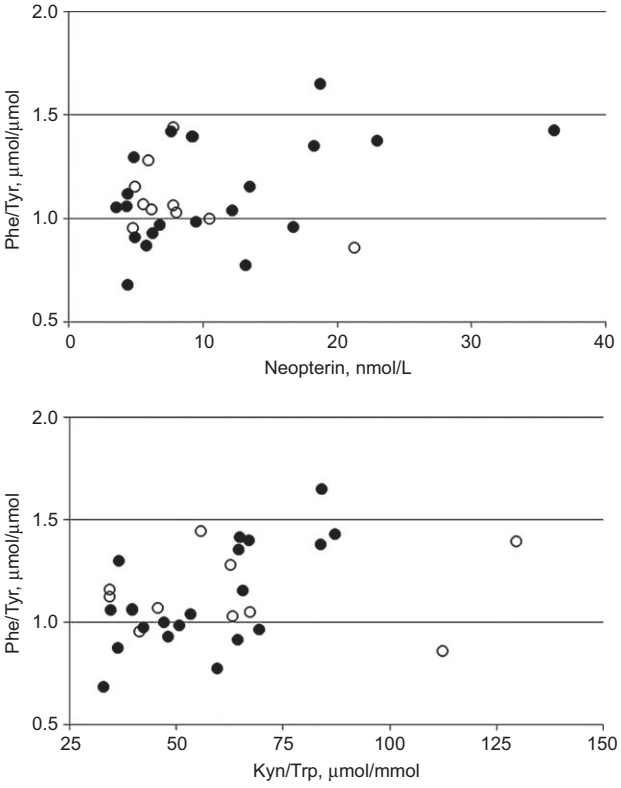

Figure 1 Associations of serum phenylalanine-to-tyrosine ratios (Phe/Tyr) with neopterin concentrations (r=0.354, p<0.05; upper graph) and with kynurenine-to-tryptophan ratios (Kyn/Trp) (r=0.396, p<0.05; lower graph) in 31 patients with coronary artery disease (open circles, 10 females; filled circles, 21 males).

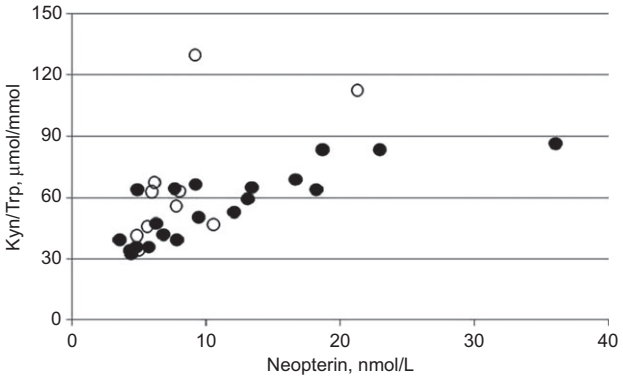

Figure 2 Association between serum kynurenine-to-tryptophan ratios (Kyn/Trp) and neopterin concentrations (r=0.617, p<0.01) in 31 patients with coronary artery disease (open circles, 10 females; filled circles, 21 males).

p<0.05), neopterin concentrations (r=0.518, p<0.02), and Kyn/Trp (r=0.617, p<0.01). Moreover, neopterin concentrations correlated with Kyn/Trp (r=0.826, p<0.001) and inversely with nitrite concentrations (r=−0.490, p<0.07). There were no significant relationships between absolute phenylalanine concentrations and any concentrations of the analytes.

Discussion

In patients with CAD, we report associations between higher Phe/Tyr and higher neopterin and Kyn/Trp concentrations, both the latter variables indicating immune activation. The positive correlation found between neopterin and Kyn/Trp is in agreement with their close immunobiological background, namely, Th1-type immune activation, and their association with higher Phe/Tyr thus indicates that a disturbed PAH functional activity may be related to immune activation. Similar positive relationships between Phe/Tyr and immune activation markers have been described earlier in patients suffering from different diseases in which immune activation plays a major role and is associated with adverse outcome such as ovarian carcinoma, HIV-1 infection, and also in patients after trauma and with sepsis [12, 13, 17]. Taking all results together, it seems fairly reasonable that immune activation leads to the increase of Phe/Tyr in CAD patients.

According to the literature, alterations of phenylalanine and of Phe/Tyr could be involved in a wide spectrum of neuropsychiatric abnormalities in patients [11, 14, 23, 24]. Previously in healthy elderly individuals, increased phenylalanine concentrations and increased Phe/Tyr have been found to be correlated with scores for depressive symptoms such as Montgomery-Asberg Depression Rating Scale (MADRS) or with a fatigue inventory or with a neurotoxicity rating scale (NRS) [14]. Additionally, in patients with HCV infection under treatment with IFN-α a significant increase of blood Phe/Tyr and phenylalanine concentrations was reported [25, 26]. Moreover, in the same patients, an association between disturbed phenylalanine metabolism and low dopamine concentrations in the cerebrospinal fluid was evident and disturbances are correlated with fatigue scores [24]. Unfortunately, neuropsychiatric test results were not available from our patients, and further studies are to be conducted to address this relationship in CAD patients.

The increase of Phe/Tyr may be due to impaired function of PAH that could result from BH4 deficiency. However, during inflammatory processes involving increased formation of IFN-γ, GTP cyclohydrolase I is induced and primarily leads to the formation of BH4. Only in humans and primates, but not in cells from other species, significant production of neopterin is detected in cells of the monocytes-macrophages lineage, whereas in other cells and in other species only the biosynthesis of BH4 is of relevance [27]. BH4, the necessary cofactor of several monooxygenases of amino acids including PAH [28], nitric oxide (NO) synthases (NOS) [29] and of glyceryl ether monoxygenase [30], is released in increased amounts from, for example, endothelial cells. Consequently, production of vasodilatory NO increases during acute immune activation processes and blood pressure falls. However, in CAD patients a paradox seems to exist because patients show signs of vasoconstriction despite their proinflammatory status [31, 32]. Therefore, supplementation of patients with BH4 is one current strategy to treat increased blood pressure, however, thus far with only very limited success [32]. Although the direct measurement of BH4 in the blood of patients is technically feasible [33], preanalytical requirements are complex and thus not easily applicable in clinical studies. Instead, measurement of phenylalanine and tyrosine and calculating Phe/Tyr may overcome this limitation and can serve as a surrogate of BH4. The measurement of Phe/Tyr could lead to a better understanding of BH4 biochemistry in patients, because any deficiency of BH4 should cause an increase of Phe/Tyr also in patients with normal genetic background of PAH [11].

When BH4 availability is disturbed, it should also affect other BH4-dependent enzymatic pathways such as NOS activity for which cofactor BH4 is an important regulator of functional activity and is required to maintain enzymatic coupling of L-arginine oxidation to produce NO [31, 32]. Loss or oxidation of BH4 to 7,8-dihydrobiopterin (BH2) is associated with NOS uncoupling that results in

the production of vasoconstrictor superoxide anion ($O_2^{\bullet-}$, hyperoxide anion) at the expense of vasodilatory NO [32]. Thus, BH4 deficiency not only impairs PAH activity and increases Phe/Tyr but at the same time diminishes NO production. Our study shows some weak inverse association between Phe/Tyr and nitrite concentrations which also points towards BH4 deficiency. It should be noted that nitrite concentrations can only serve as a rough estimate of NO production rates, but at least our conclusion is further supported by the inverse association between nitrite and neopterin concentrations and Kyn/Trp. Also, ample evidence already exists which indicates that NO production is impaired in CAD patients even though immune activation and IFN-γ production would induce inducible NOS (iNOS) gene expression [34]. This conclusion is in line with increased blood pressure often seen in patients, most probably due to vasoconstriction rather than vasodilation. Thus, our findings may help to explain the existing paradox that particular chronic inflammatory conditions such as CAD are in line with high rather than low blood pressure.

In summary, our study provides preliminary data that patients with CAD and higher serum neopterin and Kyn/Trp levels may exhibit higher Phe/Tyr ratios; a finding which is most probably due to impaired PAH activity. However, such results need to be confirmed by a larger sized investigation and also a comparison of CAD patients with healthy controls of the same age. Still, our data indicate disturbed phenylalanine metabolism in CAD patients, which could possibly be associated with neuropsychiatric disturbances because phenylalanine metabolism is closely related to neurotransmitter biosynthesis. Thus, further studies may shed more light on the background of neuropsychiatric disturbances in CAD patients. Finally, in the absence of an easy routine assessment of BH4 concentrations in patients, measurements of Phe/Tyr may allow some indirect information on the availability of BH4 and the functional activity of BH4-dependent enzymes including NOS.

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