Plasma pteridine concentrations in patients with chronic renal failure

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Abstract

Background. Pteridine metabolism is impaired in the uraemic state. This may affect cardiovascular function and contribute to malnutrition. We wished to clarify further the impact of impaired pteridine metabolism.

Methods. Using the HPLC method, the plasma concentrations of endogenous pteridines were determined in 64 patients with chronic renal failure (33 on intermittent haemodialysis (HD) treatment vs 31 not yet on renal replacement therapy), and in 18 healthy controls. The patients were classified into three groups on the basis of creatinine clearance (Ccr): group (a), Ccr > 60 ml/min; group (b), Ccre = 10–60 ml/min; group (c), all patients receiving HD.

Results. Total neopterin (NP) and biopterin (BP) levels and the NP:BP ratio (a biomarker for macrophage activity) were significantly higher, whereas tetrahydrobiopterin (BH4) dihydrobiopterin (BH2) ratio (a biomarker for nitric oxide synthase and phenylalanine hydroxylase activities) was significantly lower in group (c) (118.9 ± 11.7 ng/ml, 18.8 ± 1.2 ng/ml, 6.79 ± 0.53, and 0.26 ± 0.06) than in healthy subjects (5.17 ± 0.29 ng/ml, 2.83 ± 0.19 ng/ml, 1.92 ± 0.13, and 1.15 ± 0.11; P < 0.01). These significant differences were also observed between control and group (b) (12.4 ± 2.20 ng/ml, 4.48 ± 0.36 ng/ml, 2.81 ± 0.48, and 0.74 ± 0.08; P < 0.01). In groups (a) and (b), significant negative correlations were found between Ccr and the total NP level (r = −0.663, P < 0.01), the total BP level (r = −0.492, P < 0.01), the BH2 level (r = −0.677, P < 0.01), and the NP:BP ratio (r = −0.493, P < 0.01). Conversely, significant positive correlations were found between Ccr and the BH4:BH2 ratio (r = 0.602, P < 0.01).

Conclusion. The reduction of quinoid-type BH2 to BH4 is modified in patients with advanced chronic renal failure, before and after the initiation of regular HD treatment. These metabolic alterations may play a role in the impaired macrophage, endothelial constitutive nitric oxide synthase, or phenylalanine hydroxylase (PH) activities observed in such patients.

Keywords: chronic renal failure; dihydrobiopterin; NADH; nitric oxide; superoxide; tetrabiopterin

Introduction

The major complications for patients on haemodialysis (HD) are cardiovascular diseases and malnutrition, which often determine their mortality [1–5]. Cardiovascular diseases are known to be related to the acceleration of inflammation that may cause the nutritional state to deteriorate in these patients. Therefore, many studies have focused on the relationship between inflammatory reactions and malnutrition in HD patients, although the exact mechanism has not been elucidated.

Tetrahydrobiopterin (BH4), a substance belonging to the pteridine group, is very important for the role it plays in the metabolism of some amino acids because it acts as a coenzyme, such as the ones for phenylalanine hydroxylase (PH), tyrosine hydroxylase, and other enzymes (Figure 1) [6]. Recently, in addition to the role for amino acid metabolism, it has been reported that BH4 is related to the control of the endothelium-dependent vascular function by supporting nitric oxide (NO) production as a coenzyme of NO synthase (NOS).

In the absence of BH4 activation, the plasma levels of phenylalanine can be elevated, and active oxygen might be generated in quantities greater than that released by NO because of the oxidase activity of NOS, which activates an inflammatory reaction [7–9]. In animals, it has been reported that the impairment of BH4 causes a reduction in the activity of endothelium-derived NO [10]. This may suggest that BH4 metabolism is adversely affected in a uraemic state, because it has been reported that NO production...
and phenylalanine metabolism are impaired, and the inflammation reaction takes place in such a state.

In this study, pteridine concentrations were determined in patients with chronic renal failure to prove the relationship of pteridine metabolism to the state of renal impairment and the possible role of pteridines in cardiovascular diseases and malnutrition in these patients.

Subjects and methods

Patients and control subjects

Our subjects included 33 patients receiving haemodialysis and 31 patients with less severe chronic renal failure who were not receiving haemodialysis. As an index of renal function, the creatinine clearance (Ccr) was determined in CRF patients without HD and compared with BH₄ levels. The patients were classified into three groups on the basis of Ccr (group (a) Ccr > 60 ml/min, and group (b) Ccr = 10–60 ml/min). All patients receiving haemodialysis were in group (c) (Table 1). The primary diagnoses were chronic glomerular nephritis and renal sclerosis in haemodialysis patients and chronic glomerular nephritis in non-haemodialysis patients. No patients had diabetic nephropathy. Diabetes mellitus is known to involve other factors that regulate endothelial constitutive nitric oxide synthase activity. Our subjects had received no previous treatment with nitrate, angiotensin-converting enzyme inhibitor, or angiotensin II antagonist. Eighteen healthy persons (mean age 55.2 ± 3.90 years) were also recruited as control subjects. The study protocol was approved by the research and ethics committee of our institution.

Blood samples

Blood samples were collected in the outpatient clinic from non-HD patients and before dialysis from HD patients in the morning, because glucocorticoids, which regulate the levels of BH₄, are highest in the morning [11].

Measurement of pteridines

Levels of neopterines (NPs) and biopterines (BPs) in plasma were measured with a modification of the high-performance liquid chromatography method of Fukushima and Nixon [12]. The concentration of reduced BP, i.e. BH₄, was calculated from the total BP concentration (which equals concentrations of the reduced type plus the oxidized type) and the concentration of oxidized BP. Also, the ratio of the total NP to total BP (NP/BP) was calculated. Blood samples were collected immediately before dialysis or during outpatient consultation.

Measurements of plasma levels of Phe and Tyr

The ratio of Tyr to Phe (Tyr/Phe) was determined by measuring levels of Phe and Tyr in the plasma with the high-performance liquid chromatography–ninhydrine luminescence method in both groups of patients.

Statistical methods

Data are expressed as the means ± standard error of the mean (SEM). The two-sample t-test with Welch’s correction and the Mann–Whitney test were used to analyse differences between groups. Pearson correlations was used to analyse data for correlations. All calculations were made using StatView v.4.5 for Macintosh (Abacus Concepts, Berkeley, CA, USA). Differences were considered to be significant when P < 0.05.

Results

Serum levels of total NP and of total BP, and levels of BH₂ were significantly higher in group (c) than in groups (a), (b), or the control group. Furthermore, these same variables were significantly higher in

Table 1. Patient profiles

<table>
<thead>
<tr>
<th></th>
<th>Healthy control</th>
<th>Group (a)</th>
<th>Group (b)</th>
<th>Group (c) (haemodialysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>15</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Age (mean ± SEM)</td>
<td>55.2 ± 3.90</td>
<td>44.5 ± 3.74</td>
<td>58.5 ± 3.08</td>
<td>59.6 ± 1.74</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>11:7</td>
<td>9:6</td>
<td>11:5</td>
<td>22:11</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>78.1 ± 2.32</td>
<td>36.5 ± 3.38</td>
<td>(&gt; 60.0)</td>
<td>(10.0–60.0)</td>
</tr>
</tbody>
</table>

Ccr, creatinine clearance.

Fig. 1. Pteridine metabolism. BH₄ is generated from GTP through the enzymes GTP cyclohydrolase I (GTPCH I) and PTPS. BH₄ acts as a coenzyme of PH during transformation of Phe to Tyr and is itself oxidized and transformed to BH₂ of the quinoid type. BH₄ is also produced by the reduction of quinonoid-type BH₂ by DHPR. This recycling reaction of BH₄ includes oxidation and reduction reactions of BH₂; BH₄ is the reduced form, and BP and BH₂ are oxidized forms. When GTP is converted to 7,8-dihydrobiopterin triphosphate (BH₃P) but bypasses PTPS, it becomes NP.

Table 1. Patient profiles

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</tbody>
</table>

Ccr, creatinine clearance.
Tyr Ccr and the Tyr u

Significant positive correlations were found between Ccr and the BH4 (1034 K. Yokoyama increased, and the BH4 upteridines were investigated in patients with renal failure.

The plasma levels of pteridines in patients with renal failure were compared with healthy controls.

In order to investigate the relations between our results and renal function, the levels of pteridine and Ccr were scattered in patients with renal failure not on dialysis (group a) and group b, or the control group. The BH4/BH2 ratio was significantly lower in group c than in group a or the control group. None of these variables differed significantly between group a and the control group. After dialysis in group c, the levels of total BP and BH4 had significantly decreased, but the BH2 ratio was unchanged (Table 3). BH4 levels after HD (1.1 ± 0.4 ng/ml) were lower than those in healthy subjects (1.49 ± 0.15 ng/ml). There were no significant differences between before HD and before the next HD.

In order to investigate the relations between our results and renal function, the levels of pteridine and Ccr were scattered in patients with renal failure not on dialysis (group a) and group b; n = 31). Significant negative correlations were found between Ccr and the total NP level (r = −0.663, P < 0.01), the total BP level (r = −0.492, P < 0.01), the BH2 level (r = −0.677, P < 0.01), and the NP/BP ratio (r = −0.493, P < 0.01). Conversely, significant positive correlations were found between Ccr and the BH4/BH2 ratio (r = 0.602, P < 0.01) (Figure 2).

Levels of Thr and the Tyr/Phe ratio were significantly lower in patients receiving HD (38.9 ± 3.23 ng/ml and 0.59 ± 0.02) than in control subjects (66.4 ± 6.03 ng/ml, P = 0.0002; 0.99 ± 0.08, P < 0.0001), but levels of Phe did not differ significantly (Table 4). Significant positive correlations were found between Ccr and the Tyr/Phe ratio (P = 0.62, P < 0.05). The Tyr/Phe ratio and the BH4/BH2 ratio were positively correlated (r = 0.719, P < 0.0001; Figure 3).

Discussion

In the present study, the actions of endogenous pteridines were investigated in patients with renal impairment and those on HD. When on HD, the total plasma levels of NP and BP and the NP/BP ratio increased, and the BH4/BH2 ratio decreased significantly. In addition, Ccr was negatively correlated with the NP level and the NP/BP ratio was positively correlated with the BH4/BH2 ratio. The results showed that the dialysate contents of NP, BP, BH3, and BH4 were similar. These substances had a reduction rate of about 70%; however, the amount of generation until the next HD session was six times higher in NP than in BP, although their molecular weights were similar.

The BH4/BH2 ratio in patients receiving HD decreased significantly. As the demand for BH4 increases, BH4 is required through a reduction of oxidized quinonoid BH3 in addition to its synthesis from guanosine triphosphate (GTP) [6]. However, the fact that the level of the reduced form of BH4 is lower than those of the oxidized forms of BP and BH2 in renal failure may suggest that there is a decreased reduction of dihydropteridine reductase (DHPR) during the conversion from quinonoid BH3 to BH4.

To investigate this possibility, the DHPR activity in blood cells was determined after adding sufficient quantities of NADH. Because there was no difference in the DHPR activity between HD patients and healthy subjects, it was decided that such a change in the state of reduction might be involved in the pathophysiology of renal failure. Under oxidative stress, BH4 acts as a radical scavenger similar to ascorbic acid by being oxidized to BH2 Miyata et al. [13] reported that the degree of ascorbic acid oxidation in dialysed patients was twice as high as it was in normal subjects.

In our study, there was no difference between those patients with a renal dysfunction and healthy subjects in their BH4 level. However, it has been reported that BH2 inhibits biological activity by competitive binding with endothelial constitutive NOS (eNOS) [14]. Therefore, in patients with renal failure, although BH4 levels in the plasma did not change when the

**Table 2. The plasma levels of pteridines in patients with renal failure**

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Group (a)</th>
<th>Group (b)</th>
<th>Group (c) (haemodialysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NP (ng/ml)</td>
<td>5.17 ± 0.29</td>
<td>6.21 ± 0.49</td>
<td>12.4 ± 2.20</td>
<td>118.9 ± 11.7</td>
</tr>
<tr>
<td>Total BP (ng/ml)</td>
<td>2.83 ± 0.19</td>
<td>3.32 ± 0.19</td>
<td>4.48 ± 0.36</td>
<td>18.8 ± 1.2</td>
</tr>
<tr>
<td>BH2 (ng/ml)</td>
<td>1.33 ± 0.07</td>
<td>1.53 ± 0.10</td>
<td>2.67 ± 0.25</td>
<td>15.2 ± 0.98</td>
</tr>
<tr>
<td>BH4 (ng/ml)</td>
<td>1.49 ± 0.15</td>
<td>1.79 ± 0.15</td>
<td>1.81 ± 0.19</td>
<td>3.70 ± 0.85</td>
</tr>
<tr>
<td>Total NP/Total BP</td>
<td>1.92 ± 0.13</td>
<td>1.95 ± 0.20</td>
<td>2.81 ± 0.48</td>
<td>6.79 ± 0.53</td>
</tr>
<tr>
<td>BH4/BH2</td>
<td>1.15 ± 0.11</td>
<td>1.23 ± 0.12</td>
<td>0.74 ± 0.08</td>
<td>0.26 ± 0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Before HD</th>
<th>After HD</th>
<th>Before/next HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NP (ng/ml)</td>
<td>118 ± 12.7</td>
<td>45.5 ± 5.3</td>
<td>101 ± 12.0</td>
</tr>
<tr>
<td>Total BP (ng/ml)</td>
<td>19.3 ± 2.2</td>
<td>6.6 ± 0.6</td>
<td>16.9 ± 2.0</td>
</tr>
<tr>
<td>BH2 (ng/ml)</td>
<td>16.1 ± 1.9</td>
<td>5.5 ± 0.71</td>
<td>12.8 ± 1.9</td>
</tr>
<tr>
<td>BH3 (ng/ml)</td>
<td>3.1 ± 1.7</td>
<td>1.1 ± 0.4</td>
<td>3.4 ± 1.8</td>
</tr>
</tbody>
</table>
renal function was compromised, BH₄ was relatively low because the elevation of BH₂ resulted in a decrease in NO production [15]. From their amino-acid profile, our results showed a BH₄ deficiency in patients with renal failure. In fact, the decreased Tyr/Phe ratio indicates that the conversion of Phe to Tyr by PH also decreased. The decreased Tyr/Phe ratio before HD suggests that the BH₄ deficiency also affects amino-acid metabolism. Moreover, the Tyr/Phe and BH₄/BH₂ ratios were positively correlated \((r=0.719, P<0.0001)\).

It has been suggested that in patients with renal failure there is a relative deficiency in NO production by eNOS. Guanidino compounds, such as asymmetric dimethyl-L-arginine, accumulate in the blood of patients with chronic renal failure, inhibit eNOS, and suppress endothelium-dependent vascular dilatation [16,17]. Our results suggest that a BH₄ deficiency inhibits NOS. Kakoki et al. [10] reported that impairment of dimerization of eNOS by depleting BH₄ influences the decreased activity of endothelium-derived NO because BH₄ stabilizes eNOS in its dimeric form, which is an active form.

### Table 4. Serum levels of Tyr and Phe in patients with renal failure

<table>
<thead>
<tr>
<th></th>
<th>Healthy control</th>
<th>Haemodialysis patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr (ng/ml)</td>
<td>66.4 ± 6.03</td>
<td>38.9 ± 3.23</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Phe (ng/ml)</td>
<td>69.0 ± 7.56</td>
<td>65.2 ± 4.53</td>
<td>NS</td>
</tr>
<tr>
<td>Tyr/Phe</td>
<td>0.99 ± 0.08</td>
<td>0.59 ± 0.02</td>
<td>&lt;0.0001</td>
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</table>

### Fig. 2. The relationship between plasma levels of BH₄s and Ccr.

- Upper left: The total NP level was negatively correlated with Ccr \((r=−0.63, P<0.001)\).
- Upper right: Similarly, the NP/BP ratio was negative correlated with Ccr \((r=−0.53, P<0.001)\).
- Lower left: BH₂ was negatively correlated with Ccr \((r=−0.59, P<0.001)\).
- Lower right: The BH₄/BH₂ ratio was positively correlated with Ccr \((r=0.62, P<0.001)\).

### Fig. 3. The relationship between the BH₄/BH₂ ratio and the Tyr/Phe ratio.

The Tyr/Phe ratio and the BH₄/BH₂ ratio, which reflects BH₄ activity, were positively correlated \((r=0.719, P<0.0001)\).
When BH₄ is deficient, NOS shows only oxidase activity: thus active oxygen (O₂⁻) is generated in greater quantities than NO [18]. Consequently, inflammation increases because the macrophages and monocytes are stimulated, which in turn stimulate inducible NOS (iNOS) synthesis. In patients with renal failure, although iNOS synthesis is increased when macrophages and monocytes are stimulated, the BH₄ level undergoes a relative reduction. Thus, the unbalanced iNOS and eNOS concentrations in those with renal failure may be closely related to the disorder of pteridine metabolism.

It was reported that the expression of iNOS, eNOS, and peroxynitrite-modified proteins in experimental anti-myeloperoxidase is associated with crescentic glomerulonephritis [19]. In our study the NP/BP ratio increased in patients with renal failure, which suggests that O₂⁻ is generated as a consequence of a deficiency of BH₄. Once macrophages have been activated, BP is not generated; but NP production increases after the phosphorylation of GTP because the 6-pyruvoyl tetrahydropterin synthase (PTPS) activity is low in macrophages and monocytes [20,21].

Our results suggest that the macrophages are activated in patients with renal failure. Therefore one may speculate that the NP level is a clinically significant index of both renal function and macrophage activity. Godai et al. [22] also reported that serum NP levels and serum creatinine concentration were positively correlated. In addition and of interest, it was reported that higher levels of serum NP serve as a marker for the progression of diabetic nephropathy [23]. It is important that the relationship between a deficiency in BH₄ and superoxide impairment with renal failure be clarified. Further study is needed here.

In summary, it has been shown that, in patients with chronic renal failure and on dialysis, the BH₄/BH₂ ratio decreases and the Phe/Tyr ratio increases. This finding suggests that the reduction of quinonoid-type BH₂ is decreased, which plays a major role in causing impairment in NO production and phenylalanine metabolism. The disorder of pteridine metabolism in patients with renal failure may hold a key to the pathophysiology of cardiovascular diseases and malnutrition.

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References


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