Abnormal cyanide metabolism in uraemic patients

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Abstract

Background. We previously investigated the factors involved in uraemic neuropathy in patients undergoing regular haemodialysis and found a significant relationship between the severity of vibration sensation impairment and the patients’ smoking habits. The administration of methylcobalamin markedly improved the severity of uraemic neuropathy in terms of vibration perception thresholds. We presumed that abnormal cyanide metabolism is involved in the development of uraemic neuropathy.

Methods. Serum levels of thiocyanate (SCN⁻), the detoxication product of cyanide, were determined in 12 patients with preterminal chronic renal failure (PCRF), 30 patients undergoing regular haemodialysis (HD patients), and 13 healthy volunteers as a control group. Nine of the 30 HD patients were smokers. In addition, in 10 HD patients without smoking habits and 10 non-smoking healthy volunteers, the proportion of each vitamin B₁₂ analogue in total vitamin B₁₂ was estimated.

Results. The mean serum SCN⁻ level of the 12 PCRF patients (5.1 ± 1.5 µg/ml) was significantly higher than that of the control (2.8 ± 0.9 µg/ml) (P<0.01). The mean SCN⁻ level before haemodialysis in the 21 non-smoking HD patients was identical to that in the PCRF group, whereas the level in the nine smoking HD patients (7.2 ± 1.8 µg/ml) significantly higher than that in the non-smoking subgroup (P<0.01). In 16 HD patients with methylcobalamin treatment, serum SCN⁻ levels were lower than in those without methylcobalamin treatment (4.5 ± 0.5 µg/ml in non-smoking subgroup, P<0.05). And in the methylcobalamin-treated subgroup (n=5), the proportion of cyanocobalamin fraction (10.5 ± 2.6%) was as high as the level in Leber’s disease patients, while the proportion of methylcobalamin fraction was low. And the serum cyanocobalamin level was higher in the treated subgroup.

Conclusion. In uraemic patients, cyanide detoxication capability is impaired because of a reduced SCN⁻ clearance, and increased cyanocobalamin synthesis indicates elevation of cyanide pool, which would be related to the development of uraemic neuropathy. Methylcobalamin was considered to be utilized in cyanide detoxication process via cyanocobalamin synthesis.

Key words: chronic renal failure; uraemic neuropathy; cyanide; vitamin B₁₂; haemodialysis

Introduction

The development mechanisms of uraemic neuropathy have not been elucidated. Because clinical improvement occurs in patients who receive frequent and adequate dialysis or a successful kidney transplant, the development of uraemic neuropathy has been attributed to the accumulation of dialysable metabolites [1]. Pathologically, this neuropathy is usually observed as primary axonal degeneration with secondary segmental demyelination [2]. Neurotoxic compounds could deplete energy supplies in the axon by inhibiting the activities of nerve fibre enzymes that are necessary for energy synthesis, and thus produce pathological changes in nerves [2,3]. Various substances have been designated as putative neurotoxins, e.g., guanidine compounds [4], parathyroid hormone [5], and myoinositol [6].

To investigate the clinical symptoms of uraemic neuropathy and the underlying mechanisms, we previously conducted neurological tests, including measurement of vibration perception thresholds (VPTs), in 25 patients who were receiving haemodialysis, and found a significant relationship between the severity of vibration sensation impairment and the smoking habit [7]. Smoking was considered to be the main cause of cyanide exposure in these patients. Administration of methylcobalamin markedly improved the severity of uraemic neuropathy in terms of VPTs [7]. Based on these findings, we presumed that an abnormality in cyanide metabolism would be involved in the
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Subjects and methods

We evaluated 55 Japanese subjects in the following three groups: (i) 12 patients with preterminal chronic renal failure (PCRF group) (CCr 5.1 ± 2.3 ml/min, mean age 55 ± 16.9 years, male/female 4/8); (ii) 30 haemodialysis patients (HD group) who had received regular haemodialysis (mean age 35.0 ± 9.4 years, male/female 12/18), and (iii) 13 volunteers (control group) with normal renal function (mean age 39 ± 7.2 years, male/female 5/8). None of the PCRF and control group subjects smoked. Besides smoking, there were no factors which could result in different cyanide exposure between the study population. Nine of the 30 HD group patients were smokers: for the smoking subgroup (n = 9), mean age was 55.2 ± 3.3 years, male/female 6/3, and the duration of haemodialysis was 86.0 ± 79.0 months; for the non-smoking subgroup (n = 21), mean age was 52 ± 12 years, male/female 11/10, and the duration of haemodialysis was 75.0 ± 52.6 months.

Of the 30 HD patients, 16 had received 500 µg of methylcobalamin (Methylcobal®, Eisai Co., Ltd., Tokyo) intravenously after each haemodialysis as treatment for their neurological symptoms in the period ranging from 6 to 12 months (mean 8.6 ± 3.8). There were no marked differences in male/female ratio between each group or subgroup.

The patients were outpatients of the Nagoya Daini Red Cross Hospital, while the volunteers consisted of hospital employees or their relatives or friends. The purpose of this study was fully explained to all participants. They agreed to supply blood samples for this study and signed the consent forms.

Serum levels of thiocyanate (SCN⁻) were determined in all subjects by a modification of Bowler’s procedure [13,14]. Blood samples of HD patients were obtained before haemodialysis. Smoking on the day of blood sampling was not limited, because SCN⁻ was considered to not elevate within a short period of time after cyanide exposure, i.e. Matthew et al. [14] reported that serum thiocyanate levels increase quite slowly after cyanogen administration, and thiocyanate concentration reaches the peak level 3–5 days after the initiation of oral amylgadin (a cyanogenic glucoside) administration (0.5 g amygdalin p.o. three times daily administered 1 h before meals).

To obtain the proportion of each vitamin B₁₂ analogue in the total serum concentration of vitamin B₁₂, we evaluated blood samples from 10 of the 21 non-smoking HD patients. Five of these 10 patients had been treated with methylcobalamin (500 µg of methylcobalamin intravenously after each haemodialysis). Table 1 summarizes the background of these 10 patients; there were no marked differences in male/female ratio or in the duration of haemodialysis between the treated (with methylcobalamin) and untreated subgroups. Venous blood of these patients was collected in foil-wrapped syringes before haemodialysis, and plasma was separated in a dark room under red photographic light to avoid photolysis of the methylcobalamin (CH₃-B₁₂). deoxyadenosylcobalamin (DBCC), or cyanocobalamin (CN-B₁₂) content. The proportion of each vitamin B₁₂ analogue was determined by bioautographic analysis of chromatogram in which we used Lactobacillus leichmannii (ATCC 10586) as a test organism [15,16]. For comparison, these measurements were also conducted in six non-smoking healthy volunteers (mean age 50.2 ± 4.5 years; mean creatinine 0.9 ± 0.1, male only).

Statistical analysis

All data were expressed by mean ± SD. Student’s t test for paired and non-paired comparisons were applied. A P value less than 0.05 was considered statistically significant.

Results

Serum levels of thiocyanate (SCN⁻)

As shown in Figure 1, the mean serum levels of SCN⁻ in the 12 PCRF patients (5.1 ± 1.5 µg/ml) was significantly higher than in the controls (2.8 ± 0.9 µg/ml) (P < 0.01). The mean SCN⁻ level before haemodialysis in the 21 non-smoking HD patients was virtually identical to that in the PCRF group, whereas the level in the nine smoking HD patients significantly exceeded that in the non-smoking HD subgroup (P < 0.01). In
Table 1. Patients' background and proportion of vitamin B\textsubscript{12} analogues in sera in treated and untreated subgroups

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>Duration of HD (months)</th>
<th>Total B\textsubscript{12} content (pg/ml)</th>
<th>Proportion of vitamin B\textsubscript{12} analogues in sera</th>
<th>Content of CN-B\textsubscript{12} (pg/ml)</th>
<th>CH3-B\textsubscript{12}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH-B\textsubscript{12}</td>
<td>DBCC</td>
<td>CN-B\textsubscript{12}</td>
</tr>
<tr>
<td>T.H.</td>
<td>F</td>
<td>55</td>
<td>109</td>
<td>86900</td>
<td>7.10%</td>
<td>20.50%</td>
<td>0.30%</td>
</tr>
<tr>
<td>K.Y.</td>
<td>F</td>
<td>57</td>
<td>127</td>
<td>95900</td>
<td>5.60%</td>
<td>19.10%</td>
<td>0.60%</td>
</tr>
<tr>
<td>M.I.</td>
<td>F</td>
<td>40</td>
<td>81</td>
<td>101000</td>
<td>11.60%</td>
<td>18.70%</td>
<td>0.20%</td>
</tr>
<tr>
<td>S.M.</td>
<td>M</td>
<td>58</td>
<td>123</td>
<td>99600</td>
<td>9.60%</td>
<td>11.90%</td>
<td>0.70%</td>
</tr>
<tr>
<td>T.T.</td>
<td>M</td>
<td>46</td>
<td>113</td>
<td>102600</td>
<td>6.90%</td>
<td>26.80%</td>
<td>0.40%</td>
</tr>
<tr>
<td>Mean</td>
<td>±SD</td>
<td></td>
<td>51 ± 8</td>
<td>111 ± 18</td>
<td>8.2 ± 2.4%</td>
<td>19.4 ± 5.3%</td>
<td>0.4 ± 0.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH-B\textsubscript{12}</td>
<td>DBCC</td>
<td>CN-B\textsubscript{12}</td>
</tr>
<tr>
<td>M.M.</td>
<td>M</td>
<td>41</td>
<td>117</td>
<td>799</td>
<td>13.40%</td>
<td>19.40%</td>
<td>12.40%</td>
</tr>
<tr>
<td>K.N.</td>
<td>F</td>
<td>43</td>
<td>15</td>
<td>438</td>
<td>9.70%</td>
<td>21.20%</td>
<td>9.70%</td>
</tr>
<tr>
<td>H.K.</td>
<td>F</td>
<td>62</td>
<td>34</td>
<td>1843</td>
<td>7.40%</td>
<td>32%</td>
<td>7.90%</td>
</tr>
<tr>
<td>K.H.</td>
<td>M</td>
<td>41</td>
<td>156</td>
<td>942</td>
<td>10.10%</td>
<td>17.90%</td>
<td>14.10%</td>
</tr>
<tr>
<td>H.T.</td>
<td>F</td>
<td>63</td>
<td>24</td>
<td>1290</td>
<td>8.70%</td>
<td>21.60%</td>
<td>8.60%</td>
</tr>
<tr>
<td>Mean</td>
<td>±SD</td>
<td></td>
<td>50 ± 11</td>
<td>69 ± 63</td>
<td>9.9 ± 2.2%</td>
<td>22.4 ± 5.6%</td>
<td>10.5 ± 2.6%*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH-B\textsubscript{12}</td>
<td>DBCC</td>
<td>CN-B\textsubscript{12}</td>
</tr>
<tr>
<td>Control (healthy non-smoker; n = 6)</td>
<td>50 ± 4</td>
<td>683.8 ± 61.2</td>
<td>148 ± 0.9%</td>
<td>17.6 ± 1.7%</td>
<td>0.2 ± 0.1%</td>
<td>67.4 ± 2.1%</td>
<td></td>
</tr>
<tr>
<td>Leber's disease (non-smoker)***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.4 ± 5.2%</td>
<td></td>
</tr>
</tbody>
</table>

Treated subgroup received methycobalamin 500 μg/day after every haemodialysis before their enrolment and during this study. OH-B\textsubscript{12}, hydroxycobalamin; DBCC, deoxyadenosylcobalamin; CN-B\textsubscript{12}, cyanocobalamin; CH3-B\textsubscript{12}, methylcobalamin.

*P<0.01 vs the delta of the control, **P<0.01 vs the delta of the treated subgroup. ***Data reported by Wilson J et al. [9].
both the smoking and non-smoking HD subgroups, the mean serum levels of SCN\(^-\) decreased significantly after haemodialysis, i.e. from 7.2 ± 1.8 to 3.8 ± 1.0 µg/ml in the smoking subgroup (P < 0.01) and from 4.8 ± 0.7 to 3.2 ± 1.1 µg/ml in the non-smoking subgroup (P < 0.01).

Among the 30 HD patients, 16 received methylcobalamin treatment. Because serum SCN\(^-\) levels vary largely according to the presence or absence of smoking habit, effects of methylcobalamin on serum SCN\(^-\) levels should be examined for smoking and non-smoking subgroups. Among non-smoking HD patients (n = 21), SCN\(^-\) level in the methylcobalamin-treated subgroup (n = 10) was 4.5 ± 0.5 µg/ml, which was significantly lower (P < 0.05) than 5.2 ± 0.8 µg/ml of the untreated subgroup (n = 11). Among smoking HD patients (n = 9), SCN\(^-\) level in the treated subgroup (n = 6) was 7.0 ± 1.8 µg/ml, while it was 7.6 ± 2.1 µg/ml in the non-treated subgroup (n = 3). SCN\(^-\) level in the treated subgroup tended to be lower than in the untreated subgroup. However, there was no statistical significant difference.

**Proportion of vitamin B\(_{12}\) analogues in serum of 10 non-smoking haemodialysis patients, with or without methylcobalamin treatment**

Table 1 summarizes the proportions of vitamin B\(_{12}\) analogues in the serum of 10 non-smoking HD patients and 6 non-smoking healthy volunteers. In the untreated subgroup (without methylcobalamin), the proportion of cyanocobalamin fraction was significantly high (10.5 ± 2.6%), which is equivalent to the level reported for patients with Leber’s disease (non-smoker), and the proportion of methylcobalamin fraction was significantly lower (57.2 ± 3.4%) than the controls (67.4 ± 2.1%). In the treated subgroup, the proportions of these two fractions were equivalent to the controls, cyanocobalamin level was elevated (402 ± 208 pg/ml vs 105 ± 40.2 pg/ml in untreated subgroup), and total level of vitamin B\(_{12}\) content was extremely high.

**Discussion**

Cyanide-releasing substances are metabolized into cyanide ion and its salts which are called cyanide (CN). CN poisoning may result from the inhalation of hydrocyanic acid or from the ingestion of soluble inorganic cyanide salts or cyanide-releasing substances such as cyanamide, cyanogen chloride, and nitroprusside. Parts of many plants (chokecherry, pin cherry, wild black cherry, peach, apricot, bitter almond and so on) also contain substances such as amygdalin which release cyanide on digestion. Tobacco smoke may also cause chronic CN poisoning. CN diffuses rapidly in the cells, where it can be partly detoxified by CN sulphutransferase (known as rhodanese). Some CN react with cytochrome oxidase. CN are later slowly released from the complex with cytochrome oxidase and then detoxified by rhodanese. This process was considered because blood thiocyanate levels increase slowly. The majority of CN in vivo is enzymatically converted to SCN\(^-\), and excreted in the urine.
K. Koyama et al. (Figure 2). The remaining CN is pooled in the body and metabolized mainly via two other routes: (i) production of 2-amino-4-thiazolinecarboxylic acid from cystine and CN, and (ii) synthesis of cyanocobalamin (CN-B₁₂) via the combination of CN with some other form of vitamin B₁₂ analogue, such as hydroxycobalamin or methylcobalamin [12]. Though these two routes and other pathways function only auxiliary to the main route via thiocyanate, CN detoxication (i.e. synthesis of cyanocobalamin via the combination of CN with some other form of vitamin B₁₂ analogue, such as hydroxycobalamin or methylcobalamin) is known to be accelerated in Leber’s disease patients. The proportion of cyanocobalamin fraction increases in those patients. It is because the CN pool in Leber’s disease increases due to the decrease of SCN⁻ production. It is difficult to monitor the level of serum CN because CN diffuses rapidly in the cells. We previously tried to monitor the level of serum CN⁻ in 20 haemodialysis patients in a pilot study by using a modification of the method of Feldstein and Klendshaj [14,17], but the level was undetectable. Therefore it is considered that measuring the proportion of cyanocobalamin fraction is the only way to indicate the volume of the CN pool.

In our uraemic patients, serum SCN⁻ levels increased, the proportion of cyanocobalamin fraction was markedly increased to the level as high as the levels seen in Leber’s disease patients, and the proportion of methylcobalamin fraction also decreased (Table 1). Our evidence indicates that in chronic renal failure, SCN⁻ is accumulated due to the decrease in SCN⁻ clearance. This impairs the major metabolic pathway of CN, and as a result the CN pool increases. This increase would accelerate CN detoxication via cyanocobalamin synthesis using vitamin B₁₂, and result in an increase in the proportion of cyanocobalamin fraction and a decrease in the proportion of methylcobalamin fraction (Figure 3). Because the evidence was obtained in non-smoking uraemic patients, there could be a supply of CN-releasing substances besides smoking, such as exposure to tobacco smoke (secondhand tobacco smoke). All vitamin B₁₂ analogues are excreted in the urine, and their excretion rates do not change under the condition of impaired renal function, while changes in enzymatic activity of rhodanese in chronic renal failure have not been reported.

In our uraemic patients in the smoking subgroup, serum SCN⁻ levels more increased. That evidence suggests that CN pool would be increased by smoking and then neuropathy would be induced in uraemia.

Methylcobalamin, the active form of vitamin B₁₂, can act as a coenzyme. In a previous study we observed that the vibratory sensation in uraemic patients was improved by the intravenous administration of methylcobalamin [7]. In the present study the proportions of methylcobalamin and cyanocobalamin fractions in the serum were normal and serum SCN⁻ level were low in the methylcobalamin-treated subgroup. This indicates that the CN pool decreases in the methylcobalamin-treated subgroup. In addition, patients in the same group presented elevated serum cyanocobalamin levels. This indicated that cyanocobalamin synthesis accelerated and CN pool decreased in the methylcobalamin-treated subgroup. We considered

![Figure 2. Metabolic pathways of cyanide. CN, cyanide; Na₂S₂O₃, sodium thiosulphate; SCN⁻, thiocyanate; CN-B₁₂, cyanocobalamin; HCN, hydrocyanic acid; HCNO, cyanic acid; HCOOH, formic acid.](image-url)
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Fig. 3. Changes in cyanide detoxication pathway. In chronic renal failure, SCN\textsuperscript{−} is accumulated due to the decrease in SCN\textsuperscript{−} clearance. It impairs the major metabolic pathway of CN, and then the CN pool increases. This increase accelerates CN detoxication via cyanocobalamin synthesis using vitamin B\textsubscript{12}, and this results in the increase of the proportion of cyanocobalamin fraction and the decrease of the proportion of methylcobalamin fraction. If a patient under these conditions is exposed to CN due to smoking, etc., CN supply increases and CN cannot be detoxicated sufficiently; the accumulated CN would induce neuropathy.

that methylcobalamin is utilized in CN detoxication process via cyanocobalamin synthesis, and via the detoxication process, the clinical use of methylcobalamin could result in favourable neurological effects.

There have been no reports demonstrating methylcobalamin administration changes enzymatic activities of rhodanese.

Vitamin B\textsubscript{12} is extensively bound to specific plasma proteins called transcobalamins, which appear to be involved in the rapid transport of cobalamin to tissues. A reported positive logistic correlation between saturated binding rates and vitamin B\textsubscript{12} levels [18] indicates that a large amount of methylcobalamin is required to detoxify intracellular CN\textsuperscript{−}; therefore intravenous administration treatment of methylcobalamin is better than oral treatment of it. There were no adverse effects of methylcobalamin 500 mg intravenous administration after every haemodialysis.

We conclude that in uraemic patients, the ability to detoxify CN is impaired, and that this impairment would be related to the development of uraemic neuropathy. Methylcobalamin is considered to be utilized in CN detoxication process via cyanocobalamin synthesis, and its clinical use can result in favourable neurological effects.

References


Received for publication: 3.10.96
Accepted in revised form: 19.3.97