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The protein metabolite hypothesis, a model for the progression of renal failure: An oral adsorbent lowers indoxyl sulfate levels in undialyzed uremic patients

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The protein metabolite hypothesis, a model for the progression of renal failure: An oral adsorbent lowers indoxyl sulfate levels in undialyzed uremic patients. We have recently demonstrated that indoxyl sulfate promotes the progression of glomerular sclerosis in uremic rats. In the present study, we determined whether an oral adsorbent (AST-120) could reduce the serum and urine levels of indoxyl sulfate and suppress the progression of chronic renal failure (CRF) in undialyzed uremic patients. Twenty-five undialyzed uremic patients were given AST-120 at a dose of 6 g/day for 6 months, while 10 undialyzed uremic patients were not given AST-120. The effects of the oral adsorbent on the slope of the 1/serum creatinine (1/Sc) -time plot, and the serum and urine levels of indoxyl sulfate were evaluated. Administration of AST-120 significantly decreased the slope of the 1/Sc -time plot in the CRF patients. Among the patients in whom urinary excretion of indoxyl sulfate was reduced by AST-120, the oral adsorbent significantly improved the slope of the 1/Sc -time plot. The change in the slope of the 1/Sc -time plot showed a significant negative correlation with the change in the urine level of indoxyl sulfate. Thus, patients who showed a greater decrease of urinary indoxyl sulfate also showed more marked suppression of the progression of CRF. These results support the notion that indoxyl sulfate, a protein metabolite, is involved in the progression of CRF, and that an oral adsorbent can delay progression at least partly by reducing the serum and urine levels of indoxyl sulfate.

Circulating uremic toxins are thought to be involved in the progression of glomerular sclerosis. Motojima et al [1] recently reported that peritoneal dialysis and the administration of AST-120 slowed the progression of glomerular sclerosis. Their results support the notion that nephrectomy increases the circulating levels of substances stimulating sclerosis and that hypertrophy of remnant nephrons following nephrectomy involves circulating substances [2-4]. Glomerular hypertrophy and the development of glomerular sclerosis are reported to be closely related [5-10]. We recently demonstrated that indoxyl sulfate is one of the circulating substances promoting the progression of renal failure [11, 12]. We found that oral administration of indoxyl sulfate to uremic rats promoted the progression of glomerular sclerosis along with a dramatic decline in renal function [11]. Oral administration of indole, a precursor of indoxyl sulfate, to uremic rats also promoted the progression of glomerular sclerosis after conversion to indoxyl sulfate in the liver [12]. Because indoxyl sulfate can be removed by peritoneal dialysis [13] and its serum level can also be reduced by orally administering AST-120 [14-16], Motojima's findings can be at least partially explained by the removal of indoxyl sulfate.

Protein restriction suppresses the progression of renal disease in laboratory animals [17, 18]. Most studies in humans have also suggested that restriction of dietary protein is beneficial in retarding the progression of CRF [19-26]. An oral adsorbent (AST-120; Kremezin, Kureha-Chemical Co., Tokyo, Japan) also retards the progression of CRF in both uremic rats [27-29] and undialyzed uremic patients [30, 31]. Thus far, however, there have been no clinical reports concerning the effects of this oral adsorbent on serum and urine levels of indoxyl sulfate in undialyzed uremic patients.

We hypothesized that an overload of dietary protein metabolites such as indoxyl sulfate acting on the remnant nephrons could promote the progression of glomerular sclerosis and CRF. Thus, the protective effect of AST-120 against the progression of CRF might be explained by alleviating this overload through reduced production of dietary protein metabolites such as indoxyl sulfate. In fact, we previously demonstrated in uremic rats that both a low-protein diet and AST-120 reduced the serum and urine levels of indoxyl sulfate and protected against the progression of renal dysfunction and glomerular sclerosis [11]. We also demonstrated that a low-protein diet reduced the serum and urine levels of indoxyl sulfate in undialyzed uremic patients [32].

In the present study, we examined the clinical effects of AST-120 on the progression of CRF and on the serum and urine levels of indoxyl sulfate in undialyzed uremic patients. Our results supported the notion that indoxyl sulfate is involved in the progression of CRF, and that AST-120 suppresses CRF at least partly by reducing serum and urine levels of indoxyl sulfate.

METHODS

Patients

Twenty-five undialyzed patients with CRF [13 males and 12 females aged 57.1 ± 12.2 (sd) years] were orally given AST-120 (Kremezin) at a dose of 6 g/day for 6 months. The primary

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Table 1. Clinical data of CRF patients in the AST-120 and control groups

<table>
<thead>
<tr>
<th></th>
<th>AST-120</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After 6 months</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Serum creatinine mg/dl</td>
<td>3.86 ± 0.26</td>
<td>4.43 ± 0.40^c</td>
</tr>
<tr>
<td>Serum urea nitrogen mg/dl</td>
<td>50.6 ± 3.7</td>
<td>60.8 ± 4.7^a</td>
</tr>
<tr>
<td>Uric acid mg/dl</td>
<td>7.4 ± 0.3</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>Total protein g/dl</td>
<td>6.7 ± 0.2</td>
<td>6.7 ± 0.1</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol mg/dl</td>
<td>217 ± 13</td>
<td>219 ± 9</td>
</tr>
<tr>
<td>Triglycerides mg/dl</td>
<td>193 ± 31</td>
<td>174 ± 19</td>
</tr>
<tr>
<td>Red blood cells ×10^6/mm^3</td>
<td>354 ± 11</td>
<td>340 ± 13</td>
</tr>
<tr>
<td>Ccr ml/min</td>
<td>17.5 ± 1.9</td>
<td>15.2 ± 1.7</td>
</tr>
<tr>
<td>Mean blood pressure mm Hg</td>
<td>99 ± 3</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Serum indoxyl sulfate mg/dl</td>
<td>2.02 ± 0.28</td>
<td>1.70 ± 0.35^a</td>
</tr>
<tr>
<td>Urine indoxyl sulfate mg/day</td>
<td>66.8 ± 6.2</td>
<td>43.4 ± 9.2^d</td>
</tr>
<tr>
<td>Urine creatinine g/day</td>
<td>0.66 ± 0.08</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Urine urea nitrogen g/day</td>
<td>5.10 ± 0.57</td>
<td>5.48 ± 0.52</td>
</tr>
<tr>
<td>Protein intake g/kg body wt/day</td>
<td>0.76 ± 0.06</td>
<td>0.86 ± 0.05</td>
</tr>
</tbody>
</table>

All results are expressed as the mean ± sd. NS is not significant.

* P < 0.05, ^b P < 0.01, ^c P < 0.001 by the paired Wilcoxon test as compared with before, ^d P < 0.05 by the U-test as compared with control values.

Fig. 1. Effect of AST-120 on serum creatinine in CRF patients. The serum level of creatinine increased significantly in patients after 3 and 6 months with or without AST-120 treatment due to the progression of CRF. However, the mean serum creatinine level in the patients given AST-120 was lower than that in the control patients. * P < 0.05, ** P < 0.01, *** P < 0.001 as compared with pre-treatment levels.

Fig. 2. Effect of AST-120 on serum indoxyl sulfate in CRF patients. The serum level of indoxyl sulfate decreased significantly in the patients given AST-120 after 1, 3 and 6 months of treatment, while it did not change significantly in the control patients. * P < 0.05, *** P < 0.001 as compared with pre-treatment levels.

from the patients before treatment, and after 1, 3, and 6 months of AST-120 treatment.

The reciprocal of the serum creatinine level was plotted against time (weeks), and the progression of CRF was assessed from the regression coefficient (the slope of the regression line) determined by least squares linear regression analysis.

The protein intake was calculated from Maroni's equation [33];

\[
\text{Protein intake (g/day)} = (\text{UUN} + 0.031 \times \text{body wt}) \times 6.25
\]

where UUN is urinary urea nitrogen (g/day) and body wt is body weight (kg).

Analysis of indoxyl sulfate

Serum and urine levels of indoxyl sulfate were measured using a newly developed high-performance liquid chromatography
(HPLC) method, which is applicable to a larger-variety of compounds and is more durable than that reported previously by us [34]. The chromatograph apparatus (Japan Spectroscopic Co., Tokyo, Japan) consisted of a Model 380-FU pump, a Model 380-50 degasser, a Model 851-AS autosampler, a Model 820-FP fluorescence detector, a Model Chromatocorder 12, and a reverse phase column (Develosil PT C8-5, 300 mm x 4.6 mm I.D., from Nomura Chemical Co., Aichi, Japan) with a guard column (Develosil PT C8-5, 30 mm x 4.6 mm I.D.).

The mobile phase consisting of solution (A) (distilled water/ acetic acid, 100:0.08, vol/vol) and solution (B) (acetone/tert/acetic acid, 100:0.08, vol/vol) was delivered at a flow rate of 1 ml/min at ambient temperature. The mobile phase was programmed to change from 100% solution (A) to 62% solution (B) over 25 minutes, and then to 100% solution (B) over 1 minute. Subsequently, 100% solution (B) was maintained for 4 minutes and then changed to 100% solution (A) over 1 minute, after which 100% solution (A) was maintained for 9 minutes before the next injection. The eluate was monitored by detecting fluorescence with excitation at 295 nm and emission at 390 nm. Serum samples (5 μl) were directly injected into the apparatus.

Calibration was done using standard solutions containing indoxyl sulfate (0.01 to 10 mg/dl) in distilled water. The calibration curve (y = -0.052 + 0.0000000827x, y: indoxyl sulfate, mg/dl, x: peak area) showed a very strong correlation (r = 0.99998). Intra-assay and inter-assay coefficients of variation were 0.33% (N = 5) and 5.4% (N = 5), respectively, for a serum sample with an indoxyl sulfate level of 4.46 mg/dl.

Identification of indoxyl sulfate was confirmed by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS), which was performed by directly connecting the HPLC with a mass spectrometer (Hitachi MS-1000S). Selected ion monitoring of m/z 212 (M-H)− was used for the identification of indoxyl sulfate in the samples.

Statistical analysis

Results are expressed as the mean ± se. Statistical analysis was done using the paired Wilcoxon test or the U-test, as appropriate. Differences were considered statistically significant when the P value was less than 0.05.

RESULTS

No significant differences of serum creatinine, dietary protein intake, or other clinical data were observed between the two groups before the study (Table 1). Protein intake did not change throughout the study in both the control and AST-120 groups.

Figure 1 shows the effect of AST-120 on serum creatinine. Both groups of patients showed a significant increase of the serum creatinine level after 3 and 6 months, but the mean creatinine level was lower in the AST-120 group than in the control patients. This was not due to the intestinal adsorption of creatinine by AST-120, because urinary creatinine excretion was not decreased in the AST-120 group (Table 1). Although AST-120 has been demonstrated to adsorb creatinine in vitro, the amount is too small to decrease urinary creatinine.

Figure 2 shows the effect of AST-120 on the serum level of indoxyl sulfate. There was a significant decrease of the indoxyl sulfate level in the AST-120 group, while the control patients did not show any significant change.

Figure 3 shows the effect of AST-120 on the urinary excretion of indoxyl sulfate. In the AST-120 group, urine levels of indoxyl sulfate decreased to about 60% of the pretreatment value, while the control patients did not show any significant change.

Figure 4 shows the slope of the 1/Scr·time plot in the CRF patients before and after treatment with or without AST-120. Although the control CRF patients did not show any significant change in the slope of this plot, the patients given AST-120 showed an increased mean slope. Also, among the 20 patients in whom urinary levels of indoxyl sulfate decreased, AST-120 significantly increased the slope of the 1/Scr·time plot. Two of the other five patients given AST-120 showed an increase of urinary indoxyl sulfate, one patient showed no change, and the remaining two patients could not be assessed because urine samples were not obtained.

Figure 5 shows the correlation between the change in the urinary level of indoxyl sulfate and the change in the slope of the 1/Scr·time plot in CRF patients with and without AST-120 treatment. The change in the slope of the plot showed a significant negative correlation (r = -0.55, P < 0.01) with the change in the urine level of indoxyl sulfate. Even after excluding two patients who exhibited the most marked slowing of the progression of CRF, the correlation between the change in slope of the plot and the change of urinary indoxyl sulfate was still significant (r = -0.58, P < 0.01). In other words, patients who showed a greater decrease in the urinary excretion of indoxyl sulfate also showed more marked slowing of the progression of CRF. Even among the patients given AST-120, the change in the slope of the 1/Scr·time plot showed a significant negative correlation (r = -0.46, P < 0.05) with the change of urine indoxyl sulfate.

DISCUSSION

The present results demonstrated that an oral adsorbent (AST-120) reduced the serum and urine levels of indoxyl sulfate and slowed the progression of CRF in undialyzed uremic patients. Notably, those who showed a greater decrease in the urinary excretion of indoxyl sulfate also showed more a marked slowing of the progression of CRF. These results support the hypothesis that
the protective effects of AST-120 on the progression of CRF may be explained at least partly by reducing the overload of indoxyl sulfate on the remnant nephrons, because indoxyl sulfate has been demonstrated to stimulate the progression of glomerular sclerosis in uremic rats [11, 12].

Indoxyl sulfate is considered to be a uremic toxin, since it inhibits the binding of albumin [34], erythroid colony formation [35], lymphocyte blast formation [35], and thyroxine hepatocyte transport [36], and because it stimulates the progression of glomerular sclerosis [11, 12] and the gene expression of transforming growth factor (TGF)-β1, tissue inhibitor of metalloproteinases (TIMP)-1 and pro-α 1(I) collagen in the kidneys of uremic rats [37]. The serum level of indoxyl sulfate is markedly increased in uremic patients [11, 13, 16, 34].

A small portion of protein-derived tryptophan is metabolized into indole by tryptophanase in intestinal bacteria such as *Escherichia coli*, which are mainly present in the large bowel [38, 39], and this may explain the observation that a low-protein diet reduces urinary excretion of indoxyl sulfate in both uremic rats and undialyzed uremic patients [11, 32]. However, orally administered tryptophan is not converted to indole by intestinal bacteria [12], because it is rapidly absorbed in the small intestine and consequently does not reach the large bowel. However, we increased the serum and urine levels of indoxyl sulfate by administering tryptophan directly into the large bowel of uremic rats [12], and we thus demonstrated that indole was produced from tryptophan by intestinal bacteria.

Indole is absorbed into the blood from the intestine and is metabolized to indoxyl sulfate, which is normally excreted in the urine [40]. However, uremic patients cannot efficiently excrete indoxyl sulfate because of reduced renal clearance. Since AST-120 shows a high in vitro adsorption of indolic and phenolic compounds including indole [14], it binds indole and the other compounds in the intestine and increases their excretion in the feces, thus inhibiting absorption (Fig. 6). Consequently, AST-120 decreases the serum and urinary levels of indoxyl sulfate. We previously reported that AST-120 treatment reduced the serum levels of indoxyl sulfate in uremic rats [14, 15] and in uremic patients on hemodialysis [16]. In the present study, we demonstrated that AST-120 reduced the serum and urine levels of indoxyl sulfate and slowed the progression of CRF in undialyzed uremic patients.

In the protein metabolite hypothesis, we proposed that endogenous protein metabolites such as indoxyl sulfate play a significant role in the progression of CRF (Fig. 7) [41]. The initial insult leads
Sulfate, for example, stimulates progressive glomerular sclerosis. Sulfate is levitated by events that might be interrupted.

Indoxyl sulfate stimulates the progression of CRF by increasing synthesis of TGF-β, TIMP-1, and collagen, leading to further loss of functioning nephrons in a vicious cycle of CRF progression. If the overload of indoxyl sulfate is alleviated by AST-120 and/or protein restriction, this chain of events might be interrupted.

to a loss of functioning nephrons via a disease-specific pathophysiological process. A progressive decline in the glomerular filtration rate leads to increased circulating levels of endogenous protein metabolites such as indoxyl sulfate [11, 13, 16, 34] and the adverse effects of overload on the remnant nephrons. Indoxyl sulfate, for example, stimulates progressive glomerular sclerosis [11, 12] and the progression of CRF by increasing the gene expression of TGF-β1, TIMP-1, and procollagen [37], leading to further loss of nephrons. Thus, the vicious cycle of progressive renal injury is complete. Our present results demonstrate that an oral adsorbent can reduce the serum and urine levels of indoxyl sulfate in undialyzed uremic patients. If the overload of indoxyl sulfate is alleviated, for example, by a low-protein diet [11, 32] or by the administration of AST-120 [14-16], the chain of events leading to progression of renal damage might be interrupted. In fact, many clinical and experimental studies have demonstrated that both dietary protein restriction [17-26] and AST-120 [27-31] can suppress the progression of CRF.

![Diagram](https://via.placeholder.com/150)

**Fig. 7. Diagram of the protein metabolite hypothesis, a model for the progression of CRF.** Loss of functioning nephrons leads to an overload of protein metabolites such as indoxyl sulfate on the remnant nephrons. Indoxyl sulfate stimulates the progression of CRF by increasing synthesis of TGF-β, TIMP-1, and collagen, leading to further loss of functioning nephrons in a vicious cycle of CRF progression. If the overload of indoxyl sulfate is alleviated by AST-120 and/or protein restriction, this chain of events might be interrupted.

**Fig. 6. Metabolic pathway for the production of indoxyl sulfate and the inhibitory effect of AST-120 on its production.**