Administration of oral charcoal adsorbent (AST-120) suppresses low-turnover bone progression in uraemic rats

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Abstract

Background. Using a rat model of renal failure with normal parathyroid hormone levels, we had demonstrated previously that bone formation decreased depending on the degree of renal dysfunction, and hypothesized that uraemic toxins (UTx) are associated with the development of low-turnover bone development, complicating renal failure. In this study, focusing on indoxyl sulphate (IS) as a representative UTx, we analysed the effect of an oral charcoal adsorbent AST-120, which removes uraemic toxins and their precursors from the gastrointestinal tract, on bone turnover.

Methods. AST-120 or vehicle was administered orally to model rats with uraemia and low turnover bone. Bone turnover was analysed by histomorphometry. Expression of osteoblast-related genes and oat-3 gene was analysed by reverse transcription polymerase chain reaction.

Results. In rats treated with vehicle, serum IS level increased with time after renal dysfunction, while bone formation decreased accompanied by down-regulation of the parathyroid/parathyroid-related peptide hormone receptor, alkaline phosphatase and osteocalcin genes. Administration of AST-120 inhibited the accumulation of IS in blood and ameliorated bone formation. Bone formation rate was 2.4 ± 1.7 m3/m2/year in controls given vehicle and was 11.7 ± 2.4 m3/m2/year in rats administered with AST-120 (P < 0.05). AST-120 treatment also reversed the down-regulation of osteoblast-related genes. Gene expression of oat-3 was detected in the tibia of rats.

Conclusion. Administration of the oral charcoal adsorbent AST-120 decreases the osteoblast cytotoxicity of UTx including IS, and suppresses progression of low bone turnover in uraemic rats.

Keywords: indoxyl sulphate; low bone turnover; oral charcoal adsorbent; renal failure; uraemic toxins

Introduction

Various substances that are excreted by the kidney under normal conditions are accumulated in blood when renal function is impaired. These substances are known as uraemic retention solutes, and are classified according to molecular weight and protein binding state [1]. Accumulation of some of these uraemic retention solutes in the body has been reported to impose various impairment to different organs. When toxicity is proven, they are called uraemic toxins (UTx).

Indoxyl sulphate (IS) is one of the UTx. Tryptophan contained in food is transformed to indole by the intestinal bacteria, and the indole absorbed from the intestine is transformed in the liver into indoxyl and then to IS [2]. Since ~90% of IS in blood binds with albumin, it is excreted through the kidney mainly from the proximal renal tubules into urine. In chronic renal failure, the blood concentration of UTx increases markedly due to lowered renal clearance. Compared with healthy persons, the blood IS concentration is approximately 30 times higher in pre-dialysis patients with chronic renal failure and 80 times higher in patients before initiation of dialysis [3]. IS is known to accelerate the progression of renal failure, and is a surrogate parameter of renal function. Patients who accumulate larger amounts of IS tend to show a higher speed of progression of renal failure [4]. Administration of IS or its precursor, indole,
to rats with renal failure results in lowered renal function [5,6].

An oral charcoal adsorbent, AST-120 (Kureha Corporation, Tokyo, Japan) was developed for the treatment of end-stage renal disease by reducing UTx. It has been used clinically in Japan since 1991. AST-120 adsorbs hydrophobic uraemic substances including IS in the gastrointestinal tract, and is excreted into the feces. Clinical studies [7–9] and basic research using animal models [10–12] have demonstrated that treatment with AST-120 in patients or animals with renal failure suppresses the increase of serum creatinine and also the progression of renal failure by lowering blood UTx concentrations. AST-120 exhibits the pharmacological action presumably by adsorbing indole generated in the intestinal tract, thereby suppressing synthesis of IS in the body and preventing accumulation of IS in blood.

On the other hand, we have recently produced an animal model of renal failure with low bone turnover [13]. We have shown that in renal failure with normal parathyroid hormone (PTH) level, the expression of PTH receptor genes is down-regulated depending on the degree of renal dysfunction. This result suggests a possibility that decreased bone response to PTH may cause low bone turnover. In addition, decrease of bone turnover indicated by histomorphometric measurements depends on the degree of renal failure impairment, suggesting that UTx accumulated in the blood may be associated with low bone turnover.

Hence, we hypothesized that UTx are associated with the development of low bone turnover, complicating renal failure. Among the various UTx, we focused on IS that has been studied as a substance promoting impairment of renal function. We studied the effect of IS as one of the factors responsible for bone turnover and also the effect of oral administration of AST-120 that is known to suppress the accumulation of UTx including IS.

Materials and methods

Animal experiments

A rat model of renal failure with low bone turnover was produced using the same methods as previously reported [13]. Briefly, male Sprague-Dawley strain rats weighing around 350 g that underwent thyroparathyroidectomy (TPTx) and two-stage nephrectomy (Nx) were given continuous infusion of a physiological level of 1–34 PTH. As shown in Figure 1, six animals were sacrificed at the first Nx (time 0) to obtain baseline data. Six weeks after the second Nx (7 weeks from baseline), six TPTx and six TPTx-Nx rats were sacrificed. The remaining TPTx-Nx rats were divided into two groups. One group (n=9) was administered the oral adsorbent AST-120 (4 g/kg body weight admixed in animal feed) and the other group (n=9) was given vehicle and no AST-120. AST-120 was acquired from Kureha Corporation. As a control, the group that underwent TPTx alone was also included. The remaining rats were sacrificed at week 12 after the second Nx (13 weeks from baseline). Blood and bone samples were collected from all rats. Urine was collected before sacrifice to measure creatinine clearance.

All animal experiments were approved by the Animal Care and Use Committee of the Biomedical Research Laboratories, Kureha Chemical Industry Co., Ltd.

Serum and urine biochemistry

Serum samples were stored at −70°C until biochemical or hormonal assays. Urine samples were stored at −20°C for later analyses. Serum calcium, phosphorus, alkaline phosphatase activity and urinary calcium were measured using an autoanalyser (Hitachi 736, Hitachi Co., Ltd., Hitachi-City, Japan). Serum and urine creatinine concentrations were determined using Wako kit 277–1050, and urea nitrogen levels were measured using Wako kit 279–36201 (Wako Pure Chemicals, Tokyo, Japan). Serum PTH levels were measured with an immunoradiometric assay kit for rat PTH (Immutopics, San Clemente, CA). Serum levels of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] were measured by a radioreceptor assay using vitamin D receptors derived from calf thymocytes. Serum concentration of IS was determined with high-performance liquid chromatography (HPLC) as described previously [2]. Briefly, we used a SHISEIDO CAPCELL PAK MF ph-1 column (size: 150 × 4.6 mm ID) with a guard column (4.0 × 10 mm ID). The mobile phase [0.1 M KH2PO4/tetrahydrofuran = 95/5 (v/v)] was adjusted to pH 6.5 with 1 M NaOH was delivered at a flow rate of 1.0 ml/min at ambient temperature. The eluate was monitored by a fluorescence detector (excitation: 295 nm, emission: 390 nm) for analysis.

Bone histomorphometry

Fourteen and seven days prior to sacrifice, all animals were injected subcutaneously with calcein (8 mg/kg body weight; Wako Pure Chemical Industries, Osaka, Japan) for labelling. Rats were sacrificed by exsanguination via cardiac puncture.

Blood samples were collected for subsequent biochemical determinations. The right proximal tibia was removed from
Table 1. PCR primer sequences and their product sizes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
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<tbody>
<tr>
<td>PTH receptor</td>
<td>Forward: 5'-AGCGAGTGCCCTCAAGTTCTAT-3'</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-ACACCGCTCCCTCAAGGAG-3'</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>Forward: 5'-CTGTGAAAAAGCTTGTGAAA-3'</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CTTGGAGGAGCACAATAAGG-3'</td>
<td></td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>Forward: 5'-AGCTGACTTGTGACATCCGT-3'</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CTTAGAGCATTGCGGTGCAGT-3'</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward: 5'-TCATGAAAGTGTGACATCGGTCACTCGT-3'</td>
<td>285</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CTTAGAGCATTGCGGTGCAGT-3'</td>
<td></td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone/parathyroid-related peptide hormone receptor; ALP, alkaline phosphatase.

Each rat, fixed in 70% ethanol and embedded in glycolmethacrylate (Wako Pure Chemical Industries) without decalcification. Then, serial sections (5 μm in thickness) were cut longitudinally using a microtome (Model 2050; Reichert Jung, Buffalo, NY, USA), and the sections were further stained with toluidine blue O to discriminate between mineralized and unmineralized bone and to identify cellular components. Unstained sections 5 μm were used to visualize calcine labelling under a fluorescent light microscope. Histomorphometric analysis of secondary spongiosa of the proximal metaphysis between 1.2 and 3.6 mm distal to the growth plate-epiphysial junction was performed using a semi-automated system (Osteoplan II; Carl Zeiss, Thornwood, NY, USA), and measurements were made at 200× magnification. Dynamic parameters were determined as follows. Single-labelled and double-labelled surfaces as well as total bone surface (BS) in the secondary spongiosa were traced at 200× magnification. Then, single-labelled surface (sLS/BS, %) and double-labelled surface (dLS/BS, %) were calculated as a percentage of the total bone surface. Labelling width was determined as the average distance between the double labels. Mineral apposition rate (MAR, μm/day) was calculated by dividing the labelling width by the number of days between the two calcine administrations. Bone formation rate per bone surface (BFR/BS, μm²/μm²/year) was the product of (sLS/2 + dLS) × MAR/BS. Trabecular osteoclast surface (Oc.S/BS, %) and eroded surface (ES/BS, %) were determined as parameters of bone resorption. The nomenclature, symbols and units used in this study are those recommended by the American Society for Bone Mineral Research (ASBMR) Nomenclature Committee (Parfit et al. J Bone Miner Res 1987; 2: 595–610).

RNA extraction and reverse transcription—polymerase chain reaction (RT–PCR)

After sacrifice, the left tibia was removed. The proximal metaphysis was separated from the diaphysis. The bone was flushed with phosphate-buffered saline to remove all the bone marrow and then frozen in liquid nitrogen. Proximal metaphysis of the left tibia without bone marrow were ground to a fine powder using a mortar and pestle under liquid nitrogen in RNAase-free conditions. Total RNA was extracted using an ISOGEN kit (Wako Pure Chemicals Industries) following the manufacturer’s instruction. Total RNA (2 μg) from bone powder was used as the template for complementary DNA synthesis in a 20 μl volume using an RT-PCR kit (Takara Shuzo, Shiga, Japan) according to the manufacturer’s instructions. The reaction mixture contained 1 unit/μl Moloney murine leukaemia virus reverse transcriptase; 1 × RNA PCR buffer; 5 mM MgCl₂; 250 μM each of dATP, dCTP, dGTP and dTTP; and 1 μM random 9-mer oligonucleotide. The mixture was incubated at 30°C for 10 min, 42°C for 30 min and 100°C for 5 min, and finally stored at 4°C. The PCR reaction mixture totalled 50 μl and contained 1 μl cDNA, 0.05 units/μl native Pfu DNA polymerase (Clontech, Palo Alto, CA), 20 mM Tris–HCl (pH 8.0), 2 mM MgCl₂, 6 mM (NH₄)₂SO₄, 10 mM KCl, 0.1% Triton X-100, 10 μg BSA, 200 μM dNTP and 0.5 μM each of forward and reverse oligonucleotide primer. PCR reactions were carried out using various primers (Table 1). β-Actin was used as an internal control for efficacy of the extraction, reverse transcription and PCR steps. The thermal cycling protocol was 95°C for 50 s, 60°C for 50 s and 72°C for 1 min, for 32 cycles (Thermal Cycler, Takara Shuzo). The amplification reaction products (10 μl) were analysed using agarose gel electrophoresis and visualized by ethidium bromide staining. Semi-quantitative analysis was performed using NIH-Image version 1.62.

Expression of mRNA for organic anion transporters (OATs) in tibia

Total RNA was isolated from the tibia in TPTx rats and cDNA synthesis, and PCR reaction was carried out as described above. The sequence of the forward primer was 5'-CAATGACCTCCTGAAACAGGTG-3' and that of the reverse primer was 5'-TCCAGGTGCCCATTGCGCTCTG-3' for mOAT-1. The sequence of the forward primer was 5'-CATGACCTTCCTCGAGATTCTG-3' and that of reverse primer was 5'-TGCTGTGTGATCCAGTCAT-3' for mOAT-3. The product sizes of OAT-1 and -3 were 291 and 310 bp, respectively. Analyses of OAT-1 and OAT-3 transcripts were carried out in parallel using the following thermal cycle: 95°C for 30 s, 60°C for 45 s, and 72°C for 1 min, for 35 cycles (Thermal Cycler, Takara Shuzo). The amplification reaction products (10 μl) were analysed using agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical analysis

All data are expressed as mean ± SD. Means of groups were compared by ANOVA, and significance of difference was determined by post hoc testing using Fisher’s protected least significant difference test.
Results

Effect of oral charcoal adsorbent treatment on bone turnover

The body weights at the beginning of AST-120 administration were 442.0 ± 33.6 g in TPTx group and 427.6 ± 42.3 g in TPTx-Nx group; and the body weights at endpoint sacrifice were 618.0 ± 58.4 g in TPTx group, 508.0 ± 39.4 g in vehicle group, and 536.2 ± 59.5 g in AST group. Despite similar feed consumption, AST-120 treatment appeared to ameliorate suppressed weight gain in TPTx-Nx rats. As shown in Table 2, significant increases of serum creatinine, urea nitrogen and inorganic phosphate were observed in TPTx-Nx rats. Creatinine clearance was significantly decreased in TPTx-Nx rats compared with TPTx rats. The changes of these parameters were dependent on the time after nephrectomy. At experiment endpoint (12 weeks after second Nx), accumulation of IS in the serum was significantly increased in TPTx-Nx rats treated with vehicle. This increase was suppressed by oral charcoal adsorbent (AST-120) treatment. Treatment with oral charcoal adsorbent had no effect on renal function (Table 2), serum 1,25(OH)2D3 (42 ± 19 pg/ml in TPTx rats, 43.2 ± 13 pg/ml in TPTx-Nx rats treated with vehicle, and 46 ± 13 pg/ml in TPTx-Nx rats treated with AST) or PTH (106 ± 59 pg/ml in TPTx rats, 104 ± 63 pg/ml in TPTx-Nx rats treated with vehicle and 104 ± 61 pg/ml in TPTx-Nx rats treated with AST) at endpoint sacrifice. On the other hand, AST-120 treatment significantly ameliorated the reduction of bone formation (Table 3). The mineral apposition rate in TPTx-Nx rats treated with vehicle was reduced to 80% of the TPTx group at endpoint. This reduction was protected by administration of AST-120. Figure 2 shows the changes in bone formation rate. While the rate decreased over time in TPTx-Nx rats treated with vehicle, AST-120 administration significantly improved the bone formation rate.

Table 2. Creatinine clearance and other serum biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>CCr (ml/min)</th>
<th>Cre (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Alb (mg/dl)</th>
<th>Ca (mg/dl)</th>
<th>P (mg/dl)</th>
<th>IS (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 7 weeks</td>
<td>2.8 ± 0.4</td>
<td>0.4 ± 0.1</td>
<td>20.2 ± 3.0</td>
<td>3.0 ± 0.2</td>
<td>9.6 ± 0.8</td>
<td>7.4 ± 1.0</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>TPTx 1.2 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>19.7 ± 1.2</td>
<td>3.1 ± 0.1</td>
<td>9.4 ± 0.4</td>
<td>7.4 ± 0.8</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>TPTx-Nx 1.8*</td>
<td>0.7 ± 0.1*</td>
<td>37.7 ± 3.2*</td>
<td>2.4 ± 0.3*</td>
<td>9.6 ± 1.6</td>
<td>9.2 ± 1.0*</td>
<td>0.22 ± 0.1*</td>
<td></td>
</tr>
<tr>
<td>TPTx 3.5</td>
<td>0.6 ± 1.3</td>
<td>22.3 ± 5.6</td>
<td>2.8 ± 0.2</td>
<td>9.5 ± 0.6</td>
<td>7.5 ± 1.7</td>
<td>0.04 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>TPTx-Nx vehicle 4.8</td>
<td>1.0 ± 0.5</td>
<td>48.4 ± 12.3*</td>
<td>2.2 ± 0.2*</td>
<td>9.5 ± 0.7</td>
<td>12.5 ± 1.9*</td>
<td>0.25 ± 0.18*</td>
<td></td>
</tr>
<tr>
<td>AST 2.4</td>
<td>1.1 ± 0.3*</td>
<td>1.0 ± 0.2*</td>
<td>48.2 ± 8.3*</td>
<td>2.2 ± 0.2*</td>
<td>9.6 ± 1.2</td>
<td>10.4 ± 2.4*</td>
<td>0.07 ± 0.06*</td>
</tr>
</tbody>
</table>

TPTx, thyroparathyroidectomy; Nx, partial nephrectomy; CCr, creatinine clearance; Cre, creatinine; BUN, blood urea nitrogen; Alb, albumin; Ca, calcium; P, phosphorus; IS, indoxyl sulphate. Baseline denotes at first Nx (time 0), and 7 and 13 weeks are the times from baseline. AST group was TPTx-Nx rats treated with the oral charcoal adsorbent AST-120 starting from 6 weeks after the second Nx. Vehicle group was TPTx-Nx rats treated with vehicle without AST-120.

*P < 0.05 vs TPTx.

Table 3. Effect of oral charcoal absorbent administration on bone formation parameters

<table>
<thead>
<tr>
<th></th>
<th>Ob.S/BS (%)</th>
<th>OS/BS (%)</th>
<th>MAR (µm/d)</th>
<th>MS/BS (%)</th>
<th>BFR/BS (µm/µm²/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 7 weeks</td>
<td>20.5 ± 4.8</td>
<td>26.6 ± 12.2</td>
<td>1.5 ± 0.1</td>
<td>51.4 ± 11.9</td>
<td>27.4 ± 2.9</td>
</tr>
<tr>
<td>TPTx 17.3</td>
<td>21.1 ± 11.6</td>
<td>1.4 ± 0.2</td>
<td>48.6 ± 7.0</td>
<td>24.8 ± 7.3</td>
<td></td>
</tr>
<tr>
<td>TPTx-Nx 5.6</td>
<td>5.3 ± 1.8</td>
<td>0.6 ± 0.1*</td>
<td>26.8 ± 7.0*</td>
<td>6.9 ± 2.2*</td>
<td></td>
</tr>
<tr>
<td>TPTx 15.3</td>
<td>19.1 ± 3.5</td>
<td>1.3 ± 0.1</td>
<td>38.0 ± 12.4</td>
<td>17.2 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>TPTx-Nx vehicle 2.5</td>
<td>6.2 ± 1.4*</td>
<td>0.3 ± 0.2*</td>
<td>10.8 ± 5.3*</td>
<td>2.4 ± 1.7*</td>
<td></td>
</tr>
<tr>
<td>AST 3.8</td>
<td>1.2 ± 2.8*</td>
<td>1.1 ± 0.3*</td>
<td>29.1 ± 6.1*</td>
<td>11.7 ± 2.4*</td>
<td></td>
</tr>
</tbody>
</table>

Ob/S/BS, osteoblast surface per bone surface; OS/BS, osteoid surface per bone surface; MAR, mineral apposition rate; MS/BS, mineralized surface per bone surface; BFR/BS, bone formation rate per bone surface. AST group was given oral charcoal absorbent (AST-120) 6 weeks after the second Nx, and vehicle group received only vehicle and no AST-120.

*P < 0.05 vs TPTx group.

*P < 0.05 vs vehicle group.
Effect of oral charcoal adsorbent treatment on gene expression

To determine the inhibitory effect of UTx accumulation on the expression of genes associated with bone turnover, we performed RT–PCR using RNA samples extracted from the proximal metaphysis. Gene expression of parathyroid/parathyroid-related peptide hormone receptor (PTHR), alkaline phosphatase (ALP) and osteocalcin (OC) was down-regulated in the TPTx-Nx rats given vehicle compared with rats receiving TPTx only. Treatment with oral adsorbent almost reversed the down-regulation of these genes (Figure 3A and B).

Expression of OATs in rat tibia

Since AST administration was found to inhibit lowered bone turnover and decrease blood IS concentration, in order to obtain an insight into the molecular mechanisms of IS on bone formation, rat OAT-1 and OAT-3 expression was determined by RT-PCR using RNA extracted from rat tibia. Expression of OAT-3 was observed, whereas no expression of OAT-1 was detected in rat tibia (Figure 4).

Discussion

In our previous study, we confirmed that bone turnover decreased depending on renal function in uraemic rat without secondary hyperparathyroidism [13]. That study suggests a possibility that UTx accumulated in blood may be associated with the
Our results demonstrated marked increase of serum IS dependent on the duration of renal insufficiency in our model rats. However, the high serum IS concentration was decreased remarkably by AST-120 administration, showing that AST-120 prevents accumulation of IS in blood. At the same time, AST-120 administration suppressed the reduction of osteoblast surface, mineral apposition rate and bone formation rate in TPTx-Nx rats. Furthermore, RT-PCR using RNA extracted from tibia demonstrated that, while the expression of PTH receptor and bone formation-related genes was down-regulated in TPTx-Nx rats treated with vehicle, the down-regulation was almost reversed by AST-120 treatment. These results suggest that IS accumulation in blood might induce osteoblast dysfunction and that AST-120 treatment might ameliorate the dysfunction by preventing accumulation of IS in the circulation.

As seen in Table 2, we observed no suppression of renal dysfunction by AST-120 administration under the conditions of the present study. Niwa et al. [10] reported that, in animals with renal failure that were administered AST-120, serum IS concentration decreased even before a clear reduction of serum creatinine and blood urea nitrogen was observed. Our results may support their findings. Therefore, our observations suggest that at least in vivo IS causes down-regulation of osteoblast-related genes in bone and induces low bone turnover even before it accelerates renal dysfunction. Our results also suggest that AST-120 treatment starting from the early stage of renal dysfunction may be effective in preventing the development of low bone turnover.

On the other hand, OAT-1 and -3 are known to be molecules that have high affinity to IS and transport IS into cells [14–18]. It is reported that intracellular IS increases oxidative stress and causes cell dysfunction in renal tubular cells [14,16]. From these findings, we hypothesized that osteoblast dysfunction is associated with OAT that mediates IS uptake into cells, and examined whether OAT is present in bone by RT-PCR. We confirmed that OAT-3 gene is expressed in the tibia of rats. Although whether OAT-3 exists in osteoblasts cannot be ascertained in the present study, we can however conclude that OAT-3 exists at least in the bone cells because we extracted the RNA samples using the proximal tibia after removing the bone marrow. The functional dysfunction caused by IS reflects the data obtained from histomorphometric measurements. Further studies using cultured osteoblasts are necessary to elucidate how IS causes osteoblast dysfunction.

Addition of serum obtained from dialysis patients to osteoblastic cell cultures decreased response to PTH through down-regulation of the PTH receptor gene in osteoblastic cells [19] and reduced secretion of interleukin-6 from osteoblastic cells [20]. In these reports, however, the exact substances that induce osteoblast dysfunction were not identified. UTx including IS are candidates of the substances in uraemic sera that impair osteoblastic function. Identification of these substances may have therapeutic implication.

In conclusion, the present study demonstrates that administration of AST-120 is effective to protect against the development of low bone turnover in renal failure. This effect may be a result of the prevention of UTx accumulation in blood by AST-120. Accumulation of IS, which is one of the UTx, in blood is known to cause renal dysfunction by inducing renal tubular injury. This study suggests that accumulation of IS or other indolic compounds also lowers bone formation and decreases bone turnover in uraemic rats, probably by inducing impairment of osteoblastic function. In vitro studies using indole metabolites and various uraemic toxins other than IS will clarify whether the osteoblast cytotoxicity is IS-specific. Further studies are also required to clarify the mechanisms of UTx in inducing osteoblastic dysfunction and identify specific uraemic toxins with this effect, which may provide insight to the pathogenesis and low-turnover bone progression in uraemia.

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Conflict of interest statement. None declared.

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