In Vivo Kinetics of Indoxyl Sulfate in Humans and Its Renal Interaction with Angiotensin-Converting Enzyme Inhibitor Quinapril in Rats

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ABSTRACT

Indoxyl sulfate (IS) is an organic anion uremic toxin that accumulates in patients with chronic kidney disease (CKD). The aims of this study were to examine the kinetic profiles of IS in humans at a steady state after multiple doses of L-Trp, a precursor of IS, and the in vivo interaction of IS with the angiotensin-converting enzyme inhibitor quinapril, whose active metabolite is a substrate of organic anion transporter 3 (OAT3) in rats. First, 12-h kinetics after single doses of Trp (2, 4, and 8 g) were examined in two healthy volunteers. Second, 24-h kinetics after a single dose of 2 g of Trp was studied in six volunteers. Third, 35-h kinetics after single and multiple doses of 2 g of Trp were examined in five volunteers. In anesthetized rats, quinapril or probenecid, an inhibitor of OATs, was given intravenously before IS, and blood and urine samples were taken until 90 min. Trp and IS concentrations were determined by high-performance liquid chromatography. Ultrafiltration was used to measure serum unbound IS concentrations. Renal tubular secretion of IS accounted for more than 90% of its renal clearance in the steady state of serum IS levels after multiple doses in humans. In animals, the serum area under the curve of IS increased in conjunction with a decrease in renal clearances after coadministration of IS with quinapril or probenecid. It is concluded that quinapril may inhibit the urine excretion of IS via OAT3-mediated renal tubular transport in patients with CKD.

Introduction

Many metabolic products accumulate in patients with CKD, and those that are harmful to organs are called uremic toxins. Uremic toxins are classified into 3 groups: free water-soluble low-molecular-weight solutes, middle molecules, and protein-bound solutes (Vanholder et al., 2003). IS, one of the major uremic toxins, is characterized as a protein-bound solute and is a substrate of OAT1, OAT3, and OAT4 (Enomoto et al., 2003). IS has been found to inhibit the albumin binding of anionic drugs in vitro (Bowmer and Lindup, 1982; Mabuchi and Nakahashi, 1988), and its intracellular uptake was mostly inhibited by organic anion drugs in the rat kidney (Deguchi et al., 2004). Therefore, concomitant use of drugs that have high protein binding and are substrates or inhibitors of OATs may cause the accumulation of IS in patients with CKD. It has been demonstrated that the plasma protein binding of organic anion drugs was decreased, increasing the fraction unbound ($F_{un}$) of organic anion drugs, in chronic renal failure when GFR is less than 15 ml/min/1.73 m$^2$ (Dreisbach and Lertora, 2003).

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ABBREVIATIONS: CKD, chronic kidney disease; IS, indoxyl sulfate; OAT, organic anion transporter; GFR, glomerular filtration rate; Thio, sodium thiosulfate; PAH, sodium para-aminohippurate; RPF, renal plasma flow; AUC, area under the curve; HPLC, high-performance liquid chromatography.
contribution of renal active transport to urine excretion. Because serum levels of IS in people with normal renal function are low, L-Trp, a precursor of IS, was administered to healthy volunteers to increase the serum IS levels (Niwa and Ise, 1994). IS is produced primarily through degradation of Trp to indole by enterobacteria, and indole is subsequently oxidized and then conjugated with sulfate in the liver (Smith and Macfarlane, 1996). In this study, we examined the kinetics of IS under a steady state after multiple doses of L-Trp. Second, pharmacokinetic interactions were investigated in rats by intravenous administration of organic anion drugs and IS. In these experiments, the serum IS levels were set to be similar to those in the above-mentioned human study. Quinapril, an angiotensin-converting enzyme inhibitor, whose active metabolite quinaprilat is a substrate of OAT3 and protein-binding rate in plasma is high along with quinaprilat (approximately 97% in humans), was used as a probe drug (Yuan et al., 2009). Probenecid, an inhibitor of intracellular IS uptake (approximately 97% in humans), was used as a probe drug (Yuan et al., 2009).

Materials and Methods

Human Study

Subjects. The subjects were six healthy male volunteers. Two, six, and five volunteers participated in the first, second, and third protocol, respectively. Each volunteer is identified by a unique number in the text. The age, body weight, and body mass index of the subjects are presented in Table 1. Only subjects whose serum creatinine level was within the normal range were included in the study. Subjects were excluded for any of the following reasons: an active disease requiring treatment, clinically abnormal laboratory tests, a history of allergy, a psychiatric disease, drug abuse, digestive tract disease requiring treatment, a positive result for human immunodeficiency virus antigen or antibody, hepatitis B surface antigen, hepatitis C antibody, or a positive serological test for syphilis, a blood donation of 200 ml within 4 weeks or 400 ml within 12 weeks before the study, and participation in other clinical studies within 3 months before the present study. All subjects gave written informed consent before the study began. The studies were approved by the Institutional Review Board of Kitasato University East Hospital and were conducted at the Clinical Trial Center of Kitasato University East Hospital.

Treatment and Formulation. Trp was provided by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) as a powder of L-Trp, which is used as a food additive and is listed in the Japanese Pharmacopoeia, Fifteenth Edition. Enteric-coated capsules filled with Trp (333.3 mg L-Trp/capsule) were obtained from Sunsho Pharmaceutical Co., Ltd. (Shizuoka, Japan). Sodium thiosulfate (Thio) and sodium para-aminohippurate (PAH) were purchased from Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan) and Daiichi Sankyo Company, Ltd. (Tokyo, Japan), respectively.

Study Design. The study consisted of three separate protocols (Table 2). First, a single oral dose of L-Trp was administered to two fasting volunteers at 8:00 AM on day 1 at escalating doses of 2, 4, and 8 g with a 2-week washout period between each dose. The second protocol, a single oral dose of L-Trp, was administered to six volunteers at 8:00 AM 30 min after breakfast on day 1 at a dose of 2 g. The third protocol, a sequential two-phase study, consisted of single and multiple doses with an 11-day washout period. In the single-dose study, L-Trp at a dose of 2 g was administered at 10:00 PM on day 1. In the multiple-dose study, L-Trp at a dose of 2 g was administered at 10:00 PM daily from day 1 to day 8. A renal function test was conducted during the washout period.

Low-Trp diets were consumed on day 1 and day 1 in the first protocol, on day 1 and day 2 in the second protocol, and on day 1, day 2, day 8, and day 9 in the third protocol (Table 3). Blood and urine samples were collected at 0, 2, 3, 4, 6, 8, and 12 h after administration of L-Trp and every 2 h for 12 h, respectively, in the first protocol. Samples were collected at 0, 4, 6, 8, 10, 12, 14, 16, and 24 h after administration of L-Trp and every 2 h for 24 h, respectively, in the second protocol. In the single-dose study of the third protocol, blood samples were collected at 0, 8, 11, 14, 15, 20, 23, 26, 32, and 35 h after administration of L-Trp, and urine was collected for 0 to 8, 8 to 14, 14 to 20, 20 to 26 and 26 to 35 h. In the multiple-dose study, blood samples were collected in the evening on day 1, 4, and 6 before each administration of L-Trp and at 0, 8, 11, 14, 15, 20, 23, 26, 32, and 35 h after administration of L-Trp. Urine was collected in a manner similar to that for the single-dose study. Blood was kept at room temperature, and serum was separated from blood by centrifugation at 4000 g for 15 min. Serum and urine samples were stored at −20°C until the analysis.

Renal Function Tests. Renal function tests were performed 8 days after the single dose of L-Trp in the second protocol, in which the washout period was thought to be long enough for the elimination of IS. In brief, 80 ml of 10% Thio solution and 12 ml of 10% PAH were given for 10 min by an intravenous drip injection 30 min after intake of 500 ml of water. The subjects urinated 25 min after the injections, and this urine was discarded. Then, urine was collected for 30 min. Blood samples were collected at 10 and 20 min after urination. The clearances of Thio and PAH were calculated as eq. 1:

\[ C_{\text{Thio or PAH}} = \frac{U_{\text{Thio or PAH}}}{P_{\text{Thio or PAH}}} \times V \times 1.48 / A \]  

where \( C_{\text{Thio or PAH}} \) is the clearance of Thio or PAH, \( U_{\text{Thio or PAH}} \) is the urine concentration of Thio or PAH, \( P_{\text{Thio or PAH}} \) is the plasma concentration of Thio or PAH, \( V \) is the urine flow rate, and \( A \) is the surface area of the body.
TABLE 3
Energy and amounts of Trp in low-Trp diets

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Single or Multiple Doses of L-Trp</th>
<th>Days after Dose of L-Trp</th>
<th>Energy</th>
<th>Trp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>kcal</td>
<td>mg</td>
</tr>
<tr>
<td>1</td>
<td>Single dose</td>
<td>–1</td>
<td>2400</td>
<td>484</td>
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<td></td>
<td>1</td>
<td>1590</td>
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</tr>
<tr>
<td>2</td>
<td>Single dose</td>
<td>1</td>
<td>1705</td>
<td>296</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>898.0</td>
<td>178</td>
</tr>
<tr>
<td>3</td>
<td>Single dose</td>
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<td>2402</td>
<td>480</td>
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<td></td>
<td></td>
<td>2</td>
<td>1652</td>
<td>399</td>
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<tr>
<td></td>
<td>Multiple doses</td>
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<td>2310</td>
<td>460</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>1669</td>
<td>367</td>
</tr>
</tbody>
</table>

where \( C_{\text{Thio or PAH}} \) (milliliters per minute) is the clearance of Thio or PAH, \( U_{\text{Thio or PAH}} \) (milligrams per deciliter) is the urine concentration of Thio or PAH, \( P_{\text{Thio or PAH}} \) (milligrams per deciliter) is the calculated serum concentration of Thio or PAH at the middle time of two measured points, \( V \) (milliliters per minute) is urine volume per minute, 1.48 is a standard body surface area for Japanese (square meters), and \( A \) is body surface area. \( A \) is calculated using eq. 2:

\[
A = \text{body weight}^{0.425} \times \text{height}^{0.725} \times 0.007271
\]

\( C_{\text{Thio}} \) and \( C_{\text{PAH}} \) refer to glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively.

**Kinetic Parameters.** Mean \( C_{\text{max}}, T_{\text{max}}, \) and \( AUC_{0–\infty} \) were obtained from the serum concentration-time curves of IS for individuals in the single- and multiple-dose studies in the second protocol. Urinary excretion rates of Trp and IS were calculated as a percentage of urine excretion to the administered dose of L-Trp. Percentage of \( F_u \) of IS in the serum was calculated as the ratio of \( AUC_{0–\infty} \) for unbound IS to that for total IS. Renal clearance of IS (eq. 3) was obtained from the urine excretion of IS for 35 h divided by \( AUC_{0–\infty} \) of IS. The clearances of renal tubular secretion (CLTS) and extraction rate of IS by the renal tubules (ETS) were calculated as eqs. 3 and 4:

\[
\text{CL}_\text{T} = \frac{\text{CL}_\text{R} \times \text{GFR} \times F_u}{1 - \text{CL}_\text{R} / \text{RPF}}
\]

\[\text{ETS} = \text{CL}_\text{T} / \text{RPF} \]

The ratio of CLT to \( F_u \cdot \text{GFR} \), a measure indicating the renal elimination pattern of a drug, was also calculated. When the value is more than 1, it is inferred that active secretion is apparent in the renal elimination of a drug.

**Animal Study.**

Animals. Seven-week-old male LEW/CrlCrlj rats (Oriental Yeast Co., Ltd., Tokyo, Japan) were housed in our animal care facility under constant humidity and temperature and a 12-h light/dark cycle. The animals were maintained on a certified diet of MF pellets under constant humidity and temperature and a 12-h light/dark cycle. The animals were maintained on a certified diet of MF pellets under constant humidity and temperature and a 12-h light/dark cycle.

Reagents. IS potassium salt, quinapril, and probenecid were purchased from Sigma-Aldrich (St. Louis, MO). IS was dissolved in saline at concentrations of 0.5 and 0.05%. Quinapril was dissolved in saline at 0.2%. Probenecid was first dissolved in 1 M NaOH solution and then diluted with saline and titrated at pH 7.9 to pH 8.1 by a diluted HCl solution at a concentration of 5%. Saline was also prepared at a pH similar to the above range as the vehicle control for probenecid treatment.

Study Design. Rats were initially anesthetized with pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL) at a dose of 65 mg/kg i.p. For blood sampling with time, an Intramedic dilute HCl solution at a concentration of 5%. Saline was also prepared at a pH similar to the above range as the vehicle control for probenecid treatment. A tube inserted into the blood vessels was filled with saline solution containing heparin. Blood samples were collected via a tube before administration of the coadministered drugs and at 5, 15, 30, 45, 60, and 90 min after administration of IS. Total blood sampling volume was approximately 2 ml. A coadministered drug such as quinapril or probenecid was given 2 min before administration of IS. All drugs were given intravenously. The urinary bladder was cannulated through a small abdominal skin incision using a polytetrafluoroethylene tube (feeding needles; Fuchigami Ltd., Kyoto, Japan). Urine was collected for 90 min. Doses of quinapril after IS were set at 2 and 0.5 mg/kg, respectively, molar doses of which were comparable (4.2 and 2.3 μmol/kg, respectively). Doses of probenecid after IS were set at 50 and 5 mg/kg, respectively, by referring to the previous study, in which rats were coadministered IS and probenecid at 10 mg/kg (46.9 μmol/kg) and 50 mg/kg (175 μmol/kg), respectively, to investigate the pharmacokinetic interaction (Deguchi et al., 2003). There were 14 rats in the IS administration groups with and without quinapril, respectively, and 4 and 5 rats in IS administration groups with and without probenecid, respectively.

**Kinetic Parameters.** Mean \( AUC_{0–\infty} \) values were obtained from the serum concentration-time curves of IS and unbound IS. \( C_{\text{L}} \) was obtained from the total IS amount in urine for 90 min divided by the \( AUC_{0–\infty} \) of IS.

**High-Performance Liquid Chromatography.**

Reagents. Acetonitrile and methanol were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan), and indoxyl sulfate potassium salt was purchased from Sigma-Aldrich.

Sample Preparation. Aliquots of serum were divided into two portions, one of which was used to determine the total concentrations of Trp and IS and the other to measure \( F_u \) of IS. The samples were prepared as described previously (Lagana et al., 1986). For determination of the former, 200 μl of methanol was added to 100 μl of serum. After vortex mixing, the mixture was kept for 1 h at 4°C followed by centrifugation at 6000 g for 20 min. The resulting supernatants were filtered through a 0.45-μm cellulose acetate filter (Advantec DISMIC-13cp; Advantec Toyko Kaisha, Ltd., Tokyo, Japan) to remove large insoluble particles. For the latter, 500 μl of serum was ultrafiltered through a MICROCON YM-10 membrane (Millipore Corp., Billerica, MA) and left for 30 min at room temperature followed by centrifugation at 6000 g for 20 min. In both cases, 20- and 10-μl samples were injected. Urine was filtered through a 0.45-μm filter to remove large insoluble particles, and a 5-μl sample was injected.

Trp and IS Assay. Trp and IS concentrations in serum and urine were quantified by HPLC. The chromatograph assembly (Waters 600 controller) consisted of a Waters 600 pump, Waters 717 plus autosampler, JASCO FP-920 Intelligent Fluorescence Detector, and a reverse-phase column (Develosil column; Nomura Chemical Co., Ltd., Seto, Japan) with a guard column. The mobile phase consisted of solution A (distilled water-acetic acid, 100:0.08, v/v) and solution B (acetonitrile-acetic acid, 100:0.08, v/v) was delivered at a flow rate of 1 ml/min at ambient temperature. The mobile phase was linearly programmed from 100% of solution A to 100% of solution B within 20 min and then to 100% of solution A within 1 min and was maintained for 9 min before the next injection. The eluate was monitored by detection of fluorescence at an excitation wavelength of 295 nm and an emission wavelength of 390 nm. Calibration was obtained using standards of 10.2 and 10.7 μg/ml Trp and IS, respectively. The intra-assay and interassay coefficients of variation were 0.9 and 4.3% for Trp at a concentration of 3.4 μg/ml, 0.7 and 1.9% for IS at a concentration of 3.6 μg/ml, and 8.9 and 9.8% for IS at a concentration of 0.036 μg/ml, respectively. Regarding the unbound fraction of IS, the intra-assay and interassay coefficients of variation were 0.8 and 2.1% for IS at a concentration of 11 μg/ml and 4.3 and 8.3% for IS at a concentration of 0.11 μg/ml IS. Linearity of the standard curves was obtained at ranges from 0.16 to 10 μg/ml for Trp and 0.0083 to 11 μg/ml for IS.
**Data Analysis**

The results are expressed as the mean ± S.D. in the human study and as the mean ± S.E. in the animal study. Statistical comparisons of the kinetic variables were performed using the paired \( t \) test in the human study and the unpaired \( t \) test in the animal study. Differences in the serum concentrations of IS with time due to administration of IS alone and with coadministration of quinapril or probenecid were assessed using repeated-measures analysis of variance with the post hoc Fisher protected least significant difference test or Scheffé test, respectively. The level of statistical significance was \( P < 0.05 \) (two-sided).

**Results**

Changes in Total Concentrations of Trp and IS in Serum and Excretion Rates of Trp and IS in Urine after Single, Ascending Doses of L-Trp after Low-Trp Diets. Figure 1 presents individual data for the changes in total concentrations of Trp (Fig. 1, A–C) and IS (Fig. 1, D–F) in serum for 12 h after L-Trp administration at doses of 2, 4, and 8 g. Trp increased in the blood early after administration. At the lower two doses, Trp reached a maximum 2 h after administration and the increases were dose-dependent in both subjects. Trp levels had decreased to baseline by 12 h. The increases in Trp were biphasic for the highest dose of L-Trp. IS levels increased gradually in both subjects after administration of 2 and 4 g L-Trp. No increases in IS were observed with 8 g L-Trp in either subject. Figure 2 shows the urinary excretion rates of Trp (A) and IS (B) after single escalating doses of L-Trp. Two subjects were administered L-Trp at doses of 2, 4, and 8 g in a fasting state in the morning. Urine sampling was conducted until 12 h after administration. The urinary excretion rates of Trp and IS were calculated as a percentage of the amounts of Trp and IS in the collected urine relative to L-Trp administered.
Fig. 4. Concentration-time changes in total concentrations of IS in serum after single (○) and multiple (●) doses of L-Trp. Subjects 1 (A), 2 (B), 3 (C), 7 (D), and 8 (E) were administered L-Trp at a single dose of 2 g after multiple doses of 2 g daily for 8 days in a nonfasting state at night, with an 11-day interval between the single and multiple doses. Subject 8 was withdrawn after the single-dose study because of an incidental headache; therefore, only the single-dose data are shown for subject 8 (E). Blood sampling was conducted until 35 h after the single dose (●) and after the last dose in the multiple-dose study (○). It was also conducted on days 1, 4, and 6 before each dose in the multiple-dose study (★). The total concentrations of IS in serum were determined by HPLC. The subjects started to consume a low-Trp diet on the day of single administration of L-Trp and on the last ad-

Comparison of Changes in Total Concentrations of IS in Serum and Urinary Excretion Rates of Trp and IS between Single and Multiple Doses of L-Trp after Low-Trp Diets. Figure 4 shows the individual data for changes in total serum concentrations of IS in the single- and multiple-dose studies. Subject 8 was withdrawn from the multiple-dose study because of treatment for a moderate headache accompanying nausea during the single-dose study. Therefore, only data from the single-dose study are displayed for this subject (Fig. 4E). Serum concentrations of IS increased in four subjects after multiple doses compared with a single dose, although the increase was small in subject 4 (Fig. 4D). In both the single- and multiple-dose studies, the elevation of serum IS concentrations gradually decreased toward the basal levels within each observation period. Large intrapersonal differences in IS were also demonstrated after the single (2.1–6.6%) and multiple doses (2.8–8.4%), respectively (Fig. 5). Kinetic Parameters of IS after Single and Multiple Doses of L-Trp. Table 4 summarizes the kinetic parameters of IS after single and multiple doses of L-Trp and the results of renal function tests. Data for subject 5, who was withdrawn after the single dose study, were not included in the

Changes in Total Concentrations of IS in Serum and Excretion Rates of Trp and IS in Urine after Single Dose of L-Trp after Uncontrolled Diets. Figure 3 shows the individual data for changes in serum concentrations of IS (Fig. 3A) and for urinary excretion rates of Trp and IS (Fig. 3B) for 24 h after a single dose of L-Trp. The baseline concentrations of total IS in serum were higher in all subjects than those in the first protocol (Fig. 1, D–F). An apparent increase in IS concentrations in serum was not observed in the subjects except for subject 2. As shown in Fig. 3B, large interindividual differences in urinary excretion rates of IS were demonstrated after administration of L-Trp. The urinary excretion rates of Trp and IS ranged from 1.1 to 2.5 and 1.8 to 8.1%, respectively.

Comparison of Changes in Total Concentrations of IS in Serum and Urinary Excretion Rates of Trp and IS between Single and Multiple Doses of L-Trp after Low-Trp Diets. Figure 4 shows the individual data for changes in total serum concentrations of IS in the single- and multiple-dose studies. Subject 8 was withdrawn from the multiple-dose study because of treatment for a moderate headache accompanying nausea during the single-dose study. Therefore, only data from the single-dose study are displayed for this subject (Fig. 4E). Serum concentrations of IS increased in four subjects after multiple doses compared with a single dose, although the increase was small in subject 4 (Fig. 4D). In both the single- and multiple-dose studies, the elevation of serum IS concentrations gradually decreased toward the basal levels within each observation period. Large intrapersonal differences in serum IS concentrations were displayed in subjects 3 and 7 during the multiple doses (Fig. 4, C and D). As was shown in Fig. 3B, large interindividual differences in urinary excretion rates of IS were also demonstrated after the single (2.1–6.6%) and multiple doses (2.8–8.4%), respectively (Fig. 5).

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rates of IS at doses of 2 and 4 g did not show dose-dependent increases within the observation period.
analysis. Both mean $C_{\text{max}}$ and AUC$_{0-35}$ increased by 1.4-fold after multiple doses compared with values after a single dose, although the changes were not significant. The $F_u$ values for IS in serum after single and multiple doses were both small. $C_{\text{RT}}$ significantly decreased after the multiple doses compared with $C_{\text{RT}}$ after the single dose. More than 90% of the total renal clearance undergoes tubular secretion as indicated by the rates of $CL_{\text{T}}$ to $CL_R$ for IS. The net renal tubular secretion of IS was shown to be approximately half of the GFR. The ratios of $CL_R$ to $F_u \cdot GFR$ greatly exceeded 1 and the ETS values were low.

**Safety of L-Trp in the Human Study.** No adverse events were observed with a single dose of 2 g L-Trp in the first and second protocols. Mild fuzzy headache and sleepiness developed in subject 1 from 2 to 10 h after administration of 4 g L-Trp. After administration of 8 g L-Trp in subject 1, dull headache and sleepiness, diarrhea, and nausea occurred at 20 min, 1 h, and 2.5 h after administration, respectively, and all of these symptoms except the diarrhea disappeared by 5 h after administration. The diarrhea improved the next morning. These adverse events were mild. In subject 2, muddle, sleepiness, and nausea occurred 20 min after administration. Postural discomfort and paleness occurred from 4 to 6 h after administration. The subject was rested in a supine position because of these adverse events. Muddle, sleepiness, and nausea disappeared 7, 6, and 8 h after administration, respectively. Diarrhea appeared 4 h and 1 day after administration and resolved by the morning 2 days after administration. The nausea and postural discomfort and paleness were moderate and other effects were mild. In the single- and multiple-dose study, a headache accompanying nausea was reported in subject 5 after the single dose, and the subject took ibuprofen at 21.5 h. Because the subject experienced a headache and nausea before participation in the study, it is unlikely that they were related to study drug administration.

**Pharmacokinetic Interactions of IS with Quinapril in Rats.** As shown in Fig. 6, the serum concentrations of IS gradually decreased with time after intravenous administration of IS. The concentrations at time $-2$ min were baseline values of endogenous IS before administration of quinapril or its vehicle. The decrease was suppressed by coadministration of quinapril. With respect to the kinetic parameters of IS, the AUC$_{0-90}$ increased and $CL_R$ decreased in the quinapril coadministered group compared with the IS alone group (Table 5). The $F_u$ of IS was also low in rats, but the protein-binding rates did not differ between the IS alone group and quinapril
administered by injection into the distal ileum and cecum in rats.

above, AUC0–90 increased and CLR decreased after coadministration of IS with time after administration of IS with and without IS after multiple doses of L-Trp in humans and the pharmacokinetic interactions between IS and organic anion drugs in rats. The percentage of conversion of L-Trp to IS could be less than 10% as indicated by the 35-h urinary excretion rate of IS after multiple doses of L-Trp (Fig. 5B), assuming that 100% indole was absorbed into the blood. A higher conversion rate was demonstrated in healthy volunteers when L-Trp was administered by injection into the distal ileum and cecum through intestinal, after which the 24-h urinary excretion rate of IS was nearly 14% (Bryan, 1966).

The biphasic increases in Trp at the highest single dose may be due to suppression of intestinal absorption, because nausea was present in both subjects during the times that corresponded to the phase of the decreased concentrations of Trp (Fig. 1C). The serum IS concentrations gradually increased, followed by an increase in Trp up to the single administration of 4 g L-Trp; however, dose proportionality was not described because of an insufficient observation period. Thus, the observation period was extended to 24 h in the second protocol. However, the concentration-time curves of IS in serum were not reproduced as was demonstrated in the first protocol (Fig. 3A). This failure was probably due to insufficient dietary control of Trp intake. Indeed, the baseline concentrations of IS in serum were higher in the second protocol than in the first (Figs. 1, D–F, and 3A). The subjects started the low-Trp diet 24 h before and were fasted for 12.5 h before administration of L-Trp in the first protocol, whereas the subjects started the low-Trp diet just before administration of L-Trp and were not fasted before administration of L-Trp in the second protocol. We also measured the concentrations of indole in serum and urine to investigate possible mechanisms for the delay in the appearance of IS in serum, and no peaks were detected at the retention time for indole (16.71 min, data not shown). These results suggest that indole is rapidly conjugated with sulfuric acid in the liver, and a delay in the appearance of IS in serum may reflect the time required to reach L-Trp at the end of the intestine. Taking into consideration the fact that dietary Trp affects on the baseline concentrations of IS and serum IS appeared late after administration of L-Trp, we modified the design of the third protocol. The low-Trp diet was started 14.5 h before administration of L-Trp, the time for administration of L-Trp was moved forward from in the morning to at night, and the observation period was extended from 24 to 35 h.

Serum IS increased in each subject after multiple doses of L-Trp compared with that after a single dose; however, the increases in mean $C_{\text{max}}$ and AUC0–35 were not significantly different. This was probably due to a small number of the subjects and still higher baseline concentrations of IS compared with those in the first protocol. Thus, a desirable washout treatment before administration of L-Trp may be the 24-h low-Trp diet and 12.5-h fasting, which were adopted in the first protocol.

The decrease in CLR of free IS after multiple doses of L-Trp suggests that the renal active transport of IS was saturated at a higher concentration of IS in the blood. When the starting points of the increase in serum IS levels, focusing on subject 1 and 2, were evaluated, they were delayed after administration at night compared with those in the morning (Figs. 1 and

### Table 5

Kinetic parameters of IS coadministered with or without quinapril in animal studies

<table>
<thead>
<tr>
<th></th>
<th>IS alone</th>
<th>IS + quinapril</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–90 (mg·min/ml)</td>
<td>236 ± 18</td>
<td>374 ± 59</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AUC0–90 (mg·min/ml)</td>
<td>28.7 ± 4.7</td>
<td>34.3 ± 5.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Fu (%)</td>
<td>13.3 ± 2.9</td>
<td>11.3 ± 2.2</td>
<td>0.58</td>
</tr>
<tr>
<td>CLR, ml·min⁻¹·kg⁻¹</td>
<td>1.2 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Table 6

Kinetic parameters of IS coadministered with or without probenecid in animal studies

<table>
<thead>
<tr>
<th></th>
<th>IS alone</th>
<th>IS + probenecid</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–90 (mg·min/ml)</td>
<td>1630 ± 135</td>
<td>3553 ± 197</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AUC0–90 (mg·min/ml)</td>
<td>225 ± 28</td>
<td>577 ± 60</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fu (%)</td>
<td>14.0 ± 1.8</td>
<td>16.5 ± 2.3</td>
<td>0.41</td>
</tr>
<tr>
<td>CLR, ml·min⁻¹·kg⁻¹</td>
<td>1.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Discussion**

The aims of the present study were to examine the kinetics of IS, focusing on the protein-binding rate and renal elimination pathway, in a steady state of serum concentrations of IS after multiple doses of L-Trp in humans and the pharmacokinetic interactions between IS and organic anion drugs in rats. The percentage of conversion of L-Trp to IS could be less than 10% as indicated by the 35-h urinary excretion rate of IS after multiple doses of L-Trp (Fig. 5B), assuming that 100% indole was absorbed into the blood. A higher conversion rate was demonstrated in healthy volunteers when L-Trp was administered by injection into the distal ileum and cecum.
4). These results may be due to a circadian rhythm of intestinal motility, which decreases at night and increases while awake (Rao et al., 2001). Mild increases in serum IS concentrations at 35 h may have been due to dietary Trp.

As was represented by the high values of $\text{CL}_{\text{R}}/F_\text{R} \cdot \text{GFR}$, a major pathway of renal elimination of IS was tubular secretion (Table 4). The elimination rates of IS by renal tubules were found to be low as expressed by the low values of ETS. According to the concept of drug clearance, the clearance of a drug with a low extraction ratio is influenced by the protein binding rate and intrinsic clearance (Rowland and Tozer, 1995). The intrinsic clearance of a drug is generally defined by metabolism and transport activities in the organ of elimination. These results suggest that IS may interact with organic anion drugs, which are highly protein bound and undergo renal tubular transport in vivo.

Animal experiments were conducted to examine the hypotheses stated above. The results indicate that the kinetic interaction of IS with quinapril resulted from the inhibition of renal elimination of IS, not from the inhibition of protein binding (Fig. 6; Table 5). The reason that probenecid interacted with IS at protein binding may be the relative differences in doses of the combined drugs, i.e., IS and probenecid were 5 and 50 mg/kg and IS and quinapril were 0.5 and 2 mg/kg, respectively, so that probenecid might easily displace the protein binding of IS. On the basis of Tables 5 and 6, the inhibitory effect of probenecid on $\text{CL}_{\text{R}}$ of IS seems to be more potent (approximately 85%) than that of quinapril (approximately 50%). Given that probenecid is a general inhibitor not only for OAT1, OAT3, and OAT4 but also for other organic anion transporters, whereas the active metabolite of quinapril is an inhibitor for OAT3, the results indicate that the clearance of IS may involve such organic anion transporters. It was suggested that OAT3 may contribute to the overall urine excretion of IS by nearly 50/85 = 100 = 59% in rats.

In both the human and animal studies, the serum IS concentrations were comparable to those in patients with low creatinine clearance (1.2 ± 1.1 µg/ml, mean ± S.E.; 30–80 ml/min, $n = 27$) (Niwa and Ise, 1994). Such patients are usually referred to as having mild and moderate CKD, in which the decreases in GFR are defined as 60 to 89 and 30 to 59 ml/min/1.73 m², respectively.

Finally, regarding the safety of L-Trp, sleepiness, headache, nausea, postural discomfort, and paleness and diarrhea were observed in the subjects administered L-Trp at doses more than 4 g accompanying the increases in Trp levels in serum. Similar adverse reactions to L-Trp have been also reported (Greenwood et al., 1975; Yuwiler et al., 1981; Lieberman et al., 1984). It was concluded that a single administration of L-Trp was tolerated up to 4 g.

In summary, the results of this study have demonstrated that IS was highly bound to serum protein and the major renal elimination route for IS was renal tubular secretion in humans. Kinetic interaction was demonstrated between IS and quinapril in rats, in which serum IS levels were comparable to those in the human study described above. It is concluded that urine excretion of IS, a major organic anion uremic toxin, may be inhibited by coadministration of the organic anion drug quinapril via renal tubular transport in patients with CKD with a less progressive stage.

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Authorship Contributions

**Participated in research design:** Fujita and Majima.

**Conducted experiments:** Fujita, Ishihara, Yasuda, Nakamura, Maeda, Kobayashi, Sahashi, and Ikeda.

**Performed data analysis:** Fujita and Kumagai.

Wrote or contributed to the writing of the manuscript: Fujita.

References


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