Organic Anion Transporting Polypeptides 1B1 and 1B3 Play an Important Role in Uremic Toxin Handling and Drug-Uremic Toxin Interactions in the Liver

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ABSTRACT - PURPOSE. Organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3 contribute to hepatic uptake of numerous drugs. Thus, reduced OATP1B1 and OATP1B3 activity in chronic kidney disease (CKD) may have a major impact on the hepatic clearance of drugs. The effect of drug-uremic toxin interactions on OATP1B1 and OATP1B3 has not been well studied. In the present study, we examine the inhibitory effects of uremic toxins on OATP1B1 and OATP1B3 transport activity to evaluate the interactions between drugs and uremic toxins in patients with chronic kidney disease. **METHODS.** [^{3}H]Estron-3-sulfate, [^{3}H]taurocholate uptake and [^{3}H]methotrexate by OATP1B1 and OATP1B3 expressing HEK293 cells were performed to evaluate the inhibitory effect of uremic toxins. To clarify whether the uremic toxins that interact with OATP1B1 and/or OATP1B3 were substrates for these transporters, we performed uptake studies. **RESULTS.** Four uremic toxins, kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol, inhibited OATP1B1- and OATP1B3-mediated transport in a concentration-dependent manner, with IC_{50} values of 180, 770, 2700, and 4600 μM, respectively, for OATP1B1 and 180, 1100, 1300, and 1700 μM, respectively, for OATP1B3. [^{3}H]Methotrexate uptake by OATPs was also inhibited by the four uremic toxins in a dose-dependent manner. Uptake studies revealed that kynurenic acid is a substrate for both the OATP1B1 and OATP1B3. Moreover, OATP1B3 was involved in the transport of indoxyl sulfate. Indole-3-acetic acid and *p*-cresol were not significantly transported by OATP1B1 and OATP1B3. **CONCLUSIONS.** We showed that some uremic toxins inhibit OATP-mediated uptake in a concentration-dependent manner, and clarified OATPs contribution to uremic toxin handling in the liver. Thus, we provided basic information to estimate the inhibitory effects of uremic toxins on OATPs in CKD patients. These data suggest that the dose of drugs excreted via renal and non-renal pathways should be carefully adjusted in CKD patients.

INTRODUCTION

Organic anion-transporting polypeptides (OATPs) are a family of sodium-independent organic anion transporters found in a variety of tissues, including the liver, kidney, intestine, and brain. OATPs contribute to the transport of bile acids, thyroid hormones, steroid conjugates, anionic oligopeptides, eicosanoids, and various drugs and xenobiotic compounds across membranes (1-3). OATP1B1 and OATP1B3 are members of the liver-specific subfamily of OATPs, which are localized to the sinusoidal membrane of hepatocytes, and transport a wide variety of clinically used drugs (4-7).

Chronic kidney disease (CKD) causes a negative cycle in which an accumulation of drugs and uremic toxins in blood leads to further impairment of the renal function. The Japanese Society for Dialysis Therapy recently reported that the number of CKD patients requiring artificial dialysis increases by five to ten thousand

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a year (8). In CKD patients, uremic toxins and drugs that are typically excreted in the urine accumulate in the body owing to decreased glomerular filtration rates and impairment of renal transporters, such as organic anion transporter (OAT) 1, OAT3, and OATP4C1 (9-13). Drugs excreted in the bile are also affected by the uremic condition of CKD patients (14-16). For example, plasma concentrations of rosuvastatin, which is primarily eliminated through the liver and is a substrate of OATP1B1, are increased 3-fold in patients with end-stage renal disease (creatinine clearance, CLcr, < 30 mL/min) than in those with healthy subjects (CLcr > 80 mL/min) (17). Plasma concentrations of tadalafil, which is primarily metabolized by CYP3A4, are also reported to be elevated in patients with end-stage renal disease (18).

OATPs regulate access to hepatocellular enzymes and transport into the bile canaliculi, and could be the rate-limiting step in the overall process of hepatic drug clearance (17, 19). Although OATPs play an important role in drug handling in CKD patients, few studies have addressed the effect of drug-uremic toxin interactions on these transporters (16, 20-23).

In the present study, we examined the inhibitory effects of uremic toxins on OATP1B1- and OATP1B3-mediated transport to estimate drug-uremic toxin interactions in CKD patients. Moreover, we tried to clarify which uremic toxins are transported by OATPs.

MATERIALS AND METHODS

Materials

Indoxyl sulfate, hippuric acid, creatinine, and *p*-cresol were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Indole-3-acetic acid was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 3-Carboxy-4-methyl-5-propyl-2-furan -propanoic acid (CMPF) was purchased from Cayman Chemical Co. (Ann Arbor, MI). *trans*-Aconitate, kynurenic acid, L-kynurenine, quinolinic acid (2,3-pyridinedicarboxylic acid), uric acid, and methylguanidine hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO). H-Arg (di-Me) -OH (asymmetrical, ADMA), H-Arg (di-Me) -OH (symmetrical, SDMA), and rifampicin were purchased from Enzo Life Sciences, Inc. (Ann Arbor, MI). Sodium creatine phosphate was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). [^{3}H]Estrone-3-sulfate (E_3S) (specific activity: 20-46.5 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). [^{3}H]Taurocholate (TCA) (specific activity: 5.0-10 Ci/mmol) and [^{3}H]indole-3-acetic acid (specific activity: 20 Ci/mmol) were purchased from Muromachi Yakuhin Co., Ltd. (Tokyo, Japan). [^{3}H]Methotrexate (MTX) (specific activity: 32.3 Ci/mmol) was purchased from Daiichi Clarity Co., Ltd. (Chiba, Japan). All other chemicals were commercially available and of the highest purity possible.

Cell Culture

Human embryonic kidney (HEK293) cells transduced with OATP1B1, OATP1B3, or an empty vector were previously established (3, 24). OATP1B1/HEK293, OATP1B3/HEK293 cells, and mock cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and G418 (0.5 mg/mL) under an atmosphere of 5% CO_2 and 95% air at 37° C.

Transport Studies

The cellular uptake in monolayer cultures grown on 24-well plates was measured. After washing once, the cells were preincubated in Krebs–Henseleit (KH) buffer (118 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM *N*-(2-hydroxyethyl) piperazine – *N* ′ - 2 - ethanesulfonic acid [HEPES], 5.0 mM D-glucose, and 1.53 mM $CaCl_2$, pH 7.4). Uptake was initiated by adding either substrates ([^{3}H]E_3S, [^{3}H]TCA and [^{3}H]MTX) or uremic toxins ([^{3}H]indole-3-acetic acid, kynurenic acid, indoxyl sulfate, and *p*-cresol). At the indicated times, uptake was terminated by replacing the uptake buffer with ice-cold KH buffer, and then washing two times with ice-cold KH buffer. Intracellular accumulation of radioactivity was measured using a liquid scintillation counter (Packard, 1600TR). Kynurenic acid, indoxyl sulfate, and *p*-cresol were measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The protein content of the solubilized cells was determined by the Bradford method using a Protein Assay kit (Bio-Rad Laboratories Inc., Hercules, CA) with bovine serum albumin as a standard.

Inhibitory Effects of Uremic Toxins

We calculated the IC_{50} value of the uremic toxins that inhibited OATP1B1 and OATP1B3 over 50%. The IC_{50} values were estimated using a nonlinear

regression analysis of the competition curves with a one-compartment model using the following equation: $V = 100 \times IC_{50} / (IC_{50} + [I]) + A$, where V is the transport amount (% of control), [I] is the concentration of uremic toxins, and A is the nonspecific transport (% of control) using software Origin 8 (Lightstone Corp., Tokyo, Japan).

Assay of Uremic Toxins by LC/MS/MS

Cells were scraped and homogenized in 250 µL of water for kynurenic acid analysis and 100 µL of water for indoxyl sulfate and *p*-cresol analyses. Cell lysates were deproteinized by adding equal volumes of acetonitrile. The mixture was vortexed and centrifuged at 12,000 × *g* for 10 min at room temperature. The supernatants were used directly for indoxyl sulfate and *p*-cresol measurement. For the measurement of kynurenic acid, the supernatant was evaporated at 40-60 °C, and the residue was reconstituted in 50 µL of mobile phase. Chromatographic separation was carried out using a Shimadzu Prominence 20A System (Shimadzu, Kyoto, Japan) with a Shiseido CAPCELL PAK C18 MGIII column (2.0 mm×50 mm, 3 µm). For the determination of kynurenic acid, the column was eluted with an isocratic flow of acetonitrile/water/acetic acid (30:70:0.1, v/v/v) at a flow rate of 0.3 mL/min. The injection volume was 5 µL. Indoxyl sulfate was eluted with a binary flow of acetonitrile and water at a flow rate of 0.2 mL/min. Acetonitrile increased from 5% to 60% in a linear gradient over 2 min and held until 5 min. Acetonitrile decreased to 5% from 5 min to 5.1 min and held until 8.1 min. The injection volume was 1 µL. *p*-Cresol was also eluted with a binary flow of methanol and water at a flow rate of 0.2 mL/min. Methanol was increased from 30% to 95% in a linear gradient over 3 min and held until 5 min. Then methanol was decreased to 30% from 5 min to 5.1 min and held until 9.1 min. The injection volume was 1 µL. The column temperature was kept at 40° C. Negative ion electrospray tandem mass spectrometric analysis was carried out using an API 3200 LC/MS/MS System at unit resolution with selected reaction monitoring (*m/z* 188 > 144 for kynurenic acid, *m/z* 212 > 79.8 for indoxyl sulfate, and *m/z* 107 > 76.9 for *p*-cresol). Data were acquired and analyzed using Analyst software (version 1.5) (Applied Biosystems) (Foster City, CA, USA)).

Statistical Analysis

Data are expressed as mean ± S.E.M. When appropriate, the differences between groups were tested for significance using the unpaired Student's t-test. Statistical significance was indicated by *p* values less than 0.05.

RESULTS

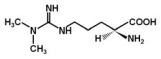
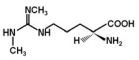
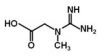
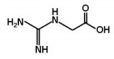
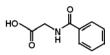
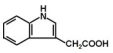
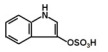
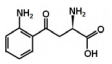
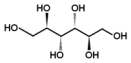
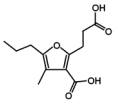
Inhibitory Effects of Uremic Toxins on OATP1B1- and OATP1B3-Mediated Transport

We first examined the inhibitory effects of uremic toxins on OATP1B1- and OATP1B3-mediated transport. Uptake experiments using [^{3}H]E_3S for OATP1B1 and [^{3}H]TCA for OATP1B3, which are known substrates for each transporter, were performed. Eighteen uremic toxins with a variety of functional groups were selected (Table 1). The results of the screening showed that 1000 µM kynurenic acid, 10000 µM indole-3-acetic acid, 10000 µM indoxyl sulfate, and 10000 µM *p*-cresol inhibited both OATP1B1- and OATP1B3-mediated transport more than 50%. CMPF (1000 µM) and urea (10000 µM) also inhibited both OATP1B1- and OATP1B3-mediated transport, but the inhibition by these uremic toxins was moderate (greater than 20%, less than 50%). L-Kynurenine (1000 µM) selectively decreased OATP1B1-mediated transport by 22%. SDMA selectively decreased OATP1B3-mediated transport by 47%, but the inhibition of SDMA was not concentration-dependent (SDMA: 1-1000 µM, data not shown). No significant inhibition to OATP1B1-mediated transport was observed with 10000 µM creatinine, 10000 µM guanidine, 1000 µM methylguanidine, 10000 µM hippuric acid, and 100000 µM mannitol. However, all of these uremic toxins moderately inhibited (greater than 20%, less than 50%) OATP1B3-mediated transport. The inhibitory effects of most uremic toxins on OATPs were higher than those observed with clinical concentrations.

Next, we evaluated the IC_{50} of the four uremic toxins (kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol) that inhibited OATPs by more than 50%. The four uremic toxins inhibited OATP1B1- and OATP1B3 -mediated transport in a concentration-dependent manner (Fig. 1 and 2).

The IC_{50} values of kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol for OATP1B1 were 180 ± 110 µM, 770 ± 130 µM, 2700 ± 290 µM, and 4600 ± 790 µM, and the corresponding values for OATP1B3 were 180 ± 20 µM, 1100 ± 330 µM, 1300 ± 420 µM, and

Table 1. Chemical Structures, Clinical Concentrations and Inhibitory Effects of Uremic Toxins on OATPs

Uremic Toxin	Chemical Structure	Concentration (μM)	Transport (%)		Clinical Conc. (μM)
			OATP1B1	OATP1B3	
Guanidines					
ADMA		100	86±14	127±14	0.69－36 [28]*
SDMA		1000	92±18	53±3.7	0.27－6.1 [28]*
Creatine		10000	98±8.6	82±9.9	48－1800 [28]*
Creatinine		10000	102±12	67±6.4	110－2100 [28]*
Guanidine		10000	102±5.9	77±11	0.20－14 [28]*
Guanidinoacetic acid		10000	104±5.5	98±18	1.2－5.9 [28]*
Methylguanidine		1000	105±4.6	69±12	0.10－25 [28]*
Hippurates					
Hippuric acid		10000	93±7.6	57±4.6	28－2600 [28]
Indoles					
Indole-3-acetic acid		1000 10000	54±2.1 24±2.9	65±11 12±5.3	0.10－52 [28]
Indoxyl sulfate		1000 10000	82±7.8 42±3.4	59±9.9 22±9.7	2.4－940 [28]
Kynurenic acid		100 1000	92±0.7 43±2.3	71±14 23±5.2	5.3－50 [28]
L-Kynurenine		1000	78±2.5	104±7.7	0.7－4.6 [28, 35]
Quinolinic acid		1000	116±3.5	93±25	0.16－20 [28, 35]
Phenols					
p-Cresol		1000 10000	96±6.5 16±1.3	84±6.4 1.8±8.4	5.6－380 [28]
Polyols					
Mannitol		10000	101±7.7	72±18	7.1－420 [28]*
Others					
CMPF		1000	79±5.6	75±12	18－390 [28]
trans-Aconitate		1000	106±7.7	86±8.6	0.5－6.5 [36]
Urea		100000	72±5.3	62±9.3	6700－77000 [28]*

CMPF: 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, ADMA: H-Arg(di-Me)-OH (asymmetrical); SDMA: H-Arg(di-Me)-OH (symmetrical); Asterisk indicates free concentration of uremic toxin (protein unbound) transport (%) are shown as mean ± S.E.M. (n =3 - 6).

1700 ± 85 μM. Indole-3-acetic acid strongly inhibited OATP1B1-mediated transport (IC_{50} 770 μM) more than that of OATP1B3 (IC_{50} 1100 μM). Indoxyl sulfate and *p*-cresol had a higher affinity for OATP1B3 than for OATP1B1. Compared to the other uremic toxins, kynurenic acid inhibited the transport activity of OATP1B1 and OATP1B3 with relatively high affinity.

Drug-Uremic Toxin Interactions on OATP1B1 and OATP1B3

We next examined the inhibitory effects of kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol on OATP1B1- and OATP1B3-mediated drug transport. We performed uptake experiments using [^{3}H]MTX as a model substrate of OATP1B1 and OATP1B3. [^{3}H]MTX uptake by OATP1B1- and OATP1B3-expressing HEK293 cells was inhibited by the four uremic toxins in a concentration-dependent manner (Fig. 3). Rifampicin, a known inhibitor of OATPs, was used as a positive control. OATP1B1- and OATP1B3-mediated [^{3}H]MTX uptake were inhibited 57 ± 30% and 4.7 ± 3.9% versus control, respectively, by 10 μM rifampicin. The uremic toxins inhibited OATP1B1- and OATP1B3-mediated [^{3}H]MTX uptake in a similar manner.

Uptake of Uremic Toxins by OATP1B1- and OATP1B3-Expressing HEK293 Cells

To clarify whether the uremic toxins that interact with OATP1B1 and/or OATP1B3 were substrates for these transporters, we performed uptake studies. Kynurenic acid uptake was significantly higher in OATP1B1- and OATP1B3-expressing HEK293 cells than in mock cells (1.1, 1.6, and 2.0 pmol/mg protein/2 min for mock, OATP1B1/HEK293, and OATP1B3/HEK293 cells, respectively) (Fig. 4A). Moreover, indoxyl sulfate uptake was significantly higher in OATP1B3-expressing HEK293 cells than in controls (11 and 14 pmol/mg protein/2 min for mock and OATP1B3/HEK293 cells, respectively), but not in OATP1B1-expressing HEK293 cells (Fig. 4C). No significant indole-3-acetic acid and *p*-cresol transport was observed by OATP1B1- or OATP1B3-expressing HEK293 cells (Fig. 4B and D).

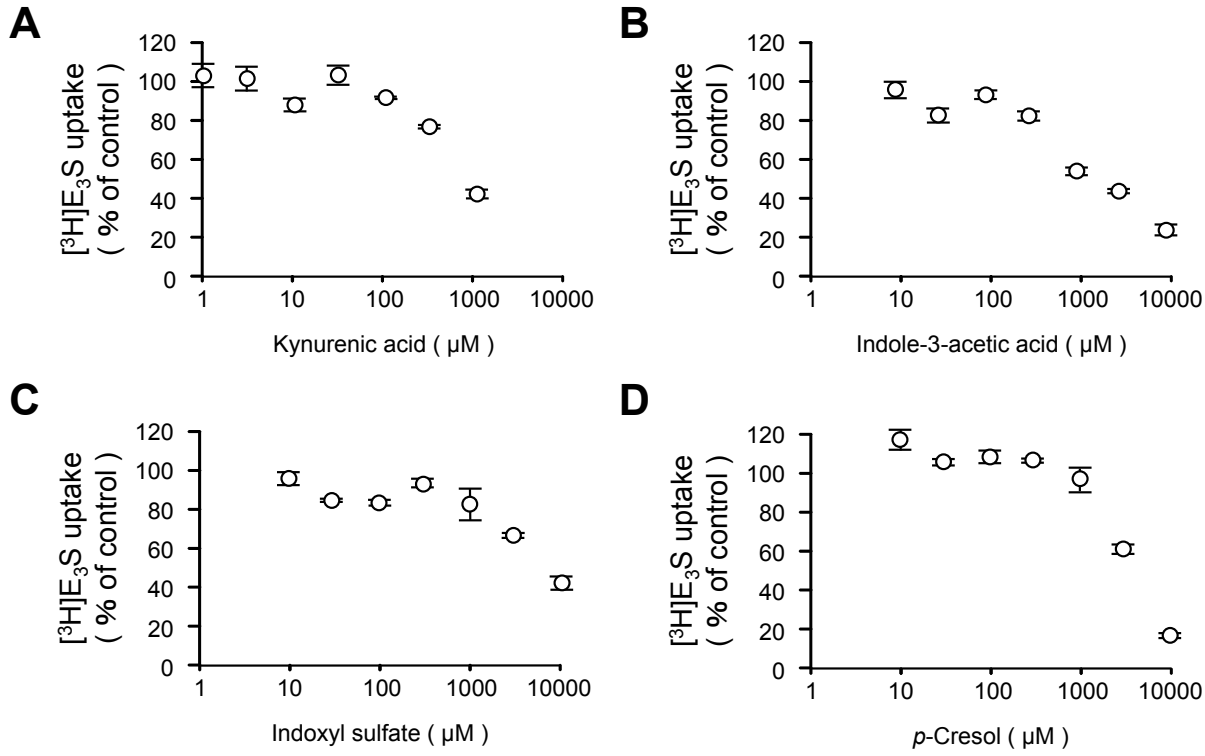


Figure 1. Inhibitory effects of uremic toxins (kynurenic acid (A), indole-3-acetic acid (B), indoxyl sulfate (C), and *p*-cresol (D)) on OATP1B1-mediated transport. Cells were incubated for 15 sec at 37 °C with 9.2-11 nM [^{3}H]E_3S in the presence or absence of uremic toxins. OATP1B1-mediated transport was calculated after subtracting nonspecific uptake by mock cells. Each point and bar represents the mean ± S.E.M. (n = 3).

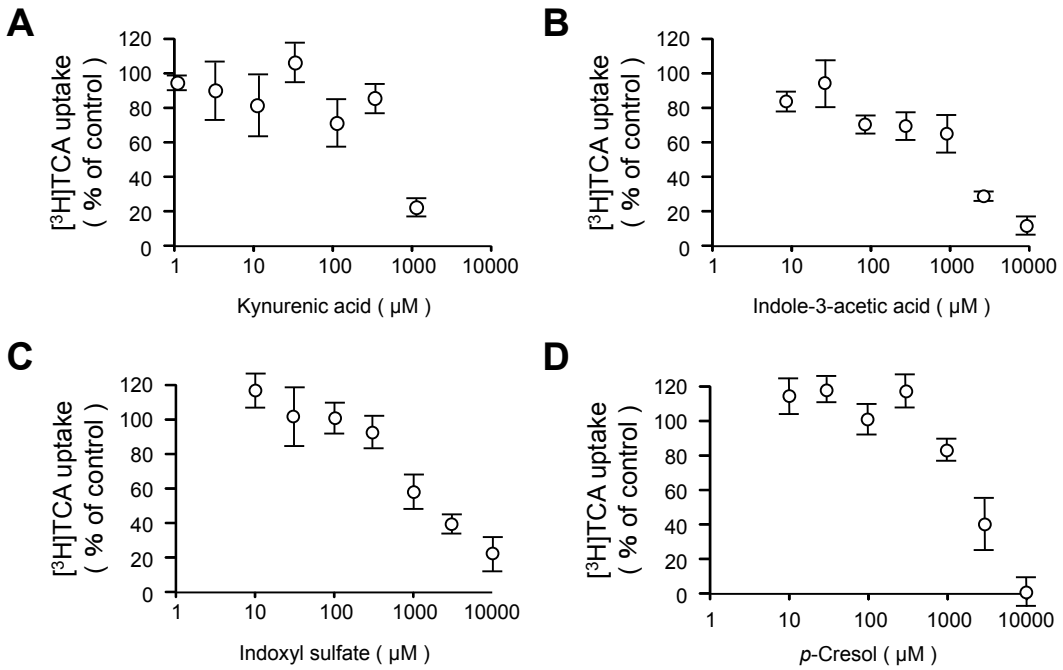


Figure 2. Inhibitory effects of uremic toxins (kynurenic acid (A), indole-3-acetic acid (B), indoxyl sulfate (C), and *p*-cresol (D)) on OATP1B3-mediated transport. Cells were incubated for 2 min at 37 °C with 50-100 nM [^{3}H]TCA in the presence or absence of uremic toxins. OATP1B3-mediated transport was calculated after subtracting nonspecific uptake by mock cells. Each point and bar represents the mean ± S.E.M. ((A), (B) and (D) (n = 3), (C) (n = 5-6)).

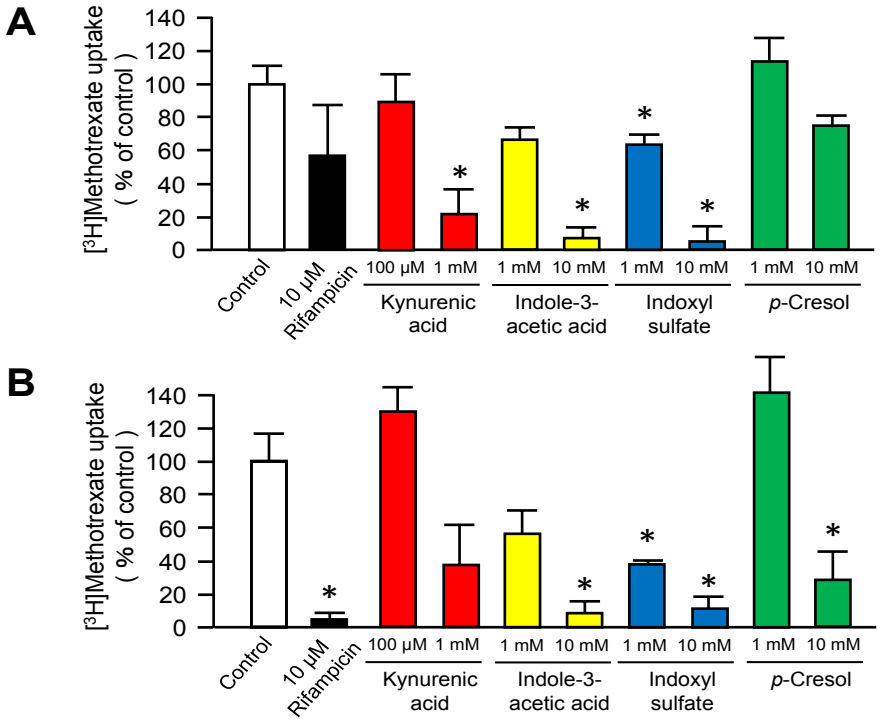


Figure 3. Inhibitory effects of uremic toxins on [^{3}H]MTX uptake by OATP1B1- (A) and OATP1B3-expressing HEK293 cells (B). Cells were incubated for 5 min at 37 °C with 16 nM [^{3}H]MTX in the presence or absence of uremic toxins and 10 μM rifampicin. Open column represents [^{3}H]MTX uptake in the absence of inhibitors (control). Closed, red, yellow, blue and green columns represent the inhibitory effects of 10 μM rifampicin, kynurenic acid, indole-3-acetic acid, indoxyl sulfate and *p*-cresol on OATP1B1- and OATP1B3-mediated [^{3}H]MTX transport. OATP1B1- and OATP1B3-mediated transport was calculated after subtracting nonspecific uptake by mock cells. Each column represents the mean ± S.E.M. (n = 3). Asterisk indicates a significant difference from control value ($p < 0.05$).

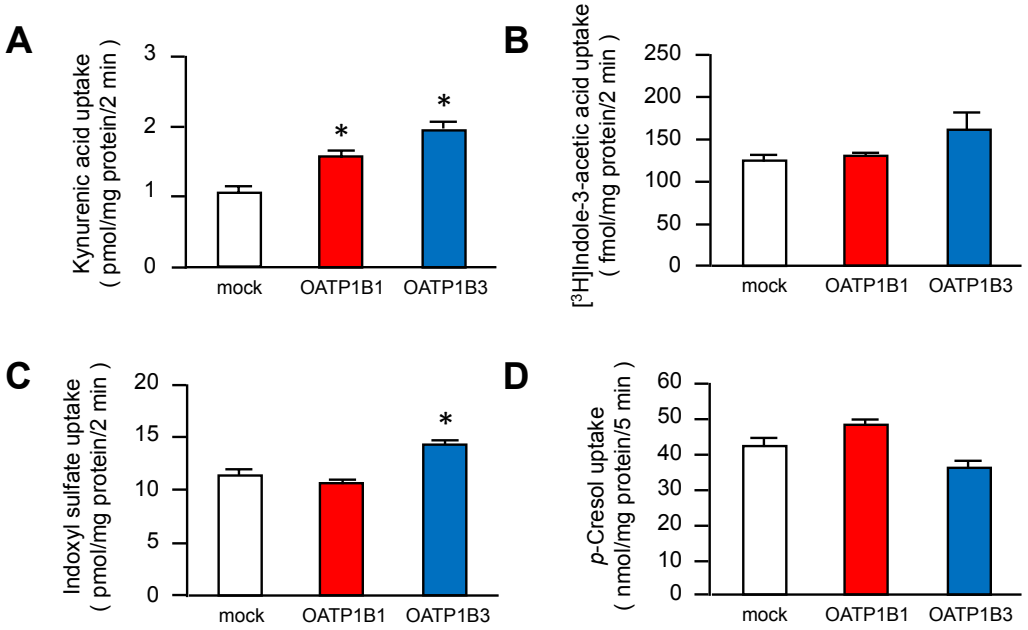


Figure 4. Uremic toxins (kynurenic acid (A), indole-3-acetic acid (B), indoxyl sulfate (C), and *p*-cresol (D)) uptake by OATP1B1- and OATP1B3-expressing HEK293 cells. OATP1B1- (red columns), OATP1B3-expressing HEK293 cells (blue columns), or mock cells (open columns) were incubated at 37 °C for 2 min with 10 μM kynurenic acid, 25 nM [^{3}H]indole-3-acetic acid, 50 μM indoxyl sulfate, 100 μM *p*-cresol. Each column represents the mean ± S.E.M. (n = 3). Asterisk indicates a significant difference from value of mock cells ($p < 0.05$).

DISCUSSION

In the present study, we evaluated the interaction of uremic toxins with the hepatic organic anion transporters OATP1B1 and OATP1B3. This is the first report to quantify the effects of four representative uremic toxins (kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol) on OATP1B1- and OATP1B3-mediated transport. Our results indicate that some uremic toxins are substrates of OATP1B1 and/or OATP1B3.

From the screening results, we found that OATP1B1-mediated [^{3}H]E_3S transport and OATP1B3-mediated [^{3}H]TCA transport were inhibited by kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol in a dose-dependent manner (Fig. 1 and 2). These four uremic toxins affected both OATP1B1 and OATP1B3, with IC_{50} values of 180 and 180 μM for kynurenic acid, 770 and 1100 μM for indole-3-acetic acid, 2700 and 1300 for indoxyl sulfate, and 4600 and 1700 μM for *p*-cresol, respectively. We also identified several uremic toxins that moderately inhibited OATP1B1- and OATP1B3-mediated transport (greater than 20%, less than 50%) (Table 1). The data showed that only L-kynurenine selectively inhibited OATP1B1-mediated transport, whereas five uremic toxins (creatinine, guanidine, methylguanidine, hippuric acid, and mannitol) selectively inhibited OATP1B3-mediated transport. Two uremic toxins (CMPF and urea) moderately inhibited both OATP1B1- and OATP1B3-mediated transport. Moreover, the effects of CMPF and urea on OATPs occurred at clinically relevant concentrations. This data suggests that indoxyl sulfate, *p*-cresol, and many uremic toxins had a stronger inhibitory effect on OATP1B3-mediated transport. These results suggest that typical and selective substrates of OATP1B3, such as telmisartan, may be affected by uremic toxins to a greater extent than substrates of both OATP1B1 and OATP1B3 in CKD patients (25).

According to previous reports, CMPF was the most potent inhibitor of OATP for the uptake of erythromycin, eprosartan, and digoxin, with a K_i value of 20 to 50 μM (16, 20, 21). In another report, it was shown that the IC_{50} values of CMPF, indoxyl sulfate, hippuric acid, and indole-3-acetic acid were >300 μM, 4750 μM, >3000 μM, and >3000 μM, respectively (26). Moreover, OATP1B1-mediated SN-38 uptake was strongly inhibited by CMPF and indoxyl sulfate, with IC_{50} values of 158 μM and 2290 μM, respectively (26). The IC_{50} values of CMPF, indoxyl sulfate,

hippuric acid, and indole-3-acetic acid were different from those obtained in our studies. The reason why the IC_{50} values of uremic toxins were different is unclear.

Next, we performed an uptake study using [^{3}H]MTX as a model drug to examine the inhibitory effects of uremic toxins on OATP1B1- and OATP1B3-mediated drug transport. [^{3}H]MTX uptake by OATP1B1- and OATP1B3-expressing HEK293 cells was significantly inhibited by the four uremic toxins examined (kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol). The uremic toxins inhibited [^{3}H]MTX uptake by OATP1B1- and OATP1B3-expressing HEK293 cells in a concentration-dependent manner (Fig. 3). Rifampicin decreased both OATP1B1- and OATP1B3-mediated [^{3}H]MTX uptake 57% and 4.7%, respectively (Fig. 3). In the previous report, the IC_{50} values of rifampicin were evaluated as 8.8 μM for OATP1B1 and 3.9 μM for OATP1B3, respectively [3]. Vavricka *et al.* have reported that the apparent K_m value of rifampicin transport was 13 μM for OATP1B1 and 2.3 μM for OATP1B3 (27). This supports our screening results, and the IC_{50} values of uremic toxins. Therefore, the IC_{50} values in our study can be used to accurately estimate the inhibitory effects of these transporters.

When we calculate the effect of uremic toxins, the binding affinities of uremic toxins, such as kynurenic acid, indole-3-acetic acid, indoxyl sulfate, hippuric acid, and CMPF for serum protein must be considered. Almost 90% of uremic toxins exist in the protein-bound form in plasma sera (28). Thus, the effects of uremic toxins on OATPs are thought to be smaller under normal conditions. However, in chronic disease states, such as CKD, uremic toxins may have a greater effect. For instance, CKD patients frequently have hypoalbuminemia, resulting in an increase in protein-unbound uremic toxins (29). Moreover, it was reported that compared to control subjects, dialyzed patients have significantly lower levels of albumin (30). Therefore, we could estimate the inhibitory effects of uremic toxins on OATPs by using the IC_{50} values in CKD patients. When we calculated the inhibitory effects using the equation ($V = 100 \times IC_{50} / (IC_{50} + [I]) + A$), kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol decreased OATP1B1 transport to 78, 94, 74, and 92% at maximum concentration of the uremic toxins in CKD patients shown in Table 1, and the corresponding values for OATP1B3 transport were 78, 96, 58, and 82%. Compared to other uremic toxins, indoxyl sulfate may strongly affect OATP1B3 transport at clinically relevant concentration. Although each uremic toxin may show weak inhibitory effect on OATPs singly, inhibitory effects of many uremic toxins on OATPs should be strengthened additively and synergistically in CKD patients. We therefore need to carefully adjust the doses of drugs excreted via both renal and non-renal pathways in CKD patients.

We also assessed the uptake of kynurenic acid, indole-3-acetic acid, indoxyl sulfate, and *p*-cresol by OATPs to better understand the affinity of uremic toxins to OATPs. Our results indicate that kynurenic acid is the substrate of both OATP1B1 and OATP1B3, and that indoxyl sulfate is the substrate of OATP1B3. Indole-3-acetic acid and *p*-cresol were not significantly transported by OATP1B1 and OATP1B3. In previous studies, some uremic toxins have been reported to inhibit CYPs (15, 31). These uremic toxins also alter the expression of hepatic transporters (32, 33). For instance, the plasma concentration of tadalafil, which is primarily metabolized by CYP3A4, is elevated in patients with end-stage renal disease (18). Further, the plasma concentration of tadalafil is affected not only by CYP3A4 but also by OATP1B1 expression (34). Although it is important to know hepatic uptake process of uremic toxin, little information is available in this regard. Previous reports and our results suggest that hepatic uptake of the uremic toxins via OATP1B1 and OATP1B3 plays an important role in access to the intracellular environment, and that the accumulation of uremic toxins via OATP1B1 and OATP1B3 can alter the expression levels of hepatocellular enzymes and transporters (14, 37, 38). Thus, additional studies about the detailed mechanisms of hepatic uremic toxins uptake are needed to inform the hepatotoxicity induced by uremic toxins and drug-uremic toxin interactions in patients with CKD.

In conclusion, we have demonstrated that some uremic toxins inhibit OATP-mediated uptake in a concentration-dependent manner, and have clarified OATPs contribution to uremic toxin handling in the liver. Thus, we provided basic information to estimate the inhibitory effects of uremic toxins on OATPs in CKD patients. Accumulated uremic toxins affect the uptake of various substrates by OATPs in the liver, and the uptake of endogenous compounds in hepatocytes may also be affected, potentially

leading to liver injury. We therefore emphasize the necessity of careful adjustment of doses for drugs excreted by renal and non-renal pathways in CKD patients.

DECLARATION

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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