Characterization of Cellular Uptake of Perfluorooctanoate via Organic Anion-Transporting Polypeptide 1A2, Organic Anion Transporter 4, and Urate Transporter 1 for Their Potential Roles in Mediating Human Renal Reabsorption of Perfluorocarboxylates

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It has been hypothesized that human renal apical membrane transporters play a key role in human renal reabsorption of perfluorooctanoate (PFO), which contributes to the long half-life of PFO in humans. In the present study, PFO uptake kinetics of human organic anion-transporting polypeptide (OATP) 1A2, organic anion transporter (OAT) 4, and urate transporter 1 (URAT1) in stably transfected cell lines was investigated. OAT4 and URAT1, but not OATP1A2, were shown to mediate saturable PFO cellular uptake. OAT4-mediated PFO uptake was stimulated by a low extracellular pH, which was evidenced as a lower Michaelis constant ($K_m$) at pH 6 (172.3 ± 45.9 µM) than that at pH 7.4 (310.3 ± 30.2 µM). URAT1-mediated PFO uptake was greatly enhanced by an outward Cl$^-$ gradient, and its $K_m$ value was determined to be 64.1 ± 30.5 µM in the absence of extracellular Cl$^-$. The inhibition of OATP1A2- or OAT4-mediated estrone-3-sulfate uptake or URAT1-mediated urate uptake has been compared for linear perfluorocarboxylates (PFCs) with carbon chain lengths from 4 to 12. A clear chain length–dependent inhibition was observed, suggesting that PFCs in general are substrates of OAT4 and URAT1 but with different levels of affinities to the transporters depending on their chain length. Our results suggest that OAT4 and URAT1 are key transporters in renal reabsorption of PFCs in humans and, as a result, may contribute significantly to the long half-life of PFO in humans.

Key Words: perfluorooctanoic acid; PFOA; renal reabsorption; OAT4; URAT1; OATP1A2.

In experimental animals, PFO is metabolically inert, distributed mainly to the liver and plasma, and excreted primarily via the urinary route (Kemper, 2003). PFO elimination is highly species dependent. Biological half-lives of PFO range from hours in female rats (Kemper, 2003) and rabbits (Kudo and Kawashima, 2003); days in male rats (Kemper, 2003); weeks in dogs (Hanjiharvi et al., 1988), mice (Lou et al., 2009), and monkeys (Butenhoff et al., 2004b) to years in humans (Olsen et al., 2007). It has been proposed that renal proximal tubular reabsorption of PFO plays a key role in renal clearance of PFO, and the PFO renal reabsorption levels in different species contribute to the large variation of biological half-lives of PFO in those species, including human (Andersen et al., 2008). Harada et al. (2005) have shown that human PFO renal clearance was only ~0.001% of the glomerular filtration rate, which validated the importance of active renal reabsorption of PFO in humans. Active tubular reabsorption of organic anions in kidney is mediated via apical membrane transport proteins, which are often species specific (El-Sheikh et al., 2008; Hilgendorf et al., 2007). In rats, proximal tubule brush border membrane–expressed organic anion-transporting polypeptide (Oatp) 1a1 has been shown to facilitate active cellular uptake of PFO (Weaver et al., 2010; Yang et al., 2009), and its potential involvement in renal reabsorption of PFO has been linked to the sex-dependent renal elimination of PFO in rats (Yang et al., 2009). In humans, OATP1A2 (also known as human OAPT-A, OATP1, or OATP) is the closest ortholog of rat Oatp1a1 based on their shared amino acid identities (67%; Kullak-Ublick et al., 1995). The expression of OATP1A2 in human kidney is localized to the apical domain of distal nephrons (Lee et al., 2005), which has been suggested to be responsible for the active tubular reabsorption of the cancer drug methotrexate (Badagnani et al., 2006). Human organic anion transporter (OAT) 4 has been identified as a renal apical transporter in the proximal tubule cells...
(Ekaratanawong et al., 2004) and facilitates the reabsorption of conjugated steroids such as estrone-3-sulfate (E3S) with high affinity (Cha et al., 2000). Nakagawa et al. (2009) has provided evidence of OAT4-mediated PFO uptake in transiently transfected human embryonic kidney 293 (HEK293) cells. Human urate transporter 1 (URAT1) shares 42% amino acid identity with OAT4 and is also localized to the apical membrane of the epithelium of proximal tubules (Enomoto et al., 2002). URAT1 was discovered as an apical urate transporter, playing a pivotal role in renal reabsorption of urate to maintain urate homeostasis (Enomoto et al., 2002). Because of their apical membrane localization in the distal and proximal tubules and demonstrated roles in renal reabsorption of organic anions, OATP1A2, OAT4, and URAT1 were good candidates as potential PFO transporters that mediate renal reabsorption in humans.

The objectives of the present study were to evaluate PFO transport potential and characterize PFO uptake kinetics of OATP1A2, OAT4, and URAT1 in stably transfected cell lines. Inhibition of marker substrate transport of OATP1A2, OAT4, or URAT1 by perfluorocarboxylates (PFCs) of different chain lengths was investigated to reveal possible chain length–dependent PFC transport via these specific membrane transporters. The findings of this study provide key mechanistic information of human renal handling of PFO, which will be a significant step toward our understanding of the long PFO half-life in humans.

MATERIALS AND METHODS

Materials. 14C-PFOA (chemical purity > 99%, labeled at the carboxyl carbon, ammonium salt; activity, 2.11 GBq/mmol) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). 14C-uric acid (1.91 GBq/mmol) was purchased from PerkinElmer (Waltham, MA). 14C-uric acid (1.91 GBq/mmol) was purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). Perfluorobutanoic acid (FPBA, C4) and perfluoropentanoic acid (PFPeA, C7) were purchased from Sigma-Aldrich (St Louis, MO). Perfluoropentanoic acid (FPFA, C5), perfluorohexanoic acid (PFHaA, C6), PFOA (C8), perfluorononanoic acid (PFNA, C9), perfluorodecanoic acid (PFDA, C10), perfluoroundecanoic acid (PFUAnA, C11), and perfluoroundecanoic acid (PFUAnA, C11), and perfluoroundecanoic acid (PFUAnA, C11), and perfluoroundecanoic acid (PFUAnA, C11), and perfluoroundecanoic acid (PFUAnA, C11) were purchased from Oakwood Product (West Columbia, SC). The purities of the perfluorocarboxylic acids were equal or greater than 97%. E3S, sulfobromophthalein (S3), and urate transporter 1 (URAT1) shares 42% amino acid identity with OAT4 and is also localized to the apical membrane of the epithelium of proximal tubules (Enomoto et al., 2002). URAT1 was discovered as an apical urate transporter, playing a pivotal role in renal reabsorption of urate to maintain urate homeostasis (Enomoto et al., 2002). Because of their apical membrane localization in the distal and proximal tubules and demonstrated roles in renal reabsorption of organic anions, OATP1A2, OAT4, and URAT1 were good candidates as potential PFO transporters that mediate renal reabsorption in humans.

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For OAT4 cis-inhibition experiments, OAT4-expressing CHO cells were incubated with either 7nM ³H-E3S or 5µM ¹⁴C-PFOA for 10 s in the absence or presence of 100µM inhibitors BSP, probenecid, glutarate, or PAH. Inhibition of passive diffusion in mock cells was negligible.

For OAT4 trans-stimulation experiments, OAT4-expressing CHO cells were preincubated with either glutarate or PAH (10µM each) for 2 h before the start of the uptake assay.

For PFCs chain length–dependent inhibition experiments, OAT4- and OATP1A2-expressing cells were incubated with 7nM ³H-E3S for 10 or 30 s, respectively, and URAT1-expressing cells were incubated with 6µM ¹⁴C-uric acid for 1 min, in the absence or presence of 100µM PFCs with chain lengths ranging from 4 to 12 (C4–C12). Mock cells were treated the same way as their corresponding transporter-expressing cells were treated.

Data analysis. Nonspecific passive diffusion of PFO to mock CHO cells can be described by a nonsaturable linear kinetics (Han et al., 2008; Yang et al., 2009):

\[ v_{\text{par}} = P_{\text{dif}} \cdot [S], \]  

where \( v_{\text{par}} \) is the velocity of PFO (picomole per minute per milligram) passive diffusion, \([S]\) is the substrate concentration (micromolar), and \( P_{\text{dif}} \) is passive diffusion constant (microliter per minute per milligram) and was obtained by linear regression to Equation 1.

The total cellular uptake of PFO in OAT4-expressing CHO cells is considered to be the sum of OAT4-mediated uptake (saturable) and the nonspecific passive diffusion (nonsaturable):

\[ v_{\text{total}} = \frac{V_{\text{max}} \cdot [S]}{K_{\text{m}} + [S]} + P_{\text{dif}} [S], \]  

where \( V_{\text{max}} \) is the initial uptake velocity (picomole per minute per milligram) in OAT4-expressing cells, \( V_{\text{max}} \) is the maximum velocity (picomole per minute per milligram) for transporter-mediated uptake, and \( K_{\text{m}} \) is the Michaelis constant (micromolar) for transporter-mediated uptake. \( V_{\text{max}} \) and \( K_{\text{m}} \) values of OAT4-mediated PFO uptake kinetics were obtained by nonlinear regression to Equation 2 with the input of \( P_{\text{dif}} \) from Equation 1.

URAT1 specifically mediated PFO uptake was described by

\[ v_{\text{URAT1}} = \frac{V_{\text{max}} \cdot [S]}{K_{\text{m}} + [S]}, \]  

where \( v_{\text{URAT1}} \) is the difference of the initial uptake velocity in URAT1-expressing HEK293 cells and in mock HEK293 cells. \( V_{\text{max}} \) and \( K_{\text{m}} \) values of URAT1-mediated PFO uptake kinetics were obtained by nonlinear regression to Equation 3.

All curve fittings of nonlinear regressions were conducted in the software package Origin (version 7.0; OriginLab, MA).

The percentage of OATP1A2- or URAT1-mediated marker substrate (E3S or urate, respectively) uptake in the presence of 100µM PFCs was calculated according to:

Percentage relative to the control = \( \frac{V_{\text{transporter, PFC}} - V_{\text{mock, PFC}}}{V_{\text{transporter}} - V_{\text{mock}}} \times 100\). 

where \( V_{\text{transporter, PFC}} \) and \( V_{\text{mock, PFC}} \) are initial uptake velocities of the substrate in the transporter cells or the mock cells, respectively, in the presence of PFCs, whereas \( V_{\text{transporter}} \) and \( V_{\text{mock}} \) are substrate uptake velocities in the absence of PFCs.

RESULTS

Reverse Transcription-PCR

Expression of OATP1A2, OAT4, and URAT1 messenger RNA has been confirmed in the transporter-expressing cells by RT-PCR. Supplementary figure 1 shows that, with corresponding gene-specific primers, the full-length PCR products of OATP1A2-, OAT4-, and URAT1-expressing cells exhibited bands for OATP1A2, OAT4, and URAT1 genes whose size were 2013, 1653, and 1646 bp, respectively (lane 3). The bands with the same sizes were also shown for the PCR products amplified using OATP1A2, OAT4, and URAT1 plasmid DNA as the templates (lane 2 in Supplementary fig. 1) but were absent from the RT-PCR products of the vector-transfected (mock) control cells (lane 4 in Supplementary fig. 1).

Organic Anion-Transporting Polypeptide 1A2

Because OATPs’ transport activities are often enhanced at a lower extracellular pH (Leuthold et al., 2009), we screened our OATP1A2-expressing HEK293 cells at pH 6, 7, and 8 for their uptake activities of E3S and PFO. Figure 1A shows that E3S was taken up by OATP1A2-expressing HEK293 cells at higher rates than mock (vector-transfected) cells, and the uptake rates were pH dependent only for OATP1A2 CDNA-transfected cells where acidic pH significantly enhanced E3S uptake rates. In contrast, PFO uptake rates were not significantly different between OATP1A2-expressing HEK293 cells and mock cells at any of the pH conditions (Fig. 1B).

The potential of PFC chain length–dependent inhibition of OATP1A2-mediated E3S uptake was investigated at pH 6. Figure 2 shows that, at 100µM PFCs, no inhibitory effect was
seen for chain length between 4 and 7, whereas ~30% of inhibition was seen by PFO. Perfluorodecanoate (PFD, C10) showed the most inhibition (~70%), but the inhibition level was reduced at longer chain lengths (for C11 and C12).

Organic Anion Transporter 4

E3S is a marker substrate for human OAT4 with reported $K_m$ values ranged from 1.01 to 21.7$\mu$M (Cha et al., 2000; Islam et al., 2007; Ugele et al., 2008; Zhou et al., 2006). In the present study, uptake of E3S in OAT4 cDNA-transfected CHO cells was shown to be time and concentration dependent (Supplementary fig. 2), and the $K_m$ value was determined to be 38.1 ± 8$\mu$M.

Hagos et al. (2007) has reported that (1) OAT4-mediated 6-carboxylfluorescein (6-CF) or E3S uptake was significantly cis-inhibited by 500$\mu$M of probenecid but much less so by glutarate and PAH and (2) both glutarate and PAH significantly trans-stimulated OAT4-mediated 6-CF and E3F uptake. We have confirmed the same observations using our OAT4-expressing CHO cells (Supplementary fig. 3).

PFO uptake in OAT4-expressing CHO cells increased as a function of time with an apparent saturation phase after 10 s of incubation (Supplementary fig. 4). Because OAT4 uptake kinetics of known substrates is pH dependent (Hagos et al., 2007), we compared OAT4-mediated PFO uptake rates at different pH values. Supplementary figure 5 shows that acidification of the uptake buffer from pH 8 to 5.5 increased PFO uptake by more than 250%. This observation is consistent with the previously reported pH-dependent trend for OAT4-mediated membrane transport (Hagos et al., 2007).

Concentration-dependent PFO uptake in OAT4-expressing cells and in mock cells was determined under pH 7.4 and 6 conditions (Fig. 3). A passive diffusion process was identified for PFO uptake in the mock cells, which was demonstrated by a linear relationship between PFO uptake velocities and PFO concentrations (Fig. 3, open circles and Equation 1). PFO uptake in OAT4-expressing cells was not saturated even at 1mM of PFO concentration (Fig. 3, filled circles) because of this passive diffusion component. The differences of PFO uptake velocities measured in OAT4-expressing cells and in mock cells exhibited saturable kinetics that can be described by a standard Michaelis-Menten equation (Fig. 3, open squares, Equation 2). $P_{\text{diff}}$, $K_m$, and $V_{\text{max}}$ values from Equation 2 were determined to be 17.5 ± 6.7 $\mu$l/min/mg, 310.3 ± 30.2$\mu$M, and 37.4 ± 12.5 nmol/min/mg, respectively, at pH 7.4, and 20.6 ± 6.6 $\mu$l/min/mg, 172.3 ± 45.9$\mu$M, and 36.6 ± 7.1 nmol/min/mg, respectively, at pH 6. Both $P_{\text{diff}}$ and $V_{\text{max}}$ values were not pH dependent, whereas the $K_m$ value was about half at pH 6 than that at pH 7.4 ($p < 0.05$).

Supplementary figure 6 shows that, analogous to OAT4-mediated E3S uptake (Supplementary fig. 2), OAT4-mediated PFO uptake was significantly (1) cis-inhibited by both BSP
and probenecid (100μM each) but not by glutarate and PAH (Supplementary fig. 6A) and (2) trans-stimulated by both glutarate and PAH (Supplementary fig. 6B) due to the asymmetric transport characteristics of OAT4 (Hagos et al., 2007).

OAT4 transport activity toward PFCs as a chemical class was investigated by inhibition of OAT4-mediated E3S uptake. The initial uptake velocities of 7nM 3H-E3S in OAT4-expressing CHO cells were determined in the absence or presence of 100μM PFCs at chain lengths ranged from 4 to 12. The percentage relative to control 3H-E3S uptake by individual PFC was compared in Figure 4. It is shown that, starting from perfluoroheptanoate (PFHp, C7), OAT4-mediated E3S uptake was inhibited with a chain length–dependent manner by the PFCs. The correlation between longer chain length and higher inhibition potential extended to PFD (C10). However, this trend was reversed for perfluoroundecanoate (PFU, C11) and perfluorododecanoate (PFDd, C12), which showed a reduction of inhibition potential compared with PFD (Fig. 4).

Urate Transporter 1

Enomoto et al. (2002) have shown that urate uptake via URAT1 is dramatically enhanced by an outward Cl⁻ gradient (intracellular Cl⁻ > extracellular Cl⁻) as a result of an exchange mechanism for urate and inorganic Cl⁻ in URAT1. In the present study, we measured URAT1-mediated urate and PFO uptake in Cl⁻-present and Cl⁻-free (chloride salts were replaced by gluconate salts) buffers.

Figure 5A shows that urate uptake rate in URAT1-expressing HEK293 cells in a Cl⁻-present buffer was significantly higher (p < 0.01) than that in the mock cells. When Cl⁻ was completely replaced by gluconate, urate uptake rate was enhanced by more than threefold in the URAT1-expressing cells but was not changed in the mock cells. Figure 5B shows that PFO uptake rate under the Cl⁻-present condition was higher in URAT1-expressing cells than in the mock cells, but their difference was not statistically significant (p > 0.01). Under the Cl⁻-free condition, PFO uptake rate was enhanced by more than fourfold in the URAT1-expressing cells but was not changed significantly in the mock control cells.

Time-dependent PFO uptake under the Cl⁻-free condition is shown in Figure 6A. PFO uptake rate in URAT1-expressing HEK293 cells increased over incubation time, whereas in mock control cells, PFO uptake rate stayed at a much lower level for the entire incubation duration (5 min). The concentration-dependent PFO uptake under the Cl⁻-free condition was also investigated. Figure 6B shows evidence of baseline PFO-specific transport in mock HEK293 cells, which was manifested by the slight curvature of the relationship between PFO uptake rates in the mock cells and PFO concentrations (open circles in Fig. 6B). Therefore, URAT1-specific uptake kinetics was
analyzed by using the difference of the uptake rates from the URAT1-expressing cells and the mock cells (solid circles in Fig. 6B; Equation 3). The \( K_m \) and \( V_{\text{max}} \) values for URAT1-mediated PFO uptake were determined to be 64.1 ± 30.5 \( \mu \text{M} \) and 323.6 ± 89.8 pmol/min/well, respectively.

PFCs exhibited chain length–dependent inhibition of URAT1-mediated urate uptake. Figure 7 shows that, at 100 \( \mu \text{M} \) of PFCs, uptake of 6 \( \mu \text{M} \) 14C-urate under the \( \text{Cl}^-/\text{C}_0 \)-free condition was inhibited the most and roughly at the same levels by PFHp (C7), PFO (C8), Perfluorononanoate (PFN) (C9), and PFD (C10). PFCs with shorter (C4–C6) or longer (C11 and C12) chain lengths showed relatively less inhibition.

**DISCUSSION**

Based on animal studies, renal elimination is a determining factor for PFO’s body clearance (Kemper, 2003). The finding that PFO has a long half-life of ~2.3 (Bartell et al., 2010) or 3.8 (Olsen et al., 2007) years in humans suggests that PFO renal elimination in humans is very inefficient when compared with other animal species in which PFO half-lives range from a few hours in rabbits (Kudo and Kawashima, 2003) and female rats (Kemper, 2003) to a few weeks in dogs (Hanhijarvi et al., 1988), mice (Lou et al., 2009), and monkeys (Butenhoff et al., 2004b).

In rats, renal handling of PFO involves active secretion via proximal tubule basolateral membrane transporters Oat1 and Oat3 (Nakagawa et al., 2008; Weaver et al., 2010) and reabsorption via proximal tubule brush border (apical) membrane transporter Oatp1a1 (Weaver et al., 2010; Yang et al., 2009). The higher expression level of Oatp1a1 in the male rat kidney has been related to the longer PFO half-life in the male rat than that in the female rat (Yang et al., 2009), which strongly indicates an important role of renal reabsorption in PFO biological half-lives in rats.

Human OAT1 and OAT3 have similar affinities to PFO compared with their rat orthologs (Nakagawa et al., 2008). Therefore, tubular secretion of PFO in human kidneys via OAT1 and OAT3 transporters is expected to be as effective as it is in rat kidneys, especially considering the high expression levels of OAT1 and OAT3 in human kidneys (Hilgendorf et al., 2007; Motohashi et al., 2002). This leaves tubular reabsorption as a plausible mechanism for species-related differences in PFO renal elimination and for the long half-life of PFO in humans. The renal tubule apical membrane transport system that is responsible for tubular reabsorption of organic anions is mainly made up by the solute carrier (SLC) proteins (El-Sheikh et al., 2008). In humans, OAT4 (SLC22A11) and URAT1 (SLC22A12) are expressed in the proximal tubule of the kidney (Ekaratanaawong et al., 2004; Enomoto et al., 2002), whereas OATP1A2 (SLCO1A2) is expressed in the kidney distal tubule (Lee et al., 2005). Our present study suggests that PFO is not a substrate, but likely an inhibitor, of OATP1A2, whereas both OAT4- and URAT1-mediated saturable cellular...
uptake of PFO, and therefore, are likely involved in tubular reabsorption of PFO.

Human OATP1A2 is mainly expressed in the blood-brain barrier (BBB; Gao et al., 2000; Lee et al., 2005), the kidney (Badagnani et al., 2006; Lee et al., 2005), and the small intestine (Glaeser et al., 2007). OATP1A2 exhibited pH-dependent uptake of the known substrate, E3S (Fig. 1A), which is consistent with the observation of other OATPs (Leuthold et al., 2009). However, OATP1A2 did not mediate PFO uptake at a pH range between 6 and 8 (Fig. 1B). This is a clear indication that OATP1A2 is not involved in PFO renal reabsorption, intestinal absorption, and BBB uptake. Because OATP1A2 is the most important influx transporter in the BBB (Lee et al., 2005; Urquhart and Kim, 2009), its lack of PFO transport activity may have at least partly contributed to the findings that PFO could not effectively pass BBB (Harada et al., 2007; Maestri et al., 2006).

Despite PFO not being an OATP1A2 substrate, PFCs exhibited a chain length–dependent inhibition of OATP1A2-mediated E3S uptake, with PFD (C10) showing the most potency (Fig. 2). For PFO, the inhibition was relatively weak and the uptake of 7nM E3S was reduced to ~70% in the presence of 100μM of PFO. In human, this potential OATP1A2 inhibitory effect by PFO is considered negligible for the general population with a mean serum PFO concentration of ~5 ng/ml (12nM) (Butenhoff et al., 2004a).

OAT4-mediated PFO uptake was pH sensitive (Supplementary fig. 5). Compared with the uptake kinetic parameters obtained at pH 7.4, the $V_{\text{max}}$ value was almost unchanged at pH 6, whereas the $K_{\text{m}}$ value was lowered to half. This suggests that a more alkaline urine would cause a lower level PFO renal reabsorption via OAT4 ($V_{\text{max}} = V_{\text{max}}/K_{\text{m}}$). We have also shown a PFC chain length–dependent inhibition of OAT4-mediated E3S uptake, with PFD (C10) exhibiting the highest inhibition potential (Fig. 4). Because PFO is both a substrate and an inhibitor of OAT4, it is reasonable to assume that other PFCs with OAT4 inhibition potencies were also substrates of OAT4 and were reabsorbed in the human kidney via OAT4. Besides being expressed in the human kidney, OAT4 is also expressed in human placenta (Cha et al., 2000; Ugele et al., 2003). This tissue localization suggests that OAT4 may be involved in transcellular transport of PFO across the placental barrier that has been documented previously (Midasch et al., 2007; Monroy et al., 2008).

URAT1 is a member of the OAT family with 42% amino acid identity as OAT4 and has been confirmed to play a key role in urate homeostasis via a renal reabsorption mechanism (Enomoto et al., 2002). In the present study, URAT1 substrate transport activity in URAT1-expressing cells was not high enough to characterize URAT1-mediated PFO uptake kinetics under a normal buffering condition that contains $\text{Cl}^-$ (Fig. 5B). Under a $\text{Cl}^-$-free buffering condition, URAT1-mediated urate or PFO uptake rate was dramatically enhanced as a result of a $\text{Cl}^-$ exchange mechanism for URAT1 (Enomoto et al., 2002). Under the $\text{Cl}^-$-free condition, the $K_{\text{m}}$ value for URAT1-mediated PFO uptake is 64.1 ± 30.5μM. Assuming $\text{Cl}^-$ is a simple exchanger for URAT1-mediated PFO uptake, this $K_{\text{m}}$ value, although measured under a $\text{Cl}^-$-free condition, should reflect the true PFO-URAT1 affinity under physiological conditions. Our data suggest that URAT1 could be a key transporter involving in human renal reabsorption of PFO. Weaver et al. (2010) have also shown enhanced PFO uptake in rat Urat1-expressing cells under a normal $\text{Cl}^-$-containing buffering condition. This indicates that the mechanism of PFO renal reabsorption via URAT1 may not be limited to humans.

We have shown that PFCs were able to cis-inhibit URAT1-mediated urate uptake with a chain length–dependent manner (Fig. 7). This result suggests a potential of PFCs to reduce urate renal reabsorption, which does not correlate well with the observation of a positive association between PFO and uric acid serum levels (Steenland et al., 2010). More importantly, it has been suggested that regulation of urate blood level in humans is not achieved by URAT1 alone but through a coordination of multiple renal transport proteins (Eraly et al., 2008), such as OAT1 (Ichida et al., 2003), OAT3 (Bakhiya et al., 2003), OAT4 (Hagos et al., 2007), OAT10 (Bahn et al., 2008), NPT1 (Uchino et al., 2000), and URATv1 (Anzai et al., 2008). Therefore, it would be inappropriate to assume a direct connection between urate blood level and the potential of PFO renal reabsorption via URAT1.

PFCs, especially those with chain lengths between 7 and 10, have been shown directly (via uptake assays; Weaver et al., 2010) or indirectly (via inhibition assays, the present study) to be substrates of OAT1, OAT3, OAT4, and URAT1. Among them, OAT1 and OAT3 locate at the basolateral membrane of the proximal tubular cells and excrete PFCs from the blood side to the urine side, whereas OAT4 and URAT1 at the brush border membrane are responsible for the reabsorption of PFCs back into the proximal tubular cells (Fig. 8). Even though cellular electrochemical gradient favors the removal of negatively charged intracellular PFCs and we have shown the significant
contribution of passive diffusion in cellular transport of PFO (Han et al., 2008; Yang et al., 2009), one would expect intracellular accumulation of PFCs without an efficient and active efflux pathway on the basolateral membrane (Fig. 8). However, such an accumulation did not occur in the kidney of the rat (Oatp1a1 instead of OAT4 and URAT1 in this case for rat renal reabsorption; Kemper, 2003; Yang et al., 2009). Therefore, we rationalize that there could be PFC efflux transporter(s) residing in the basolateral membrane that assist(s) in moving intracellular PFCs back to the systemic circulation. The potential candidate transporters that could fulfill this role were multidrug resistance–associated protein 6 (El-Sheikh et al., 2008; Scheffer et al., 2002) and organic solute transporter α/β (Ballatori et al., 2005). It would be important for future studies to investigate if these transporters, by working with OAT4 and URAT1, were involved in renal reabsorption of PFCs and, as a result, contributed to the observed long serum half-life of PFO in humans.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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