Uptake of Chemically Reactive, DNA-Damaging Sulfuric Acid Esters into Renal Cells by Human Organic Anion Transporters

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The procarcinogen 1-methylpyrene is activated by hepatic enzymes via 1-hydroxymethylpyrene to 1-sulfooxymethylpyrene (1-SMP), a highly reactive and mutagenic metabolite. Previously, high levels of 1-SMP DNA adducts were observed in rat kidneys after intraperitoneal administration of 1-hydroxymethylpyrene or 1-SMP. This study examined whether organic anion transporters (OAT) that are expressed at the basolateral membrane of proximal tubule cells are involved in uptake of SMP. Human epithelial kidney (HEK293) cells that stably express human OAT1 (hOAT1) and hOAT3 were used. Stable isomers of 1-SMP, (2-SMP and 4-SMP) competitively inhibited the uptake of characteristic substrates p-aminohippurate for hOAT1 and estrone sulfate for hOAT3. Both inhibitors exhibited high affinity for hOAT1 (K_i = 4.4 μM for 2-SMP; K_i = 5.1 μM for 4-SMP) as well as hOAT3 (K_i = 1.9 μM for 2-SMP; K_i = 2.1 μM for 4-SMP). The uptake rate of 4-SMP (at a concentration of 10 μM) by hOAT1- and hOAT3-expressing cells was 3.0 and 1.6 times higher, respectively, than in control cells. Uptake of the reactive isomer 1-SMP was investigated using as the end point the level of DNA adducts that were formed in the cells. After exposure to 1-SMP (10 μM), the DNA adduct level was 4.6 and 3.0 times higher in hOAT1- and hOAT3-expressing cells, respectively, than in control cells. The enhanced DNA adduct formation in hOAT-expressing cells was abolished in the presence of the OAT inhibitor probenecid. This study indicates that OAT can mediate the basolateral uptake of reactive sulfuric acid esters into proximal tubule cells and thereby participate in kidney cell damage by these compounds.

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Various adverse effects of chemicals are mediated by chemically reactive metabolites that covalently bind to cellular structures. For example, binding to DNA can lead to the induction of mutations, acute cytotoxicity, alterations in the cell cycle, and apoptosis. These processes are important in tumorigenesis, for example. Because many reactive molecules are short lived, they often produce greater damage in the cells in which they are generated than in remote tissues. In this study, we address the question of whether vectorial transport by transmembrane transporters also may lead to an enhanced exposure of certain cells to reactive metabolites.

Numerous polycyclic aromatic hydrocarbons are carcinogens that are activated to reactive metabolites and then form DNA adducts and induce mutations (1). Epoxides are important ultimate carcinogens/mutagens of many polycyclic aromatic hydrocarbons. An additional activation pathway has been observed with alkylated polycyclic hydrocarbons, which are abundant in the environment. Methylpyrenes, whose metabolites were investigated in this study, have been detected in cigarette smoke condensate (2); in car exhaust and cellulose pyrolysates (3); in sea sediments (4); in smoked cheese (5); as contaminants in olive oil (6); and as bio-accumulated pollutants in oysters, crabs, and finfish (7). We recently synthesized deuterated standards of the three possible monomethylpyrenes (1-, 2-, and 4-methylpyrene); their use as internal standards allowed the unambiguous identification and accurate quantification of these hydrocarbons in various matrices. For example, the level of these monomethylpyrenes in various cigarette smoke condensates consistently were approximately 10-fold higher than the level of benzo[a]pyrene, which often is used as a reference compound for measuring exposures to polycyclic aromatic hydrocarbons (A.S. et al., unpublished observations, 2005). The three isomers present were approximately in a 1:1:1 ratio. Unlike pyrene, which is not carcinogenic, 1-methylpyrene induces liver tumors in the mouse (8). It is converted via side-chain hydroxylation, yielding 1-hydroxymethylpyrene (1-HMP) (9), and subsequent sulfo conjugation (10–12) to a highly reactive metabolite, 1-sulfooxymethylpyrene.
Sulfate is a good leaving group in 1-SMP because the resulting cation is resonance stabilized. 1-SMP reacts with various nucleophilic sites of the DNA, in particular the exocyclic amino group of guanine (12).

In the rat, 1-HMP sulfotransferase activity is high in live tissues but negligible in extrahepatic tissues (12). Therefore, we expected that treatment of rats with 1-HMP would lead to the formation of DNA adducts primarily in liver. Indeed, we found substantially higher levels of DNA adducts in liver than in other tissues after intraperitoneal administration of 1-HMP to rats. However, there was a striking exception: adduct levels in the kidney were three times higher than in liver, although 1-HMP sulfotransferase activity in subcellular renal preparations amounted to <0.5% of the hepatic activity (12). The tissue distribution of DNA adducts was similar when the active metabolite, 1-SMP, instead of its metabolic precursor, 1-HMP, was administered to rats (12), indicating that the site of 1-SMP generation/administration did not determine the tissue distribution of the adducts. Moreover, we could detect 1-SMP in blood serum of animals that were treated with 1-SMP or 1-HMP (13). This finding was surprising, because the half-life ($t_{1/2}$) of 1-SMP in aqueous solution is short (3.6 min at 37°C) (13). However, $t_{1/2}$ was prolonged dramatically (to 990 and 2,310 min) in the presence of rat blood serum and physiologic levels of BSA, respectively (13). Therefore, SMP that is absorbed from the peritoneal cavity or formed and exported by the liver reaches the kidneys with the blood stream. Because of its plasma protein binding, it is unlikely that 1-SMP is filtrated in the glomeruli. This property and its negative charge also should prevent a free penetration into kidney cells. Therefore, 1-SMP presumably is subject for vectorial transport in the proximal tubules.

Two basolateral organic anion transporters (OAT), OAT1 and OAT3, are considered to be the main carriers that mediate the concentrative uptake of anionic compounds from blood into renal proximal tubule cells (14,15). Both transporters were cloned recently for several species, including rat (16–18) and human (19,20). They are highly conserved across species. They mediate the uptake of structurally diverse organic acids and some neutral compounds in exchange for intracellular α-ketoglutarate (21–23). Their substrates include many endogenous and exogenous compounds, such as dicarboxylates (20), prostaglandins (24), cyclic nucleotides and urate (16,20,23), nonsteroidal anti-inflammatory drugs (25), diuretics (26), antibiotics (27), and antiviral agents (28). On the one hand, these carriers protect the organism by secreting waste products, drugs, and other xenobiotics and their metabolites. On the other hand, their activity could be associated with proximal tubular injury as a result of the accumulation of toxins. For example, ochratoxin A (29), cephalosporins (27), and indoxyl sulfate (30) have been shown to induce nephrotoxicity via OAT1- and OAT3-mediated uptake into renal proximal tubules.

In these examples, the nephrotoxicanants are stable molecules. The goal of our study was to clarify a possible involvement of human OAT1 (hOAT1) and hOAT3 in the uptake of a reactive sulfuric ester, 1-SMP, and therefore in 1-SMP-induced DNA damage. To this end, we used HEK293 cells that stably express hOAT1 or hOAT3. 1-SMP is highly reactive and short lived in water. Its isomers, 2- and 4-SMP, are virtually stable under the same conditions ($t_{1/2}$ > 1 d at 37°C). Therefore, 2- and 4-SMP were used for conventional transport studies, whereas 1-SMP was used to monitor the influence of OAT on the induction of the primary DNA damage, the formation of DNA adducts.

**Materials and Methods**

**Chemicals**

1- and 2-SMP were synthesized as described previously (31). 4-SMP was prepared from 1,2,3,6,7,8-hexahydropyrene (Acros Organics BVBA, Geel, Belgium) as starting material. Friedel-Crafts acetylation of hexahydropyrene and subsequent dehydrogenation with 2,3-dichloro-5,6-dicyano-p-benzoquinone afforded 4-acetylpyrene, which was subjected to a haloform reaction with freshly prepared NaOBr to furnish pyrene-4-carboxylic acid. The acid was reduced with LiAlH4 to produce 4-HMP, which was transformed into 4-SMP as described for 1- and 2-SMP (31). The purity of the SMP was >99%, as determined by HPLC with fluorescence detection (chromatographic conditions, see last paragraph of subsection Uptake Studies). They were dissolved in DMSO within 3 h before use. [6,7-3H(N)]-Estrone sulfate (ES; 57.3 Ci/mmol) and [glycyl-2-3H]−p-aminohippurate (PAH; 4.18 Ci/mmol) were purchased from Perkin-Elmer (Boston, MA). The specific radioactivity of PAH was reduced to 0.5 Ci/mmol by adding unlabeled PAH.

**Figure 1.** Structural formulas of 1-sulfooxymethylpyrene (1-SMP) and its major DNA adduct (47).
**Tissue Culture**

The recombinant human epithelial kidney cell lines T-REx HEK293-hOAT1 and -hOAT3 were established by using the Flp-In expression system (Invitrogen, Karlsruhe, Germany) according to the manufacturer’s protocol. Briefly, the cDNA of hOAT1 (GenBank no. AF097490) and hOAT3 (B1706120) were cloned into the Flp-In expression vector pcDNA3/FRT, which contained an Flp recombination target site linked to the hygromycin resistance gene. These constructs were co-transfected with the Flp recombinase expression vector pOG44 into Flp-In HEK293 cells. Cells that stably expressed hOAT1 or hOAT3 were selected in the presence of hygromycin (200 μg/ml). Untransformed Flp-In HEK293 cells served as controls. Cells were grown in flasks that contained Dulbecco’s modified minimum essential medium (high glucose) supplemented with 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml), and blasticidin (5 μg/ml). Cultures were maintained in a humidified atmosphere that contained 5% CO₂ at 37°C. Cells were split in a 1:5 ratio every 3 to 4 d.

**Uptake Studies**

Initial experiments were carried out at 37°C. However, uptake was so fast that it would have been difficult—or impossible with our technical facilities—to find conditions under which uptake was linear with time, a prerequisite for kinetic analyses. We know from previous investigations using the *Xenopus* oocytes expression system that hOAT1 and hOAT3 work well at room temperature (23). Therefore, a temperature of 22°C was used in this study. The additional advantage of this modification is an extension of the life span of highly reactive substrates, such as 1-SMP.

For studying the influence of SMP on the uptake of standard substrates, cells were seeded in 24-well plates (2 × 10⁴ cells in 1 ml of medium per well) 2 d before the start of the experiment. Uptake of [³H]PAH (for OAT1) and [³H]ES (for hOAT3) was assayed in Ringer’s solution (130 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 20 mM HEPEs, 1 mM Na₂HPO₄, and 18 mM glucose [pH 7.4]) in the absence or presence of SMP. In the initial experiments, [³H]ES, [³H]PAH, and SMP were used at concentrations of 50 nM, 10 μM, and 20 μM, respectively, and the exposure time amounted to 20 min. The uptake was terminated by three washes with cold buffer (0.5 ml). Cells then were solubilized in 0.5 ml of 1 N NaOH, and after neutralization with 0.5 ml of 1 N HCl, the ³H content was assayed by liquid scintillation counting. The results were standardized by protein estimation using the bicinchoninic acid assay (Pierce, Rockford, IL) with serum albumin as a standard. After noticing drastic inhibition of the uptake of the standard substrates by SMP under these conditions, we conducted detailed kinetic studies using variable concentrations of the substrate (10 and 100 μM [³H]PAH for hOAT1; 50 and 300 nM [³H]ES for hOAT3) and the inhibitor (at least five concentrations of 2-SMP or 4-SMP in the range of 0 to 10 μM). Furthermore, the exposure time was reduced to 3 min. It was ascertained that uptake was linear with time for this period. Data were plotted and analyzed according to Dixon (32).

For determining the uptake of 4-SMP, cells were incubated in Ringer’s solution that contained 10 μM 4-SMP for 15 min. After three washes with cold Ringer’s solution, the cells were lysed with 0.25 ml of 1 N NaOH, and after neutralization with 0.25 ml of 1 N HCl and protein precipitation with 1 ml of acetone, an aliquot (usually 10 μl) of the supernatant was injected into an HPLC using a Shimadzu SPD-M10 Avp autosampler (Shimadzu Deutschland GmbH, Duisburg, Germany). The samples were separated using a Shimadzu SLC-10 Avp delivery system equipped with Phenomenex Gemini C18 column (250 × 3 mm, 5 μm). The eluent was methanol that contained 20% (vol/vol) water and 0.05% triethylamine. The flow rate was 0.2 ml/min. 4-SMP was quantified from its fluorescence signal (λex 334 nm, λem 392 nm) using a Shimadzu SPD-M10 Avp detector.

**Analysis of DNA Adducts Formed by 1-SMP**

DNA adducts were assayed using the ³²P-postlabeling technique (33) with modifications described by Phillips and Castegnaro (34). Cells were exposed to 10 μM 1-SMP for 20 min at room temperature in Ringer’s solution. After two washings, cells were collected and DNA was isolated using the DNA Midi Kit (Qiagen, Hilden, Germany). Aliquots of 5 μg of DNA were digested to 3'-mononucleotides with micrococal nuclease (Sigma, Steinheim, Germany) and spleen phosphodiesterase (Calbiochem, Bad Soden, Germany). Adducted nucleotides were enriched with nuclease P1 (MP Biomedicals, Eschwege, Germany), labeled at the 5’ position with [γ-³²P]ATP (50 μCi; Hartmann Analytic, Braunschweig, Germany). Adducted nucleotides were visualized by autoradiography using an Image Capture (Canberra Packard, Dreieich, Germany) and quantified via their specific radioactivity as described by Gupta (35).

**Results**

**Inhibition of hOAT1- and hOAT3-Mediated Uptake of Model Substrates by SMP**

To identify whether stable isomers of SMP interact with OAT, we performed competition experiments using the relatively stable SMP isomers 2-SMP and 4-SMP. They were tested for their ability to inhibit hOAT-mediated uptake of model substrates: PAH for hOAT1 and ES for hOAT3. At a concentration of 20 μM, 2-SMP and 4-SMP inhibited PAH (10 μM) uptake by hOAT1-expressing cells by 86 and 90%, respectively (Figure 2A), and ES (50 nM) uptake by hOAT3-expressing cells by 86 and 90% (Figure 2B). In the latter case, the resulting transport activity was even somewhat below the ES uptake in untransformed control cells, suggesting that endogenous transporters in addition to cDNA-expressed hOAT3 were inhibited.

After noticing these strong inhibitions, we studied the inhibition in more detail using varying concentrations of the substrates and the inhibitors. Moreover, we shortened the exposure time to 3 min and ascertained that uptake was linear with time under this condition. Kᵢ values were determined from Dixon plots. Representative plots are shown in Figure 3. Similar results were obtained in repeat experiments. 2-SMP as well as 4-SMP competitively inhibited the organic anion transport mediated by hOAT1 and hOAT3. The mean Kᵢ values of 2-SMP and 4-SMP for hOAT1 were determined at 4.4 μM (3.9 and 4.9 μM in two separate experiments) and 5.1 μM (4.5, 5.3 and 5.4 μM in three experiments). The corresponding values for hOAT3 were 1.9 μM (1.7 and 2.1 μM) with 2-SMP and 2.1 μM (1.9, 1.9 and 2.5 μM) with 4-SMP.

It has been shown that the reactive isomer 1-SMP can bind covalently to nucleophilic groups of amino acids, proteins, and nucleic acids (36). Therefore, it also might bind covalently to OAT and inhibit them irreversibly. To test this possibility, we
exposed hOAT-expressing cells to 1-SMP (5 μM) for 5 min, followed by a change of the medium, before studying the uptake of standard substrates. Controls were treated equally except that 1-SMP was omitted. The 1-SMP pretreatment reduced the uptake of PAH by hOAT1-expressing cells to 87 ± 5% (n = 3) of the control level. The corresponding figure for ES uptake by hOAT3-expressing cells was 94 ± 11% (n = 3). These changes were not statistically significant. Therefore, we did not detect a significant irreversible inhibition under the experimental conditions used.

hOAT1- and hOAT3-Mediated Uptake of 4-SMP into Cells

We then studied whether 4-SMP, added to the medium, is transported by hOAT into the cells. Because the pyrene moiety shows strong fluorescence, small amounts of 4-SMP could be detected using this property. Coupling of fluorescence detection with HPLC ensured that the compound that was recovered from the cells indeed was 4-SMP rather than a metabolite or decomposition product. The uptake rates of 4-SMP were 3.0- and 1.6-fold higher in hOAT1- and hOAT3-expressing cells, respectively, than in control cells (Figure 4). Addition of probenecid, a known inhibitor of hOAT1 and hOAT3 (37), brought the uptake rates in hOAT1- and hOAT3-expressing cells down to the level observed in untransformed control cells (Figure 4).

Influence of Expression of hOAT1 and hOAT3 on Formation of DNA Adducts by 1-SMP

The uptake of the reactive isomer 1-SMP was assayed indirectly, on the basis of its property to form DNA adducts upon getting inside the cell (38,39). Untreated cells showed no bulky DNA adducts. However, cells that were exposed to 1-SMP (10 μM) exhibited a pattern of adduct spots that is typical for 1-SMP, as observed in previous in vitro and in vivo studies (38,39). This pattern was similar, but the level of the DNA adducts varied strongly across the various HEK293-derived cell lines. Representative autoradiograms of adduct in hOAT1-expressing and control cells are shown in Figure 5A. Expression of hOAT1 and hOAT3 led to 4.6- and 3.0-fold increases, respectively, in the level of DNA adducts compared with control cells (Figure 5B). The presence of probenecid, an OAT inhibitor (37), reduced the level of adducts that were formed in the hOAT-expressing cell lines down to the level observed in control cells (Figure 5B).

Discussion

Sulfo conjugation is a common terminal step in the biotransformation of xenobiotics and usually is followed by renal excretion. Various sulfo conjugates are bound reversibly to serum proteins to a high extent and, therefore, have to be excreted into the tubules rather than filtered in the glomeruli. The basolateral import of numerous organic anions into proximal tubule cells is mediated by OAT1 and OAT3. To date, interaction with these transporters has been studied only for stable molecules. However, various metabolically formed sulfo conjugates are chemically reactive and may damage the cell via covalent binding to cellular macromolecules.

We have studied interactions of isomeric SMP with OAT. One of these isomers (1-SMP) is highly reactive, whereas the others (2- and 4-SMP) are virtually stable. The hOAT1- and hOAT3-mediated uptake of reference substrates was completely inhibited in the presence of 2- and 4-SMP, used at a relatively low concentration (20 μM). Subsequently, we showed that the inhibition is competitive and that 2-SMP and 4-SMP interact with high affinity with both hOAT. The Ki values were similar with both isomers but somewhat lower for hOAT3 (1.9 and 2.1 μM, respectively) than for hOAT1 (4.4 and 5.1 μM). This finding is in accordance with previous observations that...
some sulfo conjugates inhibit OAT3 at lower concentrations than OAT1 (14). Therefore, estrone sulfate (the model substrate for hOAT3 with a $K_m$ of 2.2 $\mu$M [40]) and dehydroepiandrosterone sulfate (20) are transported by OAT3 but not by OAT1 (14).

We then studied whether 4-SMP is transported by OAT into the cells. Positive results were obtained with both OAT. Notably, the magnitude of 4-SMP uptake by hOAT1 was approximately 2.3 times higher than that by hOAT3, despite a lower affinity of hOAT1 (represented by the $K_i$ values in the inhibition studies). We suspect that binding of 4-SMP with high affinity to hOAT3 may impede its release after the translocation into the cell and that a somewhat lower affinity (as observed with hOAT1) may be more favorable for a high translocation rate. We then proved that not only stable benzylic sulfuric acid esters, such as 4-SMP, but also the reactive congener 1-SMP are substrates of hOAT. In this case, we demonstrated the uptake indirectly, taking advantage of the observation that 1-SMP forms DNA adducts that can be detected by the postlabeling technique (38). Therefore, we demonstrated that the amount of DNA adducts upon exposure to 1-SMP was several times higher in hOAT1- or hOAT3-expressing cells than in control cells. As with the uptake of the stable isomer 4-SMP, the effect was stronger with hOAT1, compared with hOAT3, and was reduced to the level observed in control cells in the presence of probenecid. We did not investigate the mechanism of 1-SMP-mediated DNA adduct formation in control cells. It is possible that 1-SMP was taken up by unidentified endogenous transmembrane transporters (which also may be involved in the uptake of 4-SMP, PAH, and ES into control cells). Another mechanism would involve hydrolysis of 1-SMP in the extracellular compartment and uptake of the resulting, uncharged

![Figure 3. Kinetic analyses of the influence of 2-SMP and 4-SMP on organic anion uptake by hOAT1 and hOAT3. Uptake of $[^{3}H]$PAH (10 or 100 $\mu$M) and $[^{3}H]$ES (50 or 300 nM) was assayed in HEK293-derived cell lines that stably expressed hOAT1 (A) and hOAT3 (B), respectively, in the presence of varying concentrations of 2-SMP or 4-SMP. The treatment was for 3 min. Data are presented as Dixon plots. Values are means ± SE of three wells from a single experiment. From these plots, the following $K_i$ values were calculated: for hOAT1, 4.9 $\mu$M 2-SMP, 5.3 $\mu$M 4-SMP; and for hOAT3, 2.1 $\mu$M 2-SMP, 1.9 $\mu$M 4-SMP. Similar values were obtained in repeat experiments (see main text).](image-url)
1-HMP and its sulfo conjugation within the cells; we have detected that HEK293 cells express SULT1A1 protein (at a low level; N.B. et al., unpublished observations, 2005), an enzyme that is capable of converting 1-HMP to 1-SMP (12,41). Finally, 1-SMP may have reacted spontaneously with chloride anion of the Ringer's buffer to generate 1-chloromethylpyrene, an uncharged reactive molecule; it has been found that this mechanism is important for the high bacterial mutagenicity of 1-SMP (42).

DNA adducts may lead to mutations, and mutations in certain genes can lead to the development of tumors. Renal cell carcinoma accounts for approximately 3% of all adult malignancies (reviewed in [43]). Their incidence varies more than 10-fold between various countries and has been increasing in various regions of the world, suggesting an involvement of exogenous factors. The clear cell carcinoma, arising from proximal tubular cells, makes up 70 to 80% of renal neoplasias. A large proportion of these tumors has mutations in chromosome 3p, which contains the Hippel-Lindau gene (reviewed in [43]). It has been estimated that approximately 30% of renal cell carcinoma in men and 24% in women are due to cigarette smoking (reviewed in [43]). 1-Methylpyrene is present at high levels in cigarette smoke and is preferentially oxidized to 1-HMP by human cytochromes P450 and then converted by sulfotransferases to a reactive ester that preferentially forms DNA adduct in kidney (at least in the rat model) (12). The results of our study indicate that concentrative uptake by OAT may be involved in this organotropism. However, cigarette smoke contains additional components (e.g., other alkylated polycyclic aromatic hydrocarbons, aromatic amines, alkenylbenzenes, 5-hydroxymethylfurfural) that can be metabolized to mutagenic sulfo conjugates (11). We would not be surprised if some of them would be enriched in tubular cells by OAT-dependent mechanisms, as demonstrated in our study for 1-SMP.

1-Methylpyrene is present at high levels in cigarette smoke and is preferentially oxidized to 1-HMP by human cytochromes P450 and then converted by sulfotransferases to a reactive ester that preferentially forms DNA adduct in kidney (at least in the rat model) (12). The results of our study indicate that concentrative uptake by OAT may be involved in this organotropism. However, cigarette smoke contains additional components (e.g., other alkylated polycyclic aromatic hydrocarbons, aromatic amines, alkenylbenzenes, 5-hydroxymethylfurfural) that can be metabolized to mutagenic sulfo conjugates (11). We would not be surprised if some of them would be enriched in tubular cells by OAT-dependent mechanisms, as demonstrated in our study for 1-SMP.

SMP are high-affinity competitive inhibitors of hOAT1 and
smokers are unknown but certainly are several orders of magnitude below its estimated $K_m$ value, as is evident from the level to the parent hydrocarbon. Contents of methylpyrenes in cigarette smoke are high compared with those of the reference polycyclic hydrocarbon benzo[a]pyrene but low in absolute terms. Approximately 0.5 μg of each monomethylpyrene and smaller amounts of dimethylpyrenes are formed from one package of cigarettes (plus hundreds of other alkylated polycyclic aromatic hydrocarbons whose levels and biotransformation pathways have not been determined in detail [44]). Exposure to a low, unknown concentration is not unique for 1-SMP but is found with any reactive metabolites of any tobacco smoke carcinogens. Their levels are too low to be measured. Nevertheless, there is no doubt that these reactive metabolites are a major cause of smoking-associated neoplasias. In this context, it is important to know that a single reactive molecule may be sufficient to induce a mutation and that mutations can accumulate with time in a tissue or a cell clone. At low exposure levels, the efficiency ($V_{\text{max}}/K_m$) is the most critical parameter for comparing alternative transport and biotransformation pathways. A rough estimate of the efficiency of hOAT1 and hOAT3 can be made from the data of our study for the stable isomer 4-SMP when $K_m$ (5.1 μM for hOAT1 and 2.1 μM for hOAT3) is used as a substitute for $K_m$ and the transport rate at 10 μM 4-SMP in hOAT-expressing cells minus the corresponding value of the control cells is used instead of $V_{\text{max}}$. On the basis of the data presented in Figure 4, this would yield hOAT1- and hOAT3-mediated transport rates of 51 and 17 pmol/min per mg protein, respectively (or higher, if the conditions were outside the linear range). The estimates of the catalytic efficiency would be 10 and 8 × 10^{-6} L/min for hOAT1 and hOAT3, respectively, ignoring differences in expression levels. Because these values are similar and their inaccuracy may be considerable with the estimation method used, it is difficult to speculate about the relative importance of these transporters. This question would be of some interest, as their distribution in the kidney is different and may determine the susceptible structures. Distribution data that are based on immunohistochemistry are available for the rat nephron (45,46). Both transporters were localized to the basolateral membrane. OAT1 expression was strong in the proximal tubule segment S2 and weak in segment S3, whereas OAT3 was detected in proximal tubule segments S1 and S2, thick ascending limb, distal tubule, and principal cells along the collecting duct.

Conclusion
We provide evidence for cellular uptake of SMP by renal organic anion transporters hOAT1 and hOAT3. Because 1-SMP is the active metabolite of a carcinogen and because a substantial number of other carcinogens also are activated to reactive sulfo conjugates, OAT1 and OAT3 may play a hitherto unrecognized role in the development of renal carcinoma.

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References


