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Functional Consequences of Single Nucleotide Polymorphisms in the Human Organic Anion Transporter hOAT1 (*SLC22A6*)

Authors: Kelly Bleasby, Laura A. Hall, Jennifer L. Perry, Harvey W. Mohrenweiser and John B. Pritchard.

Primary laboratory of origin: Laboratory of Pharmacology & Chemistry, The National Institute of Environmental Health Sciences, NIH, RTP, NC 27709 (K.B., L.A.H., J.L.P., J.B.P.)

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Corresponding author:

Dr. John B. Pritchard
Laboratory of Pharmacology & Chemistry,
The National Institute of Environmental Health Sciences,
111 T.W. Alexander Drive, RTP, NC 27709

Phone: (919) 541-4054
Fax: (919) 541-5737
e-mail: **pritcha3@niehs.nih.gov**

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Abstract

The human organic anion transporter hOAT1 (*SLC22A6*) contributes towards the uptake of a range of small organic anions across the basolateral membrane of the renal proximal tubule and drives their urinary elimination. The aim of this study was to identify genetic variants of hOAT1 and to investigate potential effects on the functional properties of this transporter. Twenty single nucleotide polymorphisms (SNPs) in hOAT1 were identified in genomic DNA from 92 individuals of African, Asian and Caucasian origin. Two SNPs encoded changes in amino acid sequence; arginine to histidine (residue 50) and lysine to isoleucine (residue 525). Significantly, these SNPs were only present in the samples of African origin. When expressed in *Xenopus* oocytes, wildtype R50-hOAT1 and the variants R50H-hOAT1 and K525I-hOAT1 all mediated the probenecid sensitive uptake of the classic organic anion para-aminohippurate (PAH). Kinetic analysis indicated that the transport affinity (K_m) for PAH was unchanged in the variants, compared to wildtype. Interestingly, the K_m for the nucleoside phosphonate analogs adefovir, cidofovir and tenofovir appeared to be decreased in the R50H-hOAT1 variant compared to the wildtype, whereas the kinetics of K525I-hOAT1 remained unchanged. In conclusion, this is the first study to identify variation of hOAT1 in a racially diverse sample and to investigate the functional properties of the resulting variants. Since hOAT1 has been suggested as the basis of nephrotoxicity induced by nucleoside phosphonate analogs, this study raises the intriguing possibility that individuals with genetic variation in hOAT1, such as R50H, may display different handling of these drugs.

Introduction

The central role of the kidney in the elimination of potentially toxic xenobiotics from the blood into the urine is well documented. Early studies began with the whole animal observations (Rennick et al., 1977; Besseghir et al., 1981; Besseghir and Rennick, 1981), followed by many years of intact tissue (Groves et al., 1995; Chatsudthipong and Jutabha, 2001) and cellular studies (Saito et al., 1992; Chan et al., 1997). It is only recently that the molecular identity of the contributing transport proteins has been elucidated. (Burckhardt and Wolff, 2000; Wright and Dantzler, 2004).

During secretion of organic anions by the renal proximal tubule, the anionic drug or xenobiotic must cross two plasma membrane barriers. It is first removed from the circulation across the basolateral membrane into the cell and then secreted across the apical membrane into the urine for elimination. Specific transport proteins are thought to be responsible for each of these steps. At the basolateral membrane, the main mechanism for organic anion uptake was shown to be organic anion/α-ketoglutarate exchange (Pritchard and Miller, 1996). The molecular counterpart of this uptake process was, until recently, considered to be organic anion transporter 1 (OAT1), first isolated from rat kidney by expression cloning (Sekine et al., 1997; Sweet et al., 1997). Recent studies however, indicate that a second transporter OAT3, also operates by this mechanism and thus may contribute towards this step (Bakhiya et al., 2003; Sweet et al., 2003)

The first human organic anion transporter hOAT1 (*SLC22A6*), was identified by homology cloning in 1998 and identified as a 60.3kDa protein (Reid et al., 1998; Cihlar

et al., 1999; Hosoyamada et al., 1999). At the genomic level, hOAT1 is 8.2kb, located on chromosome 11q13-1 and is composed of 10 exons, separated by 9 introns (Bahn et al., 2000). Since its isolation, OAT1 transport has been characterized in great detail (Burckhardt and Wolff, 2000; Wright and Dantzler, 2004). Substrates for this transporter are wide ranging; from the classic small organic anion para-aminohippurate, to several clinically important drugs, herbicides and endogenous substances. Indeed, OAT1's uptake of the nucleoside phosphonate analogs cidofovir and adefovir has been suggested to be the basis for the nephrotoxicity induced by clinical doses of these drugs (Ho et al., 2000).

Analysis of the human genome indicates that several types of genetic variation, including deletions, insertions and single nucleotide polymorphisms (SNPs) exist. SNPs are sequence variations that occur when a single nucleotide in the genomic sequence is altered and are the most common form of variation, with approximately one SNP occurring per 1.9kb of the genome (Chakravarti, 2001). Recently, several groups have turned their efforts to the study of variation in membrane transporters (Iida et al., 2001; Ito et al., 2001; Leabman et al., 2002; Schinkel and Jonker, 2002). This study however, is the first to identify variation in a member of the OAT transporter family in a set of geographically diverse samples and to investigate the impact of this variation on transporter function.

Methods

Variant Identification

72 genomic DNA samples screened for variation in hOAT1 were obtained from the Human Genetic Cell repository, sponsored by the National Institutes of Health, housed at the Coriell Institute, Camden NJ. These samples from individuals of African (15 African-American, 9 African-Pygmy), Asian (4 Indo-Pakistani, 5 native Taiwanese, 5 mainland China, 3 Cambodian, 3 Japanese, 4 Melanesian) or Caucasian (7 Utah, 5 Druze, 7 Eastern European, 5 Russian) origin were selected for geographical diversity (Fritsche et al., 2000). An additional 20 samples were from the 'DNA Polymorphism Discovery Resource' (NIH (Collins et al., 1998)). These samples are from anonymous U.S. residents selected to represent major racial groupings of the population: European-American, African-American, Mexican-American, Native-American, and Asian-American. In total, 92 individuals/184 chromosomes were screened. As all samples came from commercially available cell lines from healthy anonymous donors, the protocol was considered exempt by the Lawrence Livermore National Laboratory Review Board under 10CRF745.101(b) 4.

The resequencing strategy was used to identify variants and is described elsewhere in detail (Mohrenweiser et al., 2002). Briefly, this involved the sequencing of the same genomic region in the 92 individuals in order to identify DNA sequence variation. PCR products containing the individual exons of hOAT1, plus adjacent intronic and non-coding regions were generated using oligonucleotide primers specific to hOAT1 (Table 1) and Pfu DNA polymerase (Stratagene, La Jolla CA). The PCR primers were located

in the introns approximately 75-100 nucleotides 5' and 3 ' of the intron/exon boundary so that at least 50 nucleotides of high quality intronic sequence could be obtained adjacent to each exon, with the exceptions noted in Table 1. Exon 1 was amplified as two overlapping products to accommodate the large size of this exon. Several introns were sufficiently small so that pairs of exons and the entire intron could be amplified as a single product. The ability to obtain high quality sequence from both DNA strands was the limitation imposed on the size of the amplified product. 5' binding sites for energy transfer (ET) DNA sequencing primers (Forward; GTTTTCCCAGTCACGACG. Reverse; AGGAAACAGCTATGACCAT) were added to each primer and thus PCR products could be directly sequenced in both directions using the DYEnamic ET primers and sequencing system (Amersham Life Science, Cleveland OH). It should be noted that complete intronic and untranslated regions were not sequenced in this study. The locations of the PCR primers and SNPs in the current genomic sequence can be obtained by searching the database with a sequence homology algorithm (e.g. BLAST) using first the reference cDNA sequence to obtain the genomic sequence for *SLC22A6* and then repeating the search using the primer sequence or the sequence surrounding the SNP.

Variant reconstruction

The variants R50H-hOAT1 and K525I-hOAT1 were reconstructed by site-directed mutagenesis of the wildtype hOAT1 (pcDNA3.1-hOAT1) (Cihlar et al., 1999) using the Quikchange mutagenesis system (Stratagene, La Jolla, CA). The mutagenic primers (Qiagen, Valencia CA.) were as follows; R50H-hOAT1 (sense) 5'-CCCACCACTGCCACCCGCCTGCCG-3', R50H-hOAT1 (antisense) 5'-

CGGCAGGCGGGTGGCAGTGGTGGG-3', K525I-hOAT1 (sense) 5'-GAGCAGGAAAGGGATACAGACGCGACAGC-3', K525I-hOAT1 (antisense) 5'-GCTGTCGCGTCTGTATCCCTTTCCTGCTC-3'. The wildtype and variant plasmids were purified (High-speed plasmid midi, Qiagen, Valencia CA.) and the sequence confirmed by automated sequencing (Dyenamic ET, Amersham Life Science, Cleveland OH) at the NIEHS core facility. The plasmids were linearized by *XbaI* digest (New England Biolabs, Beverly MA) and capped cRNA was produced by in vitro transcription (T7-message machine, Ambion, Austin TX).

Expression of variants

Wildtype and variant hOAT1 cRNAs were expressed in *Xenopus Laevis* oocytes as previously described (Cihlar et al., 1999). Briefly, stage V and VI oocytes were harvested from *Xenopus Laevis* (Xenopus One, Ann Arbor, MI) and isolated by collagenase digestion in calcium-free OR buffer (in mM; 82.5 NaCl, 2.5 KCl, 1 MgCl2, 5 HEPES, pH 7.6). 24 hours post-isolation, oocytes were injected with either 30 ng of cRNA, or water as the control. Medium was changed every 24 hours.

Transport measurements

72 hours post injection, groups of 6-8 oocytes were incubated for 20 min at room temperature in oocyte Ringer 2 (OR-2 (in mM); 82.5 NaCl, 2.5 KCl, 1 Na2HPO4, 3 NaOH, 1 CaCl2, 1 MgCl2, 1 pyruvic acid, 5 HEPES, pH 7.6) containing various concentrations of either ^{3}H-PAH (Para aminohippuric acid, 1 µCi/ml), ^{14}C-Adefovir (9-(2-Phosphonylmethoxyethyl)-adenine 1 µCi/ml), ^{3}H-Cidofovir ((S)-1-[3-Hydroxy-2(phosphonylmethoxy)propyl]-cytosine 1 µCi/ml) or ^{3}H-Tenofovir ((1R)-9-(2-

phosphonylmethoxypropyl)-adenine 1 μCi/ml). In order to calculate the diffusional component of uptake, substrate uptake at 10μM and 200 μM was also measured in the presence of 1mM probenecid. At 200 μM, the diffusional component was approximately 10% and 20% of total flux for PAH and the nucleoside phosphonates respectively. These figures were subtracted from total uptake to determine the transporter-mediated component. Previous experiments determined that 20 minute substrate uptake was within the initial linear range of hOAT1 transport. Individual oocyte radioactivity was measured by liquid scintillation spectroscopy with external quench correction. To determine transport K_m, kinetic data were corrected for diffusion and subjected to non-linear and linear (Lineweaver-Burk transformation) regression analysis.

All animal experiments were conducted under protocols approved by the NIEHS Animal Care and Use Committee.

Statistics

Means were compared using one way analysis of variance (ANOVA) with Dunnett post-test. Differences in mean values between control and test groups were considered significant when $P<0.05$. Kinetic data are presented graphically as single representative experiments using 6-8 oocytes per point and in tabular format as the mean of 2-4 independent experiments.

Chemicals

^{3}H-PAH (4 Ci/mmol) was purchased from Perkin Elmer Life Sciences, Inc. (Boston, MA). ^{14}C-Adefovir, ^{3}H-Cidofovir and ^{3}H-Tenofovir and their unlabeled equivalents were purchased from Moravek (Brea CA) or were kind gifts from Gilead Sciences, CA. Unlabeled PAH and probenecid were obtained from Sigma (St. Louis MO). All other chemicals were obtained from commercial sources and were of reagent grade.

Results

Genetic variation in *SLC22A6*

The 10 exons of the hOAT1 gene, with their flanking intronic and untranslated regions were directly sequenced from 184 chromosomes. In total, 20 single nucleotide polymorphisms (SNPs) were identified; five in untranslated regions, six in introns and nine in exons. Of the nine sequence variants found in exons, two coded for amino acid changes (non-synonymous), whereas seven did not (synonymous). At amino acid position 50, arginine (codon CGC) was changed to histidine (codon CAC) and at amino acid 525, lysine (codon AAA) was changed to isoleucine (codon ATA). The locations of the amino acid substitutions were assigned using GenBank sequence AF124373 as the reference. Table 2 shows the positions of the SNPs, the changes detected and the allele frequency. The highest frequency SNPs (0.27 and 0.30) were in the 5' untranslated region of hOAT1, and seven of the 20 SNPs were >0.01 in the total chromosomes screened. The frequency of the 2 non-synonymous SNPs, R50H and K525I, were 0.04 and 0.005 respectively in the total sample and were heterozygous in all cases. However, if the data is divided into the sub-samples of Caucasian, African and Asian origin (Table 3) it can be seen that in the samples studied, these two SNPs were only detected in the chromosomes of African origin. Within this sub-sample of 48 chromosomes, the allele frequency is increased to 0.17 and 0.02 for R50H and K525I-hOAT1 genotypes respectively. Interestingly, within the samples of African origin, the R50H variant was represented in both the African American and Pygmy groups (allele frequency of 0.28 and 0.1 within each group respectively). The K525I variant was an individual of African American descent. Previous studies have identified genetic variation in hOAT1; Iida et.

al (2001) identified eight variants in the hOAT1 gene in a Japanese sample of 96 chromosomes, and three of these SNPs were identified in the Asian samples of the present study (Table 2). The most comprehensive study to date of genetic variation in hOAT1 was carried out by the "Pharmacogenetics and Pharmacogenomics Knowledge Base" (Klein et al., 2001); www.pharmgkb.org); 31 variants were identified in an ethnically diverse population of 528-552 chromosomes. Of these variants, nine were also found in the present study, as indicated in Table 2.

Examination of the two non-synoymous SNPs observed in the present study indicates that in contrast to lysine 525, arginine 50 is an evolutionary conserved residue located within a motif of six conserved residues (Figure 1a). Within the predicted secondary structure of hOAT1, R50 appears to be located in the large extracellular loop between transmembrane domains 1 and 2, whereas K525 is located in the C-terminus (Figure 1b). The potential impact of these substitutions on protein structure and activity was explored with the "Sorting Intolerant from Tolerant" (SIFT) (Ng and Henikoff, 2002) and the "Polymorphism Phenotyping" (PolyPhen) (Sunyaev et al., 2001) algorithms. These algorithms predict the potential impact of amino acid substitutions based upon evolutionary conservation of sequence, localization within functional domains and the chemical and physical properties of the exchanged amino acid residues. Both algorithms predicted that the Ile substitution at reside 525 had a high probability of impacting function (PSIC score of 2.31 and SIFT score of 0.0), while the His substitution at residue 50 was predicted to have only a moderate negative impact (PSIC score of 1.82 and SIFT score of 0.17).

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Functional analysis of hOAT1 variants

Initial experiments were conducted to determine whether the reconstructed variants R50H-hOAT1 and K525I-hOAT1 were functional. Uptake of ^{3}H-PAH (10μM) into oocytes expressing either the wildtype hOAT1 or individual variants was measured in the presence and absence of the hOAT1 transport inhibitor probenecid (1mM). Compared to water-injected control, oocytes expressing wildtype, R50H or K525I -hOAT1 mediated significant uptake of the substrate and this uptake was almost completely abolished in the presence of probenecid (data not shown). Based on this, the transport properties of the two variants were then characterized in more detail by measuring transport kinetics for four hOAT1 substrates; PAH, adefovir, cidofovir and tenofovir.

Figure 2 shows one representative experiment of the uptake of the classic organic anion PAH mediated by wildtype hOAT1, R50H and K525I-hOAT1 over a range of concentrations (2 - 200 μM). Uptake is corrected for diffusion (measured in presence of 1mM probenecid). The calculated K_m for wild type hOAT1 in this experiment was 5.1 ± 0.2 μM, which is consistent with the previously published K_m for this transporter (Cihlar et al., 1999). Table 4 shows the mean K_m values for the R50H and K525I-hOAT1 variants were 4.9 ± 0.6 μM and 4.7 ± 0.1 μM respectively. The lack of statistical significant difference ($p>0.05$) between these kinetic values indicates no change in the transport affinity for PAH in the R50H and K525I-hOAT1 variants, when compared to wildtype hOAT1.

Kinetic studies were also conducted for three nucleoside phosphonate analogs. Figures 3, 4 and 5 show representative experiments of transporter mediated adefovir, cidofovir and tenofovir uptake for wildtype-hOAT1 and R50H and K525I-hOAT1 mutants over a range of concentrations (5-200 μM). Furthermore, Table 4 shows mean K_m values for several independent experiments. Figures 3 and 4 show that again, hOAT1 had calculated K_m values for adefovir and cidofovir consistent with the literature (Cihlar et al., 1999) of 20 ± 1.9 μM and 55 ± 9.1 μM respectively, however, the R50H-hOAT1 construct displayed significantly reduced ($p<0.01$) K_m values of 11.4 ± 0.6 μM and 16.1 ± 2.7 μM, a decrease of approximately 40% and 70% respectively. The K_m for K525I-hOAT1 remained almost identical in both cases to the wildtype transporter. Figure 5 shows a representative experiment where kinetic constants were determined for a third nucleoside phosophonate, tenofovir, which showed a similar trend. Mean Km values from multiple experiments are again shown in table 4 and it can be seen that the R50H-hOAT1 mutant K_m (14.6 ± 1.8 μM) was significantly decreased ($P<0.05$) compared to wildtype hOAT1 (22.3 ± 0.7 μM) whereas the K525I variant was similar at 21.9 ± 2.5 μM. V_{max} values for the transport of the three nucleoside phosphonate analogs were also decreased ~50-60% for the R50H variant, compared to either the wildtype hOAT1 or the K525I variant.

Discussion

The last decade has seen an explosion in the interest and understanding of epithelial transport mechanisms. Dedicated research efforts have identified many transport proteins at the molecular level and have gone on to ascertain their tissue and cellular distributions, driving forces and substrate specificities (van Montfoort et al., 2003). Now, following in the footsteps of the highly characterized cytochrome P450 enzymes, transporter research is moving in the direction of pharmacogenetics. The identification of genetic variation in transporters and the subsequent characterization of phenotype will add to the growing knowledge base in the understanding of interindividual response to xenobiotics.

The aim of the present study was to identify SNPs in the hOAT1 gene and to investigate the functional properties of any non-synonymous SNPs detected. Among the 184 chromosomes screened, 20 single nucleotide polymorphisms were detected. Ten of these SNPs are new, with three being identified previously in Japanese samples (Iida et al., 2001) and nine identified in an ethnically diverse population (Klein et al., 2001). Two of the SNPs identified in our study resulted in an amino acid change; arginine to histidine at residue 50 and lysine to isoleucine at residue 525. As has been described for polymorphisms in other transporters and enzymes (Schaeffeler et al., 2001; Leabman et al., 2002), there was an uneven distribution of SNPs across racial groups, indeed of the 184 samples studied, the two non-synonymous SNPs detected were only found in the chromosomes of African descent.

In an effort to reveal potentially subtle differences in transporter function, analysis of transport kinetics of a range of substrates was studied. R50H-hOAT1 appeared to have an increased affinity for the nucleoside phosphonate analogs cidofovir, adefovir and tenofovir compared to wildtype hOAT1, whereas its transport of PAH was unaffected. In contrast K525I-hOAT1 appeared to have similar transport characteristics to the wildtype-hOAT1, suggesting that this residue is not important in the determining the transport affinity of the substrates tested.

Both V_{max} and K_m changes were seen in the kinetics of the R50H variant relative to wildtype hOAT1 (both ~50-60% lower) or the K525I variant, which was identical to wildtype. However, while differences in V_{max} were easily seen, their significance remains less certain, since they may reflect either changes in intrinsic transporter function or simply a different level of expression in the oocyte system. As shown in Figures 2-5, all three variants were expressed in the plasma membrane and mediated transport. Unfortunately, attempts to detect differences in variant expression using antibodies against hOAT1 were not successful and both possibilities remain. However, this uncertainty serves to highlight the critical need to determine hOAT1 expression levels in people carrying the R50H SNP, before the full impact of this mutation on anionic drug transport can be determined.

There is no such complication in the interpretation of the changes in K_m. These do demonstrate a substantial increase in substrate affinity for the R50H variant. This increase appeared to follow the rank order of substrate affinity for the wildtype hOAT1,

i.e., the greatest increase in the affinity of the R50H variant was seen for those substrates demonstrating the weakest affinity for the wildtype transporter. In agreement with this data, several reports have shown that the functional properties of variant transporters vary with the substrate(s) tested (Kerb et al., 2002; Leabman et al., 2002; Ohashi et al., 2002). This study supports the clear need to study a range of substrates when investigating the phenotype of polymorphic transporters.

A recent study by Shu et. al. (Shu et al., 2003) suggested that the degree of chemical change and the evolutionary conservation of the amino acid changed in a polymorphism could be predictive of the functional consequences. For the non-synonymous SNPs identified in this study, lysine to isoleucine is considered a radical chemical change (Grantham value of 102 (Grantham, 1974), PSIC score of 2.31 (Ng and Henikoff, 2002) and a SIFT score of 0.0 (Sunyaev et al., 2001), however residue 525 does not appear to be conserved in OAT1 and OAT3 (Figure 1a). It is interesting that this substitution was predicted to disrupt function, but functional differences were not detected. In contrast however, arginine to histidine is not considered to be a substantial chemical change (Grantham value of 29, PSIC score of 1.82 and SIFT score of 0.17), but this residue does lie within a highly conserved region of hOAT1 (Figure 1a). Indeed, R50 resides within the first extra cellular loop of hOAT1 – a structure which is conserved throughout the families of organic anion and cation transporters (Burckhardt and Wolff, 2000; Wright and Dantzler, 2004). Furthermore, several residues within this loop have previously been shown to be important for transporter function (Bleasby et al., 2002). Thus in this study,

evolutionary conservation of amino acid residues appears to be more predictive of function than chemical changes.

This study has focused primarily on the two non-synonymous SNPs detected in hOAT1, however seven synonymous SNPs were also detected. A high ratio of synonymous to non-synonymous SNPs was also observed by Leabman et. al. (Leabman et al., 2002) in the organic cation transporter hOCT2 and it was suggested that this could be indicative of a lack of tolerance to changes in transporters involved in the critical role of detoxification. In addition, several SNPs were also found in the 5' and 3' untranslated and intronic regions of hOAT1, despite only a small fraction of these regions being sequenced. It is possible that SNPs in these regions of hOAT1 could have important effects on protein expression and modulation, however until more is known about the regulation of wildtype hOAT1 it is not possible to investigate the potential importance of SNPs in these regions.

As membrane transporter research moves towards the investigation of genetic variation, caution must be observed when interpreting the data and the wider picture must be taken into account. Transporter expression and function can be modulated by several mechanisms, with genetic variation being just one of them. It must also be considered that many of these transporters are multispecific and thus a particular substrate could utilize more than one transporter to traverse a membrane (e.g. OAT1 and OAT3 at the basolateral membrane). Thus, under conditions where transporters are altered by polymorphisms, the pathway utilized for transport may change, but as is often observed

with knockout animals (Jonker et al., 2003) a compensatory mechanism or alternative pathway may mean that no overall change would be detected *in vivo*. However, for an OAT1 substrate, if the rate determining step for renal elimination is uptake across the basolateral membrane, then even moderate changes in the function of OAT1 could cause significant effects. Since hOAT1 has been suggested as the basis of nephrotoxicity of the nucleoside phosphonate analogs (Ho et al., 2000) and adefovir does not appear to be a substrate for hOAT3 (Cihlar et al., personal communication), these studies raise the interesting possibility that individuals with genetic variation in hOAT1 such as R50H may handle these drugs differently.

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Footnotes

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To whom requests for reprints should be made:

Dr. John B. Pritchard
Laboratory of Pharmacology & Chemistry,
The National Institute of Environmental Health Sciences,
111 T.W. Alexander Drive, RTP, NC 27709

Phone: (919) 541-4054
Fax: (919) 541-5737
e-mail: **pritcha3@niehs.nih.gov**

Current address; Department of Drug Metabolism, Merck Research Laboratories,

Rahway, NJ 07065 (KB)

Lawrence Livermore National Laboratory, Livermore, CA 94551. Current address;

Epidemiology Division, University of California, Irvine, Irvine, CA 92697 (HWM)

Figure legends

Figure 1. (A) Partial alignments of amino acid sequences from human, rat and mouse OAT1 and OAT3 in the regions surrounding residues R50 and K525. The conservation of R50 (black shading) and the surrounding motif (grey shading) are indicated. (B) Predicted secondary structure of hOAT1 indicating the positions of R50 and K525.

Figure 2. Representative experiments of PAH transport kinetics for wildtype R50-hOAT1 (K_m 5.1 ± 0.2 μM, V_{max} 11.2 ± 0.1 pmol/oocyte/20 min), R50H-hOAT1 (K_m 4.3 ± 0.1 μM, V_{max} 11.2 ± 0.1 pmol/oocyte/20 min) and K525I-hOAT1 (K_m 4.9 ± 0.2 μM, V_{max} 6.8 ± 0.1 pmol/oocyte/20 min). Each point reflects the mean ± S.E.M of 6-8 oocytes. The Lineweaver-Burk transformation of these data is shown on the right.

Figure 3. Representative experiments of adefovir transport kinetics for wildtype R50-hOAT1 (K_m 20.0 ± 1.9 μM, V_{max} 31.7 ± 0.9 pmol/oocyte/20 min), R50H-hOAT1 (K_m 11.4 ± 1.5 μM, V_{max} 17.3 ± 0.6 pmol/oocyte/20 min) and K525I-hOAT1 (K_m 19.2 ± 1.1 μM, V_{max} 29.3 ± 0.5 pmol/oocyte/20 min). Each point reflects the mean ± S.E.M of 6-8 oocytes. The Lineweaver-Burk transformation of these data is shown on the right.

Figure 4. Representative experiments of cidofovir transport kinetics for wildtype R50-hOAT1 (K_m 55.4 ± 9.1 μM, V_{max} 25.3 ± 1.5 pmol/oocyte/20 min), R50H-hOAT1 (K_m 16.1 ± 2.7 μM, V_{max} 14.3 ± 0.5 pmol/oocyte/20 min) and K525I-hOAT1 (K_m 52.3 ± 5.7

μM, V_{max} 33.6 ± 5.7 pmol/oocyte/20 min). Each point reflects the mean ± S.E.M of 6-8 oocytes. The Lineweaver-Burk transformation of these data is shown on the right.

Figure 5. Representative experiments of tenofovir transport kinetics for wildtype R50-hOAT1 (K_m 21.1 ± 3.6 μM, V_{max} 60.3 ± 3.0 pmol/oocyte/20 min), R50H-hOAT1 (K_m 14.4 ± 1.4 μM, V_{max} 27.1 ± 0.7 pmol/oocyte/20 min) and K525I-hOAT1 (K_m 20.3 ± 4.5 μM, V_{max} 58.0 ± 3.8 pmol/oocyte/20 min). Each point reflects the mean ± S.E.M of 6-8 oocytes. The Lineweaver-Burk transformation of these data is shown on the right.

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Table 1. Oligonucleotide primers used for PCR and sequencing

Region sequenced	Forward Primer (5'- 3')	Reverse Primer (5'- 3')
Exon 1 (part 1)	TCCAGGCCAAGGATTAAAAAC	CAGTGAAGTTCTGCAGGGTGT
Exon 1 (part 2)	ATGGCCTTTAATGACCTCCTG	TTAACAAAGGCCCCCATCTT
Exons 2&3	CTGAAGCCAGGCTCATCTCT	GGGAGTGGGCTGGTAAGAAT
Exon 4	TGGAGAGAGGGGCCTTTACTA	CTACATTTGTGGGATGGGATG
Exons 5&6	AATAAAATCCCATCGCAGACC	TCTTTCACCCATTGTGTCTCC
Exons 7&8	TCCTTAAGCAGGGTACCCAAT	TTGATTTAGCTCTGGGGTCTG
Exon 9	AATCAAATGGGGAAGAGAGGA	GCTTTGGGCTAGAAGAGGAAG
Exon 10	GCTCCAGGGAGCAGTAGGTAT	AAGATGCTTTCCTGAACCACA
Sequencing[a]	GTTTTCCCAGTCACGACG	AGGAAACAGCTATGACCAT

[a]5' binding site sequence for the forward and reverse energy transfer DNA sequencing primers.

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Table 2. Single nucleotide polymorphisms identified in the hOAT1 (*SLC22A6*) gene.

Exon	Position in hOAT1 sequence[a]	cDNA Sequence 5'- 3'	Amino acid position	Amino acid change	Allele frequency[b]
	302	GTCTC(C/T)AGCAG	5' UTR	-	0.016
	418	GGCTG(C/T)TGTCC	5' UTR	-	0.005
	453	CTCCC(G/A)GAGCA[d]	5' UTR	-	0.272
	560	CAGCC(A/G)CTGGG[c,d]	5' UTR	-	0.295
1	728	CTGC**C(G/A)C**CCGC[c]	50	**Arg/His**	0.042
1	759	AAG**AA(C/T)**GGGGG[c]	60	Asn/Asn	0.006
1	831	TCC**CC(G/T)**CAGTG[c]	84	Pro/Pro	0.027
1	930	TTC**CC(A/G)**TCTAC[c]	117	Pro/Pro	0.032
3	1687	CTC**CT(C/G)**TCGGG	194	Leu/Leu	0.007
	3258	CTTAA(C/T)CCTCT[c]	Intron 3	-	0.012
5	3936	TCC**GG(G/A)**AGGCT	279	Gly/Gly	0.006
5	3972	AGA**GT(C/T)**GCCCG	291	Val/Val	0.006
	4053	AACCT(C/T)CCAGG[c,d]	Intron 5	-	0.056
	4151	GATCC(C/T)GGGCC[c]	Intron 5	-	0.011
	7749	AGCCT(G/A)AGCTG	Intron 8	-	0.083
	7761	CACCC(T/C)CCAAA	Intron 8	-	0.005
	7793	CATGC(C/T)GGAAC	Intron 8	-	0.120
9	7941	ATC**TA(C/T)**GGTGC[c]	490	Tyr/Tyr	0.005
10	8347	AGGG**A(A/T)A**CAGA	525	**Lys/Ile**	0.005
	8472	GGTCC(T/C)ACAGG	3' UTR	-	0.005

[a]Variant position is relative to accession number AJ249369. The common nucleotide in the cDNA sequence is followed by the variant nucleotide and is enclosed in parentheses, the codon of the amino acid is underlined and bold. [b]Allele frequency is the alleles observed/number of chromosomes tested. [c]SNP reported by PharmGKB. [d]SNP reported by Iida et al. (2001).

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Table 3. Allele frequency[a] of SNPs by racial group

Position in hOAT1 sequence	Ancestry			
	Asian (n=48)	African (n=48)	Caucasian (n=48)	Anonymous[b] (n=40)
302	0.0	0.0	0.04	0.03
418	0.0	0.0	0.02	0.0
453	0.21	0.54	0.10	0.23
560	0.19	0.54	0.15	0.28
728	0.0	0.17	0.0	0.0
759	0.0	0.02	0.0	0.0
831	0.0	0.04	0.04	0.05
930	0.0	0.06	0.04	0.05
1687	0.06	0.17	0.0	0.0
3258	0.0	0.04	0.0	0.08
3936	0.0	0.02	0.0	0.0
3972	0.0	0.02	0.0	0.0
4053	0.14	0.0	0.0	0.08
4151	0.0	0.02	0.0	0.03
7749	0.0	0.29	0.0	0.05
7761	0.0	0.0	0.0	0.03
7793	0.21	0.02	0.08	0.10
7941	0.0	0.02	0.0	0.0
8347	0.0	0.02	0.0	0.0
8472	0.0	0.0	0.0	0.03

[a]Allele frequency is the alleles observed/number of chromosomes tested. [b]Anonymous samples represent individuals of unknown racial group from the NIH DNA Polymorphism discovery resource (Collins et al., 1998).

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Table 4. Summary of mean K_m values (μM) for hOAT1 substrates.

	PAH	Adefovir	Cidofovir	Tenofovir
hOAT1	4.9± 0.9	17.2 ± 2.7	51.8 ± 4.4	22.3 ± 0.7
R50H-hOAT1	5.5 ± 0.6	11.4 ± 0.6*	21.3 ± 2.8*	14.6 ± 1.8*
K525I-hOAT1	4.9 ± 0.2	18.9 ± 1.3	45.7 ± 3.9	21.9 ± 2.5

* Indicates statistical difference ($p<0.05$) when compared to hOAT1 within each substrate group. Data are mean determinations from three (PAH, cidofovir and tenofovir) and four (adefovir) different animals.

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Figure 1. Conservation of amino acid residues and predicted location

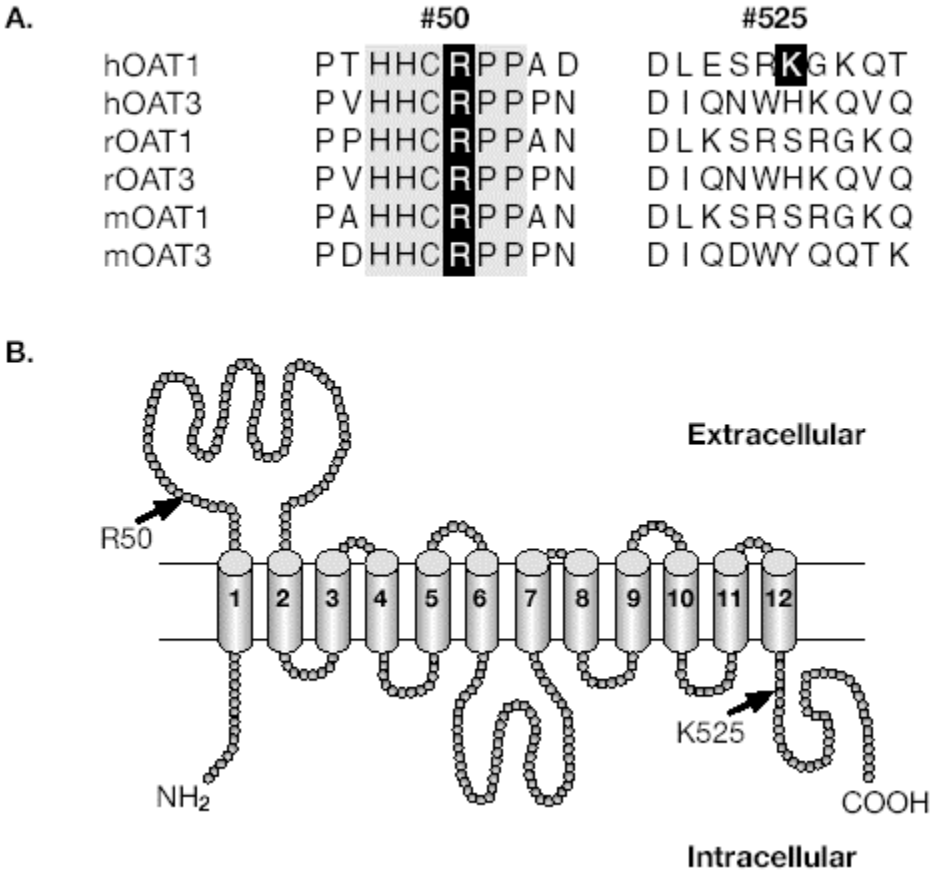

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Figure 2. PAH transport kinetics

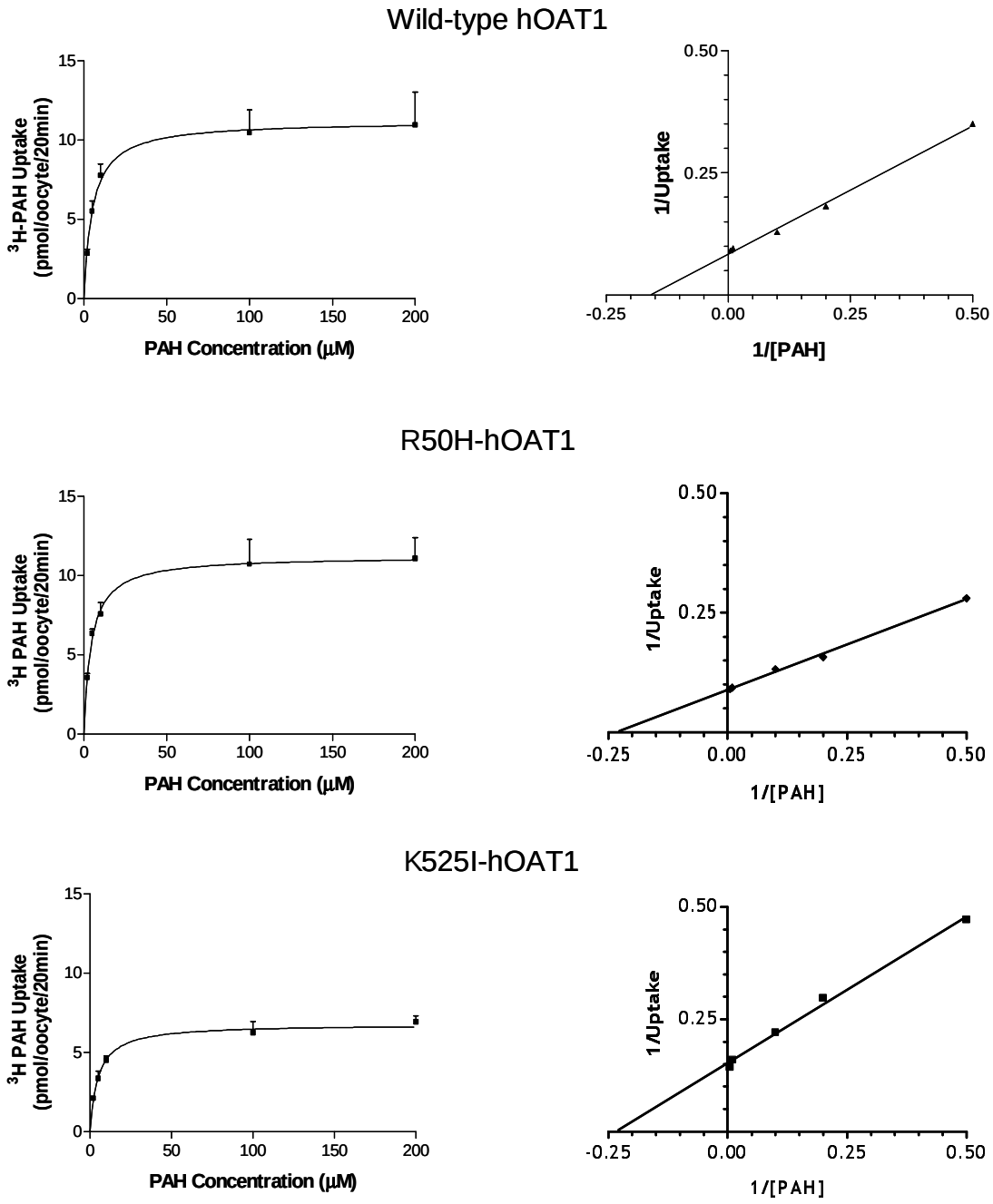

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Figure 3. Adefovir transport kinetics

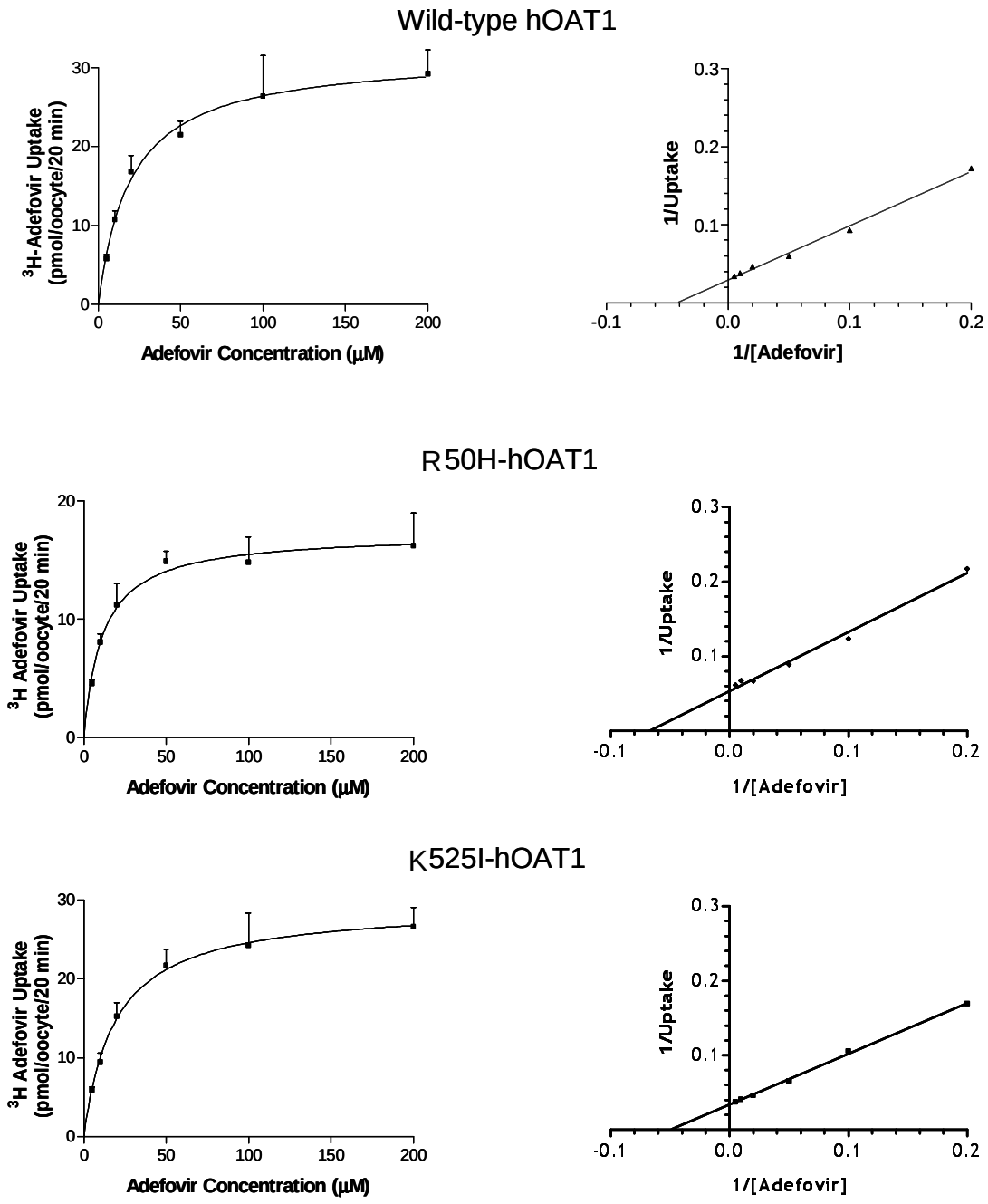

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Figure 4. Cidofovir transport kinetics

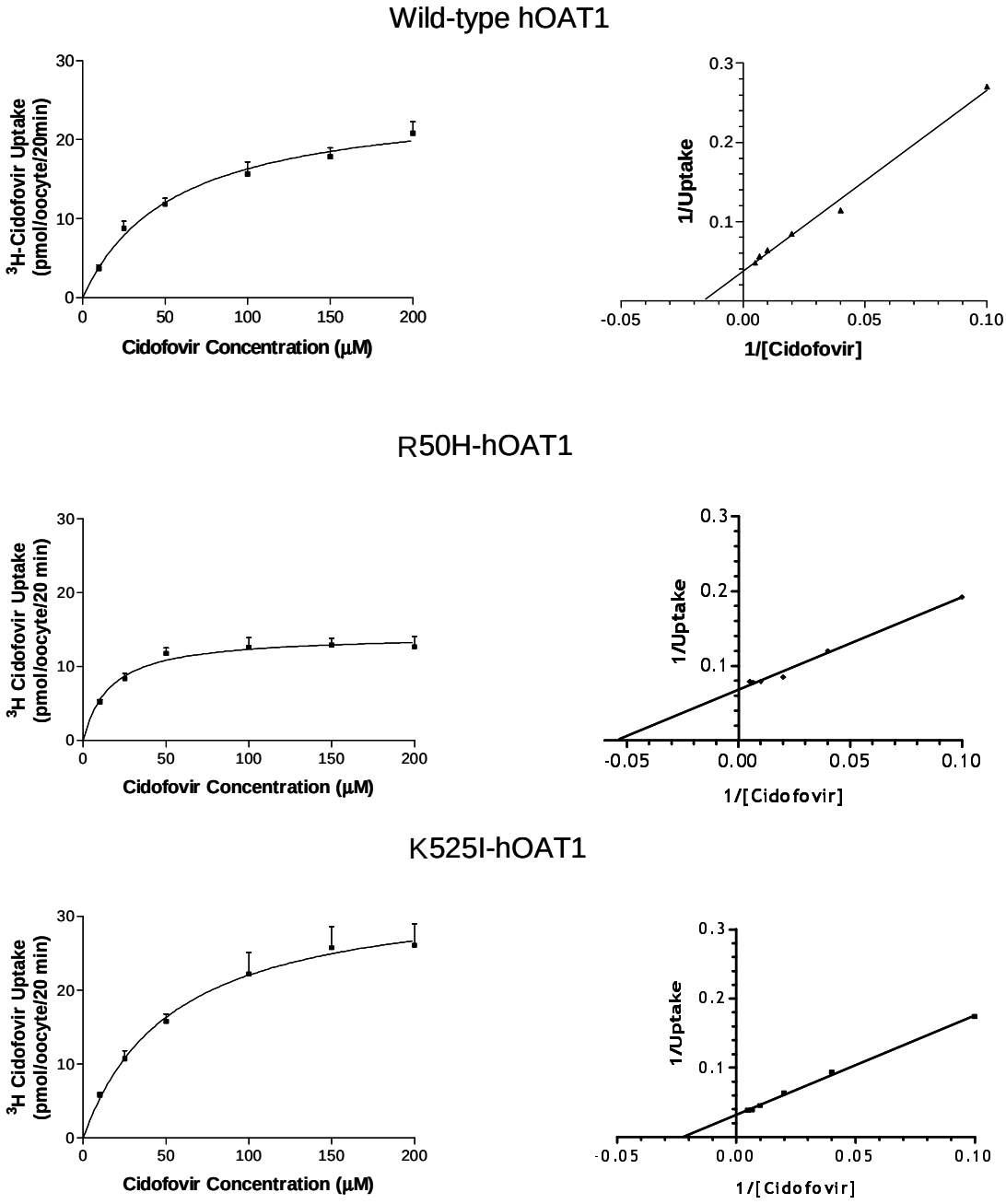

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Figure 5. Tenofovir transport kinetics