Nucleoside Phosphonate Interactions with Multiple Organic Anion Transporters in Renal Proximal Tubule

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ABSTRACT

The interactions of two antiviral, acyclic nucleoside phosphonates, adefovir and cidofovir, with xenobiotic transporters was studied in intact killifish (Fundulus heteroclitus) renal proximal tubules by using fluorescent substrates, confocal microscopy, and quantitative image analysis. Both drugs reduced in a concentration-dependent manner the transport of fluorescein on the classical organic anion system and transport of fluorescein-methotrexate on multidrug resistance-associated protein 2 (Mrp2). Neither drug inhibited transport of a fluorescent cyclosporin A derivative on P-glycoprotein. Inhibition of Mrp2-mediated transport was abolished by 50 μM p-aminohippurate, indicating that adefovir and cidofovir entered the cells at the basolateral membrane on the classical organic anion transport system (OAT1). Comparison of the inhibitory potencies of the nucleoside phosphonates with other substrates and inhibitors showed them to be moderate inhibitors of OAT1- and Mrp2-mediated transport.

The vertebrate renal proximal tubule is responsible for the excretory transport of a large number of potentially toxic chemicals, including waste products of normal metabolism, drugs, environmental pollutants, and drug and pollutant metabolites. These chemicals are handled by specific secretory xenobiotic transport systems that remove substrates from the blood, transport them across the tubular epithelium, and concentrate them in urine (Pritchard and Miller, 1993, 1996). Twelve years ago, only two transport systems for xenobiotics had been characterized in renal proximal tubule, one for organic anions and the other for organic cations. At that time it was clear that both of these “classical” systems possessed separate basolateral and luminal transporters and that each step in transport was both concentrative and directly or indirectly tied to cell metabolism (Fig. 1). The specificities of the two systems were sufficiently different that only limited overlap in substrates transported was expected or found (Pritchard and Miller, 1993).

Since then, the number of xenobiotic transporters known to be present in proximal tubule has increased substantially. For example, two members of the ABC superfamily of transporters, P-glycoprotein and multidrug resistance-associated protein 2 (Mrp2), have been localized to the luminal membrane of proximal tubule cells (Thiebaut et al., 1989; Schaub et al., 1997) and shown to mediate active excretory transport of xenobiotics in intact tubules (Miller, 1995; Schramm et al., 1995; Masereeuw et al., 1996, 1999). At the molecular and functional levels, these transporters are very different from those that make up the classical systems in that they 1) are ATP-driven; 2) handle larger, more lipophilic substrates; and 3) exhibit unusually broad specificities (Ford and Hait, 1990; Konig et al., 1999). Although exceptions have been noted, P-glycoprotein mediates transport of larger organic cations and some neutral compounds, and Mrp isoforms mediate transport of larger organic anions and some neutral compounds. However, there appears to be considerable overlap in specificities between these ABC transporters and among the ABC transporters and the classical systems. Furthermore, the tools of molecular biology have identified transporter families for organic anions (OATs and organic anion-transporting polypeptides) and organic cations (organic transporter cations) that are expressed in proximal tubule. Although OAT1 appears to be the basolateral transporter (anion exchanger) for the classical organic anion system (Sweet et al., 1999) and organic cation transporter 2 may be the basolateral transporter for the classical organic cation system (Sweet et al., 2000), it is still not clear where and how several of the transporters identified at the molecular level function within proximal tubule cells.

It is clear however that at each face of the renal proximal tubule cell xenobiotics encounter multiple transporters, some of which have broad specificity limits. This suggests interesting complexities with regard to multiple routes of transport and multiple sites of substrate-transporter interaction. For

ABBREVIATIONS: ABC, ATP-binding cassette; Mrp2, multidrug resistance-associated protein 2; OAT, organic anion transporter; FL-MTX, fluorescein-methotrexate; FL, fluorescein; NBD-CSA, [N-(4-nitrobenzofurazan-7-yl)-o-Lys8]-cyclosporin A; PAH, p-aminohippurate; CSA, cyclosporin A; LTC4, leukotriene C4.
example, we recently demonstrated that the fluorescent organic anion lucifer yellow entered proximal tubule cells on the basolateral transporter for the Na\(^+\)-dependent organic anion transport system (OAT1), but was transported from cell to tubular lumen on two transporters, one of which was Mrp2 (Masereeuw et al., 1999). Similarly, the weak base daunomycin was found to enter the cells on the basolateral transporter for the organic cation system and exit on an anion transport system (OAT1), but was transported from tubular lumen by Mrp2 (6). NBD-CSA enters the cells by simple diffusion and is pumped into the lumen by P-glycoprotein (7). For the evidence supporting these mechanisms, see Miller et al. (1993, 1996), Miller and Pritchard (1991, 1994), Schramm et al. (1995), and Masereeuw et al. (1996, 2000).

To begin to sort out the complexities of xenobiotic excretion in intact renal proximal tubules, we have developed a powerful experimental system based on isolated, killifish tubules. Renal tissue from certain marine teleost fish offers several advantages for the study of mechanisms of xenobiotic secretion (Miller and Pritchard, 1991). The nephron of these animals is composed primarily of proximal tubules, which are easily isolated and which retain viability for long periods when maintained in a simple physiological saline. During tubule isolation, broken ends reseal and form a closed, fluid-filled luminal compartment that is separated from the medium by the epithelium. By using fluorescent substrates, confocal microscopy, and image analysis, xenobiotic uptake by cells and secretion into the lumen can be visualized and measured (Miller and Pritchard, 1994; Masereeuw et al., 1996; Miller et al., 1996). Finally, we have identified fluorescent substrates and nonfluorescent inhibitors that can be used as tools to distinguish specific pathways of xenobiotic excretion in killifish tubules.

The present studies have two purposes: first, to develop further the killifish tubule model by extending the range of xenobiotics tested as inhibitors of transport; and second, to use the system to investigate interactions of two antiviral, acyclic nucleoside phosphonates, adefovir and cidofovir (Fig. 2), with renal xenobiotic transporters in an intact proximal tubule. Both drugs are actively secreted by the kidney (Cundy et al., 1995a,b) and both have been shown to be substrates for OAT1 in cell lines transfected with the transporter (Cihlar et al., 1999; Mulato et al., 2000). Their handling by intact proximal tubule is of particular interest, because tubular nephrotoxicity limits use in the clinic at the high doses used for acquired immunodeficiency syndrome therapy (Lalezari et al., 1997; Kahn et al., 1999), and experiments with transfected cell lines showed enhanced toxicity with increased OAT1 function and reduced toxicity with inhibited OAT1 function (Ho et al., 2000; Mulato et al., 2000). However, at lower doses used to treat hepatitis B infection, adefovir appears to exhibit no nephrotoxicity (Perrillo et al., 2000).

Materials and Methods

Chemicals. Fluorescein-methotrexate (FL-MTX) and fluorescein (FL) were obtained from Molecular Probes (Eugene, OR). The fluorescent cyclosporin A derivative NBD-CSA was synthesized as described previously (Schramm et al., 1995). Adefovir and cidofovir were obtained from Dr. John Pritchard (National Institute on Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC). All other chemicals were obtained from commercial sources at the highest purity available.

Animals and Tissue Preparation. Killifish were obtained near the Duke University Marine Laboratory (Beaufort, NC) and maintained at the National Institute on Environmental Health Sciences in tanks with artificial seawater. Renal proximal tubules were prepared in marine teleost saline (Forster and Taggart, 1958) containing 140 mM NaCl, 2.5 mM KCl, 1.5 mM CaCl\(_2\), 1.0 mM MgCl\(_2\), and 20 mM Tris, pH 8.0. To obtain tubules, kidney tissue was teased under a dissecting microscope with fine forceps to remove adherent hematopoietic tissue. Individual proximal tubules were dissected and transferred to an aluminum foil-covered Teflon incubation chamber containing 1.5 ml of marine teleost saline with fluorescent compound and added effectors. The chamber floor was a glass coverslip to which the tubules adhered lightly and through which the tissue could be viewed by means of an inverted confocal laser scanning microscope. The fluorescent compounds were dissolved in water or dimethyl sulfoxide and added to the incubation medium. Prelim-

Fig. 1. Xenobiotic transport mechanisms in killifish renal proximal tubules. FL entry into the cells at the basolateral membrane is driven by indirect coupling to the Na\(^+\) gradient, through organic anion exchange (3) and sodium-dicarboxylate (a-ketoglutarate, a-KG) cotransport (2). Exchange is driven by the gradient for a-KG, which is also produced by the mitochondria. The Na\(^+\) gradient is maintained by the Na\(^+\), K\(^+\)-ATPase (1). FL exit at the luminal membrane is mediated by an as yet unidentified transporter (5). FL-MTX entry is mediated by an as yet unidentified transporter (4). FL-MTX is pumped into the lumen by Mrp2 (6). NBD-CSA enters the cells by simple diffusion and is pumped into the lumen by P-glycoprotein (7). For the evidence supporting these mechanisms, see Miller et al. (1993, 1996), Miller and Pritchard (1991, 1994), Schramm et al. (1995), and Masereeuw et al. (1996, 2000).

Materials and Methods

Chromatics. Fluorescein-methotrexate (FL-MTX) and fluorescein (FL) were obtained from Molecular Probes (Eugene, OR). The fluorescent cyclosporin A derivative NBD-CSA was synthesized as described previously (Schramm et al., 1995). Adefovir and cidofovir were obtained from Dr. John Pritchard (National Institute on Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC). All other chemicals were obtained from commercial sources at the highest purity available.

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<table>
<thead>
<tr>
<th>Compound</th>
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<tr>
<td>Cidofovir</td>
<td>Cytosine</td>
<td>-CH(_2)OH</td>
</tr>
<tr>
<td>Adefovir</td>
<td>Adenine</td>
<td>-H</td>
</tr>
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Fig. 2. Structures of the nucleoside phosphonates.
inary experiments showed that the concentrations of dimethyl sulfoxide used (<1%) had no significant effects on the uptake and distribution of the fluorescent-labeled test compounds as measured by confocal and epifluorescence microscopy.

**Confocal Microscopy.** The chamber containing the tubules was mounted on the stage of a Zeiss model 410 or model 510 laser scanning confocal microscope (inverted) and viewed through a 40× water immersion objective (numerical aperture = 1.2). A 488-nm laser line, a 510-nm dichroic filter, and a 515-nm long-pass emission filter were used. Low laser intensity (1–3% of maximum) was used to avoid photobleaching of the dyes. With the photomultiplier gain set to give an average luminal fluorescence intensity of 100 to 200 (full scale, 0–255), tissue autofluorescence was undetectable. For measurements, tubules in the chamber were first viewed under reduced, transmitted light illumination. A suitable field with several tubules was then selected and a confocal image was acquired by averaging eight frames. The image was saved to disk for later analysis.

Fluorescence intensities were measured from stored images using NIH Image 1.61 software as described previously (Miller, 1998). For each tubule, two to four adjacent cellular and luminal areas (100–300 pixels each) were selected. The background fluorescence intensity was subtracted and the average pixel intensity for each area was calculated. The values used for that tubule were the means for all selected areas. Video and confocal microscopy of glass capillary tubes filled with solutions of known concentrations of fluorescein and other dyes has shown that the relationship between image fluorescence intensity and concentration is approximately linear (Miller and Pritchard, 1991; D. S. Miller, unpublished data). However, because there are large uncertainties in relating cellular fluorescence to the actual concentration of a fluor within cells, data are reported here as average pixel intensities rather than as estimated concentrations. Data are presented as mean ± S.E. Means were considered statistically different when the probability value (P) was less than 0.05 by use of an unpaired t test.

**Results**

For the present experiments, three fluorescent xenobiotics were used as substrates: FL, FL-MTX, and NBD-CSA. Previous studies from this laboratory have shown that each of these fluorescent compounds is transported across renal proximal tubule by a different mechanism; the mechanisms of transport are summarized in Fig. 1.

In teleost renal proximal tubules, FL is a substrate for the Na⁺–dependent, ouabain-sensitive classical renal organic anion transport system that is driven by indirect coupling to Na⁺ at the basolateral membrane (Miller and Pritchard, 1991, 1994; Fig. 1). Uptake is most likely mediated by a teleost form of OAT1, the only member of the OAT family that supports organic anion/dicarboxylate exchange (Burckhardt and Wolff, 2000), and hence the only family member that can indirectly couple organic anion uptake to the Na⁺ gradient. Figure 3 shows a typical confocal micrograph of a tubule after a 30-min incubation in medium containing 1 μM FL. Clearly, this dye is concentrated in the tissue with fluorescence in the tubular lumen (urinary space) > cells > medium. This is the same fluorescence distribution pattern seen previously for FL and is taken to indicate that secretion is a result of two uphill transport steps arranged in series (Fig. 1). Because of this series arrangement of transporters, we would expect treatments that reduced cellular accumulation of FL to have also reduced luminal accumulation. Consistent with this, the organic anions PAH and probenecid caused a concentration-dependent decrease in FL accumulation in the cells and tubular lumen (Fig. 4). With increasing concentrations of PAH or probenecid, cellular and luminal fluorescence fell in parallel. Estimated IC₅₀ values for PAH and probenecid were 5 to 10 μM (Table 1).

Like PAH and probenecid, adefovir and cidofovir reduced FL transport in a concentration-dependent manner (Fig. 5). Both drugs reduced cellular and luminal accumulation of FL.
Adefovir was a more potent inhibitor of FL transport than cidofovir, but neither drug was as potent as PAH or probenecid (Table 1). Luminal accumulation of FL-MTX in killifish proximal tubules is a result of two uphill-mediated steps arranged in series (Fig. 1). A substantial body of evidence indicates that neither of these steps is shared with FL and other small organic anions. Unlike FL, transport of FL-MTX is relatively insensitive to Na\(^+\)/H\(^+\) depletion and is not affected by ouabain. 

Substrate and inhibitor specificity profiles and immunostaining with antibodies directed at mammalian Mrp2 indicate that the luminal step in FL-MTX transport across killifish proximal tubule is mediated by a teleost form of Mrp2 (Masereeuw et al., 1996, 1999, 2000). Although kidney does express several Mrp isoforms, Mrp2 is the only one localized to the luminal pole of epithelial cells, including renal proximal tubule (Konig et al., 1999).

Two inhibition patterns have been observed with FL-MTX as substrate. Certain large organic anions, e.g., methotrexate, reduce both cellular and luminal accumulation, indicating at a minimum action at the basolateral membrane (Masereeuw et al., 1996). Other large organic anions, at submicromolar to micromolar concentrations, only reduce luminal accumulation of FL-MTX (Masereeuw et al., 1996, 1999, 2000). Figure 6 shows dose-response curves for two such large organic anions, LT\(_C\)\(_4\) and MK571. Both were particularly effective inhibitors of FL-MTX transport from cell to lumen. From these data we estimate \(I_{50}\) values to be 0.3 and 1 \(\mu\)M for LT\(_C\)\(_4\) and MK571, respectively (Fig. 6; Table 1). At these same low concentrations, these large organic anions did not inhibit FL transport (Table 1). In contrast, 10 to 100 \(\mu\)M PAH did not significantly reduce FL-MTX transport, although 500 \(\mu\)M PAH did inhibit (Fig. 7). Taken together, these results are consistent with a low affinity of Mrp2 for PAH and a high affinity for substantially larger organic anions.

Both adefovir and cidofovir reduced luminal accumulation of FL-MTX, but neither compound affected cellular accumulation (Fig. 8). Of the two drugs, cidofovir was the more potent inhibitor of FL-MTX transport. The \(I_{50}\) value for cidofovir was one-fifth of that of adefovir (Table 1). Clearly, based on the dose-response curves, both nucleoside phosphonates were more effective inhibitors of FL-MTX transport than of FL transport.

Irrespective of the mechanism involved, adefovir and cidofovir had to enter the cells to reduce FL-MTX transport across the luminal membrane. If entry was mediated by the

![Fig. 5. Inhibition of FL transport by adefovir and cidofovir. Tubules were incubated in medium containing 1 \(\mu\)M FL without and with the indicated concentrations of drug. After 30 min, confocal images were obtained and analyzed as described under Materials and Methods. Data are given as mean fluorescence intensities for 10 to 11 tubules; variability is shown as S.E. bars. Statistical comparisons: **\(P < 0.01\).](image)

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![Fig. 6. Inhibition of FL-MTX transport by LT\(_C\)\(_4\) and MK571. Tubules were incubated in medium containing 1 \(\mu\)M FL-MTX without and with the indicated concentrations of drug. After 30 min, confocal images were obtained and analyzed as described under Materials and Methods. Data are given as mean fluorescence intensities for 10 to 15 tubules; variability is shown as S.E. bars. Statistical comparisons: **\(P < 0.01\).](image)

**TABLE 1**

Inhibition of transporter function in killifish renal proximal tubules

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>OAT1</th>
<th>Mrp2</th>
<th>P-glycoprotein</th>
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<tbody>
<tr>
<td>PAH</td>
<td>10</td>
<td>500</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Probenecid</td>
<td>5</td>
<td>&gt;5</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>LT(_C)(_4)</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>MK571</td>
<td>&gt;10</td>
<td>1</td>
<td>&gt;10</td>
</tr>
<tr>
<td>PSC833</td>
<td>&gt;30</td>
<td>&gt;10</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>10</td>
</tr>
<tr>
<td>Verapamil</td>
<td>&gt;100</td>
<td>&gt;50</td>
<td>10</td>
</tr>
<tr>
<td>CSA</td>
<td>&gt;10</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>&gt;10</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>&gt;50</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Adefovir</td>
<td>100</td>
<td>50</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Cidofovir</td>
<td>250</td>
<td>10</td>
<td>&gt;250</td>
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basolateral classical organic anion system, blocking that system with a competitor organic anion should reduce inhibition of FL-MTX transport at the luminal membrane. To test this supposition, tubules were incubated in medium containing nucleoside phosphonates without and with 50 μM PAH. This concentration of PAH greatly reduced transport on OAT1 at the basolateral membrane (Fig. 4) without affecting transport of FL-MTX at the luminal membrane (Fig. 7). Figure 9 shows that adefovir and cidofovir at 50 μM caused the expected reduction of luminal FL-MTX accumulation and that PAH abolished that reduction.

In killifish renal proximal tubules, NBD-CSA enters cells by simple diffusion but is pumped into the lumen by P-glycoprotein (Fig. 1; Schramm et al., 1995). Luminal accumulation of NBD-CSA is inhibited by micromolar concentrations of several P-glycoprotein substrates, including CSA, PSC833 (Table 1), and several Ca²⁺ channel blockers, including, verapamil and nifedipine (Fig. 10). At 250 μM, neither adefovir nor cidofovir reduced transport of NBD-CSA (Fig. 11).

Discussion

By mediating active drug excretion, xenobiotic transporters play a major role in determining drug concentrations reaching sensitive sites within an organism. Along with drug-metabolizing enzymes, these transporters are important determinants of drug effectiveness on the one hand and of xenobiotic toxicity on the other hand. Moreover, because of their wide specificity limits, these transporters provide a mechanism, competition for transport, by which chemicals with very different structures may interact to alter xenobiotic excretion rates, plasma concentration profiles, and tissue distribution patterns. Thus, it is important to characterize the individual transporters that drive excretion, understand how xenobiotics interact with these membrane proteins, and be able to determine the molecular routes that chemicals follow during transport from blood to urine. Using killifish renal proximal tubules, confocal microscopy, and image analysis, we have developed a battery of pathway-specific fluorescent substrates and nonfluorescent inhibitors that not only allow us to identify specific transporters responsible for excretion of fluorescent xenobiotics but also, through transport inhibition experiments, to identify transporters with which nonfluorescent compounds interact. There are two important caveats that must be considered when interpreting
results of these inhibition experiments. First, although the experiments can demonstrate direct interactions with the transporters, such interactions only imply routes of transport for the nonfluorescent inhibitors. Whether a compound is actually handled by a given transporter can only be established when that compound is used as substrate. Second, the present results only address interactions with transporters that handle the three fluorescent compounds used as substrates. Other transporters are clearly present in proximal tubules, and the extent to which the nucleoside phosphonates interact with and are transported by these is unknown. For example, recent studies have shown that adefovir can interact with Mrp4 and Mrp5 (Schuetz et al., 1999; Wijnholds et al., 2000) and that at least one other OAT, OAT3, is expressed in human but not rat proximal tubule (Cha et al., 2001). OAT3, however, does not appear to be capable of supporting Na⁺-driven organic anion transport or organic anion exchange, so its role in renal secretion is not yet certain.

In the present study, we determined the effects of two nonfluorescent drugs on the transport of three fluorescent substrates, each of which is transported from bath to lumen by a different sequence of steps, i.e., by different transporters (Fig. 2). For two of the substrates, FL and FL-MTX, secretion into the tubular lumen is a result of mediated basolateral and luminal transport steps arranged in series. For the third, NBD-CSA, only the luminal step is mediated. Both adefovir and cidofovir reduced the transport of FL and FL-MTX in a concentration-dependent manner. However, neither drug reduced the luminal accumulation of the P-glycoprotein substrate NBD-CSA. This latter result is important for two reasons. First, it rules out interactions of the compounds with luminal P-glycoprotein. Second, it indicates that the nucleoside phosphonates do not inhibit transport by

Fig. 10. Inhibition of NBD-CSA transport by nifedipine and verapamil. Tubules were incubated in medium containing 1 μM NBD-CSA without and with the indicated concentrations of nifedipine and verapamil. After 60 min, confocal images were obtained and analyzed as described under Materials and Methods. Data are given as mean fluorescence intensities for 10 to 12 tubules; variability is shown as S.E. bars. Statistical comparisons: *P < 0.05, **P < 0.01.
disrupting cell function, e.g., by interfering with cellular metabolism, opening tight junctions, or altering cell signaling. All of the transporters involved in secretion of the three fluorescent substrates mediate uphill and energy-dependent transport. As a result, they are particularly sensitive to metabolic inhibitors (Miller and Pritchard, 1994; Schramm et al., 1995; Masereeuw et al., 1996) and their accumulation within the lumen is dependent on intact tight junctions. In addition, all three are sensitive to activation of protein kinase C, which reduces transport function (Miller, 1998; Miller et al., 1998; Masereeuw et al., 2000). If the nucleoside phosphonates inhibited energy metabolism, increased tight junctional permeability, or activated protein kinase C, we would have expected to see all transporters affected similarly. This was not the case, because transport on P-glycoprotein was not reduced by a high concentration (250 μM) of either drug.

The nucleoside phosphonates did reduce both cellular and luminal accumulation of FL, suggesting that at a minimum interactions with the basolateral transporter for small organic anions. Based on function, i.e., Na$^+$ dependence, ouabain sensitivity, and glutarate stimulation, this appears to be a killifish form of OAT1, an organic anion exchanger that has been cloned from rat, human, and flounder kidney (Burckhardt and Wolff, 2000). Inhibition of FL transport in the intact tubule was expected, because both compounds are known to be substrates for and competitive inhibitors of human and rat OAT1 expressed in heterologous systems, i.e., in Xenopus oocytes injected with OAT mRNA and in Chinese hamster ovary cells transfected with OAT cDNA (Cihlar et al., 1999; Ho et al., 2000). In those systems, the affinity of the transporter for adefovir was higher than for cidofovir. In the present experiments, adefovir was the more potent inhibitor of FL transport, but it was not as potent an inhibitor of transport as PAH or probenecid. This result is consistent with data showing that apparent $K_m$ values for transport of PAH and probenecid on OAT1 are lower than corresponding values for the two nucleoside phosphonates (Cihlar et al., 1999; Ho et al., 2000).

In killifish tubules, both adefovir and cidofovir reduced the luminal accumulation of FL-MTX, a process mediated by the ATP-driven drug export pump Mrp2, located on the luminal membrane of renal proximal tubule cells. Neither drug affected the cellular accumulation of FL-MTX, indicating a specific effect on the luminal step in transport. These are the first data showing these drugs interact with Mrp2. They suggest that, like the fluorescent organic anion lucifer yellow (Masereeuw et al., 1999), adefovir and cidofovir enter the renal cells on OAT1 but exit on Mrp2. This sequence of events is consistent with experiments presented here showing that PAH abolished the interactions of the nucleoside phosphonates with Mrp2. That is, PAH, at a concentration that did not affect FL-MTX transport, prevented inhibition of cell to lumen transport of FL-MTX by adefovir and cidofovir, presumably by preventing their cellular accumulation mediated by OAT1.

Table 1 summarizes results from a large number of inhibition experiments that used fluorescent substrates for OAT1, Mrp2, and P-glycoprotein, nonfluorescent drugs, and killifish proximal tubules. The drugs tested show a variety of inhibition patterns. Some, such as PSC833 and LTC4, are potent and specific inhibitors of a single transporter. Others, e.g., CSA, ritonavir, and saquinavir, inhibit more than one transporter with a range of potencies. Adefovir and cidofovir clearly interact with more than one transporter, but they are no where near as potent as the classical substrates for OAT1 (PAH and probenecid) or Mrp2 (LTC4 and MK571).

Nephrotoxicity limits use of adefovir and cidofovir in the treatment of human immunodeficiency virus (Kahn et al., 1999). Experiments with rabbits show that both drugs are actively secreted by the kidney and both accumulate to high levels in renal proximal tubules. Toxicity of these drugs appears to correlate with OAT1-mediated cellular accumulation because 1) probenecid reduced nephrotoxicity in monkeys treated with cidofovir (Lacy et al., 1998), 2) transfecting cell lines with OAT1 greatly increased adefovir and cidofovir uptake and cytotoxicity (Cihlar et al., 1999), and 3) organic anions reduced cytotoxicity in OAT1-expressing cell lines (Ho et al., 2000; Mulato et al., 2000). However, drug accumulation in renal proximal tubule cells is a function of both uptake at the basolateral membrane and efflux at the luminal membrane and treatments that block efflux, like those that enhance uptake, should increase both accumulation and toxicity. The present results suggest that the ATP-driven drug efflux pump Mrp2 is one transporter responsible for nucleoside phosphate efflux from renal cells. Mrp2 is a transporter with very wide specificity limits and thus handles a number of xenobiotics and xenobiotic metabolites (anionic drug conjugates) as well as endogenous compounds (Konig et al., 1999). If Mrp2 mediates efflux of adefovir and cidofovir from proximal tubule cells, competitive interactions at the transporter could result in reduced efflux and increased tox-

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**Fig. 11.** Lack of effect of adefovir and cidofovir on NBD-CSA transport. Tubules were incubated in medium containing 1 μM NBD-CSA without and with the indicated concentrations of drug. After 60 min, confocal images were obtained and analyzed as described under Materials and Methods. ■, lumen; □, cells. Data are given as mean fluorescence intensities for 9 to 15 tubules; variability is shown as S.E. bars. Neither drug significantly affected NBD-CSA transport.
icity. Conversely, the nucleoside phosphonates could also interfere with the excretion of other potentially toxic chemicals handled by OAT1 or Mrp2 and thus increase retention and possibly toxicity.

References


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