Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs

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Background: Various drug transporters of the ATP-binding cassette (ABC) family restrict the oral bioavailability and cellular, brain, testis, cerebrospinal fluid and fetal penetration of substrate drugs. MDRI P-glycoprotein (P-gp) has been demonstrated to transport most HIV protease inhibitors (HPI) and to reduce their oral bioavailability and lymphocyte, brain, testis and fetal penetration, possibly resulting in major limiting effects on the therapeutic efficacy of these drugs.

Objectives: To investigate whether the ABC transporters MRP1, MRP2, MRP3, MRP5 and breast cancer resistance protein 1 (Bcrp1) are efficient transporters of the HPI saquinavir, ritonavir and indinavir.

Methods: Polarized epithelial non-human (canine) cell lines transduced with human or murine complementary DNA (cDNA) for each of the transporters were used to study transepithelial transport of the HPI.

Results: MRP2 efficiently transported saquinavir, ritonavir and indinavir and this transport could be enhanced by probenecid. Sulfinpyrazone was also able to enhance MRP2-mediated saquinavir transport. In contrast, MRP1, MRP3, MRP5, or Bcrp1 did not efficiently transport the HPI tested.

Conclusions: Human MRP2 actively transports several HPI and could, based on its known and assumed tissue distribution, therefore reduce HPI oral bioavailability. It may also limit brain and fetal penetration of these drugs and increase their hepatobiliary, intestinal and renal clearance. MRP2 function and enhancement of its activity could adversely affect the therapeutic efficacy, including the pharmacological sanctuary penetration, of HPI. \textit{In vivo} inhibition of MRP2 function might, therefore, improve HIV/AIDS therapy.

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Introduction

MDRI P-glycoprotein (P-gp) is a drug-extruding ATP-binding cassette (ABC) transporter that efficiently transports most HIV protease inhibitors (HPI) [1–4]. P-gp is present in the apical membrane of many critical epithelia and endothelia. Because of its localization and distribution, P-gp limits the oral bioavailability and brain, testis and fetal penetration of HPI and in all likelihood lymphocyte penetration as well [1,3–8]. P-gp activity thus contributes to lower plasma levels and pharmacological sanctuary sites for HPI and may in this manner limit their therapeutic efficacy against HIV/AIDS.

Given the impact of P-gp on in vivo HPI pharmacology, it is important to assess whether other drug transporters of the ABC family can also efficiently transport HPI. For instance, MRPI1 encodes a widely expressed transporter [9] that, when present in epithelial cells, is found primarily in the basolateral membrane. In mice, it greatly reduces the accumulation of the substrate drug etoposide in cerebrospinal fluid [10,11]. The related transporter MRP2 is found in the apical membrane of several epithelia [12–14]. In rats, MRP2 contributes to hepatobiliary, intestinal and renal drug excretion and to the reduction of oral availability of its substrates [15,16]. Based on parallels with P-gp, it might reduce fetal and brain penetration of its substrates.

The breast cancer resistance protein (BCRP) is also an apical transporter in epithelia [17,18]. Pharmacological inhibition experiments in mice indicate that the mouse BCRP homologue Bcrp1 reduces oral bioavailability, limits fetal penetration and mediates hepatobiliary excretion of its substrates [19]. Clearly, if HPI are efficiently transported substrates of MRPI1, MRP2 and/or BCRP, this may well affect their pharmacological disposition and thus their therapeutic efficacy.

Some reports suggest that MRPI1 may be a transporter of saquinavir, ritonavir and perhaps indinavir [20–22]. There is, however, no direct experimental evidence that MRP2 transports HPI, although there are some indications that HPI can inhibit MRP-like transport in fish renal tubules and rat brain microvessels [13,23].

In order to assess directly whether HPI are transported substrates for MRPI1, MRP2 and Bcrp1, a well-defined system was used consisting of polarized non-human (canine) MDCKII cells, transduced with human MRPI1 or MRP2 complementary DNA (cDNA) or with murine Bcrp1 cDNA [19,24,25]. More recently discovered members of the MRP family include MRP3 and MRP5. Expression of MRPI1 is found in liver, adrenal gland, pancreas, kidney and intestine, whereas MRP5 is expressed in most tissues. Transport was also studied in transduced cell lines overexpressing human MRP3 and MRP5 [26,27]. Both MRP3 and MRP5 localize basolaterally in epithelial cells, but their in vivo pharmacological function is as yet unclear [28].

Materials and methods

Chemicals

Ritonavir (Norvir, 80 mg/ml) was from Abbott Laboratories Inc. (Abbott Park, Illinois, USA). [3H]-Ritonavir and [3H]-indinavir were obtained from Moravek (Brea, California, USA). Indinavir was obtained from Merck (West Point, Pennsylvania, USA). [14C]-Saquinavir and saquinavir were provided by Roche Discovery Welwyn (Welwyn Garden City, UK). PSC833 [29] was a gift of Novartis Pharma Inc. (Basel, Switzerland) and GF120918 [30] was provided by GlaxoSmithKline (Research Triangle Park, North Carolina, USA). MK571 was from Biomol (Plymouth, Pennsylvania, USA) and probenecid was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Cyclosporin A (Neoral, 100 mg/ml) was from Novartis Pharma B.V. (Arnhem, the Netherlands).

Cell lines and transport assays

Generation and culturing of the polarized non-human (canine) MDCKII [31] cell lines stably expressing human MRPI1, MRP2, MRPI3, MRP5 and murine Bcrp1 have been previously described [19,24–27,32]. Transport assays were performed as described previously [8]. The amount of HPI in the cell layer at the end of the experiment was determined by liquid scintillation counting of the excised filter, after washing with ice-cold phosphate-buffered saline.

Statistics

P values for increased net drug transport were determined by the Student’s t-test (one-tailed, assuming equal variance). The net drug translocation (i.e., the difference between apically and basolaterally directed transport) after 4 h was used to compare transport rates.

Results

Transport of protease inhibitors by multidrug resistance proteins

MDCKII parental cells and derived cell lines stably transduced with different MRP cDNA were cultured on porous membranes in Transwell plates to test for their capacity to mediate transepithelial transport of 5 μmol/l [14C]-saquinavir, [3H]-ritonavir, or [3H]-indinavir (Fig. 1). These cells form a polarized monolayer in which the rate of basolaterally or apically directed translocation can be followed after adding drug
to the apical or basolateral side of the monolayer. Without active transport, passive translocation of drug will be similar in both directions. Based on the subcellular localization of each transporter in polarized cells, MRP1, MRP3 and MRP5 should transport their substrates in the basolateral direction, and MRP2, Bcrp1 and P-gp in the apical direction. The P-gp inhibitor GF120918 (1 μmol/l) was present to suppress the moderate apically directed HPI transport mediated by endogenous P-gp in the parental cell line (Fig. 1a–c). Even 5 μmol/l GF120918 did not significantly inhibit MRP1 or MRP2 [33]. Under these circumstances, the MRP1-, MRP3- and MRP5-transduced lines did not mediate substantial polarized transport of any HPI (Fig. 1d–f, j–l and m–o, respectively). However, MRP2 was clearly able to mediate substantial apically directed transport of all tested HPI (Fig. 1g–i; P < 0.005). Of the three HPI, saquinavir was transported most efficiently by MRP2, and ritonavir least. Drug concentrations were determined by high-pressure liquid chromatography coupled to an electrospray ionization sample inlet and two quadrupole mass
transport of saquinavir was studied in the presence of probenecid and sulfinpyrazone. These compounds were used at concentrations that were previously found to inhibit MRP2-mediated saquinavir transport, but without causing cytotoxicity. The following compounds were tested up to the maximum dissolvable concentration of 5 mM. It is apparent that sulfinpyrazone enhanced MRP2-mediated saquinavir transport in a wide concentration range of probenecid (Fig. 2m). A Neomycin-transduced subclone of MDCKII cells (Neo-line), which is known to contain hardly any endogenous canine MRP2 (not shown), was also included.

The relative rates of saquinavir transport were determined (i.e., the percentage of apically directed transport divided by basolaterally directed translocation after 4 h; Fig. 2m). Without probenecid, this relative rate was 6.3 in the MRP2 line, compared with 2.0 and 1.3 in the parental and the Neo-line, respectively. The maximum relative rates of saquinavir transport with probenecid in the parental, Neo-line and MRP2 line were, respectively, 5.6, 1.2 and 32. These data are in agreement with the relative levels of MRP2 expression.

Another drug that enhanced MRP2-mediated saquinavir transport is the clinically used uricosuric agent sulfinpyrazone (Fig. 2n), which was tested over a concentration range from 0 to its maximum dissolvable concentration of 5 mM. It is apparent that sulfinpyrazone enhanced MRP2-mediated saquinavir transport at concentrations between 1 and 5 mM/l, but in a less dramatic way than probenecid. Sulfinpyrazone also enhanced saquinavir transport in the parental line, again most likely through enhancement of endogenous MRP2 activity.

Discussion

Our data show that human MRP2 is a relatively efficient transporter of saquinavir, ritonavir and indinavir and that this transport can be markedly enhanced by the drugs probenecid and sulfinpyrazone. Human MRP1, MRP3 and MRP5 and murine Bcrp1 are not efficient transporters of the tested HPIs.

The fact that most tested HPI are transported by MRP2 can have important implications for their pharmacological use. In rats, Mrp2 has been demonstrated to contribute to hepatobiliary, renal and direct intestinal excretion of its substrates and to limit their oral bioavailability [12,15,16]. Human MRP2 activity is, therefore, likely to reduce the plasma levels of HPI by these same processes. One might consider applying MRP2 inhibitors, in order to improve plasma levels of these drugs. However, suitable specific and efficient MRP2 inhibitors are not yet available. The risk of unpredicted inhibition of MRP2 in patients as a result of coadministered drugs is presumably limited, as we found that a number of candidate inhibitors of MRP2 were not able to inhibit MRP2-mediated saquinavir transport.

Unexpectedly, our in vitro data show that MRP2-
Fig. 2. Transepithelial transport of protease inhibitors. (a–l) Transepithelial transport of 50 µmol/l [14C]-saquinavir, [3H]-ritonavir, or [3H]-indinavir in MDCKII (parent) or MDCKII–MRP2 monolayers in the presence of 1 µmol/l GF120918, either without (a–f) or with (g–l) 500 µmol/l probenecid. At the start of the experiment, the radioactive drug was applied to one compartment (basolateral or apical), and the percentage of radioactivity appearing in the opposite compartment was measured at 1, 2, 3 and 4 h. Results are expressed as mean values (n = 3), with bars indicating the SD. Translocation from the basolateral to the apical compartment (b); translocation from the apical to the basolateral compartment (c). Numbers in the panels indicate the percentage of radioactivity retrieved from the cell layer 4 h after application of the drug to the apical (Ap) or basolateral (Bl) compartment. (m,n) Relative transport rates of [14C]-saquinavir (5.0 µmol/l) measured after 4 h in the presence of 1 µmol/l GF120818 with increasing concentrations of (m) probenecid in MDCKII (low expression endogenous MRP2), MDCKII–MRP2 (overexpression of human MRP2) and MDCKII–Neo (very low expression of endogenous MRP2) and (n) sulfinpyrazone in MDCKII and MDCKII–MRP2 cells (n = 1). Relative transport rates were determined by dividing the percentage of apically directed transport by the percentage of basolaterally directed translocation.
mediated HPI transport can be boosted substantially by probenecid and somewhat by sulfinpyrazone. It may be that drugs other than probenecid can also enhance MRP2-mediated transport of HPI and could negatively influence oral bioavailability, plasma pharmacokinetics and sanctuary penetration of HPI.

One can envisage a relatively simple mechanism by which probenecid and sulfinpyrazone enhance MRP2-mediated HPI transport. Evers et al. [32] demonstrated that MRP2-mediated glutathione export can be enhanced by sulfinpyrazone and that sulfinpyrazone and glutathione are both transported by MRP2. They proposed that MRP2 has two substrate binding and transport sites, one binding the anionic glutathione (G-site) and the other binding drugs like sulfinpyrazone (D-site). Simultaneous occupation of both sites could then result in efficient export of both compounds. Probenecid and sulfinpyrazone are organic anions and it may be that they bind to the G-site and HPI to the D-site, resulting in efficient cotransport.

Our finding that MRP1 is not an efficient transporter for saquinavir, ritonavir and indinavir, appears to contrast with some previous reports [20–22]. Srinivas et al. [20] found that saquinavir and ritonavir can partially enhance doxorubicin sensitivity in an MRP1-overexpressing line, but the efficacy of these HPI in suppressing HIV replication in these cells was not substantially altered. The data, therefore, suggest that saquinavir and ritonavir can perhaps inhibit MRP1 but are not efficiently transported by it.

The studies of Jones et al. [21,22] were performed in CEM300 cells overexpressing MRp1 as a result of extended selection with the cytotoxic drug etoposide, a procedure known frequently to result in a multitude of alterations in the cell, including upregulation of multiple different drug exporters [23]. It is, therefore, difficult to exclude that upregulation of other MRP-like HPI transporters (possibly MRP2) may have affected the data obtained.

In contrast, a study by van der Sandt et al. [34] presented data from polarized cells transfected with MRP1 cDNA that was consistent with MRP1 not being a transporter of ritonavir and indinavir. They found that transport of ritonavir and indinavir towards the basolateral compartment was not increased relative to the untransfected parental cells [35]. Based on the available evidence and our own data, we do not want to exclude that MRP1 (and perhaps MRP3, MRP5 and Bcrp1 as well) are moderately active transporters of HPI, but it is clear that MRP2 is much more efficient. We, therefore, expect that the impact of MRP2 on HPI pharmacology will be more pronounced than that of the other transporters.

It might be worthwhile to determine if some herbal products or conventional drugs enhance MRP2 function in the way probenecid does, since this might lead to seriously decreased HPI exposure. If it turns out that in vivo MRP2 function has a prominent effect on HPI oral bioavailability and sanctuary penetration, as appears to be the case for P-gp, it may be useful to take this into account in further HIV/AIDS drug development programmes.

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