INTERACTIONS BETWEEN HIV-1 PROTEASE INHIBITORS AND ATP-DRIVEN DRUG EFFLUX PUMPS IN TELEOST RENAL PROXIMAL TUBULE

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A major strategy used to fight infection with human immunodeficiency virus type 1 (HIV-1) is treatment with protease inhibitors, several of which demonstrate high antiretroviral potency to HIV-1 *in vitro*. However, the low and variable oral bioavailability of clinically used protease inhibitors is a significant impediment to their use. It is becoming clear that several pharmacokinetic mechanisms contribute to this problem. Initially, low bioavailability was attributed to metabolism by the cytochrome P450 3A4 isoform (CYP3A4; Eagling et al.,*Brit. J. Clin. Pharmacol.*44:190-194, 1997), but recent studies have implicated another process: active extrusion mediated by the ATP-driven, drug efflux pump, p-glycoprotein (Kim et al., *J. Clin. Invest.* 101:289-294, 1998; Alsenz et al., *Pharm. Res.* 15:423-428, 1998).

Here we used renal proximal tubules from killifish (*Fundulus heteroclitus*), fluorescent substrates and confocal microscopy as a model experimental system to study interactions between the HIV protease inhibitors, saquinavir and ritonavir, and the drug transporting ATPases, p-glycoprotein and multidrug resistance-associated protein isoform 2 (Mrp2). Both saquinavir and ritonavir inhibited luminal accumulation of a fluorescent cyclosporin A derivative (a substrate for p-glycoprotein) and of fluorescein methotrexate (a substrate for Mrp2). Neither drug affected cellular accumulation of the fluorescent substrates. Of the two protease inhibitors, ritonavir was the more potent inhibitor of transport by at least a factor of 20. In fact, ritonavir was inhibitory in the submicromolar concentration range and was at least as good an inhibitor of p-glycoprotein- and Mrp2-mediated transport as cyclosporin A and leukotriene C4 (LTC_4), respectively. Inhibition of p-glycoprotein and Mrp2-mediated transport was not due to toxicity or impaired metabolism, since neither saquinavir nor ritonavir inhibited transport of fluorescein on the renal organic anion system. Experiments with a fluorescent saquinavir derivative showed strong secretion into the tubular lumen that was inhibited by verapamil, LTC_4, saquinavir and ritonavir. Together, the data demonstrate that saquinavir and especially ritonavir are potent inhibitors of p-glycoprotein- and Mrp2-mediated transport. The experiments with the fluorescent saquinavir derivative suggest that these compounds may also be substrates for both p-glycoprotein (inhibition by verapamil) and Mrp2 (inhibition by LTC_4).

These results have important implications with regard to the clinical use of protease inhibitors. First, they demonstrate that saquinavir and ritonavir interact not only with p-glycoprotein, but also with Mrp2. Thus, both major families of ATP-driven drug transporters may contribute to the drugs' low oral bioavailability and their inability to act at sites of HIV infection in the central nervous system. Second, the data for ritonavir show it to be among the most potent known inhibitors of p-glycoprotein and Mrp2. This suggests that use of protease inhibitor therapy may require adjustment of dose levels of other administered drugs. It also suggests clinical uses outside of HIV therapy, e.g., to reverse multidrug resistance. (Supported by the MDIBL CMTS (ES 03828), NATO CRG 960281 and DFG, FR1211).