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Both P-gp and MRP2 mediate transport of Lopinavir, a protease inhibitor

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Abstract

Polarized epithelial non-human (canine) cell lines stably transfected with human or murine complementary DNA (cDNA) encoding for various efflux transporters (P-gp/MDR1, MRP1, MRP2, and Bcrp1) were used to study transepithelial transport of Lopinavir (LVR) and compare results with the MDCKII-wild type cells. These transmembrane proteins cause multidrug resistance by decreasing the total intracellular accumulation of drugs. Lopinavir efflux was directional and was completely inhibited by MK-571, a selective MRP family inhibitor in the MDCKII-MRP2 cell line. Similarly, LVR efflux was also inhibited by P-gp inhibitors P-gp-4008 and GF120918 in the MDCKII-MDR1 cell line. The efflux ratios of LVR in the absence of any efflux inhibitors in the MDCK-wild type, MDCKII-MDR1, MDCKII-MRP1 and MDCKII-MRP2 cell monolayers were 1.32, 4.91, 1.26 and 2.89 respectively. The MDCKII-MDR1 and MDCKII-MRP2 cells have significantly increased LVR efflux ratio relative to the parental cells due to the apically directed transport by MDR1 and MRP2 respectively. The efflux ratios in MRP2 and MDR1 transfected cell lines were close to unity in the presence of MK-571 and P-gp-4008, respectively, indicating that LVR efflux by MRP2 and P-gp was completely inhibited by their selective inhibitors. MDCKII-MRP1 cells did not exhibit a significant reduction in the LVR efflux relative to the parental cells, indicating that LVR is not a good substrate for MRP1. Transport studies across MDCKII-Bcrp1 cells indicated that LVR is not transported by Bcrp1 and is not a substrate for this efflux protein. In conclusion, this study presents direct evidence that LVR is effluxed by both P-gp and MRP2 which may contribute to its poor oral bioavailability and limited penetration into the CNS.
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Keywords: MDCKII-MDR1; MDCKII-MRP2; MDCKII-MRP1; MDCKII-Bcrp1; MDCKII-WT; P-glycoprotein (P-gp); Multidrug resistance protein (MRP); Breast cancer resistance protein (BCRP); Lopinavir (LVR); Uptake; Transport; Permeability; Efflux ratio (ER)

1. Introduction

HIV protease inhibitors (PIs) have revolutionized the treatment of HIV infection (Roberts et al., 1990; Vacca et al., 1994; Danner et al., 1995; Kempf et al., 1995; Patick et al., 1996). Due to limited oral bioavailability and poor pharmacokinetics of many of the currently available PIs, additional efforts have been made to design more potent PIs with improved pharmacokinetic properties. Lopinavir (LVR), an analog of ritonavir (RVR) is a potent inhibitor of wild type and mutant HIV protease ($K_i = 1.3 - 28$ pM) (Kumar et al., 2004). Its structure is outlined in Fig. 1. The LVR:RVR combination (KALETRA) has been shown to be effective in the treatment of HIV infection and is approved for clinical use (Hurst and Faulds, 2000; Miller, 2000). LVR, as such, is extensively metabolized by CYP3A4 and produces low systemic availability when administered alone. RVR potently inhibits CYP3A4 and is used in combination with LVR to enhance the systemic exposure of LVR (Kumar et al., 1999). This combination results in LVR concentrations that greatly exceed those necessary *in vitro* to inhibit both wild-type and PI-resistant HIV isolates. (Kaletra® Prescribing Information, Abbott Laboratories, January 2002).

The low oral bioavailability of LVR was attributed to high first-pass metabolism (Kumar et al., 2004). *In vitro* investigations with human liver microsomes have shown that cytochrome P450 3A plays a predominant role in the metabolism of LVR. High first pass metabolism can also occur due to intestinal efflux which can lead to increased exposure time to metabolizing enzymes (Wacher et al., 1995, 2001; Katragadda et al., 2005). We have hypothesized that the low oral bioavailability of LVR

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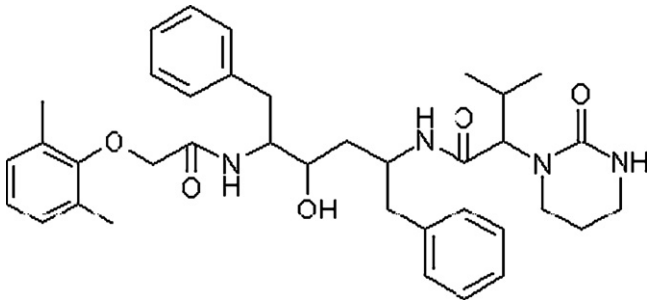

Fig. 1. Structure of lopinavir.

and possibly limited brain penetration could be in part due to efflux of LVR by several efflux pumps such as P-glycoprotein (P-gp), multidrug-resistance related proteins (MRPs) and breast cancer resistance protein (BCRP) present on intestinal epithelial and blood capillary endothelial cells. Potential interaction between efflux transporters in the GIT and CYP3A4 metabolizing enzymes may be a source of variation associated with LVR absorption and distribution (Williams and Sinko, 1999). In humans, CYP3A4 is the principal enzyme involved in the hepatic and intestinal drug metabolism, and there is a striking overlap of substrate specificites among CYP3A4, P-gp and MRPs. The coordinated function of both CYP3A and P-gp, MRPs can dramatically lower oral bioavailability of compounds which are substrates for both (Van Asperen et al., 1997; Wacher et al., 1998) and this may also be true for LVR.

P-gp-mediated efflux of LVR is known (Vishnuvardhan et al., 2003; Woodahl et al., 2005), but interactions of LVR with other adenosine triphosphate-binding cassette (ABC) efflux transporters such as MRPs and BCRP have not yet been investigated. Therefore, it is important to delineate quantitatively if these latter efflux transporters can restrict, at least in part, the permeation of LVR at both the intestinal and blood brain barrier (BBB) membranes.

ABC transporters comprise one of the largest membrane-bound protein families. These proteins transport substrates against a concentration gradient with ATP hydrolysis as a driving force across the membrane. P-gp, a multiple drug-resistant (MDR) gene product, transports a wide range of compounds, including anticancer drugs, steroids, calcium channel blockers and antihistamines (Endicott and Ling, 1989; Borst et al., 1993, 2000; Germann et al., 1993; Pal and Mitra, 2006). P-gp-mediated efflux reduces the intracellular accumulation of these compounds, thereby diminishing drug efficacy. P-gp is present on the apical membrane of many absorptive epithelial and endothelial cells. Because of its localization and distribution, P-gp limits the oral absorption and bioavailability of PIs across intestine, brain, testis and placenta (Kim et al., 1998; Polli et al., 1999; Smit et al., 1999; Choo et al., 2000; Huisman et al., 2001, 2002).

Recent studies have demonstrated that the PIs are also substrates for the MRPs, belonging to the same ABC transporter family (Huisman et al., 2002; Bachmeier et al., 2005). So far, eight MRP homologs have been identified for ABC proteins, MRP1–8. MRP1 is a widely expressed transporter. When present in epithelial cells, this protein is found primarily in the basolateral membrane (Hipfner et al., 1999). However, it has been reported that MRP1 does not mediate substantial polarized transport of PIs in MDCKII-MRP1 cells (Huisman et al., 2002). In contrast to MRP1, MRP2 is localized on the apical membrane of several epithelia. Functionally, it is similar to P-gp-mediated elimination of toxic compounds in gut and placenta (Kruh and Belinsky, 2003). It has been fairly established that MRP2 effluxes PIs (Huisman et al., 2002; Williams et al., 2002).

Human BCRP/MXR is a relatively new ABC efflux transporter. Like P-gp, BCRP confers high levels of resistance to anthracyclines, mitoxantrone and the camptothecins by enhancing drug efflux from the cell to extracellular space (Litman et al., 2000; Bates et al., 2001; Ejendal and Hrycyna, 2002). BCRP is expressed in larger amounts than P-gp in the intestine (Taipalensuu et al., 2001). The expression of a BCRP homologue, known as brain multidrug resistance protein (BMRP), has also been reported in porcine brain capillary endothelial cells (Eisenblatter et al., 2003). Both BCRP and BMRP possess one half of the MDR1 P-gp structure with only six transmembrane domains and one ATP-binding domain (Doyle et al., 1998). In addition to this structural similarity, most known substrates for BCRP/BMRP are similar to P-gp (i.e., hydrophobic, amphiphilic xenobiotics), suggesting that PIs may also interact with BCRP/BMRP (Litman et al., 2001; Doyle and Ross, 2003). In fact, results from a recent study suggest that saquinavir (SQV), RVR and nelfinavir (NFV) may serve as inhibitors of BCRP (Gupta et al., 2004).

Thus, P-gp and MRPs, can play an important role in lowering intestinal absorption and brain penetration of LVR. Because these efflux transporters are oriented in the secretory (i.e., out of the organ or tissue) direction, high efflux will lead to lower net absorption for LVR. Sub-therapeutic concentrations of PIs in the sanctuary sites like brain, testes and bone marrow may cause persistence of viral infections leading to drug resistance (Williams and Sinko, 1999). Therefore, the purpose of this study is to assess the affinity of LVR for the efflux transporters using a well-defined system consisting of polarized non-human (canine) MDCKII cells, singly transfected with human MDR1, human MRP1/MRP2 complementary DNA (cDNA) or murine Bcrp1 cDNA and also to delineate quantitatively whether efflux limits permeation of LVR across intestinal and BBB absorptive cells.

2. Materials and methods

2.1. Materials

Unlabeled (ulb) LVR, Fumitremorgin-C (FC) and GF120918 (GF) were generous gifts from Abbott Laboratories Inc., National Institutes of Health AIDS Research and Reference Reagent Program (National Institutes of Health, Bethesda, MD) and GlaxoSmithKline Ltd respectively. [3H] LVR (1 Ci/mmol) and [3H] Mitoxantrone (MX) (4 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA, USA). P-gp-4008 (P4) and MK-571 (MK) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Biomol (Plymouth meeting, PA, USA) respectively. High-performance liquid chromatography grade DMSO

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