Interaction of Lopinavir with efflux proteins: MDR1, MRP1,2 and 3

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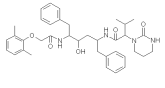
PURPOSE

- To test whether Lopinavir (Lop/LVR), a protease inhibitor used currently in the anti-HIV therapy, is a substrate for efflux proteins MDR1, MRP1,2,3 which might contribute to its low oral and brain bioavailability.
- To devise methods to bypass this efflux if it exists, and try to get increased oral and brain bioavailability of Lopinavir

RATIONALE

- HIV protease inhibitors (PIs) have revolutionized the treatment of HIV infection and acquired immune deficiency syndrome (AIDS)
- The oral bioavailabilities of the human immunodeficiency virus (HIV) protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir) are low and/or variable, with limited penetration into the central nervous system (CNS)
- Sub-therapeutic concentrations of PIs in the sanctuary sites like brain, lung and bone-marrow cause persistence of viral infections and may lead to viral resistance.

LOPINAVIR



- Lopinavir (LVR) is a novel protease inhibitor (PI) developed from Ritonavir. LVR is a potent inhibitor of wild type and mutant HIV protease (Ki =1.3-28 pM)
- Its molecular formula is: C37H48N4O5, and its molecular weight is 628.80.
- Lopinavir is given along with Ritonavir and the brand name for this combination is Kaletra, Abbott Laboratories
- Lopinavir, the active component of this combination, is extensively metabolized by CYP3A4 and produces low systemic concentrations when used alone. Ritonavir potently inhibits CYP3A4 and is used to enhance the systemic exposure of lopinavir.
- In a study where the kinetic and thermodynamic characterization of different HIV-1 PIs was studied, Lopinavir had fast association and the highest affinity compared to the other PIs, and the interaction kinetics were less temperature-dependent as compared with the other inhibitors
- The low oral bioavailability of LVR in animal models was attributed to high first-pass metabolism

MDR1

- MDR1 P-glycoprotein (P-gp) is a drug-extruding ATP-binding cassette (ABC) transporter that efficiently transports most HIV PIs.
- P-gp is an ATP-dependent drug efflux pump typically associated with MDR in cancer chemotherapy.
- 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 PIs

MRP1

- The expression and localization of MRPs makes it extremely important to study the interaction of therapeutic drugs with these proteins.
- MRP1 is a widely expressed transporter that, when present in epithelial cells, is found primarily in the basolateral membrane. High levels of MRP1 are present in the epithelium of the choroid plexus
- MRP1 is able to confer resistance to anthracyclines, vinca alkaloids, campothecins and methotrexate

MRP2

- In contrast to MRP1, MRP2 is found in the apical membrane of several epithelia.
- Its expression is found in liver canaliculi, with lower levels in renal proximal tubules, gut enterocytes, syncytiotrophoblast cells of the placenta and possible brain capillaries.
- Therefore, it is functionally similar to P-gp in its involvement in the terminal elimination of compounds and its role as a barrier in gut and placenta.
- In rats, MRP2 contributes to hepatobiliary, intestinal and renal drug excretion and to the reduction of oral availability of its substrates. It is now known that PIs are transported by MRP2

MRP3

- More recently discovered members of the MRP family include MRP3, MRP4 and MRP5.
- Expression of MRP3 is found in liver, adrenal gland, pancreas, kidney and intestine, whereas MRP5 is expressed in most tissues.
- Both MRP3 and MRP5 localize basolaterally in epithelial cells, but their in vivo pharmacological function is as yet unclear.

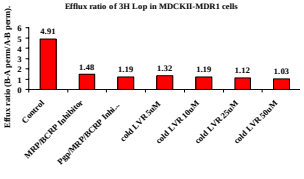
CELL LINES USED

- Polarized epithelial non-human (canine) cell lines transduced with human or murine complementary DNA (cDNA) for each of the transporters (P-gp/MDR1, MRP1, MRP2, MRP3) were used to study transepithelial transport of Lopinavir (LVR) in comparison with the MDCK-Wild type cell line.
- These transmembrane proteins cause multidrug resistance either by decreasing the total intracellular retention of drugs or redistributing intracellular accumulation of drugs away from target organelles.

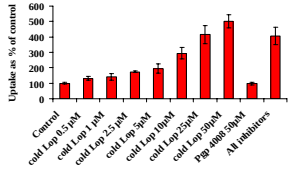
INHIBITORS USED

- MRP family transporters were selectively inhibited with MK-571, a specific leukotriene D4 (LTD4) receptor antagonist. MK-571 specifically inhibits at least MRP1 and MRP2, but not P-gp.
- P-gp transporter was selectively inhibited with P-gp 4008, a specific inhibitor of MDR1/P-gp mediated efflux.
- Fumitremorgin-C (FC) is a selective inhibitor of the BCRP protein.
- Cold (unlabeled) Lopinavir was a gift from Abbott Laboratories Inc.

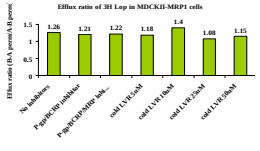
Interaction of Lopinavir with MDR1



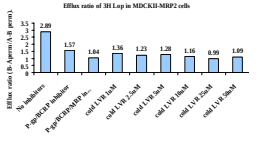
Intracellular accumulation of 3H Lopinavir after incubation and efflux with cold Lopinavir and inhibitors in MDCKII-MDR1 cell line



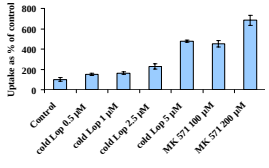
Interaction of Lopinavir with MRP1



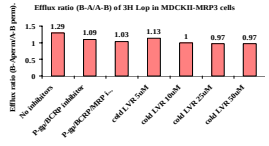
Interaction of Lopinavir with MRP2



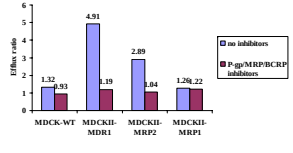
Intracellular accumulation of 3H Lopinavir after incubation and efflux with cold Lopinavir and inhibitors in MDCKII-MRP2 cell line



Interaction of Lopinavir with MRP3



Efflux ratios for 3H Lopinavir transport across various MDCK cell lines



DISCUSSION AND CONCLUSION

- Lopinavir efflux was directional and was completely inhibited by MK-571, a selective MRP family inhibitor (100 and 200 µM) in the MRP transfected cell lines.
- Lopinavir efflux was directional and was completely inhibited by P-gp 4008, a selective P-gp inhibitor (50 µM) in the P-gp transfected cell line.
- The MDCKII-MRP1 cells have a reduced LVR efflux relative to the parental cells, due to basolaterally directed transport by MRP1.
- The MDCKII-MDR1/MRP2 cells have a significantly increased LVR efflux ratio relative to the parental cells due to the apically directed transport by MDR1 and MRP2 respectively.
- The efflux ratios were close to unity in the presence of MK-571 in the MRP-2 transfected cell line and in the presence of P-gp 4008 in the MDR1 transfected cell line indicating that LVR efflux by MRPs and MDR1/P-gp was completely inhibited by MK-571 and P-gp 4008 respectively.
- The efflux ratios in the presence of MK-571 were not significantly different from those of control in the MDCKII-MRP1/MRP3 cell lines indicating that Lopinavir is not a good substrate for these efflux proteins.
- Thus, Lopinavir was found to be a substrate for efflux proteins which in turn may lead to its increased metabolism because the drug will be exposed to more metabolizing enzymes
- Drug-drug interactions may occur since drugs that induce efflux protein activity would be expected to increase the clearance of lopinavir, resulting in lowered plasma concentrations of lopinavir.
- Therefore it is desirable to modify Lopinavir in such a way that its efflux is minimal and influx into the intestinal and brain cells is increased to get higher bioavailability and in turn greater activity.

FUTURE WORK

- Our strategy to bypass the efflux of Lop would be to synthesize prodrugs of Lop which will:
  - evade the efflux pumps substantially
  - be taken up by the intestinal and brain cells with the help of nutrient influx transporters like peptide transporters (PepT1 and 2) or vitamin transporters (SMVT or Folate or Biotin)
  - have lesser protein binding as compared to the parent drugs
  - have atleast the same or higher anti-viral activity when compared with the parent drug.
- We also want to check for interaction of Lop with other efflux proteins like MRP4,5 and BCRP which might play an important role in the low oral bioavailability of Lop.