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Anti-HIV Protease Inhibitors May Aggravate Rifampicin Induced Liver Injury Through Multifaceted Interactions On Hepatic Transporters

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OPTIVIA BIOTECHNOLOGY

ABSTRACT

BACKGROUND: Rifampicin (RIF) is known to cause liver injuries through blocking transporter-mediated hepatic bile salt (BS) excretion. Compared to using RIF alone, higher incidences of severe liver injuries were observed in HIV-infected tuberculosis patients taking RIF with protease inhibitors (PIs). This study aims to elucidate potential hepatic transporter interactions that could contribute to such adverse drug reactions.

METHODS: Inhibitory potencies of RIF, lopinavir (LPV) and ritonavir (RTV) and their major metabolites on major hepatic BS transporters were tested for mechanistic assessment of their ability to block BS biliary excretion; PIs were tested for modulating P-glycoprotein (P-gp) mediated efflux and cellular retention of RIF and its metabolite desacetyl rifampicin (des-RIF); BS transcellular flux and intracellular accumulation were measured with cells expressing hepatic BS transporters, with and without P-gp, treated with RIF/des-RIF alone or together with PIs.

RESULTS: 1) RIF and des-RIF are potent inhibitors of BSEP but not NTCP, whereas LPV and RTV inhibit both; 2) P-gp attenuated BSEP inhibition by RIF and des-RIF through reducing their intracellular concentrations by 5 to 13 fold; 3) LPV/RTV at clinical concentrations aggravated the inhibitory effects of RIF/des-RIF on BS transport through additive, direct inhibition of NTCP and BSEP, and indirectly through inhibiting P-gp, which led to increased RIF/des-RIF intracellular concentrations for more BSEP inhibition.

CONCLUSION: Hepatic P-gp may attenuate rifampicin's liver toxicity through reducing its hepatocellular concentration; protease inhibitors could aggravate rifampicin's liver toxicity directly through further inhibiting bile salt transporters and indirectly through increasing rifampicin's liver level.

INTRODUCTION

The enterohepatic circulation of bile salts is governed by transporters. Reabsorption of bile salts is very efficient in healthy humans, with <10% loss on a daily basis. Cholestasis is any condition in which the flow of bile from the liver is slowed or blocked. The condition can be relatively mild (canalicular bile salt flow slowed), or can be quite severe if the hepatocellular bile salt level is elevated (hepatitis), leading to potential liver failure. Drugs are often involved and can cause "**drug induced liver injury**" (**DILI**), as a result of inhibition of a variety of transporters. If the drug inhibits hepatic uptake transporters, this could result in cholestasis, as the uptake of bile salts would slow. If however, the drug inhibits efflux transporters such as BSEP, toxic bile salts can build up within the hepatocytes and cause hepatitis.

The antibiotic rifampicin is often used as part of a combination therapy in the treatment of tuberculosis (TB). Rifampicin is known to cause DILI in some patients. HIV infected TB patients treated with rifampicin and protease inhibitors such as lopinavir/ritonavir (LPV/R) demonstrate unexpectedly high rates of DILI [1,2]. This may be due to the effect of protease inhibitors in blocking transporters involved in the clearance of bile salts, and rifampicin and its major metabolite from liver cells.

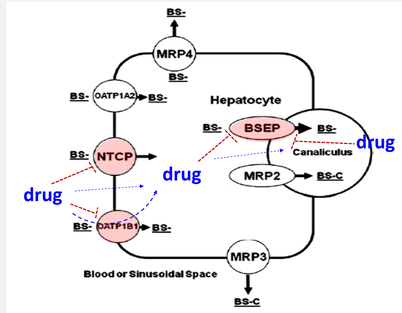
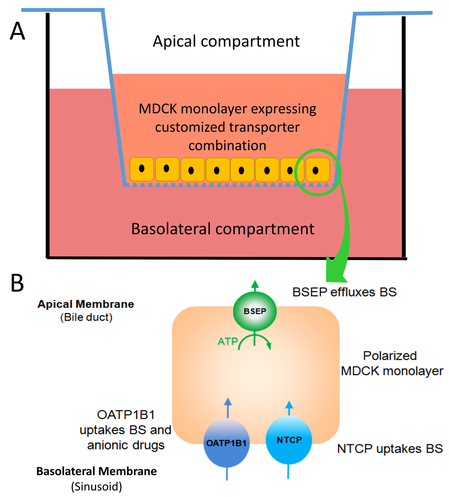


Figure 1. Sites of Inhibition of hepatic bile salt transporters. Adopted from Morgen, R.E., et al, Tox Sci 118(2), 485–500 (2010).

MATERIALS AND METHODS

Figure 2. Experimental setup using A) Transwell™ cell culture apparatus and B) customized drug transporter expressing MDCK monolayer.



1. Cell culture: MDCK-II cells and/or MDCK-II-MDR1 cells were seeded in Millipore Millicell® 96-well insert plate (PCF-0.4 µm).

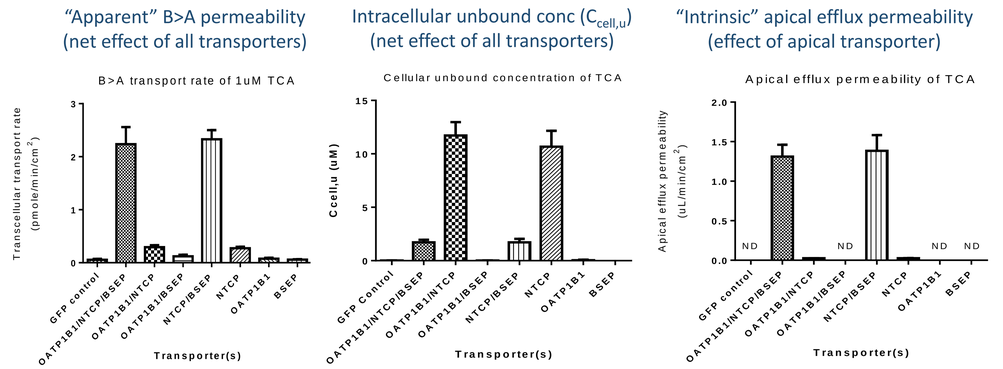
2. Transfection: Approximately 24 hr. later, cells were transfected using a novel *in situ* transfection technology, Opti-Expression™, which allows consistent and effective transfection of polarized cell monolayers. Cells were transfected with a mixture of plasmids encoding all or part of OATP1B1, NTCP, and BSEP. The relative expression levels were tailored to mimic the in vivo levels (OATP1B1≈NTCP ≈ 2X BSEP).

3. Transcellular bile salt transport assay: Assays were conducted 48 hr. after transfection. Substrates such as ^{3}H-taurocholate, were applied to the basolateral side (sinusoidal uptake) while inhibitors were applied to either or both apical and basolateral sides. Paracellular transport was negligible.

4. Intracellular RIF/des-RIF measurement: RIF and des-RIF were separated by HPLC with polar-RP column (Phenomenex, 75x3.0mm) and detected by API4000 mass spectrometer. MRM mode with m/z 823.4->791.5, 781.4->749.4, and 272.0->156.2 for RIF, des-RIF, and an internal standard (carbutamide), respectively.

RESULTS

Figure 3. Contribution of various combinations of transporters. The substrate used is 1 µM taurocholate. The apparent B->A transcellular permeability is $P_{app}=J_{apical}/C_{dosing,u}$ and the intrinsic efflux permeability is $P_{eff,int}=J_{apical}/C_{cell,u}$.



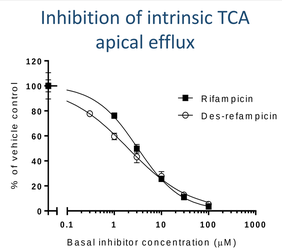


Figure 4. Effect of RIF and des-RIF on BSEP mediated apical TCA efflux in the OATP1B1/NTCP/BSEP model (IC_{50}s are 3.14 µM and 1.98 µM, respectively). While both RIF and des-RIF do not inhibit NTCP, they are potent BSEP inhibitors, potentially leading to elevated bile salt levels in hepatocytes.

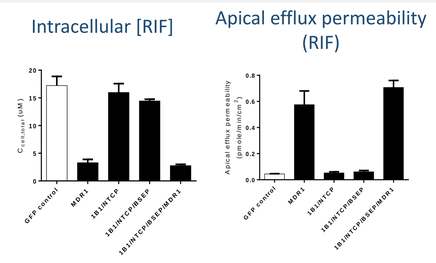


Figure 5. Assessment of active transport of 1µM RIF. RIF is a substrate of P-gp, but is not transported by either NTCP or BSEP. As a result, if P-gp is absent or inhibited, RIF intracellular concentrations [RIF] increased. Similar results were obtained with 1 µM des-RIF.

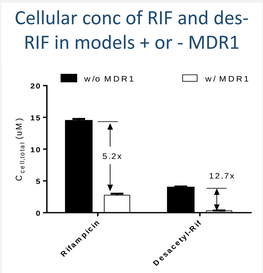


Figure 6. P-gp dramatically reduces the intracellular concentrations of RIF and des-RIF in an OATP1B1/NTCP/BSEP/MDR1 model. P-gp could be a key determinant of the hepatocellular concentrations of RIF and des-RIF.

RESULTS (CONTINUED)

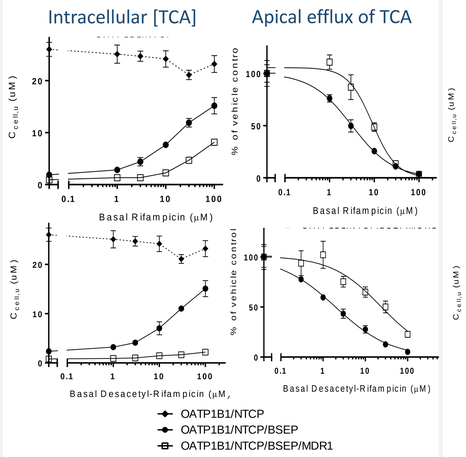


Figure 7. Effect of P-gp on drug induced inhibition of 1µM [3H]-TCA transport. P-gp drastically attenuated cellular accumulation of TCA, and increased the apparent IC_{50} values of RIF (2.9X) and des-RIF (11.9X) on apical efflux.

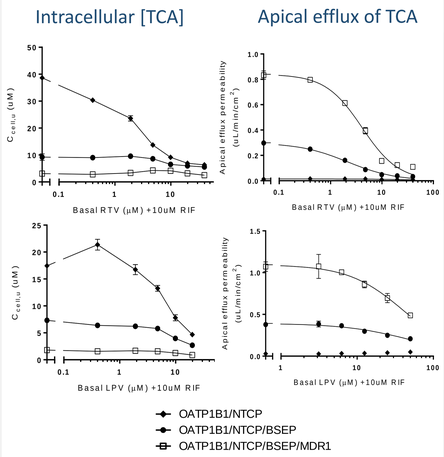


Figure 8. Effect of protease inhibitors on TCA transport in the presence of RIF. Both ritonavir (RTV) and lopinavir (LPV) inhibited NTCP and BSEP at clinically relevant concentrations.

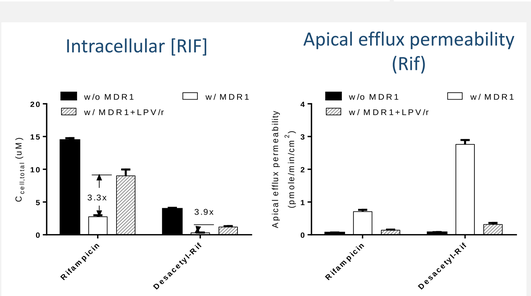


Figure 9. Potential synergistic effect of lopinavir and ritonavir (LPV/r) on RIF hepatotoxicity through P-gp Inhibition. The protective effect of P-gp is reduced when protease inhibitors are present, leading to higher intracellular (Left) and less apical efflux (Right) of RIF and des-RIF.

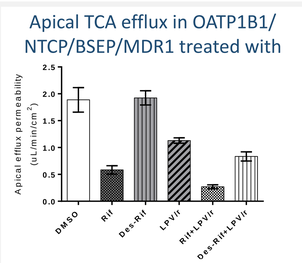


Figure 10. Lopinavir and ritonavir (LPV/r) directly inhibited apical TCA efflux through acting on BSEP and P-gp, and sensitizes the inhibitory effects of RIF and des-RIF through blocking their efflux by P-gp. All drugs were at 10 µM.

CONCLUSIONS

Rifampicin (RIF) and its metabolite desacetyl-rifampicin (des-RIF) are potent inhibitors of BSEP, but not NTCP. As a result, rifampicin treatment has the potential to cause cholestatic hepatitis by increasing the hepatocellular bile salt concentration. P-gp may act to alleviate these effects by transporting RIF and des-RIF from hepatocytes into the bile. These results demonstrate the intracellular concentrations of RIF and des-RIF are dramatically reduced in the presence of active P-gp, increasing the apparent BSEP inhibition IC_{50} of des-RIF by 11.9X. P-gp, NTCP and BSEP can be inhibited by HIV protease inhibitors lopinavir and ritonavir (LPV/r). The "protective" effect of P-gp may be reduced when co-administered protease inhibitors block P-gp mediated removal of RIF and des-RIF from the hepatocytes, leading to increased inhibition of BSEP by RIF and des-RIF, which in turn results in increasing hepatocellular levels of toxic bile salts and damaging liver.

Many drugs are reported to cause liver toxicity by interfering with hepatic bile salt clearance, through inhibition of either sinusoidal uptake or canalicular efflux. Drugs that affect the hepatocellular disposition can indirectly modulate the bile salt secretion. By using well-defined multi-transporter models to conduct mechanistic studies of the drug effects on bile salt transport, we may be able to better predict which drugs or drug combinations will have increased chances of cholestasis or other types of hepatotoxicity.

References

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[3] Warren WS, et al., ISSX Meeting, P234, TX, Oct 2012
[4] Morgan RE, et al., Toxicol Sci. 2010 Dec;118(2): 485–500.