



P-Glycoprotein and MRP-1 Interactions with Rifampicin

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BACKGROUND:

Rifampicin (RIF) is a potent inducer of CYP P450 and P-glycoprotein (P-gp) in the gut and liver and thereby significantly reduces plasma concentrations of HIV PIs (>90%) and NNRTIs (30-50%).

Both tuberculosis (TB) and HIV are intracellular pathogens, therefore drug accumulation within target cells is important for their activity.

We examined the effect of RIF on P-gp and MRP1 expression on peripheral blood mononuclear cells (PBMCs) (as a surrogate for alveolar macrophages) and characterised the cellular accumulation of RIF *in vitro* and *in vivo*.

METHODS:

CEM (parental), CEM_{VBL100} (P-gp over-expressing), CEM_{E1000} (MRP1 over-expressing) cells were gifts from Dr R. Davey, (University of Queensland, Australia). MDCKII_{CTL}, MDCKII_{MDR1} (P-gp over-expressing) and MDCKII_{MRP1} (MRP1 over-expressing) cells were gifts from Prof. P. Borst (Netherlands Cancer Institute, Amsterdam).

Cell Line Accumulation: Cellular accumulation ratio (CAR) of rifampicin (1µM, ³H-RIF) was determined in CEM, CEM_{VBL100} and CEM_{E1000} and repeated in the presence of XR9576 (P-gp inhibitor: 1µM) and MK571 (MRP1 inhibitor: 50µM).

Trans-Epithelial Transport (TET): Apically (BL→AP) and basolaterally (AP→BL) directed flux of rifampicin (1µM, ³H-RIF) was measured over 4 hr in MDCKII_{CTL}, MDCKII_{MDR1} and MDCKII_{MRP1} cells in the presence and absence of XR9576 (1µM) and MK571 (50µM).

Rifampicin Pharmacokinetic Study: Eight rifampicin receiving patients [for TB (n=4) and staphylococcal infection (n=4)] were recruited into a pharmacokinetic study that was approved by the local ethics committee.

Plasma and PBMCs were isolated from blood taken at 0.5, 1, 2, 4, 6 and 8 hr post dosing.

Cellular associated and plasma rifampicin concentrations were measured by a validated HPLC-MS method.

P-gp, MRP1 and BCRP transporter expression were assessed by flow cytometry.

Data Analysis: all *in vitro* results were analysed using the Mann-Whitney U statistical test.

RESULTS:

Figure 1: The CAR of [³H]-rifampicin (1 µM) in CEM, CEM_{VBL100} and CEM_{E1000} cells in the presence and absence of a P-gp inhibitor (XR9576: 1µM) and the MRP1 inhibitor (MK571 50µM). Data are expressed as mean ± SD (n=4). *** P > 0.001, * P > 0.05.

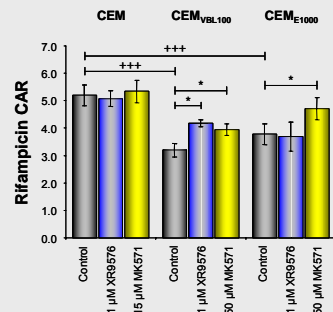
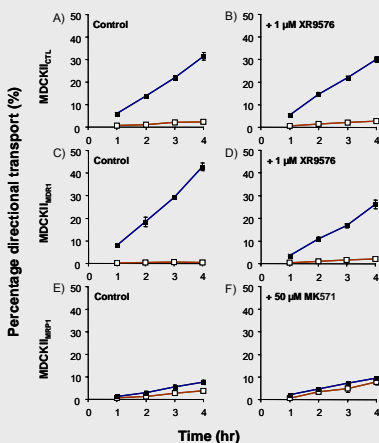


Figure 2: TET of 1 µM [³H]-rifampicin in MDCKII_{CTL} (A, B) MDCKII_{MDR1} (C, D) and MDCKII_{MRP1} (E, F) in the absence (A, C, E) and presence of 1 µM XR9576 (B, D) and 50 µM MK571 (F). Apically (BL→AP: —■—) and basolaterally (AP→BL: -□-) directed transport are expressed the % of RIF in the receiver vs. donor compartment per hr. Data are expressed as mean ± SD (n=4).



Cell line accumulation: RIF CAR (mean ± SD) in CEM_{VBL100} (3.19 ± 0.24, p<0.05) and CEM_{E1000} (3.77 ± 0.38, p<0.05) was significantly lower than in CEM (5.19 ± 0.38; n=4) (Fig 1).

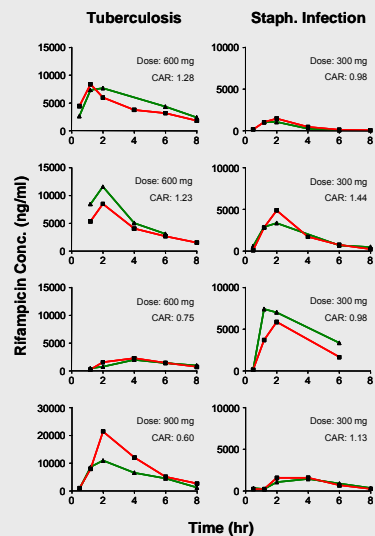
XR9576 (1 µM) and MK571 (50 µM) significantly increased RIF accumulation in CEM_{VBL100} (4.18 ± 0.13, p<0.05) and CEM_{E1000} (4.71 ± 0.41, p<0.05), respectively, compared to the inhibitor free control (n=4) (Fig 1).

MK571 however did also increase the CAR of RIF in CEM_{VBL100}, which may be due to the presence of MRP1 in CEM_{VBL100} (data not shown) or the lack of specificity of MK571.

Trans-epithelial transport of RIF was significantly greater in MDCKII_{MDR1} than in MDCKII (P<0.05; Fig 2).

XR9576 significantly decreased this transport in MDCKII_{MDR1} (P<0.05). AP→BL transport of RIF in MDCKII_{MRP1} (P<0.05) was significantly higher than in MDCKII but was not decreased by MK571 (n=4; Fig 2).

Figure 3: Plasma (—■—) and cellular (—▲—) rifampicin PK profiles from RIF receiving patients (tuberculosis: left column, staphylococcal infections: right column). Each profile represents one patient. CAR values below are calculated by dividing the cellular AUC by the plasma AUC.

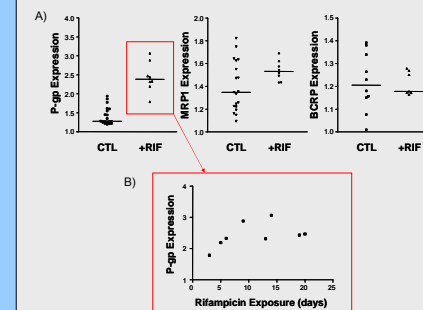


Rifampicin Pharmacokinetic Study (Fig 3): Plasma AUC ranged from 4382 – 83184 ng.hr/ml.

Cellular AUC ranged from 3293 – 53772 ng.hr/ml.

The CAR of RIF in patients receiving RIF ranged from 0.60-1.44.

Figure 4: (A) Drug transporter expression in rifampicin receiving patients (n=8) vs. healthy controls (n=20). (B) P-gp expression in the rifampicin receiving patients vs number of days receiving rifampicin dose (exposure).



A significant increase in P-gp expression was observed in PBMCs isolated from RIF receiving patients P < 0.0001 vs. those from healthy volunteers (1.42 ± 0.23; n=20) (Fig 4a).

There appears to be a positive relationship between days of RIF exposure and P-gp expression (Fig 4b).

No difference was observed for MRP-1 or BCRP expression (Fig 4a).

CONCLUSIONS:

RIF is a substrate for both P-gp and MRP1 and a potent inducer of P-gp expression on PBMCs *in vivo*.

We have validated an assay for the measurement of cellular RIF accumulation *in vivo*.

We have shown for the first time the cellular PK or RIF

Alterations in cellular efflux transporter expression may impact on the CAR of HIV drugs.

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