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Abstract

Background: The efflux transporter MRP1 is involved in active transport of ARVs and reduces their intracellular accumulation. In vitro studies have demonstrated that NNRTIs and some NRTIs inhibit MRP1 and increases concentrations of some ARVs in cells. However, this mechanism of intracellular PK interaction has not been demonstrated in clinical settings.

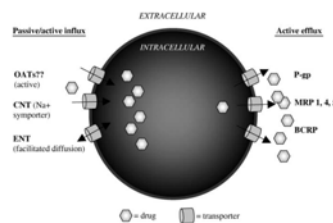
Methods: 7 healthy volunteers were recruited for a darunavir (DRV) + efavirenz (EFV) drug interaction study. PBMCs were collected from these subjects at baseline, 10 days after administration of DRV 900 mg QD/ritonavir (RTV) 100 mg QD, and 14 days after administration of EFV 600 mg QD. Flow cytometry was performed for MRP1 protein expression using a FITC-conjugated antibody against the MRP1 m6 epitope, after cell permeabilization. MRP1 expression was compared between CD4+ and total PBMCs. MRP1 efflux function was assessed by incubating PBMCs with carboxyfluorescein diacetate (CFDA) with and without the MRP1 inhibitors MKS71 and probenecid, and quantifying intracellular CFDA fluorescence. MRP1 mRNA expression was measured by real time PCR.

Results: MRP1 protein expression was reduced after DRV/RTV administration (Geometric Mean Ratio of 0.58 [95% confidence interval 0.51-0.65], $P < 0.001$) but not significantly after EFV administration (GMR 0.82 [0.64-1.06] $P = 0.10$, $N = 6$). MRP1 protein expression was 41% higher in CD4+ cells compared to all PBMCs. MRP1 efflux function was increased after EFV administration (GMR 3.13 [2.73-3.59], $P < 0.001$) but not after DRV/RTV administration (GMR 1.06 [0.80-1.42], $P = 0.42$). MRP1 mRNA expression was increased after DRV/RTV administration (GMR 2.80 [1.45-5.41], $P = 0.009$) but not significantly after EFV administration (GMR 1.75 [0.87-3.51], $P = 0.10$).

Conclusions: DRV/RTV decreased MRP1 protein expression but not mRNA expression or efflux function in vivo. EFV increased MRP1 efflux function but not expression. MRP1 could play a larger role in CD4+ lymphocytes which expressed more MRP1 protein. This clinical study suggests that in vitro studies and measuring plasma concentrations alone in pharmacokinetic studies may underestimate the complex intracellular interactions of combination ARVs.

Introduction

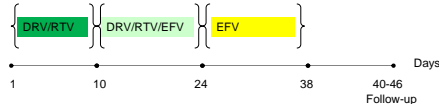
- MRP1 is an efflux transporter that pumps antiretroviral (ARV) drugs out of cells
- In vitro studies show that ARVs can inhibit MRP1 and that can increase the intracellular concentration of ARVs in cells¹
- We study this mechanism of intracellular interaction in a longitudinal study



Methods

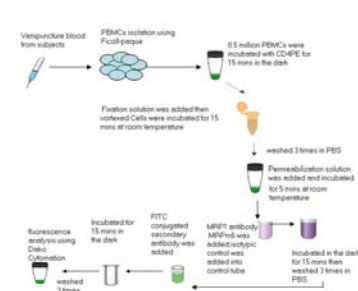
STUDY DESIGN

- Seven healthy volunteers: 4 males 3 females, ages 24-38 years, weight 54-83 kg
- Volunteers all given darunavir (DRV) 900 mg and ritonavir (RTV) 100 mg daily or/and efavirenz (EFV) 600 mg once daily as in the scheme below:

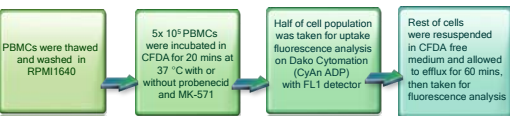


- 10 mL of blood was taken on days 0 (baseline), 9 (after DRV/RTV) and 37 (after EFV) for measurement of MRP1

MEASUREMENT OF MRP1 PROTEIN EXPRESSION²



MEASUREMENT OF MRP1 EFFLUX FUNCTION



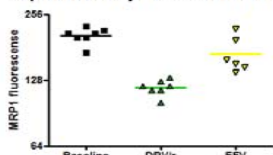
MEASUREMENT OF MRP1 mRNA EXPRESSION



Results

MRP1 PROTEIN EXPRESSION

Expression assay for MRP1 in total PBMCs

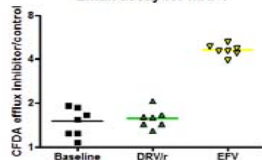


	Baseline	DRV/RTV	EFV
Mean ± SD	205 ± 17	118 ± 9.7	168 ± 32
GMR vs baseline (95% CI)		0.58 (0.51-0.65)	0.81 (0.64-1.06)
P value		<0.001	0.10

- MRP1 expression was 41% higher (geometric mean ratio 1.41) in CD4 cells compared to total PBMCs

MRP1 EFFLUX FUNCTION

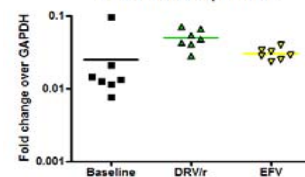
Efflux assay for MRP1



	Baseline	DRV/RTV	EFV
Mean ± SD	1.50 ± 0.33	1.58 ± 0.25	4.62 ± 0.41
GMR vs baseline (95% CI)		1.06 (0.80-1.42)	3.13 (2.73-3.59)
P value		0.62	<0.001

MRP1 mRNA EXPRESSION

MRP1 mRNA expression



	Baseline	DRV/RTV	EFV
Mean ± SD	0.025 ± 0.03	0.050 ± 0.02	0.030 ± 0.006
GMR vs baseline (95% CI)		2.80 (1.45-5.41)	1.75 (0.87-3.51)
P value		0.009	0.10

CORRELATIONS

- No significant correlation were found between MRP1 protein expression and efflux function
- No correlation between MRP1 protein and mRNA expression

Conclusions

- DRV/RTV markedly reduced MRP1 protein expression
- There was a non-significant trend for EFV reducing MRP1 protein expression
- Efflux function increased significantly after EFV, but is non-specific and may be due to upregulation of other transporters
- Induction of mRNA did not correlate with other measures

- Inhibition of MRP1 may lead to synergistic interactions especially in CD4 cells where MRP1 expression is higher
- This clinical study suggests that in vitro studies and measuring plasma concentrations alone in pharmacokinetic studies may underestimate the complex intracellular interactions of combination ARVs.
- Results should be confirmed in HIV-infected patients

Acknowledgements

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References:
 1. Weiss, J. D., Thekk, et al. (2007). "Inhibition of MRP1/ABCC1, MRP2/ABCC2, and MRP3/ABCC3 by nucleosides, nucleotides, and non-nucleoside reverse transcriptase inhibitors." *Drug Metab Dispos* 35(3): 360-4.
 2. Meenan, S. R., P. G. Hoogwerf, et al. (2002). "Determination of P-gp and MRP1 expression and function in peripheral blood mononuclear cells in vivo." *J. Immunol Methods* 262(1-2): 159-69.