

Effect of Efavirenz and Nevirapine on P-gp Expression: the Impact on Protease Inhibitor Fluxing

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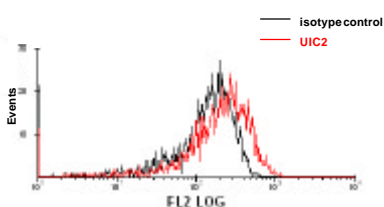
INTRODUCTION

- The efflux transporter P-glycoprotein (P-gp) is expressed in many cells and is thought to play a role in multidrug resistance to anti-HIV drugs.
- The protease inhibitors (PIs), and possibly the nucleoside analogues (NRTIs), are substrates for P-gp, which may lead to sub therapeutic cellular concentrations.
- The PIs are also inducers of P-gp, potentially altering the cellular distribution of themselves and other drugs.
- C3435T, a single nucleotide polymorphism (SNP) located in exon 26 of the Pgp gene (MDR-1), has been associated with high (CC), low (TT) and intermediate (CT) expression of P-gp protein.
- Here the effect of the NNRTIs, efavirenz (EFV) and nevirapine (NVP) on P-gp expression in PBMCs from healthy volunteers (genotyped for the C3435T polymorphism) was examined. The effect of altered P-gp on the uptake of the PI lopinavir (LPV) was also investigated.

METHODS

- PBMCs were isolated from 16 healthy volunteers (aged 21-54 years) and resuspended at 5×10^6 /ml before incubating with either EFV (10 μ M), NVP (10 μ M) or vehicle alone (VEH; DMSO) for 72 h.
- Cells were stained for P-gp with primary (isotype control or UIC2) and secondary (PE-conjugated) antibodies. Fluorescence was determined by flow cytometry.
- Histograms from isotype control and UIC2 antibodies were overlaid (Figure 1) and the % P-gp positive cells calculated, using FCS expressTM software.

Figure 1 Representative histograms showing fluorescence (FL-2) against the number of events.

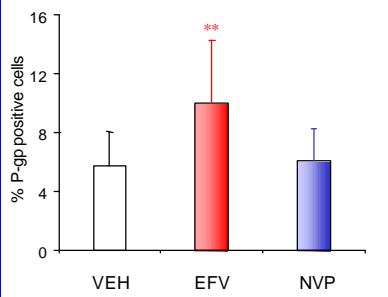


- DNA was isolated from all 16 healthy volunteers and C3435T genotype determined by RLFP. Mbo1 was the restriction enzyme used.
- C3435T genotype was then related to the change in % P-gp positive cells following exposure to EFV or NVP compared to VEH.
- Cellular uptake of the radiolabelled LPV was measured in CEM and CEM_{VBL} (which overexpress P-gp) cell lines, using oil stop methodology as previously described.

RESULTS

- EFV significantly ($p < 0.01$; Kruskal Wallis) upregulated P-gp on the surface of PBMCs, compared to control. No change in P-gp was observed with NVP (Figure 2).

Figure 2 Cell surface P-gp following *in vitro* exposure (72 h) to VEH (0.5% DMSO; n=16), EFV (10 μ M; n=16) or NVP (10 μ M; n=15). Data are expressed as mean \pm s.d.; ** $p < 0.01$.



- No difference in the induction of Pgp was observed between different C3435T genotypes (Kruskal Wallis; Figures 3 & 4).

Figure 3 Change in P-gp following incubation with EFV. Data expressed as the difference in P-gp positivity between VEH and drug treated cells. The bar indicates the median.

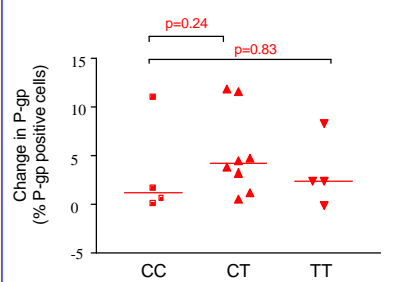
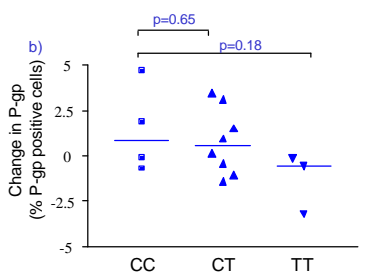
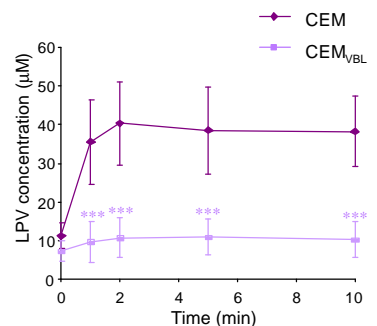


Figure 4 Change in P-gp following incubation with NVP. Data expressed as the difference in P-gp positivity between VEH and drug treated cells. The bar indicates the median.



- Intracellular accumulation of LPV was significantly reduced ($p < 0.0001$; Kruskal Wallis) in CEM_{VBL} cells compared to CEM cells (Figure 5).

Figure 5 Intracellular accumulation of ¹⁴C LPV in CEM and CEM_{VBL} cell lines (n=6). Data are expressed as mean \pm s.d.; *** $p < 0.0001$.



CONCLUSION

- EFV, but not NVP, increased P-gp expression on the surface of PBMCs *in vitro*.
- The effect of EFV and NVP on P-gp appears to be independent of C3435T genotype status.
- P-gp modulates the cellular uptake of LPV.
- Further studies are in progress to determine if the NNRTIs are substrates and/or inhibitors of P-gp and to examine their effect on transporter expression *in vivo*.
- The effect of NNRTIs on the intracellular accumulation of other drugs *in vivo* will also be investigated.

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