

Induction of P-glycoprotein in peripheral blood mononuclear cells (PBMC) *in vitro*

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Background

- P-glycoprotein (P-gp) is a transport protein expressed in lymphocytes and other tissues. HIV protease inhibitors (PIs) are substrates for P-gp [1] raising the possibility that P-gp mediated efflux may contribute to sub-therapeutic drug levels and treatment failure.
- Recent studies have demonstrated a correlation between a polymorphism at position 3435 in exon 26 of the MDR1 gene and expression of P-gp in both the intestine [2] and PBMC [3].
- In this *in vitro* study, the effects of PI incubation on P-gp expression in PBMC has been investigated along with the influence of the C3435T polymorphism in exon 26 of MDR1.

Methods

- PBMC were isolated from healthy individuals and resuspended in Hank's balanced salt solution at a concentration of 5×10^6 cells.mf⁻¹. Cells were incubated with the PIs ritonavir (RTV), nelfinavir (NFV), lopinavir (LPV), indinavir (IDV) or amprenavir (APV) at 10 (n=14) or 100µM (n=10) or vehicle (VEH) alone for 72h and fixed.
- P-gp expression was assessed by flow cytometry using the anti-P-gp antibody UIC2 and phycoerythrin labelled secondary antibody. The % positivity for P-gp was calculated using histogram subtraction of isotype controls with FCS Express software.
- Genotyping for the C3435T polymorphism was carried out on DNA extracted from whole blood using restriction fragment length polymorphism analysis (RFLP) using MboI restriction endonuclease. Induction of P-gp was calculated by subtraction of P-gp % positivity in VEH controls from PI incubated samples. These were then compared between genotypes.
- Statistical analysis was carried out by non-parametric analysis of variance (ANOVA, Kruskal-Wallis).

Figure 1. Representative gel showing products of RFLP. Lane 1 shows a homozygous mutant sample, lane 2 shows a heterozygous sample and lane 3 a homozygous wild-type sample.

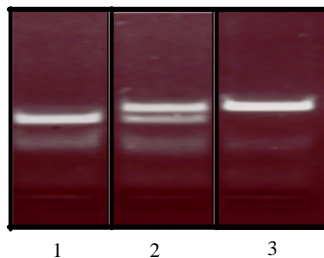


Figure 2. P-gp % positivity of PBMC following 72h incubation with 10 (■) or 100µM (■) PI compared to cells alone (CA) or VEH treated controls.

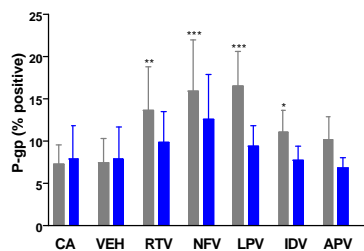
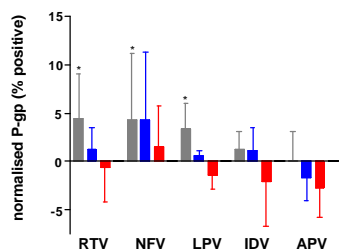


Figure 3. P-gp % positivity normalised to VEH following 72h incubation with 10µM PI in PBMC isolated from C3435T heterozygotes (■), homozygous wild-type (■) and homozygous mutant (■) individuals.



Results

- Incubation with 100µM RTV, NFV, LPV and IDV led to a significant increase in P-gp % positivity compared to VEH controls. (difference from VEH ? ? p<0.05, ? ? ? p<0.01, ? ? ? p<0.001, Figure 2 mean ± s.d, n=10)
- Incubation with the PIs tested at 10µM led to a trend towards increased P-gp % positivity which was not significant (Figure 2, mean ± s.d, n=14).
- Of the 14 healthy volunteers genotyped, 6 were heterozygous for the C3435T polymorphism, 4 were homozygous wild-type and 4 were homozygous mutant. This fits Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1.02$).
- PBMC isolated from heterozygotes showed significant P-gp induction following incubation with 10µM RTV, NFV and LPV (Difference from 0, ? ? p<0.01, ? ? ? p<0.001. Figure 3, mean ± s.d.). PBMC isolated from homozygous wild-type volunteers showed a trend towards P-gp induction with all PIs tested except APV. PBMC isolated from homozygous mutant volunteers showed a trend towards decreased P-gp % positivity with all PIs tested except NFV.

Conclusions

- In this model, the PIs induced P-gp in a concentration dependent manner.
- C3435T genotype may be predictive of P-gp response to PI in PBMC although subject numbers must be increased to demonstrate the reproducibility of these findings.
- Further studies will examine the clinical relevance of these observations and the interaction between HIV infection and P-gp expression.

References

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