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Biochemical Pharmacology 68 (2004) 783–790
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

# Rapid induction of P-glycoprotein expression by high permeability compounds in colonic cells in vitro: a possible source of transporter mediated drug interactions?

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Received 22 January 2004; accepted 5 May 2004

**Abstract**

P-glycoprotein (PGP) substrates with high membrane permeability, such as propranolol and verapamil, are considered to be essentially ''transparent'' to PGP since the transporter does not significantly limit their absorption or elimination. However, the question of whether such compounds can modulate PGP expression in epithelial cells following short-term exposure, with potential consequences for drug interactions, has not been addressed.

LS180 colonic epithelial cells were exposed to propranolol or verapamil at concentrations (50–300 μM) consistent with those likely to be present in the gut lumen during oral dosing. Both compounds stimulated four to six-fold increases in MDR1 mRNA and PGP protein expression measured by quantitative real-time PCR and immunoblotting, respectively. These changes were accompanied by an induction in transporter activity measured by rhodamine 123 efflux. In contrast, metoprolol, a compound with similar permeability but no affinity for PGP had no effect on PGP expression. The induction of PGP by propranolol and verapamil was rapid with significant increases occurring within 3 h with maximal stimulation after 6 h exposure. Rifampicin, shown to cause clinical drug interactions via a PXR-mediated increase in PGP expression, exhibited a very similar time-course and extent of induction.

In conclusion, verapamil and propranolol, whose trans-epithelial permeability are unaffected by PGP, appear to be effective inducers of PGP expression in gut epithelial cells in vitro. While the in vivo significance of these observations is unknown, this questions whether high permeability, ''PGP-transparent'' compounds, currently favoured in drug selection strategies, should be evaluated in terms of their potential for transporter-mediated drug interactions.
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*Keywords:* Transporter induction; P-glycoprotein; Drug absorption; LS-180 cells; Drug permeability

## 1. Introduction

P-glycoprotein (PGP) is located on epithelial and endothelial surfaces throughout the body including the liver, kidney, gastrointestinal tract and blood–brain barrier where it plays an important role in the absorption, elimination and distribution of many xenobiotics including a wide range of therapeutic drugs [1,2]. In the intestine, the high levels of PGP expressed may directly limit oral drug absorption [3–6] and also result in variable and non-linear pharmacokinetics [7,8]. In addition, PGP is a source of clinically significant drug–drug interactions [9]. Recent studies have shown that PGP expression can be induced in gut epithelium as a result of prolonged or repeat exposure to compounds such as rifampicin (RIF) and St John's Wort, resulting in impaired absorption of co-administered drugs [10]. For example, repeat oral administration of RIF over a 10-day period in healthy volunteers decreased plasma levels of the PGP substrate digoxin an effect that could be correlated with an induction of duodenal PGP expression [11]. In this and other examples, PGP induction was accompanied by increased expression of CYP enzymes suggesting that these factors can be co-ordinately regulated [12]. Recent evidence suggests that the nuclear response element, pregnane X receptor (PXR), which is abundantly expressed in the intestine, may be the primary mediator of drug-induced intestinal PGP and CYP expression, [13,14] although other mechanisms are also likely to be involved [15].

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0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.05.006

One group of compounds for which relatively little is known regarding their potential for PGP-mediated interactions, specifically involving transporter induction, are those that interact with PGP but have high membrane permeability. This group, which includes propranolol (PRO) and verapamil (VER), do not exhibit asymmetric permeability in in vitro models of drug absorption and there is no evidence that PGP has a significant effect on their absorption or elimination in vivo [16,17]. Nevertheless, it is clear that such compounds do interact with the transporter. For example, VER is one of the most effective stimulators of PGP ATPase activity and inhibits the PGP-mediated efflux of several drugs [17,18]. One explanation of these properties is that verapamil diffuses so rapidly across the cell membrane that, even though the drug is being actively transported out of the cell by PGP, it immediately diffuses back [19]. A similar explanation may hold for PRO and progesterone, which also act as inhibitors of PGP mediated efflux, although there is some controversy regarding their ability to activate PGP-associated ATPase [17,20]. As a result, high permeability compounds are generally regarded to be ''transparent'' to PGP and unlikely to exhibit any pharmacokinetic issues resulting from transporter interaction, and the possible effect of such compounds on transporter expression via PXR or other mechanisms is poorly defined.

The aim of this study was, therefore, to investigate the ability of PRO and VER to alter the molecular and functional expression of PGP in human colonic cells in vitro. Evidence is presented that both compounds cause a rapid up-regulation of the transporter with characteristics similar to that of RIF, a known inducer of PGP expression. The possible implications for unexpected drug–drug interactions involving high permeability compounds are discussed.

## 2. Materials and methods

### 2.1. Materials

[*N*-methyl-$^3$H]-verapamil hydrochloride (2220–3145 GBq/mM) and [L-4-$^3$H]-propranolol (555–1110 GBq/mM) were obtained from Perkin-Elmer Life Sciences (Boston, MA). All non-labelled drugs were obtained from Sigma-Aldrich (Poole, UK). Tissue culture consumables were obtained from Invitrogen Life Technologies (Paisley, UK). Caco-2 and LS-180 cell lines were obtained from the American Type Culture Collection, Mannassas, USA.

### 2.2. Analysis of drug transport in Caco-2 monolayers

The colonic epithelial cell line, Caco-2 (passage 25–35) was cultured in Dulbecco's Modified Eagles Medium supplemented with 10% fetal calf serum, 1% non-essential amino acids, 2 mM glutamine and 50 IU/ml penicillin/50 ug/ml streptomycin as previously described [21]. For transport studies, cells were seeded onto polycarbonate Falcon membranes (0.4 μm pore size; 0.33 cm$^2$ diameter; BD Biosciences Oxford, UK) at a density of 2–3 × 10$^5$ cells/cm$^2$ and cultured for 22–27 days prior to use. At the end of this period, monolayers were washed free of culture medium and equilibrated in Hank's Balanced Salt Solution pH 7.5 at 37 °C for transport studies. After a 20 min equilibration period, drug transport was measured over 3 × 40 min periods in the apical to basolateral (A–B) and basolateral to apical (B–A) directions under ''sink'' conditions using a protocol similar to that described previously [22]. Analysis of drug concentrations in donor and receiver compartments was by liquid scintillation counting (PRO, VER) or LC–MS (MET, RIF) using standard methods.

Drug permeability in each direction was measured as apparent permeability ($P_{app}$) (cm s$^{-1}$) obtained according to the following equation:

$$P_{app} = \frac{dQ/dt}{CA} \quad (1)$$

where $dQ/dt$ is the rate at which the compound appears in the receiver compartment, $A$ is the surface area of the tissue, and $C$ is the initial concentration of the compound in the donor compartment.

### 2.3. Drug activation of PGP ATPase

The stimulation of ATPase hydrolysis by the compounds was tested using membranes from high five (BT1-TN5B1-4) cells expressing high levels of PGP (Gentest, Boston, MA) according to the manufacturers instructions. Briefly, membranes were incubated with or without test compounds in a medium containing 50 mM 2-morpholinoethanesulfonic acid, 50 mM KCl, 2 mM dithiothreitol, 2 mM ethyleneglycol bis(2-aminoethyl ether)-tetraacetic acid, 2 mM Tris–HCl and 5 mM $NaN_3$. The reaction was initiated by addition of ATP (4 mM final concentration) and incubated at 37 °C for 20 min in the presence and absence of the PGP ATPase inhibitor vanadate. Following termination of the reaction by addition of 10% sodium dodecyl sulphate, inorganic phosphate (Pi) formed during the reaction was assayed spectrophotometrically (655 nm), after addition of a solution containing 1.3% sulphuric acid, 0.2% ammonium molybdate, 2.3% trichlorlacetic acid and 1% ascorbic acid. Drug-mediated activation of PGP-ATPase was defined as the amount of Pi formed in the absence of vanadate minus that formed in the presence of vanadate and expressed as nmol Pi/mg protein/min.

### 2.4. P-glycoprotein induction studies in LS180 colonic cells

The ability of drugs to induce P-glycoprotein expression and function was studied in the LS180 human colonic cell

line. LS180 cells were cultured in Eagle's minimal essential medium (MEM) supplemented with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate and 10% fetal bovine serum. Cells seeded at a density of $1 \times 10^5/cm^2$ in T-25 $cm^2$ flasks were grown to approximately 90% confluence and exposed to drugs at the concentration indicated for varying periods of time (1.5–24 h). Cells were then either analysed for changes in PGP activity using the Rhodamine 123 efflux assay or processed for mRNA or immunoblot analysis of PGP expression as described below.

### 2.5. Real time quantitative PCR analysis of MDR1 mRNA

Total RNA was isolated from LS180 cultures by scraping cells into TriZol reagent and processing according to the manufacturers instructions. Samples were treated with DNAase I (Ambion, Huntingdon UK) and RNA integrity checked by ethidium bromide agarose electrophoresis and purity by measurement of the 260/280 nm ratio, which was routinely in the range 1.8–2.0. DNA contamination was excluded by performing PCR reactions on non-transcribed RNA with primers specific for human β-actin. Absence of actin bands was an indication that samples were free of significant genomic DNA contamination. Then 5 μg of each RNA sample was reverse transcribed by a commercial first-strand cDNA synthesis kit (Amersham Biosciences, Little Chalfont, UK) using random hexamers.

Relative quantification of gene expression was performed by Taqman real-time quantitative PCR analysis (single-reporter, FAM dye) using an ABI Prism 7700 sequence detection system (Applied Biosystems). Reverse transcribed RNA corresponding to 50–250 ng of total RNA extracted from LS180 cells was used in each reaction along with gene specific primers (12 pmol/reaction) and probes (3 pmol/reaction) for human MDR1 and the reference gene, villin in a total volume of 20 μl. The primer and probe sequences used were as described by Taipalensuu et al. [23].

MDR1:
Forward primer—CAGACAGCAGGAAATGAAGTTGAA
Reverse primer—TGAAGACATTTCCAAGGCATCA
Probe—TTTCACTTTTGGATTCATCAGCTGCATTTTCTA

Villin:
Forward primer—CATGAGCCATGCGCTGAAC
Reverse primer—TCATTCTGCACCTCCACCTGT
Probe—TCATCAAAGCCAAGCAGTACCCACCAAG

Templates for standards were prepared from Caco-2 cDNA in dilutions corresponding to 0.1–1000 ng of the original RNA. Two standard curves were prepared, one for the endogenous reference gene villin and one for the target MDR1 gene. The assay results were normalised to the endogenous control. For each experimental sample, the amount of target and endogenous reference was determined from the appropriate standard curve. Then, the target MDR1 amount was divided by the villin reference amount to obtain a normalised value of MDR1 expression relative to villin.

### 2.6. Immunoblot analysis of P-glycoprotein expression

Epithelial preparations were suspended in lysis buffer (120 mM NaCl, 5 mM HEPES (pH 7.5), 1% Triton-X100, 2 mM EDTA, 25 mM NaF, 1 mM $NaVO_4$) containing a cocktail of protease inhibitors (50 μl/ml buffer, Sigma, Catalogue number P8340). The cell suspension was left on ice for 30 min, sonicated for 3 s and centrifuged at 13,000 × *g* for 30 min at 4 °C. The resulting supernatant was retained and the protein concentration determined by the BCA method (Pierce, UK). Then 20 μg aliquots of supernatant protein were fractionated on 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were transferred to Hybond ECL nitrocellulose membrane (Amersham-Pharmacia Biotech UK Ltd., Little Chalfont, UK). Membranes were soaked in TBS-T (50 mM Tris–HCl (pH 7.9), 150 mM NaCl and 0.05% (v/v) Tween 20) containing 2% (w/v) milk protein at room temperature overnight. Membranes were subsequently washed in TBS-T and incubated overnight with monoclonal anti-PGP antibody (C219, 1:400 dilution in TBS-T, Alexis Biochemicals, San Diego, USA) and with monoclonal anti-villin antibody (Clone 12, dilution 1:1000 in TBS-T, Transduction Laboratories, Lexington, USA) for 2 h at room temperature. The secondary antibody was goat anti-mouse IgG horseradish peroxidase-conjugated antibody (BioRad Laboratories Ltd., Hemel Hempstead, UK) used at a dilution of 1:5000. Blots were developed using the Enhanced Chemiluminescence System (ECL) system (Amersham-Pharmacia Biotech UK Ltd., Little Chalfont, UK). Bands for PGP (MW 170 kDa) and villin (MW 95 kDa) were digitised and quantified using Scion Image for Windows (Scion Corporation, Frederick, USA).

### 2.7. Rhodamine 123 efflux assay

Following drug treatment as described above, LS-180 cells in 35 mm culture dishes were washed 5 times with HBSS and incubated for 90 min with 5 μM Rhodamine 123 (R123) in the same medium containing 10% serum. Incubations were performed in the presence or absence of 10 μM verapamil to inhibit PGP function. Cells were then rapidly washed five times with ice-cold HBSS and lysed in 1.5 ml of a solution containing 0.5% deoxycholate, 1% Triton-X100, 1 mM phenyl methyl sulfonyl fluoride and 50 mM $KH_2PO_4$ pH 7.4 for 10 min at 37 °C followed by 20 min of shaking. Cells were scraped off the plastic and

briefly homogenised to ensure complete lysis. A sample of the lysate was taken for protein determination by the Bradford method (Pierce, UK) and the remainder was centrifuged at 13,000 × *g* for 10 min. The level of R123 in the resulting supernatant was measured fluorimetrically ($\lambda_{ex} = 492$ nm, $\lambda_{em} = 535$ nm) using a Victor MultiLabel Counter and standardised to protein content. The ratio of intracellular R123 fluorescence in the absence and presence of 10 μM verapamil is indicative of the activity of PGP.

### 2.8. Data analysis

Values are expressed as mean ± S.D. for the number (*n*) of observations indicated. Statistical analyses were performed using Student's *t* test with a significance level of 5%.

## 3. Results

Three high permeability compounds, propranolol (PRO), verapamil (VER) and metoprolol (MET) were investigated for their ability to modulate PGP expression in human intestinal cells. Initial studies defined the nature of their interaction with PGP in terms of their ability to activate vanadate-sensitive ATPase activity in PGP over-expressing membranes (Fig. 1A) and exhibit bi-directional permeability across Caco-2 monolayers, an assay of active PGP mediated transport (Fig. 1B). Comparative studies were also undertaken with the low to medium permeability compound, rifampicin (RIF), which is a prototypical PGP substrate. PRO and VER stimulated PGP ATPase but showed no asymmetric permeability ($R_{B-A/A-B} = 1$) across Caco-2 consistent with their high permeability ($P_{app} > 15 \times 10^{-6}$ cm/s). In contrast RIF, which has a much lower A–B permeability ($2 \times 10^{-6}$ cm/s) stimulated PGP ATPase and was also actively effluxed ($R_{B-A/A-B}$ of 4.5 ± 0.6) in Caco-2, an effect that could be blocked by the PGP inhibitor, GF120918 (data not shown). The third high permeability compound MET showed no asymmetry across Caco-2 and was unable to activate ATPase activity suggesting that it is not a substrate for PGP.

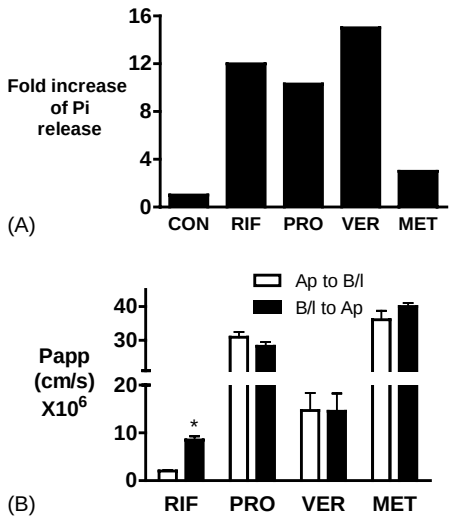


Fig. 1. Effects of high permeability compounds on (A) MDR1 ATPase activity and (B) unidirectional permeability across Caco-2 monolayers. Drugs were used at the following concentrations: rifampicin, 20 μM (RIF); propranolol, 200 μM (PRO); verapamil, 50 μM (VER) and metoprolol 100 μM (MET) for ATPase activation studies. In permeability studies, all drugs were used at the same concentration (20 μM). Papp values are the mean of 3 × 40 min flux periods in each direction. ATPase data represents duplicate determinations with a single membrane preparation data. Permeability values are mean ± S.D. for three to four monolayers in each group. $^{*}P < 0.01$ compared to the permeability in the A–B direction.

The effect of these compounds on cellular PGP levels was investigated using LS-180 human colonic cells at concentrations that had no effect on cell viability (data not shown). Incubation of LS-180 with PRO (200 μM) or VER (50 μM) for 24 h induced a marked (four to six-fold) increase in MDR1 mRNA levels compared to control cells (Fig. 2). RIF (20 μM), which has previously been shown to up-regulate PGP expression via activation of the xenobiotic response element PXR in these cells, also induced a significant increase in MDR1 expression. However, MET had no effect on MDR1 mRNA levels (Fig. 2). Similar effects were observed on membrane expression of P-glycoprotein (Fig. 3) with PRO and VER inducing an approximately 4-fold increase in P-glycoprotein levels normalised against the epithelial marker villin, a similar effect to that seen with RIF. These changes in molecular expression were mirrored in an up-regulation of P-glycoprotein transport activity measured using the Rhodamine 123 efflux assay (Fig. 4). Under control conditions, LS180 exhibit a very low level of transporter function that was significantly increased following incubation with PRO, VER or RIF. The effects of PRO on PGP activity in LS-180 cells increased progressively over the range 20–300 μM (Fig. 5) with significant induction occurring at concentrations as low as 50 μM.

The time course of induction is an important consideration when assessing the potential for this effect to occur in

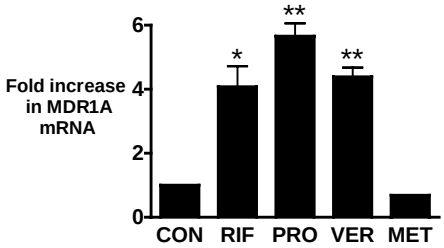


Fig. 2. Influence of compounds on MDR1A mRNA expression in LS180 colonic cells. The drugs indicated were incubated with LS180 cells at the concentrations shown in Fig. 1 for 24 h. MDR1 mRNA was quantified using real-time PCR analysis (TaqMan) standardising against the epithelial marker protein, villin in each case as described in Section 2. The data was normalised to DMSO-treated controls and expressed as the mean (±S.D.) increase in MDR1 mRNA compared to controls. $^{*}P < 0.05$, $^{**}P < 0.01$. $n = 4$–5 in each group.

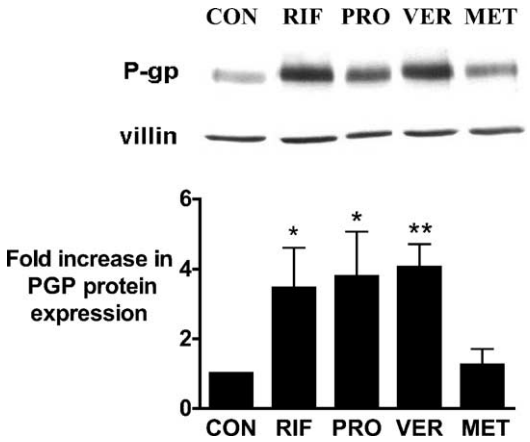


Fig. 3. PRO and VER up-regulate membrane expression of PGP in LS180 cells. Cells were exposed to the indicated drugs for 24 h at the concentrations shown in Fig. 1. PGP protein expression was analysed by immunoblotting with specific antibodies to MDR1 and villin as described in Materials and Methods. Inset shows a typical immunoblot of LS180 membranes. Bargraph shows mean data (±S.D.) from three to five separate membrane preparations normalised to DMSO treated controls. $^{*}P < 0.05$, $^{**}P < 0.01$.

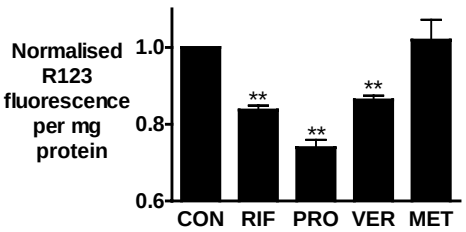


Fig. 4. Exposure to PRO and VER increases PGP transport activity in LS180 cells. PGP mediated rhodamine efflux was measured after 24 h incubation with the indicated drugs or DMSO as control as described in Section 2. Data shows the change in verapamil-sensitive intracellular rhodamine concentration normalised to DMSO controls—a decrease in intracellular fluorescence is indicative of an increase in PGP activity. Data are mean ± S.D. for four observations in each group. $^{*}P < 0.05$, $^{**}P < 0.01$.

vivo due to the transient nature of enterocyte exposure to luminal contents. Fig. 6 shows the change in MDR1 mRNA levels relative to control after exposure to PRO (200 μM) and VER (50 μM) for varying periods from 1.5 to 24 h in LS-180 cells. PRO-treated cells showed a marked (five-

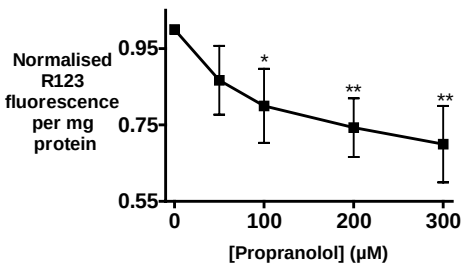


Fig. 5. Dose dependence of PRO-mediated induction of PGP activity. LS180 cells were exposed for 24 h to different concentrations (50–300 μM) of PRO. PGP activity was assessed using the Rhodamine 123 efflux assay. Data are mean ± S.D. for three to four observations in each group. $^{*}P < 0.05$, $^{**}P < 0.01$.

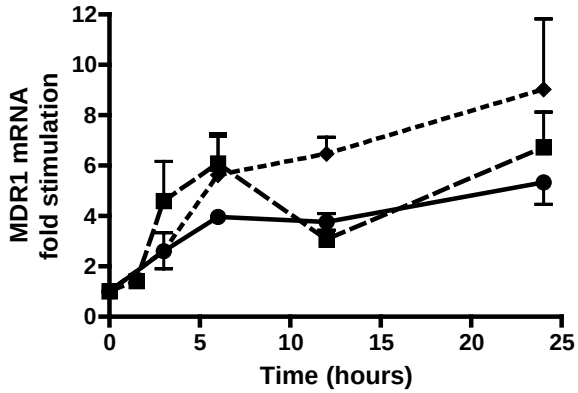


Fig. 6. Time course of MDR1 induction in LS180. Cells were incubated with either 200 μM PRO (■), 50 μM VER (◆) or 20 μM RIF (●) for varying periods (3–24 h) and MDR1 mRNA levels quantified by real-time PCR. Data is presented relative to DMSO treated controls and represents mean ± S.D. of three to six separate experiments in each group.

fold) up-regulation of MDR1 expression after 3 h that was not significantly increased by longer exposure. A similar profile was seen with VER, although the initial increase in MDR1 expression appeared to occur more slowly and continued to rise over 24 h exposure. Interestingly, the time course of MDR1 expression induced by these compounds was similar to that caused by RIF, which also elicited a marked increase in MDR1 expression during the first 6 h of exposure.

## 4. Discussion

In the present study, we have investigated the effects of high permeability PGP substrates on PGP expression in a human colonic epithelial cell line. Interaction with PGP will generally be a negative indicator in drug selection since such compounds are more likely to exhibit absorption problems or adverse drug–drug interactions. It is generally acknowledged that the higher the lipophilicity and membrane permeability of PGP substrates, the less effective the transporter will be in limiting their absorption and permeability across the gut. As a result, these compounds are often assumed to be ‘‘transparent’’ to PGP. However, the data from the present study suggests that compounds such as PRO and VER, which fall into this category, are capable of significantly increasing PGP expression in a gut-derived cell line and that this occurs even after relatively short periods of exposure (3–6 h). The implication is that high permeability substrates may have the potential to cause adverse drug interactions mediated by transporter induction. It has also been shown that nefazodone, trazone and some non-nucleoside HIV-1 reverse transcriptase inhibitors (such as nevirapine and efavirenz) that do not show up as being PGP substrates in a Caco-2 transport assay, probably due to their relatively high permeability can also up-regulate the expression of PGP in LS-180 cells if exposed over a period of 3 days [24,25].

The effects of PRO and VER on PGP expression shown here were substantial and verified by changes in mRNA and protein levels, as well as functionally, by an increase in verapamil sensitive rhodamine efflux. There appeared to be a close correlation between these parameters in LS180 cells suggesting that changes in MDR1A mRNA levels are likely to be a quantitative indicator of alterations in PGP transporter activity. For example, a 24-h exposure of LS-180 cells to 200 μM PRO, elicited four to six-fold increases in all three parameters, although it is difficult to quantify accurately the increase in PGP function using the rhodamine technique. This effect was similar to that elicited by RIF, albeit at a lower concentration (20 μM). The membrane permeability of RIF is at least 10 fold lower than either PRO or VER and, unlike these drugs, PGP significantly limits its intestinal permeability. RIF is also considered to be a prototypical inducer of PGP expression in gut cells both in vitro and in vivo via a mechanism involving activation of PXR [13]. While the present study has not specifically defined the mechanism of PGP induction by PRO and VER in LS180 cells, the similarities with RIF-mediated induction may indicate a role for PXR. In common with RIF, neither PRO nor VER induced PGP expression in the Caco-2 cell line (data not shown), which has been shown to express little or no PXR [26]. In addition, all three compounds exhibited a similar time course for effects on PGP expression causing rapid induction of MDR1 gene expression within 3–6 h of exposure, which is consistent with a common mechanism of action. However, we are not aware of studies reporting PRO-mediated induction of CYP expression, which is also controlled by PXR, so an alternative pathway may be responsible for the effects on PGP expression shown here. Activation of other signalling processes has been implicated in induction of PGP expression, including NF-κB [27], protein kinase C [28] and the PI3-kinase/Akt pathway [29]. It is known that PRO and VER modulate several signalling components [30]. Identification of the specific mechanisms involved in PRO- and VER- induction of PGP is an important area for future study.

To our knowledge this is the first report showing that high permeability PGP substrates have the capacity to induce significant up-regulation of PGP expression after short exposure (3–6 h). Recent studies [31,32] have also reported increased expression of PGP following exposure of LS-180 cells to verapamil but after a much longer period (72 h). The finding of rapid induction may be relevant to the likely in vivo significance of this effect, because even during chronic oral administration the intestinal concentration of a high permeability compound is likely to decrease rapidly as the drug is absorbed, meaning that intestinal tissues may only be exposed to ''inducing'' concentrations for a few hours. A previous study reported that Caco-2 cells, exhibited significantly higher PGP expression when cultured for extended periods (≈7 days) with a variety of compounds including verapamil [33]. However, such data can be difficult to interpret in terms of specific induction of PGP. In extended culture, higher PGP expression may result from selection of PGP-expressing cells within a heterogenous population due to low-level toxicity of compounds, unless this has been specifically excluded.

Although, the present study offers strong evidence that high permeability PGP substrates have the capacity to rapidly up-regulate PGP activity in intestinal cells in vitro, the in vivo significance of such findings remains to be determined. In the case of PRO and VER, the concentrations that caused induction were relatively high but these are not incompatible with the doses at which these drugs are commonly administered in man. For example, the recommended daily oral maintenance dose of PRO for the treatment of hypertension is 160–320 mg (BNF). This would result in luminal concentrations of >500 μM PRO, assuming a fed upper GI tract volume of 1 l. Similarly, VER administered at a dose of 240 mg might be expected to produce luminal concentrations in excess of 200 μM. Physiologically based modelling of propranolol absorption, based on observations in man [34] suggest that an oral dose of 120 mg will be absorbed at a linear rate for at least 3 h. This extends to approximately 10 h for a slow release formulation of PRO [34,35]. Given the capacity of PRO to rapidly up-regulate MDR1 expression within a few hours, as shown here, coupled with the high intracellular drug concentrations likely to persist during absorption it is feasible that enterocyte PGP activity could be significantly affected.

The potential for induction at non-intestinal sites such as the liver should also be considered. Highly permeable compounds are often associated with high hepatic concentrations that may be sufficient to induce transporters. In an extensive study of hepatocytes taken from 62 human livers it was reported that those treated with omeprazole, an orally administered high permeability drug, exhibited approximately five times higher expression of the MRP3 transporter compared to the rest of the population [36]. The study reported here suggests that similar induction could also occur for PGP.

A link between rifampicin-mediated increase in intestinal PGP expression and reduced absorption of a co-administered compound (digoxin) has been demonstrated [11]. The question of whether high permeability compounds have the capacity to produce similar drug interactions has not been defined, although there are contradictory observations. Pre-treatment of rats with VER for 14 days did not significantly alter talinolol absorption but effects on tissue PGP expression were not measured [37]. However, in a clinical study, Pedersen et al. [38] reported that the initial increase in digoxin concentration which occurs on co-administration with VER, presumable due to an acute interaction with PGP, gradually reduces and returns to base line levels after six weeks, which is consistent with an induction of PGP. Similarly, it has also been shown that

chronic administration of VER leads to a significant increase in its own elimination after repeat administration [39].

These reports indicate the potential for PGP induction to produce clinically important drug interactions, however, currently it is not clear how widespread such interactions might be [40]. Nevertheless, it is important, during drug selection, to identify compounds that have the potential for transporter induction and these data suggest that PGP screens based simply on changes in drug permeability may not identify all compounds with this potential.

In conclusion, we have shown that high permeability PGP substrates, whose trans-epithelial permeability are unaffected by PGP, nevertheless, have the ability to induce a rapid increase in expression of the transporter in vitro by a mechanism that probably involves the nuclear receptor PXR. Although the in vivo significance of this effect has not been addressed, these data suggest that high permeability, ''PGP-transparent'' compounds, of the type favoured by current drug selection strategies, may need to be re-evaluated in terms of their potential to cause drug interactions mediated by up-regulation of PGP and possibly other transporters.

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