Predicting P-Glycoprotein Effects on Oral Absorption: Correlation of Transport in Caco-2 with Drug Pharmacokinetics in Wild-Type and mdr1a(–/–) Mice *in Vivo*

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Purpose. Cell-based permeability screens are widely used to identify drug–P-glycoprotein (PGP) interaction *in vitro*. However, their reliability in predicting the impact of PGP on human drug pharmacokinetics is poorly defined. The aim was to determine whether a quantitative relationship exists between PGP-mediated alterations in Caco-2 permeability and oral pharmacokinetics in mice.

Methods. Two indicators of drug efflux were measured in Caco-2 for a group of 10 compounds, the ratio of A-B and B-A transport ($R_{B\text{-}A/A\text{-}B}$) and the ratio of A-B transport in the presence and absence of a PGP inhibitor, GF120918 (R_{GF}). These data were correlated with ratios of oral plasma levels in either mdr1a(–/–) or mdr1a/1b(–/–) and wild-type mice ($R_{KO/WT\ in\ vivo}$) calculated from literature data on these compounds.

Results. A significant, positive correlation ($r^2 = 0.8$, $p < 0.01$) was observed between R_{GF} and $R_{KO/WT\ in\ vivo}$. In contrast, $R_{B\text{-}A/A\text{-}B}$, a more commonly used *in vitro* measure, showed a much weaker correlation with *in vivo* data ($r^2 = 0.33$, $p = 0.11$). A strong correlation with R_{GF} was also observed after correction of *in vivo* data for PGP effects on IV clearance.

Conclusion. The increase in A-B drug permeability following inhibition of PGP in Caco-2 allows a reasonable prediction of the likely *in vivo* impact that PGP will have on plasma drug levels after oral administration.

KEY WORDS: Caco-2; drug absorption; mouse; P-glycoprotein.

INTRODUCTION

During the last few years, there has been a dramatic increase in the identification and molecular characterization of drug efflux proteins (1). These proteins are localized on epithelial and endothelial surfaces throughout the body including the liver, kidney, gastrointestinal tract, and blood-brain barrier and have the capacity to modulate the absorption, distribution, and excretion of a wide range of therapeutic drugs (2,3). In the intestine, the high levels of P-glycoprotein (PGP) expressed may directly limit oral drug absorption and cause variable and nonlinear pharmacokinetics (4). In addition, intestinal PGP has been shown to be a site of clinically significant drug-drug interactions (5). Identification of potential PGP substrates and inhibitors has therefore become an important part of drug development and selection with several *in vitro* screening assays available, including ATPase activation, accumulation of fluorescent substrates, and bidirectional permeability across cell monolayers (6–8). The usefulness of these assays in characterizing drug-PGP interactions and strategies for their use at different stages of the drug discovery/development process has recently been discussed (9,10). Despite this, interpretation of such assays in terms of the likely *in vivo* impact of interaction with PGP on, for example, oral absorption remains problematic, particularly because a large number of chemical entities interact with PGP but in most cases this does not lead to significant *in vivo* effects. Therefore, there is a clear need to establish *in vitro-in vivo* correlations that can define the predictive value of *in vitro* PGP data.

A major obstacle to establishing such correlations for oral absorption is the lack of definitive *in vivo* data. Poor absorption may result from a combination of factors making it difficult to define the importance of PGP-mediated transport, particularly in man. One approach to this problem is to use pharmacokinetic data from mdr1a(–/–) mice in which intestinal PGP function has been specifically and completely ablated (11–13). Comparison of mdr1a(–/–) mice with wild-type (WT) animals allows the contribution of intestinal PGP to be quantitatively defined without recourse to inhibitors that often exhibit specificity and affinity problems *in vivo*. (12) A similar strategy has recently been reported by Adachi and co-workers (14) who showed that reductive *in vitro* screens may be useful in predicting the *in vivo* penetration of PGP substrates across the blood-brain barrier (BBB).

The aim of the current study was to investigate whether a quantitative relationship also exists between PGP-mediated changes in Caco-2 permeability and PGP effects on oral pharmacokinetics for a group of 10 compounds where data was available in WT and PGP knockout (PGP-KO) mice.

MATERIALS AND METHODS

Materials

All non-radiolabeled drugs were from Sigma (Poole, UK) except saquinavir (a gift from F. Hoffmann-La Roche Ltd. Basel, Switzerland), UK224,671 (Pfizer Global Research and Development, Sandwich, Kent, UK), tacrolimus (Fujisawa Pharmaceutical Co, Osaka, Japan), topotecan (Glaxo-SmithKline, King of Prussia, PA, USA), and S 09788 (Servier Research and Development, Fulmer, UK). The following [^{3}H]-labeled drugs were also used: verapamil (2220–3145 GBq/mM), digoxin (555–1111 GBq/mM) (NEN Life Science Products Inc., Boston, MA, USA), paclitaxel (544 GBq/mM, Moravek Biochemicals, Inc. CA, Brea, USA), saquinavir (496 GBq/mM, Hoffmann-La Roche Ltd., Basel, Switzerland), and UK224,671 (33.2 μCi/mg, Pfizer Global Research and Development, Sandwich, UK). Tissue culture consumables were obtained from Invitrogen Life Technologies (Paisley, UK).

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ABBREVIATIONS: P_{app}, apparent permeability; PGP, P-glycoprotein; R_{GF}, ratio of apical to basolateral drug permeability (P_{app}) across Caco-2 in the presence and absence of the PGP inhibitor GF120918 and in control conditions; $R_{B\text{-}A/A\text{-}B}$, ratio of basolateral to apical and apical to basolateral drug permeability (P_{app}) across Caco-2.

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Methods

In vitro Assays of Drug Transport in Caco-2 Monolayers

The colonic epithelial cell line, Caco-2 (passage 25-35), was cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum, 1% nonessential amino acids, 2 mM glutamine, and 50 IU/ml penicillin–50 μg/ml streptomycin as previously described (15). 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 to 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 transport studies conducted in Hank's Balanced Salt Solution pH 7.5 (Sigma, Poole, UK) at 37°C. After an initial 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 (15). Analysis of drug concentration in donor and receiver compartments was by liquid scintillation counting in the case of radiolabeled compounds and by LC-MS-MS in the case of non-radiolabeled compounds (described below).

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

$$P_{app} = \frac{dQ/dt}{C \cdot A} \quad (1)$$

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.

Using this approach, two indicators of active transport were assessed as shown in Fig. 1. $R_{B\text{-}A/A\text{-}B}$ represents the ratio of transport in the B-A compared to the A-B direction and indicates the net apically directed secretion of the drug. R_{GF} represents the ratio of A-B permeability measured in the presence and absence of the PGP inhibitor, GF120918 (20 μM), and represents the change in absorptive permeability when the influence of P-glycoprotein is removed. This concentration of GF120918 was found to completely and consistently ablate PGP-mediated transport in both Caco-2 monolayers and intestinal tissues (data not shown).

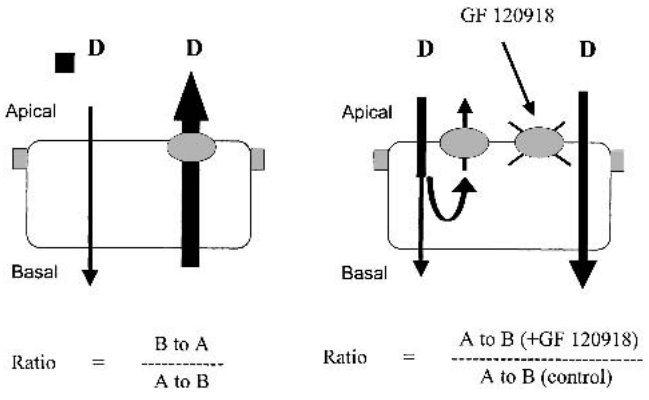

Fig. 1. Schematic representation of *in vitro* measures of PGP modulated drug permeability used in this study.

For each of the 10 drugs studied, permeability in both directions was initially analyzed over a concentration range shown in Table I and the net efflux of the drug was obtained by subtracting the A-B from the B-A flux. This was plotted against the drug concentration so that estimates of K_m and V_{max} for the active transport component could be determined. Data were obtained from four separate observations and kinetic analyses were performed by nonlinear regression using GraphPad Prism V3.00 (GraphPad Software Inc, San Diego, CA, USA). Values for $R_{B\text{-}A/A\text{-}B}$ and R_{GF} were then subsequently measured at a concentration approximately equal to 50% of the K_m value, up to a concentration of 40 μM. This concentration was also used for the compounds in which the flux did not saturate due to solubility problems. For all Caco-2 flux experiments, a sample from the donor compartment was taken at the beginning and end of each experiment so the recovery of each compound could be calculated. The recovery was greater than 95% for all drugs except the A-B flux of S 09788 (87%), topotecan (84%), and tacrolimus (93%).

Use of Literature Data on Oral Pharmacokinetics in WT and PGP-KO Mice

Information comparing oral pharmacokinetic data in wild-type and PGP-KO mice for the 10 compounds used in this study was extracted from published studies except for S 09788, which was kindly provided by Dr. Marie-Helene Brillanceau of Servier Research and Development (Fulmer Hall, UK). Table II summarizes the information gathered for each compound. The KO/WT plasma ratio indicates the ratio of plasma drug levels in the absence and presence of PGP at the indicated time point after oral administration. As indicated in Table II, data for seven of the compounds were taken from studies using mdr1a(–/–) mice. Data on the remaining three compounds were obtained using mice in which both mdr1a and mdr1b genes had been deleted [mdr1a/1b(–/–)]. Intravenous (IV) data were only available for drugs studied in mdr1a(–/–) mice. Throughout this report, both single [mdr1a(–/–)] and double [mdr1a/1b(–/–)] knockout strains of mice are defined collectively as PGP-KO except where specifically identified.

In studies without full AUC data, the most common time point quoted was 4 h and this was used where available. However, for topotecan, talinolol, and rifampicin, the 4-h time point was not given and, in these cases, plasma levels at different time points were used as indicated. If full AUC was reported, the ratio of AUC in PGP-KO and WT animals was calculated. Where data were available for both oral and IV administration, oral plasma levels were corrected for the effects of PGP on systemic clearance by dividing the KO (or WT) plasma concentration following oral administration by the KO (or WT) plasma concentration following IV administration at the same time point. The ratio of the IV corrected values for KO and WT mice represents the effects of PGP on oral bioavailability (Table II).

In all cases except verapamil, IV and oral data were obtained from the same study. The oral dose for each compound is shown except for verapamil and talinolol where none was quoted.

Table I. Experimental Data Showing the Permeability Characteristics of 10 PGP Substrates Across Caco-2 Monolayers

Drug	K_m* (μM)	V_{max}* (nmol/hr/cm^2)	Conc.† (μM)	P_{app} (cm/s)	R_{GF}	$R_{B\text{-}A/A\text{-}B}$
Verapamil	No net secretion		20	14.7 ± 3.3	1.1 ± 0.8	1.0 ± 1.1
Paclitaxel	65 ± 30 (1–70)	4.9 ± 0.1	30	2.1 ± 0.9	2.6 ± 0.5	4.1 ± 0.7
Digoxin	73 ± 17.4 (2–100)	3.4 ± 0.1	30	1.1 ± 0.3	2.2 ± 0.3	6.5 ± 0.5
Saquinavir	15.4 ± 4.3 (2–50)	1.3 ± 0.1	7.5	2.2 ± 0.4	3.2 ± 0.9	5.5 ± 1.2
Topotecan	170 ± 10 (5–300)	7.2 ± 0.3	40	1.0 ± 0.3	2.6 ± 0.5	3.5 ± 1.1
Talinolol	100 ± 7.6 (5–300)	5.5 ± 0.2	40	1.5 ± 0.4	2.5 ± 0.7	7.0 ± 1.4
Rifampicin	55 ± 10.3 (2–300)	4.3 ± 0.4	20	2.0 ± 0.5	2.5 ± 0.3	4.2 ± 1.3
UK224,671	301 ± 113 (15–300)	9.9 ± 2.1	40	0.3 ± 0.2	15.0 ± 2.2	20.2 ± 3.2
Tacrolimus	No saturation (0–40)		40	2.1 ± 0.6	4.0 ± 0.7	7.5 ± 2.2
S 09788	No saturation (1.5–50)		40	1.2 ± 0.4	2.74 ± 0.7	7.7 ± 1.7

* K_m and V_{max} are calculated from the net secretion of each drug. The concentration range (in μM) used to determine the K_m for each drug is noted in brackets beneath the K_m value.

† Concentration is that used to obtain P_{app}, R_{GF}, and $R_{B\text{-}A/A\text{-}B}$ calculated as defined in the "Materials and Methods" section.

Compound Analysis

For the Caco-2 permeability studies, the transepithelial flux of verapamil, paclitaxel, digoxin, saquinavir, and UK 224,671 was monitored by spiking the drug stock solution with approximately 0.1 μCi of ^{3}H isotope of the drug. In the case of talinolol, rifampicin, tacrolimus, topotecan, and S 09788, the flux was monitored using LC-MS analysis. Tacrolimus and S 09788 using verapamil as internal standard, talinolol and topotecan using each other as internal standard, and rifampicin using talinolol as internal standard were separated on a Luna C18(2) 50 × 4.6 mm 3μm column (Phenomenex, Macclesfield, UK) at 40°C using a quaternary gradient maintained at 1 ml/min by a Waters Alliance 2790 HT LC system (Milford, MA, USA). For talinolol and topotecan, an initial mobile phase of 90% 0.01 M ammonium acetate/10% acetonitrile was ramped linearly to 82% acetonitrile/18% 0.002 M formic acid from minutes 1 to 4 following which the initial ratio was immediately reestablished and equilibrated from minutes 4 to 5. The retention times were approximately 3.4 (talinolol) and 2.9 (topotecan) min. For rifampicin, an initial mobile phase of 90% 0.01 M ammonium acetate/10% acetonitrile was ramped linearly to 90% acetonitrile/10% 0.01M ammonium acetate from minutes 0.5 to 3 and held between minutes 3 to 4 following which the initial ratio was immediately reestablished and equilibrated from minutes 4 to 5. The retention times were approximately 3.2 (rifampicin) and 3.7 (talinolol) min. For tacrolimus, an initial mobile phase of 66% acetonitrile/33% 0.01M ammonium acetate was changed to 66% acetonitrile/17% 0.01 M ammonium acetate/17% 0.002 M formic acid at 1 min and to 90% acetonitrile/5% 0.01 M ammonium acetate/5% 0.002 M formic acid at 2 min and held between minutes 2 to 3, following which the initial ratio was immediately reestablished and equilibrated from minutes 3 to 4. The retention times were approximately 4.0 (tacrolimus) and 3.3 (verapamil) min. For S 09788, an initial mobile phase of 10% acetonitrile/90% 0.002 M formic acid was ramped linearly to 90% acetonitrile/10% 0.002 M formic acid between minutes 1 and 4 following which the initial ratio was immediately reestablished and equilibrated from minutes 4 to 5. The retention times were approximately 3.7 (S 09788) and 3.9 (verapamil).

The compounds were detected and quantified by atmospheric pressure electrospray ionization MS/MS using a Micromass Quattro Ultima triple quadropole mass spectrometer (Marche, UK). The LC column eluate was split and ¼ was delivered into the MS where the desolvation gas (nitrogen) flow rate was 600 l/h, the cone gas (nitrogen) flow rate was

Table II. Literature Data Comparing Drug Pharmacokinetics in Wild-Type and PGP-KO Mice for 10 Compounds

Drug	Time (h)	Dose* (mg/kg)	KO/WT plasma ratio	KO/WT AUC ratio	KO/WT plasma ratio (IV correction)	Ref.
Verapamil*	4	NA	1.0	NA	1.0‡	16, 17
Paclitaxel	4	10	4.4	6.4	2.2	18
Digoxin	4	0.2	2.8	2.4†	2.01	19
Saquinavir	4	5	4.1	6.5	4.1	20
Topotecan*	2	1	2.3	2.0†	NA	21
Talinolol*	1	NA	2.9	NA	NA	22
Rifampicin	24	5	3.5	NA	NA	23
UK224,671	4	2	>32	NA	>32	24
Tacrolimus	4	2	8.3	8.2	3.5	25
S 09788	4	10	3.4	2.4	3.4	

KO, knockout; WT, wild type; AUC, area under curve; IV, intravenous.

* Data taken from studies in mdr1a/1b(–/–) mice. All other data was from mdr1a(–/–) mice.

† AUC data calculated from published time vs. plasma concentration data.

‡ IV data taken from a separate study.

150 l/h, and the source temperature was 125°C. Using positive ion mode, protonated molecular ions were formed using a capillary energy of 3.5 kV and a cone energy of 51 V (topotecan), 60 V (rifampicin, verapamil), or 65 V (tacrolimus, talinolol). Product ions were monitored as ion chromatograms that were subsequently integrated and quantified by quadratic regression of standard curves.

Statistical Analysis

Experimentally derived *in vitro* data are shown as mean ± SD (n = 4 to 6). Errors associated with *in vitro* ratio values were calculated according to the rules for propagation of error relating to two variables.

RESULTS

In vitro permeability data in Caco-2 monolayers were obtained for the 10 compounds for which *in vivo* pharmacokinetic data were available in WT and PGP-KO mice. In each case, the net secretion of the drug (ie $Flux_{B-A} - Flux_{A-B}$) was determined over the range of concentrations shown in Table I to determine the approximate K_m for active transport. The drugs were subsequently used at approximately half the K_m concentration to determine the ratio of transport in the B-A and A-B directions ($R_{B-A/A-B}$) and the ratio of A-B transport in the presence or absence of the potent PGP inhibitor, GF120918 (R_{GF}) for each drug. This is illustrated in Fig. 2 for saquinavir, where a saturable, apically directed net secretion of saquinavir was observed in Caco-2 monolayers with a K_m of 15.4 μM (Fig. 2A). A concentration (7.5 μM) equivalent to approximately 50% of the K_m was used subsequently in studies to determine the experimental values for R_{GF} and $R_{B-A/A-B}$ in Caco-2 (Fig. 2B), which for saquinavir were 3.2 ± 0.9 and 5.49 ± 1.2, respectively. Similar data were derived for 10 other PGP substrates and are summarized in Table I. In all cases, apart from verapamil, which showed no net secretion, the compounds exhibited an apically directed secretion that was saturable for all compounds, except tacrolimus and S 09788, with K_m values ranging from 15.4 to 300 μM. R_{GF} represents the increase in A-B permeability when PGP function is abolished by the inhibitor GF120918. All compounds, with the exception of verapamil, exhibited R_{GF} values greater than 1 in the range 2.2 to 15, indicative of the degree to which PGP is influencing their absorption across Caco-2. Values for the more widely used measure of *in vitro* drug efflux, $R_{B-A/A-B}$, were consistently higher than R_{GF} within the range 3.5 to 20.2. $R_{B-A/A-B}$ values in the presence of GF 120918 were not significantly different from 1 (indicating ablation of active efflux) for all drugs except rifampicin that had a ratio of 1.5 and S 09788 for which it is 2.

Table II summarizes the published *in vivo* data comparing pharmacokinetics in WT and PGP-KO mice for these same group of compounds. The primary indicator of PGP effects in this model is the ratio of plasma drug concentration in PGP-KO and WT strains ($R_{KO/WT\ in\ vivo}$) following oral administration. The data to calculate the values were available 4 h postadministration for 7 of the compounds in the group and at 1, 2, and 24 h for the remaining 3 compounds. Calculated $R_{KO/WT\ in\ vivo}$ values covered a wide range from 1 for verapamil (i.e., no effect of PGP) to >32 in the case of UK224,671.

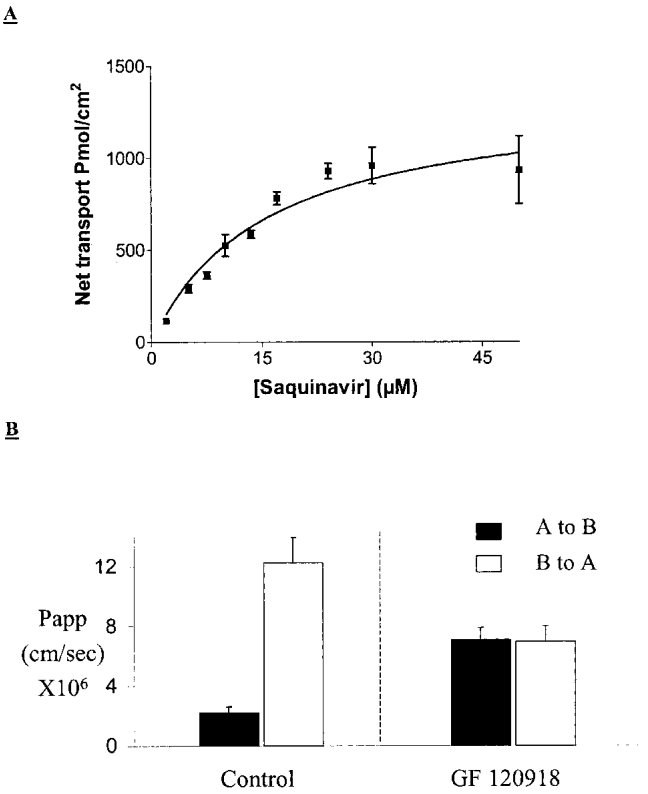

Fig. 2. Kinetic analysis of saquinavir transport across Caco-2. Figure shows, using saquinavir as an example of the *in vitro* data obtained for the 10 compounds used in this study: (A) concentration dependence of net saquinavir flux across Caco-2 monolayers. Data are mean ± SD for 4 monolayers at each concentration. (B) Bidirectional permeability of saquinavir in the presence and absence of the PGP inhibitor GF120918 (20μM). $R_{B-A/A-B}$ and R_{GF} were calculated directly from these data as described in "Materials and Methods." Data are mean ± SD for six monolayers in each group.

The availability of both *in vivo* and *in vitro* measures of transport function for this group of PGP substrates allowed the correlation between these parameters to be examined. Initial correlations excluded UK224,671, a consistent outlier in both *in vitro* and *in vivo* measures of PGP function (R_{GF} = 15.0, $R_{B-A/A-B}$ = 20.2, and $R_{KO/WT}$ = >32), which were much higher than any other compound in the group. Figure 3 shows a plot of R_{GF} in Caco-2 against $R_{KO/WT}$ in wild-type and PGP-KO mice for 9 compounds omitting UK224,671. There is a significant, positive correlation between the increase in absorption across Caco-2 when PGP is inhibited and the increase in plasma concentration for the drug in PGP-KO mice compared to wild type mice (r^2 = 0.80; $p < 0.01$). The relationship between $R_{B-A/A-B}$, the alternative measure of efflux in Caco-2 with $R_{KO/WT}$ *in vivo*, for the same group of compounds is shown in Fig. 4. Again, there is a positive correlation between these parameters, although in this case, the value of r^2 is much lower and the correlation fails to reach statistical significance (r^2 = 0.33; p = 0.10). Figure 5 shows the effect of inclusion of UK224,671 on the correlation between R_{GF} in Caco-2 and $R_{KO/WT}$ in mice. Inclusion of UK224,671 improves the correlation between R_{GF} and $R_{KO/WT}$ (r^2 = 0.991; $p < 0.001$) and between $R_{B-A/A-B}$ and $R_{KO/WT}$ (r^2 = 0.889; $p < 0.001$) (graph not shown).

Data on plasma levels following IV administration in

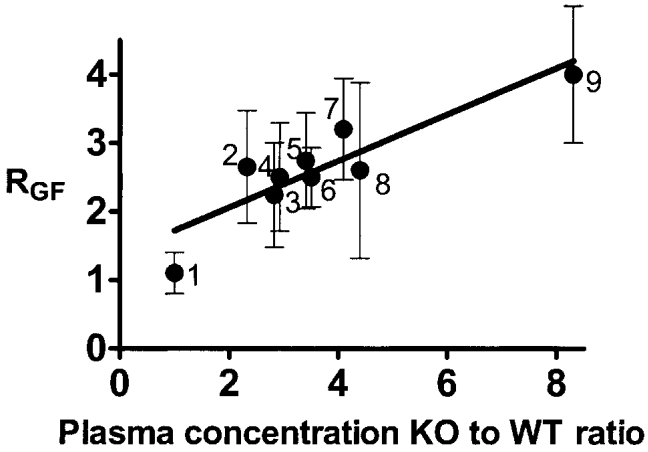

Fig. 3. Relationship between the ratio of plasma concentration after oral administration in PGP-KO and wild-type mice and R_{GF} in Caco-2 using data taken from Tables II and I, respectively. Data points are 1. verapamil, 2. topotecan, 3. digoxin, 4. talinolol, 5. S 09788, 6. rifampicin, 7. saquinavir, 8. paclitaxel, 9. tacrolimus. R_{GF} is shown as mean ratio ± SD calculated according to the law of propagation of errors. Plasma concentration ratios shown are mean literature data. Correlation line analysis: $r^2 = 0.80$; $p < 0.01$.

wild-type and PGP-KO mice were available for six of the compounds (verapamil, paclitaxel, digoxin, saquinavir, tacrolimus, and S 09788) used in Figs. 3 and 4. Using this information, it was possible to correct oral plasma levels for PGP-mediated effects on drug clearance in mice. Figure 6 replots the R_{GF} vs $R_{KO/WT}$ data for these compounds after correction of the $R_{KO/WT}$ *in vivo* data for PGP-mediated clearance measured 4 h after IV administration. This plot, which represents the relationship between *in vitro* permeability and oral bioavailability in the presence and absence of PGP, shows a significant positive correlation between these parameters ($r^2 = 0.79$; $p < 0.05$).

DISCUSSION

One of the major problems in developing *in vitro-in vivo* correlations of PGP function is the paucity of *in vivo* data

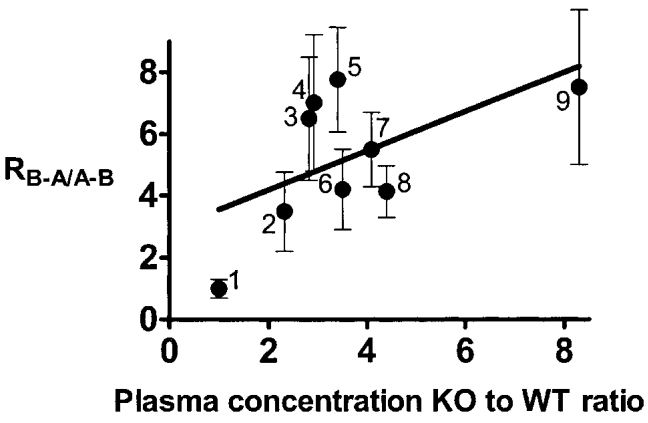

Fig. 4. Relationship between the ratio of plasma concentration after oral administration in PGP-KO and wild-type mice and $R_{B\text{-}A/A\text{-}B}$ in Caco-2 using data taken from Tables II and I, respectively. Data points are as shown in Fig. 3. $R_{B\text{-}A/A\text{-}B}$ is shown as mean ratio ± SD. Correlation line analysis: $r^2 = 0.33$; $p = 0.106$.

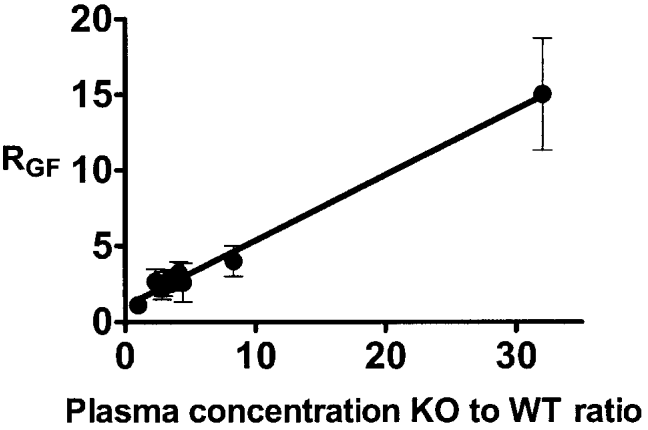

Fig. 5. Relationship between the ratio of plasma concentration after oral administration in PGP-KO and wild-type mice and R_{GF} in Caco-2 as shown in Fig. 3 but with the inclusion of UK224,671. Correlation line analysis: $r^2 = 0.991$; $p < 0.001$.

showing an unequivocal effect of the transporter on oral pharmacokinetics. This is because interaction with PGP is just one of many factors that will influence the efficiency of absorption—others include drug solubility, membrane permeability, metabolism and other transporters (26)—and it is often difficult to separate these experimentally, particularly in view of the overlap in substrates for PGP and CYP3A4 (27). To address this, the current study has examined the relationship between two different markers of *in vitro* PGP function in Caco-2 cells and oral pharmacokinetic data in PGP-KO and wild-type mice, taken from literature studies. Mice lacking PGP have been used extensively to define the role of PGP in modulating oral absorption without recourse to transport inhibitors that suffer from problems of specificity and efficacy, particularly *in vivo* (12). Intestinal tissues isolated from PGP-KO mice have also been shown to be a useful *in vitro* tool for resolving the roles of PGP and non-PGP transporters on drug permeability in different regions of the gut (13,28).

The approach used here is similar to that adopted by

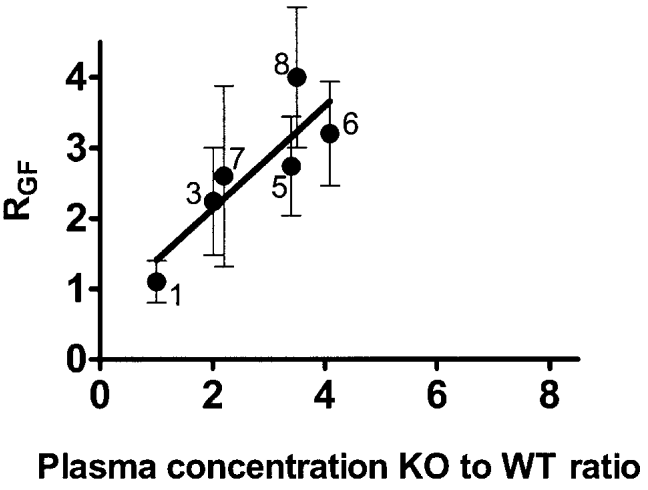

Fig. 6. Correlation of R_{GF} in Caco-2 with oral plasma concentration in PGP-KO and WT mice after IV correction for effects of PGP on drug clearance. Data points are 1. verapamil, 3. digoxin, 5. S 09788, 6. saquinavir, 7. paclitaxel, 8. tacrolimus. Correlation line analysis: $r^2 = 0.79$; $p < 0.05$.

Adachi *et al.* (14) to correlate transport function in human mdr1 expressing LLC-PK1 cells with the ability of PGP to limit penetration of drugs across the blood-brain barrier (BBB) in mice. This study showed that the ratio of drug concentration in the brain of mdr1a/1b knockout and wild-type mice correlated well with the transport ratio ($R_{B\text{-}A/A\text{-}B}$) across transfected and parental LLC-PK1 monolayers. It would be expected that the role of PGP in modulating brain penetration of drugs would be simpler to model than its effects on oral plasma levels because of the relative complexities of the processes involved in each case. PGP modulation of brain penetration is dependent on the expression of the transporter at a single barrier, the BBB. In contrast, plasma concentrations of a drug after oral administration will be influenced by a combination of factors; absorption across the intestine, hepatic and biliary clearance, and distribution of the drug into tissues such as the brain. All these factors may be affected by PGP expressed at the respective tissues involved. Factors such as variable expression of PGP along the intestine (28,29) and the possibility of differences in the kinetics of drug-PGP interaction at different sites add to the potential complexity. It is interesting, therefore, that for the group of 10 drugs used in this study, PGP-mediated changes in drug permeability across a simple "one-barrier" model, Caco-2 appears to give a good estimate of the likely effect of PGP on the plasma level of the drug after oral administration in the mouse. The ability of Caco-2 to model a multicomponent system in which the combined effects of PGP-mediated transport at several different barriers (e.g., intestine, liver) influence the plasma concentration of the drug suggests that the basic characteristics of transport at each site are similar. However, because most of the *in vivo* studies were performed in mdr1a(–/–) mice (see Table II), we are not able to account for possible effects of the mdr1b gene, which is co-expressed with mdr1a in rodents at nonintestinal sites. However, evidence suggests that mdr1a is responsible for the majority of PGP mediated drug transport effects with mdr1b playing a relatively minor role (8,11).

In addition, though drug metabolism is a potential confounding factor, it is unlikely to have a major influence on the correlations described here. Caco-2 have very low levels of metabolic enzymes while the data from the animal studies is based on analysis of the bioactive, parent compound, in all cases except for digoxin and saquinavir in which radioisotopes were used. In this study, we have examined the effect of PGP on the plasma concentration of drugs after oral administration by comparing PGP KO to WT animals so the effect of metabolism will have little effect on this analysis. However, there is one report that identified differences in CYP3A4 activity in WT and PGP-KO mice under some conditions but this was not a consistent finding (30).

The availability of both IV and oral data from mdr1a(–/–) and WT mice provides a basis for separating the effects of this transporter on oral absorption from its effects on systemic clearance and distribution. Despite the low number of compounds for which these data were available, it was possible to show a significant correlation between R_{GF} and $R_{KO/WT\ in\ vivo}$ where the *in vivo* ratio had been corrected with IV data. Significant PGP effects on clearance leading to a reduction in the $R_{KO/WT\ in\ vivo}$ were only observed for three of the compounds (paclitaxel, saquinavir, tacrolimus) and this had little or no effect on the correlation coefficient ($r^2 = 0.79$ vs. 0.80 without IV correction). The fact that the correlation was similar, both with and without IV correction, reinforces the hypothesis that Caco-2 may be a valid model of PGP-mediated transport for multiple tissues. These data also implies that increased clearance caused by PGP may be a relatively minor component of the *in vivo* PGP effect in most cases. However, there is a second proviso, the mouse studies analyzed here have followed the accepted protocol of administering a similar concentration of drug in both oral and IV studies. The higher plasma concentrations achieved after IV administration [e.g., 100-fold for paclitaxel (17)] could result in a greater concentration of drug in the liver compared to oral administration, particularly for compounds with low to medium intestinal permeability. It is possible that these concentrations could saturate the transporter and so produce an underestimation of the effects of PGP on drug clearance and disposition after IV administration.

The present study suggests that R_{GF} is a more reliable *in vitro* predictor of the *in vivo* effects of PGP than the more commonly used $R_{B\text{-}A/A\text{-}B}$, particularly when UK224,671 was excluded from the correlation. In terms of its A-B permeability, R_{GF}, $R_{B\text{-}A/A\text{-}B}$ values and saturability, UK224,671 is very atypical of the rest of the compounds in this study and tends to skew both R_{GF} and $R_{B\text{-}A/A\text{-}B}$ data in favor of positive correlation. UK224,671 was a development compound which was not progressed due to anomalous absorption in man (24) and literature data on these type of compounds in PGP-KO mouse are extremely rare. A greater availability of such data would add much to our understanding of the *in vivo* effects of PGP.

R_{GF}, which estimates the change in unidirectional absorptive flux when PGP function is ablated by GF120918, may approximate more closely to the physiological change in membrane permeability caused by PGP than a measure of bi-directional permeability such as $R_{B\text{-}A/A\text{-}B}$. A second factor is that the $R_{B\text{-}A/A\text{-}B}$ value can be difficult to assess accurately particularly in circumstances where the A-B flux of a PGP substrate is low, leading to problems of analytical sensitivity and poor recovery. Under these circumstances, inaccuracies in the measurement of low A-B permeability may result in a significant overestimation of the $R_{B\text{-}A/A\text{-}B}$ value but would be expected to have a much smaller impact on R_{GF}. A recent study has investigated the relative merits of bi-directional efflux ratio and absorptive flux in the presence and absence of a PGP inhibitor as indicators of PGP effects on absorption across cell monolayers (31). The authors conclude that efflux ratio should be avoided when assessing the effects of PGP on absorption; data presented here lends support to this idea. GF120918 has been used as a reasonably specific inhibitor of PGP to generate R_{GF} values but is reported to also inhibit breast cancer resistance protein (BCRP) (21). We cannot, therefore, exclude a possible contribution from BCRP in the transport of some substrates in Caco-2 (i.e., topotecan), although it is unclear whether this transporter is functionally expressed in Caco-2 cells.

It is interesting that a good *in vitro-in vivo* correlation was maintained despite the considerable variation in oral dose administered in the *in vivo* studies (Table I). However, the fact that the dose used produced significant differences in plasma concentrations in PGP KO compared to FVB for each drug, excepting verapamil, suggests that the concentrations in the intestinal lumen were below that required to saturate

PGP function. In the *in vitro* studies, the drug concentrations were set at less than the K_m value to ensure that PGP would not be saturated.

A recent study by Adachi and co-workers has addressed the relationship between PGP-drug interaction in LLCPK1 cells transfected with human MDR1 and intestinal permeability using *in situ* perfusion in mice (32). This study showed a positive correlation between PGP-mediated alterations in drug permeability across cell monolayers and the ratio of compound absorbed across perfused jejunal segments from double-knockout, mdr1a/1b(–/–) mice. This demonstrates that the ability of PGP to alter drug permeability in simple cell monolayers is related quantitatively to its effects on permeability across the intestine. The results of the current study suggest that cell models may also be able to predict the extent to which P-glycoprotein will modify drug plasma levels after oral administration *in vivo.*

In conclusion, the current study, albeit using a relatively limited data set, has established a quantitative relationship between PGP-induced changes in absorption across Caco-2 and PGP-induced decreases in plasma concentrations of orally administered drugs in mice. This correlation was maintained when the oral pharmacokinetic data were corrected for PGP effects on systemic drug clearance suggesting that Caco-2 mimics PGP-induced changes in drug permeability not only in the intestine but also at other sites. The relevance of this finding to PGP function in man remains to be determined, although there appears to be a close similarity between substrate recognition and transport efficacy for PGP between the two species (14). Establishing quantitative relationships between *in vitro* and *in vivo* indicators of PGP function will be useful in developing models that can predict the likely *in vivo* impact of drug-PGP interactions identified by reductive *in vitro* screens.

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