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**Author post-print (accepted) deposited in CURVE September 2011**

## **Original citation & hyperlink:**

Janneh, O. , Anwar, T. , Jungbauer, C. , Kopp, S. , Khoo, S.H. , Back, D.J. and Chiba, P. (2009) P-glycoprotein, multidrug resistance-associated proteins and human organic anion transporting polypeptide influence the intracellular accumulation of atazanavir. *Antiviral Therapy*, volume 14 (7): 965-974.

<http://dx.doi.org/10.3851/IMP1399>

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## Original article

# P-glycoprotein, multidrug resistance-associated proteins and human organic anion transporting polypeptide influence the intracellular accumulation of atazanavir

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**Background:** Drug efflux (for example, P-glycoprotein [P-gp], multidrug resistance-associated proteins [MRPs] and breast cancer resistance protein [BCRP]) and influx (for example, human organic anion transporting polypeptide [hOCTP] or human organic anion transporting polypeptide [hOATP]) transporters alter the cellular concentrations of some HIV protease inhibitors (HPIs). Here, we studied the lipophilicity and uptake of [<sup>3</sup>H]-atazanavir (ATV) in CEM (parental), CEM<sub>VBL</sub> (P-gp-overexpressing), CEM<sub>E1000</sub> (MRP1-overexpressing) and peripheral blood mononuclear cells (PBMCs), and evaluate the effects of modulators of drug transporters on uptake.

**Methods:** Lipophilicity was measured by octanol/saline partition method. The influence of influx/efflux transporters on uptake was evaluated in the absence and presence of inhibitors of P-gp (GPV031), P-gp/BCRP (tariquidar and GF120918), P-gp/MRP1 (dipyridamole and daidzein), MRP1/2 (frusemide and genistein), hOATP/hOCTP (estrone-3-sulfate [E-3-S]) and hOATP/hOCTP/MRP (probenecid). The effects of a number of HPIs on

uptake were also evaluated. Data from digitonin permeabilized cells allowed the evaluation of the contribution of cellular binding to total drug uptake, whereas the inhibitory effect of ATV on P-gp was assessed by daunomycin efflux/uptake assays.

**Results:** [<sup>3</sup>H]-ATV is lipophilic and accumulates in the cultured cells as follows: CEM > CEM<sub>E1000</sub> > CEM<sub>VBL</sub>. Tariquidar, GF120918 and daidzein significantly increased the uptake of [<sup>3</sup>H]-ATV in the cultured cells. By contrast, only daidzein and tipranavir significantly increased uptake in PBMCs, with tariquidar and frusemide devoid of effects, whereas dipyridamole, E-3-S, GPV031 and genistein significantly decreased accumulation. ATV inhibits P-gp activity; manipulation of uptake with digitonin suggests binding of [<sup>3</sup>H]-ATV to P-gp.

**Conclusions:** [<sup>3</sup>H]-ATV is lipophilic, a P-gp, MRP and hOATP substrate and an inhibitor of P-gp. Concomitant administration of ATV with drugs and dietary components (for example, daidzein and genistein) that interact with these transporters could alter its pharmacokinetics.

## Introduction

HIV protease inhibitors (HPIs) in combination with other antiretrovirals (for example, reverse transcriptase inhibitors) play a pivotal role in the management of HIV-infected patients. The therapeutic effects of these drugs is dependent on their cellular accumulation, which is in part affected by the expression of ATP binding cassette family of drug efflux transporters, such as P-glycoprotein (P-gp), multidrug resistance-associated

proteins (MRPs) and breast cancer resistance protein (BCRP) [1–3]. In addition, there are some known anionic and cationic transporters, such as human organic anion transporting polypeptide (hOATP) and human organic cation transporters (hOCTs), respectively, which mediate the influx/elimination of many clinically important drugs and xenobiotics, and these have been shown to limit the accumulation of some HPIs [2,4–6].

All these transporters are differentially expressed in the liver, kidney, circulating lymphocytes, brain, testes and intestines – sites important in drug disposition, or which might act as sanctuary for the replicating virus and/or support viral replication. Clearly, low intracellular/plasma drug concentration or altered distribution within these sites could affect clinical outcome.

The transporters work in concert to control the bioavailability of clinically important drugs and endogenous compounds. Thus, together with the cytochrome P450 enzymes, these transporters are important determinants of drug–drug interactions, drug efficacy and toxicity [7]. Indeed, previous studies have shown the oral availability, brain, and fetal penetration of saquinavir (SQV) to be limited by P-gp [8,9]. Similarly, the intracellular accumulations of a number of HPIs have been shown to be limited by P-gp, MRP1, MRP2 and BCRP [1,2,8–19]. There is ample evidence that some HPIs also induce and inhibit the expression of drug efflux transporters, such as P-gp, MRP1/2 and BCRP [12,16,20–25]. However, the role of BCRP in the efflux of HPIs is equivocal [1,3,24–27]; there is evidence that BCRP induces resistance to nucleoside reverse transcriptase inhibitors [27].

We have shown that the intracellular concentrations of HPIs are amenable to manipulation by coadministration with inhibitors of drug efflux transporters [1,2,13]. A potential use of such agents would be to enhance intestinal drug absorption and increase drug penetration to biologically important protective barriers, such as the blood–brain, blood–cerebrospinal fluid, maternal–fetal barriers, testes, and within circulating lymphocytes that might be impenetrable to these drugs. To this end, because ritonavir-mediated inhibition of cytochrome P450 (CYP3A4/CYP2D6) and possibly P-gp has been shown to increase exposure to coadministered HPIs, ritonavir (RTV)-boosted HPIs are a standard component of antiretroviral regimen [28]. Interestingly, we and others have recently shown that other HPIs, such as atazanavir (ATV), can pharmacoenhance the intracellular accumulation and area under the curve (AUC) of coadministered HPIs [2,29]. Published evidence to date suggests that ATV might be a substrate, or induces or inhibits P-gp, MRP and BCRP activities [2,3,23,25,30,31], but does not alter lymphocyte expression of P-gp, and MRP1 [3,31]. The current study aims to characterize the determinants of the cellular transport and sequestration of [<sup>3</sup>H]-ATV in cultured CD4<sup>+</sup> T-cells and in primary human lymphocytes – an important site to study the transport and intracellular concentration of antiretrovirals. Experiments were designed to understand the lipophilicity and sequestration of [<sup>3</sup>H]-ATV within cells and the efflux and influx pumps relevant for the vectorial transport and retention of the drug.

## Methods

### Reagents

[<sup>3</sup>H]-ATV and [<sup>14</sup>C]-mannitol (specific activities 3.1 Ci/mmol and 50 mCi/mmol, respectively) were purchased from Moravек Biochemicals and Radiochemicals (Brea, CA, USA). RTV and lopinavir (LPV) were obtained from Abbott Laboratories (North Chicago, IL, USA); nelfinavir (NFV), indinavir (IDV), SQV, amprenavir (APV), ATV and tipranavir (TPV) were obtained from Agouron Pharmaceuticals (San Diego, CA, USA), Merck (Rahway, NJ, USA), Roche (Welwyn Garden City, UK), Glaxo Wellcome (Middlesex, UK), Bristol–Myers Squibb (Hounslow, UK) and Boehringer Ingelheim (Berkshire, UK), respectively. Tariquidar was donated by Xenova Group Plc (Berkshire, UK). The peripheral blood mononuclear cells (PBMCs) were isolated from blood buffy coats, which were obtained from the Regional Blood Transfusion Centre (Liverpool, UK). CEM (parental), CEM<sub>VBL</sub> (VBL) and CEM<sub>E1000</sub> (E1000) cell lines were from R Davey (Bill Walsh Cancer Research Laboratories, Royal North Shore Hospital, Sydney, Australia). Lymphoprep was purchased from Axis Shields (Oslo, Norway). GPV031 was a gift from G Ecker (Department of Medicinal Chemistry, University of Vienna, Vienna, Austria). All other chemicals were supplied by Sigma Chemical Co. (Poole, UK).

### Cell culture

The cultured cells used in this study were CEM (a CD4<sup>+</sup> T-cell line), VBL (P-gp overexpressing; selected using vinblastine) and E1000 (MRP-1 overexpressing; selected using epirubicin). The expression of these transporters has been previously validated in our laboratory [13]. The cells were maintained at 37°C, 5% CO<sub>2</sub> in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS).

### Isolation of PBMCs

PBMCs were isolated from blood buffy coats using Lymphoprep according to the manufacturer's instructions.

### Octanol/saline partition coefficient

As the cellular association of a drug within target cells is a composite of active influx/efflux, passive diffusion and ion trapping, the lipophilicity of [<sup>3</sup>H]-ATV was measured as described previously [6] to establish if simple physicochemical characteristics were major determinants of intracellular accumulation.

### Transport of [<sup>3</sup>H]-ATV and the effects of specific inhibitors on transport in CEM and CEM<sub>VBL</sub> cells

Initial experiments examined the optimal concentrations of inhibitors for maximal inhibitory effects on Pgp, MRP and hOATP activity. Cells (CEM and its variant

cells) were incubated in the absence or presence of varying concentrations of XR9576 (0.01–1  $\mu\text{M}$ ; P-gp/BCRP inhibitor [32–34]), MK571, dipyridamole (1–100  $\mu\text{M}$ ; P-gp/MRP inhibitor [35,36]), frusemide (1–100  $\mu\text{M}$ ; MRP1/2 inhibitor [1], GF120918 (1–100  $\mu\text{M}$ ; P-gp/BCRP inhibitor [13,37]), GPV031 (1–100  $\mu\text{M}$ ; P-gp inhibitor [38]), probenecid (1–100  $\mu\text{M}$ ; MRP/hOATP [14,39] and E-3-S (1–100  $\mu\text{M}$ ; hOATP/hOCTP inhibitor [6,40]) as described [1].

In subsequent experiments, the transport and accumulation of [ $^3\text{H}$ ]-ATV (20 nM) was measured by incubating the cells ( $2.5 \times 10^6$  cells/ml) in the absence and presence of fixed concentrations of inhibitors: tariquidar (1  $\mu\text{M}$ ), frusemide (50  $\mu\text{M}$ ), dipyridamole (50  $\mu\text{M}$ ), E-3-S (30 and 100  $\mu\text{M}$ ), probenecid (30 and 100  $\mu\text{M}$ ), GPV031 (30 and 100  $\mu\text{M}$ ) and GF120918 (50  $\mu\text{M}$ ). We also investigated the interaction of soy-derived phytoestrogens daidzein and genistein (at 1 and 5 mM) known to interact with P-gp, MRP, BCRP and hOATP [41–47] on the cellular accumulation ratio (CAR) of [ $^3\text{H}$ ]-ATV. The interaction of the HPis, APV, IDV, LPV, NFV, RTV, SQV and TPV (all at 10 and 30  $\mu\text{M}$ ) on the transport and accumulation of [ $^3\text{H}$ ]-ATV was also investigated in CEM and its variant cells. All experiments were carried out in RPMI-1640 media supplemented with 10% FBS as described previously [1]. The amount of drug associated with the cells was quantified by taking a ratio of the amount of drug in the cell pellets to the amount of drug in a similar volume of the bathing media after incubation, taking cell volume (1 pl) into consideration. This ratio was defined as the CAR.

#### Accumulation of [ $^3\text{H}$ ]-ATV in isolated PBMCs and the effects of modulators of accumulation

The assay was carried out essentially as described above. Briefly, isolated PBMCs ( $5 \times 10^6$  cells/ml; cell volume 0.4 pl) were incubated in the absence or presence of fixed concentrations of the inhibitors: tariquidar (1  $\mu\text{M}$ ), GF120918 (50  $\mu\text{M}$ ), frusemide (50  $\mu\text{M}$ ), dipyridamole (50  $\mu\text{M}$ ), GPV031 (30 and 100  $\mu\text{M}$ ), E-3-S (30 and 100  $\mu\text{M}$ ), and APV, IDV, LPV, NFV, RTV, SQV and TPV (all at 10 and 30  $\mu\text{M}$ ). In addition, we investigated the interaction of daidzein and genistein (1 and 5 mM, respectively) on the CAR of [ $^3\text{H}$ ]-ATV.

#### Effects of digitonin permeabilization on the transport of [ $^3\text{H}$ ]-ATV

In order to characterize the energy dependence and accumulation of [ $^3\text{H}$ ]-ATV, drug transport assays were carried out in cells pretreated without or with 20  $\mu\text{M}$  digitonin as described previously [48] followed by steady-state drug accumulation assay. Cells were incubated with 20  $\mu\text{M}$  digitonin prior to the addition of [ $^3\text{H}$ ]-ATV (20 nM), after which cells were incubated for a further 15 min. In other experiments, the cells were

permeabilized by further incubating the incubation mixture with 20  $\mu\text{M}$  digitonin for 5 min once steady-state was reached (15 min).

#### Effects of ATV on daunomycin accumulation and efflux in cultured cells

Daunomycin is a substrate of P-gp and its accumulation and efflux were performed as described previously [38]. Concentration–response curves were fitted to the data points using non-linear least-squares and 50% effective concentration values were calculated as described previously [38].

#### Statistical analyses

Data are expressed as mean  $\pm$ SD and the distribution of data was assessed using the Shapiro–Wilk test. Statistically significant differences between controls and drug-treated samples were further assessed using the Mann–Whitney U test of the StatsDirect statistical software (version 2.6.3; Cheshire, UK). Differences between means were considered to be significant for  $P < 0.05$ .

## Results

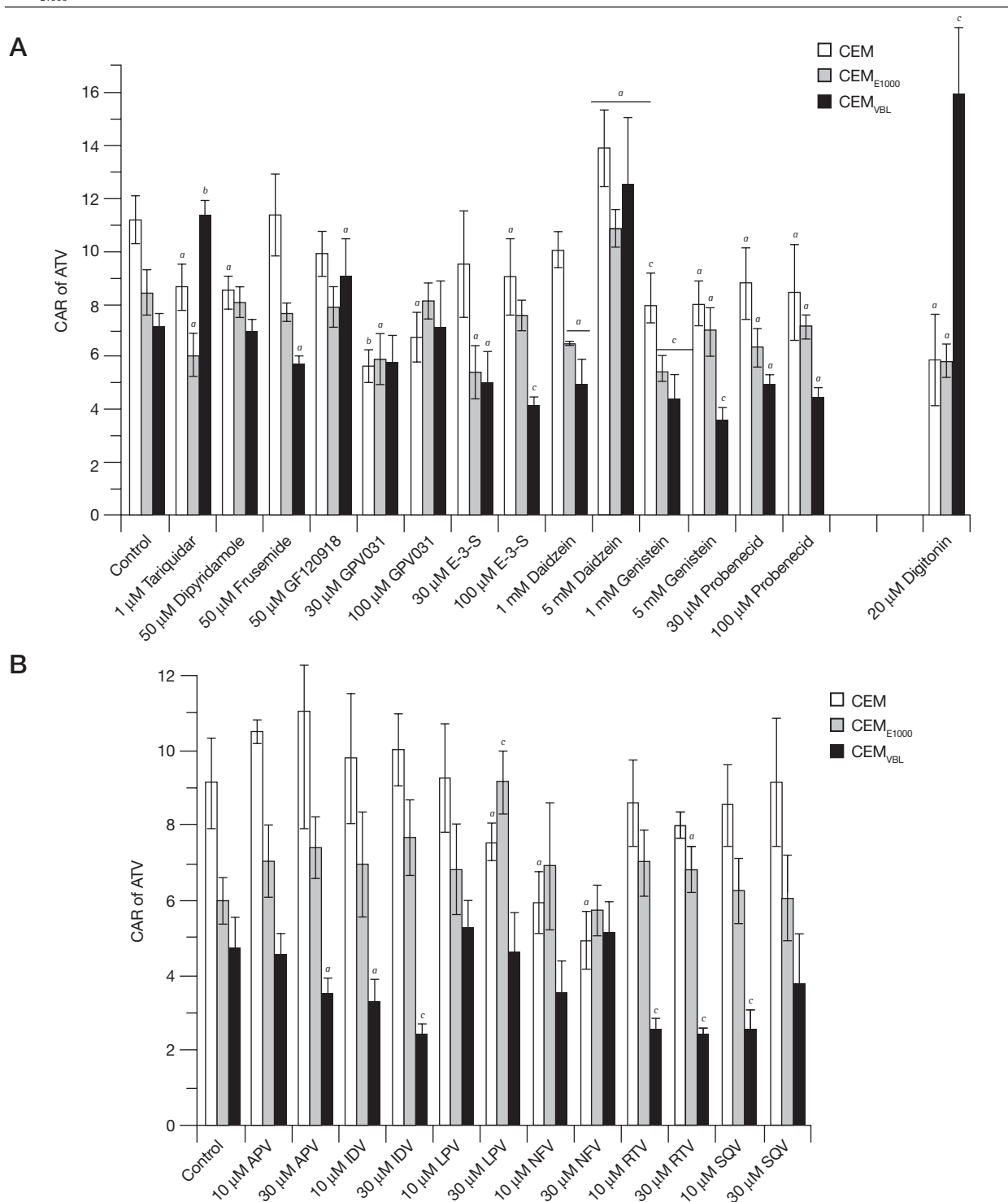
#### Octanol/saline partition coefficient

The octanol/saline partition coefficient for [ $^{14}\text{C}$ ]-mannitol (used as control) and [ $^3\text{H}$ ]-ATV were  $0.002 \pm 0.001$  and  $29.90 \pm 4.12$ , respectively.

#### Transport of [ $^3\text{H}$ ]-ATV and the effects of inhibitors of drug efflux/influx transporters and digitonin on accumulation

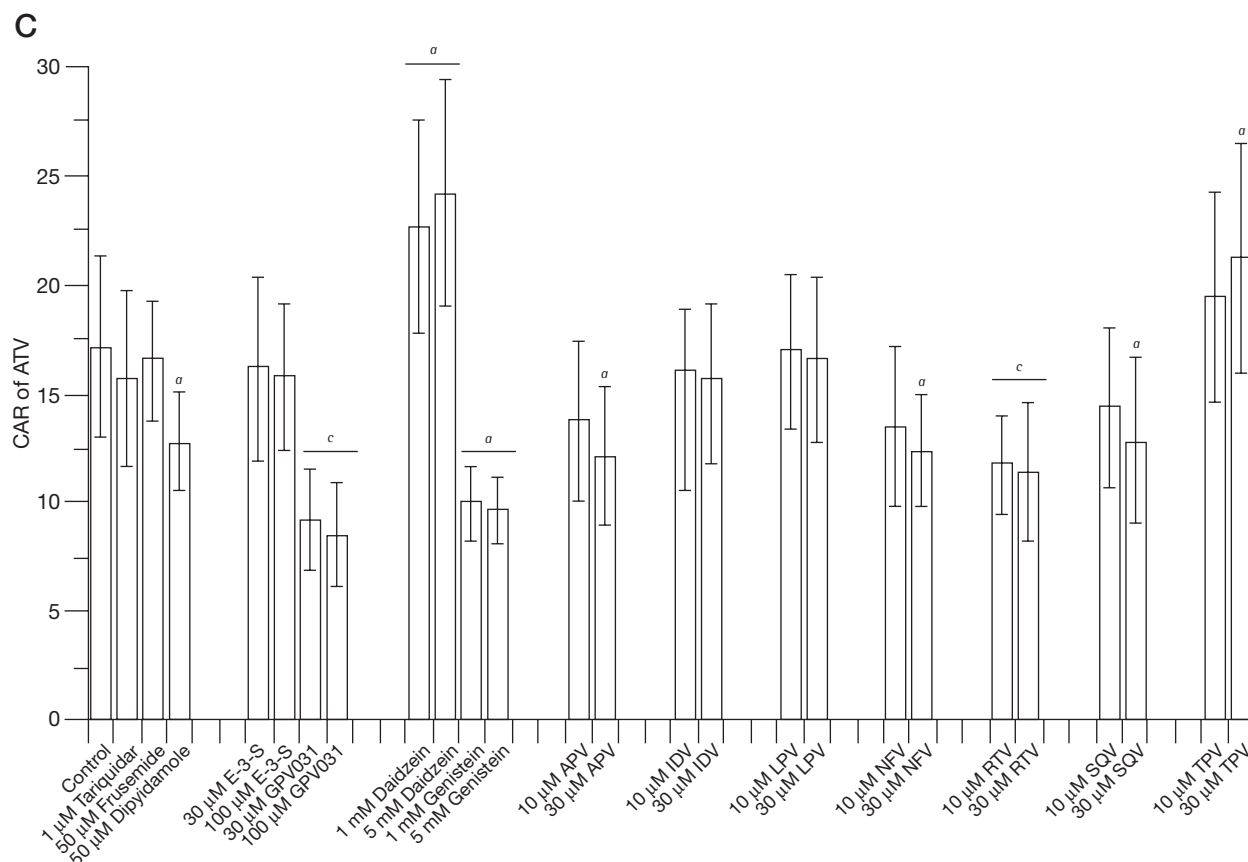
As can be seen in Figure 1A, we observed differential accumulation of [ $^3\text{H}$ ]-ATV in the CEM, E1000 and VBL cells with the baseline CAR of CEM, E1000 and VBL cells being  $11.2 \pm 0.9$ ,  $8.4 \pm 0.9$  and  $7.0 \pm 0.6$ , respectively. Tariquidar, dipyridamole and GPV031 (at 30  $\mu\text{M}$ ) significantly decreased ( $P \leq 0.05$ ) the CAR of ATV in the CEM cells. Similarly, tariquidar and GPV031 (at 30  $\mu\text{M}$ ), and daidzein (at 1 mM), significantly ( $P \leq 0.05$ ) decreased the CAR of [ $^3\text{H}$ ]-ATV in the E1000 cells. Genistein significantly ( $P \leq 0.05$ ) decreased the CAR of [ $^3\text{H}$ ]-ATV in the CEM and its variant cells. Tariquidar and GF120918 significantly ( $P \leq 0.05$ ) increased the CAR of [ $^3\text{H}$ ]-ATV in the VBL cells, and daidzein (at 5 mM) increased the CAR of [ $^3\text{H}$ ]-ATV in CEM and its variant cells ( $P < 0.05$ ). Frusemide, daidzein (at 1 mM) and genistein significantly ( $P \leq 0.01$ ) decreased the CAR of [ $^3\text{H}$ ]-ATV in the VBL cells. E-3-S (at 30  $\mu\text{M}$ ) reduced the CAR of [ $^3\text{H}$ ]-ATV in E1000 and VBL cells ( $P < 0.05$ ), with 100  $\mu\text{M}$  E-3-S causing a significant ( $P \leq 0.01$ ) decrease in the CAR of [ $^3\text{H}$ ]-ATV in CEM and VBL cells. To characterize the contribution of specific cellular binding to total the accumulation of ATV, we investigated the effects of digitonin permeabilization on

**Figure 1.** Effects of modulators of drug transporters, digitonin and HPIs on the accumulation of [<sup>3</sup>H]-atazanavir in CEM, CEM<sub>E1000</sub>, CEM<sub>VBL</sub> and PBMCs



Effects of (A) modulators of drug transporters (tariquidar, dipyridamole, frusemide, GF120918, GPV031, daidzein, genistein, estrone-3-sulphate [E-3-S] and probenecid), digitonin and (B) HIV protease inhibitors (HPIs; amprenavir [APV], indinavir [IDV], lopinavir [LPV], nelfinavir [NFV], ritonavir [RTV] and saquinavir [SQV]) on the accumulation of [<sup>3</sup>H]-ATV in CEM, CEM<sub>E1000</sub> and CEM<sub>VBL</sub> cells. (C) Modulators of drug transporters (tariquidar, frusemide, dipyridamole, E-3-S, GPV031, daidzein and genistein) and HPIs (APV, IDV, LPV, NFV, SQV and tipranavir [TPV]) on the accumulation of [<sup>3</sup>H]-ATV in isolated peripheral blood mononuclear cells (PBMCs) from blood buffy coats. Bars indicate mean  $\pm$  SD ( $n=4$ , with 4 independent observations from cultured CEM and its variant cells and  $n=6$ , with 4 independent observations from each buffy coat PBMC sample). Results are expressed as cellular accumulation ratio (CAR), which is the ratio of the amount of [<sup>3</sup>H]-ATV associated with the cell pellets to the amount in a similar volume of media after incubation.  $P$ -values of  $^aP<0.05$ ,  $^bP<0.001$  and  $^cP<0.01$  indicate statistically significant differences (increases/decreases) in the CAR of [<sup>3</sup>H]-ATV as a result of coincubation with the compounds compared with control. Data were analysed by the Shapiro-Wilk test followed by the Kruskal-Wallis test.

Figure 1. Continued



accumulation. Here, cells were either initially permeabilized with 20 μM digitonin prior to the accumulation studies or subsequent to the accumulation studies. We observed that permeabilization of CEM and E1000 cells with digitonin significantly ( $P < 0.05$ ) reduced the accumulation of [ $^3\text{H}$ ]-ATV in these cells by 47% and 30%, respectively. Unexpectedly, permeabilization of VBL cells with digitonin caused a significant ( $P < 0.01$ ) increase in accumulation (from  $7.0 \pm 0.6$  to  $15.9 \pm 2.6$ ; Figure 1A). Identical results were obtained in experiments in which the effect of digitonin was assessed subsequent to the accumulation studies (data not shown).

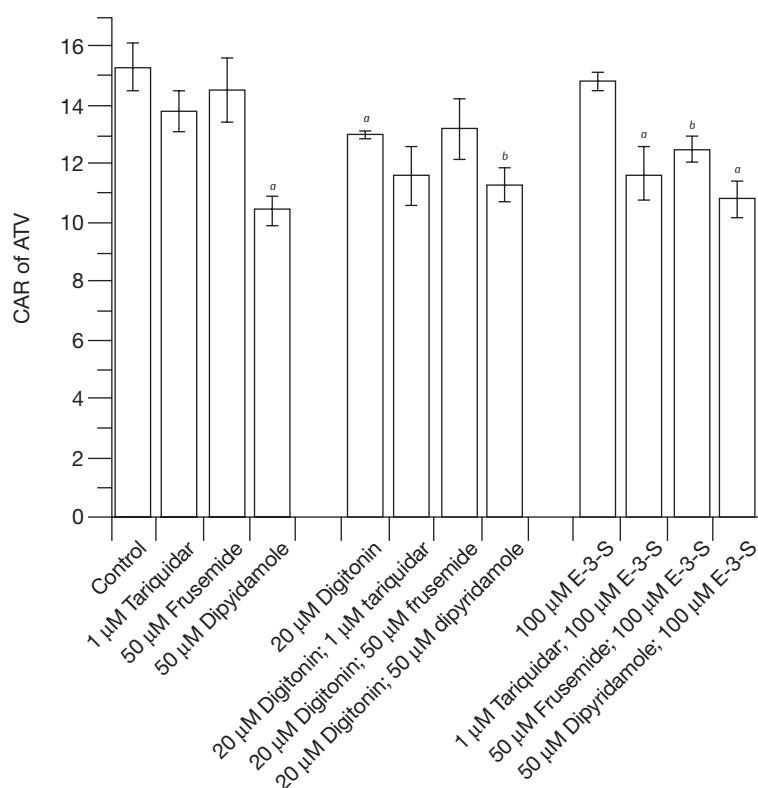
Effects of various HPIs on the accumulation of [ $^3\text{H}$ ]-ATV RTV is used to boost the plasma concentration of a number of HPIs. Here, we investigated whether RTV and other HPIs could modulate the transport and accumulation of [ $^3\text{H}$ ]-ATV in the CEM and its variant cells. Of the HPIs tested, only LPV (at 30 μM) significantly ( $P < 0.01$ ) increased the association of [ $^3\text{H}$ ]-ATV to the E1000 cells. We observed differential accumulation of [ $^3\text{H}$ ]-ATV in CEM, E1000 and VBL cells, with the following rank order: CEM > E1000 > VBL. LPV and NFV

(at 30 and 10 μM, respectively) significantly ( $P < 0.05$ ) decreased the accumulation of [ $^3\text{H}$ ]-ATV in the CEM cells. By contrast, LPV and RTV (at 30 μM) increased the accumulation of [ $^3\text{H}$ ]-ATV in the E1000 cells. In the VBL cells, the accumulation of [ $^3\text{H}$ ]-ATV was significantly ( $P < 0.01$ ) reduced by APV, IDV and RTV (at 30 μM). The uricosuric agent, probenecid (at 100 μM) significantly ( $P < 0.05$ ) increased the accumulation of [ $^3\text{H}$ ]-ATV in the E1000 cells, but had no effects on accumulation in CEM and VBL cells (Figure 1B).

Transport of [ $^3\text{H}$ ]-ATV in PBMCs and the effects of drug efflux inhibitors and HPIs on accumulation

We observed variable accumulation of [ $^3\text{H}$ ]-ATV in the PBMCs ranging from  $13.6 \pm 1.5$  to  $24.9 \pm 1.9$ . We previously showed that PBMCs express P-gp, MRP, BCRP and hOATP [1,6]. Dipyridamole, E-3-S and GPV031 caused a modest decrease in accumulation (Figure 1C). Interestingly, daidzein significantly increased ( $P < 0.05$ ), whereas genistein caused a significant ( $P < 0.01$ ) decrease in the CAR of [ $^3\text{H}$ ]-ATV. Unexpectedly, of all the HPIs tested, only TPV (at 30 μM) caused a modest ( $P < 0.05$ ) increase in the CAR of [ $^3\text{H}$ ]-ATV. APV, NFV, RTV and

**Figure 2.** Effects of modulators of drug transporters and digitonin, alone and in combination, on the accumulation of [<sup>3</sup>H]-ATV in PBMCs



Data were tested for normality of distribution by the Shapiro-Wilk test followed by a parametric paired *t*-test. Each bar represents the mean  $\pm$  SD ( $n=3$ , with 4 incubations per treatment). <sup>a</sup> $P<0.01$  and <sup>b</sup> $P<0.05$  compared with respective controls. ATV, atazanavir; CAR, cellular accumulation ratio; E-3-S, estrone-3-sulphate; PBMCs, peripheral blood mononuclear cells.

SQV caused a decrease ( $P<0.05$ ) in the CAR of [<sup>3</sup>H]-ATV, either at both of the concentrations tested or at higher concentrations.

#### Effects of digitonin permeabilization on the transport of [<sup>3</sup>H]-ATV

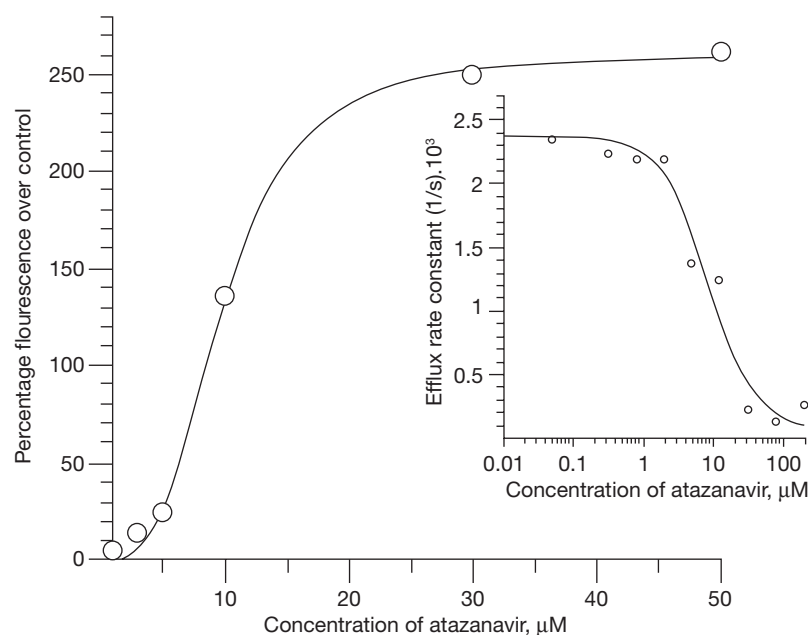
Here, cells were either initially permeabilized with 20  $\mu$ M digitonin prior to the accumulation studies (in the absence or presence of inhibitors) or subsequently permeabilized upon carrying out the accumulation studies in the absence or presence of the inhibitors at steady state. As shown previously (Figure 1C), tariquidar and frusemide did not alter the accumulation of [<sup>3</sup>H]-ATV, whereas dipyridamole significantly ( $P<0.01$ ) decreased the accumulation of [<sup>3</sup>H]-ATV (Figure 2). Permeabilization of the cells with digitonin significantly reduced ( $P<0.01$ ) the accumulation of [<sup>3</sup>H]-ATV (from  $15.3 \pm 0.8$  to  $13.0 \pm 0.1$ ). Of the digitonin-pretreated cells that were subsequently coincubated with tariquidar or frusemide, we observed no significant decrease in the CAR of [<sup>3</sup>H]-ATV compared

with digitonin-treated samples. By contrast, there was a significant ( $P<0.05$ ) decrease in the accumulation of [<sup>3</sup>H]-ATV in cells pretreated with digitonin followed by dipyridamole over cells treated with digitonin alone. Identical results were obtained with cells that were initially pretreated with the inhibitors and subsequently permeabilized with digitonin (data not shown). We also investigated the effects of E-3-S (alone and in the presence of tariquidar-, frusemide- or dipyridamole-treated cells) on the accumulation of [<sup>3</sup>H]-ATV. E-3-S alone did not alter the CAR of [<sup>3</sup>H]-ATV. However, there was a significant ( $P\leq 0.01$ ) decrease in the CAR of [<sup>3</sup>H]-ATV in cells pretreated with tariquidar, dipyridamole and frusemide, followed by subsequent E-3-S coincubation (Figure 2).

Atazanavir increases the accumulation and decreases the efflux of daunomycin in the cultured cells

ATV increased the accumulation and decreased the efflux of daunomycin in a concentration-dependent manner, with a 50% inhibitory concentration of 9.8  $\mu$ M

Figure 3. Concentration-dependent effects of atazanavir on the accumulation and efflux of daunomycin in CEM<sub>vBL</sub> cells



Concentration-dependent effects of atazanavir (0–50 μM) on the accumulation and efflux (inset; tariquidar at 0–200 μM) of daunomycin in CEM<sub>vBL</sub> cells. Initial efflux rates were determined from the slope of linear regression line fitted to mean fluorescence intensity values determined after 1, 2, 3 and 4 min. Initial daunomycin efflux rates are given as a percentage of the fluorescence values determined for the zero time point. Data points represent the mean of two independently performed experiments. The 50% inhibitory concentration  $IC_{50}$  values for the inhibition of efflux (8.5 μM; inset) and enhancement of accumulation (9.8 μM; inset) of daunomycin were calculated from the concentration–response curves using Grafit (Erithacus Software Ltd, Surrey, UK).

and 8.5 μM, respectively (Figure 3). However, ATV had no effects on the accumulation and efflux of daunomycin in the CEM line (data not shown).

## Discussion

Here, we investigated the contribution of lipophilicity, efflux/influx mechanisms and embedding/sequestration in cell membranes to the accumulation of [<sup>3</sup>H]-ATV. We observed [<sup>3</sup>H]-ATV to be approximately 7× less lipophilic than [<sup>14</sup>C]-LPV, but 1.7× more lipophilic than [<sup>3</sup>H]-SQV [6]. The octanol/saline partition coefficient determined for [<sup>14</sup>C]-mannitol (our positive control) was similar to that reported previously [49].

Drug transporters are important determinants of variable drug accumulation, drug–drug interactions, efficacy, resistance and toxicity. There is ample evidence that some HPIs are substrates, inhibitors and inducers of drug transporters such as P-gp, MRP1/2, BCRP and hOATP/hOCTP [1,2,4,7–25,50]. However, the role of drug transporters in the accumulation of ATV has not been well documented [2,3,25,30,31]. In order to characterize the transport profile of ATV, we studied its accumulation in cultured cells and in primary human cells, with known transporter expression profiles [1,13]. These data provide evidence that ATV is a substrate of P-gp,

and MRP1. This stems from the observations that there was differential accumulation of [<sup>3</sup>H]-ATV between CEM and its variant P-gp- and MRP1-overexpressing lines and that inhibitors of P-gp/BCRP (that is, tariquidar and GF120918) and P-gp/MRP (daidzein) increased the accumulation of [<sup>3</sup>H]-ATV in CEM and its variant cells. However, in contrast to the data obtained on other HPIs [1,2,13–15,51], the observation that tariquidar, dipyrindamole, frusemide, GF120918 and GPV031 all significantly decreased or did not alter the accumulation of [<sup>3</sup>H]-ATV in some or all of the cells (Figure 1A) was unexpected (Figure 1A and 1C). Although we found no direct evidence that the influx of HPIs are sensitive to these drugs, and given that none of the drugs (at the concentrations and incubation times employed) are cytotoxic to the cells (data not shown), we postulate that the observed effects might be the result of inhibition of influx, competition for accumulation, or displacement of [<sup>3</sup>H]-ATV from cell membranes. Indeed, there is evidence that dipyrindamole and frusemide influence the fluxes or exchange of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>/H<sup>+</sup> and Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> [52,53]. Given that E-3-S, a substrate/inhibitor of hOATP/hOCT [4,54], significantly decreased the accumulation of [<sup>3</sup>H]-ATV, it is plausible that the accumulation of [<sup>3</sup>H]-ATV might be linked to the fluxes/exchange of these cations. Despite the recent observation that tariquidar does not inhibit



hOATP1B3-mediated uptake of paclitaxel [55], it could also be possible that the effects of tariquidar on some of the cells might be the result of inhibition of an influx transporter not described elsewhere.

As with the cultured CEM and its variant cells, the effects of inhibitors of drug transport proteins on the intracellular accumulation of drugs in PBMCs might depend on the relative expression of the transporters. We recently showed the expression of several drug efflux transporters on PBMCs and demonstrated that the accumulation of HPIs is amenable to modulation by inhibitors of drug efflux and influx transporters [1,2,6]. Here, inhibitors of drug efflux transporters, such as tariquidar, dipyrindamole, frusemide and E-3-S, produced contrasting results to that measured for SQV and LPV [1,2]. The following possibilities might explain these observations: reduced cell surface expression of the efflux and influx transporters; important drug–drug interactions; the transporter profile of ATV differs from other HPIs – ATV is acid-sensitive to absorption, inhibits hepatic uridine 5'-diphospho-glucuronosyltransferase and induces CYPs [56,57]; and identification of some additional properties of some of the inhibitors used, such as frusemide and dipyrindamole, might have potential to inhibit drug uptake transporters. Clearly, the decrease in the accumulation of [<sup>3</sup>H]-ATV by drugs such as frusemide, dipyrindamole and GPV031 (all known inhibitors of efflux transporters) warrants further investigations.

Soy-derived phytoestrogens, such as daidzein and genistein, have been reported to interact with some major drug transporters, including P-gp, MRP, BCRP and hOATP [42–47,58]. However, their interactions with HPIs are largely undefined. We observed that daidzein had dual effects, with the agents causing modest but significant decrease (at 1 mM) and increase (at 5 mM) in accumulation of [<sup>3</sup>H]-ATV ( $P < 0.05$ ) in the cultured cells. In PBMCs, genistein decreased the accumulation of [<sup>3</sup>H]-ATV, whereas daidzein increased it. The observed effect of genistein is in agreement with the evidence that it inhibits an influx transporter, showing that it inhibits hOATP1B1-mediated uptake of [<sup>3</sup>H]-dehydroepiandrosterone sulfate [45]. Furthermore, it has also been shown that genistein induces the depolarization of membrane potential [59], which might ultimately prevent the cellular accumulation of [<sup>3</sup>H]-ATV. As serum concentrations of phytoestrogens could be high [60], these data suggest that intake of soy tablets and or diets rich in soy might influence the accumulation, efficacy and toxicity of ATV. Taken together, these data suggest that these phytoestrogens are both inhibitors of influx and efflux transporters and that ATV might be a substrate of transporters that are affected by these phytoestrogens [42–47,58].

The evidence that E-3-S significantly decreased the accumulation of [<sup>3</sup>H]-ATV in the cultured cells suggests that ATV, like SQV and LPV [6] is also a

substrate of hOATP. Although we observed that E-3-S alone did not decrease the accumulation of [<sup>3</sup>H]-ATV in the PBMCs (Figure 1C and 2), contrary to the lack of effects of tariquidar and frusemide alone (Figure 1C and 2), coincubating tariquidar-, frusemide- and dipyrindamole-treated cells with E-3-S significantly decreased the accumulation of [<sup>3</sup>H]-ATV (Figure 2). These observations indicate the potential for significant drug–drug and drug–endogenous-substance interactions. We also observed that ATV enhances both P-gp-mediated accumulation and inhibits P-gp-mediated efflux of daunomycin in VBL cells (Figure 3). This is in some agreement with previous studies, which show that although ATV does not induce P-gp or MRP expression [31,61], it is an inhibitor of P-gp (and MRP [2,23,30] and is also able to increase the plasma concentration of coadministered HPIs [29] via blunting the activities of P-gp/MRP [2] and possibly cytochrome P450 enzymes [30].

RTV and other HPIs, including ATV, have been shown to modulate the plasma and intracellular concentrations of other HPIs [2,28,29] via inhibition of P-gp/MRP and cytochrome P450. Of the HPIs tested, only LPV and RTV (at 30  $\mu$ M) increased the accumulation of [<sup>3</sup>H]-ATV in E1000 cells (Figure 1B), whereas TPV (at 30  $\mu$ M) significantly increased accumulation of [<sup>3</sup>H]-ATV in PBMCs (Figure 1C). There is evidence that some antiretrovirals, such as IDV, RTV, SQV and efavirenz, are potent inhibitors of uptake transporters (for example, hOATP1B1) or hOCTs [5,62,63]. Some of the HPIs (for example, APV, IDV and SQV) reduced the accumulation of ATV in the P-gp overexpressing VBL cells (Figure 1B and 1C). In a previous investigation, we observed that of the hOATP isoforms studied, only VBL cells express hOATP1A2 [6]. Given that some of these HPIs are also substrates of hOATP [6], it is probably that the inhibitory effects of some of the HPIs on the accumulation of ATV are a direct result of their competition for entry into the cells with ATV. Overall, these observations plus that observed with probenecid suggest that ATV might be a substrate of uptake transporters. Thus its coadministration with some HPIs or probenecid might result in alterations in its cellular accumulation.

Investigations that aim to characterize the role of drug transporters and the influence of drug embedding/sequestering into cell membranes on the accumulation of [<sup>3</sup>H]-ATV in the cells using digitonin produced interesting, but unexpected, findings. This manipulation decreased CEM-, E1000- and PBMC-associated [<sup>3</sup>H]-ATV by 47%, 30% and 15%, respectively. However, a similar manipulation led to a significant increase in VBL-associated [<sup>3</sup>H]-ATV, suggesting the possibility that [<sup>3</sup>H]-ATV binds differentially to cells expressing different and varying amounts of transporters, with the drug binding more avidly to P-gp- than MRP-overexpressing

cells. Another possibility is that [<sup>3</sup>H]-ATV might not be efficiently transported by P-gp and MRP. Coincubations containing digitonin-treated cells followed by subsequent treatment with tariquidar, frusemide or dipyridamole were without any additional inhibitory effects over that observed with the inhibitors alone, suggesting lack of interaction between the coadministered agents.

In conclusion, we found that ATV is lipophilic, is a substrate of P-gp, MRP and hOATPs and is an inhibitor of P-gp, making its disposition susceptible to alteration by coadministered drugs and dietary components that interact with these transporters.

## Acknowledgements

The authors thank The Royal Irish Academy/Austrian Science Academy (OJ), the National Institute of Health Research (Department of Health) and the Northwest Development Agency (SHK and DJB) for infrastructural and project support and the Austrian Science Fund (SFB35; PC). The authors are grateful to PG Bray for helpful discussions during the preparation of this manuscript.

## Disclosure statement

DJB and SHK have received grant/research support from Abbott, Bristol-Myers Squibb, GlaxoSmithKline and Roche Pharmaceuticals. They have served as consultants for Gilead Sciences, GlaxoSmithKline and Vertex. All other authors declare no competing interests.

## References

- Janneh O, Owen A, Chandler B, *et al.* Modulation of the intracellular accumulation of saquinavir in peripheral blood mononuclear cells by inhibitors of MRP1, MRP2, P-gp and BCRP. *AIDS* 2005; **19**:2097–2102.
- Janneh O, Jones E, Chandler B, Owen A, Khoo SH. Inhibition of P-glycoprotein and multidrug resistance-associated proteins modulates the intracellular concentration of lopinavir in cultured CD4 T cells and primary human lymphocytes. *J Antimicrob Chemother* 2007; **60**:987–993.
- Bousquet L, Roucaïrol C, Hembury A, *et al.* Comparison of ABC transporter modulation by atazanavir in lymphocytes and human brain endothelial cells: ABC transporters are involved in the atazanavir-limited passage across an *in vitro* human model of the blood–brain barrier. *AIDS Res Hum Retroviruses* 2008; **24**:1147–1154.
- Su Y, Zhang X, Sinko PJ. Human organic anion-transporting polypeptide OATP-A (SLC21A3) acts in concert with P-glycoprotein and multidrug resistance protein 2 in the vectorial transport of Saquinavir in Hep G2 cells. *Mol Pharm* 2004; **1**:49–56.
- Zhang L, Gorset W, Washington CB, Blaschke TF, Kroetz DL, Giacomini KM. Interactions of HIV protease inhibitors with a human organic cation transporter in a mammalian expression system. *Drug Metab Dispos* 2000; **28**:329–334.
- Janneh O, Hartkoorn RC, Jones E, *et al.* Cultured CD4T cells and primary human lymphocytes express hOATPs: intracellular accumulation of saquinavir and lopinavir. *Br J Pharmacol* 2008; **155**:875–883.
- Izzedine H, Launay-Vacher V, Deray G. Renal tubular transporters and antiviral drugs: an update. *AIDS* 2005; **19**:455–462.
- Huisman MT, Smit JW, Wiltshire HR, Hoetelmans RM, Beijnen JH, Schinkel AH. P-glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir. *Mol Pharmacol* 2001; **59**:806–813.
- Park S, Sinko PJ. P-glycoprotein and multidrug resistance-associated proteins limit the brain uptake of saquinavir in mice. *J Pharmacol Exp Ther* 2005; **312**:1249–1256.
- Kim AE, Dintaman JM, Waddell DS, Silverman JA. Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein. *J Pharmacol Exp Ther* 1998; **286**:1439–1445.
- Lee CG, Gottesman MM, Cardarelli CO, *et al.* HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* 1998; **37**:3594–3601.
- Washington CB, Duran GE, Man MC, Sikic BL, Blaschke TF. Interaction of anti-HIV protease inhibitors with the multidrug transporter P-glycoprotein (P-gp) in human cultured cells. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **19**:203–209.
- Jones K, Bray PG, Khoo SH, *et al.* P-Glycoprotein and transporter MRP1 reduce HIV protease inhibitor uptake in CD4 cells: potential for accelerated viral drug resistance? *AIDS* 2001; **15**:1353–1358.
- Huisman MT, Smit JW, Crommentuyn KM, *et al.* Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. *AIDS* 2002; **16**:2295–2301.
- Agarwal S, Pal D, Mitra AK. Both P-gp and MRP2 mediate transport of lopinavir, a protease inhibitor. *Int J Pharm* 2007; **339**:139–147.
- Gutmann H, Fricker G, Drewe J, Toeroek M, Miller DS. Interactions of HIV protease inhibitors with ATP-dependent drug export proteins. *Mol Pharmacol* 1999; **56**:383–389.
- Ronaldson PT, Lee G, Dallas S, Bendayan R. Involvement of P-glycoprotein in the transport of saquinavir and indinavir in rat brain microvessel endothelial and microglia cell lines. *Pharm Res* 2004; **21**:811–818.
- Bachmeier CJ, Spitzenberger TJ, Elmquist WF, Miller DW. Quantitative assessment of HIV-1 protease inhibitor interactions with drug efflux transporters in the blood–brain barrier. *Pharm Res* 2005; **22**:1259–1268.
- Williams GC, Liu A, Knipp G, Sinko PJ. Direct evidence that saquinavir is transported by multidrug resistance-associated protein (MRP1) and canalicular multispecific organic anion transporter (MRP2). *Antimicrob Agents Chemother* 2002; **46**:3456–3462.
- Shiraki N, Hamada A, Yasuda K, Fujii J, Arimori K, Nakano M. Inhibitory effect of human immunodeficiency virus protease inhibitors on multidrug resistance transporter P-glycoproteins. *Biol Pharm Bull* 2000; **23**:1528–1531.
- Chandler B, Almond L, Ford J, *et al.* The effects of protease inhibitors and nonnucleoside reverse transcriptase inhibitors on P-glycoprotein expression in peripheral blood mononuclear cells *in vitro*. *J Acquir Immune Defic Syndr* 2003; **33**:551–556.
- Dupuis ML, Flego M, Molinari A, Cianfriglia M. Saquinavir induces stable and functional expression of the multidrug transporter P-glycoprotein in human CD4 T-lymphoblastoid CEMrev cells. *HIV Med* 2003; **4**:338–345.
- Storch CH, Theile D, Lindenmaier H, Haefeli WE, Weiss J. Comparison of the inhibitory activity of anti-HIV drugs on P-glycoprotein. *Biochem Pharmacol* 2007; **73**:1573–1581.
- Gupta A, Zhang Y, Unadkat JD, Mao Q. HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). *J Pharmacol Exp Ther* 2004; **310**:334–341.
- Weiss J, Rose J, Storch CH, *et al.* Modulation of human BCRP (ABCG2) activity by anti-HIV drugs. *J Antimicrob Chemother* 2007; **59**:238–245.

26. Colombo S, Soranzo N, Rotger M, *et al.* Influence of ABCB1, ABCC1, ABCC2, and ABCG2 haplotypes on the cellular exposure of nelfinavir *in vivo*. *Pharmacogenet Genomics* 2005; 15:599–608.
27. Wang X, Furukawa T, Nitanda T, *et al.* Breast cancer resistance protein (BCRP/ABCG2) induces cellular resistance to HIV-1 nucleoside reverse transcriptase inhibitors. *Mol Pharmacol* 2003; 63:65–72.
28. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. *Clin Pharmacokinet* 1998; 35:275–291.
29. Ford J, Boffito M, Maitland D, *et al.* Influence of atazanavir 200 mg on the intracellular and plasma pharmacokinetics of saquinavir and ritonavir 1600/100 mg administered once daily in HIV-infected patients. *J Antimicrob Chemother* 2006; 58:1009–1016.
30. Perloff ES, Duan SX, Skolnik PR, Greenblatt DJ, von Moltke LL. Atazanavir: effects on P-glycoprotein transport and CYP3A metabolism *in vitro*. *Drug Metab Dispos* 2005; 33:764–770.
31. Chinn LW, Gow JM, Tse MM, Becker SL, Kroetz DL. Interindividual variability in the effect of atazanavir and saquinavir on the expression of lymphocyte P-glycoprotein. *J Antimicrob Chemother* 2007; 60:61–67.
32. Pick A, Muller H, Wiese M. Structure-activity relationships of new inhibitors of breast cancer resistance protein (ABCG2). *Bioorg Med Chem* 2008; 16:8224–8236.
33. Kuhnle M, Egger M, Muller C, *et al.* Potent and selective inhibitors of breast cancer resistance protein (ABCG2) derived from the P-glycoprotein (ABCB1) modulator tariquidar. *J Med Chem* 2009; 52:1190–1197.
34. Mistry P, Stewart AJ, Dangerfield W, *et al.* *In vitro* and *in vivo* reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* 2001; 61:749–758.
35. Damle BD, Sridhar R, Desai PB. Dipyridamole modulates multidrug resistance and intracellular as well as nuclear levels of doxorubicin in B16 melanoma cells. *Int J Cancer* 1994; 56:113–118.
36. Verstuyft C, Strabach S, El-Morabet H, *et al.* Dipyridamole enhances digoxin bioavailability via P-glycoprotein inhibition. *Clin Pharmacol Ther* 2003; 73:51–60.
37. Kawamura K, Yamasaki T, Yui J, *et al.* *In vivo* evaluation of P-glycoprotein and breast cancer resistance protein modulation in the brain using [(11)C]gefitinib. *Nucl Med Biol* 2009; 36:239–246.
38. Chiba P, Ecker G, Schmid D, *et al.* Structural requirements for activity of propafenone-type modulators in P-glycoprotein-mediated multidrug resistance. *Mol Pharmacol* 1996; 49:1122–1130.
39. Tahara H, Kusuhara H, Chida M, Fuse E, Sugiyama Y. Is the monkey an appropriate animal model to examine drug–drug interactions involving renal clearance? Effect of probenecid on the renal elimination of H2 receptor antagonists. *J Pharmacol Exp Ther* 2006; 316:1187–1194.
40. Sakamoto K, Mikami H, Kimura J. Involvement of organic anion transporting polypeptides in the toxicity of hydrophilic pravastatin and lipophilic fluvastatin in rat skeletal myofibres. *Br J Pharmacol* 2008; 154:1482–1490.
41. Jager W, Winter O, Halper B, *et al.* Modulation of liver canalicular transport processes by the tyrosine-kinase inhibitor genistein: implications of genistein metabolism in the rat. *Hepatology* 1997; 26:1467–1476.
42. Bobrowska-Hagerstrand M, Wrobel A, Rychlik B, *et al.* Monitoring of MRP-like activity in human erythrocytes: inhibitory effect of isoflavones. *Blood Cells Mol Dis* 2001; 27:894–900.
43. Evans AM. Influence of dietary components on the gastrointestinal metabolism and transport of drugs. *Ther Drug Monit* 2000; 22:131–136.
44. Cooray HC, Janvilisri T, van Veen HW, Hladky SB, Barrand MA. Interaction of the breast cancer resistance protein with plant polyphenols. *Biochem Biophys Res Commun* 2004; 317:269–275.
45. Wang X, Wolkoff AW, Morris ME. Flavonoids as a novel class of human organic anion-transporting polypeptide OATP1B1 (OATP-C) modulators. *Drug Metab Dispos* 2005; 33:1666–1672.
46. Nguyen H, Zhang S, Morris ME. Effect of flavonoids on MRP1-mediated transport in Panc-1 cells. *J Pharm Sci* 2003; 92:250–257.
47. Enokizono J, Kusuhara H, Sugiyama Y. Effect of breast cancer resistance protein (Bcrp/Abcg2) on the disposition of phytoestrogens. *Mol Pharmacol* 2007; 72:967–975.
48. Hodges M, Yikilmaz E, Patterson G, *et al.* An iron regulatory-like protein expressed in *Plasmodium falciparum* displays aconitase activity. *Mol Biochem Parasitol* 2005; 143:29–38.
49. Gibbs JE, Thomas SA. The distribution of the anti-HIV drug, 2',3'-dideoxycytidine (ddC), across the blood-brain and blood-cerebrospinal fluid barriers and the influence of organic anion transport inhibitors. *J Neurochem* 2002; 80:392–404.
50. Sanchez Mdel C, Lopez P, Velez R, Yamamura Y. P-glycoprotein expression in HTLV-III cells after treatment with HIV-1 protease inhibitors. *Ethn Dis* 2008; 18 Suppl 2:S2-60–64.
51. Profit I, Eagling VA, Back DJ. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. *AIDS* 1999; 13:1623–1627.
52. Cremaschi D, Meyer G, Rossetti C, Botta G, Palestini P. The nature of the neutral Na<sup>+</sup>-Cl<sup>(-)</sup>-coupled entry at the apical membrane of rabbit gallbladder epithelium: I. Na<sup>+</sup>/H<sup>+</sup>, Cl<sup>(-)</sup>/HCO<sub>3</sub><sup>(-)</sup> double exchange and Na<sup>+</sup>-Cl<sup>(-)</sup> symport. *J Membr Biol* 1987; 95:209–218.
53. Joiner CH, Jiang M, Claussen WJ, Roszell NJ, Yasin Z, Franco RS. Dipyridamole inhibits sickling-induced cation fluxes in sickle red blood cells. *Blood* 2001; 97:3976–3983.
54. Letschert K, Faulstich H, Keller D, Keppler D. Molecular characterization and inhibition of amanitin uptake into human hepatocytes. *Toxicol Sci* 2006; 91:140–149.
55. Smith NF, Acharya MR, Desai N, Figg WD, Sparreboom A. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. *Cancer Biol Ther* 2005; 4:815–818.
56. Zhang D, Chando TJ, Everett DW, Patten CJ, Dehal SS, Humphreys WG. *In vitro* inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to *in vivo* bilirubin glucuronidation. *Drug Metab Dispos* 2005; 33:1729–1739.
57. Lankisch TO, Moebius U, Wehmeier M, *et al.* Gilbert's disease and atazanavir: from phenotype to UDP-glucuronosyltransferase haplotype. *Hepatology* 2006; 44:1324–1332.
58. Chen J, Lin H, Hu M. Absorption and metabolism of genistein and its five isoflavone analogs in the human intestinal Caco-2 model. *Cancer Chemother Pharmacol* 2005; 55:159–169.
59. Versantvoort CH, Rhodes T, Twentymann PR. Acceleration of MRP-associated efflux of rhodamine 123 by genistein and related compounds. *Br J Cancer* 1996; 74:1949–1954.
60. Maubach J, Depypere HT, Goeman J, *et al.* Distribution of soy-derived phytoestrogens in human breast tissue and biological fluids. *Obstet Gynecol* 2004; 103:892–898.
61. Bousquet L, Roucairol C, Hembury A, *et al.* Comparison of ABC transporter modulation by atazanavir in lymphocytes and human brain endothelial cells: ABC transporters are involved in the atazanavir-limited passage across an *in vitro* human model of the blood-brain barrier. *AIDS Res Hum Retroviruses* 2008; 24:1147–1154.
62. Campbell SD, de Morais SM, Xu JJ. Inhibition of human organic anion transporting polypeptide OATP 1B1 as a mechanism of drug-induced hyperbilirubinemia. *Chem Biol Interact* 2004; 150:179–187.
63. McRae MP, Lowe CM, Tian X, *et al.* Ritonavir, saquinavir, and efavirenz, but not nevirapine, inhibit bile acid transport in human and rat hepatocytes. *J Pharmacol Exp Ther* 2006; 318:1068–1075.

Accepted for publication 21 June 2009