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Title page

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Title: **The influence of passage number for Caco2 cell models when evaluating P-gp mediated drug transport.**

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Abstract

Caco2 cells are a human adenocarcinoma cell line that forms tight junctions and are widely used to examine bidirectional drug transport as well as P-glycoprotein mediated efflux. Unfortunately Caco2 cell lines can be very heterogeneous in nature. Our aim was to improve the Caco2 cell model for determination of P-glycoprotein mediated drug transport. Young passage Caco2 from ATCC had inadequate expression of P-glycoprotein, therefore three approaches were adopted to up regulate Caco2 P-glycoprotein expression to mimic that *in-vivo*; a) incubation of mature Caco2 monolayer with rifampicin b) Prolonged exposure of Caco2 cells to vinblastine (generating the Caco2 VIN line) and c) splitting cells every 7 to 9 days until late passage numbers (over P80) were available. Upon development of the models, P-gp expression and activity was determined using western blotting and bidirectional transport studies of rhodamine123. All 4 models exhibited P-gp mediated efflux transport for rhodamine123. Incubation with rifampicin didn't alter bidirectional transport compared to Passage 44 cells. Increased passage number altered P-glycoprotein expression and the efflux ratio increased to 4.7 for passage 80 from 1.4 of passage 44. The highest basolateral to apical transport was observed for both passage 89 Caco2 and the Caco2 VIN model with an efflux ratio of 13 to 14. Western blot images confirmed the increased P-glycoprotein expression of late passage and Caco2 VIN. Caco2 cells are not ready for P-gp related research when first acquired from ATCC (Passage 18). Late passage Caco2 cell monolayers or Caco2 VIN models are needed to determine P-gp mediated efflux transport.

1. Introduction

Permeability of a drug in the gastrointestinal tract depends on multiple factors which include physiochemical parameters of drugs, partitioning coefficients, ionization, solubility and active and passive transport mechanisms (Rowland and Tozer 2011; Potts and Guy 1995; Barthe et al. 1999; Artursson et al. 2001). Efflux transporters such as P-glycoprotein (P-gp) which facilitate serosal to luminal directional transport of a drug could also alter the gastrointestinal permeability of a drug (Hunter et al. 1993; Hunter and Hirst 1997; Evers et al. 1998). P-glycoprotein, a 170 kDa membrane protein is not only expressed in the epithelial or endothelial cells in the intestine but also in liver, brain, kidney and placental body tissues (Fojo et al. 1987; Thiebaut et al. 1987; Cordon-Cardo et al. 1990; Gatmaitan and Arias 1993). Therefore P-glycoprotein efflux transport can result in altered drug absorption, distribution, metabolism and elimination. In addition, pharmacokinetic drug-drug interactions are likely due to concurrent administration of drugs which interact with P-gp in some way.

Caco2 cells are derived from a human colorectal adenocarcinoma cell line (Fojo et al. 1987). This is the most widely used *in-vitro* cell line for determination of gastrointestinal permeability and identification of P-gp substrates and P-gp inducers or repressors. However it is known that P-gp expression in Caco2 cells can vary depending on the cell origin, cell culturing condition and the passage number (Walter and Kissel 1994; Anderle et al. 1998; Artursson et al. 2001). Hence it is important to ensure adequate expression of P-gp in Caco2 cell monolayer to develop assays for rapid determination of P-gp mediated efflux of tested drugs.

It has been found that some anticancer drugs, such as vinblastine, cultured with Caco2 cells can induce P-gp expression (Anderle et al. 1998; Perloff et al. 2003). Shirasaka and colleague also used vinblastine to upregulate the P-gp expression in Caco2 cells where a short term culture method was adopted (Shirasaka et al. 2006). Similarly, rifampicin has also shown P-gp inducing properties in Caco2 cell lines (Westphal et al. 2000).

The index for P-gp mediated efflux transport in *in-vitro* Caco2 cell models and *in-vivo* rodent models has been determined using the fluorescent dye, rhodamine123 (Perloff et al. 2003) which shows a good correlation between fluorescent rhodamine123 efflux and P-gp expression across multiple cell lines (Lee et al. 1994). Therefore our aims were to optimize the Caco2 cell model for adequate expression of P-gp for assay of drugs for P-gp substrate and inhibitory properties using rhodamine123 as our reference P-gp substrate.

2. Investigations and results

2.1 Bidirectional transport studies

Apparent permeability values for rhodamine123 through passage 44 Caco2 cells and 4 cell models; Caco2 RIF, Caco2 VIN and late passage Caco2s (passage 80) and (passage 88) are given in Table 1. The baseline cells and four models exhibited higher basolateral to apical transport (equivalent to serosal to luminal directional transport (efflux)) of rhodamine123 rather than apical to basolateral transport (uptake) of rhodamine123. The efflux transport of rhodamine123 was inhibited when exposed to the P-gp inhibitor PSC 833 and the Rhodamine123 efflux ratio dropped to one indicating active efflux was replaced with bidirectional diffusion as shown in Figure 1 for all four cell models. Therefore it is evident that P-gp transporter proteins were expressed in all cells tested from early passage 44, Caco2 RIF, Caco2 VIN and late passage Caco2 cell monolayers, although the amounts expressed were different.

The Ap-Bas directional transport of rhodamine123 in Caco2 VIN and late passage Caco2 cell models was around $0.4-0.5 \times 10^{-6}$ cm/sec and the cell intake was about 4 fold lower than the passage 44 and Caco2 RIF models where P_{app} was in the range of $2.1-2.2 \times 10^{-6}$ cm/sec. The efflux ratio for late passage Caco2 cells increased up to 14.2 from 1.4 of passage 44, the earlier passage cells and this increase mainly accounted to the drop in uptake direction transport in late passage Caco2 cells rather than increase in basolateral to apical transport

The attempt to force a consistent P-gp over expression by using a continuous low level of vinblastine exposure to stress the Caco2 cells was effective and resulted in equivalent rhodamine123 efflux to that developing naturally during late passage 88 cultures. Caco2 VIN showed high P-gp activity with an efflux ratio of 13 for rhodamine123 compared to 14 for passage 88 cells. The highest basolateral to apical transport (efflux) was observed for Caco2 VIN model and was 6.6×10^{-6} cm/sec.

P-glycoprotein induction using rifampicin exposure did not result in notable increase of rhodamine123 compared to equivalent passage 44 Caco2 cells and no change in efflux ratio was observed.

2.2 Western blotting

Cell lysates for day 21, 24 and 28 for Caco2 VIN and late passage cells, day 21 and 24 for passage 44 and day 28 for Caco2 RIF were subjected to western blotting and the image is shown in Figure 2. The highest P-gp expression was for day 24 Caco2 VIN model and day 28 had slightly lower P-gp expression indicating possible down regulation of P-gp following prolonged exposure.

The P-gp expression has gradually increased over day 21 to 28 for passage 80 Caco2 cells, however was lower compared to the Caco2 VIN model. The day 21 P-gp expression for late passage Caco2 cells was 3 fold lower compared to the day 24 Caco2 VIN model P-gp expression. This was also evident in the rhodamine123 efflux ratio where day 21 passage 80 Caco2 showed an efflux ratio only one third of the day 24 Caco2 VIN monolayers. The blots for passage 44 and Caco2 RIF were faint in the western blot, much lower than the expression in the other Caco2 types.

3. Discussion

In-vitro cell lines are used for determination of drug permeability in early drug development and also to establish P-gp mediated efflux transport of drugs. The Caco2 cell line is the most widely used epithelial cell monolayer for drug transport studies and is available from international cell banks. These cells have some advantages over other cell lines that form tight junctions such as the Canine cell line, Madin-Darby canine kidney (MDCK) cells which can be transfected with human MDR1. These transfected cell lines require usage agreements with the laboratories that created them as they are modified cells, rather than native cells, and can be time consuming to create de novo. Also, unlike the Caco2 cells the MDCK cell line is not human, being canine in origin. The MDCK cell lines need to be maintained using selection pressures to maintain the high human P-gp levels and tight junctions as most transfections to date are not stable without continual selection.

A good correlation between ABC transporter protein expression of *in-vitro* Caco2 cells and *in-vivo* jejunum is established (Taipalensuu et al. 2001), hence *in-vitro* and *in-vivo* correlation for P-gp expression can be anticipated. Most importantly, the column shape and microvilli architecture of the Caco2 cells allows a good correlation between the *in-vitro* drug permeability of Caco2 cells and *in-vivo* drug absorption, which enables good predictions of *in-vivo* drug absorption from apparent permeability values (Fogh et al. 1977; Thiebaut et al. 1987; Artursson 1991; Crowe et al. 2006). These predictions are based on plasma membrane properties of the Caco2 cells and generally apply to drugs of diffusion where physico-chemical properties of the drug and transcellular bidirectional transport provide equivalent bidirectional transport.

However, the main limitation of *in-vitro* Caco2 cells is its heterogenic nature between labs (Artursson and Borchardt 1997). These changes could be cell characteristics in relation to the origin of the cell line, passage number and the culturing condition within and between laboratories. Hence in order to minimise variability, consistent cell culture conditions need to

be adopted within the laboratory. The passage 18 cells obtainable from the ATCC had little functional P-gp expression and have poorer tightness between cells (results not shown). This means that labs considering Caco2 bidirectional efflux studies to examine P-gp substrates activity of drugs are likely to be disappointed with results if they use low passage number Caco2 cells.

Other laboratories that have already used low passage Caco2 cells have shown little evidence of active efflux. One bidirectional transport study from 1998 that examined peptidomimetics using passage 35-50 cells concluded that the drugs themselves were not P-gp substrates as their transport was equivalent in both directions (Camenisch et al. 1998), while our data suggests that P-gp may not have been as active in cells of that passage masking any real P-gp mediated efflux. Other groups that have used low passage number Caco2 monolayers have focused on permeability characteristics that have little to do with active efflux proteins (Faust and Albers 1988; Dulfer et al. 1996), and such data would be expected to be relatively consistent irrespective of the passage used. In addition, in our hands very low passage Caco2 cells, around passage 20-25 had low TEER values, of between 250 and 350 ohm.cm² after 3 weeks of seeding at 65,000 cells per cm² (results not shown) which was one of the primary reasons for continuously progressing the passage number after acquisition from ATCC. Previous work from our laboratory used late passage Caco2 cells with much higher TEER values on millicell inserts (600-800 ohm.cm²).

To overcome these limitations, three interventions were trialled in our laboratory with the ATCC cells; Caco2 RIF, Caco2 VIN and continuous passaging were adopted to improve P-gp expression of early passage Caco2 cells. Passage 41 cells were used as the baseline cells. A change in P-gp expression was observed over 47 additional passages of Caco2 cells from this point, Ap-Bas transport of rhodamine123 was altered in late passage cells (passage 80+) compared to earlier passages (P > 40s). Both early and late passage cells had cell differentiation, monolayer tightness and therefore formed a good cell barrier for drug transport. It should be noted that we still needed to wait until passage numbers in the late

30's before there was consistency in tight junctions above 400 ohm.cm². The early passage cells had high cell uptake of rhodamine123 (2.2×10^{-6} cm/sec) and this dropped to less than $0.4-0.5 \times 10^{-6}$ cm/sec for late passage cells increasing the efflux ratio up to 10 fold. The change in rhodamine123 uptake could be attributed to increased expression of P-gp in late passage Caco2 cell monolayer and/or formation of tighter monolayers. Western blotting imaging and quantification showed a clear increase in P-gp expression in late passage Caco2 cells. Thus, in keeping with the current literature (Artursson and Borchardt 1997), an increase in the P-gp expression over almost 50 passages was observed which would affect the cell uptake of potent P-gp substrate such as rhodamine123. It is evident that over a large number of passages there is an observable increase in P-gp expression and it is not merely an increase in monolayer tightness. Therefore later stage Caco2 monolayers are more likely to identify low to moderately effluxed P-gp substrate and thus are a better model than early passage Caco2 cells, which would only be able to identify strongly effluxed drugs, and even these would not reach the internationally accepted efflux ratio of 2, needed to justify the title of P-gp substrate (Crivori et al. 2006; Hayeshi et al. 2008). Hence it is important to periodically study the bidirectional transport of a standard P-gp substrate (such as rhodamine123) with the increase in passages number (especially when the early passage Caco2 cells were obtained from ATCC) (Artursson et al. 1996; Artursson et al. 1996).

A small increase in P-gp expression was also observed for Passage 80 cells over day 21, 24 and 28 with the highest P-gp expression on day 28. This is consistent with the current literature where Hosoya *et al.* et al. 1996 observed a significantly higher P-gp expression on day 27 compared to day 17.

Other groups have shown rifampicin to have P-gp inducing properties (Westphal et al. 2000). However the attempt to induce P-gp expression using rifampicin did not alter the P-gp expression of passage 44 cells to a notable level compared to those treated with normal media (The efflux ratio increased from 1.4 to 1.5). Therefore use of rifampicin to establish a model for rapid determination of P-gp properties of a drug was limited. Leslie Benet's group

used grapefruit juice in passage 30 to 40 Caco2 cells to induce extra P-gp expression alongside their MDCK-MDR1 transfected cells (Soldner et al. 1999). We would suggest that much lower P-gp expression observed in the Caco2 cells than the MDCK-MDR1 cells in this study was related to the low passage number of the Caco2 cells (Soldner et al. 1999). Certainly our own lack of rifampicin effect on low passage Caco2 cells also suggests that not only do low passage Caco2 cells have much lower P-gp expression than late passage Caco2 cells, but that induction from short term co-incubation with an inducer, such as grapefruit juice, or rifampicin is not enough to counter the inherently low P-gp levels observed in these passages of Caco2.

However, longer term generation of increased P-gp was successful in the current study. For our Caco2 VIN model, cells were exposed to a potent P-gp substrate, vinblastine at nanomolar concentration for an extended period in increasing concentrations. Shirasaka et al. 2006 used vinblastine for induction of P-gp, where a short term culture method was adopted using differential medium to induce P-gp expression. This was not implemented in the current study as this could reduce the required monolayer tightness and cells were grown for 24 days.

Out of the 3 distinct models, Caco2 VIN model showed similar P-gp expression to passage 88 cells with an efflux ratio of 13. This was also visually apparent in western blots where day 24 P-gp expression for Caco2 VIN was 3 fold higher than the day 21 passage 80 Caco2. Hence increases in efflux were directly related to the increased P-gp expression. The P_{app} values for Apical to Basolateral transport of rhodamine123 was similar for both late passage Caco2 cells and the Caco2 VIN model, while Caco2 VIN increased the saturation threshold for the P-gp substrate of interest. This allowed identification of low affinity P-gp substrates. Although this approach took over a month of culturing in low concentration vinblastine, and through 3 passages of cells, it did provide P-gp activity equivalent to a further 10 months of passaging. Thus, the ability to have a cell line capable of determining P-gp mediated efflux from lower passage Caco2 cells by encouraging Caco2 cells to ramp up production of P-gp

for its own survival by using the cytotoxic vinblastine at low levels is a potent tool when acquiring Caco2 cells at a low passage number.

The early passage cells had comparatively lower P-gp expression; therefore this cell model is unlikely to be useful for identification of low affinity P-gp substrates. Further, extensive cell uptake could be observed for potent P-gp substrates. Thus, when P-gp was not likely to be involved and the cell monolayer is only representing a physiologically relevant barrier that reflects physicochemical properties of the drugs, then irrespective of early or late passages, the Caco2 model may be acceptable for drug studies as results between this large spread of passages are not too dissimilar. Hence the early and late passage Caco2 cell models compared well to determine the passive permeability of compounds not shown to be P-gp substrates. However, if a research group wants to examine P-gp mediated efflux in low passage Caco2 cells then we recommend vinblastine exposure over 3 passages prior to those experiments. However, our optimal recommendation would be to acquire late passage Caco2 cells, above passage 85 for active efflux studies.

In conclusion, a clear increase in P-gp expression was observed over almost 50 passages of Caco2 cells from passage 40 to 88 and late passage cells are suitable to use as a cell model for assay of the P-gp mediated drug transport of drugs. Exposure of Caco2 cells to the P-gp inducer, rifampicin did not result in a notable increase in the P-gp efflux of rhodamine123. The Caco2 VIN model had equivalent P-gp expression to passage 88 Caco2 cells and can function as an *in-vitro* model to identify P-gp substrates with low affinity for P-gp if low passage Caco2 cells are the only cells available to a research group.

4. Experimental

4.1 Drugs and Chemicals

Vinblastine hydrochloride was purchased from ICN biochemicals (Seven hills, NSW, Australia). PSC 833 was donated from Novartis Pharma (Basel, Switzerland). Mini protease inhibitor tablets were supplied by Thermo Scientific (Rockford, USA). Ethanol was supplied from Fisher Scientific (Fair Lawn NJ, USA). Dimethyl sulfoxide (DMSO) was purchased from Ajax Finechem (NSW, Australia). TRIS hydrochloride was purchased from Ultrapure bio-reagents (NJ, USA). Sodium Chloride, sodium potassium tartrate and copper (II) sulphate were purchased from Chem-Supply (Gillman, SA). Rhodamine123, rifampicin, sodium dodecyl sulphate, sodium carbonate, casein, potassium dihydrogen ortho-phosphate, trifluoroacetic acid, phosphoric acid, formic acid and nonidet P40 substitute were supplied by Sigma Aldrich (MO, USA). Sodium hydroxide and Folin & Ciocalteu's reagent were supplied by BDH Merck Pvt Ltd (Victoria, Australia). All other chemicals were of analytical grade.

4.2 Tissue culture reagents

The human colon carcinoma cell lines (Caco2) were obtained from American Type Culture Collection (ATCC), University Boulevard (Manassas, VA, USA) at passage 18. High glucose Dulbecco's modified eagle medium (DMEM), Dulbecco's phosphate buffered saline (PBS), L-Glutamine, Hanks buffered salt solution (HBSS), N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid (HEPES), trypan blue stain (0.4%) and TrypLE Express were supplied by Gibco [Life technologies (NY, USA)]. Glucose was purchased from APS Finechemicals (NSW, Australia). Non-essential amino acid, penicillin G (10,000 u/mL) and streptomycin (10,000 µg/mL) were purchased from Trace Biosciences (Castle Hill, NSW, Australia). Foetal calf serum (FCS) was obtained from the SerANA (Bunbury, Western Australia).

NuPAGE MOPS running buffer (20X), SeeBlue Plus2 Pre-stained Standard, Bolt Sample reducing agent (10X), Bolt 4-12% Bis-Tris Plus 15 well gels, NuPage transfer buffer (20 X), Nupage LDS sample buffer (4X) and NuPage antioxidant were purchased from Novex by Life Technologies (Melbourne, Australia).

MDR1 mouse monoclonal antibody was purchased from Santa Cruz biotechnology Inc. (Santa Cruz, CA, USA). Mouse monoclonal Anti-beta-Actin antibody was supplied by Sigma Aldrich (MO, USA). Goat anti-mouse secondary antibodies were purchased from Jackson ImmunoResearch Laboratories

Inc (West Grove, PA, USA). Clarity Western ECL substrate was supplied by BIORAD Laboratories Inc (Gladesville, NSW, Australia).

4.3 Development of cell models

The Caco2 cell line passage 18 was maintained in high glucose DMEM supplemented with 10% FCS, penicillin, streptomycin, glutamine and non-essential amino acid, incubated at 37 °C, 95% humidity and 5% CO₂. Medium was replaced twice a week and cell splitting was done using 50:50 TrypLE Express: PBS (1.5 mL each) upon 90% confluency, in about 7-10 days of post seeding.

Three interventions were adopted to improve P-gp transporter protein expression in Caco2 cell lines. Passage 41 cells froze in liquid nitrogen were regrown in normal media and baseline characteristics were determined in passage 44 cells.

Exposure of Caco2 cells to rifampicin (Caco2 RIF): Passage 44 Caco2 cells were grown in normal media on 0.6 cm² diameter 0.45 µm pore size Millipore PCF filter inserts at a cell density of 65,000 cells/cm². On day 24, cells were exposed to medium supplemented with 10 µM rifampicin for 96 hours. On day 28, cells were washed with PBS three times and incubated in normal medium for 4 hours and bidirectional transport of rhodamine123 was done

Exposure of Caco2 cells to increasing concentration of vinblastine (Caco2 VIN): Caco2 cells (passage 41) were seeded in 25cm² flasks and maintained in medium supplemented with 10 nM vinblastine till passage 43 was reached. Passage 44 cells were seeded onto Millipore PCF filter inserts at the cell density of 100,000 cells/cm² and were grown on high glucose DMEM supplemented with 10 nM vinblastine for first 17 days and 50 nM for the last 7 days. Fresh vinblastine was added every medium replacement, which was every 2 to 3 days. Bidirectional transport studies for rhodamine123 was done on day 24 following washing and incubation with normal medium.

Regular passaging of cells to get a late passage cell: Cells were regularly passaged over about one and half years and maintained in normal medium. Once the cells reached passage 80 and above, cells were grown on filter inserts and the rhodamine123 transport studies were done within 21 to 24 days.

4.4 Bidirectional transport studies of rhodamine123

Upon cell maturation, the trans epithelial electrical resistance (TEER) across Caco2 cell monolayer was measured before and after the experiment using an epithelial voltage/ohm meter (EVOM) and the ENDOHM 12 chamber (World precision instruments, Sarasota, FL). Transport studies were allowed to progress if TEER values above 300 Ω .cm² were evident. TEER values were also taken at the completion of the study to ensure membrane integrity was maintained during the 2 to 3 hour time course of the study. In the determination of apical to basolateral (Ap-Bas) directional transport, the medium in the apical (donor) compartment was replaced with 300 μ L of 5 μ M rhodamine123 or rhodamine123 plus 4 μ M PSC 833 in HBSS while the basolateral (receiver) compartment had 600 μ L of fresh HBSS or HBSS plus 4 μ M PSC833. The basolateral to apical (Bas-Ap) directional transport was determined by placing the drug in the basolateral compartment with the volumes as mentioned above (300 μ L in the apical and 600 μ L in the basolateral). Transport medium was collected from the receiver compartment and was replaced with the same volume of fresh HBSS or HBSS+ inhibitors at 30, 60, 90 and 120 minutes. At the end of the experiment, 100 μ L of sample was removed from the donor compartment and washed cells were sonicated in deionized water and centrifuged to collect the supernatant.

Rhodamine123 levels were quantified using a Perkin Elmer Enspire multi-mode plate reader (Waltham, MA, USA) and measurements were done using fluorescence detection at excitation and emission wavelengths of 485 and 525 nm respectively. The apparent permeability (P_{app}) value was determined by the methods described by Crowe and Lemaire 1998 with the mean and standard error values (SEM using N=3) were calculated.

4.5 Western blotting

Caco2 RIF, Caco2 VIN and late passage Caco2 cells were grown concurrently with the cells used in the rhodamine123 studies in 6 well plates using the above described procedures. Cells were lysed on days 21, 24 and 28 and passed through 21 gauge needle for maximum protein yield. The protein

content was determined using the micro Lowry protein assay and had electrophoresis undertaken by adding Nupage sample buffer and reducing agent to the soluble protein sample. The reduced proteins were loaded in 4 -12% Bis Tris Plus 15 well Nupage BOLT gels, along with SeeBlue precolored molecular weight markers. Gels were run using Novex Bolt Mini Gel Tank (Life Technologies, CA, USA) and transfer was done in a Xcell II Bolt Module (Novex, CA, USA). The membrane was blocked using 2% casein in TBS and washed with TBST. The primary antibody; Mdr (G-1) mouse monoclonal IgG2b 200 and mouse anti-beta actin were used as the antibody for β -actin, the reference protein. HRP linked goat anti mouse antibody was used as the secondary antibody. The washed membrane was incubated in BIORAD's clarity chemiluminescent system was read and semi quantified using a Chemidoc MPT imager with Image Lab™ software from BIORAD (Gladesville, NSW, Australia). The relative abundance of P-gp expression was given as a ratio of P-gp expression to β -actin.

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Figure legends

Figure 1: Ap-Bas (■) and Bas-Ap (◆) transport of rhodamine123 alone and Ap-Bas (□) and Bas-Ap (◇) transport of rhodamine123 when combined with P-gp inhibitor PSC 833 through (A) Caco2 RIF, (B) Caco2 VIN, (C) P-80 and (D) P-88 Caco2 cell monolayers (N=3, Mean ± SEM)

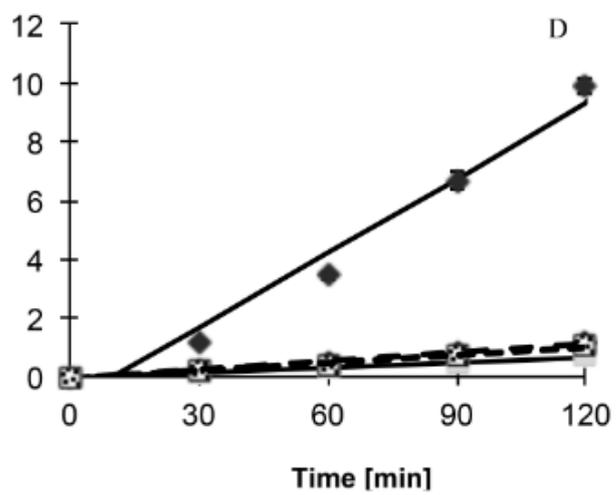
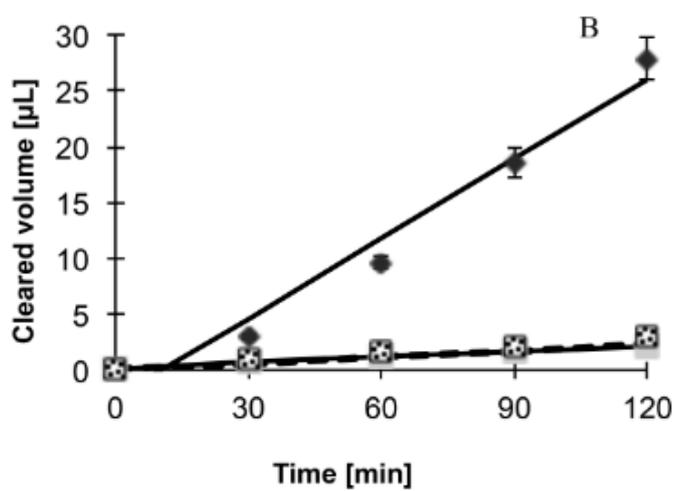
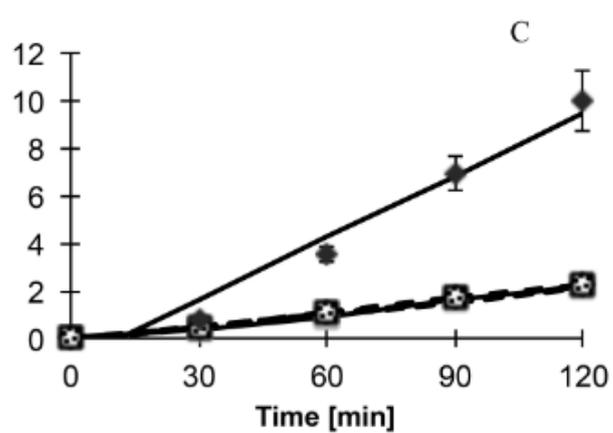
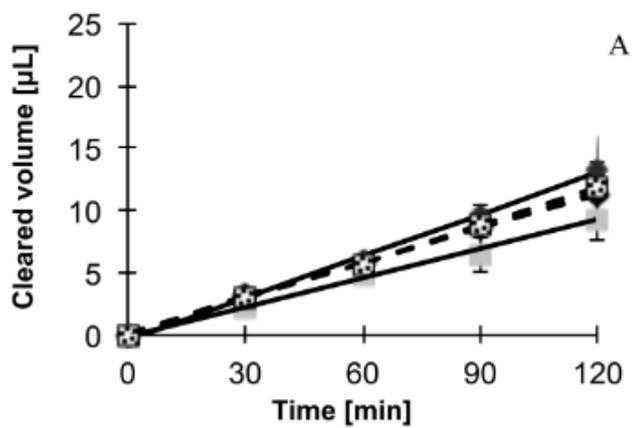
Figure 2: Western blot analysis and P-gp expression for MDR1 P-glycoprotein (170Kda) and β -actin (42 Kda) for three Caco2 cell models. Lanes 1 & 2: Passage 44 days 24 and 28, lane 3: Passage 44 Caco2 RIF, lanes 4-6: Passage 80 day 21, 24 and 28, lane 7-9: Caco2 VIN days 21, 24 and 28 and lanes 11 and 12 are molecular weight marker and P-gp transfected Hela-MDR1 cell positive controls respectively

Tables

Table 1: Apparent permeability ($P_{app} \pm SEM$) for Rhodamine123 (5 μ M Rh123) alone and combined with PSC 833 (4 μ M) for different Caco2 cell monolayer models

Caco2 monolayer models	Drug	Ap-Bas (10^{-6} cm/s)	Bas-Ap (10^{-6} cm/s)	Efflux ratio	Net transport ^a (P value)
P- 44	Rh123	2.24±0.07	3.17±0.22	1.4	Efflux (0.02)*
	Rh123 + PSC	2.29± 0.22	2.29 ± 0.08	1.0	Diffusion (0.98)
Caco2 RIF	Rh123	2.10±0.04	3.13±0.26	1.5	Efflux (0.01)*
	Rh123 + PSC	2.76±0.22	2.60±0.14	0.9	Diffusion (0.57)
Caco2 VIN	Rh123	0.50±0.03	6.61±0.44	13.2	Efflux (<0.001)**
	Rh123 + PSC	0.69±0.05	0.63±0.04	0.9	Diffusion (0.31)
P- 80	Rh123	0.51±0.05	2.42±0.29	4.7	Efflux (0.003)**
	Rh123 + PSC	0.54±0.05	0.53±0.04	1.0	Diffusion (0.96)
P-88	Rh123	0.38±0.04	5.40±0.09	14.2	Efflux (<0.001)**
	Rh123 + PSC	0.31±0.01	0.44±0.02	1.4	Efflux (0.005)*

* Significant at the level of $P < 0.05$, ** Significant at the level of $P < 0.005$, ^aThe P_{app} of Bas-Ap direction was divided by the Ap-Bas direction to obtain efflux ratio



1 2 3 4 5 6 7 8 9 10 11

