Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 99–105
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Utility of 96 well Caco-2 cell system for increased throughput of P-gp screening in drug discovery

Praveen V. Balimane*, Karishma Patel, Anthony Marino, Saeho Chong

Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Princeton, NJ, USA

Received 4 December 2003; accepted in revised form 23 February 2004

Available online 13 April 2004

Abstract

The use of Caco-2 cells for screening of discovery compounds for their permeability characteristics and P-glycoprotein interactions is well established and used routinely in pharmaceutical industries world-wide. The screening model involves growing cells on 12 or 24 well transwell format. In this manuscript, we report the use of Caco-2 cells grown on 96 well transwell plates for screening compounds for their potential to interact with P-gp. Bi-directionality studies were performed with known P-gp substrates such as saquinavir, indinavir, vinblastine, vincristine, verapamil, digoxin and taxol. P-gp inhibition studies were also conducted using radiolabeled digoxin as the probe. The results demonstrated that P-gp substrates had efflux ratios (P_c (B to A)/P_c (A to B)) in the 96 well format that were comparable to the ratios seen in 12 and 24 well format. Inhibition of digoxin efflux transport in presence of the test compounds (P-gp substrates) demonstrated that 96 well cells express adequate amounts of efflux transporters and perform as well as the 12 and 24 well Caco-2 cells. Thus, the 96 well Caco-2 cell set-up presents a higher throughput permeability model capable of identifying compounds that interact with P-gp and has the potential to significantly increase the efficiency of P-gp screening in early drug discovery.

Keywords: Permeability; Efflux transporter; Caco-2; High throughput; P-glycoprotein; Inhibitor; Substrate

1. Introduction

For a new chemical entity (NCE) to become a successful drug there are a multitude of desirable characteristics it should possess: potency to a biological target, selectivity, good stability and physico-chemical properties, minimal toxicity and adequate ADME profile. There are diverse screens that are set-up in order to select the compounds with the most favorable 'developability' characteristics with regards to ADME. Good permeability through intestinal membranes that can lead to adequate systemic absorption is a property highly desirable in NCEs. Various assay systems are used in early discovery to screen the permeability properties of compounds [1]. Commonly used permeability assay systems include cell-based models such as Caco-2, MDCK, LLC-PK1 cells; tissue-based models such as Ussing chamber, single pass perfusion; whole animal models such as in vivo screens; and even non-empirical in silico models. Combinatorial chemistry and other advances in synthetic chemistry have led to a tremendous inflow of discovery compounds being fed into the screens for permeability assessment. Some key characteristics desirable in a permeability screen are: high efficiency, high accuracy, time, cost and space effectiveness, and capability for high throughput. Caco-2 cells grown on 12 or 24 well transwell plates have been the staple of the pharmaceutical industry for high-throughput permeability of discovery compounds. Since P-gp is known to play a significant role in the pharmacokinetics of compounds, bi-directional studies in Caco-2 cells are routinely done for identifying compounds that interact with P-gp.

Caco-2 cells are a sturdy cell line that have been used to predict permeability and absorption of compounds in human [2–4]. Traditionally, these cells are grown on 12 and 24

Abbreviations: A, to B, apical to basolateral; ADME, absorption, distribution, metabolism, elimination; B, to A, basolateral to apical; BCRP, breast cancer resistance protein; HBSS, Hank's balanced salt solution; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; LRP, lung cancer resistance protein; MRP, multi-drug resistance protein; NCE, new chemical entity; P-gp, P-glycoprotein; P_c, permeability co-efficient; TEER, trans-epithelial electrical resistance.

* Corresponding author. Address: Bristol-Myers Squibb, P.O. Box 4000, Mailstop: F.13-07, Princeton, NJ, USA. Tel.: +1-609-52-4401; fax: +1-609-252-6802.

E-mail address: praveen.balimane@bms.com (P.V. Balimane).

0939-6411/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.02.014

wells and have been extremely successful in screening discovery compounds. However, advances in the field of molecular biology and combinatorial chemistry have shifted the bottle-neck in drug discovery from the compound synthesis step to compound selection step. There is a tremendous challenge to improve the throughput of existing screening techniques to keep up with compound synthesis. Increasingly a lot of companies have incorporated sophisticated levels of automation into these assays to make it amenable to higher throughput. The throughput is normally a staggering 100s and even 1000s of compounds per week through this screen. However, there are a large number of reasons why even further miniaturization (i.e. 96 well Caco-2) would help in such permeability assays. Apart from the obvious increase in throughput it would lead to tremendous cost reduction (by virtue of decreased cost of media, plates and buffer). One other key advantage over 12 or 24 well set-up is also that much less discovery compound would be required for 96 well to perform the same assay. This can be a significant advantage keeping in mind the hectic pace at which diverse chemotypes are synthesized in early stages of discovery.

One typical problem that has been encountered during miniaturization of any assay has been the increased variability and progressive drop in the 'quality' of data generated because of increased 'quantity'. In general, the levels of expression of transporters in cell models are known to be expressed more consistently in higher surface area wells (6 or 12 well) compared to smaller wells. Since the objective of this study was to optimize the use of 96 well Caco-2 cells, it was important to demonstrate the following week over week: (1) P-gp (and other efflux transporters such as MRP, BCRP and LRP) were consistently expressed to equivalent levels and (2) cell monolayer maintained their integrity. Studies done with reference compounds (metoprolol, cimetidine, mannitol, digoxin) demonstrated that the cells were functional and maintained their utility between passage numbers 40 and 80 (data not included in the manuscript).

The use of 96 well Caco-2 cell system that can perform as well as the 12 or 24 well Caco-2 can significantly increase the productivity needed in early drug discovery. Moreover, the fact that the 96 well Caco-2 cells retains adequate as well as consistent expression of efflux transporter (i.e. P-gp) make them an attractive model for P-gp screening.

2. Experimental

2.1. Materials and methods

Caco-2 cells (passage # 17) were obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle medium, non-essential amino acids and antibiotic–antimiotic were purchased from JHR Biosciences (Lenexa, KS). Fetal bovine serum was obtained from Hyclone Lab. Inc. (Logan, Utah). HTS-Transwell® inserts (surface area: 1, 0.33 and 0.1 cm^2 for 12, 24 and 96 well, respectively) with a polycarbonate membrane (0.4 μm pore size) were purchased from Costar (Cambridge, MA). Hank's balanced salt solution (HBSS) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) were purchased from Sigma Chemical Co. (St Louis, MO). All solvents were analytical grade. ^{3}H-digoxin, ^{14}C mannitol and ^{14}C taxol were obtained from Perkin–Elmer Life Sciences (Boston, MA). All other test compounds were obtained from Sigma Chemical Co. (St Louis, MO).

2.2. Caco-2 cell culture procedure

Caco-2 cells were seeded onto filter membrane at a density of ~60,000, ~100,000 and ~100,000 cells/cm^2 for 12, 24 and 96 well plates, respectively. The cells were grown in culture medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, 100 U/ml penicillin-G, and 100 μg/ml streptomycin. The culture medium was replaced every 2 days and the cells were maintained at 37 °C, 95% relative humidity, and 5% CO_2. Permeability studies were conducted with the monolayers cultured for approximately 21 days with the cell passage numbers between 50 and 80. Physiologically and morphologically well-developed Caco-2 cell monolayers with trans-epithelial electrical resistance (TEER) values greater than 400 Ω cm^2 were used for the studies reported in this manuscript.

2.3. Bi-directional P-gp substrate assay (12, 24 and 96 well Caco-2)

The transport medium used for the bi-directional studies was modified HBSS buffer containing 10 mM HEPES. The pH of both the apical and basolateral compartments was 7.4. Prior to all experiments, each monolayer was washed twice with buffer and TEER was measured to ensure the integrity of the monolayers. The concentration of test compounds was typically 50 μM in this assay. The bi-directional permeability studies were initiated by adding an appropriate volume of buffer containing test compound to either the apical (for apical to basolateral transport; A to B) or basolateral (for basolateral to apical transport; B to A) side of the monolayer. Volume of the apical and basolateral compartment was maintained at 0.8 ml in 12 well plates (apical and basolateral volumes was 0.2 and 0.6 ml, respectively, in the 24 well plates and 0.15 and 0.3 ml, respectively, in the 96 well plates). The monolayers were then placed in an incubator for 2 h at 37 °C. Samples were taken from both the apical and basolateral compartment at the end of the 2 h period and the concentrations of test compound were analyzed by a high performance liquid chromatography method [2]. Permeability coefficient (P_c)

was calculated according to the following equation:

$$P_c = dA/(dtSC_o),$$

where dA/dt is the flux of the test compound across the monolayer (nmole/s), S is the surface area of the cell monolayer, and C_o is the initial concentration (50 μM) in the donor compartment. The P_c values were expressed as nm/s.

A set of well characterized and well studied compounds were selected to run the bi-directional assay. Mannitol (probe to confirm the integrity of the monolayer and also a P-gp non-interactor) was used as a control. Standard P-gp substrates (saquinavir, vincristine, indinavir, digoxin, vinblastine, taxol and verapamil) [5] were some of the compounds studied as positive control.

2.4. P-gp inhibition assay (24 and 96 well Caco-2)

The transport medium used for the P-gp inhibition studies was also modified HBSS buffer containing 10 mM HEPES. The pH of both the apical and basolateral compartments was 7.4. Prior to all experiments, each monolayer was washed twice with buffer and TEER was measured to ensure the integrity of the monolayers. The model incorporated the use of inhibition of transport of digoxin, a P-gp substrate, in Caco-2 cells. Both the apical to basolateral (A to B) transport as well as the basolateral to apical (B to A) transport of [^{3}H]-digoxin was measured in the absence and presence of the test compound. The concentration of digoxin used was 5 μM, which was much below its K_m value of ~60 μM [6]. The concentration of test compounds was chosen to be 10 μM in this assay. The studies were initiated by adding an appropriate volume of buffer containing digoxin to either the apical (apical to basolateral transport) or basolateral (basolateral to apical transport) side of the monolayer (the volumes of the apical and basolateral compartment for each plate format has been provided in Section 2.4). The compound whose potential to inhibit P-gp needs to be assessed was added to both sides of the monolayer at 10 μM concentration. The monolayers were then incubated for 2 h at 37 °C. Samples are taken from either the apical (basolateral to apical transport) or basolateral (apical to basolateral transport) compartment at the end of the 2-h period and analyzed for [^{3}H]-digoxin using the radio-labeled liquid scintillation counter.

The apical to basolateral as well as the basolateral to apical permeability coefficient (P_c) of digoxin was calculated in the presence and absence of the test compound. Results have been reported as 'degree of inhibition' of digoxin transport by the test compound as listed in the equation below [7]:

$$\text{Degree of inhibition} = [1 - ((\text{iBA} - \text{iAB})/(\text{dBA} - \text{dAB}))] \times 100$$

where dBA and dAB are the B to A and A to B permeability of digoxin alone. iBA and iAB are the B to A and A to B permeability of digoxin in presence of the test compound.

3. Results

3.1. Bi-directional P-gp substrate assay results

A set of well-characterized and well-studied compounds were selected to test the validity of the P-gp substrate assay in Caco-2 cells grown on 12, 24 and 96 wells. The bi-directional permeability values for these compounds across the Caco-2 monolayers grown on 12 well format is shown in Fig. 1. Mannitol permeability values were very low (<25 nm/s) in both direction suggesting that the cells had intact monolayers and that mannitol was not interacting with any efflux or influx transporter systems. Compounds such as saquinavir, digoxin, vincristine, vinblastine, indinavir and taxol demonstrated much higher efflux (B to A) permeability as compared to influx (A to B) permeability. The efflux ratio (P_c (B to A)/P_c (A to B)) for these compounds varied from 3 to 25. This demonstrated that the cells expressed adequate amounts of P-gp and other additional efflux transporters thus leading to a sufficiently adequate efflux ratio for these compounds. Verapamil, a well established P-gp substrate demonstrated similar permeability in both direction. Even though it is a substrate for efflux, it also has a very high intrinsic permeability that dominates over P-gp efflux component. Therefore, verapamil and other such compounds that have very high intrinsic permeability values are generally difficult to screen using the cell based bi-directional assay. This is consistent with similar observations made by other investigators [8,9]

Fig. 2 shows the bi-directional permeability values for these compounds across the Caco-2 monolayers grown on

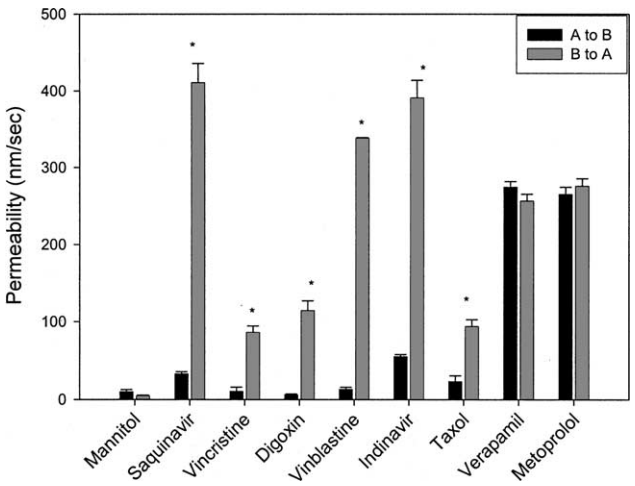

Fig. 1. Bi-directional permeability values in the 12 well Caco-2 cell transwells. All the compounds were studied at 50 μM concentration at 37 °C for 2 h. Each column represents the mean ± SD of three to six data points. (*, B to A permeability is significantly higher than A to B; $P < 0.01$).

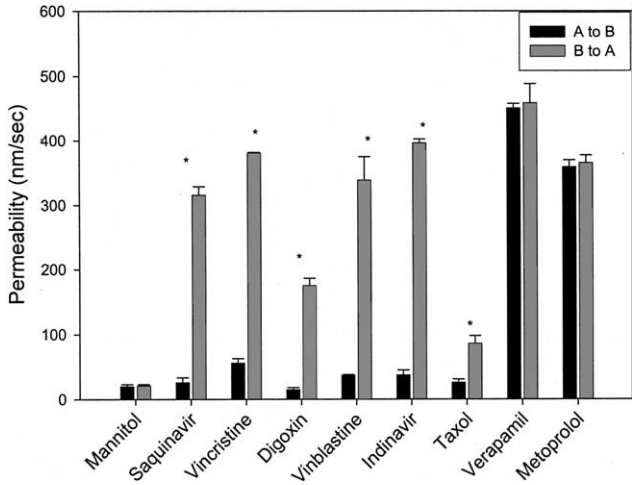

Fig. 2. Bi-directional permeability values in the 24 well Caco-2 cell transwells. All the compounds were studied at 50 μM concentration at 37 °C for 2 h. Each column represents the mean ± SD of three to six data points. (*, B to A permeability is significantly higher than A to B; $P < 0.01$).

24 well format. Mannitol permeability was again low and the typical P-gp substrates demonstrated much higher efflux (basolateral to apical) permeability as compared to influx (apical to basolateral) permeability. The efflux ratio (P_c (B to A)/P_c (A to B)) for the compounds varied from 3 to 12. Similarly, Fig. 3 shows the bi-directional in 96 well Caco-2 cells. Mannitol permeability was again low and the typical P-gp substrates demonstrated much higher efflux (basolateral to apical) permeability as compared to influx (apical to basolateral) permeability. The efflux ratio (ratio of efflux to influx permeability) for the compounds varied from 4 to 15. These results demonstrate that in spite of miniaturization, the cells expressed adequate amounts of P-gp and other additional efflux transporters in the 96 well Caco-2 cells

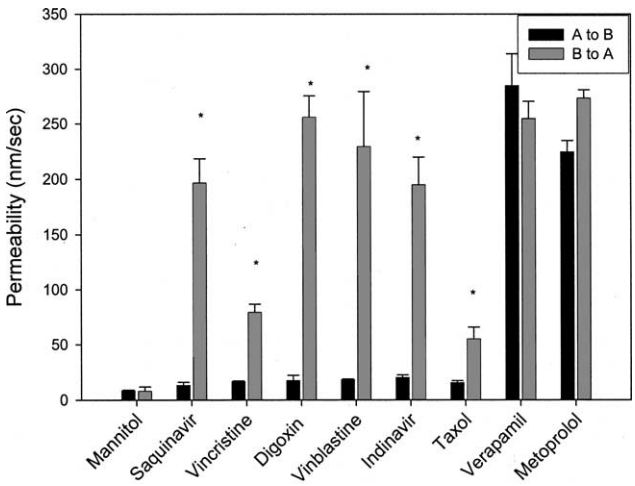

Fig. 3. Bi-directional permeability values in the 96 well Caco-2 cell transwells. All the compounds were studied at 50 μM concentration at 37 °C for 2 h. Each column represents the mean ± SD of three to six data points. (*, B to A permeability is significantly higher than A to B; $P < 0.01$).

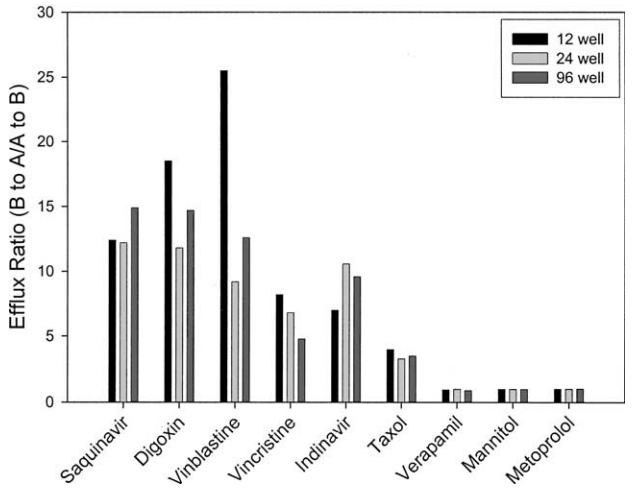

Fig. 4. Comparison of efflux ratio for various compounds across 12, 24 and 96 well Caco-2 cell transwells. The bi-directional permeability studies for all the compounds were performed at 50 μM concentration at 37 °C for 2 h.

thus leading to a sufficiently adequate efflux ratios for these compounds. Miniaturization can provide some obvious advantages at the drug discovery stage: less compound requirement, less solvents, improved throughput and reduced analytical and experimental expenses. However, the fact that the quality of the data generated with 96 well Caco-2 was un-compromised makes it a very attractive feature for implementation in discovery settings. Fig. 4 provides a direct comparison of the efflux ratios obtained for the compounds in 12 well, 24 well and 96 well plates. Compounds like saquinavir, digoxin and vinblastine constantly demonstrate efflux ratio greater than 10 across the three plate architectures. Taxol, vincristine and indinavir have efflux ratio between 3 and 10 across the plates. Finally, verapamil, because of its very high intrinsic permeability had an efflux ratio of ~1 in all plates architecture in spite of being a P-gp substrate. Controls such as metoprolol and mannitol also demonstrated ratios ~1.

3.2. P-gp inhibition assay results

Digoxin is a well established probe that is routinely used to identify compounds that have the potential to inhibit P-gp [10]. Fig. 5 presents the results obtained from the digoxin inhibition study in the 24 well Caco-2 format. The bi-directional permeability of digoxin was assessed in the presence and absence of 10 μM test compound. GF120918, cyclosporin and ketoconazole are all identified as very potent inhibitors of P-gp [11] and they all had significant effects on the transport characteristics of digoxin in both direction. In the presence of these inhibitors, the influx permeability (A to B) of digoxin increased significantly along with a significant decrease in the efflux permeability (B to A). Effectively, in the presence of these inhibitors digoxin demonstrated permeability values that were similar in both direction. The efflux pump was completely knocked out and the degree of inhibition observed was greater than

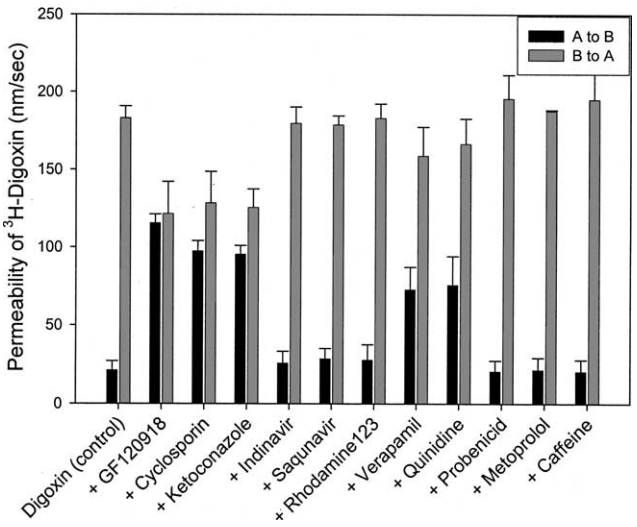

Fig. 5. Effect of various compounds on bi-directional permeability of ^{3}H-digoxin in the 24 well Caco-2 cell transwells. Digoxin concentration was 5 μM and the test compounds were 10 μM in both compartments. Permeability studies were performed at 37 °C for 2 h. Each column represents the mean ± SD of three data points.

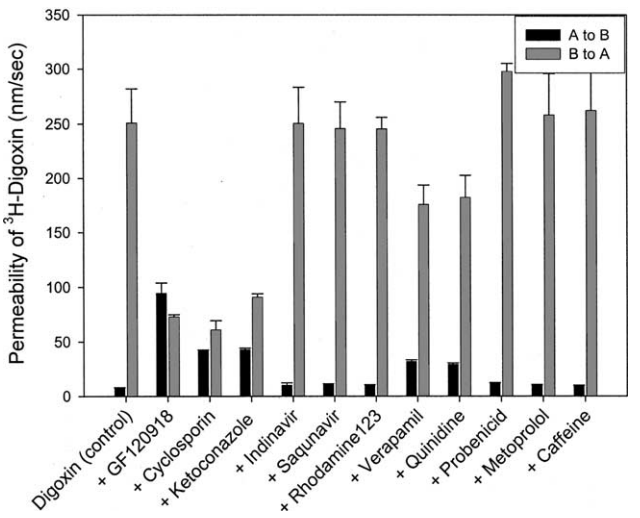

Fig. 6. Effect of various compounds on bi-directional permeability of ^{3}H-digoxin in the 96 well Caco-2 cell transwells. Digoxin concentration was 5 μM and the test compounds were 10 μM in both compartments. Permeability studies were performed at 37 °C for 2 h. Each column represents the mean ± SD of three data points.

80% (ketoconazole and cyclosporin demonstrated 81% inhibition and for GF120918 it was 96%). In the presence of verapamil and quinidine (both good substrates/inhibitor of P-gp) there was again a significant increase in influx permeability and decrease in efflux permeability of digoxin. The degree of inhibition observed for this category of compounds was between 41 and 44%. Compounds such as indinavir, saquinavir and rhodamine123 (normally established to be weak P-gp substrates with K_m values of >20 μM) demonstrated very slight effect on digoxin permeability values thus leading to less than 10% degree of inhibition. Finally, the negative controls used in the assay such as metoprolol, caffeine and probenacid had absolutely no impact on digoxin permeability. Similar digoxin bi-directional studies were also performed using the 96 well Caco-2 format and the data is presented in Fig. 6. Multiple repeats of digoxin study confirmed the vectorial (B to A $\gg$ A to B) nature of its transport across cells. GF120918, cyclosporin and ketoconazole significantly increased the influx permeability of digoxin and simultaneously also decreased the efflux permeability significantly. The degree of inhibition observed for these compounds was 80–100% (ketoconazole 80%; cyclosporin 92%; and GF120918 100%). Verapamil and quinidine demonstrated degree of inhibition between 37 and 41% and weak substrates such as indinavir, saquinavir and rhodamine 123 lead to less than 10% inhibition. Metoprolol, caffeine (transcellular compounds) and probenacid (probable OAT, MRP substrate) had no effect at all on digoxin permeability in the 96 well plates. Thus, it was demonstrated that in spite of the miniaturization the levels of expression of P-gp efflux transporter remained adequate in the 96 well format and the cells can be used for screening compounds that potentially can inhibit P-gp in human. Fig. 7 compares the quality of inhibition data obtained from the various architectures of Caco-2 plate. The quality of inhibition data obtained from the 96 well Caco-2 cell system was equivalent to the 24 well Caco-2 for a diverse set of P-gp substrates/inhibitors and non-substrates. Thus, the 96 well Caco-2 cell transwells with their increased throughput and reduced expenses can be an attractive model in early drug discovery for improved efficiency of P-gp screening.

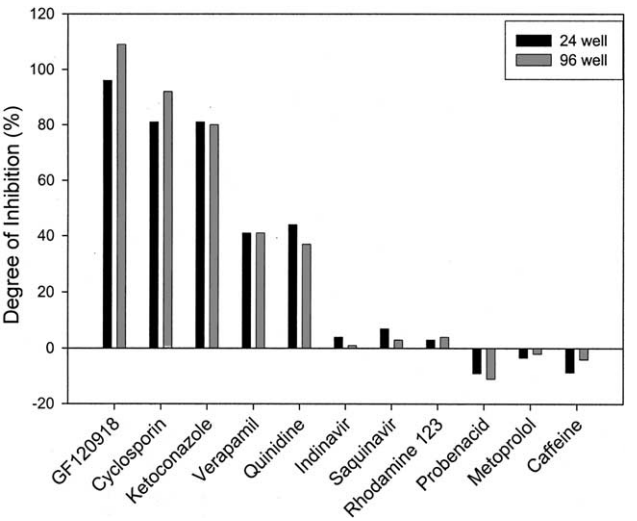

Fig. 7. Degree of inhibition of digoxin transport by various compounds across 24 and 96 wells Caco-2 cell transwells. Digoxin concentration was 5 μM and the test compounds were 10 μM in both compartments. Permeability studies were performed at 37 °C for 2 h. The degree of inhibition was calculated using the formula provided in the text. Each column represents the mean of three data points.

4. Discussions

P-gp is one of the most extensively studied ATP-binding cassette transporter which is known to operate as a defense mechanism by expelling toxic xenobiotics out of the system. It is a ubiquitous transporter which is present on the apical surface of the enterocytes, canalicular membrane of hepatocytes, and on the apical surface of kidney, placenta and endothelial cells of brain membrane. Due to its strategic location in some of these key tissues it is widely recognized that P-gp plays a pivotal role in dictating the pharamcokinetics of a wide array of drug substances. There are ample examples from clinical setting that demonstrate the role played by P-gp in drug absorption, drug distribution (brain, placenta) and drug excretion (kidney and biliary) [12–14]. Direct evidence of P-gp limiting the oral absorption of paclitaxel is provided by in vivo studies performed on double knockout *mdr1a* (−/−) mice [15]. The three fold increase in bioavailability of paclitaxel in the knockout mice clearly demonstrates the role of P-gp in limiting the its oral absorption. In clinical studies, the bioavailability of paclitaxel and docetaxel have been shown to increase significantly on co-administration with cyclosporin A (a well documented inhibitor of MDR1), clearly implicating P-gp as an efflux barrier at the absorption stage. Similarly, P-gp expressed at the blood–brain barrier limits the entry of drugs into the brain. Brain uptake studies performed on amprenavir in mice with or without treatment of PSC 833 or GF120918 (potent and specific inhibitors of P-gp) clearly demonstrated significantly higher accumulation in brain in the absence of P-gp inhibitors [16]. The impact of P-gp in modulating the biliary elimination of compounds is demonstrated by a significant decrease in vincristine's biliary elimination in presence of verapamil (a known P-gp substrate) [17]. Thus, it is clear that P-gp plays a critical role in dictating the pharmacokinetics of a wide array of compounds by impacting them in various stages of their absorption, distribution, metabolism and elimination. Because of its importance in pharmacokinetics, the screening of discovery compounds for their potential to interact with P-gp either as a substrate or as an inhibitor is becoming very critical. The use of digoxin as a probe in the P-gp inhibition studies is supported by the fact that it is a well established P-gp substrate that undergoes minimal metabolism and thus does not have any confounding interactions with metabolizing enzymes [10]. Digoxin has been demonstrated to have significant clinical drug–drug interactions with other potential P-gp inhibitors. A significant increase in absorption and simultaneous decrease in excretion of digoxin was observed on co-administration with quinidine providing evidence of digoxin's clinical interactions with P-gp inhibitors [18]. Another study where PSC 833 (a potent P-gp inhibitor) was co-administered with oral digoxin also demonstrated clinically relevant pharmacokinetic change [19]. Thus, there are several studies with digoxin as a model compound demonstrating significant drug–drug interactions with P-gp substrates and/or inhibitors making it an ideal probe for in vitro P-gp interaction.

The present study highlights the utility of Caco-2 cells grown on 96 well transwells in providing a fast, reliable and accurate screening model for identifying compounds that are P-gp substrates or inhibitors. Traditionally, transport studies performed on 12 well or 24 well transwells have been the work-horse of P-gp screening studies performed in discovery. Since the quality of the data obtained from the 96 well Caco-2 was comparable to the 12 or 24 well Caco-2, the 96 Caco-2 transwells provide a very attractive screening paradigm for identifying compounds that can interact with P-gp.

The bi-directional transport methodology presents a well-established functional assay used to identify P-gp substrates. Though the 96 well Caco-2 works as well as the other architectures, it is quite clear that there are some areas in which the principle of the assay itself fails. First, P-gp substrates that happen to have very high intrinsic permeability values are normally not picked up in the standard bi-directional assay. Verapamil used in this manuscript is such an example. If the efflux ratio (P_c (B to A)/P_c (A to B)) is strictly used, verapamil with a ratio of ~ 1 would be identified as a non-substrate of P-gp. There are several ways to optimize and fine tune this assay to prevent such false negatives. Use of low concentrations of test compound and decreased incubation time can magnify the difference between the directional permeability values and help identify compounds with vectorial transport characteristics better. Studies performed in our laboratory at sub micromolar concentrations of verapamil with a incubation time of 10 min lead to an efflux ratio of >2.5 contrary to ratios of ~ 1 seen in a typical study (50 or 100 μM for 2 h) [8,9]. Lowering the starting concentration as well as shortening the study time prevents any saturation problems with the low capacity P-gp transporter. Thus, the assay can be modified for greater sensitivity, if needed. Second problem with this assay is that P-gp substrates that have very poor intrinsic permeability values also cannot be identified as a substrate with reasonable accuracy. Compounds with poor intrinsic permeability (e.g. famotidine, ranitidine) fail to get into the lipid bilayer and thus cannot be preferentially effluxed out in spite of being P-gp substrates. Alternate models such as binding assays normally avoid this complication. Nevertheless, the cell based bi-directional assay is functional assay and has generally been touted as the model of choice in drug discovery to identify P-gp substrates early on [8]. The validation studies presented here demonstrate that Caco-2 cells grown on 96 well transwells can be used with as much confidence as 12 or 24 well model.

The use of digoxin as a P-gp substrate probe to identify potential inhibitors is also a well established idea. In spite of the fact that there are multiple binding sites both on ATP as well as the P-gp [20], the use of digoxin probe in such assays is the most optimal. The use of radiolabeled digoxin preempts any analytical bottle-neck at the back end of the study

and makes it amenable for high throughput. Studies performed with a set of compounds known to have various levels of inhibition of P-gp and demonstrating that they perform identically in 24 well vs 96 well Caco-2 confirms the utility of 96 well model. The rank ordering of these compounds were consistent across the two architectures thus presenting an effective, accurate and higher throughput model for P-gp inhibition screening.

In summary, a methodology is proposed to increase the productivity and throughput of P-gp screening assays in drug discovery. The use of 96 well Caco-2 cell model provides a fast, sensitive, cost-effective and high-throughput model in early discovery for identifying compounds that can have potential P-gp implications in the clinic.

Acknowledgements

This work was supported by Bristol-Myers Squibb-Pharmaceutical Research Institute, Princeton. Special thanks to Melissa Yarde for providing excellent technical support with cell culture.

References

[1] P. Balimane, S. Chong, R. Morrison, Current methodologies used for evaluation of intestinal permeability and absorption, J. Pharmacol. Toxicol. Methods 44 (2000) 301–312.
[2] S. Chong, S. Dando, K. Soucek, R. Morrison, In vitro permeability through Caco-2 cells is not quantitatively predictive of in vivo absorption for peptide-like drugs absorbed via the dipeptide transporter system, Pharm. Res. 13 (1996) 120–123.
[3] K. Hillgren, A. Kato, R. Borchardt, In vitro systems for studying intestinal drug absorption, Med. Res. Rev. 15 (1995) 83–109.
[4] P. Artursson, Cell cultures as models for drug absorption across the intestinal mucosa, Crit. Rev. Ther. Drug Carrier Syst. 8 (1991) 305–330.
[5] Y. Adachi, H. Suzuki, Y. Sugiyama, Comparative studies on in vitro methods for evaluating in vivo function of MDR1 P-gp, Pharm. Res. 18 (2001) 1660–1668.
[6] R. Stephens, C. O'Neill, A. Warhurst, L. Carlson, M. Rowland, G. Warhurst, Kinetic profiling of P-gp mediated drug efflux in rat and human intestinal epithelia, J. Pharmacol. Exp. Ther. 296 (2001) 584–591.
[7] R. Kim, C. Wendel, B. Leake, M. Cvetkovic, M. Fromm, P. Dempsey, M. Roden, F. Belas, A. Chaudhary, D. Roden, A. Wood, G. Wilkinson, Interrelationship between substrates and inhibitors of human CYP3A and P-gp, Pharm. Res. 16 (1999) 408–414.
[8] J. Polli, S. Wring, J. Humphreys, L. Huang, J. Morgan, L. Webster, C. Serabjit-Singh, Rational use of in vitro P-gp assays in drug discovery, J. Pharmacol. Exp. Ther. 299 (2001) 620–628.
[9] B. Johnson, W. Charman, C. Porter, The impact of P-gp efflux on enterocyte residence time and enterocyte-based metabolism of verapamil, J. Pharm. Pharmacol. 53 (2001) 1611–1619.
[10] M. Sababi, O. Borga, U. Bengtsson, The role of P-gp in limiting intestinal absorption of digoxin in rats, Eur. J. Pharm. Sci. 14 (2001) 21–27.
[11] F. Tang, R. Borchardt, Characteristics of efflux transporter(s) responsible for restricting intestinal mucosa permeation of an acycloxyalkoxy-based cyclic prodrug of the upload peptide DAD-DLE, Pharm. Res. (2002) 19.
[12] J. Lin, Drug–drug interaction mediated by inhibition and induction of P-glycoprotein, Adv. Drug Deliv. Rev. 55 (2003) 53–81.
[13] J. Lin, M. Yamazaki, Role of P-glycoprotein in pharmacokinetics, Clin. Pharmacokinet. 42 (2003) 59–98.
[14] C. Matheny, M. Lamb, K. Brouwer, G. Pollak, Pharmacokinetic and pharmacodynamic implications of P-gp modulation, Pharmacotherapy 21 (2001) 778–796.
[15] A. Sparreboom, J. Asperen, U. Mayer, A. Schinkel, J. Smit, D. Meijer, P. Borst, W. Nooijen, J. Beijen, O. Tellingen, Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-gp in the intestine, Proc. Natl Acad. Sci. USA 94 (1997) 2031–2035.
[16] J. Polli, J. Jerrett, J. Studenberg, J. Humphreys, S. Dennis, K. Brower, J. Wooley, Role of P-gp on CNS disposition of amprenavir, an HIV protease inhibitor, Pharm. Res. 16 (1999) 1206–1212.
[17] T. Watanabe, S. Miyauchi, Y. Sawada, T. Iga, M. Hanano, M. Inaba, Y. Sugiyama, Kinetic analysis of hepatobiliary transport of vincristine in perfused rat liver: possible roles of P-gp in biliary excretion of vincristine, J. Hepatol. 16 (1992) 77–88.
[18] E. Leahey, J. Reiffel, R. Drusin, R. Heissenbuttel, W. Lovejoy, J. Bigger, Interaction between quinidine and digoxin, J. Am. Med. Assoc. 240 (1978) 533–534.
[19] J. Kovarik, L. Rigaudy, M. Guerret, C. Gerbeau, K. Rost, Longitudnal assessment of a P-gp mediated drug interaction of valspodar on digoxin, Clin. Pharm. Ther. 66 (1999) 391–400.
[20] S. Ekin, R. Kim, B. Leake, A. Dantzig, E. Schuetz, L. Lan, K. Yasuda, R. Shepard, M. Winter, J. Schuetz, J. Wikel, S. Wrighton, Application of three dimensional quantitative structure activity relationship of P-gp inhibitors and substrates, Mol. Pharm. 61 (2002) 974–981.