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Co-treatment with grapefruit juice inhibits while chronic administration activates intestinal P-glycoprotein-mediated drug efflux

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P-Glycoprotein (P-gp) mediated efflux is recognized as a significant biochemical barrier affecting oral absorption for a number of drugs. Various conflicting reports have been published regarding the effects of grapefruit juice (GFJ) on P-gp-mediated drug efflux, in which GFJ has been shown both to inhibit and activate it. Hence, the present study adopted a two-way approach, involving both co-treatment and chronic administration. Bi-directional transport of paclitaxel (PCL) was carried out in the absence and presence of GFJ extract, in rat everted ileum sac. Further, the effect of chronic administration of GFJ to rats was characterized by permeability studies with indinavir (INDI). Co-treatment of GFJ extract at 100% concentration reduced the asymmetric transport of PCL (efflux ratio $= 20.8$) by increasing absorptive ($A \rightarrow B$) transport by 921% and reducing secretory ($B \rightarrow A$) transport by 41%. Further, GFJ showed a concentration dependent effect on PCL permeability. Imipramine, a passive permeability marker with absorptive permeability of $15.33 \pm 4.26 \times 10^{-6}$ cm/s showed no asymmetric transport and also no significant ($P < 0.05$) change in permeability in the presence of GFJ. Chronic administration of GFJ resulted in a significant decrease in absorptive transport of indinavir, which was even greater than that produced by rifampicin pretreatment. No change in permeability of propranolol, a passive permeability marker, was observed. Further, the decrease in absorptive transport of INDI was reversed by the P-gp inhibitor verapamil. In conclusion, GFJ extract inhibited P-gp-mediated efflux in co-treatment, whereas chronic administration led to increased levels of P-gp expression, thus having a profound effect on intestinal absorption and GFJ-drug interactions *in vivo*.

1. Introduction

The number of drugs found to exhibit interactions with grapefruit juice (GFJ) has been increasing since the discovery of its effect on the bioavailability of felodipine in 1991 (Bailey et al. 1991). Reasons for this kind of effect were proposed to be down-regulation of proteins responsible for the biochemical barrier present in the gastrointestinal tract in the form of CYP 3A4 (cytochrome P 450 3A) and P-gp (P-glycoprotein) (Langguth and Langguth 2001). Initially, compounds known to interact with GFJ belonged to the categories of dihydropyridine calcium antagonists, immunosuppressants, benzodiazepines, antihistamines and others; most of which were substrates to CYP 3A. Therefore, the mechanism proposed for such a drug-food interaction was the enzyme inhibitory activity of components present in GFJ (Schmiedlin-Ren et al. 1997). However, recent reports have indicated that absorption of drugs like saquinavir, digoxin, vinblastine, talinolol, rhodamine123 and fexofenadine has been affected by GFJ, though they are not metabolized by CYP 3A (Tian et al. 2002). This suggests the role of transport proteins, P-gp, as responsible for such interactions.

P-gp is an ATP-dependent efflux membrane transporter apically expressed throughout the GIT and acting as a major biochemical barrier in limiting oral bioavailability (Varma et al. 2003). P-gp is present in a broad spectrum of tissues viz., biliary canalicular surface of hepatocytes, luminal surface of cells of jejunum and colon, apical surface of proximal tubular cells of kidney, endothelial cells of blood brain barrier, apical membrane of fetal-membrane barrier function in placenta and in other organs such as lungs, adrenals, prostate, skin, spleen, heart and skeletal muscle. P-gp at these sites can be viewed as a barrier, which prevents entry of xenobiotics into the body or, removes them once they have entered, protects cells and keeps toxic substances into circulation, especially when certain tissues are sensitive to adverse effects (Lin 2003). The clinical significance of P-gp mediated efflux can be appreciated from the fact that it affects each and every step at which a drug is transported during its stay in the body. It influences absorption through intestinal carriers, which expel drug molecules back into the lumen; distribution, by preventing entry into tissues like the brain; metabolism, as it acts synergistically with CYP 3A; excretion,

by affecting both biliary and renal tubular function (Ambudkar et al. 1999). Overall, P-gp has serious implications for deciding a drug's pharmacokinetics and its delivery to target tissues like brain and hematopoietic cells.

Various studies have been done with the purpose of elucidating the proposed concerted role of CYP3A and P-gp in producing GFJ effects, though the relative contributions of two differ depend on the type of substrate. With cyclosporin A, there was found to be a 55% increase in area under the curve (AUC) and a 35% increase in time of maximum absorption (T_{max}) when GFJ was administered (Edwards et al. 1999). The role of P-gp was proved from the observation that no clinical effects were produced with orange juice although it contained CYP inhibitory components. Uptake experiments of vinblastine into Caco-2 also proved that GFJ ethyl acetate extract contains specific inhibitors of P-gp while on the other hand, methanolic extract has CYP inhibitory components (Ohnishi et al. 2000). Observations supporting this hypothesis have also been reported with dextromethorphan (Marco et al. 2002) and saquinavir (Kupferschmidt et al. 1998). However, these studies could not serve the purpose of apportioning the CYP and P-gp effects as the compounds selected had overlapping substrate specificity. Rhodamine123, fexofenadine and saquinavir were selected and the inhibitory effect on P-gp mediated efflux was demonstrated in rat jejunum and ileum (Tian et al. 2002). Daunorubicin, a marker of active transport function, showed a decrease in fluorescence with GFJ in overexpressed cell-lines (Wang et al. 2001). Also, concentration dependent P-gp inhibition occurred on digoxin transport in Caco-2 cell monolayers (Xu et al. 2003). In contrast to these studies that proved an inhibitory effect with GFJ, Soldner et al. have demonstrated activation of transport with vinblastine, cyclosporin A, losartan (both CYP 3A and P-gp substrates); digoxin, fexofenadine (P-gp substrates only); and no effect on nifedipine or felodipine (primary CYP 3A substrates) (Soldner et al. 1999). Similarly, a 13% decrease in mean AUC and a 5% decrease in peak plasma concentration values of celiprolol when administered with GFJ was reported. The mechanism sought involved physico-chemical factors in addition to the role of active transporters (Lilja et al. 2003).

It can thus be hypothesised that the reason for such inconsistent effects could be a difference in duration of exposure to GFJ. In the light of these contradictory reports a two-way approach, involving both co-treatment and chronic administration, was adopted in the present study. The objective of this work was to investigate the potential effects of GFJ on bi-directional transport in a rat-everted sac model via both pre-treatment and co-treatment. Determination of absorptive and secretory transport served as a measure of net permeation, which is ultimately a determinant of bioavailability. The dose-dependent effect of GFJ was also studied and compared with respect to verapamil, a potent P-gp inhibitor. Pre-treatment studies were performed with a view to finding the therapeutic relevance of these drug-drug or drug-food interactions. Rifampicin, a known P-gp inducer served as a positive control (Sandstrom and Lennernas 1999).

2 Investigations and results

2.1. Bi-directional transport of PCL

PCL is an established probe for evaluating P-gp mediated drug transport (Sparreboom et al. 1997). Absorptive permeability ($P_{app,\,A\rightarrow B}$) of PCL is $0.43 \pm 0.13 \times 10^{-6}$ cm/s and the secretory permeability ($P_{app,\,B\rightarrow A}$) is $8.90 \pm 0.59 \times 10^{-6}$ cm/s. Transport studies in the presence of verapamil showed the absorptive permeability increased to $3.15 \pm 0.71 \times 10^{-6}$ cm/s and the secretory permeability reduced to $4.13 \pm 0.36 \times 10^{-6}$ cm/s. Fig. 1 shows the asymmetric transport of PCL, which was reduced by the P-gp inhibitor, verapamil (200 μM). Functional P-gp transporters in the apical membrane of the intestine secrete intracellular PCL back into the apical chamber, which retards drug permeation in the absorptive direction but facilitates drug transport in the secretory direction, with the consequence that PCL exhibits an ER much greater than 1 (ER = 20.8). Here verapamil, a positive control for P-gp inhibition, suppressed the secretion of PCL. This caused A → B PCL flux to increase and the B → A flux to fall, thereby equalizing the P_{app} in both directions (ER ∼ 1). GFJ extract showed a similar effect to that of verapamil in bi-directional transport studies indicating that GFJ has a profound effect on the intestinal permeability of P-gp substrates (Fig. 1).

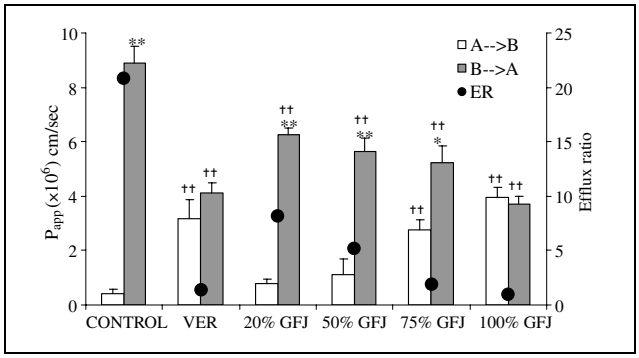

Fig. 1: Influence of grapefruit juice (GFJ) concentration on absorptive (A → B) and secretory (B → A) transport of PCL (20 μM), with respect to verapamil (VER, 200 μM), across rat ileum.
Ethyl acetate extract of GFJ in escalating concentrations was used for this study. Details are provided in methods. Secondary axis shows the efflux ratio (ER) of each determination. Data represented as mean ± SEM of three experiments. $^{**}P < 0.01$; $^{*}P < 0.05$, with respect to corresponding absorptive permeability, $^{\dagger\dagger}P < 0.01$; $^{\dagger}P < 0.05$, with respect to control in the same transport direction

2.2. Dose-dependent effect of GFJ extract on PCL transport

The effect of GFJ extract on the transepithelial flux of PCL in both directions was studied at four levels of extract concentration (20–100%). GFJ extract showed a concentration dependent inhibitory response which is consistent with the results of Xu et al. (2003). The increase in the A → B transport can be linearly correlated with juice concentration, suggesting that there is concentration dependent inhibition of P-gp (Fig. 1). As the concentration of GFJ increased, an increase in absorptive permeability and decrease in secretory permeability was observed. The absorptive permeability was increased by 921% of the control, whereas secretory transport was reduced to 41% of control, at 100% GFJ extract concentration. It is also obvious from the figure that GFJ extract at 100% concentration showed bi-directional permeabilities similar to those in the presence of verapamil.

Using $P_{app,\,A\rightarrow B}$ and $P_{app,\,B\rightarrow A}$ in the presence of verapamil as P_{PD} for the corresponding directions, AQ and SQ values of PCL were calculated for all the concentrations of GFJ (Fig. 2). AQ values indicated that the component of P-gp responsible for decreasing absorptive passive diffusion is reduced from 0.86 to 0.12 over the concentration

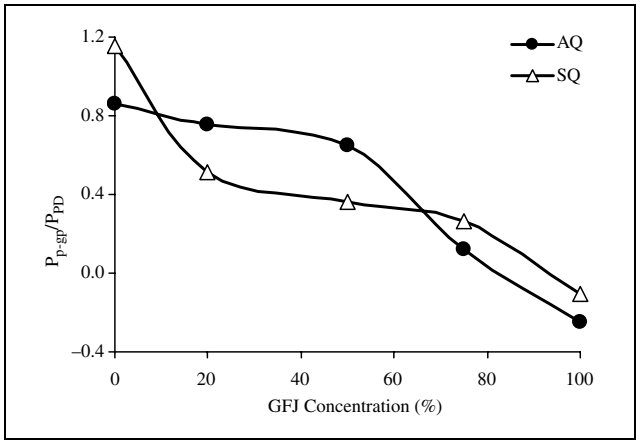

Fig. 2: Absorptive quotient (AQ) and secretory quotient (SQ) of [^{14}C]-paclitaxel across rat ileum as a function of grapefruit juice (GFJ) extract concentration present during drug transport. AQ and SQ calculated in accordance with the equation explained in section 2

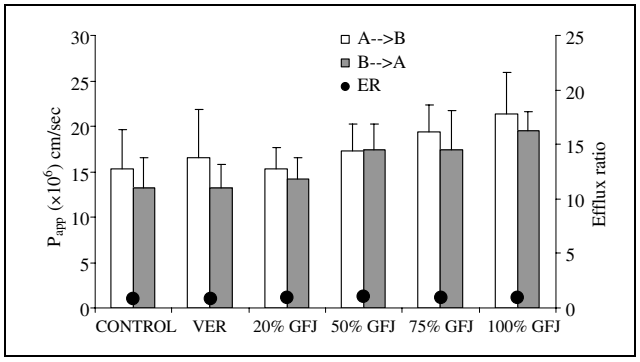

Fig. 3: Effect of P-gp inhibitor verapamil (200 μM) and GFJ extract at various concentrations on permeability of imipramine

range of 0–75% GFJ extract. This indicates that 86% of PCL passive transport is being attenuated by P-gp, which in the presence of 75% GFJ extract was reduced to only 12% attenuation. Reduction in SQ was also noted, from 1.15 to 0.26. Overall, these data indicated that GFJ reduced transport attenuation by P-gp.

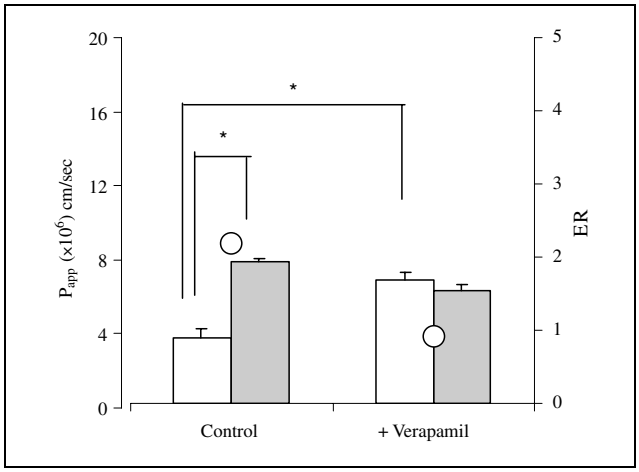

Fig. 4: Bi-directional transport of indinavir across rat ileum without and with inhibitor (verapamil 200 μM). A → B permeability (open bars) and B → A permeability (crossed bars). Secondary axis represents the Efflux Ratio (ER, open circles). Inhibitor solutions applied to apical side and amount permeated into receptor compartment measured at indicated time points. Each value is the mean ± SEM of three determinations. $^*P < 0.05$; Efflux ratio (ER) is the ratio of $P_{app,\,B\to A}/P_{app,\,A\to B}$

Permeability of the passively transported marker imipramine was monitored along with PCL to check the effect of GFJ components on the membrane bilayer and to check the integrity of the intestine. No significant change in the permeability of imipramine was observed; however, a small increase was observed as the GFJ extract concentration increased (Fig. 3).

2.3. Inhibition of indinarir efflux with verapamil

Indinarir showed asymmetric bi-directional permeability with $P_{app,\,A\to B}$ $3.57 \pm 0.47 \times 10^{-6}$ cm/s and $P_{app,\,B\to A}$ $7.78 \pm 0.18 \times 10^{-6}$ cm/s. The inhibition studies performed showed that verapamil is effective in blocking the asymmetric bi-directional transport of INDI (Fig. 4). Verapamil added at 200 μM concentration significantly inhibited INDI B → A transport and improved A → B transport. There was a 20.26% decrease in secretory permeability whereas

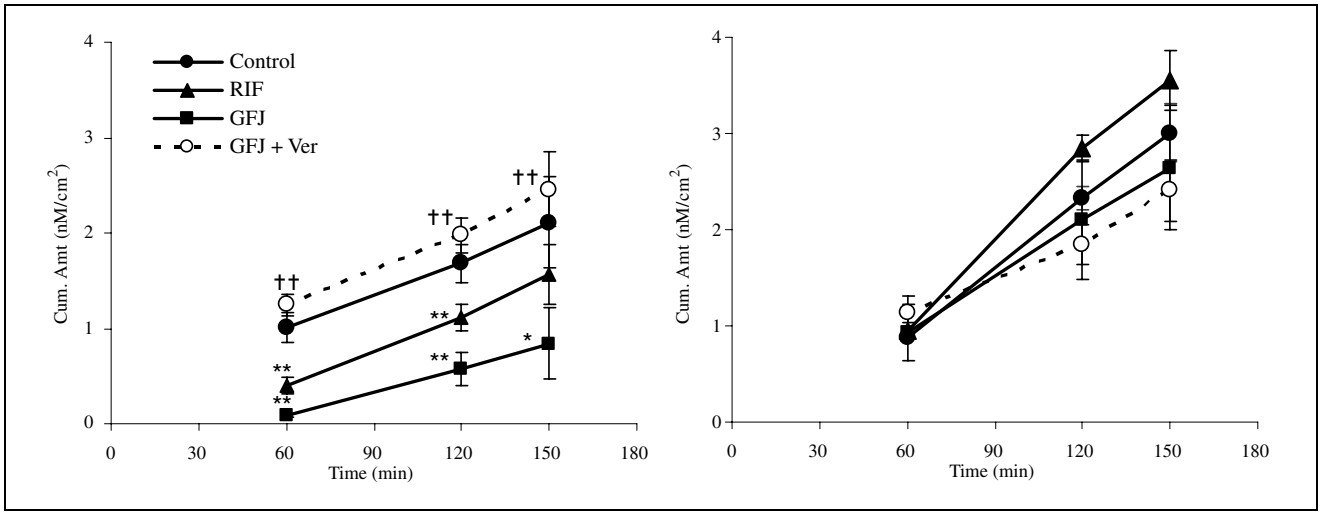

Fig. 5: Cumulative amount of indinavir transported in (a) A → B direction and (b) B → A direction in everted sac obtained from rats pretreated with rifampicin (RIF) and GFJ. SD rats (300 gm) pretreated for 7 days with either RIF or GFJ and permeability characterization carried out after 2 days washout period (See methods). Control animals received water by oral gavage. $^{**}P < 0.01$; $^*P < 0.05$, one-way ANOVA with respect to control, $^{\dagger\dagger}P < 0.01$ one-way ANOVA with respect to GFJ pretreatment

an increase of 88.37% in absorptive permeability was observed. A change of 29% in the cumulative amount transported in the basal to apical direction and an enhancement of 43.49% in the absorptive direction was also noted.

2.4. Effect of pretreatment with rifampicin and GFJ extract on INDI transport

Both GFJ and rifampicin caused induction of P-gp as was evident from a decrease in the cumulative amount of drug permeated in the absorptive (A → B) direction (Fig. 5a). However, as seen in Fig. 5b, no significant difference was observed for the amount of INDI permeated in the secretory direction when compared to control, after chronic administration both of rifampicin and GFJ. The permeability ($15.21 \pm 2.36 \times 10^{-6}$ cm/s) of propranolol, a passively transported marker, showed no difference (±10% deviation) in the absence and presence of verapamil with both untreated and treated animal intestinal sac. This indicates the integrity of the model and lack of effect of GFJ extract on the passive permeability of the drugs.

Absorption transport studies of PCL in the presence of verapamil with ileum segments obtained from animals pretreated with GFJ extract showed the cumulative amount permeated was more than that of control, indicating complete inhibition of P-gp-mediated efflux. However, no significant change in secretory transport was observed. Overall, these data indicated that the effect produced by chronic administration of GFJ extract to live animals is due to the induction/overexpression of P-gp on the apical surface of the intestine.

3. Discussion

The rat has been shown to be a good predictor of human intestinal absorption (Chiou and Barve 1998) and rat-everted intestine sac is regarded as a reliable model to investigate drug transport across the gastrointestinal tract. This model also shows P-gp activity that corresponds well with that assessed *in vivo* (Tian et al. 2002; Yumoto et al. 1999). Drug efflux mediated by P-gp is reported to increase from the proximal to distal intestine in mice, rats and humans thus demonstrateing maximum P-gp activity (Stephens et al. 2002). Considering all these factors, we used a rat everted sac model to evaluate the effect of co-treatment and chronic treatment of GFJ extract on the functional activity of P-gp.

The present study used two compounds known to interact with P-gp, PCL and INDI, to investigate the effect of GFJ on P-gp-mediated transport. Large increase in PCL permeability was observed between absorptive and secretory permeabilities. In contrast, PCL secretion was completely abolished in the presence of verapamil (200 μM), a known P-gp inhibitor (Fig. 1). These data confirm P-gp as the sole mediator of PCL secretion in the ileum. To determine whether the differences in permeability unmasked by P-gp inhibition are also seen with other drugs, bi-directional transport of an HIV protease inhibitor, INDI was studied both in the absence and presence of a P-gp inhibitor (Fig. 4). The effect of P-gp on indinavir transport was minimal with an efflux ratio ∼2, which however was reduced to ∼1 in the presence of the P-gp inhibitor verapamil (200 μM).

PCL absorptive permeability was significantly increased by GFJ extract at 100% concentration while secretory permeability was decreased. Further, GFJ extract showed a concentration dependent effect on the transport of PCL. Quantification of P-gp activity (AQ and SA) showed that GFJ has a concentration dependent inhibitory effect on drug efflux in both the absorptive and secretory directions (Fig. 2). The abolition of PCL secretion in the presence of GFJ extract confirms the ability of components of GFJ to interact with P-gp, the modulating the intestinal transport process. The increased absorption transport and the decreased secretory transport were not caused by cell damage or bilayer disruption, as no change in the permeability of the passive permeability marker imipramine was observed (Fig. 3).

Chronic administration of GFJ extract in live rats and evaluation of P-gp activity indicated that on long exposure GFJ tends to activate P-gp and thus increase P-gp-mediated transport. A distinct change in the absorptive permeability of INDI was observed (Fig. 5a). However, no significant change was observed in secretory transport (Fig. 5b). The absorptive transport of INDI increased much more markedly resulting in a significant and apparent increase in net secretion. This activating effect of GFJ extract on P-gp is inhibitable, since the increased net secretion due to GFJ exposure was neutralized and completely abolished in the inhibition studies, as we observed that absorptive permeability of INDI in the presence of verapamil is similar to that in the control. It is interesting to observe that the effect of GFJ is confined to the absorptive direction and no significant change in permeability characteristics of INDI was shown in the secretory direction. However, this is consistent with bi-directional transport studies of INDI, where inhibition of P-gp by verapamil had a minimal effect on secretory permeability (Fig. 4).

Previous studies have demonstrated that recurrent GFJ ingestion resulted in increased oral bioavailability of saquinavir and felodipine (Lown et al. 1997), with the mechanism being due to selective loss of CYP 3A4 protein from small intestinal epithelial cells with no changes in P-gp expression levels. There was found to be dose-dependent inactivation (suicidal inhibition) of CYP 3A4 from accelerated degradation of the enzyme due to the furanocoumarins present in GFJ. However, these studies do not rule out the possible involvement of P-gp, as the modulatory effect produced is substrate, duration and concentration dependent. Several hypotheses have been proposed to account for the different kinds of effects produced by GFJ. Our approach of investigating potential P-gp-mediated GFJ-drug interactions, clearly revealed that GFJ co-treatment has a P-gp inhibitory role as it interacts with P-gp and blocks the transport process, while chronic administration of GFJ shows P-gp activation as a result of increased P-gp expression.

4. Experimental

4.1. Materials

^{14}C Paclitaxel (52.3 mCi/mmol) and ^{3}H-Imipramine were purchased from Sigma Chemical Co. (MO, USA). Verapamil, flurescein sodium and imipramine HCl were from Sigma Co. (MO, USA). Indinavir, paclitaxel, propranolol, cyclosporin and rifampicin were kindly provided by Matrix Labs (Hyderabad, India), Dabur India Ltd. (New Delhi, India), Sun Pharma Ltd. (Vadodara, India), Dabur Research Foundation (New Delhi, India) and Macleods Pharmaceuticals (Mumbai, India), respectively. Grapefruit juice was obtained from Berry Ltd., Australia. All the solvents used were of HPLC grade (J. T. Baker, Mexico) and reagents were of analytical grade. Purified water obtained by reverse osmosis (USF ELGA) filtered through a 0.45 μm membrane filter was used for chromatographic analysis.

4.2. Extraction of GFJ

An aliquot of 250 ml of GFJ was mixed with 500 ml of ethyl acetate and shaken vigorously for 10 min by hand. The organic phase was separated

and evaporated to dryness in a Rotavapour at 45 °C. The residue was dissolved in 1 ml of dimethyl sulfoxide (DMSO) and different concentrations were prepared (i.e. 100% GFJ extract indicates 1 ml of reconstituted residue dissolved in 250 ml of medium), keeping the final DMSO concentration less than 1% (Tian et al. 2002).

4.3. Rat-everted sac studies

4.3.1. Surgical procedure

All animal studies were done according to the guidelines of the Institutional Animal Ethics Committee (IAEC) of the National Institute of Pharmaceutical Education and Research (NIPER), Punjab. Male Sprague Dawley rats (230–250 g) were housed under standard laboratory conditions and fasted for 16 h before the experiment with water *ad libitum*. After a mild anesthesia with urethane (1.5 mg/kg), they were sacrificed by cervical dislocation. The abdomen was cut open along the mesenteric border. Intestinal tissue from the region of interest was then immediately removed and the lumen flushed with ice-cold bicarbonate-buffered Kreb's Ringer solution (KRB) containing NaCl (7.0 g), KCl (0.35 g), $CaCl_2$ (0.28 g), $MgSO_4$ (0.28 g), $NaHCO_3$ (2.10 g), KH_2PO_4 (0.16 g) and D-glucose (5.05 g), with continuous bubbling of 5% CO_2/95% O_2.
Intestinal ileum segments (approximately 5 cm above the ileoceceal junction) were washed with cold KRB solution. Everted sac preparation was performed as reported earlier (Sharma et al. 2002). The everted sac was then suspended in 10 ml of preoxygenated KRB without the drug or inhibitor. 3 ml of KRB was placed in the serosal compartment. After incubation for 15 min, KRB on either side (mucosal or serosal) was replaced by KRB solution with or without drug or inhibitor. The drug – containing compartment was referred to as the donor compartment and the other as the receptor compartment. Throughout the experiment, the sac was maintained at 37 °C and gas (5% CO_2/95% O_2) was circulated continuously. Integrity of the sac was assessed using propranolol or imipramine.

4.3.2. Transport, efflux and inhibition studies

For investigation of apical-to-basal (A → B) transport, drug solution in KRB was placed on the mucosal side and for investigation of basal-to-apical (B → A) transport the drug was placed on the serosal side. The concentrations employed were 100 μM for propranolol; 100 μM, imipramine; 20 μM, paclitaxel (PCL); and 50 μM, indinavir (INDI). Inhibition of A → B and B → A transport of P-gp substrates was investigated in the intestinal segments in the presence of a known inhibitor, verapamil (200 μM). During inhibition studies, the inhibitor in the specified concentration was placed in the apical chamber. These inhibitors acted as positive controls that helped in the evaluation of the effect produced by GFJ.

4.4. Pre-treatment of rats with P-gp modulators

Prior to the everted sac permeability studies, male Sprague Dawley rats (body weight, 200–230 g) were treated for 7 days with the selected agents. Rats received either 100% GFJ extract (2 ml, p.o., b.i.d), or rifampicin (10 mg/kg/d, p.o.), or just 2 ml of water (placebo). Doses were selected on the basis of therapeutic doses given to man. Rifampicin was given as a positive control as it has been reported to induce expression of P-gp (Hanafy et al. 2001). Following a washout period of two days, everted-sac studies were performed as already described which involved determination of the cumulative amount transported and the effective permeability of indinavir. An aliquot of 1.0 ml was sampled from the receptor phase at the indicated time points and replaced with the same volume of buffer. The collected samples were analyzed by an appropriate bioanalytical method.

4.5. Bio-analytical assays

4.5.1. High Performance Liquid Chromatography for INDI

Reversed phase high performance liquid chromatography (RP-HPLC) with UV detection was used for quantitative estimation of INDI and propranolol concentrations in samples obtained from *ex vivo* intestinal sac experiments (Panchagnula et al. 2004). Shimadzu liquid chromatographic system comprising an LC-10AT VP solvent pump, DGU-14AM on line-degasser, SIL-10AD VP autoinjector with temperature control, CTO-10AS VP column oven and SPD-10AVP UV-Vis spectrophotometric detector was used. Mobile phase consisting of 50 mM phosphate buffer (pH 5.0) and acetonitrile (62 : 38) was pumped in isocratic mode at a flow rate of 1 ml/min at ambient temperature. The analytical wavelength was set at 210 nm. Sample preparation involved addition of 1 ml of ACN to 500 μl of sample, followed by centrifugation at 13000 rpm for 30 min. A fixed volume (1.4 ml) of supernatant was taken into another micro centrifuge tube and evaporated to dryness in a Centrivac (Maxi dry lyo, Denmark). The residue thus obtained was reconstituted with 200 μl of mobile phase and 50 μl was injected.

4.5.2. Radio-chemical analysis for PCL

For radioactivity measurements, 400 μl of each sample was mixed with 3 ml of scintillation cocktail (Amersham, U.K), in diffuse plastic scintillation vials (Tarson, India) and counts were taken for 3 min in a liquid scintillation counter (Wallac, Finland).

4.6. Calculations and statistical analysis

4.6.1. Efflux ratio and intestinal permeability

Results can be expressed as transepithelial apparent permeability (P_{app}) in cm/s, given by:

$$P_{app} = (dQ/dT)/C_0^* A \quad (1)$$

where dQ/dT is the rate of appearance of compound in the receptor chamber, C_0 is the substrate concentration in the donor chamber and A is the cross-sectional area of the tissue (Stephens et al. 2002).
The efflux ratios (ER) were calculated using the following equation:

$$P_{ER} = P_{app,\,A\to B}/P_{app,\,B\to A} \quad (2)$$

Where ER is efflux ratio, $P_{app,\,A\to B}$ is effective permeability in A → B direction and $P_{app,\,B\to A}$ permeability in B → A direction.

4.6.2 Absorptive quotient (AQ) and secretory quotient (SQ)

Inhibition studies allowed the calculation of the kinetic parameters, absorptive quotient (AQ) and secretory quotient (SQ), which reflect the extent to which P-gp affects absorptive and secretory transport respectively. These parameters illustrate explicitly the asymmetric effect of P-gp on efflux and influx of a compound. The various parameters were deduced from the following equations:

$$P_{app,\,A\to B} = P_{PD,\,A\to B} - P_{P\text{-}gp,\,A\to B} \quad (3)$$

$$P_{app,\,B\to A} = P_{PD,\,B\to A} + P_{P\text{-}gp,\,B\to A} \quad (4)$$

Where, $P_{app,A\to B}$ and $P_{app,B\to A}$ are the permeabilities in the absorptive and secretory directions, respectively. P_{PD} is the passive diffusion component of absorptive and secretory transport mediated by P-gp efflux.
AQ and SQ values were calculated as below (Troutman and Thakker 2003b):

$$AQ = P_{P\text{-}gp,\,A\to B}/P_{PD,\,A\to B} \quad (5)$$

$$SQ = P_{P\text{-}gp,\,B\to A}/P_{PD,\,B\to A} \quad (6)$$

$P_{P\text{-}gp,\,B\to A}$ and $P_{P\text{-}gp,\,A\to B}$ denote permeability due to P-gp mediated efflux in the secretory and absorptive directions, respectively.

4.6.3. Statistical tests

Data were reported as mean ± standard error of mean (SEM) unless otherwise noted. One-way ANOVA were performed on permeability data. A P-value of 0.05 was used as the significance level for all tests. All statistical tests were performed using Jandel Sigma stat version 2.0.

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