

The effect of diltiazem at different concentrations on propranolol intestinal absorption in rats[†]

Issam Abushammala^{1,*}, Hannan Fayyad¹, Ihab El-masri², Mohammed Taleb¹,
Ahmed Elquedra² & Elham Abuwaked¹

¹ Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Gaza, Palestine; ² Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Gaza, Palestine.

*Corresponding author: i.shammala@alazhar.edu.ps

Received: (14/6/2020), Accepted: (24/9/2020)

ABSTRACT

Propranolol is a synthetic β -adrenergic receptor-blocking drug. After oral administration, propranolol has complete and rapid gastrointestinal absorption. The bioavailability of propranolol is approximately 25%. Propranolol may be a substrate for a distributed P-glycoprotein (P-gp), which might lead to the efflux the drug back to the lumen of the intestine. The aim of this study was to investigate the influence of Diltiazem as P-gp inhibitor at different concentrations on intestinal absorption of Propranolol, namely on absorption rate constant (K_a). Single Pass Intestinal Perfusion (SPIP) technique in rats was conducted on three groups of Wistar albino rats (n=6 per group). The first group was perfused with Propranolol HCl (75 $\mu\text{g/mL}$) alone, meanwhile the second and the third groups were perfused with Propranolol HCl (75 $\mu\text{g/mL}$) in presence of Diltiazem HCl (250 and 1000 $\mu\text{g/mL}$) respectively. The whole small intestinal segment of anaesthetized rats was cannulated and perfused with Propranolol HCl in normal saline at 37 °C in the absence and the presence of Diltiazem HCl. Intestinal samples were taken from outlet tubing of small intestine at different time intervals and analyzed using a simple, rapid and validated spectrophotometric method. The K_a values of the drug for the rats in the three groups were calculated. The mean K_a values of Propranolol HCl in the first group was $0.81 \pm 0.014 \text{ hr}^{-1}$, meanwhile, K_a values of Propranolol HCl in the second and third groups (250 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$) were $0.778 \pm 0.012 \text{ hr}^{-1}$ and $0.857 \pm 0.030 \text{ hr}^{-1}$ respectively. Statistically, insignificant differences were found when the three groups were compared with ($P > 0.05$). The expected explanation for the lack effect of Diltiazem as P-gp inhibitor at both concentrations on the absorption constant rate of Propranolol is that P-gp plays a minimal role in the in situ intestinal absorption process of Propranolol HCl.

Keywords: Propranolol, Absorption Rate Constant, Diltiazem, P-Glycoprotein.

[†] This paper was extracted from a master's thesis in pharmacy by student Hannan Fayyad entitled " The Effect of Diltiazem on Propranolol Absorption by Using in situ Single-Pass Intestinal Perfusion Technique in Rats ", which was defended on 03/07/2017.

INTRODUCTION

β -blockers have been used for a tremendous array of indications such as the treatment of hypertension, angina pectoris, and cardiac arrhythmias, as well as for a number of other indications including migraine, hyperthyroidism and tremors [1].

According to the Biopharmaceutical Classification System (BCS), the drug absorption is classified on the basis of solubility and membrane permeability [2]. P-gp, multi-drug resistance protein 1 (MDR1), is an efflux transporter which is expressed in different barriers such as intestine, liver, kidneys,

brain, testis, placenta and lung and it is also found in ocular tissue [3-5]. P-gp is the major efflux transporter protein, responsible for reduced absorption of many drugs [6].

Propranolol, a β -blocker drug, is considered one of characterized P-gp substrate [7-9]. Propranolol was the first prototype drug for β -blockers and it is still used over the world [1]. Propranolol pharmacokinetics have been studied quite extensively [10]. The possibility of Propranolol transport by P-gp in intestine playing a significant role in its pharmacokinetics by extruding it from their intended site of action [7].

Absorption is a complex kinetics process that is dependent on many physiological, physicochemical and dosage form factors. Ho and co-authors announced that, the absorption characteristics of a drug are determined by the physicochemical properties of the drug as well as by the bio-physicochemical properties of the gastrointestinal barrier membrane [11].

Limited drug delivery to the systemic circulation which is a common cause of decreased absorption of Propranolol, may be due to the over expression of adenosine triphosphate (ATP)-driven drug efflux pumps at the intestine barrier like P-gp.

Specific inhibition of P-gp with verapamil significantly improves the absorption of Propranolol; therefore, Propranolol combination with P-gp inhibitors may be a promising therapeutic strategy to improve the gastrointestinal drugs absorption [12].

Several well-established methods are available to determine K_a , among these methods, as Single Pass Intestinal Perfusion (SPIP) technique. The *in-situ* rat intestinal perfusion technique is a commonly used for the assessment of permeability of drugs, K_a and the functional role of permeability (P-gp) on kinetics of drug transport through the whole intestine [13-15].

Diltiazem was identified as P-gp inhibitor, which could be setting off an opportunity in improving absorption of P-gp substrate by inhibiting P-gp in intestine [16].

The objective of present research study is to investigate the influence of Diltiazem as P-gp inhibitor at different concentrations on the intestinal absorption of Propranolol, namely on absorption rate constant.

MATERIALS AND METHODS

Chemicals and Instrumentation

Propranolol HCl and Diltiazem HCl standards were purchased from Sigma-Aldrich Company. Normal saline (0.9 % w/v) was obtained from B. Braun Melsungen AG (Germany). Thiopental sodium (500 mg vial) was obtained from Rotex medica (Germany). Shimadzu double beam UV-VIS spectrophotometer (UV-1601) was used. Centrifugation was made with Kokusan (H-103N) Series Centrifuge.

Animals and Study Design

Eighteen healthy Wistar albino male rats (weighted: 250-300 g, 7-9 weeks aged) were purchased from Center of Experimental Animals, Harlan Laboratories (Jerusalem). Animals were housed 4 per cage in an air-conditioned room under constant temperature ($22 \pm 2^\circ\text{C}$) with free access to food and drinking water [17]. Rats were maintained on a 12 h light-dark cycle [18]. The normal life conditions for the animals were kept based on the International Animal Ethics Committee. All experiments with rats were conducted according to the Canadian guide for the use of laboratory animals [19].

An *in-situ* intestinal perfusion procedures were performed in rats according to the methods described previously [20-22]. Rats had been fasted for 12-18 h before experiment with free access to water (*Ad Lipitum*). Then they were anaesthetized by administration of an intraperitoneal thiopental (50 mg/kg). Anaesthetized rats were placed on the fixing plate under a heating lamp keeping a normal body temperature (37°C) of the rats during all experiments. The surgical procedure was initialized by a midline abdominal incision of approximately 10 cm to expose small intestine and, then two L-shaped cannulas were inserted carefully through small narrow open at the initiate of duodenum and end of ileum. The cannulas were secured by ligation with silk suture and the biliary duct was also ligated. Then, the small intestine was returned to abdominal cavity to maintain its integrity. The intestinal lumen was rinsed using a syringe containing normal saline (37°C) that pumped slowly through the gut *via* the inlet duodenum cannula and out the ileal cannula until the effluent solution was clear and free of feces. After cleaning the intestine, the remaining perfused solution was expelled from the intestine by air pumped *via* syringe and 10 mL of drug solution was immediately introduced into the small intestine segment by the syringe. In the first group 10 mL prepared solution, containing Propranolol alone at concentration (75 $\mu\text{g/mL}$) in normal saline (0.9% w/v) was perfused into small intestine segment of six rats. The second and third groups of rats were perfused with 10 mL solution containing Propranolol (75 $\mu\text{g/mL}$) in combination with

Diltiazem (250, 1000 µg/mL) respectively. The surgical area was covered with a wet cotton pad, and drops of normal saline (37°C) were added to the cotton to prevent disturbing the circulatory system and dryness of intestine.

Perfused samples (300 µL) were collected from both sides alternatively, each 5 minutes for a total of 30 minutes. The collected samples were transferred into 2 mL Eppendorf tubes, centrifuged at 5000 rpm for 10 minutes and then, Propranolol concentrations were determined spectrophotometrically on the same day.

Analytical procedures

200 µL of the collected supernatant were transferred and diluted to 3 mL with normal saline. Subsequently, the absorbance was measured at 319 nm against blank and then the concentration of each sample was determined using calibration curve to determine K_a of Propranolol. The samples were taken in triplicate. The spectrophotometric method used for analysis of Propranolol in intestinal fluid samples was validated for specificity, linearity, precision, accuracy, and stability by previous study carried out by Abushammala and collaborators [12].

Pharmacokinetic Analysis

Intestinal absorption of Propranolol was evaluated using its apparent first-order rate constant k_{ap} calculated according to the following equation:

$$\ln C_t = \ln C_0 - k_{ap} \cdot t$$

C_t: Concentration of drug after t time of perfusion, C₀: Initial drug concentration, k_{ap} : Apparent absorption rate constant and t: Time.

Statistical Analysis

The data obtained were treated and analyzed by using Statistical Package of Social Science (SPSS) program (Version 22). One-way ANOVA and Duncan tests were applied in this study. Results were assumed to be statistically significant with a P-value < 0.05.

RESULTS AND DISCUSSION

The absorption of orally administered Propranolol is essentially complete in the intestine with no intestinal metabolism [23].

Although, the physiological role of P-gp is still not fully cleared, the function of this efflux transporter in pharmacokinetics is highly appreciated. P-gp can minimize the absorption of orally administered drugs and reduce bioavailability [24-26], but co-administration of P-gp inhibitors such as verapamil, nifedipine, and diltiazem can enhance bioavailability of many drugs by inhibiting P-gp efflux activity [24, 27]. In the present study, Propranolol was selected as an example of a P-gp substrate with limited oral bioavailability [7-9]. By co-administration of Propranolol with Diltiazem at two different concentrations, we sought to increase Propranolol bioavailability by pass P-gp mediated secretory efflux, thereby increasing its absorptive drug transport. Diltiazem was chosen for P-gp inhibition because it has been observed to effectively inhibit efflux activity [27]. The first-generation inhibitor, Diltiazem, has successfully antagonized P-gp efflux activity both *in-vitro* and *in-vivo* [3].

The analysis of Propranolol in intestinal luminal fluids collected from rats was performed using direct spectrophotometric assay of collected intestinal fluid samples at $\lambda_{max} = 319$ nm. At the selected wavelength, no interferences from intestinal components or Diltiazem HCl were recorded since the λ_{max} of Diltiazem HCl is far away from that of Propranolol HCl (Diltiazem HCl showed zero absorbance at 319 nm). Furthermore, a linear relationship between concentration and absorbance of Propranolol HCl, at 319 nm, was established over a concentration range of 10-75 µg/mL ($R^2 = 0.999$) and the limits of detection (LOD) and quantification (LOQ) were 1.0 and 5.0 µg/mL, respectively. The measured concentrations of Propranolol HCl, as shown in table 1, were within the range of linearity of the validated calibration curve.

According to our obtained results the means of ln remnant concentrations of Propranolol from the three groups after intestinal perfusion of Propranolol (75 µg/mL) alone or in combination with Diltiazem at both concentrations (250 and 1000 µg/mL) showed a low inter-individual variation and are represented in table 1 and graphically shown in figure 1.

Table (1): The mean of ln remnant concentrations of data obtained experimentally for the three groups.

Time (min)	Propranolol HCl alone	With (250 µg/mL) Diltiazem HCl	With (1000 µg/mL) Diltiazem HCl
0.0	4.305±0.04	4.304±0.08	4.303±0.03
5.0	4.141±0.05	4.149±0.05	4.139±0.05
10.0	4.098±0.07	4.071±0.06	4.063±0.06
15.0	4.026±0.06	4.012±0.04	3.991±0.06
20.0	3.933±0.05	3.922±0.07	3.926±0.05
25.0	3.893±0.06	3.890±0.06	3.843±0.04
30.0	3.810±0.07	3.822±0.05	3.790±0.05

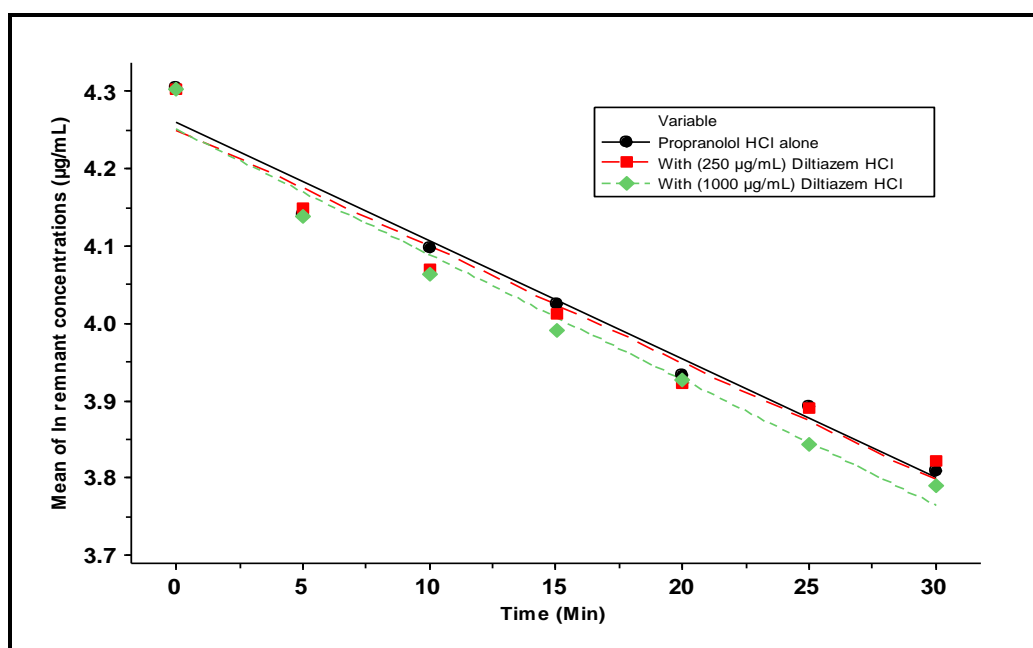


Figure (1): Graphical representation of the fit of the apparent first-order equation to the obtained mean data (remaining luminal concentrations for the three groups).

The K_a mean values of Propranolol HCl (75 µg/mL) perfused alone in intestine using SPIP in rats was $0.81 \pm 0.014 \text{ hr}^{-1}$. The gradual decrease of remnant Propranolol concentrations throughout time indicates that Propranolol absorption followed first order kinetic. It was demonstrated that, absorption rate constant mean values K_a of Propranolol

HCl (75 µg/mL) co-perfused with Diltiazem HCl (250 and 1000 µg/mL) were $0.778 \pm 0.012 \text{ hr}^{-1}$ and $0.857 \pm 0.030 \text{ hr}^{-1}$ respectively, suggesting that P-gp plays a minimal role in the *in-situ* intestinal absorption process of Propranolol with high water solubility and high membrane permeability (table 2).

Table (2): Calculated parameters of Propranolol in the three groups.

Calculated parameters	Propranolol HCl alone	With (250 µg/mL) Diltiazem HCl	With (1000 µg/mL) Diltiazem HCl
$k_a \text{ (hr}^{-1}\text{)}$	0.81 ± 0.014	0.778 ± 0.012	0.857 ± 0.030
$A_0 \%$	96.19 ± 0.006	97.11 ± 0.016	96.19 ± 0.015
R	0.968 ± 0.034	0.987 ± 0.006	0.985 ± 0.023

ka: Absorption rate constant, **A₀%**: Estimated inclination of the absorption line, **R**: coefficient of correlation.

The statistical evaluation of the data is shown in table 3 and represents the results of the statistical significance of homogeneity within each group of the three groups (Dun-

can test) and results of one-way ANOVA between the three groups with statistical significance level when ($P < 0.05$).

Table (3): shows the results of statistical evaluation of the data obtained experimentally after the application of a parametric test (Duncan test) to evaluate the homogeneity within each group of the three groups and the One-way ANOVA test. ($P > 0.05$).

Rat groups	Number of rats	F-value	Duncan test	One-way ANOVA test
First group	6	1.023	0.422	0.693
Second group	6	1.198	0.334	
Third group	6	0.693	0.633	

A validated spectrophotometric method was used for intestinal fluid samples analysis. The absorbance of Propranolol HCl was measured at λ_{\max} 319 nm, without any interferences from Diltiazem HCl [12].

The *in-situ* intestinal perfusion method used in the present study provides experimental conditions closer to what is encountered following oral administration and it has used extensively to elucidate absorption mechanism [29]. The SPIP model in rats measures the disappearance of the drug from perfused intestinal segment, directly describes its uptake into the enterocyte [29] and studies the intestinal absorption of drugs that may be affected by intestinal efflux transporters [30]. Propranolol absorption behavior, when it was co-perfused with Diltiazem using *in-situ* SPIP, is compared to this obtained by using Propranolol alone. The inhibition studies by using Diltiazem as P-gp inhibitor were performed to validate the P-gp transporter effect on intestinal absorption of Propranolol. The present research indicates that P-gp is playing a minimal role and statistically insignificant in the reuptake of Propranolol from the intestine (table 3).

Caco-2 cells are a well-established cell model that has been widely used to investigate P-gp efflux function [31]. Wang et al. observed that, Propranolol enantiomer transport in Caco-2 cells with changes in pH and attempted to inhibit transport of Propranolol by P-gp inhibitors (Verapamil and Rifampin) [9]. Wang and collaborators refer the

lack of effect of P-gp inhibitors on “the possibility that passive transcellular diffusion dominated the absorptive transport behavior of Propranolol”, consistent with the BCS class 1 assumption [9]. The finding of previous study may elucidate the null effect of P-gp inhibition on intestinal absorption of Propranolol.

CONCLUSION

The probable explanation for the insignificant effect of Diltiazem as P-gp inhibitor on Propranolol constant rate of absorption is that, P-gp plays a minimal role in the *in-situ* intestinal absorption process of Propranolol. Further, clinical studies must be conducted to confirm the obtained results.

ACKNOWLEDGMENT

I wish to express my deepest gratitude and thanks to all staff in the Faculty of Pharmacy at Al-Azhar University-Gaza (AUG) for their kind support and help.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- 1) Brunton L, Lazo J, Parker K. The pharmacological basis of therapeutics. Goodman and Gilman's Mc Graw-Hill, New York 2006.
- 2) Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutical drug classification: the

- correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* 1995; 12(3); 413-420.
- 3) Litman T, Druley TE, Stein WD, Bates SE. From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. *Cell. Mol. Life Sci.* 2001; 58(7); 931-959.
 - 4) Leslie EM, Deeley RG, Cole, SP. Multi-drug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol. Appl. Pharmacol.* 2005; 204(3); 216-237.
 - 5) Senthilkumari S, Velpandian T, Biswas NR, Sonali N, Ghose S. Evaluation of the impact of P-glycoprotein (P-gp) drug efflux transporter blockade on the systemic and ocular disposition of P-gp substrate. *J Ocul Pharmacol Ther.* 2008; 24(3); 290-300.
 - 6) Matheny CJ, Lamb MW, Brouwer KL, Pollack GM. Pharmacokinetic and Pharmacodynamic Implications of P-glycoprotein Modulation. *Pharmacotherapy.* 2001; 21(7); 778-796.
 - 7) Yang JJ, Kim KJ, Lee VH. Role of P-glycoprotein in restricting propranolol transport in cultured rabbit conjunctival epithelial cell layers. *Pharm. Res.* 2000; 17(5); 533-538.
 - 8) D'Emanuele A, Jeyprasesphant R, Penny J, Attwood D. The use of a dendrimer-propranolol prodrug to bypass efflux transporters and enhance oral bioavailability. *J Control Release.* 2004; 95(3); 447-453.
 - 9) Wang Y, Cao J, Wang X, Zeng S. Stereoselective transport and uptake of propranolol across human intestinal Caco-2 cell monolayers. *Chirality.* 2010; 22(3); 361.
 - 10) Ludden TM. Nonlinear pharmacokinetics. *Clin Pharmacokinet.* 1991; 20(6); 429-446.
 - 11) Ho Norman FH, Park JY, Morozowich W, Higuchi WI. Physical model approach to the design of drugs with improved intestinal absorption. Design of biopharmaceutical properties through prodrugs and analogs. *J Am Pharm Assoc.* 1977; 136-227.
 - 12) Abushammala I, Ramadan M, El-Qedra A. The Effect of P-Glycoprotein on Propranolol Absorption Using in Situ Rats Single-Pass Intestinal Perfusion. *Int. J. Pharm. Sci. Rev. Res.* 2013; 22(1); 161-165.
 - 13) Acra SA, Ghishan FK. Methods of investigating intestinal transport. *JPEN* 1991; 15(3); 93-98.
 - 14) Barthe L, Woodley J, Houin G. Gastrointestinal absorption of drugs: methods and studies. *Fundam Clin Pharmacol.* 1999; 13(2); 154-168.
 - 15) Salphati L, Childers K, Pan L, Tsutsui K, Takahashi L. Evaluation of a single-pass intestinal-perfusion method in rat for the prediction of absorption in man. *J. Pharm. Pharmacol.* 2001; 53(7); 1007-1013.
 - 16) Cornwell MM, Pastan I, Gottesman MM. Certain calcium channel blockers bind specifically to multidrug-resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. *J. Biol. Chem.* 1987; 262(5); 2166-2170.
 - 17) Song N, Li Q, Liu C. Intestinal permeability of metformin using single-pass intestinal perfusion in rats. *World J. Gastroenterol.* 2006; 12(25); 4064-4070.
 - 18) Zakeri-Milania P, Valizadeha H, Tajerzadehc H, Azarmia Y, Islambolchilala Z, Barzegara S. Predicting human intestinal permeability using single-pass intestinal perfusion in rat. *J Pharm Pharm Sci.* 2007; 10(3); 368-379.
 - 19) Ernest D, Alfert ED, Brenda M, Cross BM, McWilliam AA. Guide to the care and use of experimental animals. Canadian Council on Animal Care, 2ed edition, volume 1, Bradda Printing Services Inc, Ottawa. 1993; 15-52.
 - 20) Doluisio J, Billups N, Dittert L, Sugita E, Swintosky J. Drug absorption I: An in-situ rat gut technique yielding realistic

- absorption rates. *J. Pharm. Sci.* 1969; 58(10); 1196-1200.
- 21) Sanchez-Pico A, Peris-Ribera JE, Tolodano C, Torres-Molina F, Casabo VG, Martin-Villodre A. Nonlinear intestinal absorption kinetics of cefadroxil in the rat. *J. Pharm. Pharmacol.* 1989; 41(3); 179-185.
- 22) Ruiz-Balaguer N, Nacher A, Casabo V, Matilde Merino. Nonlinear intestinal absorption kinetics of cefuroxime axetil in rats. *Antimicrob. Agents Chemother.* 1997; 41(2); 445-448.
- 23) Shand D, Rangno R. The disposition of propranolol. *Pharmacology.* 1972; 7(3); 159-168.
- 24) Trambas CM, Muller HK, Woods GM. P-glycoprotein mediated multidrug resistance and its implications for pathology. *Pathology* 1997; 29(2); 122-130.
- 25) Lin JH. Drug–drug interaction mediated by inhibition and induction of P-glycoprotein. *Adv. Drug Deliv. Rev.* 2003; 55(1); 53-81.
- 26) Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics. *Clin Pharmacokinet.* 2003; 42(1); 59-98.
- 27) Aszalos A. Drug–drug interactions affected by the transporter protein, P-glycoprotein (ABCB1, MDR1): II. Clinical aspects. *Drug Discov Today.* 2007; 12(19); 838-843.
- 28) Chiou WL, Barve A. Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. *Pharm. Res.* 1988; 15; 1792-1795.
- 29) Amidon GL, Sinko PJ, Fleisher D. Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. *Pharm. Res.* 1988; 5(10); 651-654.
- 30) Wang H, Kawashima H, Strobel HW. cDNA Cloning of a Novel CYP3A from Rat Brain. *Biochem. Biophys. Res. Commun.* 1996; 221(1); 157-162.
- 31) Sun D, Lennernas H, Welage L S, Barnett JL, Landowski CP, Foster D, Amidon G. L. Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. *Pharm. Res.* 2002; 19(10); 1400-1416.