Inhibitory effect of clemastine on P-glycoprotein expression and function: an in vitro and in situ study

Mehran Mesgari Abbasi 1, 2, Hadi Valizadeh 1, Hamed Hamishekar 1, Leila Mohammadnejad 3, Parvin Zakeri-Milani 4*

1 Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2 Students Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
3 Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4 Liver and Gastrointestinal Diseases Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

Objective(s): Transporters have an important role in pharmacokinetics of drugs. Inhibition or induction of drug transporters activity can affect drug absorption, safety, and efficacy. P-glycoprotein (P-gp) is the most important membrane transporter that is responsible for active efflux of drugs. It is important to understand which drugs are substrates, inhibitors, or inducers of P-gp to minimize or avoid unwanted interactions. The aim of this study was to investigate the effects of clemastine on the expression and function of P-gp.

Materials and Methods: The effect of clemastine on P-gp function and expression was evaluated in vitro by rhodamine-123 [Rho 123] efflux assay in Caco-2 cells and Western blot analysis. Rat in situ single pass intestinal permeability model was used to investigate the clemastine effect on digoxin P<sub>eff</sub> as a known P-gp substrate. Digoxin levels in intestinal perfusates were assayed by high performance liquid chromatography (HPLC) method.

Results: The Caco-2 intracellular accumulation of Rho 123 in clemastine and verapamil treated cells was 90.8 ± 9.8 and 420.6±25.4 pg/mg protein, respectively which was significantly higher than that in control cells (50.2±6.0; P<0.05). Immunoblotting results indicated that clemastine decreased expression of P-gp in Caco-2 cells in vitro. Moreover, effective intestinal permeability (P<sub>eff</sub>) of digoxin in the presence of clemastine, was significantly increased compared to control group.

Conclusion: Findings of our study suggested dose dependent P-gp inhibition activity for clemastine in vitro and in situ. Therefore co-administration of clemastine with P-gp substrates may result in unwanted interactions and side effects.

Introduction

In recent years, transporter-mediated drug-drug and food-drug interactions are being reported with increasing frequency. It is clear that transporters have an important role in absorption, distribution, metabolism, and elimination of drugs. Induction or inhibition of drug transporters can affect drug absorption, safety, and efficacy. Therefore, study of potential interactions during the process of drug development has been noticed (1, 2). Drug transporters are classified into: solute carriers (SLC) and ATP-binding cassettes (ABC) (3).

P-glycoprotein (P-gp) is an ABC transporter and the most important membrane transporter that is responsible for active efflux (secreting) of passively diffused xenobiotics and drugs, out of the cell (4, 5). P-gp is expressed in the epithelia cells of gastrointestinal tract and many other organs. It is involved in the efflux of various kinds of drugs and substances with different chemical structures, out of the cells. After oral administration followed by complete dissolution of a drug, inhibition of P-gp by the drug can result in increased absorption of any co-administered drug that is P-gp substrate (1, 2). Therefore, P-gp-related interactions have critical clinical role and it is important to understand which drugs are substrates, inhibitors, or inducers of P-gp to minimize or avoid unwanted interactions (5, 6).

Clemastine (Figure 1), a competitive antagonist of H<sub>1</sub> histamine receptor, is a widely used, first-generation antihistamine. The drug is an ethanolamine derivative with the following formula: (++)-2-[p-chloro-a-methyl-a-phenylbenzyl]oxo[ethyl]-1-ethylpyrrolidine). Clemastine inhibits the vasodilator and vasoconstrictor effects of histamine, and the constriction of respiratory and gastrointestinal smooth muscles; therefore, it is
The optical densities of digoxin, as a well-known P-gp substrate, were measured by Western blot method. Expression of P-gp in Caco-2 cells, after treatment with clemastine and verapamil, was assessed using single pass intestinal perfusion (SPIP) technique in rats.

The current study was designed to investigate the in vitro and in situ effects of different concentrations of clemastine on the membrane transporter function of P-gp, and its expression. In vitro study was performed by assaying rhodamine-123 (Rhod123) accumulation in Caco-2 (heterogeneous human epithelial colorectal adenocarcinoma) cells after treatment with clemastine and verapamil. Rhod123 and verapamil are known as P-gp substrate and inhibitor, respectively. Expression of P-gp in Caco-2 cells, after treating with clemastine and verapamil is also assessed by Western blot method. Moreover intestinal effective permeability (Pe) of digoxin, as a well-known P-gp substrate, is investigated in situ in the presence and absence of clemastine and verapamil, using single pass intestinal perfusion (SPIP) technique in rats.

**Materials and Methods**

**Materials**

Clemastine fumarate, digoxin, verapamil, Rhod123, 3-(4,5-dime-thylthiazol-2-thiazolyl)-2,5-diphenyltetrazoliumbromide (MTT), penicillin-streptomycin, and protease inhibitor cocktail were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anti-P-gp mouse monoclonal antibody (ab80594) and rabbit polyclonal antibody to beta Actin (ab16039) were purchased from Abcam (USA). Horseradish peroxidase (HRP) conjugated anti-mouse immunoglobulin (IgG) (AP8036) and anti-rabbit IgG (AP7181) were purchased from Razi Biotech (Iran). Dulbecco’s modified Eagle medium (DMEM), trypsin-EDTA (0.25%), fetal calf serum (FCS) were purchased from Gibco (Carlsbad, CA, USA). Tissue culture flasks and other disposable cell culture items were purchased from TPP Co. (Switzerland). Enhanced chemiluminescence (ECL) Western blot detection kit, medical X-ray film, pre-stained protein ladder (10-250kDa), and protein assay kit were purchased from Amersham (GE Healthcare, Chalfont St. Giles, UK), Fuji (Tokyo, Japan), Cinagen, and Pars Azmoon (Iran), respectively. All other chemicals were purchased from Merck Co. (Darmstadt, Germany).

**Cell culture**

The Caco-2 cells were obtained from Pasteur Institute of Iran (Tehran, Iran). The cells were routinely cultured in DMEM with 10% heat inactivated fetal bovine serum, 1% penicillin-streptomycin, and 1% non-essential amino acids. Cells were placed in a CO2-incubator (Membnet, Germany), at 37 °C with 90% relative humidity and 5% CO2 atmosphere. The culture medium was replaced 2-3 times per week. After reaching 80-90% confluency, the cells were detached from the culture flask by adding 0.25% trypsin-EDTA solution to seed them in well-plates or flasks (2).

**In vitro cytotoxicity study (MTT assay)**

The cytotoxic effect of different concentrations of clemastine (0.01, 0.1, 0.5, 1.5, 5, and 10 µM) on Caco-2 cells were determined, using MTT assay. The Caco-2 cells were seeded into 96-well plates at a density of 15×10⁴ cells per well. After 24 hr, the medium was replaced with 200 µl per well of clemastine at different concentrations diluted with complete culture medium. After 24 hr incubation, the cells were washed by PBS, and 50 µl of the MTT solution (2 mg/ml) was added to the wells and incubated for 4 hr at 37 °C in a CO2 incubator. Then MTT solution was removed and the resulting for mazan crystals were solubilized with 200 µl/well of DMSO and 25 µl/well Sorensen’s phosphate buffer (mix 80.4 ml of 133 mM NaHPO₄ and 19.6 ml of 133 mM KH₂PO₄, pH = 7.4). The optical densities (ODs) were detected with an ELISA microplate reader (Statfax-2100, Awareness, USA) at 570 nm with background subtraction at 630 nm. The following formula was used for calculating the percentage of cell viability (10). The MTT assay was performed in triplicate for control and each concentration of test agents.

\[
\text{Cell viability} \% = \frac{\text{OD value of test}}{\text{OD value of control}} \times 100
\]

The cell viability was calculated for each concentration and analyzed statistically. The results were expressed as mean ± standard deviation (SD).

**In vitro Rhod123 efflux assay**

P-gp function in Caco-2 cells was assayed by measurement of intracellular accumulation of Rhod123, a known P-gp substrate, which is inversely proportional to P-gp activity. Caco-2 cells were seeded in 24-well plates and allowed to attach for 24...
hr in CO₂-incubator. The old medium was removed and cells were washed with PBS. New culture media containing clemastine (0.01, 0.5, and 10 µM) or verapamil (300 µM) was added to wells. After 24 hr, the old medium was removed and cells were washed three times with PBS. Rho₁₂₃ solution (DMEM containing 10 mM HEPES (pH=7.4) and 5 µM Rho₁₂₃) was added and incubated for 3 hr at 37 °C, followed by washing three times with ice-cold PBS (pH = 7.4). Cells were lysed in 1% Triton X-100 and centrifuged (3-18k, Sigma, UK) at 1500 g for 10 min. Supernatant was used for measuring Rho₁₂₃ and protein contents. The intracellular accumulation of Rho₁₂₃ in samples were determined quantitatively by fluorescence spectrophotometry at exciting wavelength of 485 nm and emission wavelength of 532 nm (FP-750, Jasco, Japan). Protein content of the samples was evaluated by a commercial protein assay kit (Pars Azmoon, Iran), and Rho₁₂₃ values were normalized by the protein content of each sample (supernatant) (11, 12).

**Western blot analysis of P-gp expression in Caco-2 cells**

Expression of P-gp in Caco-2 cells was assessed by Western blot after the cells were treated with clemastine, and verapamil. Caco-2 cells were seeded in a 6-well plate at the density of 10⁶ cells per well. After 24 hr, the cells were treated with clemastine (10 µM), and verapamil (300 µM) (n = 4). Culture medium alone was considered the control (n = 4). After 48 hr, solutions were discarded, the cells were washed (PBS), and incubated for 5 min with 0.25% trypsin-EDTA at 37 °C. The detached cells were washed twice with PBS. Lysis buffer (Triton X-100 50 mM, NaCl 150 mM, EDTA 5 mM, 1% protease inhibitor cocktail, and Tris-HCl, pH=7.4) was added, and cell suspension was centrifuged at 1500 g for 5 min. Proteins were resolved by 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)(80 V, 120 min) and electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane using a semi-dry Western blotting system (Bio-Rad, USA). All membranes were blocked for nonspecific binding, by incubation in 3% nonfat-dried milk for 1 hr, at room temperature. Then, the membrane was washed 3 times with Tris buffered saline with 0.1% Tween 20 (TBS-T), and incubated overnight with mouse monoclonal anti-P-gp antibody (1/1000 in TBS) (ab80594, Abcam, USA). The next day, membranes were washed with TBS, and then incubated with HRP-conjugated rabbit anti-mouse secondary antibody (AP8036, Razi Biotech, Iran), for 2 hr. The membranes were washed and the proteins were then detected using an ECL kit (Amersham, GE Healthcare, Italy). The proteins were visualized by exposing the membrane to a medical x-ray film (Fuji, Japan) for 5 min in a dark room. β-Actin was considered as the internal standard and detected using rabbit polyclonal anti-β-actin as primary antibody (ab16039, Abcam, USA). HRP-conjugated goat anti-rabbit IgG was the secondary antibody (AP7181, Razi Biotech, Iran). P-gp expression is presented as the ratio of intensity of P-gp band to β-actin band in the same blot run (P-gp/β-actin) (13).

**In situ study**

**Animals**

Male Sprague-Dawley rats (200–250 g) were supplied from Pasteur Institute of Iran. The animals were housed in standard cages with a 22±2 °C temperature, a 12/12 hr light/dark cycle, 55–60% relative humidity, and free access to standard rodent chow and clean water. Prior to experimentation, rats were fasted for 12-16 hr (water ad libitum). All experiments were conducted in accordance with the humanity and animal ethic protocols. The animal study protocols were approved by the Research Ethical Committee of Tabriz University of Medical Sciences (Ref No: TBZMED. REC.1394.378). In all animal studies “Guide to the care and use of experimental animals” by Canadian Council on Animal Care, was followed.

**In situ SPIP method**

The animals were anesthetized with an IP injection of thiopental sodium (60 mg/kg) (LogiChem, UK). A 3-4 cm incision was made in abdominal midline and approximately 9-11 cm of the jejunum segment of the intestine was selected and cannulated at both ends with polypropylene tubes. The exposed segment was kept moist with body tempered saline during the experiment. Before injection of the main drug solution, body tempered normal saline was passed through the cannulated segment to wash and clear the lumen of the segment. PBS (pH = 7.4) containing 50 mg/l phenol red without drug (blank solution) was pumped through the segment, at a constant flow rate of 0.2 ml/min (Qm). Blank perfused solution samples were collected and used to prepare digoxin calibrator solutions for HPLC and also for stability studies.

The SPIP method was carried out in anesthetized rats, as described above, and the blank solution containing the substance of interest was perfused at a constant flow rate of 0.2 ml/min, using a volumetric infusion pump (Argus Medical AG, Heimberg, Switzerland). After reaching the C_out/C_in ratio to a steady state (Figure 2), each perfusion experiment lasted for 90 min, and perfusates were quantitatively collected at 10 min intervals (2 ml). Some changes, such as water absorption and secretion, during perfusion may cause errors in the calculated permeability values. Therefore, 50 mg/l phenol red was added as a non-absorbable marker to correct the results. Digoxin (20 µM, a typical substrate of P-gp), verapamil (300 µM, a typical P-gp

Iran J Basic Med Sci, Vol. 19, No. 4, Apr 2016
Effects of Clemastine on P-gp function in vitro and in situ

Figure 2. Plots of inlet/outlet concentrations ratio ($C_{in}/C_{out}$) of digoxin (20 µM) versus time in the presence of (A) 0.01, (B) 0.5, and (C) 10 µM clemastine in single pass intestinal permeability (SPIP) study ($n = 3$, error bars represent mean ± SD)

Inhibitor), and different concentrations of clemastine (0.01, 0.5, and 10 µM), each one in a blank solution with 20 µM digoxin, were administered, as described above (14, 15). The SPIP method was performed in at least three rats for each concentration of each drug (9 for clemastine, 3 for verapamil, and 3 for digoxin alone) and the blank solution. At the end of procedure, the length of the segment was measured (cm) and the animal was euthanatized. Samples were stored in an ultra-low temperature freezer (JalTajhiz Production, Karaj, Iran), at -70 °C until performing HPLC and phenol red analysis. The concentration of phenol red in the perfused (outlet) samples was measured at 560 nm using an ultraviolet-visible (UV-VIS) spectrophotometer (Ultra-spec 2000, Pharmacia, Pfizer, New York, NY, USA) (16). The amount of digoxin in the perfused samples was detected by HPLC.

HPLC analysis of digoxin in intestinal perfused samples

HPLC system (Smartline manager 5000, Smartline UV Detector 2600, and Smartline Pump 1000, Knauer Advanced Scientific Instruments, Berlin, Germany) was used to detect the amount of digoxin in the perfused samples. The mobile phase was 35% v/v of acetonitrile in water, which was filtered through a sintered glass filter P5 (1.0-1.6 µm, Winteg, Germany) and degassed in a sonicator. The mobile phase was pumped in isocratic mode at ambient temperature. The flow rate was 2 ml/min. UV detection was performed at 218 nm, and the injection volume of samples was 20 µl. The HPLC column was Knauer-15VE081ESJ-150X 4.6 mm with precolumn-Eurospher 100-5 C8 (14).

Data analysis

$P_{eff}$ values were calculated from concentrations of compounds in the outlet perfused samples, at the steady state. The steady state, as shown in Figure 2, was confirmed by the ratio of the outlet to inlet concentrations (corrected for phenol red concentrations) versus time (14, 15).

Phenol red correction

Corrected outlet concentration of the digoxin ($C_{out}$) was calculated using the following equation (17).

$$C_{out(corr)} = C_{out} \times \frac{CPR_{in}}{CPR_{out}}$$

Where:

- $C_{out(corr)} = corrected$ outlet concentration of the drug
- $C_{out} = outlet$ concentration of the drug
- $CPR_{in} = concentration$ of phenol red entering the intestinal segment.
- $CPR_{out} = concentration$ of phenol red leaving the intestinal segment.

Calculations were based on outlet perfusate steady state concentrations after the selected time points. The steady state intestinal $P_{eff}$ was calculated according to this equation:

$$P_{eff} = \frac{-Q_{in} \times ln (C_{out}/C_{in})}{2 \times 60 \pi rl}$$

Where:

- $P_{eff} = effective$ permeability (cm/s)
- $Q_{in} = perfusion$ rate (0.2 ml/min)
- $C_{in} = concentrations$ of the test drug entering the segment

426
Effects of Clemastine on P-gp function in vitro and in situ

Mesgari Abbasi et al

Iran J Basic Med Sci, Vol. 19, No. 4, Apr 2016

427

Figure 3. Effects of different concentrations of clemastine on cell viability in Caco-2 cells. Bars show mean ± SD of at least 3 measurements. * P<0.05 was considered as significance level.

\[ C_{\text{out}} = \text{concentrations of the test drug leaving the segment} \]

\[ r = \text{the radius of the intestinal segment (≈ 0.18 cm)} \]

\[ l = \text{the length of the intestinal segment (cm)} \]

Figure 4. Intracellular concentrations of rhodamine-123 in Caco-2 cells after treating with clemastine (10 µM) and verapamil (300 µM) (n=4). Bars show mean ± SD. * (P<0.05) and ** (P<0.01), significantly different from control cells (untreated cells).

Effect of clemastine on the efflux function of P-gp in Caco-2 cells (Rho\textsubscript{123} uptake)

Rho\textsubscript{123} efflux activity of P-gp in Caco-2 cells was used to investigate the effect of clemastine on the function of P-gp as an efflux pump. The cells were treated with 10 µM clemastine and 300 µM verapamil, in addition to Rho\textsubscript{123}. Intracellular concentration of Rho\textsubscript{123} was significantly increased from 50.2 ± 6.0 pg/mg protein (mean±SD) of control (non-treated) to 90.8 ± 9.8 (P<0.05) and 420.6±25.4 (P<0.01) pg/mg protein of clemastine and verapamil treated cells, respectively (Figure 4).

Immunoblotting

Expression of P-gp in clemastine and verapamil treated Caco-2 cells were investigated using immunoblotting method. The β-actin protein expression was considered as internal immunoblotting control. P-gp expression was evaluated as the P-gp band intensity to β-actin band intensity ratio (P-gp/β-actin), and was compared with P-gp bands of verapamil treated and non-treated Caco-2 cells bands in the same blot run (14). As illustrated in Figure 5, low density of Western blot bands of clemastine treated cells related to those of untreated cells, is evidence of low expression of P-gp, which indicates the inhibition effect of clemastine on P-gp expression.

Figure 5. Western blot of P-gp and β-actin in clemastine (10 µM) and verapamil (300 µM) treated, and control (non-treated) Caco-2 cells (bands of 2 samples of each are shown).

Figure 6. Effects of different concentrations of clemastine (0.01, 0.5, and 10 µM) and verapamil (300 µM) on the effective permeability (P\textsubscript{e}) of digoxin compared to the control group (digoxin) (n=3). Bars show mean±SD. * (P<0.05) and ** (P<0.01)**, significantly different from control (digoxin) group.

Results

Preliminary experiments showed that no considerable adsorption of compounds on the tubing and syringe took place. The experiments also demonstrated stability of the drugs during the experiments.

In vitro cytotoxicity study (MTT assay)

The results of MTT cytotoxicity tests showed that clemastine had no cytotoxic effect on Caco-2 cells in the range of used concentrations (Figure 3).
**Discussion**

Based on data from the *in vitro* Rhod 123 uptake study, treatment of Caco-2 cells with 10 mM clemastine increased the accumulation of Rhod 123 by 80.9% compared with control (Figure 4), suggesting that clemastine inhibits P-gp *in vitro*. Protein expression of P-gp in clemastine treated Caco-2 cells was lower than the control cells (Figure 5), confirming the inhibition of P-gp by clemastine. We also found *in situ* that, 0.5 and 10 mM clemastine significantly increased digoxin P_{eff} by about 65% and 165%, respectively (Figure 6). The results of the *in situ* study showed a dose dependent effect of clemastine, in the range of 0.01-10 µM, on P-gp. The enhancement can be resulted from inhibition of efflux activity of P-gp.

Putting together, our results strongly suggest that, clemastine is a P-gp inhibitor. This is in agreement with the prediction of Broccatellia *et al* using a reliable *in silico* model (18). The model was established after studying 1200 compounds structure and using various Molecular Interaction Fields (MIF)-based technologies. The pharmacophoric features and membrane partitioning related physico-chemical properties were also considered in creating the model (18). Clemastine was also present in the *in silico* structure-based screening of P-gp inhibitor library of Shityakov and Förster (19). The list was provided by the gradient optimization method, using polynomial empirical scoring functions. In their study the hydrophobic interactions between P-gp and selected drug substances were assessed as the main forces involved in the inhibition effect (19). Clemastine was tested *in vitro* in the MDR-MDCK cell model and *in situ* in the rat brain perfusion model by Obradovic *et al*. The *in vitro* model showed that the presence of cyclosporine-A (a typical inhibitor of P-gp) decreased efflux of clemastine, which indicates that clemastine is a P-gp substrate. But, the *in situ* perfusion results of the study demonstrated that the brain penetration of clemastine was not increased by co-perfusion with cyclosporine-A. Therefore, P-gp-mediated efflux appears not to be a limiting factor to the CNS penetration of clemastine (20). The *in vitro* result of the study was in contrary with the findings of Varma *et al*. In their study, clemastine was placed in non-P-gp-substrate list (21). On the other hand, the most frequent and significant adverse effects of clemastine reported in the literature are those that are related to CNS. Because of high expression of P-gp in blood-brain barrier cells, it is probable that clemastine is a substrate or inhibitor of P-gp and may red blood plasma to CNS. Because of strong expression of P-gp in brain tissue, it is probable that clemastine is not a substrate or inhibitor of P-gp (7) and therefore none of the studies are in contrary of our findings.

The therapeutic level of clemastine in blood plasma is 0.7-2 ng/ml (~1.5-4.4 nM) (23). Our findings demonstrated no P-gp inhibition activity for 0.01 µM solution of clemastine (Figure 6). Therefore, clemastine does not have P-gp inhibition activity in its plasma therapeutic levels. On the other hand, the maximum recommended oral dose for clemastine is 2.68 mg. Considering the intestinal fluid volume, after orally receiving the dose, the intestinal concentration of the clemastine is about 7.8 µM, that is more than 0.5 µM and is expected to show P-gp inhibition activity based on our findings (Figure 6). Despite widespread use of clemastine, there are only a few reports on the pharmacokinetics and metabolism of clemastine (8). The P-gp inhibitory effect of clemastine *in vitro* and *in situ* to our knowledge, has not been previously reported.

In human intestine, P-gp is highly expressed on the brush-border membrane of epithelial cells. Nowadays, the majority of drugs are administered orally and it is important to keep in mind the potential for co-administration of compounds that are the P-gp substrates or inhibitors. The P-gp-mediated drug-drug interaction may result in increasing the permeability and drug concentration in body fluids, followed by unwanted adverse effects (24). Therefore, P-gp inhibition effect of clemastine may provide useful information for clinicians and researchers. However, the molecular mechanisms of the P-gp inhibition effect of clemastine is not clear and further investigations are necessary to find them out.

**Conclusion**

In conclusion, present study demonstrated that clemastine treatment can down-regulate the expression of P-gp *in vitro* and inhibit function of P-gp *in vitro* and *in situ*. The study provides useful information for predicting potential drug-drug interactions when clemastine is co-administered with drugs transported by P-gp and may reduce unwanted reactions. Further studies are recommended to verify the P-gp inhibition roles of clemastine and clarify the molecular mechanisms of the inhibition.

**Acknowledgment**

The authors appreciate the members of Drug Applied Research Center and Immunology Research
Effects of Clemastine on P-gp function in vitro and in situ

Mesgari Abbasi et al

Center of Tabriz University of Medical Sciences, Tabriz, Iran. The results described in this paper were part of Mehran Mesgari Abbasi's PhD thesis and was financially supported by the Drug Applied Research Center of Tabriz University of Medical Sciences (Grant No. 91-123).

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Lumen AA, Li L, Li J, Ahmed Z, Meng Z, Owen A, et al. Transport inhibition of digoxin using several common P-gp expressing cell lines is not necessarily reporting only on inhibitor binding to P-gp. PLoS One 2013; 8:e69394.
2. Kishimoto W, Ishiguro N, Ludwig-Schwellinger E, Ebner T, Schaefer O. In Vitro predictability of drug-drug interaction likelihood of P-glycoprotein-mediated efflux of dabigatran etexilate based on [I]2/IC50 threshold. Drug Metab Dispos 2014; 42:257–263.
3. Kobor T, Harada S, Nakamoto K, Tokuyama S. Mechanisms of P-glycoprotein alteration during anticancer treatment: role in the pharmacokinetic and pharmacological effects of various substrate drugs. J Pharmacol Sci 2014; 125:242–254.
4. Kobor T, Harada S, Nakamoto K, Tokuyama S. Functional alterations of intestinal P-glycoprotein under diabetic conditions. Biol Pharm Bull 2013; 36:1381–1390.
5. Li Y, Huang L, Zeng X, Zhong G, Huang M, et al. Down-regulation of P-gp expression and function after mulberroside a treatment: potential role of protein kinase C and NF-kappa B. Chem Biol Interact 2014; 213:44–50.
6. Wessler JD, Grip LT, Mendell J, Giugliano RP. The P-glycoprotein transport system and cardiovascular drugs. J Am Coll Cardiol 2013; 61:2495–2502.
7. Han S, Karłowicz E, Bondesson U, Tourneke K, Hedeland M. Identiﬁcation of some new clemastine metabolites in dog, horse, and human urine with liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2004; 18:2267–2272.
8. Minigh J. Clemastine. xPharm: The Comprehensive Pharmacology Reference 2008; 1–6.
9. Han y, Chin Tan TM, Lim L. In vitro and in vivo evaluation of the effects of piperine on P-gp function and expression. Toxicol Appl Pharmacol 2008; 230:283–289.
10. Zrieî A, Farinotti R, Buyse M. Cyclooxygenase-2 inhibitors prevent trinitrobenezene sulfonic acid-induced P-glycoprotein up-regulation in vitro and in vivo. Eur J Pharmacol 2010; 636:189–197.
11. Zastreja J, Jackson J, Bajwaa M, Ligginsb R, Iqbal a F, Burta H. Enhanced cellular accumulation of a P-glycoprotein substrate, rhodamine-123, by caco-2 cells using low molecular weight methoxypolyethylene glycol-block-polypropionate diblock copolymers. Eur J Pharm Biopharm 2002; 54:299–309.
12. Wanga XD, Menga MX, Gasa LB, Liua T, Xub Q, Zenga S. Permeation of astilbin and taxifolin in Caco-2 cell and their effects on the P-gp. Int J Pharm 2009; 378:1–8.
13. Patel JR, Barve KH. Intestinal permeability of lamivudine using single pass intestinal perfusion. Indian J Pharm Sci 2012; 74:478–481.
14. Zakeri Milani P, Valizadeh H, Tajerzadeh H, Azarmi Y, Islambolchilar Z, Barzegar S, et al. Predicting human intestinal permeability using single-pass intestinal perfusion in rats. J Pharm Pharm Sci 2007; 10:360-379.
15. Valizadeh H, Mehtari M, Zakeri-Milani P. Evidence for enhanced intestinal absorption of digoxin by P-glycoprotein inhibitors. Trop J Pharm Res 2012; 11:939–945.
16. Prasad N, Bhasler S. Characterization of intestinal transport of Vinristine in rats applying in situ single pass intestinal perfusion. Pharmacologia 2012; 3:617-621.
17. Broccatellia F, Carosatia E, Nerib A, Frosinib M, Goraecic L, Opread TI, et al. A novel approach for predicting P-glycoprotein (ABCB1) inhibition using molecular interaction fields. J Med Chem 2011; 54:1740–1751.
18. Shityakov S, Förster C. In silico structure-based screening of versatile P-glycoprotein inhibitors using polynomial empirical scoring functions. Adv Appl Bioinform Chem 2014; 7:1–9.
19. Obradovic T, Dobson GG, Shingaki T, Kungu T, Hidalgo IJ. Assessment of the first and second generation antihistamines brain penetration and role of P-glycoprotein. Pharm Res 2007; 24:318–327.
20. Varma MVS, Satesh K, Panchagnula R. Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport. Mol Pharm 2004; 2:2-21.
21. Wessler JD, Grip LT, Mendell J, Giugliano RP. The P-glycoprotein transport system and cardiovascular drugs. J Am Coll Cardiol 2013; 61:2495-2502.
22. Drug information online, http://www.drugs.com/cdi/clemastine.html
23. Zakeri-Milani P, Valizadeh H. Intestinal transporters: enhanced absorption through P-glycoprotein-related drug interactions. Expert Opin Drug Metab Toxicol 2014; 10:859–871.