APB Advanced Pharmaceutical Bulletin, 2014, 4(3), 219-224
doi: http://dx.doi.org/10.5681/apb.2014.032
http://apb.tbzmed.ac.ir/
TUOMS PG

Reduced ABCB1 Expression and Activity in the Presence of Acrylic Copolymers

Ramin Mohammadzadeh[1,2], Behzad Baradaran[3], Hadi Valizadeh[1], Bahman Yousefi [3], Parvin Zakeri-Milani[4]*

[1] *Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*

[2] *Students Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.*

[3] *Immunology Research Center and School of Medicine, 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.*

ARTICLEINFO

Article Type:
Research Article

Article History:
Received: 21 December 2013
Revised: 4 January 2014
Accepted: 4 January 2014
ePublished: 7 February 2014

Keywords:
ABCB1
P-glycoprotein
Intestinal efflux pump
Rhodamine
Eudragit

ABSTRACT

Purpose: P-glycoprotein (P-gp; ABCB1), an integral membrane protein in the apical surface of human intestinal epithelial cells, plays a crucial role in the intestinal transport and efflux leading to changes in the bioavailability of oral pharmaceutical compounds. This study was set to examine the potential effects of three Eudragits RL100, S100 and L100 on the intestinal epithelial membrane transport of rhodammine-123 (Rho-123), a substrate of P-gp using a monolayer of human colon cancer cell line (Caco-2).
Methods: The least non-cytotoxic concentrations of the excipients were assessed in Caco-2 cells by the MTT assay. Then the transepithelial transport of Rho-123 across Caco-2 monolayers was determined with a fluorescence spectrophotometer. Besides, the expression of the P-gp in cells exposed to the polymers was demonstrated using Western-blotting analysis.
Results: Treatment of cells with Eudragit RL100 and L100 led to a very slight change while Eudragit S100 showed 61% increase in Rho-123 accumulation (P<0.001) and also reduced transporter expression.
Conclusion: Our studies suggest that using proper concentrations of the Eudragit S100 in drug formulation would improve intestinal permeability and absorption of p-gp substrate drugs.

Introduction

Although oral route for drug administration is the most convenient and favored choice for patients, most hydrophilic drugs and some high molecular weight hydrophobic drugs show poor intestinal permeability and absorption which is a key factor that determines the pharmacokinetics of oral drug compounds and alters their both bioavailability and pharmaceutical effects.[1-4] P-gp, also known as ABCB1, is a plasma-membrane associated efflux pump in humans with an energy dependent function which plays a crucial role in the intestinal transport and is known to be responsible for the occurrence of drug resistances.[5-7] This effect of P-gp, which is encoded by MDR1 gene, is considered to be one defense against toxic agents where it can reduce the bioavailability of a wide range of pharmaceutical compounds as well.[8-10] Caco-2 cells which are human colonic adenocarcinoma cell line and expressing P-gp appear to be used widely for in vitro permeability studies.[11] Previous studies have revealed that some drugs and commonly used substances in drug formulations can alter the ability of P-gp in pumping its substrates.[12,13] That means excipients, substances other than the pharmacologically active drugs, are not considered to be inert components and may have an important effect on drug metabolism and efflux.[11,14] Polyacrylate polymers have been used widely to achieve the desired drug release profile with the drug being released at the right place and time or, if necessary, over a desired period of time. Other important uses are taste and odor masking to increase patient compliance and also protection from external influences like moisture. Eudragit® polymers are copolymers derived from esters of acrylic and methacrylic acid, whose physicochemical properties are determined by functional groups. Eudragit S100 is an anionic polymer showing a pH-dependent solubility and has been utilized for oral drug delivery because of its solubility and consequently drug release at pH above 7. Also Eudragit L100, which is an enteric anionic copolymer, is based on methacrylic acid and methyl methacrylate. On the other hand Eudragit RL100 is a positively charged acrylate polymer which is

***Corresponding author:** Parvin Zakeri-Milani, Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. 51664. Tel: +98 (411) 3392593, Fax: +98 (411) 3344798, E-mail: pzakeri@tbzmed.ac.ir

Copyright © 2014 by Tabriz University of Medical Sciences

extensively used in pharmaceutical sciences i.e. sustained release film coating, etc.[15-22] This study has been conducted in order to test the ability of the named excipients, whose influence on the expression of P-gp has not been reported until now, in down regulating the P-gp efflux transporter which would probably lead to enhancement in drug bioavailability.

Materials and Methods

Materials

Human carcinoma colorectal (Caco-2) cell line was purchased from National cell bank of Iran, Pastur institute, Iran. All cell culture disposable equipments were obtained from Orange, Belgium. RPMI 1640 – Powdered Cell Culture Medium was from PAA Co, Austria. Fetal Bovine Serum (FBS) was aquired from Gibco, Invitrogen, USA. Dimethylsulfoxide (DMSO) was from Merck, Germany. Penicillin and streptomycin were obtained from Sigma, Germany. MTT reagent (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was purchased from Roche Diagnostics GmbH, Germany. Trypsin was provided from Gibco, Invitrogen, USA.

Cell culture

All operations were performed via standard sterile conditions under a laminar flow cabinet. The cabinets were habitually sterilized overnight by exposure to ultra-violet radial ion and then washed in 70% alcohol before use. Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg/ml streptomycin and 100 U/ml penicillin. Cells were incubated in a humidified incubator having 5% CO_2 at 37 °C. Then cells were subcultured into 96-well plates and 6-well plates during various steps of the study.[23]

MTT assay

Cytotoxicity of the excipients were measured in Caco-2 cells via the MTT reagent (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide according to the manufacturer's procedure. This method is based on the ability of viable cells to metabolize yellow tetrazolium salt MTT to purple formazan crystals by mitochondrial dehydrogenases. The cells were seeded in 96-well plates with a density of 10^4 cells/well and incubated for 24 h at 37°C and 5% CO_2. The cells were treated with various concentrations of solvent extracts (10, 20, 50, 100, 150, 200, 300, 400 μg/ml) and 0.2 % (v/v) DMSO as a negative control. After 12, 24 and 48 h treatment 10 μl of MTT labeling reagent was added to all wells. The plates were incubated at 37°C and 5% CO2 for 4 hours. Then, 100μl of the solubilization solution was added to each well and followed by incubation overnight at 37°C to dissolve formazan crystals. Finally, absorbance was read using an ELISA plate reader (Bio Teck, Germany) at a wavelength of 570 nm. The percentage of cytotoxicity and cell viability were estimated using following equation[24]:

% Cytotoxicity = 1- [mean absorbance of treated cells/ mean absorbance of negative control]
% Viability = 100 - % Cytotoxicity.

Assessing Uptake of Rhodamine-123

For the uptake studies Caco-2 cells were seeded into 24-well plates; and left for 24 hours. On the other day old medium was removed and cells were washed by PBS. Then new culture media containing different concentrations of excipients and 0.3 mM verapamil, as P-gp inhibitor, were added and left for another 24 hours. On day 3 of experiment, the old medium was removed and cells were washed three times with PBS and Rho-123 solution (RPMI containing 10 mM HEPES (pH=7.4) and 5 μM Rho-123) were added and incubated in 37 °C for 3 hours. After incubation period, Rho-123 solution was removed and cells were washed three times with ice-cold PBS. Cells were lysed in 1% Triton X-100 and centrifuged in 1000 rpm for 5 minutes. Supernatant was used to measure the fluorescence and total protein content. Quantity of Rho-123 was calculated using the obtained calibration curve (R^2=1). Then cellular Rho-123 accumulation was normalized to total protein content determined by protein assay kit.[25]

Western Blotting

Cells were moved to 6-well plate in density of 10^6 cells per well and treated with excipients for 24 hours. Solutions were removed and cells were washed by PBS then incubated in 37°C for 5 minutes with Trypsin/EDTA 0.25%. Supernatant was removed and cell sediment was washed twice with PBS. Lysis buffer was added and cell suspension was centrifuged in 15000 rpm for 5 minutes. The proteins were separated by electrophoresis through SDS-polyacrylamide gel on 12.5% running gel and 4% stacking gel at 80 V for 120 min. The gel was electro blotted to Polyvinylidene difluoride (PVDF) membrane using semi-dry western blotting. 3% non-fat dry milk was used to block the membrane for 1 hour at room temperature and membrane was washed 3 times with PBS-Tween 20 0.1% and then incubated overnight with primary monoclonal antibody (Anti-β-actin), diluted 1/1000 in PBS containing 0.1% tween 20. After washing with PBS-Tween 20 0.1%, the membrane was incubated with horseradish peroxidase-conjugated Rabbit anti-mouse secondary antibody for two hours. Membrane was washed and solution A and B of Enhanced chemiluminescence (ECL) kit was added, then membrane was exposed to X-ray film. Membrane was washed twice and was incubated with MDR1 Antibody (C219) overnight. After washing, membrane was put into horseradish peroxidase-conjugated Rabbit anti-mouse secondary antibody for two hours. Membrane was washed and then solution A and B of ECL kit was added, then membrane was exposed to X-ray film.[26,27]

Copyright © 2014 by Tabriz University of Medical Sciences

Results

Cytotoxicity of excipients on cells was estimated via MTT test assay. MTT test assay of excipients specified the proper concentration of excipient which should be used in western blotting and Rho-123 uptake experiment. Optical density (OD) value obtained from ELISA reader was divided to that of control and cell viability was calculated for each excipient after 24 hours exposure to different polymer concentrations. Two maximum nontoxic concentrations were selected for western blotting and Rho-123 uptake test. According to the results, Eudragit RL100 in concentrations more than 0.5% was toxic for Caco-2 cells (P<0.001). Therefore we used its lower concentrations (0.25% and 0.1% (w/v)) for the subsequent studies. Eudragit S100 and L100 caused significant decrease in cell viability in concentrations more than 0.1% (w/v) and 0.25% (w/v), respectively. Thus lower concentrations were selected for uptake study and also western blotting. The MTT assay results are shown in Figure 1.

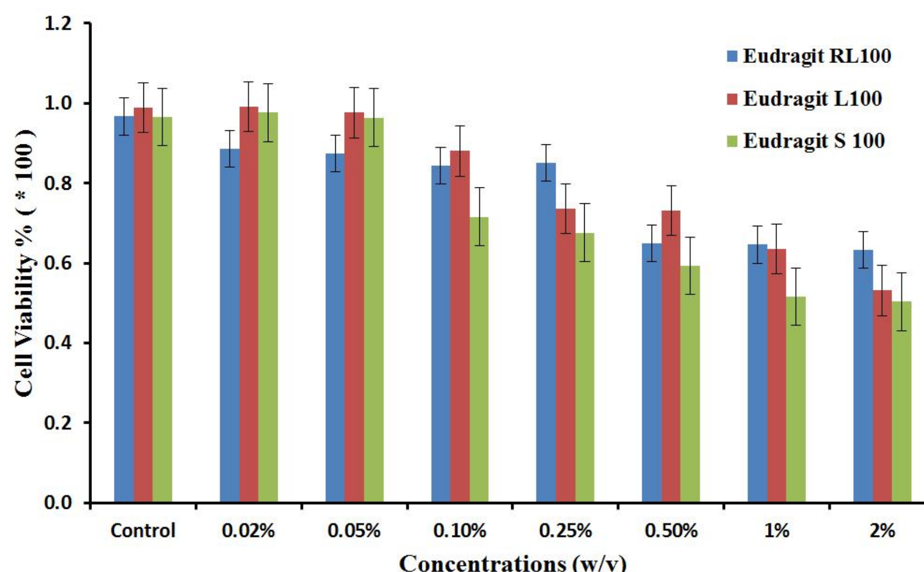

Figure 1. Effects of Eudragit RL100, L100 and S100 on cell viability in Caco-2 cells. Data are expressed as the mean of percent cell viability compared to control after exposure for 24 hours ± standard deviation (n=3).

In order to investigate the functional activity of P-gp, Caco-2 cells were incubated in 48-well plates with different concentrations of the excipients for 24 hours. Afterwards cells were washed with PBS and then exposed to Rho-123 (5 µM) for 3 hours. Cells were lysed and accumulated Rho-123 in cells was measured (excitation at 485 nm and emission measured at 530 nm) for each sample. The protein content of the aliquots was measured by protein assay and cellular Rho-123 accumulation was normalized with respect to the total protein in each well.

Eudragit S100 enhanced Rho-123 uptake significantly into Caco-2 cells as shown in the Figure 2.

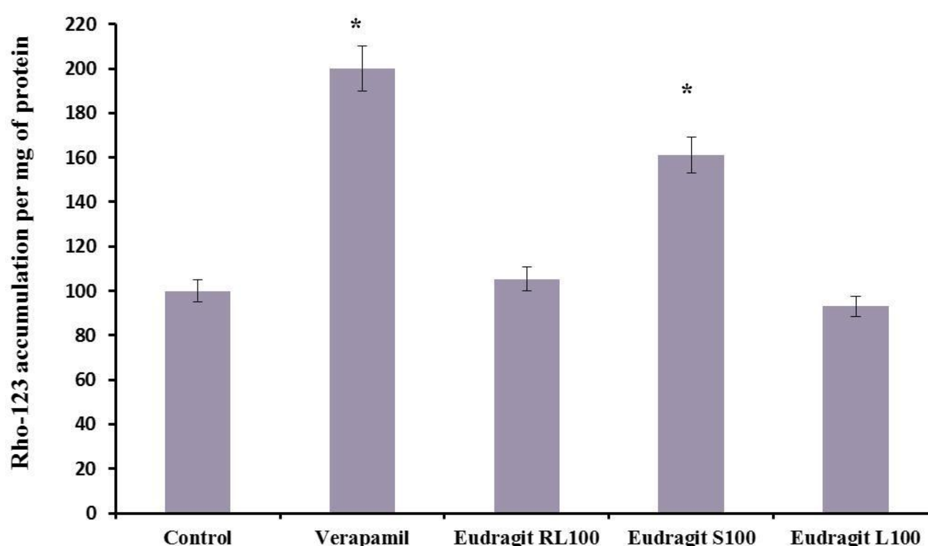

Figure 2. Effect of Eudragit RL100, Eudragit L100 and Eudragit S100 on Rho-123 uptake in Caco-2 cells. Data are expressed by the ratio of quantity of Rho-123 (mg×10^6/mL) to total protein (mg/mL) in each well. Values are versus control as compared with control group using one way ANOVA with Student-Newman-Keuls post hoc test (*P<0.001).

Copyright © 2014 by Tabriz University of Medical Sciences

P-glycoprotein expression was measured in Caco-2 cells which had been treated for 24 hours with excipients and compared to that of control. The protein was separated by electrophoresis on 12.5% running gel and 4% stacking gel. Electrophoretic transfer of separated proteins in gel was transferred to a PVDF membrane using semi-dry blotting. The membrane was blocked in PBS containing 0.1% tween-20 and 3% dried skim milk at room temperature for 1 h and washed three times for 15 min in PBS containing 0.1% tween-20. Encountering with primary and secondary antibody, the bands were visualized using ECL Western Blotting Detection Reagents and exposed to an X-ray film. As shown in the Figure 3 Eudragit S100 had inhibited the P-gp expression which leads to the inhibition of the efflux pump. Other excipients used in the current work had a similar effect as the control group.

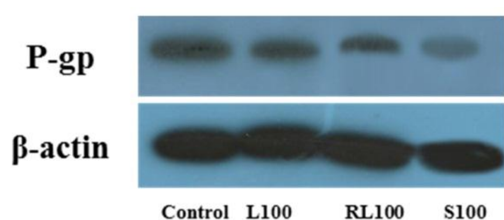

Figure 3. P-gp protein expression after 24 hours exposure to the excipients. Expression in treatment groups were compared with P-gp expression in untreated control cells. 20 µg of total protein were separated by SDS polyacrylamide gel electrophoresis and immunoblotted with monoclonal antibody C219 for P-gp and I-19 for actin.

Discussion

The results of this study showed that there are excipients which could down regulate MDR1 gene and P-gp protein expressions which can lead to enhancement in drug bioavailability. The present study characterizes the effects of the polyacrylate excipients on P-gp expression and activity in Caco-2 monolayer. Sub-toxic concentrations of excipients were prepared using MTT test assay and in the accumulation studies a known P-gp substrate, Rho-123, uptake was tested. Furthermore western blotting confirmed Rho-123 uptake data. Eudragit S100 that was able to increase Rho-123 accumulation decreased P-gp expression either. This study aimed to access a rational drug formulation development strategy for oral dosage forms based on Caco-2 monolayer as an in vitro screening model.

The oral delivery of drugs is generally the most suitable route to administer drugs, as it is painless and easy to use, and therefore it is followed by high patient compliance.[28,29] On the other hand, MDR proteins belonging to ABC transporters are a part of membrane transport proteins that detoxify cells from external substrates. These proteins are identified to limit absorption through biological membranes such as intestinal, brain and cancer cells.[30-32] Several papers have reported data on the effects of different agents and excipients other than Eudragits on the P-gp. For instance a report states that tween 80 can decrease the percentage of serosal-mucosal transport to mucosal-serosal transport of Rho-123 across rat jejunal membrane in vitro and caco-2 cell monolayer, suggestive of p-gp inhibition.[33] In addition, the in vitro absorption of digoxin across an everted rat gut sac (a p-gp substrate) showed a deep increase after treating with 0.5% (w/v) tween 20 and tween 80.[34] Also, digoxin given with tween showed an increase in AUC and C_{max} in rats. Another study has claimed that cremophor EL (0.1%, w/v) only partially inhibits P-gp activity in Caco-2 cells.[35] Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells.[25] On the other hand there are many other reports on the effects of inhibition of P-gp by co administered drugs on intestinal permeability of drugs.[36,37] Granting these findings, there was no direct study to be conducted on the changes of the expression of MDR1 gene or P-gp induced by Eudragits. In this study, concentrations of 0.1% and 0.25% (w/v) for Eudragit RL100 had no toxic effect on Caco-2 cells so used in the Rho-123 uptake assay. When compared to control group (Intracellular Rho = 2612 pg/mL; total protein = 26 mg/mL), Eudragit RL100 led to a very slight increase in the accumulation of Rho in cells which was not significant (Intracellular Rho = 2754 pg/mL; total protein =96 mg/mL). Moreover from the results of MTT test, Eudragit L100 at 0.25% (w/v) and 0.5% (w/v) concentrations and Eudragit S100 at 1% (w/v) and 2% (w/v) were found to be non-toxic to Caco-2 cells and were subjected to Rho-123 uptake assay and western blotting analysis. Treatment of cells with the Eudragit L100 caused to 7%, decrease in Rho-123 accumulation. (Intracellular Rho = 2429 pg/mL; total protein =93 mg/mL). Eudragit S100 showed significant increase in Rho-123 accumulation in cells. This increase was 61%. (Intracellular Rho = 4205 pg/mL; total protein =89 mg/mL). According to the obtained results, the present study demonstrated the efficacy of Eudragit S100 as an excipient in decreasing the P-glycoprotein expression and consequently its possible role as an inhibitory factor in the efflux process in different absorption regions. Using this polymer as an efflux pump inhibitor would be novel and it apparently will improve the bioavailability and help both substrate drug intestinal permeability and its absorption. Although the study is fulfilled in vitro, the data leave no doubt that Eudragit S100 has beneficial effects in inhibiting P-gp efflux activity.

Conclusion

Taken together, this paper suggests that using proper concentrations of the Eudragit S100 excipient would probably advance the bioavailability and help drug intestinal permeability and absorption which can plausibly have a significant impact on both drug efflux

Copyright © 2014 by Tabriz University of Medical Sciences

process and metabolism. Of course some other factors including P-gp structure, P-gp environment and substrate partitioning into the tissues have to be understood fully to decide on the ability of these commonly used excipients to inhibit P-gp activity in vitro and to further describe the effect of them on both activity and expression of P-gp. Therefore further tests for example, gut perfusion or pharmacokinetic studies in animals or more specific assays which target specific binding sites on P-glycoprotein should be carried out, later.

Acknowledgements

The authors would like to thank the authorities of Drug Applied Research Center, Tabriz University of Medical Sciences, for their financial support. This article is based on a thesis submitted for PharmD degree (No. 3664) in Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Conflict of Interest

The Authors report no conflict of interests in the present study.

References

1. Sugihara N, Toyama K, Michihara A, Akasaki K, Tsuji H, Furuno K. Effect of benzo[a]pyrene on P-glycoprotein-mediated transport in Caco-2 cell monolayer. *Toxicol* 2006;223(1-2):156-65.
2. Rekha MR, Sharma CP. Oral delivery of therapeutic protein/peptide for diabetes--future perspectives. *Int J Pharm* 2013;440(1):48-62.
3. Jiang L, Long X, Meng Q. Rhamnolipids enhance epithelial permeability in Caco-2 monolayers. *Int J Pharm* 2013;446(1-2):130-5.
4. Amin ML. P-glycoprotein inhibition for optimal drug delivery. *Drug Target Insights* 2013;7:27-34.
5. Arora A, Seth K, Kalra N, Shukla T. Modulation of p-glycoprotein-mediated multidrug resistance in k562 leukemic cells by indole-3-carbinol. *Toxicol Appl Pharmacol* 2005;202(3):237-43.
6. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, et al. Membrane transporters in drug development. *Nat Rev Drug Discov* 2010;9(3):215-36.
7. Xie Z, Cao L, Zhang J. miR-21 modulates paclitaxel sensitivity and hypoxia-inducible factor-1alpha expression in human ovarian cancer cells. *Oncol Lett* 2013;6(3):795-800.
8. Bodo A, Bakos E, Szeri F, Varadi A, Sarkadi B. The role of multidrug transporters in drug availability, metabolism and toxicity. *Toxicol Lett* 2003;140-141:133-43.
9. Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. *Annu Rev Pharmacol Toxicol* 2003;43:285-307.
10. Sugano K, Kansy M, Artursson P, Avdeef A, Bendels S, Di L, et al. Coexistence of passive and carrier-mediated processes in drug transport. *Nat Rev Drug Discov* 2010;9(8):597-614.
11. Johnson BM, Charman WN, Porter CJ. An in vitro examination of the impact of polyethylene glycol 400, Pluronic P85, and vitamin E d-alpha-tocopheryl polyethylene glycol 1000 succinate on P-glycoprotein efflux and enterocyte-based metabolism in excised rat intestine. *AAPS pharmSci* 2002;4(4):E40.
12. Shen Q, Lin Y, Handa T, Doi M, Sugie M, Wakayama K, et al. Modulation of intestinal P-glycoprotein function by polyethylene glycols and their derivatives by in vitro transport and in situ absorption studies. *Int J Pharm* 2006;313(1-2):49-56.
13. Wempe MF, Wright C, Little JL, Lightner JW, Large SE, Caflisch GB, et al. Inhibiting efflux with novel non-ionic surfactants: Rational design based on vitamin E TPGS. *Int J Pharm* 2009;370(1-2):93-102.
14. Ursino MG, Poluzzi E, Caramella C, De Ponti F. Excipients in medicinal products used in gastroenterology as a possible cause of side effects. *Regul Toxicol Pharmacol* 2011;60(1):93-105.
15. Akhgari A, Abbaspour MR, Pirmoradi S. Preparation and evaluation of pellets using acacia and tragacanth by extrusion-spheronization. *Daru* 2011;19(6):417-23.
16. Aigner D, Ungerbock B, Mayr T, Saf R, Klimant I, Borisov SM. Fluorescent materials for ph sensing and imaging based on novel 1,4-diketopyrrolo-[3,4-c]pyrrole dyes. *J Mater Chem C Mater Opt Electron Devices* 2013;1(36):5685–93.
17. Roy H, Brahma CK, Nandi S, Parida KR. Formulation and design of sustained release matrix tablets of metformin hydrochloride: Influence of hypromellose and polyacrylate polymers. *Int J Appl Basic Med Res* 2013;3(1):55-63.
18. Tsai SW, Yu DS, Tsao SW, Hsu FY. Hyaluronan-cisplatin conjugate nanoparticles embedded in Eudragit S100-coated pectin/alginate microbeads for colon drug delivery. *Int J Nanomedicine* 2013;8:2399-407.
19. Thakral NK, Ray AR, Majumdar DK. Eudragit S-100 entrapped chitosan microspheres of valdecoxib for colon cancer. *J Mater Sci Mater Med* 2010;21(9):2691-9.
20. Thakral NK, Ray AR, Bar-Shalom D, Eriksson AH, Majumdar DK. The quest for targeted delivery in colon cancer: Mucoadhesive valdecoxib microspheres. *Int J Nanomedicine* 2011;6:1057-68.
21. Thakral S, Thakral NK, Majumdar DK. Eudragit: A technology evaluation. *Expert Opin Drug Deliv* 2013;10(1):131-49.
22. Verma P, Gupta RN, Jha AK, Pandey R. Development, in vitro and in vivo characterization of Eudragit RL 100 nanoparticles for improved ocular bioavailability of acetazolamide. *Drug Deliv* 2013;20(7):269-76.

Copyright © 2014 by Tabriz University of Medical Sciences

23. Valiyari S, Baradaran B, Delazar A, Pasdaran A, Zare F. Dichloromethane and Methanol Extracts of Scrophularia oxysepala Induces Apoptosis in MCF-7 Human Breast Cancer Cells. *Adv Pharm Bull* 2012;2(2):223-31.
24. Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnol Annu Rev* 2005;11:127-52.
25. Sachs-Barrable K, Thamboo A, Lee SD, Wasan KM. Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. *J Pharm Pharm Sci* 2007;10(3):319-31.
26. Zibaei M, Firoozeh F, Bahrami P, Sadjjadi SM. Investigation of anti-Toxocara antibodies in epileptic patients and comparison of two methods: ELISA and Western blotting. *Epilepsy Res Treat* 2013;2013:156815.
27. Ferroglio E, Zanet S, Mignone W, Poggi M, Trisciuoglio A, Bianciardi P. Evaluation of a rapid device for serological diagnosis of Leishmania infantum infection in dogs as an alternative to immunofluorescence assay and Western blotting. *Clin Vaccine Immunol* 2013;20(5):657-9.
28. Gradauer K, Dunnhaupt S, Vonach C, Szollosi H, Pali-Scholl I, Mangge H, et al. Thiomer-coated liposomes harbor permeation enhancing and efflux pump inhibitory properties. *J Control Release* 2013;165(3):207-15.
29. Lee CK, Choi JS, Bang JS. Effects of Fluvastatin on the Pharmacokinetics of Repaglinide: Possible Role of CYP3A4 and P-glycoprotein Inhibition by Fluvastatin. *Korean J Physiol Pharmacol* 2013;17(3):245-51.
30. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv Drug Deliv Rev* 2003;55(1):3-29.
31. Sharma M, Prasad R. The quorum-sensing molecule farnesol is a modulator of drug efflux mediated by ABC multidrug transporters and synergizes with drugs in Candida albicans. *Antimicrob Agents Chemother* 2011;55(10):4834-43.
32. Shaikh SA, Li J, Enkavi G, Wen PC, Huang Z, Tajkhorshid E. Visualizing functional motions of membrane transporters with molecular dynamics simulations. *Biochemistry* 2013;52(4):569-87.
33. Rege BD, Kao JP, Polli JE. Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers. *Eur J Pharm Sci* 2002;16(4-5):237-46.
34. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. *Int J Pharm* 2004;278(1):119-31.
35. Huang J, Si L, Jiang L, Fan Z, Qiu J, Li G. Effect of pluronic F68 block copolymer on P-glycoprotein transport and CYP3A4 metabolism. *Int J Pharm* 2008;356(1-2):351-3.
36. Zakeri-Milani P, Valizadeh H, Islambulchilar Z, Damani S, Mehtari M. Investigation of the intestinal permeability of ciclosporin using the in situ technique in rats and the relevance of P-glycoprotein. *Arzneimittelforschung* 2008;58(4):188-92.
37. Zakeri-Milani P, Mehtari M, Valizadeh H. Evidence for the enhanced intestinal absorption of digoxin by p-glycoprotein inhibitors. *Trop J Pharm Res* 2012;12(6):939-45.

 Copyright © 2014 by Tabriz University of Medical Sciences