Drug Development and Industrial Pharmacy

http://informahealthcare.com/ddi
ISSN: 0363-9045 (print), 1520-5762 (electronic)

Drug Dev Ind Pharm, Early Online: 1–4
 DOI: 10.3109/03639045.2013.851210

informa
healthcare

RESEARCH ARTICLE

Effect of anionic macromolecules on intestinal permeability of furosemide

Hadi Valizadeh[1], Hadi Fahimfar[2], Saeed Ghanbarzadeh[3], Ziba Islambulchilar[2,4], and Parvin Zakeri-Milani[5]

[1]Faculty of Pharmacy and Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Student Research Committee, [3]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [4]Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran, and [5]Faculty of Pharmacy and Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Context: Furosemide is an anionic molecule and has very low absorption in gastro intestinal tract.
Objective: The aim of this study was to investigate the effect of anionic macromolecules on the intestinal permeability of Furosemide.
Materials and methods: The intestinal permeability of Furosemide was determined using single-pass intestinal perfusion technique in rats. Briefly a jejunal segment of ~10 cm was isolated and cannulated in both ends for inlet and outlet solution. The perfusate was collected every 10 min and samples were analyzed using the RP-HPLC method. Test samples containing furosemide and two anionic macromolecules, sodium carboxy methyl cellulose and sodium alginate, at different concentrations were used.
Results: The obtained data showed that existence of Sodium carboxy methyl cellulose significantly increased the P_{eff} values in all three investigated concentrations ($p < 0.05$) but sodium alginate only in concentrations <0.1% increased drug permeability.
Discussion: It is concluded that the anionic macromolecules at specific concentrations could alter the permeability of anionic drugs across the biological membranes.
Conclusions: Donnan phenomenon and chelating property of macromolecules could be attributed to the observed effect.

Keywords

Donnan effect, furosemide, single-pass intestinal perfusion

History

Received 24 July 2013
Revised 27 September 2013
Accepted 30 September 2013
Published online 6 November 2013

Introduction

Furosemide (FZM), 5-(aminosulfonyl)-4-chloro-2-[(furanylmethyl) amino] benzoic acid (CAS No: 54-31-9), is a diuretic and antihypertensive drug which is indicated for congestive heart failure, chronic renal failure and hepatic cirrhosis. Following oral administration FZM is rapidly but incompletely absorbed mostly in the stomach and upper small intestine, probably due to its weak acidic properties (pKa = 3.93). FZM also undergoes first pass metabolism which leads to its low bioavailability (43–50%). The biological half-life of FZM is 1–2 h[1,2]. To increase its bioavailability, FZM was formulated in different dosage forms using different excipients[1,3–5]. Excipients are considered ideally non-reactive with the drug and inert in the human body. However some of them may alter the solubility or permeability of drugs. For example in the case of FZM, macromolecules can have different effects on drug absorption through complexation, increasing viscosity, interfacial adsorption and Donnan effect[5–9]. On the other hand FZM could be administered in patients with other drugs such as P-gp efflux pump inhibitors. P-gp inhibitors have high effect on the permeability and as a result on the bioavailability of FZM[1,8,10–13].

Several techniques have been reported in the literature to investigate the drug intestinal permeability including in situ intestinal perfusion, the Caco-2 cell model and the everted intestinal sacs model. Apart from clarifying the mechanisms of drug absorption, prediction of oral drug absorption in humans is the ultimate goal of such studies[14]. The influence of P-gp inhibitors on FZM intestinal absorption and the effect of excipients on some other drugs absorption were investigated previously[1,15,16]. The effect of macromolecules on FZM transport from dialysis membrane was also studied in our previous work[17]. The aim of this study was to investigate the effect of anionic macromolecules on intestinal permeability of FZM using SPIP technique in rats.

Materials and methods

Materials

Furosemide was purchased from Shasun Chemicals and Drugs Ltd. (Pondicherry, India). NaCMC was purchased from Fluka Company (Helsinki, Finland) and Phenol red was obtained from Sigma (St. Louis, MO). Na-Alginate was purchased from

Address for correspondence: Parvin Zakeri-Milani, Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran. Tel: +98 411 3392593. Fax: +98 411 334 4798. E-mail: pzakeri@tbzmed.ac.ir

BOH Chemicals Ltd. (Poole, England). Methanol, Acetonitrile, Na_2HPO_4, NaH_2PO_4 and NaOH were prepared from Merck Company (Darmstadt, Germany).

Methods

Stability test for FZM and phenol red

For stability test of FZM, solution containing 20 μM FZM and 250 mg/l phenol red was prepared and kept at 37°C for at least 90 min. Then every 10 min 2-ml samples were taken and analyzed by the HPLC method which was developed and validated previously by Zakeri-Milani et al[18–20]. Since furosemide is susceptible to photodegradation, all solutions were protected from light with aluminum foil[21].

Intestinal perfusion technique

Perhaps the most used classic technique; employed in the study of intestinal absorption of compounds has been the Single-pass Intestinal Perfusion (SPIP) model. SPIP studies in rats were performed using established methods adapted from the literature. Briefly, male Wistar rats (200–300 g, 7–10 weeks) were fasted 12–18 h before experiment. However drinking water was readily accessible until 1 h before surgery. It has been reported that barbiturates have the least effect on intestinal permeability in rats. Therefore we have used pentobarbital (50 mg/kg) as an anesthetic agent in all experiments[11,12,22]. The rats were anaesthetized and placed on a heated pad to keep normal body temperature. By making a midline abdominal incision, an ~10 cm section of the proximal rat jejunum was cannulated gently with plastic tubing rinsed with saline (37 °C) and attached to the perfusion set which consisted of a syringe pump (Palmer, England) and a 60-ml syringe connected to it. The entire surgical area was then covered with parafilm to reduce evaporation. The phosphate buffered saline (PBS) (pH 7.2) was used as perfusion medium. About 10 ml of phenol red tock solution (2.5 g/l) was added to the 100 ml of each test solution as a non-absorbable marker in each experiment. In the SPIP experimental procedure modifications can be made to the flow rate, length of perfused intestine, concentration of the drug and composition of formulation. Loss of compound, as determined by the difference between the inlet and outlet concentrations, is attributed to absorption but only after preliminary studies such as stability studies in the buffer[1,6,10,11,23–28]. Because water absorption and secretion during the perfusion may introduce errors in the calculated absorption, various water absorption or secretion (flux) correction methods have been published. In these methods, the drug concentration in outlet tubing is corrected for volume change in the segment using phenol red concentration in inlet and outlet tubing.

The effective permeability coefficients (P_{eff}) were calculated from following equation:

$$P_{\text{eff}} = \frac{-Q\left(\ln\left(\frac{C_{Out}}{C_{in}}\right)\right)}{2\pi rL} \quad (1)$$

where C_{in} and C_{out} is the concentration of tested drug in the inlet and outlet tubing respectively; 2πrL is the area of the mass transfer surface (cm^2) within the intestinal segment which is assumed to be a cylinder area[10,11,23,27,29]. Injection of blank PBS buffer collected from outlet tubing (before perfusion of the drug solution) onto HPLC column showed that no interfering peak could be observed on chromatogram. Therefore, there was no matrix effect for the measurement of compounds studied.

Furosemide solutions were prepared in concentrations of 10 and 20 μM in PBS buffer. NaCMC and Na-Alginate (Na-ALG) solutions were prepared in concentrations of 0.1%, 0.2% and 0.5% (w/v %) in PBS buffer. Test samples (10 and 20 μmol FZM) were infused at a flow rate of 0.2 ml/min by a syringe pump for 90 min without sampling until 20 min. Outlet samples were collected every 10 min in microtubes. The volume of sample for each time interval was 2 ml and samples were stored at −20 °C until analysis. There were two control groups which only received 10 and 20 μmol FZM, and 12 test groups which received NaCMC or Na-ALG, at concentrations of 0.1%, 0.2% and 0.5% in addition to FZM. There were four rats in each group. When the experiment was completed the animals were killed with a cardiac injection of air and the intestine was removed and the exact length of intestine was measured. The radius of the intestine was considered to be 0.18 cm[20,30].

Analytical method

Furosemide in samples was measured by a previously validated reverse-phase HPLC method using a C18 column (Shimpack VP-ODS, 250 mm × 4.6 mm, 7 μm) at 37 °C. Analysis was carried out at a wavelength of 280 nm. The mobile phase consisted of a mixture of acetonitril and water (41.5:57.4 v/v%) with 0.9 ml acetic acid glacial and 0.1 ml triethylamine (adjusted to pH 5.6). The flow rate was 1 ml/min and injection volume was 30 μl. Retention times for FZM and phenol red were 9.8 and 4.5 min respectively. Area under the peak data were used for calculations and r^2 was 0.999 for both calibration curves. The detector response to phenol red did not change with the size of the sample volume. This method was simple, rapid and reliable RP-HPLC method with acceptable precision (<3.9%), accuracy (98.2–103.4%), and linearity and was used for simultaneous determination of phenol red and furosemide.

Results

Stability test

Stability study of FZM and phenol red was performed at 37 °C. The results showed that mean (±SD) remained percent of FZM and phenol red during 90 min was 99.92 ± 0.59 and 99.97 ± 0.20 respectively (Figure 1).

Intestinal perfusion technique

In drug permeability studies, in situ methods offer advantages over in vitro models. Although the animal is anaesthetized and surgically manipulated, neural, endocrine, lymphatic and mesenteric blood supplies are intact and therefore all the transport mechanisms present in a live animal are functional[10,11,31,32]. In the present study Single pass intestinal perfusion technique in rat jejunum was used to determine intestinal permeability (P_{eff}) of FZM alone and also in the presence of NaCMC or Na-ALG

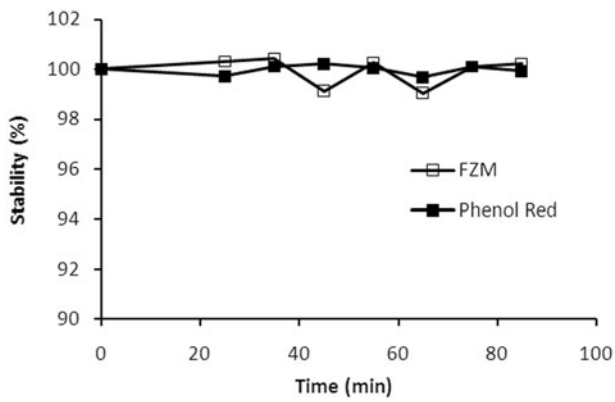

Figure 1. Stability of FZM and phenol red at 37 °C.

in the concentrations of 0.1%, 0.2% and 0.5%. As an important aspect, the potential age dependency of rat intestinal permeability should be considered. Although an age-dependent intestinal permeability might be valid for very young and very old rats, no influence of age on the jejunal permeability in the rat within the age interval of 5–30 weeks has been reported[22].

Mean P_{eff} values for control groups which received FZM in concentrations of 10 and 20 μM were 2.42×10^{-5} and 2.44×10^{-5} cm/s. Calculated P_{eff} values for FZM in samples containing FZM (10 μM) and NaCMC in concentration of 0.1%, 0.2% and 0.5% were 3.46×10^{-5}, 4.7×10^{-5} and 6.31×10^{-5} cm/s. Corresponding values for samples containing Na-ALG were 3.01×10^{-5}, $2.32 \times 10^{-5}$and 1.52×10^{-5} cm/s. The results for samples containing 20 μM FZM are illustrated in Figure 2. Results showed that existence of NaCMC increased the P_{eff} values in all three investigated concentrations but Na-ALG only in concentrations <0.1% increased FZM permeability (Figure 2).

In our pervious in vitro study it was shown that NaCMC up to 5% and Na-ALG up to 0.5% could increase FZM dialysis rate and in higher concentrations viscosity effect overcomes to electrical repulsion effect of macromolecules[17].

Figure 3 shows the influence of macromolecules concentration on the ratio of corrected concentration of FZM in the outlet solution to FZM concentration in inlet solution versus time.

Discussion

There are different mechanisms to give explanation for interactions between anionic macromolecules and FZM. One of these mechanisms is Donnan phenomenon[17,33]. Higuchi et al, investigated the effect of anionic macromolecules, NaCMC and Na-ALG, and poly-acrylic sodium salicylate on penetration of potassium benzyl penicillin through cellophane membrane. They showed that in the presence of anionic macromolecules absorption and permeability of anionic drugs across the membrane were increased. The first mechanism that has a negative effect on the permeability is the viscosity of solution. Solution viscosity is increased with increasing macromolecules concentration and the drug release to the environment is slowed down and thereby the permeability is decreased. The reason behind this is the well-known Stokes–Einstein relation which indicates an inverse dependency between diffusion coefficients of drug and solution viscosity. That means the macromolecules could effectively serve as physical barriers that reduces ionic diffusion rates[34]. In our previous study[35] we found that the existence of anionic macromolecules such as NaCMC and Na-ALG could increase dialysis rate of FZM which can be explained with Donnan effect. Moreover the use of Methyl cellulose as nonionic macromolecule with viscosity increasing effect resulted in decreased dialysis rate of FZM. Existence of cationic sodium ion, which can interact with anionic macromolecules and existence of anionic phosphate ion which also can have Donnan effect are other interfering factors. In this study as shown in Figure 2, increasing NaCMC concentration, led to increased drug permeability values, however in the case of Na-ALG, increasing concentration of macromolecule from 0.1% to 0.5%, decreased the drug effective permeability from 3.16–1.4×10^{-5} cm/s. This could be attributed to the dominance of the viscosity to Donnan effect. Another mechanism is the influence of macromolecules on the tight junctions. Tight junctions are connections between the intestinal epithelial cells which in normal condition are closed and do not allow to the substances to pass. There are different mechanisms for the functioning of the tight junctions. One of these mechanisms is related to calcium ions. If calcium ions in the extracellular environment react or make complex with substances like EDTA, calcium ion concentration is decreased and adhesion between cells is reduced and as a result, tight junctions loose and let the polar materials pass. Thus, temporary shortage of calcium induces opening of tight junctions through the breaking of adhesion between cells[36–40]. Considering the negative charge of NaCMC and Na-ALG they can hold calcium ions by negative/positive charge attraction, increasing the absorption of the drug through the opened tight junctions. On the other hand the existence of anionic phosphate ion which can bond to calcium ions or can have Donnan effect is another interfering factor[41–43].

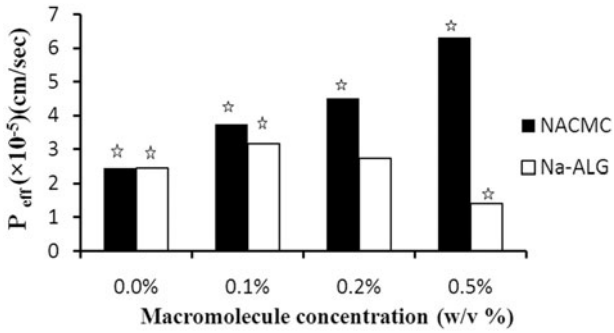

Figure 2. Comparative graph for permeability of FZM (20 μM) in the presence of various concentrations of NaCMC and Na-AlG.

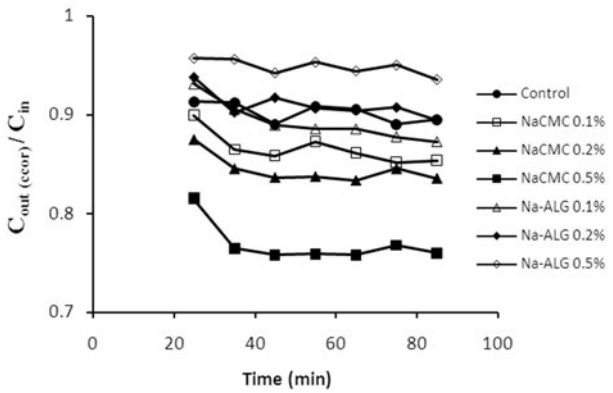

Figure 3. Influences of macromolecules on the outlet/inlet concentration of FZM.

Conclusions

In conclusion the viscosity increasing caused by macromolecules has opposite effect with Donnan phenomenon on permeation of drugs. However at low concentrations of the anionic macromolecules, they can be used to enhance the permeability of FZM. The effect of macromolecules on tight junctions increases the intestinal permeability of FZM. To distinguish the difference between the Donnan effect and the effect of macromolecules on tight junctions it is recommended to use a nonionic chelating molecule which could chelate the calcium ion without having Donnan effect and effect on viscosity. Considering the existence of cationic sodium ion which can interact with anionic macromolecules, and anionic phosphate ion which also has Donnan effect, it is necessary to investigate macromolecules effect in the absence of any ion.

Acknowledgements

The authors would like to thank the authorities of the Faculty of Pharmacy, Tabriz University of Medical Sciences for providing analytical facilities.

Declaration of interest

The authors report no declarations of interest. Authors thank Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran, for financial support. This paper is based on a Pharm D thesis (number 3479) submitted in Faculty of Pharmacy, Tabriz University of Medical Sciences.

References

1. Al-Mohizea AM. Influence of intestinal efflux pumps on the absorption and transport of furosemide. Saudi Pharm J 2010;18: 97–101.
2. Motz SA, Schaefer UF, Balbach S, et al. Permeability assessment for solid oral drug formulations based on Caco-2 monolayer in combination with a flow through dissolution cell. Eur J Pharm Biopharm 2007;66:286–95.
3. Devarakonda B, Otto DP, Judefeind A, et al. Effect of pH on the solubility and release of furosemide from polyamidoamine (PAMAM) dendrimer complexes. Int J Pharm 2007;345:142–53.
4. Perioli L, DeAlba G, Pagano C. New oral solid dosage form for furosemide oral administration. Eur J Pharm Biopharm 2011;80: 621–9.
5. Zvonar A, Berginc K, Kristl A, Gae¡perlin M. Microencapsulation of self-microemulsifying system: improving solubility and permeability of furosemide. Int J Pharm 2011;388:151–8.
6. Chitneni M, Peh KK, Darwis Y, et al. Intestinal permeability studies of sulpiride incorporated into self-microemulsifying drug delivery system (SMEDDS). Pak J Pharm Sci 2011;24:13–121.
7. Csáki KF. Synthetic surfactant food additives can cause intestinal barrier dysfunction. Med Hypotheses 2010;76:676–81.
8. Li M, Si L, Pan H, et al. Excipients enhance intestinal absorption of ganciclovir by P-gp inhibition: assessed in vitro by everted gut sac and in situ by improved intestinal perfusion. Int J Pharm 2011;403: 37–45.
9. Zambito Y, Zaino C, Uccello-Barretta G, et al. Improved synthesis of quaternary ammonium-chitosan conjugates (N+-Ch) for enhanced intestinal drug permeation. Eur J Pharm Sci 2008;33: 343–50.
10. Sun M, Zhai X, Xue K, et al. Intestinal absorption and intestinal lymphatic transport of sirolimus from self-microemulsifying drug delivery systems assessed using the single-pass intestinal perfusion (SPIP) technique and a chylomicron flow blocking approach: linear correlation with oral bioavailabilities in rats. Eur J Pharm Sci 2011; 43:132–40.
11. Zakeri-Milani P, Valizadeh H, Islambulchilar Z, et al. Investigation of the intestinal permeability of ciclosporin using the in situ technique in rats and the relevance of P-glycoprotein. Arzneim Forsch 2008;58:188–92.
12. Zakeri-Milani P, Valizadeh H, Islambulchilar Z, et al. Effect of erythromycin and clarithromycin on the intestinal transport of digoxin. Pharm Sci 2009;15:75–82.
13. Zhang Y, Zhu HX, Guo LW. Intestinal absorption of berberine alone and in combinations by rats single pass intestinal perfusion in situ. Yaoxue Xuebao 2010;47:233–8.
14. Li H, Jin H, Shim W, Shim C. An improved prediction of the human *in vivo* intestinal permeability and BCS class of drugs using the *in vitro* permeability ratio obtained for rat intestine using an Ussing chamber system. Drug Dev Ind Pharm 2013;39:515–22.
15. Lo YL. Relationships between the hydrophilic-lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. J Control Release 2003;90:37–48.
16. Ma L, Wei Y, Zhou Y, et al. Effects of Pluronic F68 and Labrasol on the intestinal absorption and pharmacokinetics of rifampicin in rats. Archiv Pharm Res 2010;34:1939–43.
17. Barzegar Jalali M, Valizadeh H, Ebrahimpoor S, et al. Effect of sodium carboxy methylcellulose on dialysis rate of furosemide. Pharm Sci 2009;15:323–8.
18. Singh N, Gupta P. Formulation and evaluation of oral multi drug delivery system of ceftriaxone sodium by using intestinal absorption enhancers. J Pharm Pharm Sci 2012;4:123–7.
19. Zakeri-Milani P, Barzegar-Jalali M, Tajerzadeh H, et al. Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation. J Pharm Biomed Anal 2005;39: 624–30.
20. Zakeri-Milani P, Valizadeh H, Azarmi Y, et al. Simultaneous determination of metoprolol, propranolol and phenol red in samples from rat in situ intestinal perfusion studies. Daru 2006; 14:102–8.
21. Asker AF, Ferdous AJ. Photodegradation of furosemide solutions. PDA J Pharm Sci Technol 1996;50:158–62.
22. Zakeri-Milani P, Valizadeh H, Tajerzadeh H, et al. Predicting human intestinal permeability using single-pass intestinal perfusion to rat. J Pharm Pharm Sci 2007;10:368–79.
23. Chen XM, Li JS, Li W, et al. Intestinal absorption of the effective components of Schisandra chinensis Baill by rats single-pass perfusion in situ. Yaoxue Xuebao 2010;45:652–8.
24. Kang MJ, Kim HS, Jeon HS, et al. *In situ* intestinal permeability and *in vivo* absorption characteristics of olmesartan medoxomil in self-microemulsifying drug delivery system. Drug Dev Ind Pharm 2012; 38:587–96.
25. Liu ZJ, Jiang DB, Tian LL, et al. Intestinal permeability of forskolin by in situ single pass perfusion in rats. Planta Med 2012;78: 698–702.
26. Paixao P, Gouveia LF, Morais JAG. Prediction of the in vitro permeability determined in Caco-2 cells by using artificial neural networks. Eur J Pharm Sci 2011;41:107–17.
27. Song NN, Li QS, Liu CX. Intestinal permeability of metformin using single-pass intestinal perfusion in rats. World J Gastroenterol 2006;12:4064–70.
28. Wang J, Wang P, Yang Y, et al. Intestinal absorption of aloe-emodin using single-passintestinal perfusion method in rat. Zhongguo Zhongyao Zazhi 2011;36:2393–8.
29. Ingels F, Beck B, Oth M, Augustijns P. Effect of simulated intestinal fluid on drug permeability estimation across Caco-2 monolayers. Int J Pharm 2004;274:221–32.
30. CCAC, Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care 1993;1:15–52.
31. Saha P, Kou JH. Effect of bovine serum albumin on drug permeability estimation across Caco-2 monolayers. Eur J Pharm Biopharm 2002;54:319–24.
32. Salphati L, Childers K, Pan L, et al. Evaluation of a single-pass intestinal-perfusion method in rat for the prediction of absorption in man. J Pharm Pharmacol 2001;53:1007–13.
33. Bele MH, Derle DV. Effect of polacrilin potassium as disintegrant on bioavailability of diclofenac potassium in tablets: a technical note. AAPS PharmSciTech 2012;13:756–9.
34. Bentz AP, Peltz MA, Snyder KA, Davis JM. Verdict: viscosity enhancers reducing diffusion in concrete technology. Concr Int 2009;31:31–6.
35. Zakei-Milani P, Islambolchilar Z, Ebrahimpoor S, et al. Investigation of furosemide dialysis rate in the presence of anionic polymers. Dissolution Technol 2012;19:21–5.
36. Deli MA. Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. Biochim Biophys Acta 2009;1788:892–910.
37. Salamat-Miller N, Johnston TP. Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium. Int J Pharm 2005;294:201–16.
38. Sonaje K, Lin K, Tseng MT, et al. Effects of chitosan-nanoparticle-mediated tight junction opening on the oral absorption of endotoxins. Biomaterials 2011;32:8712–21.
39. Ward PD, Tippin TK, Thakker DR. Enhancing paracellular permeability by modulating epithelial tight junctions. Pharm Sci Technol Today 2000;3:346–58.
40. Yu Q, Wang Z, Li P, Yang Q. The effect of various absorption enhancers on tight junction in the human intestinal Caco-2 cell line. Drug Dev Ind Pharm 2013;39:587–92.
41. Gibaldi M. Biopharmaceutics & clinical pharmacokinetics. Philadelphia: Febiger L; 1991.
42. Reppas C, Eleftheriou G, Macheras P, et al. Effect of elevated viscosity in the upper gastrointestinal tract on drug absorption in dogs. Eur J Pharm Sci 1998;6:131–9.
43. Shargel L, Wu-Pong S, Andrew BCY. Applied biopharmaceutics & pharmacokinetics. New York: McGraw-Hill; 2004.