School of Pharmacy[1], Zhengzhou University, Zhengzhou School of Pharmacy[2], Shenyang Pharmaceutical University, Shenyang, China

An investigation on intestinal absorption of a new anticancer drug, 2-methoxyestradiol

Xin-Hong Guo[1,2], Nan Zhang[1], Fu-De Cui[2], Bin Du[1], Zhen-Zhong Zhang[1]

Received April 24, 2009, accepted May 25, 2009

Zhen-Zhong Zhang, School of Pharmacy, Zhengzhou University, Science Road No. 100, Zhengzhou, 450001, China submitpaper@126.com

Pharmazie 64: 748–751 (2009) *doi: 10.1691/ph.2009.9134*

In this paper, the intestinal absorptive property of a new anticancer drug, 2-methoxyestradiol (2ME2) was investigated by *in situ* rat intestinal recirculation perfusion experimental techniques. The results indicated that the concentrations of 2ME2 had no influence on absorption rate constant (ka) of 2ME2 and the small intestinal absorption of 2ME2 was a first-order process with passive diffusion mechanism within the concentration tested. 2ME2 was well absorbed at each intestinal segment except duodenum and the best site of intestinal absorption of 2ME2 was the ileum. In addition, the PH of drug perfusate and the concentration of SDS had significant effects on absorption kinetics. Faintly basic environment profitted small intestinal absorption of 2ME2. Tween 80 and SDS could not enhance the intestinal absorption of 2ME2. The above study provided a theoretical foundation for developing effective oral preparations with higher bioavailability to treat cancers. In addition, the improved ligation way for studying the best site of intestinal absorption of 2ME2, should be used extensively in related studies because of decreasing number of experimental rats and saving time.

1. Introduction

2-Methoxyestradiol (2ME2) is a naturally occurring metabolite of estradiol in the human body which has been shown to be a potent antiangiogenic and antitumor agent in preclinical models through its apoptotic activity and antimicrotubule activity (Fotsis et al. 1994; Lakhani et al. 2003; Klauber et al. 1997; Schumacher et al. 1999). 2ME2 has been tested clinically in a number of phase I and phase II trials in patients with metastatic breast cancer, prostate cancer and various other solid tumors (Dahut et al. 2006; James et al. 2006; Lakhani et al. 2003). The results indicated that: 2ME2 is well tolerated with evidence of anticancer activity in patients with refractory metastatic breast cancer, but the therapeutical outcome of 2ME2 remains unsatisfactory, such as very low oral bioavailability and evident inter-individual variability, even though high doses above 400–600 $mg \cdot d^{-1}$ is administered. To the best of our knowledge, the causes generating the above outcome are poor water solubility, liver first pass effect and low intestinal absorption of 2ME2. Obviously it is very essential to master the above property of 2ME2 for developing new effective preparations with higher oral bioavailability.

We have learned that 2ME2 has poor water solubility and shows a liver first pass effect after oral administration (Dahut et al. 2006; James et al. 2006; Lakhani et al. 2003; Nehal and Lakhani 2007). So far we have not seen reports on intestinal absorption of 2ME2. So in this paper, an investigation of intestinal absorption of 2ME2 has been done in a rat *in situ* intestinal perfusion model to provide theoretical reference for developing new effective preparations with higher bioavailability.

2. Investigations and results

2ME2 has a water solubility of 5.34 nM in water and 6.23 nM in Krebs-Ringer nutrient solution, respectively, and its partition coefficients (logP) of 2ME2 in water and Krebs-Ringer nutrient solution are 2.87 and 3.47, respectively.

No significant difference between 2ME2 concentrations in the Krebs-Ringer nutrient solution and in two blank perfusates was found, revealing that 2ME2 was stable in the two blank perfusates.

In this study, DMSO was selected as solubilizer of 2ME2 and the effects of concentration of DMSO on the intestinal absorption of 2ME2 were investigated. The results (Table 1) were that no significant difference between these k_a values was found, which indicated that the absorption of 2ME2 was not influenced by concentration of DMSO (0.08%–0.50%). Therefore all the following assays were carried out in the presence of 0.5% DMSO.

The concentrations of 2ME2 had no significant effect on the absorption kinetics of the small intestine (Table 2), which suggested that the mechanism of intestinal absorption of 2ME2 was a passive and unsaturable transport at least at the concentrations tested (16–96 nM). 2ME2 was well absorbed from the small intestine, based on higher apparent absorption percentage (67.41%–70.61%) and very low tissue uptakes percentage (0.73%–0.77%).

At the same concentration of 2ME2 in the perfusate, 2ME2 was well absorbed at each intestinal segment except duodenum and the best site of intestinal absorption of 2ME2 was the ileum (Table 3). It was noticed that 2ME2 was also well absorbed at the colon as compared with the duodenum.

Table 1: Effect of concentration of 2ME2 on k_a from the small intestine

Concentration of DMSO (v%)	k_a (1/h)	r
0.08	0.2427 ± 0.0287	0.9866
0.25	0.2387 ± 0.0231	0.9902
0.50	0.2520 ± 0.0202	0.9892

Data were presented as mean ± S.D. of four rats. r represented correlation coefficient. No significant difference was found between k_a. Statistical significance was assessed by Student's *t*-test of the unpaired observations
k_a was estimated using perfusate (pH 7.4) containing 16 nM 2ME2

The PH of perfusate had significant effects on intestinal absorption of 2ME2 (Table 4). Better absorption of 2ME2 was found at PH of 7.4 than 6.2 and 5.0.
The concentration of SDS had significant effect on intestinal absorption of 2ME2 (Table 5). The intestinal absorption of 2ME2 was inhibited with increase of concentation of SDS from 0.01% to 0.06%. But the concentration of Tween 80 had no significant effect on intestinal absorption of 2ME2 within the concentrations tested (from 0.01% to 0.10%) (Table 5). It was noticed that the surfactants such as SDS and Tween 80 did not enhance the intestinal absorption of 2ME2.
Different ligation way had no significant effect on the absorption kinetics at each intestinal segment (Table 6), which indicated that absorption of 2ME2 at each intestinal segment was independent.

3. Discussion

In order to confirm that the amount of drug disappeared resulted from the absorption rather than other losses, preliminary studies were necessary. So the stability of 2ME2 in the blank perfusates was studied *in vitro*. 2ME2 was stable (data not shown) in the blank perfusates, which supplied the foundation for investigating intestinal absorption of 2ME2. In addition, it also indicated that the pre-absorption luminal metabolism of 2ME2 by the action of digestive enzymes did not exist. As we know, metabolism of some drugs inside the intestinal solution is one of the primary reasons for low oral bioavailability (Vasu 2006). Therefore the luminal metabolism, one of the reasons of low oral bioavailability of 2ME2 could be evacuated.
Due to poor solubility of 2ME2 in Krebs-Ringer nutrient solution, it was necessary to increase the solubility of 2ME2 for the following investigations. DMSO could be used as solubilizer to investigate the intestinal absorption of poor water soluble medicine (Muñoz et al. 2005; Oda et al. 2004). Muñoz et al. (2005) reported that four ritonavir solutions (40, 27, 13 and 7 mM) in the presence of 1% DMSO were perfused in the small intestine of anaesthetised rats, and the effects of DMSO on the intestinal permeability were investigated using solutions containing antipyrine 1.33 mM and ritonavir 7 mM with and without 1% of DMSO, indicating that antipyrine and ritonavir transport was not influenced under the conditions of the presence of 1% DMSO. In this study, the absorption of 2ME2 was not influenced by the concentration of DMSO (0.08%–0.50%) (Table 1). So 0.5% DMSO was selected as solubilizer of 2ME2.
The mechanism of intestinal absorption of 2ME2 was a passive and unsaturable transport at least within the concentrations tested and 2ME2 was well absorbed at the small intestine, which provided a theoretical foundation for developing effective new preparations with higher oral bioavailability by increasing solubility of 2ME2. At the same concentration of 2ME2 in the perfusate, 2ME2 absorption increased from the duodenum to the ileum. This may be related to the higher distal permeability due to the presence of more permeable pores in this region or to an increase in paracellular permeability as suggested by Pantzar et al. (1995). In an *in vitro* study, higher transport was also found in the ileum than in the jejunum due to the quercetin glycoside whose absorption is suggested to occur by simple diffusion via tight junctions (Matsumoto et al. 2004). In addition, a better absorption of 2ME2 was found in the colon of rats, which provided the theoretical foundation for developing colon targeting preparations to treat colon carcinoma (Carothers et al. 2002).
The intestinal absorption of 2ME2 was influenced by pH of perfusate and some surfactants. Better absorption of 2ME2 was found at pH of 7.4 than 5.0 and 7.4. It was concluded that a faintly basic environment led to a better small intestinal absorption of 2ME2. In addition, Tween 80 and SDS all could not enhance the absorption of 2ME2. Moreover, the absorption of 2ME2 decreased in the presence of 0.06% (w%) SDS. So 0.01%–0.1% (V%) Tween 80 and 0.01% (w%) SDS could be used as a solubilizer of 2ME2. In this study, the influencing ways of SDS and Tween 80 on intestinal absorption of 2ME2 was in agreement with previous publications (Feng et al. 2006; You and Yang 2004; Zhu et al. 2006). So important references for selecting proper excipients to develop new oral preparations were received.
Ligation way for studying the best site of intestinal absorption was improved. As we know, because rat *in situ* intestinal perfusion techniques were readily carried out in laboratories only with little equipments such as a infusion pump and a water bath, this method is widely used for studying the oral absorption of many drugs (Feng et al. 2006; Mikihisa et al. 2004; Le Corre et al. 1998; You and Yang 2004; Zhu et al. 2006). When absorptive properties at different intestinal segments of rat were studied with this method, usually only one intestinal segment such as duodenum, ileum, jejunum or colon from one anaesthetized rat was ligated, which exactly was one of the reasons for no more extensive application of this method as compared with the inverted intestine gut method. According to absorptive theory, drug diffused through the intestinal epithelium cell membrane and entered to the mesenterium vascellum which assembled in precava and eventually entered to liver. As mesenterium vascellum from every gut segment was independent, so it could be inferred that absorption of every gut segment could not been interacted by other gut segments to some extent. It was feasible

Table 2: Effect of concentration of 2ME2 on k_a, percentage of apparent absorption and percentage of tissue uptake from the small intestine

Concentration (nM)	k_a (1/h)	r	Percentage of apparent absorption (%)	percentage of intestinal tissue uptake (%)
16	0.2367 ± 0.0287	0.9879	67.41 ± 0.66	0.76 ± 0.08
48	0.2497 ± 0.0162	0.9927	69.28 ± 0.53	0.77 ± 0.04
96	0.2667 ± 0.0264	0.9975	70.61 ± 0.44	0.73 ± 0.06

Data were presented as mean ± S.D. of four rats. No significant difference was found between k_a, percentage of apparent absorption and percentage of tissue uptake. Statistical significance was assessed by Student's *t*-test of the unpaired observations
k_a was estimated using perfusate (pH 7.4)

Table 3: k_a at each intestinal segment of rats

Intestinal segment	k_a (1/h)	r
Duodenum	0.073 ± 0.0041	0.9912
Jejunum	0.124 ± 0.0042	0.9902
Ileum	0.150 ± 0.0162	0.9742
Colon	0.134 ± 0.0101	0.9832

Data were presented as mean ± S.D. of four rats. The significant difference was found between k_a at two different intestinal segments except jejunum and colon. No significant difference was found between k_a at jejunum and colon. Statistical significance was assessed by Student's *t*-test of the paired observations
k_a was estimated using perfusate (pH 7.4) containing 16 nM 2ME2

Table 4: Effects of pH of perfusates containing 2ME2 16 nM on k_a from the entire small intestine

pH	k_a (1/h)	r
5.0	$0.1800 \pm 0.0221^*$	0.9928
6.2	$0.1620 \pm 0.0256^*$	0.9951
7.4	0.2367 ± 0.0287	0.9467

Data are presented as mean ± S.D. of four rats
* $p < 0.05$, compared with k_a at pH of 7.4. Statistical significance was assessed by Student's *t*-test of the paired observations

that two gut segments of one anesthetized rat (such as duodenum and ileum, jejunum and colon) were simultaneously ligated to determine k_a at the two different gut segment simultaneously, leading to saving time and decreasing number of experimental rats. The results (Table 5) demonstrated that our hypothesis was correct. The improved ligation way did not influence absorption of 2ME2 at every intestinal segment and is therefore worth recommending.

4. Experimental

4.1. Animal handling

Wistar rats (Experimental Animal Center, Zhengzhou University, Zhengzhou, China), equal numbers of males and females with a body weight of 230–270 g were used. The animals were maintained under controlled temperature (20 ± 2 °C) and daily light intensity (12 h of light), and had free access to standard rodent diet and tap water. All experimental protocols were in accordance with local institutional guidelines for animal care of Zhengzhou University.

4.2. Determination of the solubility and partition coefficient (P) of 2ME2

Equilibrium solubility of 2ME2 (purity ≥ 99.0%, home-made in School of pharmacy, Zhengzhou University, China) was determined by the shake-flask method (n = 6). Excess amount of drug was added into water and Krebs-Ringer nutrient solution (in gram per liter: 7.8 NaCl, 0.35 KCl, 0.37 $CaCl_2$, 0.02 $MgCl_2$, 1.37 $NaHCO_3$, 0.32 NaH_2PO_4 and 1.4 glucose, pH 7.4), respectively, equilibrated at 37 ± 0.2 °C with vigorous shaking in shaker water bath for 48 h. Samples were filtered through 0.45 μm Millipore membrane. The first 15% of the filtrate was discarded to avoid any potential loss of the drug and the subsequent filtrate was collected. All procedures were conducted at the test temperature to avoid any precipitation of the drug. Each filtrate was determined according to the analytical procedure (4.7).
Partition coefficient (P) was determined according to the method of shaking bottles (n = 6). n-octyl alcohol solution (1 mL) containing 2ME2 (quantified 2ME2 was dissolved in 10 ml n-octyl alcohol saturated by water) and 99 ml water solution saturated by n-octyl alcohol were added into 100 mL Erlenmeyer flask and were shaken for 3 days at 25 ± 0.1 °C on a shaker (150 r·min^{-1}) to equilibrate. After equilibration, the mixture was placed quietly in a thermostatic water bath at 25 ± 0.1 °C for 1 day. Supernatant oil phase (0.5 mL) was taken out to be determined according to the analytical procedure (4.7) after being properly diluted by methanol. Then average partition coefficients of 2ME2 in water and Krebs-Ringer nutrient solution were obtained in triplicate.

Table 5: Effects of surfactants on k_a from the entire small intestine

Surfactants	k_a (1/h)	r
0.01%Tween80	$0.2100 \pm 0.0232^*$	0.9867
0.10%Tween80	$0.2200 \pm 0.0216^*$	0.9834
0.01%SDS	$0.2640 \pm 0.0267^*$	0.9944
0.06%SDS	$0.1860 \pm 0.0287^{**}$	0.9939

Data are presented as mean S.D. of four rats. k_a was estimated using perfusate (pH 7.4) containing 16 nM 2ME2
* $P > 0.05$, in comparison to k_a at the absence of Tween 80 and SDS
** $P < 0.05$, in comparison to k_a at the absence of Tween 80 and SDS
Statistical significance was assessed by Student's *t*-test of the paired observations

Table 6: Effects of ligation way on k_a at each intestinal segment

Intestinal segment	After improvement		Before improvement	
	k_a (1/h)	r	k_a (1/h)	r
Duodenum	0.076 ± 0.0082	0.9862	0.073 ± 0.0041	0.9912
Jejunum	0.112 ± 0.0122	0.9721	0.124 ± 0.0042	0.9902
Ileum	0.164 ± 0.0232	0.9806	0.150 ± 0.0162	0.9742
Colon	0.124 ± 0.0113	0.9912	0.134 ± 0.0101	0.9832

Data are presented as mean ± S.D. of four rats. No significant difference was found between two k_a at each intestinal segment by Student's *t*-test of the paired observations
k_a was estimated using perfusate (pH 7.4) containing 16 nM 2ME2

4.3. Stability of 2ME2 in blank perfusate

The stability of 2ME2 in blank perfusate was investigated *in vitro* (Wang 2007). Krebs-Ringer nutrient solution was recirculated through the whole small intestine and colon at 37 ± 0.1 °C for 4 h to obtain blank perfusates, respectively. 2ME2 32 nM was incubated in Krebs-Ringer nutrient solution and two blank perfusate in a total volume of 50 ml at 37 ± 0.1 °C for 3 h in triplicate, respectively.

4.4. Rat in situ intestinal perfusion experiments

After 12 h overnight fast, under pentobarbital sodium anesthesia administered by intraperitoneal injection (40 mg·kg^{-1}), the rats were fixed and *in situ* intestinal perfusion experiments (Xi 2007; Yuan and Li 2004) were performed (n = 4). Briefly, the intestinal tract was rinsed with 0.9% NaCl and Krebs-Ringer nutrient solution for 10 min by turns. The whole small intestine (from 1 cm after pylorus to 1 cm before cecum, 100 ml perfusate, for studying the influencing factors such as concentration of 2ME2, pH of perfusate and surfactants) and different intestinal segments (50 mL perfusate) such as duodenum (from 1 cm to 11 cm after pylorus), jejunum (from 15 cm to 25 cm after pylorus), ileum (from 20 cm to 10 cm before cecum) and colon (from 1 cm to 11 cm after cecum) were ligated (roughly 10 cm), respectively. An infusion pump was linked to the cannula. The perfusate containing 2ME2 with 0.5% dimethylsulfoxide (DMSO) in a water bath (37 °C) was recirculated, at the flow rate of 4 mL·min^{-1} for 10 min and then 2 mL·min^{-1} through the whole small intestine or each intestinal segment. During recirculation, 2 mL of samples were taken out at 0.0, 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 h by 5 mL of injector for the concentration determination of 2ME2 and phenol red in the perfusate (simultaneously, adding 2 mL of blank perfusate). The sample was filtered through a 0.45 μm filter membrane. Filtrate (0.5 mL) was used for detecting the concentration of phenol red and the rest was used for detecting concentrations of 2ME2. Finally, each intestinal segment was cut along the cannula and placed on a glass board with buffer (37 °C) for length measurement.

4.5. Treatment of small intestinal tissue

The procedure was applied as described by Andlauer et al. (2000). Briefly, after freeze drying, the tissue was powdered with a mortar and pestle and defatted twice by extraction with 10 mL hexane. The supernatants were combined and extracted with methanol to rule out any loss of 2ME2. The pellet was extracted 3 times with methanol and centrifuged at 2800 × g for 20 min. The extracts were pooled and adjusted to 10 mL. The samples were then stored at −80 °C until analysis.

4.6. Improving ligation way used for studying the best site of intestinal absorption

The ligation way used for studying the best site of intestinal absorption was improved. The two segments (such as duodenum and ileum, or jejunum and colon) instead of only one segment (such as duodenum, ileum, jejunum or colon) from only one rat were simultaneously ligated. Except the ligation way, the other experimental procedure was the same as during the *in situ* intestinal perfusion experiments (4.4).

4.7. Analytical procedure

The perfusate samples were assayed for determination of concentrations of 2ME2 and phenol red using a HPLC method (Nehal et al. 2004) (Shimadzu LC-10A with a UV detector) and UV spectrophotometry, respectively. In determination of concentrations of 2ME2, the standard stock solution was prepared in methanol (Tianjin Chemical Regent Factory, China), a serial of concentrations were prepared by using Krebs-Ringer nutrient solution, and a Kromasil C_{18} column (5 μm, 4.6 × 150 mm, Bohus, Sweden) was used. The flow rate was at 1.0 mL min^{-1} and the column temperature was set at 30 °C. The mobile phase consisted of acetonitrile (Tianjin Chemical Regent Factory, China) – water (50:50) and the UV detection wavelength was 205 nm. The concentrations of 2ME2 were calculated using an external standard method. The method had a good relation over the range of 0.80–48.00 nM, a recovery of 97.75–102.28 % and intra- and inter-day precision values of RSD of 0.95–4.29 %.

The method for determining partition coefficient was same as the above except the mobile phase consisted of acetonitrile–water (45:55). The method had specificity and a good relationship over the range of 0.80–48 nM, a recovery of 98.75–101.82 % and intra- and inter-day precision values of RSD of 0.92 % – 3.12 %.

In determination of concentrations of phenol red, dual wavelength spectrophotometry was used for correcting water volume at the detection wavelength and the reference wavelength of 558 nm and 598 nm, respectively. In a 10 mL test tube, 0.5 mL of filtrate and 5 mL of 0.2 M sodium hydroxide were added by turns. Mixed liquor was vortexed for 30 s and then was detected. The method had a linear range of 3.20–96.00 nM, a recovery of 92.75–102.58 %.

4.8. Statistical analysis

Statistical evaluations were performed by Student *t*-test of the unpaired observations to analyze the difference between the concentrations of 2ME2 and Student *t*-test of the paired observations to analyze the difference between the intestinal segments, pH of perfusate, surfactants and ligation way, P values <0.05 were considered to indicate significant differences. Data are expressed as means ± SD (n = 4).

References

Andlauer W, Kolb J, Fürst P (2000) Absorption and metabolism of genistin in the isolated rat small intestine. FEBS Lett 475: 127–130.

Carothers AM, Hughes SA, Ortega D, Bertagnolli MM (2002) 2-Methoxyestradiol induces p53-associated apoptosis of colorectal cancer cells. Cancer Lett 187: 77–86.

Dahut WL, Lakhani N, Gulley JL, Arlen PM, Kohn EC, Kotz H, McNally D, Parr A, Nguyen D, Yang X, Steinberg SM, Venitz J, Sparreboom A, Figg WD (2006) Phase I clinical trial of oral 2-methoxyestradiol, anantiangiogenic and antiapoptoticagent, inpatients with solid tumors. Cancer Biol Ther 5: 22–27.

Feng L, Jiang XH, Zhou J, Yang JY (2006) Studies on absorption kinetics of sanchinoside R1 and ginsenoside Rg1 in rat intestine. Chin Pharm J 41: 1097–1102.

Fotsis T, Zhang Y, Pepper MS (1994) The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature 368: 237–239.

James J, Murry DJ, Treston AM, Storniolo AM, Sledge GW, Sidor C, Miller KD (2006) Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer. Invest New Drugs 25: 41–48.

Klauber N, Parangi S, Flynn E (1997) Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and Taxol. Cancer Res 57: 81–86.

Lakhani N, Sarkar MA, Venitz J, Figg WD (2003) 2-Methoxyestradiol, a promising anticancer agent. Pharmacotherapy 23: 165–172.

Le Corre P, Dollo G, Chevanne F, Le Verge R (1998) Influence of hydroxypropyl-cyclodextrin and dimethyl-cyclodextrin on diphenhydramine intestinal absorption in a rat in situ model. Int J Pharm 169: 221–228.

Matsumoto M, Matsukawa N, Mineo H, Chiji H, Hara H (2004) A soluble flavonoid-glycoside, aG-rutin, is absorbed as glycosides in the isolated gastric and intestinal mucosa. Biosci Biotechnol Biochem 68: 1929–1934.

Mikihisa T, Yuka K, Hiromi N, Teruo M, Ryoko Y (2004) Segment-selective absorption of lysozyme in the intestine. Eur J Pharmacol 502: 149–155.

Muñoz MJ, Merino-Sanjuán M, Lledó-García R, Casabó VG, Máñez-Castillejo FJ, Nácher A. (2005) Use of nonlinear mixed effect modeling for the intestinal absorption data: Application to ritonavir in the rat. Eur J Pharm Biopharm 61: 20–26.

Nehal J, Lakhani AC, Alex SA, William L, Dahut B, Jürgen VC, William D, Figg AC (2004) Determination of the antiangiogenesis agent 2-methoxyestradiol in human plasma by liquid chromatography with ultraviolet detection. J Chromatogr B 806: 289–293.

Nehal J, Lakhani AC (2007) Characterization of in vitro and in vivo metablic pathways of the investigational anticancer agent,2-methoxyestradiol. Pharmacokin Pharmacodyn Drug Metabol 7: 821–831.

Oda M, Saito H, Kobayashi M, Aungst BJ (2004) Cyclodextrin as a suitable solubilizing agent for in situ absorption study of poorly water-soluble drugs. Int J Pharm 280: 95–102.

Pantzar N, Lundin S, Westrom BR (1995) Different properties of the paracellular pathway account for the regional small intestinal permeability to the peptide desmopressin. J Pharm Sci 84: 1245–1248.

Schumacher G, Kataoka M, Roth J (1999) Potent antitumor activity of 2-methoxyestradiol in human pancreatic cancer cell lines. Clin Cancer Res 5: 493–499.

Vasu KK, Vinod AB, Arvind KB (2006) Investigation of factors responsible for low oral bioavailability of cefpodoxime proxetil. Int J Pharm 317: 155–160.

Wang L, Jiang XH, Xu WJ, Li CR (2007) Complexation oftanshinone IIA with 2-hydroxypropyl-cyclodextrin: Effect on aqueous solubility, dissolution rate, and intestinal absorption behavior in rats. Int J Pharm 341: 58–67.

Xi L, Qian Z, Du P, Fu J (2007) Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. Phytomedicine 14: 633–636.

You BG, Yang MS (2004) Studies on the intestinal absorption kinetics of nitrendipine in rats. Chin Pharm J 39: 214–217.

Yuan Q, Li XR (2004) The absorption kinetics of silymarin microemulsion in rat intestine. Acta Pharm Sin 39: 631–634.

Zhu, LL, Li J, Wang GJ (2006) Studies on the intestinal absorption mechanism of diazepam in rat. J China Pharm Univ 37: 507–511.