Effect of various absorption enhancers based on tight junctions on the intestinal absorption of forsythoside A in Shuang-Huang-Lian, application to its antivirus activity

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ABSTRACT

Background: Forsythoside A (FTA), one of the main active ingredients in Shuang-Huang-Lian (SHL), possesses strong antibacterial, antioxidant and antiviral effects, and its pharmacological effects was higher than that of other ingredients, but the absolute bioavailability orally was approximately 0.72%, which was significantly low, influencing clinical efficacies of its oral preparations seriously. **Materials and Methods:** *In vitro* Caco-2 cell and *in vivo* pharmacokinetics study were simultaneously performed to investigate the effects of absorption enhancers based on tight junctions: sodium caprate and water-soluble chitosan on the intestinal absorption of FTA, and the eventual mucosal epithelial damage resulted from absorption enhancers was evaluated by MTT test and morphology observation, respectively. The pharmacological effects such as antivirus activity improvement by absorption enhancers were verified by MDCK damage inhibition rate after influenza virus propagation. **Results:** The observations from *in vitro* Caco-2 cell showed that the absorption of FTA in SHL could be improved by absorption enhancers. Meanwhile, the absorption enhancing effect of water-soluble chitosan may be almost saturable up to 0.0032% (*w*/*v*), and sodium caprate at concentrations up to 0.64 mg/mL was safe, but water-soluble chitosan at different concentrations was all safe for these cells. In pharmacokinetics study, water-soluble chitosan at dosage of 50 mg/kg improved the bioavailability of FTA in SHL to the greatest extent, and was safe for gastrointestine from morphological observation. Besides, treatment with SHL with water-soluble chitosan at dosage of 50 mg/kg prevented MDCK damage after influenza virus propagation better significantly than that of control. **Conclusion:** Water-soluble chitosan at dosage of 50 mg/kg might be safe and effective absorption enhancer for improving the bioavailability of FTA and the antivirus activity *in vitro* in SHL.

Key words: Absorption enhancers, antivirus activity, Forsythoside A, Shuang–Huang–Lian, tight junction

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INTRODUCTION

Compound prescriptions are the commonly used forms of traditional Chinese medicine, and oral administration is one of the most popular ways of compound prescriptions application. Shuang–Huang–Lian (SHL), a traditional Chinese formula containing *Flos Lonicerae* (FL), *Radix Scutellariae* (RS) and *Fructus Forsythiae* (FF), is shown to remove toxic heat and induce diaphoresis, and it has been widely used for treating acute upper respiratory tract infection, acute bronchitis, and light pneumonia.[1] Its preparations, such as Shuang-Huang-Lian oral liquid, Shuang-Huang-Lian tablet, etc., are also extensively used for treating infectious diseases of the respiratory tract caused by virus or bacterial infection in clinical practice. However, it was reported that their efficacy were usually not satisfactory compared with that of injections, which becomes one of the most limited points in the development of compound preparations. The presumptions that

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whether the low bioavailability orally of SHL resulted in the poor efficacy or whether efficacy could be improved as the absorption of active ingredients in SHL was enhanced are all yet to be investigated.

Our previous studies have shown that the chemical ingredients in SHL included phenylethanoid glycosides, lignans, quinic acids, saponins, and flavonoids.[2] Phillyrin (PR), chlorogenic acid (CA), and baicalin (BC), three representative active ingredients, are used officially as the marker compounds to characterize SHL preparations.[3] It was found that PR played an important role in the effects of anti-inflammatory,[4] antiviral,[5] and anti-obesity[6] etc., but Li *et al.,* (2011)[7] reported that the absorption of PR could be neither carried out in the gastrointestine, nor improved by absorption enhancers such as SDS, bile salt, borneol and carbomer, etc., Besides, we also found that about 100% of the apically loaded PR was retained on the apical side from Caco-2 cell transport (unpublished). Conceptually, all compounds in the pharmaceutical product that are bioavailable in the systemic circulation can be considered as relevant active markers for pharmacological effect.[8] Therefore, the real active ingredients of ligans in FF may be ligan aglycone, not lignan glycosides like PR. However, it was reported that forsythoside A (FTA), another pharmacological active ingredient of caffeyol glycosides in FF, showed strong antibacterial, antioxidant, and antiviral activities,[9-11] but its absolute bioavailability in SHL orally was approximately 0.72%, significantly low.[12] And it was found that FTA was survived positive correlation better than other ingredients like CA and BC between dose and effects of antibacterial and antiviral activities via pharmacokinetic/pharmacodynamic (PK/PD) combined with partial least squares (PLS) in SHL, and the antibacterial and antiviral activities effects were improved as the content of FTA in SHL was enhanced (unpublished). Besides, Tian *et al.,* (2012)[13] reported that the antibacterial effect against *Staphylococcus aureus* (G^+) and *Candida albicans* of FTA was similar to that of BC, higher than that of CA and PR, and the antibacterial activity against *Escherichia coli* (G^-) of FTA was higher than that of BC, CA, and PR, although the content of FTA in SHL was 500 μg/mL, significantly lower than that of BC in SHL. Therefore, FTA could be considered as one of the most important antibacterial and antivirus markers to control the quality of SHL preparations. How to improve the bioavailability of FTA would be related directly to the pharmacological efficacy improvement of SHL.

According to our previous study, it was shown that the intestinal absorption mechanism of FTA was mainly involved paracellular route transport governed by the tight junctions (TJs).[14] And the modulation of the TJs by absorption enhancers would enhance the paracellular drug transport.[14]

Therefore, the present study is to clarify the effects of various absorption enhancers based on tight junctions such as sodium caprate[15] and water-soluble chitosan[16] on the intestinal absorption of FTA in SHL via *in vitro* and *in vivo* models and to find suitable enhancers to improve the oral bioavailability of FTA.

MATERIALS

SHL extract was purchase from Harbin third pharmaceutical factory. To assure the homogeneity of the formulation and to prepare consistent hatched, the HPLC figureprint profile [Figure 1] of the SHL extract was analyzed, and the chromatographic analysis was described by Cao *et al.*, (2006) and Zhou *et al.*, (2011).[17,18] All voucher specimens were deposited in our laboratory for future reference. FTA (98% pure) was purchased from Shanghai Nature Standard Co, Ltd. Ribavirin, phillyrin, chlorogenic acid, baicalin, and scutellarin (using as internal standard, IS) were purchased from National Institute for the Control of Pharmaceutical and Biological Products. Water-soluble chitosan (degree of deacetylation 91.7%, Molecular weight 73KDa) and sodium caprate were purchased from Moldepot Co, Ltd. Heparin sodium injection was purchased from Changzhou Qianhong Bio-pharma Co., Ltd. Methanol and acetonitrile (HPLC grade) were purchased from Merck (Merck, Germany), and water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade.

METHODS

In vitro Caco-2 cell model

Preparation of transport buffer

Hank's balanced salt solution (HBSS) was used as the transport buffer for the transport study in Caco-2 cell monolayer model. It was prepared by dissolving 9.5 g of commercial available HBSS powder in 1000 mL water. The pH value of the buffer was adjusted to pH 6.0 by 85% of phosphoric acid.

Cell culture

Caco-2 cells were cultured in high glucose Dulbecco's modified eagle's medium (DMEM, Gibco, Bethesda, MD) with 10% fetal bovine serum (Gibco), 1% nonessential amino acids (Gibco). Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C . After reaching 80% confluence, Caco-2 cells were harvested with 0.25% trypsin-EDTA solution and seeded in Transwell inserts (Nalge Nunc International. Roskilde, Denmark) in 6-well plates at a density of 1.0×10^5 cells/cm^2. The protocols for cell culture in Transwell inserts were similar to those described previously.[19]

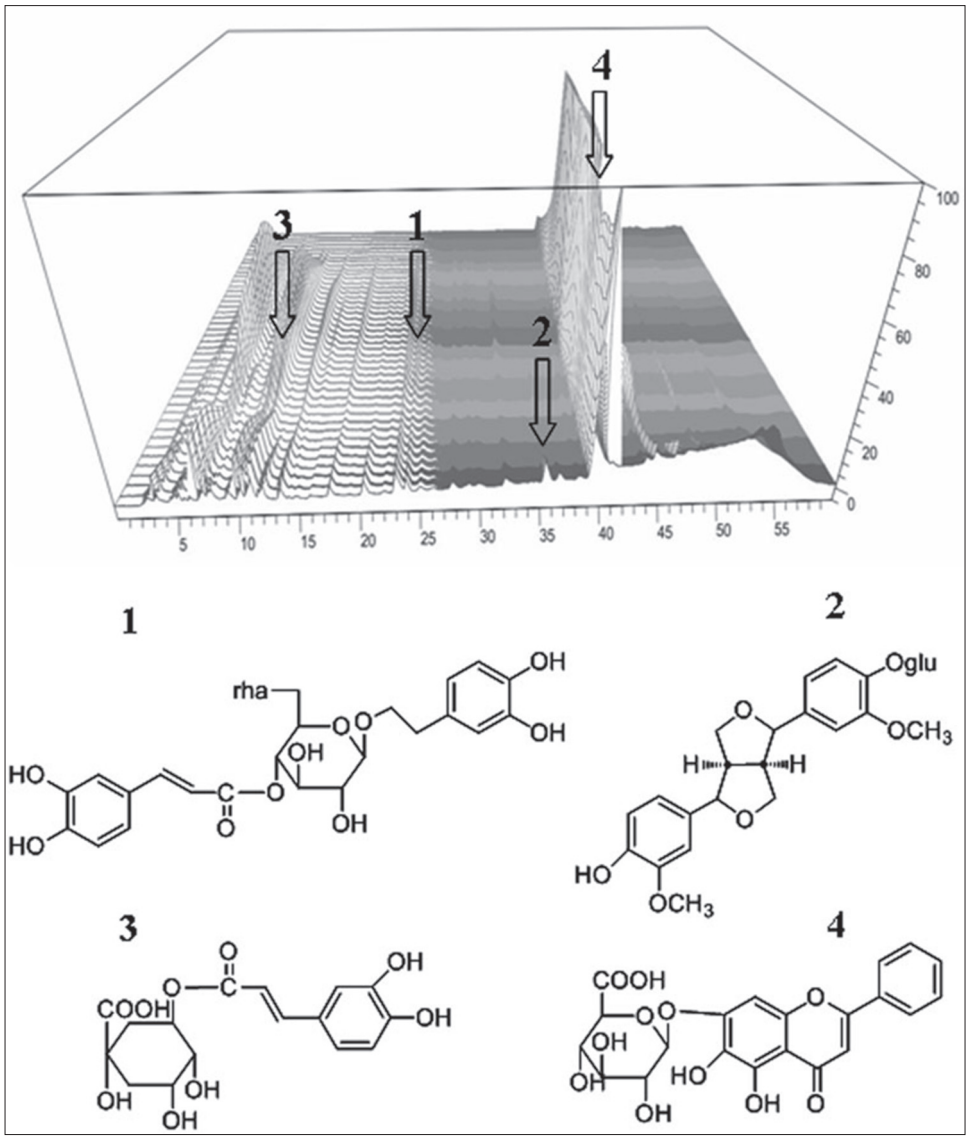

Figure 1: 3D HPLC-profile of meOH soluble portion of SHL. 1, Chlorogenic acid (CA); 2, Forsythoside A (FTA); 3, Phillyrin (PR); 4, Baicalin (BC). The concentration of BC, CA, PR, and FTA were determined to be 10 mg/mL, 1.2 mg/mL, 600 μg/mL, and 500 μg/mL, respectively

Cytotoxicity tests of SHL with different absorption enhancers on Caco-2 cells

MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) test was used to estimate the potential cytotoxicities of the studied weeping forsythia extract with different absorption enhancers toward Caco-2 cells. The Caco-2 cells were seeded onto a 96-well plate at a seeding density of 5×10^4 cells/well in DMEM culture medium and cultured at 37°C for 24 h. Subsequently, the culture medium was replaced with SHL or absorption enhancers (combined with or without SHL) dissolved in HBSS (pH 6.0) at different studied concentrations (6.25, 12.5, 25, 50, 100 μM for FTA in SHL; 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 mg/mL for absorption enhancers with or without addition of 10 μM of FTA in SHL). Blank HBSS (pH 6.0) was employed as a negative control. Then the 96-well plate was incubated at 37°C for 24 h. Thereafter, 20 μL of 5 mg/mL MTT solution in HBSS was added to each well and the plate was incubated for another 4 h. The solutions in each well were then removed followed by dissolving the remained formazan crystals in the cells with 200 μL of DMSO. The absorbance of the mixture in the 96-well plate was then measured with a Kinetic microplate reader (Molecular Devices) at 570 nm. The cytotoxicity of each compound was calculated as the percentage of the absorbance relative to that of the negative control.

Effect of absorption enhancers on the absorption of FTA in SHL

Cell culture experiments were described previously. Briefly, after culture medium was aspirated, the cell monolayers were washed three times blank HBSS. The transepithelial

electrical resistance (TEER) values of cell monolayers were measured, which were more than 250 Ω × cm^2. The monolayers were incubated with the blank HBSS for 1 h with 37°C . Thereafter the incubation medium was aspirated. Afterwards, a solution containing the compound was loaded onto the apical side. Donor samples (400 μL) (Apical side) and receiver samples (400 μL) (Basolateral side) were taken at different times (typically 1 h), followed by the addition of 400 μL drug donor solution to the donor side (AP) and 400 μL of blank buffer to the receiver side (BL). The samples were taken at 0, 1, 2, 3, and 4h after incubation. At the end of the transport experiment, integrity of the monolayer was monitored by TEER value. The concentrations of the test compound were analyzed immediately using the UPLC-MS method described by our previous report.[14] The effects of absorption enhancers of different concentrations on the absorption of FTA in SHL were investigated.

Rat *in vivo* pharmacokinetics study

Effect of absorption enhancers on the oral bioavailability of FTA in SHL

Male Sprague–Dawley rats (~250 g) were obtained from Experimental Animal Center of Nanjing University of Chinese Medicine and kept in an environmentally controlled breeding room (temperature: 20 ± 2C, humidity: 60 ± 5%) for 1 week. The animals were fasted for 12 h prior to drug administration.

The rats were randomly divided into nine groups with five rats in each group to receive various oral administrations. In Group 1, rats received SHL extract (equivalent to 10 mg/kg of FTA). In Group 2, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 25 mg/kg sodium caprate orally. In Group 3, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 50 mg/kg sodium caprate. In Group 4, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 100 mg/kg sodium caprate. In Group 5, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 150 mg/kg sodium caprate. In Group 6, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 25 mg/kg water-soluble chitosan. In Group 7, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 50 mg/kg water-soluble chitosan. In Group 8, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 100 mg/kg water-soluble chitosan. In Group 9, rats received SHL extract (equivalent to 10 mg/kg of FTA) with the addition of 150 mg/kg water-soluble chitosan.

After dosing for 0, 5, 10, 15, 20, 30, 40, 55, 70, 100, 160, 250, 480 min, blood was collected from the pre-intubated catheter and put into tubes with heparin sodium injection (10 μL) and ascorbic acid (2 μg) at predetermined time points. Subsequently, plasma was prepared by centrifugation at 5000 rpm for 7 min and stored at -80°C for further analysis.

Evaluation of gastrointestinal membrane toxicity caused by absorption enhancers via morphological observation

Preparation for microscopy

Morphological observation of gastrointestinal tissues after the exposure of 8h was performed after oral administration of SHL with or without absorption enhancers by oral gavage as described in section

Microscopy

On each glass slide two sections from each tissue were prepared and all slides were investigated in this study. All slides were coded to avoid the observer "bias" and were observed at a magnification of ×400 using a light microscope.

Effect of SHL with or without water-soluble chitosan on influenza virus

MDCK cells were grown as described[20] in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS) and 1% Pen/Strep at 37°C in a humidified incubator. The media was changed two to three times per week. The influenza virus strain, A/PR8/34(H1N1) was purchased from Chinese Academy of Preventive Medicine. It was propagated in MDCK cells in the presence of 1 μg/mL of Trypsin_TPCK (Tosylamide Phenylethyl Chloromethyl Keton-treated Trypsin) (Sigma, USA) to create the working stock. During antiviral evaluations, media supplemented with FBS was sucked out and the cell washed with PBS and then it was treated as needed. The media supplemented with Trypsin_TPCK was added.

Serum after administration orally into SHL with or without water-soluble chitosan was added to the MDCK cells after the propagation with influenza virus. Ribavirin was used as positive control. The cells were incubated at 37°C for 48 hours before viability testing by measuring the conversion of MTT as describe in section 3.1.3.

Calculation

For Caco-2 monolayer model, the apparent permeability coefficient (P_{app}) was calculated as $P_{app} = [(dQ/dt)]/[A \times C_0]$, dQ/dt (μg/S) was the flux rate, A was the effective surface area of the cell monolayer (4.2 cm^2), and C_0 (μg/mL) was the initial drug concentration in the donor chamber. Inhibition rate = [OD (drug)−OD (model)]/[OD (control)−OD (model)].

Pharmacokinetic analysis

The peak concentrations (C_{max}) and the time to reach the peak concentrations (T_{max}) were determined directly from the plasma concentration–time profiles. The area under the curve (AUC) was calculated by the trapezoidal method from time zero to the final sampling. The absorption enhancement ratios of drugs with or without enhancers were calculated as $Absorption\ enhancement\ ratio = AUC_{with\ enhancer}/AUC_{control}$

Statistical analysis

Statistical significance in the P_{app} value and pharmacokinetic parameters was estimated by the analysis of variance (Student *t*-test) or one-way ANOVA. A *P* value of less than 0.05 was considered to be significantly different. All data were expressed as mean ± SD.

RESULTS

In vitro Caco-2 monolayer model

Cytotoxicity tests of SHL with different absorption enhancers on Caco-2 cells

Caco-2 cells were exposed to various concentrations of absorption enhancers (0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 mg/mL) with addition of SHL for 24 h. It was shown that sodium caprate at concentrations up to 0.64 mg/mL was safe for the Caco-2 cells, but water-soluble chitosan at different concentrations was all safe for these cells.

Effect of different absorption enhancers on the P_{app} value of FTA in SHL in the apical-to-basolateral (AP-BL) direction from in vitro Caco-2 monolayer model

As shown in Figure 2, the P_{app} values increased significantly to 106% (4.93 ± 0.33)×10^{-6} cm/s, 187% (8.69 ± 0.36) ×10^{-6} cm/s and 155% (7.23 ± 1.07) ×10^{-6} cm/s following exposure of the Caco-2 cells to 10, 32, and 250 μg/mL of the water-soluble chitosan. Additionally, the P_{app} values also increased significantly to 127% (5.90 ± 0.74) ×10^{-6} cm/s, 156% (7.25 ± 0.79)×10^{-6} cm/s and 203% (9.44 ± 0.59) ×10^{-6} cm/s following exposure of the Caco-2 cells to 10, 32 and 80 μg/mL of the sodium caprate. The results indicated that the absorption of FTA in SHL can be improved by absorption enhancers based on tight junctions. Meanwhile, the absorption enhancing effect of water-soluble chitosan may be almost saturable up to 0.0032% (*w*/*v*).

In vivo pharmacokinetics study

Effects of absorption enhancers on the bioavailability of FTA in SHL

As shown in Table 1 and Figure 3, 150 mg/kg sodium caprate and 50 mg/kg water-soluble chitosan displayed the largest AUC values in four groups; On the other hand, 25 mg/kg sodium caprate and 25 mg/kg water-soluble chitosan did not improve the absorption of FTA so much. The rank order of the absorption-enhancing ability was 150 mg/kg sodium caprate >50 mg/kg water-soluble chitosan >100 mg/kg sodium caprate >50 mg/kg sodium caprate >150 mg/kg water-soluble chitosan >25 mg/kg sodium caprate ≥100 mg/kg water-soluble chitosan ≥25 mg/kg water-soluble chitosan. However, sodium caprate at the dosage of 150 mg/kg might result in the serious intestinal damage as described below. Therefore, these findings indicated that 50 mg/kg water-soluble chitosan, not 150 mg/kg sodium caprate would be the promising enhancer for improving the bioavailability of FTA in rats.

Effect of absorption enhancers on the gastrointestinal membrane toxicity

Morphological observation of the intestinal mucosa after administration orally SHL and absorption enhancers were shown in Figure 4 (A1-A11: Stomach, B1-B11: Jejunum, C1-C11: Colon). The results clearly indicated that 25, 50, 100 mg/kg sodium caprate and 25, 50, 100, 150 mg/kg water-soluble chitosan did not cause any significant change in the morphology of the gastrointestinal membrane, although we found that above 100 mg/kg sodium caprate could cause mucosal damage.

Effect of SHL with or without water-soluble chitosan at the dosage of 50 mg/kg on influenza virus

As shown in Figure 5, the antivirus model was built successfully, and the inhibition rate of water-soluble chitosan group was no significant compared with that of PBS group, although there was a remarkable increase in inhibition rate value after administrating orally 20 mg/kg ribavirin as a positive control. However, the difference of antivirus activity between SHL with or without water-soluble chitosan was significant from Figure 5. The inhibition rate of SHL

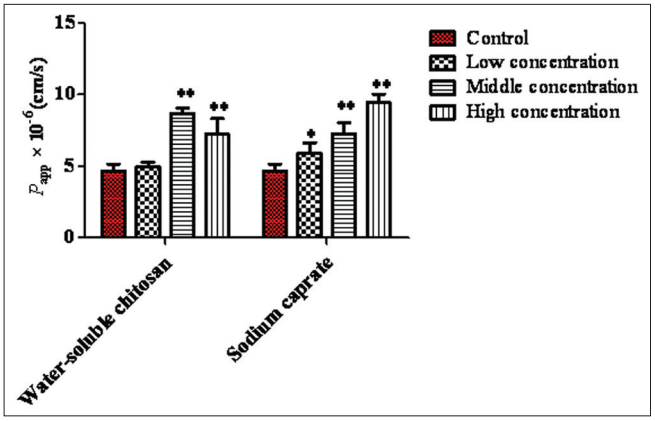

Figure 2: Effects of various absorption enhancers based on tight junctions on absorption parameters of FTA in Caco-2 cell *in vitro* model. Results are expressed as the mean ± S.D. (*) $P < 0.05$ and (**) $P < 0.01$ compared with the control group

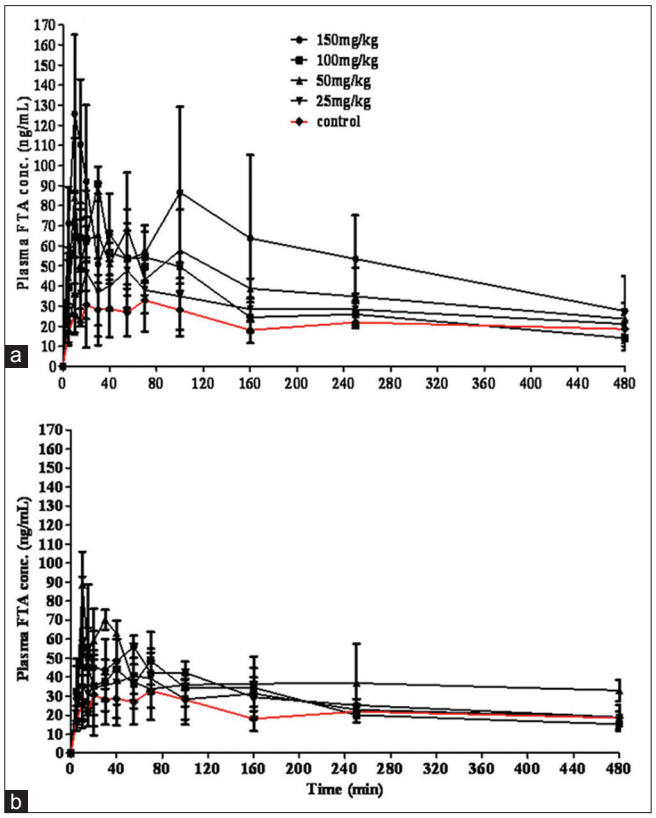

Figure 3: Plasma concentration-time profiles of FTA in SHL (equivalent to 10 mg/kg of FTA) with absorption enhancers after administration to the rat gastrointestine by *in vivo* pharmacokinetics study. Results are expressed as the mean ± S.D. of 3-5 experiments (a: sodium caprate, b: water-soluble chitosan)

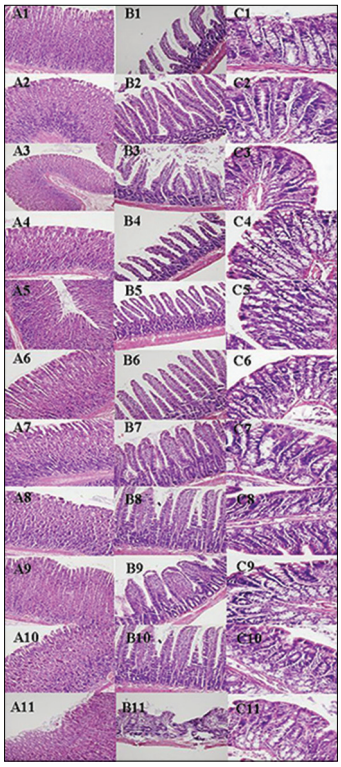

Figure 4: HE photomicrographs of rat gastric and intestinal tissue sections after oral administration of absorption enhancers of different dosages and SHL. All panels represent cross-sections of gastric and intestinal tissues. Jejunum represents small intestine. The original magnification was ×20 object lens for stomach and jejunum, ×40 object lens for colon. A, B, and C represent stomach, jejunum, and colon, respectively. A1-C1 (PBS); A2-C2 (SHL); A11-C11 (200 mg/kg Triton X-100); A3-C3, A4-C4, A5-C5 and A6-C6 (150 mg/kg, 100 mg/kg, 50 mg/kg, and 25 mg/kg sodium caprate); A7-C7, A8-C8, A9-C9 and A10-C10 (150 mg/kg, 100 mg/kg, 50 mg/kg, and 25 mg/kg water-soluble chitosan)

with water-soluble chitosan at dosage of 50 mg/kg was higher than that of without water-soluble chitosan, which indicated that the pharmacological effects such as antivirus effect of SHL can be significantly improved by addition of water-soluble chitosan.

DISCUSSION

In the present study, it was reported by Illum *et al.*, (1994)[21] that the mechanism of absorption enhancement of water-soluble chitosan was a combination of mucoadhesion and a loosening effect on the tension of the tight junctions through ionic interactions with negatively charged groups of glycocalix. Lindmark *et al.*, (1998)[15] proposed that the mechanism of absorption enhancing effect of sodium caprate was proposed to be an effect on tight junctions through PLC-dependent IP3/DAG pathways. Furthermore, we found that the absorption enhancing effect of sodium caprate and water-soluble chitosan for improving the intestinal absorption of FTA was affected by their concentrations, and the absorption of FTA increased as the concentration of sodium caprate was enhanced by *in-vitro* and *in-vivo* models [Figures 2, and 3a and Table 1]. Besides, a maximal absorption enhancing effect of water-soluble chitosan was observed at a dosage of 50 mg/kg, not higher doses *in vivo* model, and the absorption enhancing effect may be almost saturable up to 0.0032% (*w*/*v*) *in vitro* model [Figures 2 and 3b and Table 1]. Gao *et al.*, (2008)[17] reported that the absorption enhancing effect of chitosan hexamer for FD4 was dependent on its concentration, but its absorption enhancing effect was almost saturable up to 0.5% (*w*/*v*). Therefore, our present result is consistent with the previous report and chitosan has some optimal concentrations to show the greatest absorption enhancing effects for improving the intestinal absorption of FTA in SHL, although the type of chitosan was different. The reasons that the greatest absorption enhancing effects of chitosan was dependent on some optimal concentrations might be illustrated by the previous report that the chitosan of low concentration cannot enhance the absorption of drugs because of loss of interactions with negatively charged groups of

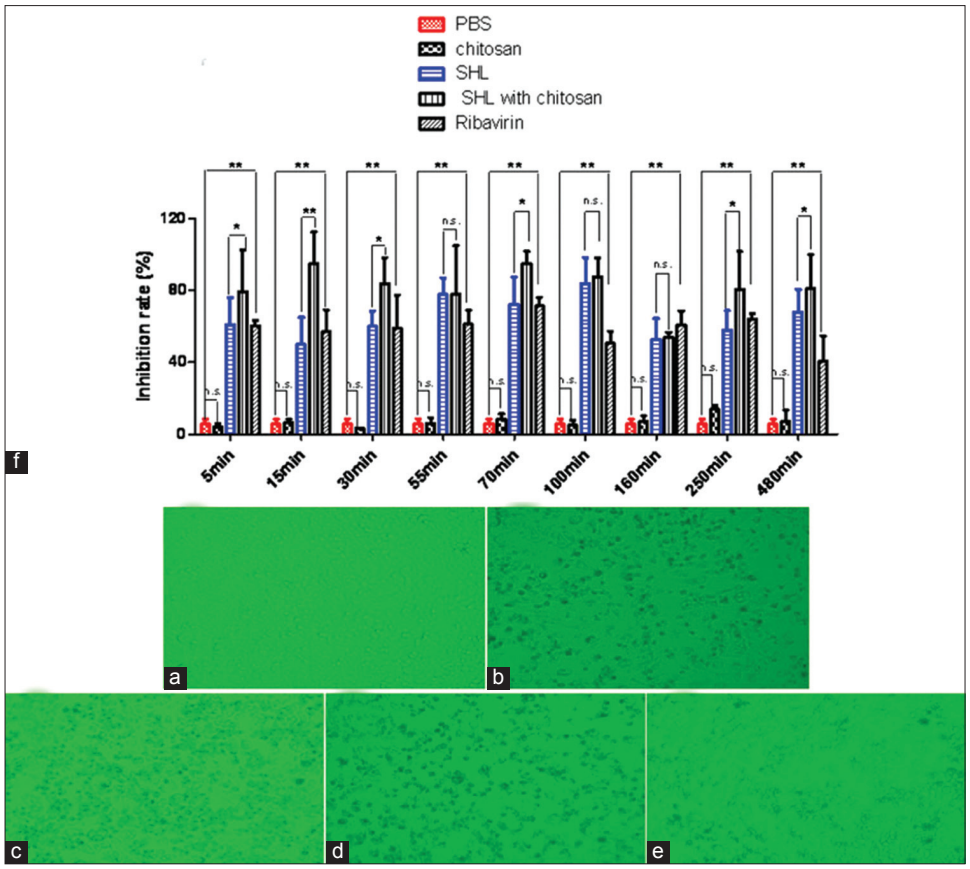

Figure 5: Effect of SHL with or without water-soluble chitosan at the dosage of 50 mg/kg on influenza virus. (A: Normal group; B: Virus group; C: PBS group; D: water-soluble chitosan group; E: Ribavirin as positive control group; F: Inhibition rate of PBS, SHL with or without water-soluble chitosan and ribavirin on influenza virus). Inhibition rate was assayed with MTT 48 hours later and expressed as percentage of controls (data ± S.D. $n = 8$). (*) $P < 0.05$, (**) $P < 0.01$ and (N.S.) no significant difference

Table 1: Effects of absorption enhancers on the absorption of FTA in SHL (10 mg/kg) by *in vivo* pharmacokinetics study

	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0\text{-}600\ min}$ (ng min/mL)	Enhancement ratio
Control	32.9±15.5	73.3±20.8	9047.5±82.7	-
Sodium caprate				
150 mg/kg	125.7±39.3	16.7±11.5	26028±6649.8**	2.88±0.73
100 mg/kg	90.8±8.6	20.0±14.1	16684.5±2372.3**	1.84±0.26
50 mg/kg	84.1±29.4	10.0±0.0	16366±1830*	1.81±0.59
25 mg/kg	47.7±19.8	55±0.0	11761.8±2486.2^{ns}	1.35±0.23
Water-soluble Chitosan				
150 mg/kg	55.9±5.9	55±0.0	14196.7±368.6*	1.56±0.04
100 mg/kg	55.3±37.4	10±0.0	12586.3±775.7^{ns}	1.39±0.09
50 mg/kg	88.4±0.8	10±0.0	16955.7±2524.6**	2.03±0.28
25 mg/kg	40.7±17.1	55±0.0	11942.7±601.0^{ns}	1.32±0.06

(**) P<0.01, (*) P<0.05, (n. s.) no significant difference, compared with the control (mean±S.D., n=5)

glycocalix, but chitosan of high concentration decreased the absorption rate of drugs owning to its high viscosity.[22]

When absorption enhancers are applied in clinical practice, their potential local toxicity should be considered. We observed the morphological changes of gastrointestinal mucosa in the presence or absence of absorption enhancers. It can be found from Figure 4 that there was no significant damage in the gastro and colon tissues with sodium caprate at different dosages, but there was significant damage in the small intestine in the presence of 150 mg/kg sodium caprate, which was consistent with the previous report shown by Motlekar *et al.*, (2005)[23] that above 100 mg/kg sodium caprate

could cause mucosal damage. However, there was no significant change in the presence of water-soluble chitosan at different dosages.

The present study also demonstrated that the absorption of FTA in SHL can be improved to the greatest extent, and was safe for gastrointestine by sodium caprate at dosage of 100 mg/kg and water-soluble chitosan at dosage of 50 mg/kg, respectively. However, as shown in Table 1, we found that enhancing effect of water-soluble chitosan at dosage of 50 mg/kg was better than that of sodium caprate at dosage of 100 mg/kg. Meanwhile, Gao *et al*., (2008)[17] reported the effect of chitosan oligomers on the absorption of FD4, and found that the bioavailability of FD4 can be increased by 2.5-fold by chitosan hexamer from *in situ* loop method. Thanou *et al.,* (2001;2007)[24,25] showed the effect of low viscosity grade Mono-*N*-Carboxymethyl Chitosan (LMCC), high low viscosity grade N-sulfonato-N, O-carboxymethy-lchitosan (SNOCC-60) on the absorption of low molecular weight heparin (LMWH), and found that the *AUC* value can be increased by 7 and 18-fold, respectively by intraduodenally administration. Therefore, chitosan derivatives as absorption enhancers need to be further investigated in order to improve the pharmacological effects of SHL formulation better. Besides, Because of complexity of Chinese medicine, not only FTA, but also other ingredients like CA, caffeic acid, etc., in SHL could also be affected by the absorption enhancers based on tight junctions, although their pharmacological effects were weaker than that of FTA. Therefore, in order to control the quality of preparation better, the study involved in the CA, caffeic acid etc., improved by absorption enhancers based on TJs need to be also further investigated.

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