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Research Article

Enhancement of Sodium Caprate on Intestine Absorption and Antidiabetic Action of Berberine

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Abstract. Berberine, a plant alkaloid used in traditional Chinese medicine, has a wide spectrum of pharmacological actions, but the poor bioavailability limits its clinical use. The present aim was to observe the effects of sodium caprate on the intestinal absorption and antidiabetic action of berberine. The *in situ*, *in vitro*, and *in vivo* models were used to observe the effect of sodium caprate on the intestinal absorption of berberine. Intestinal mucosa morphology was measured to evaluate the toxic effect of sodium caprate. Diabetic model was used to evaluate antidiabetic effect of berberine coadministrated with sodium caprate. The results showed that the absorption of berberine in the small intestine was poor and that sodium caprate could significantly improve the poor absorption of berberine in the small intestine. Sodium caprate stimulated mucosal-to-serosal transport of berberine; the enhancement ratios were 2.08, 1.49, and 3.49 in the duodenum, jejunum, and ileum, respectively. After coadministration, the area under the plasma concentration–time curve of berberine was increased 28% than that in the absence of sodium caprate. Furthermore, both berberine and coadministration with sodium caprate orally could significantly decrease fasting blood glucose and improve glucose tolerance in diabetic rats ($P<0.05$). The hypoglycemic effect of coadministration group was remarkably stronger, and the areas under the glucose curves was decreased 22.5%, compared with berberine treatment group ($P<0.05$). Morphologic analysis indicated that sodium caprate was not significantly injurious to the intestinal mucosa. The study demonstrates that sodium caprate could significantly promote the absorption of berberine in intestine and enhance its antidiabetic effect without any serious mucosal damage.

KEY WORDS: berberine; intestinal absorption enhancer; sodium caprate; type 2 diabetes.

INTRODUCTION

Berberine is an isoquinoline alkaloid extracted from the genera *Berberis* and *Coptis*. These two plants have antibacterial and anti-inflammatory actions and have been used to cure gastroenteritis and secretory diarrhea as traditional Chinese medicines for over two millennia. An extract agent berberine also has been used for many decades (1). Recently, a wealth of data has described the therapeutic effect of berberine on other diseases, such as congestive heart failure, cardiac arrhythmia, hypertension, diabetes, hyperlipemia, and cancer (2). At 1999, Lee and Yuan already reported the beneficial effect of berberine on the treatment of diabetes clinically, and other investigators also proved its role in the treatment of type 2 diabetes in clinic (3,4).

Presently, berberine has provoked great interest due to its various bioactivities and low toxicity. The pharmacologic action of berberine has been extensively examined by animal experiments. However, the studies on the absorption of berberine demonstrated that it has poor bioavailability (<5%) (5). High dose (0.9–1.5 g/day in clinic) of berberine usually causes gastrointestinal side effects, due to its poor absorption and long-term administration for diabetes treatment, which greatly limit its clinical application. Presently, few studies have focused on improving the low bioavailability of berberine. We have postulated that berberine coadministrated with an absorption enhancer might increase the bioavailability, enhance its action, and attenuate adverse effects.

Sodium caprate, a medium chain fatty acid, is a well-recognized absorption enhancer. It increases the paracellular permeability through enlarging the tight junctions, thereby expanding paracellular routes for water-soluble, low lipophilic, and poorly absorbable drugs. It is reported that sodium caprate have demonstrated a decrease in transepithelial resistance (R_m) (6) and increase in membrane capacitance, reflecting wider tight junctions and enlargement of the basolateral membrane surface area, respectively (7). Meanwhile, it has been shown that sodium caprate increases the intracellular calcium level by interaction with phospholipase C in the cell membrane, inducing calcium release from the calcium ion pool (8). Sodium caprate can also induce myosin light chain kinase activation by binding calcium ion and calmodulin, which consequently enhance permeability by

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opening tight junctions through contraction of the calmodulin-dependent actin and cadherin endocytosis (9). Furthermore, it has been found that sodium caprate inhibits the excretion pump function of P-glycoprotein (10). It has been used clinically to enhance the rectal absorption of the low molecular weight drug ampicillin (11), and it has been extensively shown to improve the bioavailability of drugs which are offer poorly absorbed (12–14).

Evaluating drug absorption is one of the first steps in documenting pharmacological actions. Therefore, the aim of the present study was to determine the pharmacokinetic characteristics of berberine and to investigate the effect of sodium caprate on the intestinal absorption and antidiabetic action of berberine, our aim being to provide new strategies for broadening the clinical use of berberine.

MATERIALS AND METHODS

Materials

Chemical Reagents

Reference grade berberine (purity quotient >99.8%) was purchased from the Institute for the Northeast Tragacanth, Changchun, China. Sodium caprate was purchased from Sigma Chemical Company. Streptozotocin (STZ) was purchased from Sigma. Glucose test kit was obtained from Beijing BHKT Clinical Reagent Co., Ltd., Beijing, China. Other reagents were purchased from Beijing General Chemical Reagent Factory, Beijing, China.

Chromatographic System and Instrumentation

A Shimadzu high-performance liquid chromatography (HPLC) system equipped with LC-10AT VP pump, DGU-14AM online degasser, SIL-10AD VP refrigerated autosampler, CTO-10AS VP column oven, and SPD-10AVP UV–VIS detector was used. Shimadzu CLASS-VP software was used for data acquisition and mathematical computations. Mettler Toledo AG 245 electronic balance, Branson 3210 sonicator, Minisart NML (0.45 μm) Sartorius filters for samples, Nichipet Nichiryo (10–100 and 100–1,000 μl) micropipette, hypodermic syringes, microliter syringes from Hamilton, Remi Magnetic stirrers with thermostatic controls (1 MLH), Beckman UV–VIS spectrophotometer 640i, and USF ELGA for preparation of reverse osmosis water were used in the study.

Animals

The experimental procedures were approved by the intuitional animal ethical committee and were approved by the Animal Care Committee of Jilin University (certificate number: scxk2007-0003). Male Wistar rats (160–180 g) were purchased from the Experimental Animal Holding of Jilin University. The animals were housed in standard polypropylene cages (three rats per cage) and maintained under controlled room temperature and humidity with 12:12 h light/dark cycle. The rats were acclimated for at least 5 days and fasted before the experiments.

In Situ Intestinal Perfusion Experiment

Recirculating Intestinal Perfusion in Rats

Experiments were carried out as described previously (15), with minor modification. Rats were fasted for 16 h, anesthetized with 20% urethane (5 mL kg^{-1}), and affixed supine on a surface under a surgical lamp to maintain body temperature. The intestine was exposed by a midline abdominal incision. Two glass tubes (outer diameter 5 mm, inner diameter 3 mm) were inserted through small slits at the top of the duodenum and ileum ends. To clear the gut, saline solution at 37°C was slowly passed through it until the effluent was clear; then, prepared phosphate buffered solution (PBS) was circulated for 20 min at 5 mL min^{-1}. The remaining perfusate was completely expelled by air. Berberine (50, 100, and 200 μmol L^{-1}) dissolved in 100 mL PBS was circulated through the gut at 1.0 mL min^{-1}. At 0, 0.5, 1.0, 2.0, 3.0, and 4.0 h, 1.5-mL perfusate solution was removed from the reservoir for analysis of berberine and replaced with equal volumes of PBS. Fresh PBS was prepared with 114.3 mM NaCl, 4.2 mM KCl, 24.9 mM Na_2HPO_4, and 0.3 mM KH_2PO_4 and adjusted to pH 7.4 with carbogen (95% O_2, 5% CO_2) bubbling. In this experiment, phenol red (56 μmol L^{-1}) was added into the blank PBS buffer to act as a nonabsorbable marker to identify any transfer of water into or out of the intestine and to monitor the structural integrity of the intestinal segment.

Animal Groups

Thirty rats were randomly divided into six groups: low-dose berberine group (50 μmol L^{-1}) with sodium caprate (BCL) or without sodium caprate (BL), middle-dose group (100 μmol L^{-1}) with (BCM) or without sodium caprate (BM), and high-dose group (200 μmol L^{-1}) with (BCH) or without sodium caprate (BH). The concentration of sodium caprate was 0.2% (*w*/*v*).

HPLC Method for Detecting the Concentration of Berberine in the Circulating Fluid

HPLC Analysis. The HPLC measurements were carried out using chromatographic column: Zorbax Extend-C18; (4.6× 250 mm, 5 μm). The mobile phase was a mixture of 10 mM ammonium acetate (0.1% formic acid) and acetonitrile (72:28). The flow rate was 1.2 mL min^{-1}. The detection wavelength was 346 nm. The column temperature was 25°C. The mobile phase was filtered through a type HA, 0.45 μm nuclepore membrane filter (Millipore Corporation), and deaerated. Twenty microliters of each sample was injected into the HPLC system for analysis. Six samples of 0.3, 5, and 30 μg mL^{-1} berberine were analyzed for 3 days in a row to generate a standard curve that could be used to calculate the concentration of samples and to determine the accuracy and precision of our method.

Data Analysis

According to the absorption process, which fits Fick's formula, the absorptive parameters of berberine in the rat

intestine were obtained from the absorptive rate constant (K_a) and the absorptive fraction in unit time ($P\%$) which was calculated by the formula: $Q = Q_0 e^{-K_a t}$, where Q represents the remaining berberine detected in the perfusate at each sampling time, t is the time of circulation of the perfusate, Q_0 is the calculated amount at zero time, and K_a is the absorption rate constant of the drug (15).

In the common way (15), the absorptive fraction in unit time ($P\%$) was calculated by the equation: $P\% = (C_0V_0 - C_tV_t)/C_0V_0 \times 100\%$, where C_0 is the initial berberine concentration in the perfusate, V_0 is the initial volume of the perfusate, C_t is the final berberine concentration in the perfusate, V_t is the final volume of the perfusate, and t is the time of circulation of the perfusate.

In Vitro Transport Across Everted Intestinal Sacs

Preparation of the Everted Rat Gut Sacs

The absorption parameters of berberine in the three intestinal segments (duodenum, jejunum, ileum) were tested by *in vitro* method as described (14,16). *In vitro* permeation studies were performed using everted small intestine segments. Rats were fasted for 16 h and then anesthetized with 20% urethane (5 mL kg^{-1}). After making a midline abdominal incision, the whole small intestine was perfused with 50 mL PBS prewarmed to 37°C, excised, and removed into a beaker containing ice-cold PBS solution, which was continuously aerated. Approximately 8-cm-long segments of duodenum (2 cm distal to the pylorus), jejunum (immediately distal to the ligament of Treitz), and ileum (immediately proximal to the cecum) were selected. One of the distal ends of each segment was tied and everted with the help of a glass rod. The proximal end was attached to a cannula then suspended in a tube containing 35 mL PBS with 50 mM berberine, with or without sodium caprate (0.2%, *w*/*v*). Two milliliters of PBS was placed into the everted intestinal segment. Samples (1 mL) were collected at 15, 30, 60, and 90 min and replaced with equal volumes of drug-free buffer. The sample was analyzed by liquid chromatography–mass spectrometry (LC–MS) methods. The medium was maintained at 37°C and stirred by bubbling with O_2/CO_2 (95/5%) throughout the process.

LC–MS Method for Detecting the Concentration of Berberine in Sacs

LC–MS Analysis. Using an API-4000 four-of-three series mass spectrometer, with an ESI-Analyst 1.3 source and data processing software, the IonSpray Voltage was set at 2,500 V for negative ionization. The nitrogen curtain gas was adjusted to a constant pressure of 15 psi, source of gas 1 (GS1, N2) and gas 2 were both set to 50 psi, and the source temperature (at set point) was 500°C. Detect mode was set to positive ion detection, and the scan mode was set to monitor multiple reactions. Parameters for quantitative analysis from m/z 336.1 to 278.1 with a declustering potential of 70 V and collision energy of 56 eV.

Data Processing

The cumulative amount of drug that had permeated through the sac per unit area (micrograms per square centimeter) was plotted against time (minutes). The apparent permeability coefficient (P_{app}, centimeters per second) was calculated using the following equation: $P_{app} = [V/(A \times C_0)] \times dC/dt$, where V is the liquid volume in the everted intestinal sacs, A is the surface area of the exposed intestine membrane (square centimeters), C_0 is the initial concentration of berberine outside the intestinal sacs, and dC/dt is the changed concentration rate of berberine at which the dose appears in the chorion compartment. The enhancement ratio (ER) represents the ability capability of sodium caprate to promote the absorption of berberine in each intestinal segment: $ER = P/P_0$, where P and P_0 are P_{app} of berberine added with and without sodium caprate.

In Vivo Experiments

In Vivo Evaluation

Male Wistar rats weighting 180–230 g were fasted for about 16 h and randomly divided into two experimental groups. We administered berberine at 100 mg kg^{-1} with or without the addition of 50 mg kg^{-1} sodium caprate via intragastric administration. Blood samples (0.2 mL) were taken from the tail vein with heparinized syringes at 0, 0.5, 1, 2, 4, and 6 h, respectively. The plasma sample was collected after centrifugation at 12,000 rpm for 3 min. The plasma concentration of berberine was determined by HPLC. Each experiment was replicated in six rats.

Berberine Level Analysis in Plasma

The peak concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined directly from the plasma concentration–time profiles. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal method from beginning to the final sampling time.

HPLC Method for Detecting the Concentration of Berberine in the Plasma

HPLC Analysis. The mobile phase was methanol–H_2O–triethylamine (75:25:0.5 *v*/*v*), adjusting pH to 6.8 by phosphoric acid. The flow rate was 1.0 mL min^{-1}. The detection wavelength was 340 nm; the column temperature was 25°C. The mobile phase was filtered through a type HA, 0.45 μm nuclepore membrane filter and de-aerated. The sample size was 20 μl.

Pretreatment of Blood Samples. A 0.5-mL acetonitrile was added to a 0.2-mL portion of plasma, for precipitin the plasma proteins. After exposure to ultrasound for 1 min, it was centrifuged at 12,000 rpm min^{-1} for 10 min. The supernatant of the sample was transferred to another clean tube and then filtrate it through a 0.45-μm oil-system nuclepore membrane filter.

Histopathological Evaluation of Local Toxicity

The ileum was carved out after washing the lumen using saline. The segment was then removed and immersed in 10% neutral buffered formalin. A vertical section was prepared

and stained with hematoxylin–eosin, examined by light microscopy. This evaluation was carried out by an experienced veterinary histopathologist in a blinded fashion.

Beneficial Effect of Berberine on the Diabetic Rats

Modeling of Type 2 Diabetes and Drug Administration

Eighty Wistar rats were randomly divided into four groups: control group, diabetes model group, berberine treatment group (BER), and berberine coadministrated with sodium caprate treatment group (BC). The method for developing diabetic rat model was used as we previously described (17,18). Control group was fed with regular chow, and another three groups were given high-fat diet for 4 weeks and then intraperitoneally injected with 30 mg/kg dose of STZ. After 1 week, fasting blood glucose (FBG) was measured, the rats with FBG<7.8 mmol L^{-1} were injected with 30 mg kg^{-1} STZ again, while the control rats were given vehicle citrate buffer (pH 4.4) in a dose volume of 0.25 mL kg^{-1}, respectively. After 4 weeks of STZ injection, the rats with the fasting blood glucose of ≥7.8 mmol L^{-1} twice were considered diabetic. Berberine (100 mg kg^{-1} body weight) with or without sodium caprate (50 mg kg^{-1} body weight) was administered orally as suspension by mixing with vehicle 1% Na-CMC at a dose volume of 0.5 mL kg^{-1} body weight of rats in treatment group for another 4 weeks. At the end of the study, FBG and intraperitoneal glucose tolerance test (IPGTT) were carried out in these four groups.

Measurement of FBG and Intraperitoneal Glucose Tolerance Test

After an overnight fast (12–16 h), the rats were ip injected with 40% glucose (2 g kg^{-1} body weight). Blood samples were collected by cutting tail at 0, 30, 60, and 120 min for measurement of glucose. Blood glucose was measured by using commercially available colorimetric diagnostic kits according to the instruction.

Statistical Analysis

Results were expressed as mean value ± standard deviation (SD). Statistical analyses were performed using the Student's *t* test or analysis of variance. $P<0.05$ was considered statistically significant differences.

RESULTS

The Accuracy and Range of Determination of Berberine by HPLC and LC–MS

Concentration of Berberine in the Circulating Fluid

The regression equation for berberine concentration in sample response peak area determined by HPLC, ranging from 0.1 to 60 μg mL^{-1}, was $S = 31.763C - 1.4038 (r^2 = 0.9996)$. Precision assay showed that the average of the relative SD within 1 day was 0.75% and intraday was 1.06%. The mean recovery was 99.2±0.48% ($n=3$). These parameters indicated that the methods fulfilled analytical requirements with an adequate repeatability (<2%). The retention time of berberine was about 5 min. PBS buffer did not interfere with the determination of berberine (Fig. 1a).

The Concentration of Berberine in the Samples from the Everted Gut Sacs

The samples from the *in situ* model were evaluated by LC–MS (Fig. 1b). The concentration of berberine in the *in vitro* experiment was much lower than the detection limit of HPLC, as described in the present. Therefore, those samples were determined using LC–MS which is more sensitive.

The regression equation for berberine concentration in sample response peak area as determined by LC–MS, ranging from 0.3 to 500 ng mL^{-1}, was $A = 1.92e^4C \pm 1.98e^3 (r^2 = 0.9994)$. It indicated that there was good linear correlation in the limit.

Berberine Level in Plasma

The regression equation for berberine concentration in sample response peak area as determined by HPLC, ranging from 0.1 to 1.6 μg mL^{-1}, was $A = 12.043C - 764.97 (r^2 = 0.9996)$. The drug peak was not affected by any of the absorption enhance or blood artifacts (Fig. 1c).

Effects of Sodium Caprate on the Intestinal Absorption of Berberine in the *In Situ* Experiment

The intestinal absorption of berberine generally followed dose-dependent and time-dependent characteristics (Table I). The absorption rates of berberine in the small intestine at 4 h were 9.3%, 10.0%, and 10.2% at the dosage of 50, 100, and 200 μmol L^{-1}, respectively, suggesting that absorption of berberine was low. In addition, there was no significant difference of the absorption rate constant (K_a) among these groups ($P>0.05$; Table I) and $r^2>0.9$ in each group, indicating that there was good linear correlation between the drug absorption and the incubation time. According to the absorption process, which fits Fick's formula, the absorption of berberine belongs to first-order absorption and the absorption pattern is that of passive diffusion.

In order to improve the intestinal absorption of berberine, the absorption enhancer sodium caprate was added. The absorption rates at 4 h were significantly increased to 18.5%, 13.1%, and 20.1%, respectively (Table I). The most notable promoting effect of sodium caprate on absorption was observed at berberine concentration of 50 μmol L^{-1} [(526.6± 30.9) ng *vs.* (272.7±50.0) ng, $P<0.05$]. However, there were no differences in K_a between berberine combined with sodium caprate and berberine alone ($P>0.05$).

The Influence of Sodium Caprate on the Transport of Berberine Across the Sac *In Vitro* Evaluation

In order to determine the possible effect and promoting site of sodium caprate on berberine absorption in the intestine, co-incubative tissue culture tests with berberine and sodium caprate (0.2%, *w/v*) were conducted using an everted intestinal sac model (19). As shown in Fig. 2, the cumulative amount of drug transported through the sac was

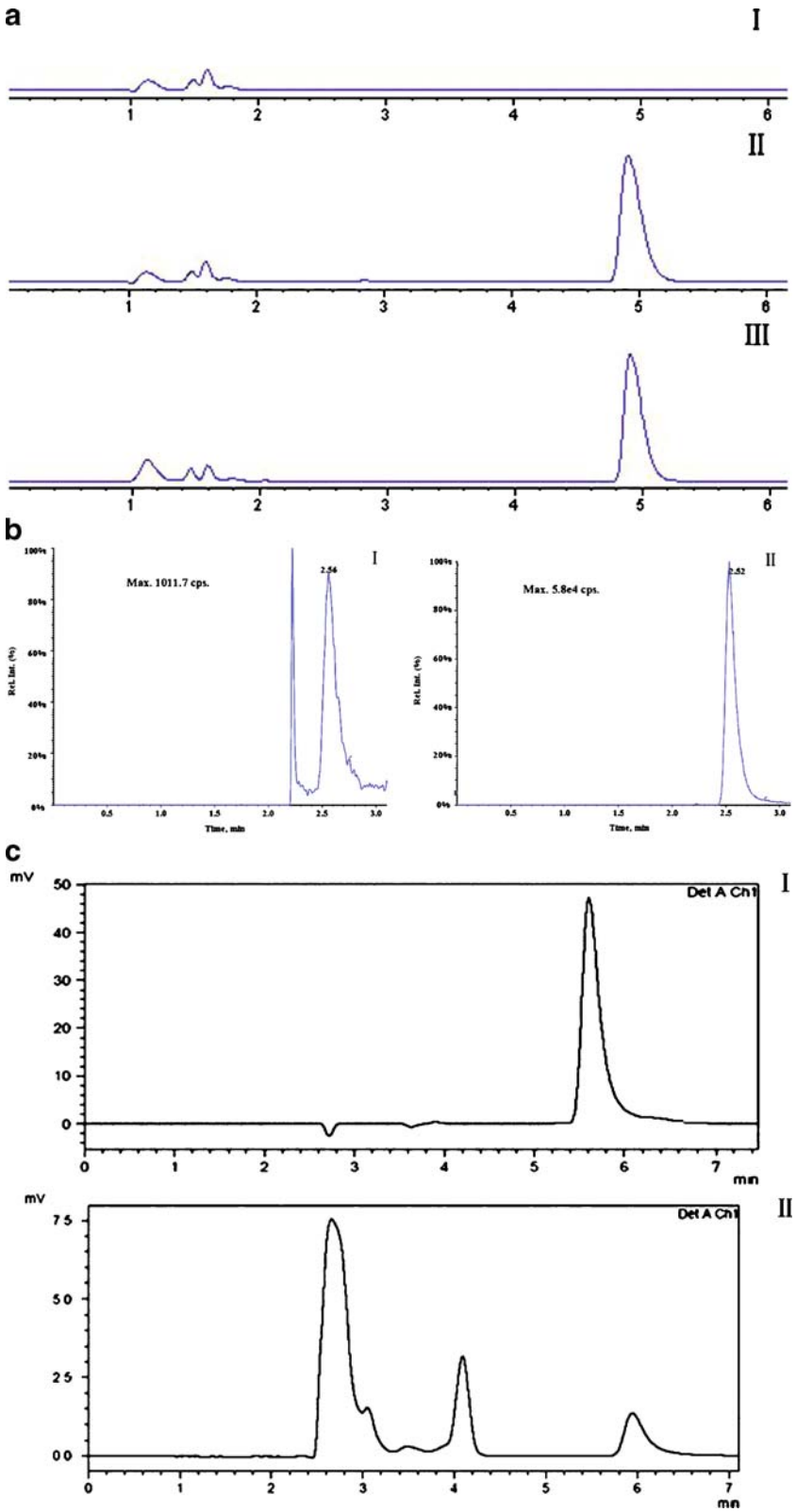

Fig. 1. **a** HPLC chromatogram of berberine in the circulating fluid *I* Blank intestinal circulation solution. *II* Standard sample (0.1 μg mL^{-1}) in PBS. *III* Sample solution. **b** LC–MS spectrum of berberine in samples from the everted gut sacs. *I* Standard preparation in PBS (3 ng mL^{-1}). *II* Sample solution. **c** HPLC chromatogram of berberine in the plasma. *I* Standard sample (0.1 μg mL^{-1}) in the plasma. *II* Sample solution

Table I. Absorbed Amount and K_a of Berberine from the Circulating Fluid

Group	Absorbed amount(ng)					Absorption rate (%)	K_a (h^{-1})
	30 min	60 min	120 min	180 min	240 min		
BL	173.3±12.8	146.4±41.6	211.1±57.3	246.6±55.1	272.7±50.0	9.3	0.034±0.0065
BCL	343.7±27.6*	467.0±38.0**	501.5±39.2*	503.6±24.5*	526.6±30.9**	18.5	0.039±0.0054
BM	372.8±72.1	540.5±98.2	571.9±96.1	592.6±49.6	654.1±86.1	10.0	0.036±0.0048
BCM	486.6±63.4	565.5±33.8	662.7±53.0	896.6±78.9*	987.3±179.8*	13.1	0.040±0.0076
BH	756.9±166.4	965.9±155.6	1,275.5±257.4	1,680.6±128.1	1,798.2±261.8	10.2	0.045±0.0095
BCH	1,493.3±367.2*	1,833.7±330.2*	1,907.7±416.1	2,233.4±399.5	2,333.1±279.3*	20.1	0.047±0.0064

The uptake of berberine was measured in the presence or absence of 0.2% sodium caprate. The absorptive rate constant (K_a): $Q = Q_0 e^{-K_a t}$, where Q represents the remaining berberine found in the perfusate at each sampling time, t is the time of circulation of the perfusate. Q_0 is the calculated intercept at zero time. Each point represents the mean ± SD. $n=5$

BL low-dose group of berberine (50 μmol L^{-1}), *BCL* low-dose group of berberine with sodium caprate, *BM* middle-dose group of berberine (100 μmol L^{-1}), *BCM* middle-dose group of berberine with sodium caprate, *BH* high-dose group of berberine (200 μmol L^{-1}), *BCH* high-dose group of berberine with sodium caprate

*$P<0.05$; **$P<0.01$ *vs.* berberine alone

plotted against time (minutes). The absorption of berberine gradually increased with incubation time up to 90 min, and it was absorbed at various intestinal segments although the absorbed dose was small. Absorption was the greatest in the jejunum, followed by the duodenum and ileum. However, there was no significant difference between the various intestinal segments. The P_{app} of berberine was 3.56×10^{-7}, 4.64×10^{-7}, and 1.88×10^{-7} cm s^{-1} in the various intestinal segments (Table II).

Sodium caprate was able to promote the absorption of berberine significantly at various intestinal segments ($P<0.05$ or $P<0.01$; Fig. 2). The absorption of berberine increased approximately about 1.5–5.2-fold, when berberine was co-incubated with sodium caprate for 90 min at concentrations of 0.2% (*w*/*v*). The ileum showed the highest sensitivity to sodium caprate followed by the duodenum and the jejunum. P_{app} increased in the three tested intestinal segments when sodium caprate was added ($P<0.05$, $P<0.01$), and the enhancement ratios were 2.08, 1.49, and 3.49 in the duodenum, jejunum, and ileum, respectively (Table II).

Effects of Sodium Caprate on the Pharmacokinetics of Berberine *In Vivo*

The plasma concentration–time curves of berberine after oral administration of berberine solution (100 mg kg^{-1}) and berberine containing sodium caprate (50 mg kg^{-1}) in rats are shown in Fig. 3, and the pharmacokinetic parameters are summarized in Table III. The C_{max} of berberine was significantly increased after coadministration while T_{max} was delayed from 30 to 60 min. The area under the serum concentration–time curve ($AUC_{0-6\ h}$) was increased 28%. The results indicated that sodium caprate could increase the absorption of berberine.

Histopathological Evaluation of Local Toxicity

Photomicrographs of intestinal mucosa exposed to berberine and berberine combined with sodium caprate are shown in Fig. 4. The Ileum cavity was chosen as the site of testing because of its greater sensitivity to sodium caprate. As indicated in the Fig. 4, epithelium of each group was undamaged, and villus structure was intact. Only a few villi showed slight edema at the tip. There was no discernible significant difference between berberine alone and with sodium caprate compared with the control groups.

Effects of Sodium Caprate on the Hypoglycemic Effect of Berberine on the Diabetic Rats

To evaluate the effects of sodium caprate on the antidiabetic action of berberine, we carried out IPGTT on diabetic rat models. As shown in Table IV and Fig. 5a, after 4 weeks of treatment, both berberine (100 mg kg^{-1}) orally administration (BER group) and coadministration with sodium caprate (BC group) could significantly decrease fasting blood glucose and improve impaired glucose tolerance. The areas under the glucose curves (millimoles per liter minute) decreased 27% and 43% in BER and BC groups, compared with diabetic model group (Fig. 5b; Table IV). The hypoglycemic effect of BC group was remarkably increased, and the areas under the glucose curves were decreased 22.5%, compared with BER group ($P<0.05$). This data also demonstrated that sodium caprate might enhance the antidiabetic action of berberine by increasing its bioavailability.

DISCUSSION

Berberine is an isoquinoline alkaloid extracted from Rhizoma Coptidis. It was initially used as an anti-inflammatory drug in clinical practice. Recently, many investigations have reported that it has a wide spectrum of pharmacological actions in the treatment of diabetes, cardiovascular diseases,

Fig. 2. Influence of sodium caprate on the cumulative berberine transport in the everted rat gut sac system. **a** The amount of berberine transported through the sac, incubated in the duodenum (*I*), jejunum (*II*), and ileum (*III*), respectively. **b** Cumulative berberine transport across the three tested intestinal segments at 90 min. *BL* incubated with berberine (50 mmol L^{-1}) alone, *BCL* co-incubated with sodium caprate (0.2%, *w*/*v*). $n=5$. *$P<0.05$, **$P<0.01$ *vs.* BL group

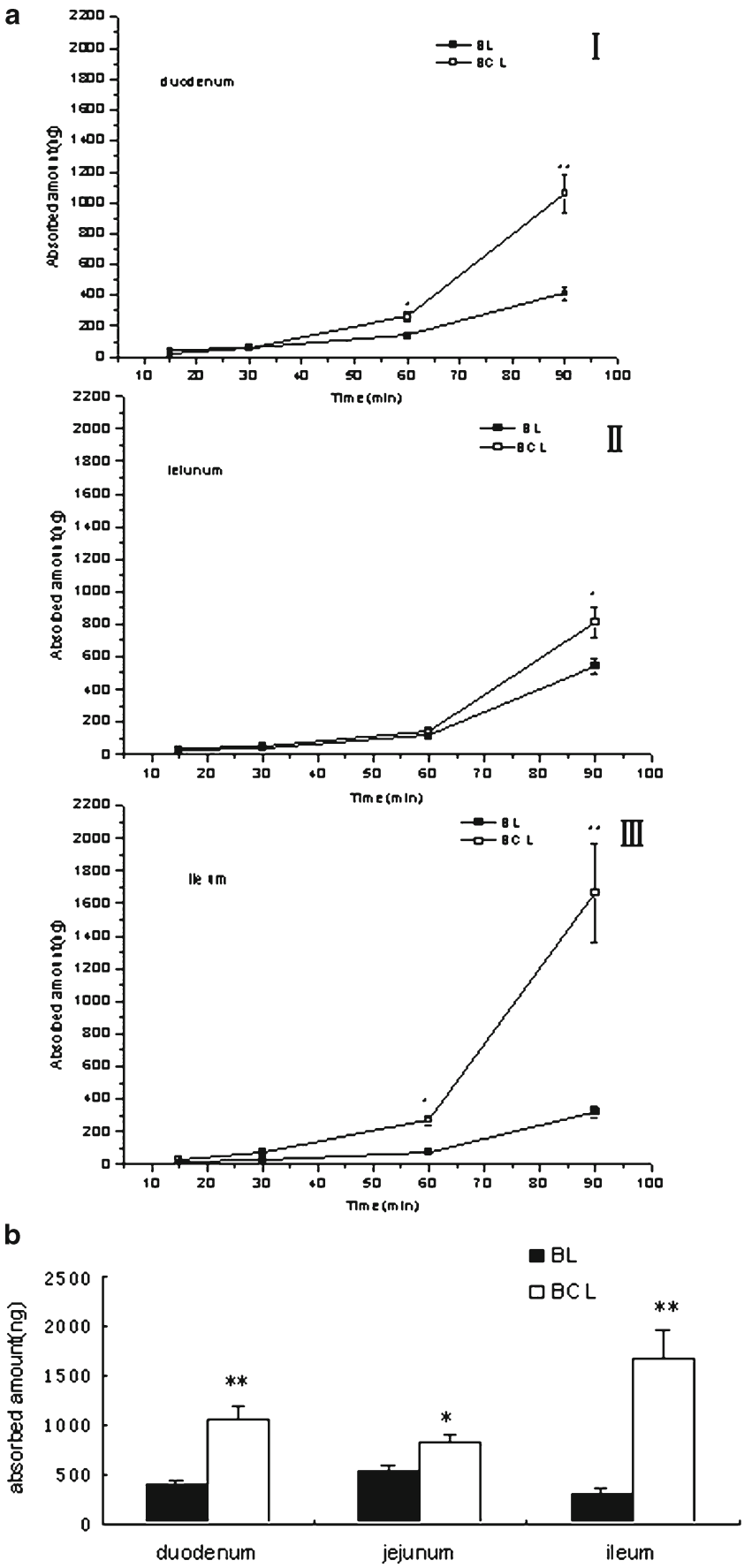
a
I
duodenum
BL
BCL
Absorbed amount(ng)
Time(min)
II
jejunum
III
ileum
b
BL
BCL
absorbed amount(ng)
duodenum
jejunum
ileum

Table II. Papp of Berberine in the Three Intestinal Segments (centimeters per second)

	P_{app}		
Group	Duodenum	Jejunum	Ileum
BL	3.56×10^{-7}	4.76×10^{-7}	2.82×10^{-7}
BCL	7.42×10^{-7}*	7.09×10^{-7}*	9.85×10^{-7}**
ER	2.08	1.49	3.49

The $P_{app}=[V/(A\times C_0)]\times dC/dt$, where V is the liquid volume in the everted intestinal sacs, A is the surface area of the exposed intestine membrane (square centimeters), C_0 is the initial concentration of berberine outside the intestinal sacs, dC/dt is the changed concentration rate of berberine at which the dose appears in the chorion compartment. The $ER=P/P_0$, where P and P_0 are P_{app} of berberine added with or without sodium caprate

BL incubated with berberine (50 μmol L^{-1}) alone, *BCL* co-incubated with sodium caprate (0.2%, *w/v*), *ER* enhancement ratios, P_{app} apparent permeability coefficient

*$P<0.05$; **$P<0.01$ *vs.* BL group

hypertension, hypercholesterolemia, and tumors (1,20–23). Therefore, the development of new application for use of berberine is of potential widespread interest.

However, berberine also displays numerous limitations or side effects including low bioavailability, poor intestinal absorption, and the need for repeated administration. It has been reported that berberine is a xenobiotic with poor bioavailability (<5%) (24). Consequently, its clinical application has been greatly limited. Therefore, development of dosage forms to enhance its bioavailability is extremely important. Coadministration with absorption enhancer is a good way. Sodium caprate is a well-recognized absorption enhancer without any serious toxicity (11). A low bioavailability of berberine may be related to the action of P-glycoprotein, a drug efflux pump (24), and sodium caprate has been reported to inhibit the excretion pump function of P-glycoprotein (10). Therefore, we expect the coadministration with sodium caprate to increase the bioavailability of berberine, decrease the clinical dose, and avoid its side effects.

The pharmacokinetic behavior of berberine and the effect of sodium caprate on absorption of berberine were firstly observed to determine whether sodium caprate can enhance the absorption of berberine, using the recirculating perfusion model. The results showed the small intestine absorption rates of berberine at 4 h was 9.3% at the dosage of 50 μmol/L. Pan *et al.* (25) also proved the poor absorbability of berberine. They stated that berberine was absorbed only small amount (2.5%) after 160 min at the same dose. Compared with berberine alone, the absorption rate in the small intestine at 4 h was increased to 18.5% when sodium caprate co-incubated. The uptake of berberine was also elevated at a different level in medium- and high-dose groups when it was combined with sodium caprate. Therefore, it is clear that sodium caprate can significantly increase the absorption of berberine in the small intestine.

Furthermore, in order to guide clinical application and the search for new forms of medication, the effects of sodium caprate on the intestinal transport and the site of action of berberine were examined *in vitro* studies using the everted rat gut sac system. Our data showed that berberine alone was absorbed in only small amounts at various intestinal segments. The order of absorption amount is jejunum > duodenum > ileum. Whereas the cumulative amount of berberine taken in through the sac was fairly low, berberine was rapidly absorbed when treated with sodium caprate after 90 min incubation. The increase was significant in all the three tested intestinal segments, and P_{app} was also enhanced when sodium caprate was added. The site in which sodium caprate promoted the greatest absorption of berberine was the ileum where berberine absorption is the weakest. The ER was 3.49 in ileum, followed by the duodenum and jejunum. The results from vitro studies indicated that the best site of absorption enhancement of sodium caprate is different with that of berberine absorption.

Several reports have shown that the most sensitive segment of intestine for the action of sodium caprate is colon (26). However, we found that berberine is scarcely absorbed in the colon segment *in vitro* experiment (data were not shown). Therefore, we only investigate the promoting function of sodium caprate in duodenum, jejunum, and ileum segments.

It is recognized that studies in the *in vivo* models are necessary to confirm the utility of the enhancer and to determine the influence of physiological variables, although *in situ* and *in vitro* studies are useful for early screening to select a potential promoter (27). Sometimes the effect of absorption enhancer *in vitro* is significant, but *in vivo*, their effect is not as noticeable as *in vitro* experiment or even undetectable because of the complex environment of whole organism. Therefore, we further verified the enhancement of

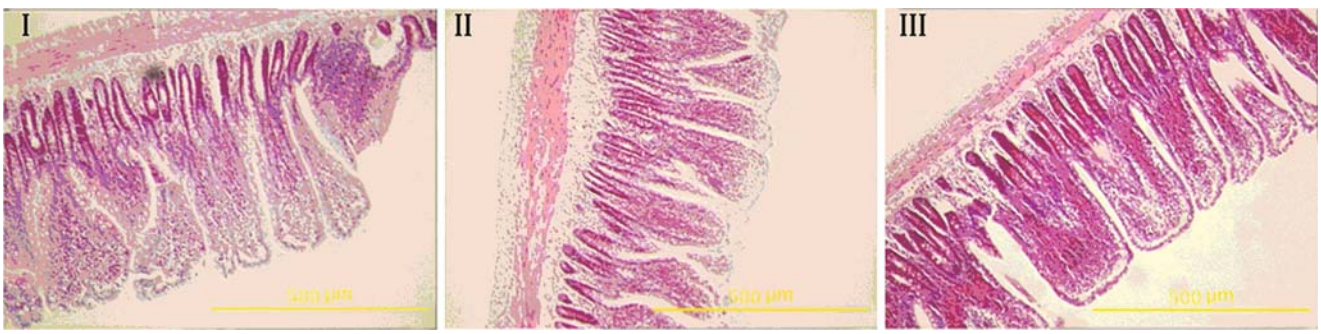

Fig. 3. Histopathological comparison between treatment and control. Light micrographs were taken at 6 h after intragastric administration of drugs (original magnification ×200). *I* Control (intragastric administration of normal saline). *II* Intragastric administration of berberine (100 mg kg^{-1}). *III* Intragastric administration of berberine (100 mg kg^{-1}) with sodium caprate (50 mg kg^{-1})

Table III. Pharmacokinetic Parameters of Berberine with and Without Sodium Caprate

	B	BC
$AUC_{0-6\ h}$ (ng mL^{-1} h^{-1})	2,641.024±230.8	3,385.22±131.4*
Tmax(min)	30	60
Cmax(ng mL^{-1})	721.39±53.45	988.84±135.56*

Berberine was oral administration at the dose 100 mg kg^{-1} with or without sodium caprate (50 mg kg^{-1}). Each value represents the mean ± SD of more than three experiments. n=6

AUC area under the serum concentration–time curve, which was calculated from 0 to 6 h following the trapezoidal rule, C_{max} maximum serum concentration of berberine, T_{max} time to achieve C_{max} after the administration of berberine, *B* oral administration of berberine without sodium caprate, *BC* oral administration of berberine with sodium caprate

*P<0.05 *vs.* B group

sodium caprate on berberine absorption through the *in vivo* experiments. In the present study, our *in vivo* studies with unrestrained conscious rats demonstrated that with coadministration of sodium caprate, the peak plasma concentration of berberine in rats was elevated, the peak time was delayed and the $AUC_{0-6\ h}$ was increased, and it indicated that sodium caprate could improve the bioavailability of berberine. However, the increase level of AUC (28%) *in vivo* model is not as noticeable as the vitro models (above 100%), owing to the complex environment of the whole organism. The concentration of sodium caprate was diluted by food or water in the intestine. Otherwise, all the conditions, such as pH, temperature, or secretions, could influence the drug absorption.

Presently, many studies reported that berberine plays noticeably hypoglycemia effect in animal experiment and clinical trials (3,4). The mechanism of berberine on antidiabetic actions has also been extensively studied (28–30). In the present study, we chose the dose of berberine as 100 mg/kg based on the clinical studies (0.9–1.5 g/days); this dose also has been used in the other studies in rats (28–30). We also found that berberine could decrease fasting blood glucose and improve impaired glucose tolerance of diabetic model rats, which were consistent with other studies (3). As we have found that sodium caprate could enhance the bioavailability of berberine in the first section of present study, the treatment effect of berberine coadministrated with sodium caprate on hyperglycemia has further confirmed this point. Berberine with sodium caprate could remarkably decrease the blood glucose level and the areas under the glucose curves, compared with berberine treatment group. This result might indicate that the coadministration of sodium caprate was an effective way for the utilization of berberine in diabetes.

The local toxicity of sodium caprate in the small intestine is one of the major concerns in relation to the use of intestinal enhancer in pharmaceutical products. Lindmark *et al.* (11) has reported that ampicillin suppositories containing 5% of sodium caprate caused nonspecific damage to the rectal mucosa. This result is in agreement with the report by other reporters (31–33), suggesting that it does not cause serious cytotoxicity and its effects is reversible. This is in agreement with our current investigations. We examined the *in vivo* effect of sodium caprate on the mucosal morphology in the ileum. Discernible morphological alteration was not observed in the rat ileum mucosa after the delivery of sodium caprate. Sodium caprate thus seems to be a good candidate for a formulation excipient to enhance the oral curative effect of berberine. However, our toxicity evaluation of berberine and/or sodium caprate has certain limitations. Further data about acute and chronic toxicity were necessary before clinical experiments.

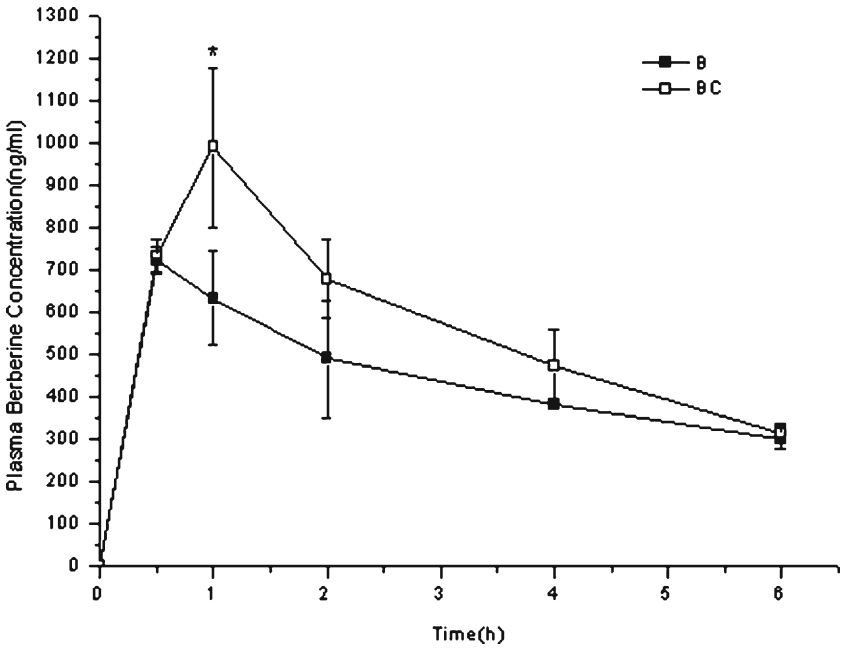

Fig. 4. Plasma berberine concentration–time profile after oral administration with or without sodium caprate in rats. All data are expressed as mean ± SD. n=6. *B* berberine administration (100 mg kg^{-1}) without sodium caprate, *BC* coadministration with sodium caprate (50 mg kg^{-1}). *P<0.05 *vs.* B group

Table IV. Plasma Glucose During IPGTT

Group	Plasma glucose (mmol/L)				AUC (mmol L^{-1} min)
	0 min	30 min	60 min	90 min	
CON	4.71±0.64	13.21±1.33	11.62±2.20	8.16±1.04	1,234.48±166.61
DM	9.05±0.88*	33.36±3.35	33.92±2.48	27.30±3.58	3,482.07±245.28*
BER	7.93±1.00	25.92±2.19**	26.37±1.18**	15.46±2.22**	2,547.24±176.56**
BC	5.89±0.62**	21.59±3.06**	18.02±2.22**·****	14.29±2.11**	1,975.68±230.03***·****

Plasma glucose concentration during IPGTT in CON, DM, BER, and BC after 4 weeks treatment. Data shown are means ± SE (n=6–10 rats per group per time point)

CON control group, *DM* diabetes model group, *BER* berberine treatment group, *BC* berberine coadministration with sodium caprate treatment group, *AUC* area under the curve during IPGTT in these four groups

*P<0.001 *vs.* control group; **P<0.05 *vs.* DM group; ***P<0.01 *vs.* DM group; ****P<0.05 *vs.* BER group

CONCLUSION

The enhancement of sodium caprate on intestinal absorption of berberine was investigated in the *in situ*, *in vitro*, and *in vivo* systems. The results demonstrated that berberine was a drug with poor absorption situation in the rat small intestine, and the overall absorptive profile of it can be increased obviously by coadministrated with sodium caprate,

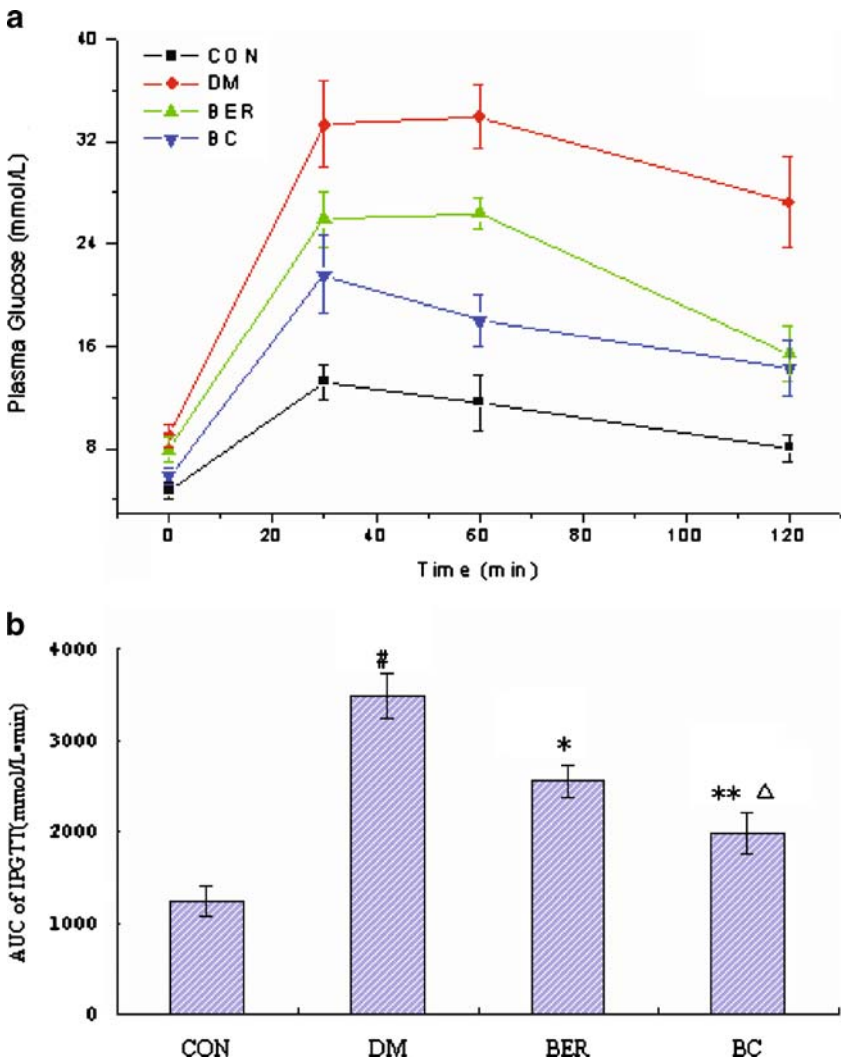

Fig. 5. **a** Plasma glucose during intraperitoneal glucose tolerance test (*IPGTT*) in control group (*CON*), diabetes model group (*DM*), berberine treatment group (*BER*), and berberine coadministration with sodium caprate treatment group (*BC*) after 4 weeks treatment. **b** Area under the curve during IPGTT in these four groups. Data shown are means ± SE (n=6–10 rats per group per time point). #P<0.001 *vs.* control group; *P<0.05, **P<0.01 *vs.* DM group; △P<0.05 *vs.* BER group

moreover enhance its antidiabetic action, without causing significant damage to the intestinal mucosa. High dose of berberine oral administration usually causes gastrointestinal side effects, which greatly limit its clinical application on diabetes mellitus. Therefore, combination with sodium caprate could lower the dose of berberine, which may offer advantages of better pharmacological action and lower adverse reaction.

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