

Berberine suppresses intestinal disaccharidases with beneficial metabolic effects in diabetic states, evidences from in vivo and in vitro study

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Abstract Clinical reports have demonstrated that berberine is a potential antidiabetic agent, but the underlying mechanism is unclear. The purpose of this study was to investigate if berberine exerts its hypoglycemic action via inhibiting intestinal disaccharidases using in vivo and in vitro experiments. Streptozotocin-induced diabetic rats received berberine (100 or 200 mg/kg) orally once daily or acarbose (40 mg/kg) orally twice daily for 5 weeks. Disaccharidase activities and sucrase–isomaltase (SI) complex messenger RNA (mRNA) expression in intestinal regions were assessed. The same treatment was operated in normal rats. Sucrose and maltose loading tests were also documented. In addition, Caco-2 cells were cultured in medium containing berberine or berberine plus chelerythrine. Compound C or H-89 for 5 days, disaccharidase activities, and SI complex mRNA levels were measured. The animal experiments showed that berberine significantly decreased the disaccharidase activities and SI complex mRNA expression both in diabetic rats and normal rats. Berberine can also significantly lower postprandial blood glucose levels induced

by sucrose or maltose loading in normal rats. The cellular results showed that berberine may suppress disaccharidase activities and downregulate SI complex mRNA expression in a concentration-dependent manner. Only H-89, an inhibitor of protein kinase A (PKA), may reverse the decrease in disaccharidase activities and SI complex mRNA expression induced by berberine. In conclusion, berberine suppresses disaccharidase activities and SI complex mRNA expression with beneficial metabolic effects in diabetic states. The inhibitory effect, at least partly, involves the PKA-dependent pathway.

Keywords Diabetes mellitus · Disaccharidase · Berberine · Sucrase–isomaltase complex · PKA

Introduction

Carbohydrates are necessary for energy and ultimately digested by α -glucosidase and disaccharidases in the small intestine. Carbohydrate digestion directly induces the increase of postprandial blood glucose levels (Levin 1994). Sucrase and isomaltase, located in the brush border membrane of small intestine (Hauri et al. 1985), are two major components of disaccharidases involved in the final carbohydrate digestion and absorption of glucose. They are produced by posttranslational process of its sucrase–isomaltase (SI) complex by pancreatic proteases (Hauri et al. 1980).

Diabetes mellitus is associated with increased intestinal digestion of carbohydrates, proteins, fats as well as absorption of glucose, amino acids, and fatty acids. Intestinal hyperplasia and hypertrophy and related increase in disaccharidases are observed in diabetic condition of

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both experimental animals (Tormo et al. 2002; Adachi et al. 2003) and patients (Dyer et al. 2002), which indicates that the increase in disaccharidase activities may be one of the factors resulting in postprandial hyperglycemia in diabetic states. The postprandial hyperglycemia is considered to be a high-risk factor resulting in the development of chronic complications of diabetes mellitus (Lebovitz 2001). The effective management of diabetes mellitus is to prevent an excess of postprandial rise in blood glucose levels (Rendell and Jovanovic 2006). An effective way to control postprandial hyperglycemia is to inhibit α -glucosidase in the intestine (Holman et al. 1999). In fact, a number of the enzymatic inhibitors have been successfully developed for treatment of diabetes mellitus (Tewari et al. 2003).

Berberine has been demonstrated antidiabetic effects in animals (Lee et al. 2006) and humans (Zhang et al. 2009; Yin et al. 2008; Zhang et al. 2008). A series of reports showed that berberine may stimulate glucose uptake (Cheng et al. 2006), modulate lipids metabolism (Kong et al. 2004), enhance insulin sensitivity, and stimulate insulin secretion (Ko et al. 2005), but the facts that berberine has characteristics of poor absorption (Pan et al. 2002) and low plasma concentration (Lu et al. 2006) cannot explain its antihyperglycemic effect. However, poor absorption may result in high exposure of berberine in intestinal tract, which in turn affects metabolism of glucose before absorption. Our previous study showed that berberine treatment may lower blood glucose levels in streptozotocin (STZ)-induced diabetic rats, accompanied by attenuated intestinal disaccharidases (Liu et al. 2008a, b). The purpose of this study was to test the hypothesis that berberine exerts its antidiabetic effect, at least in part, via suppressing intestinal disaccharidases. Further investigations about disaccharidase activities and SI complex messenger (mRNA) levels were documented to verify the hypothesis using both diabetic rats and normal rats.

The Caco-2 cells, derived from human colon adenocarcinoma, are described to display some intestinal epithelial characteristics including a small-bowel phenotype with microvilli, dome formation, and the expression of SI complex (Traber 1998; Deschênes et al. 2001; Laprise et al. 2002). In the present study, the Caco-2 cells were also used to further study the effects of berberine on disaccharidase activities and SI complex mRNA expression. Effects of some pharmacological inhibitors on disaccharidase activities and SI complex mRNA expression were observed to investigate which signal pathways are involved in the inhibitory effects of berberine on disaccharidase activities and SI complex mRNA expression. It is expected to obtain an improved understanding about regulation of intestine disaccharidase by berberine, thereby providing an alternative prospect for the use of berberine in the treatment of diabetes mellitus.

Materials and methods

Animals

Male Sprague Dawley rats, weighing 180–200 g, were supplied by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The rats were maintained in an air-conditioned animal quarter at a temperature of $22\pm 2^\circ\text{C}$ and a relative humidity of $50\pm 10\%$. Water and food (laboratory rodent chow, Nanjing, China) were allowed ad libitum. The animals were acclimatized to the facilities for 5 days and fasted with free access to water for 8 h prior to experiment. The studies were approved by the Animal Ethics Committee of China Pharmaceutical University, and every effort was made to minimize stress to the animals.

Diabetic rats induced by streptozotocin

The diabetic rats were induced by an intraperitoneal administration of 55 mg/kg of streptozotocin (Sigma-Aldrich Co., Shanghai, China) dissolved in sodium citrate buffer, pH4.5. Age-matched normal rats were injected with the vehicle (sodium citrate buffer, pH4.5). On day 7 post-STZ injection, the fasting blood glucose levels were measured using commercially available glucose kit (Jian-cheng Biotech Co., Nanjing, China) based on glucose oxidase method. Rats with fasting blood glucose levels in excess of 11.1 mM were considered to be diabetic rats (Liu et al. 2008a, b).

Drug treatment

On the seventh day post-STZ injection, the diabetic rats were randomly divided into four groups. Group 1 was served as diabetic control and only received vehicle. Group 2 and group 3 were orally dosed with 100 and 200 mg/kg of berberine (purity >99%, Qing-Ze Pharmaceutical Co., Nanjing, China) once daily, respectively. Group 4 was orally dosed with 40 mg/kg of acarbose twice daily. Age-matched normal rats were served as normal control and only received vehicle. The experimental rats were treated with the test drugs for 5 weeks. The fasting blood glucose, food uptake, and body weight were monitored on day 7, 14, 21, 28, and 35 of drug treatment.

On day 35 of the treatment, rats were fasted for 8 h. Two hours after drug treatment, blood was collected in tube via orbital vein under ether anesthesia. Serum samples were obtained, and insulin levels were measured using an ELISA kit (Millipore Co., Billerica, MA, USA). On day 36, the rats fasted for 8 h were sacrificed under ether anesthesia; three regions (duodenum, jejunum, and ileum) of intestine were immediately removed, flushed with ice-cold physiological saline, frozen in liquid nitrogen, and stored at -80°C for disaccharidase assay and RT-PCR analysis.

In parallel to the treatment of the diabetic rats with berberine, the normal rats were randomly divided into four groups and received the same treatment as described above for 37 days. On days 36 and 37 of drug treatment, oral sucrose and maltose loading tests were operated, respectively. Briefly, 30 min after treatment, rats orally received 2 g/kg of sucrose or maltose. Blood samples were collected via orbital vein under ether anesthesia before and 15, 30, 60, 90, and 120 min post sucrose or maltose administration. Plasma samples were obtained, and the blood glucose levels were measured as described above. On day 38, the rats fasted for 8 h were sacrificed under ether anesthesia, and intestinal samples were immediately obtained for disaccharidase assay and RT-PCR analysis.

Measurement of disaccharidase activities in small intestine

The crude enzyme solution from small intestine was prepared according to the following procedure (Yasuda et al. 2003). Briefly, the mucosa of 2 cm of each intestinal region was collected by scraping with a glass slide and homogenized in 1 ml of 100 mM ice-cold potassium phosphate buffer, pH 6.8. After centrifugation at 3,000×g for 10 min, the supernatant was used as crude enzyme solution. The activities of intestinal disaccharidase were measured by determination of glucose released from maltose and sucrose (Dahlqvist 1968). Protein content was measured using Bradford dye assay, using bovine serum albumin as a standard.

In parallel, direct effect of berberine on disaccharidase activities was investigated in crude enzyme solution from small intestine of normal rats according to the literature method (Mariko et al. 2006).

RT-PCR analysis

Total RNA was extracted from frozen tissue or cells using Trizol reagent (Invitrogen Co., Carlsbad, CA, USA) following the standard RNA isolation method. The quality of RNA was checked using the DU640 nucleic acid analyzer (Beckman Co., Gaithersburg, MD, USA). For rats, primers used for the amplification of the cDNAs of interest were forward 5'-GGAGGTTACATTCTACCATGTCAAG-3' and reverse 5'-CCAGGTGATTTGTATTGGTTCATCA-3' for rat SI complex gene (GenBank: L25926.1, 420 bp), forward 5'-GGTGCTGAGTATGTCGTGGAG-3' and reverse 5'-ATGCAGGGATGATGTTCTGG-3' for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (365 bp; Sheng-Xing Sci-Tech Co., Nanjing, China), and for human Caco-2 cells, forward 5'-GAGGGATTGTAATTTCTCGTTCCC-3' and reverse 5'-GGGGTAACCATAAATGCTGG-3' for human SI complex gene (GenBank:

X63597.1, 481 bp), forward 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' and reverse 5'-CTAGAAGCATTGCGGTGGACGATGGAGGG-3' for human GAPDH gene (258 bp) (Sheng-Xing Sci-Tech Co., Nanjing, China). After denaturation at 95°C for 5 min, the amplification was obtained by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min each. A final extension step at 72°C for 5 min was employed. The PCR products were subjected to 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The amplification of GAPDH for each sample was performed and used as an internal control. The relative target gene levels were normalized with GAPDH.

Cell culture

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). The Caco-2 cells (passages 23–36) were grown in DMEM (Invitrogen Co., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen Co., Carlsbad, CA, USA), 1% nonessential amino acids, 4 mmol/L of glutamine, 1×10^5 U/L of penicillin, and 100 mg/L of streptomycin according to a previous study (Ogawa et al. 2000).

Effect of berberine on disaccharidase activities in Caco-2 cells

Cells were seeded at a density of 1×10^5 cells per well in six-well culture plates (Corning Co., Lowell, MA, USA). Cells were cultured for 2 days in the free-drug medium, then cultured for another 5 days in medium containing different concentrations of berberine (2, 10, and 50 μ M) or acarbose (50 μ M). Then the cells were collected for disaccharidase assay and RT-PCR analysis. 3-(4, 5-Dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide assay showed that the tested agents used in the study had no damage on the viability of cells within the tested concentrations.

The activities of sucrose and maltase were measured using a published method (Pan et al. 2003) with minor modification. In brief, cell monolayers were washed with ice-cold phosphate-buffered saline and then collected. The collected cells underwent ultrasonic disruption in 0.1 ml glucose-free Hanks' balanced salt solution, and 0.1 ml of maltose or sucrose solution (56 mM) was added. The mixture was incubated at 37°C for 60 min. The reaction was stopped by adding 0.1 ml of methanol. The amount of glucose released from the substrates was measured using an HPLC-UV method as previously described (Honda et al. 1989). The amount of glucose (micromole) produced in 1 hour is defined as unit of disaccharidase activities.

Effect of pharmacological inhibitors on disaccharidase activities mediated by berberine in Caco-2 cells

Cells were seeded at a density of 1×10^5 cells per well in six-well culture plates. Cells were cultured for 2 days in the free-drug medium, then were cultured for another 5 days in the medium containing berberine (10 μM) coadministered with compound C (10 μM), chelerythrine (10 μM), or H-89 (10 μM). The cells were collected for disaccharidase assay and RT-PCR analysis.

Data analysis

Results are expressed as mean \pm standard deviation (SD). Statistical differences among groups were evaluated by one-way analysis of variance. If analysis was significant, the differences between groups were estimated using Student–Newman–Keuls multiple comparison post hoc test. A *p* value of less than 0.05 indicated a significant difference.

Result

Effects of berberine on disaccharidase activities and SI complex mRNA levels in intestine of diabetic rats

As expected, a single administration of STZ to rats produced diabetic symptoms including loss of body weight, significant increases in both blood glucose level ($p < 0.01$), and food uptake ($p < 0.01$), as well as decrease in intrinsic insulin ($p < 0.01$). Berberine treatment significantly decreased the fasting blood glucose levels, accompanied by the improvement of body weight and reduction of food intake (Fig. 1). Moreover, berberine treatment showed an increase in serum insulin level in diabetic rats (Table 1). The results further verified antidiabetic effects of berberine.

Both sucrase and maltase were selected for evaluating disaccharidase activities. It was found that activities of sucrase and maltase in the interested intestinal regions (duodenum, jejunum, and ileum) of diabetic rats were significantly higher than those in normal control rats (Fig. 2). Compared with normal control rats, diabetic states resulted in 2.42-, 1.85-, and 7.27-fold increase in sucrase activity and 1.45-, 1.58-, and 2.56-fold increase in maltase activity of duodenum, jejunum, and ileum, respectively.

Berberine treatment significantly decreased disaccharidase activities in intestinal regions of diabetic rats. Interestingly, berberine treatment almost restored maltase activities in duodenum of diabetic rats to the level of normal control rats, although significant decreases were found in other regions. Berberine treatment also significantly lowered sucrase activity in the interested intestinal

regions of diabetic rats. All these results demonstrated that diabetic states may induce significant increase in intestinal disaccharidase activities, and berberine treatment may attenuate the increase in disaccharidase activities under diabetic states.

In parallel, the RT-PCR analysis was used to measure SI complex mRNA levels in the intestinal regions. It was found that the measured levels of SI complex mRNA in the duodenum and jejunum of diabetic rats were 1.41- and 1.37-fold of that in the normal control rats, respectively (Fig. 3). Berberine treatment reversed the increase in SI complex mRNA expression induced by diabetic states in a dose-dependent manner. Further analysis showed that SI complex mRNA levels in diabetic rats treated with berberine were lower than those in normal control rats. For example, SI complex mRNA levels in duodenum of rats treated with 100 and 200 mg/kg of berberine were only 76% and 62% of that in normal control rats, respectively. Lower SI complex mRNA levels were also found in jejunum of rats treated with berberine (Fig. 3). Similarly, acarbose treatment decreased SI complex mRNA levels in both duodenum and jejunum of diabetic rats.

Effect of berberine on the postprandial blood glucose levels induced by sucrose or maltose loading in normal rats

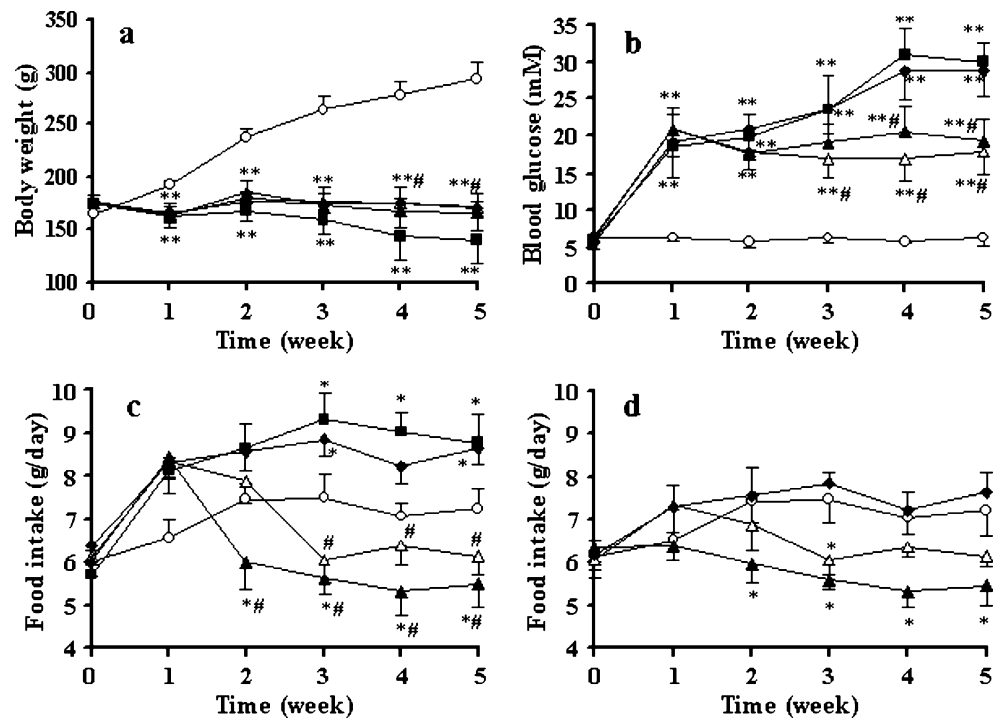
Physiological and biochemical parameters were also observed during berberine treatment in normal rats. Although body weight and blood glucose levels were similar among experimental rats, berberine treatment significantly suppressed food uptake (Fig. 1d), accompanied by slight increase in serum insulin levels (Table 1).

The postprandial blood glucose levels induced by sucrose or maltose loading in normal rats were investigated on days 36 and 37 of berberine treatment (Fig. 4), respectively. It was found that plasma glucose levels increased sharply in the experimental rats, reaching the maximum at about 60 min after oral administration of sucrose or maltose. At 60 min after sucrose or maltose loading, plasma glucose levels of the groups treated with berberine were slightly higher than those of rats treated with acarbose, but postprandial plasma glucose levels of rats treated with berberine were significantly suppressed compared to those of normal control rats. The effect continued through the experimental period, resulting in lower $\text{AUC}^{0-120\text{min}}$ values (Fig. 4).

Effects of berberine on disaccharidase activities and SI complex mRNA levels in intestine of normal rats

Intestinal disaccharidase activities of normal rats were measured on day 38 of berberine treatment (Fig. 5). In accordance with the findings in diabetic rats, berberine

Fig. 1 Effects of berberine on body weight (a), fasting blood glucose level (b), food intake (c) during the 5-week treatment in diabetic rats, and food intake in normal rats (d). Symbols represent as follows: Circle=normal control rats, filled square=diabetic control rats, open square=rats treated with 100 mg/kg of berberine, filled triangle=rats treated with 200 mg/kg of berberine, and filled diamond=rats treated with 40 mg/kg of acarbose. The results are expressed as mean±SD ($n=6$), * $p<0.05$, ** $p<0.01$ vs normal control rats, # $p<0.05$, ### $p<0.01$ vs diabetic control rats



treatment significantly suppressed intestinal disaccharidase activities in normal rats. The most effective inhibition was found in duodenum. Acarbose also showed inhibitory effect on intestinal disaccharidase activities in normal rats. It was noticed that the basal levels of both sucrase and maltase activities were inconsistent in Figs. 2 and 5. The difference may come from two batches of experiments.

Data from RT-PCR analysis showed that berberine dose-dependently suppressed SI complex mRNA expression in duodenum and jejunum of normal rats (Fig. 6). The SI complex mRNA levels in the duodenum and jejunum of rats treated with 200 mg/kg were 46% and 51% of that in normal control rats, respectively. In parallel, 100 mg/kg of berberine also induced similar reduction in both duodenum

and jejunum. Rats treated with acarbose showed significant decrease in SI complex mRNA levels in interested intestinal regions. All the results indicated that berberine suppressed intestinal disaccharidase activities and SI complex mRNA expression in both diabetic rats and normal rats.

To investigate whether berberine directly inhibits disaccharidase activities, an in vitro disaccharidase inhibition test was performed. Acarbose was also used as a positive control. The result showed that berberine had no inhibitory effects on both sucrase and maltase activities within the tested concentrations (Fig. 7). However, acarbose effectively inhibited the sucrase and maltase activities with IC_{50} of 5.96 and 25.37 μ M, respectively. These values are in agreement with a previous report (Kim et al. 2008).

Table 1 Effects of berberine on serum insulin level after 5-week treatment in diabetic or normal rats

Diabetic groups	Serum insulin level (ng/ml)	Normal groups	Serum insulin level (ng/ml)
Normal control	2.61±0.49*	Normal control	2.47±0.60
Diabetic control	1.41±0.40**	Normal+berberine (100 mg/kg)	3.41±0.52***
Diabetic+berberine (100 mg/kg)	2.20±0.54****	Normal+berberine (200 mg/kg)	2.99±0.65
Diabetic+berberine (200 mg/kg)	2.11±0.48****	Normal+acarbose (40 mg/kg)	2.37±0.29
Diabetic+acarbose (40 mg/kg)	1.78±0.62**		

The serum insulin level was measured 2 h following last treatment. The results are expressed as mean±SD ($n=6$)

* $p<0.01$ vs diabetic control rats

** $p<0.01$ vs normal control rats

*** $p<0.05$ vs normal control rats

**** $p<0.05$ vs diabetic control rats

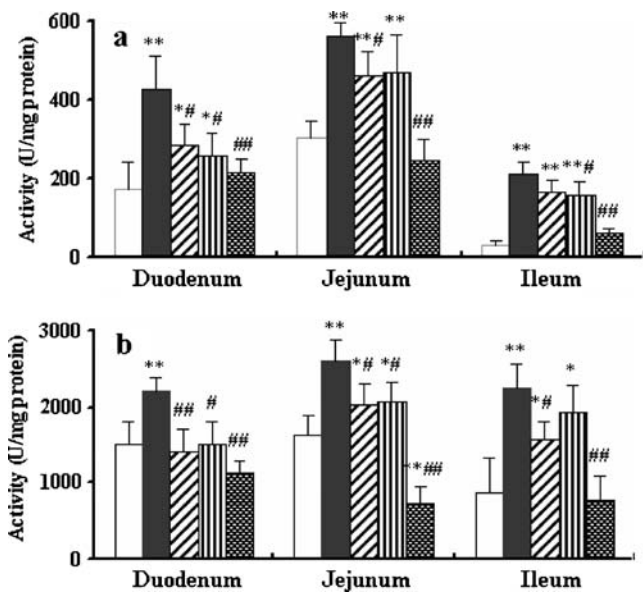


Fig. 2 Effects of 5-week treatment on sucrase (a) and maltase activity (b) in the small intestine of diabetic rats. Symbols represent as follows: white bar=normal control rats, shaded bar=diabetic control rats, diagonally striped bar=diabetic rats treated with 100 mg/kg of berberine, vertically striped bar=diabetic rats treated with 200 mg/kg of berberine, and hatched bar=diabetic rats treated with 40 mg/kg of acarbose. The results are expressed as mean±SD (n=6), *p<0.05, **p<0.01 vs normal control rats; #p<0.05, ###p<0.01 vs diabetic control rats

Effects of berberine on disaccharidase activities and SI complex mRNA levels in Caco-2 cells

To determine the effect of berberine on disaccharidase activities in Caco-2 cells, the cells were cultured in medium containing different concentrations of berberine (2, 10, and 50 μM) for 5 days. Disaccharidase activities were measured

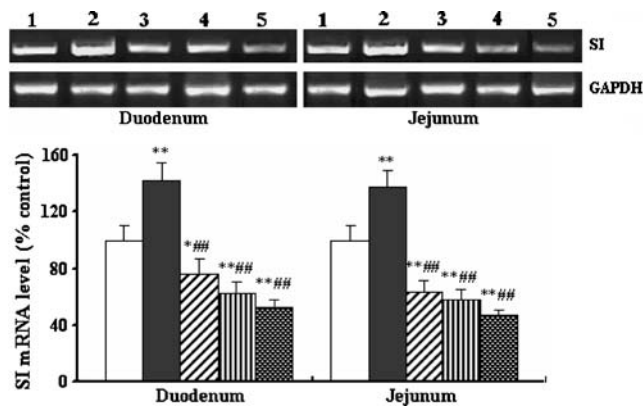


Fig. 3 Effects of 5-week treatment on SI complex mRNA expression in the small intestine of diabetic rats. Symbols represent as follows: white bar=normal control rats, shaded bar=diabetic control rats, diagonally striped bar=diabetic rats treated with 100 mg/kg of berberine, vertically striped bar=diabetic rats treated with 200 mg/kg of berberine, and hatched bar=diabetic rats treated with 40 mg/kg of acarbose. The results are expressed as mean±SD (n=4), *p<0.05, **p<0.01 vs normal control rats; ###p<0.01 vs diabetic control rats

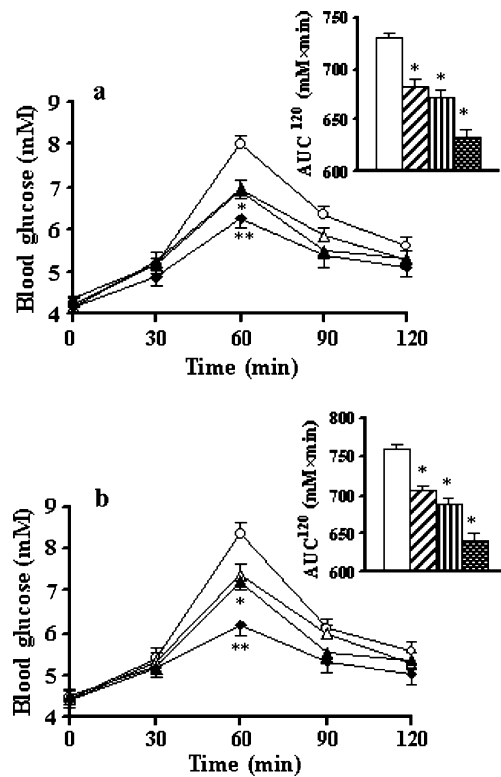


Fig. 4 Effects of berberine treatment on the postprandial blood glucose level and its exposure of glucose (AUC¹²⁰) after oral administration 2 g/kg of sucrose (a) or maltose (b) in normal rats. Symbols represent as follows: circle, white bar=normal control rats; open triangle, diagonally striped bar=rats treated with 100 mg/kg of berberine, filled triangle, vertically striped bar=rats treated with 200 mg/kg of berberine, and filled diamond, hatched bar=rats treated with 40 mg/kg of acarbose. The results are expressed as mean±SD (n=6), *p<0.05 **p<0.01 vs normal control rats

(Fig. 8a and b). It was found that berberine inhibited sucrase and maltase activities in a concentration-dependent manner. Significant inhibition on disaccharidase activities was found in cells treated with both 10 and 50 μM of berberine. Similarly, chronic exposure of acarbose to Caco-2 cells led to 45% and 48% decrease in sucrase and maltase activities, respectively.

SI complex mRNA levels in Caco-2 cells treated with berberine were investigated. It was found that berberine attenuated SI complex mRNA expression in Caco-2 cells. Significant reduction in SI complex mRNA expression was showed in cells cultured with both 10 and 50 μM of berberine (Fig. 9). However, acarbose did not affect SI complex mRNA expression in Caco-2 cells.

Effects of pharmacological inhibitors on disaccharidase activities and SI complex mRNA expression mediated by berberine in Caco-2 cells

Caco-2 cells were cultured in medium containing berberine coadminstrated with different inhibitors. Data for disacchari-



Fig. 5 Effects of 5-week treatment on sucrase (a) and maltase activity (b) in the small intestine of normal rats. Symbols represent as follows: white bar=normal control rats; diagonally striped bar=rats treated with 100 mg/kg of berberine, vertically striped bar=rats treated with 200 mg/kg of berberine, and hatched bar=rats treated with 40 mg/kg of acarbose. The results are expressed as mean±SD ($n=6$), * $p<0.05$, ** $p<0.01$ vs normal control rats

dase activities showed that only H-89, a PKA inhibitor, reversed the decrease in disaccharidase activities induced by berberine, which resulted in 23% and 17% improvement of sucrase and maltase activity, respectively (Fig. 8c and d). H-89 also reversed the decrease in SI complex mRNA expression induced by berberine (Fig. 9). The inhibitory effect of acarbose on disaccharidase activities was not

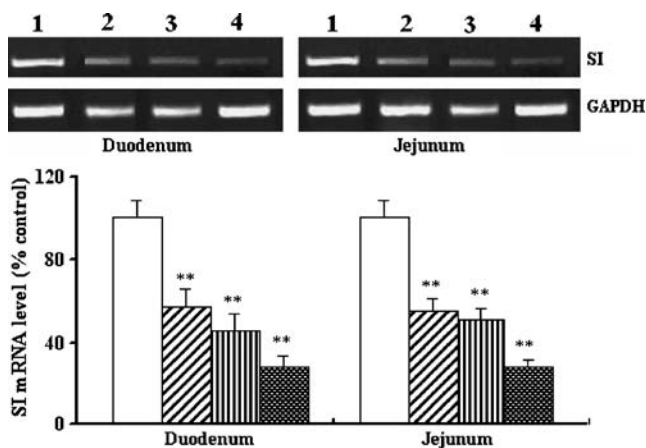


Fig. 6 Effects of 5-week treatment on SI complex mRNA expression in the small intestine of normal rats. Symbols represent as follows: white bar=normal control rats; diagonally striped bar=rats treated with 100 mg/kg of berberine, vertically striped bar=rats treated with 200 mg/kg of berberine, and hatched bar=rats treated with 40 mg/kg of acarbose. The results are expressed as mean±SD ($n=4$), ** $p<0.01$ vs normal control rats

reversed by the three inhibitors. Further investigations showed that these pharmacological inhibitors themselves showed no inhibitory effects on disaccharidase activities and SI complex mRNA expression.

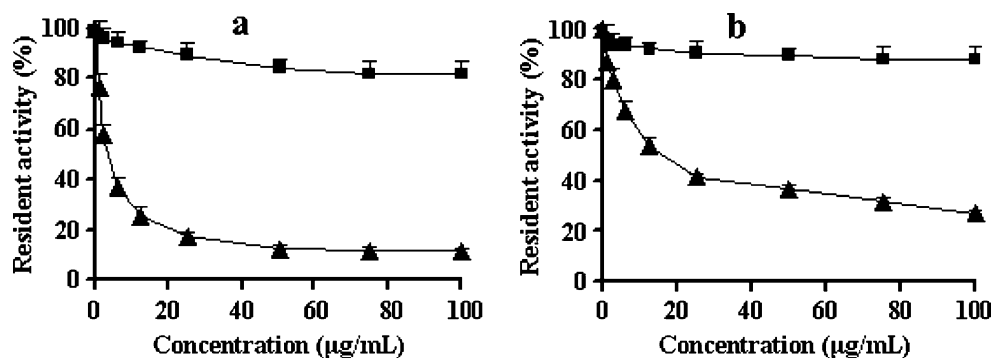
Discussion

Carbohydrates are digested into glucose by α -glucosidase and disaccharidases in the small intestine, which directly induces the increase in postprandial blood glucose levels. The significant increase in disaccharidase activities is observed in experimental animals (Mcanuff-Harding et al. 2006; Malaisse et al. 2004) and diabetic patients (Dyer et al. 2002; Tandon et al. 1975), which becomes an important factor leading to the elevated blood glucose levels. The increase in disaccharidase activities under diabetic condition may result from abnormal intestinal hyperplasia and hypertrophy (Tormo et al. 2002; Adachi et al. 2003). Insulin is considered to play an important role in regulating disaccharidase activities. Its deficiency in diabetic states may result in an increase in the enzyme activity (Tandon et al. 1975; Takenoshita et al. 1998). The present study showed that the increase in disaccharidase activities is accompanied by low insulin levels in diabetic rats, indicating a critical role of insulin in suppressing disaccharidase activities under diabetic states.

Berberine has been reported as a potential antidiabetic agent. However, poor oral bioavailability and low plasma concentration of berberine (Lu et al. 2006; Qiu et al. 2008) make it hard to understand the mechanisms for the clinical application of berberine in diabetes mellitus (Zhang et al. 2009; Yin et al. 2008; Zhang et al. 2008). Our previous study showed that berberine may attenuate the increase in disaccharidase activities in experimental diabetic rats (Liu et al. 2008a, b), indicating that berberine may exert its antidiabetic effects partly via suppressing disaccharidase activities. The present study was focused on extending the previous findings and further providing direct evidences using in vivo and in vitro experiments.

In vivo studies showed that berberine may significantly reverse the increase in activities of sucrase and maltase, two main disaccharidases in the intestinal mucosa of diabetic rats. The reduction in disaccharidase activities is associated with a decrease of fasting blood glucose and improvement of diabetes symptoms. The results indicated that berberine may reduce intestinal disaccharidase activities, resulting in the decrease of blood glucose levels in diabetic rats, which may be beneficial for the amelioration of diabetic states (Zhang et al. 2009; Yin et al. 2008; Zhang et al. 2008). The most important was that berberine significantly decreased the disaccharidase activities both in diabetic rats and normal rats, which may be one of the mechanisms for berberine as

Fig. 7 The inhibitory effect of berberine (filled square) and acarbose (filled triangle) on sucrase (a) or maltase (b) activity in crude enzyme solution from intestinal mucosa of normal control rats. Resident activity (percent) is defined as ratio of enzymatic activity in presence of inhibitors to that without inhibitors. The results are expressed as mean±SD ($n=4$)



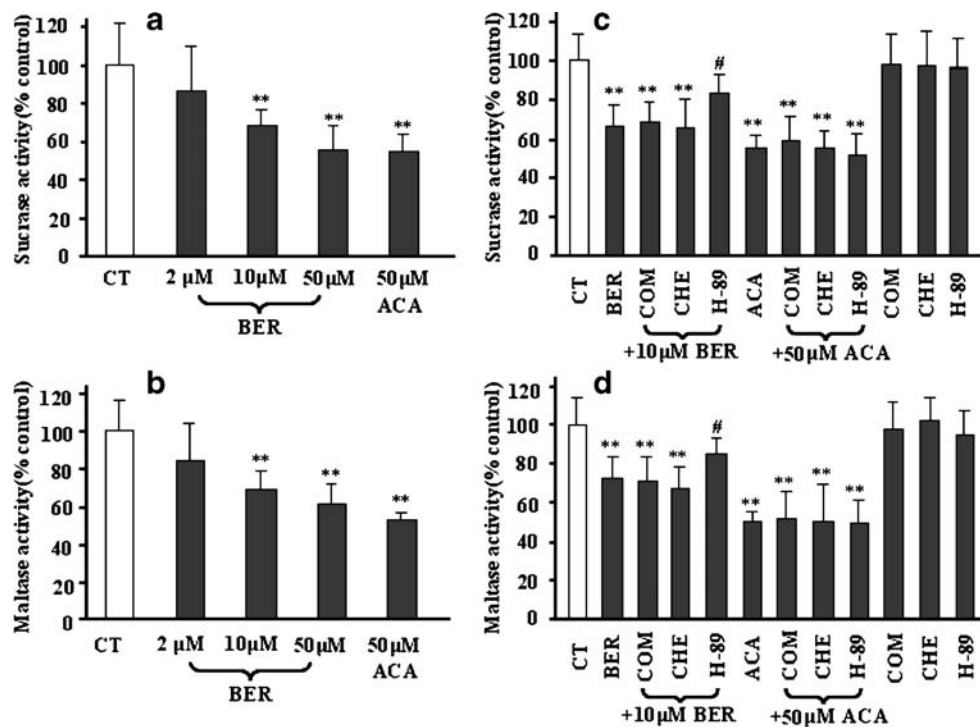
an antihyperglycemic agent. Several reports indicated that diabetic patients are accompanied with gastrointestinal symptoms including diarrhea and dysbacteriosis (Shakil et al. 2008; Rozanova et al. 2002). This indicated that berberine's antidiarrheal (Cheng et al. 2009) and antimicrobial (Freile et al. 2003) effects may also partly contribute to its antidiabetic actions.

Postprandial blood glucose levels after oral administration of sucrose or maltose to normal rats were used to investigate the contribution of the decreased disaccharidase activities induced by berberine to hypoglycemic effect. Postprandial blood glucose levels were significantly suppressed in rats treated with berberine or acarbose. Further study showed that berberine also suppressed disaccharidase activities in normal rats. The decrease in disaccharidase activities is associated with the decrease of postprandial blood glucose levels. These results also support our

hypothesis that berberine suppresses disaccharidase activities, which in turn decreases blood glucose levels.

The data from RT-PCR analysis demonstrated that SI complex mRNA levels in diabetic rats treated with berberine were significantly lower than those in diabetic control rats and were even lower than those in normal control rats. Similar suppression was found in normal rats treated with berberine. The decrease in SI complex mRNA levels is in line with the decrease in disaccharidase activities, which indicates that the decrease in disaccharidase activities induced by berberine partly results from suppression of SI complex mRNA expression. Similar reductions in both disaccharidase activities and SI complex mRNA levels were found in rats treated with acarbose, in agreement with a previous study (Goda et al. 1982). Berberine itself did not show direct inhibition on disaccharidase activities in the in vitro disaccharidase inhibition test,

Fig. 8 Effects of berberine (BER) and acarbose (ACA) on disaccharidase activities in Caco-2 cells (a, b). Effects of pharmacological inhibitors compound C (COM, 10 µM), chelerythrine (CHE, 10 µM), and H-89 (10 µM) on disaccharidase activities mediated by berberine (BER, 10 µM) or acarbose (ACA, 50 µM) in Caco-2 cells (c, d). The Caco-2 cells were cultured in medium containing test agents for 5 days. The results are expressed as mean±SD ($n=6$), ** $p<0.01$ vs control cells (CT); # $p<0.05$ vs cells treated with BBR (10 µM) alone



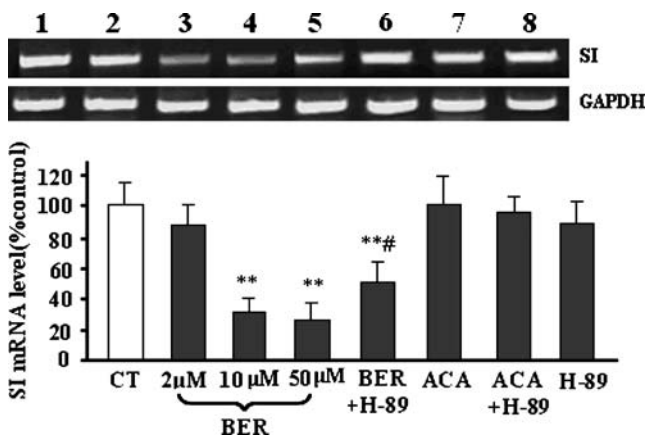


Fig. 9 The expression of SI mRNA level when Caco-2 cells were cultured in medium containing berberine (*BER*), acarbose (*ACA*, 50 μM), and a PKA inhibitor H-89 (10 μM) for 5 days. The results are expressed as mean±SD ($n=4$), ** $p<0.01$ vs control cells (*CT*); # $p<0.05$ vs cells treated with *BER* (10 μM) alone

which revealed that inhibitory effect of berberine on disaccharidase activities may be different from acarbose.

The Caco-2 cells, which has been used as an in vitro model for the studies of α -glucosidase inhibitors (Toda et al. 2000; Hansawasdi and Kawabata 2006), was also introduced into the present study. As we expected, berberine suppresses both disaccharidase activities and SI complex mRNA expression. Good association between low disaccharidase activities and low SI complex mRNA levels were found in cells treated with berberine, indicating that the suppression of SI complex mRNA expression may account for the decrease in disaccharidase activities. These results further support our findings in vivo. However, acarbose did not alter SI complex mRNA levels in the cells, which suggested that acarbose may directly inhibit disaccharidase activities in Caco-2 cells.

Accumulating evidences have showed that some signal pathways including AMPK (Hallows 2005), PKC (Frey et al. 2000), and PKA (Boucher et al. 2005; Martin-Latil et al. 2004) are involved in proliferation, differentiation as well as SI complex expression in intestinal epithelial cells. Several studies reported that berberine exerts its multi-pharmacological effects via affecting these signal pathways which are considered to be the targets of berberine. Berberine stimulates glucose uptake, inhibits lipid synthesis, and depresses proinflammatory responses via activation of AMPK (Brusq et al. 2006; Turner et al. 2008; Jeong et al. 2009). It reduces insulin resistance and upregulates insulin receptor expression through PKC-dependent pathway (Kong et al. 2009). Our study demonstrated that the inhibitory effects of berberine on disaccharidase activities, and SI complex mRNA expression may also be involved in these signal pathways. To support the in vivo data, disaccharidase activities were measured in Caco-2 cells

cultured in medium containing berberine and specific pharmacological inhibitors. It was found that only H-89, a PKA inhibitor, may reverse the decrease in disaccharidase activities and SI complex mRNA expression induced by berberine. Compound C (an AMPK inhibitor) and chelerythrine (a PKC inhibitor) showed no effects on disaccharidase activities and SI complex mRNA expression induced by berberine. The three pharmacological inhibitors themselves showed no inhibitory effects on disaccharidase activities and SI complex mRNA expression. These results suggested that the PKA-dependent pathway may be involved in the inhibitory effects of berberine on disaccharidase activities and SI complex mRNA expression.

A report showed a crucial role of PKA-dependent pathway in the human intestinal epithelial differentiation (Basson and Hong 1996). Another study using Caco-2 cell model also showed that 8Br-cAMP, a diffusible cAMP, suppresses the expression of the enterocyte markers SI complex and villin, accompanied by the decrease of cell polarization and brush border formation (Laprise et al. 2002). Octreotide stimulates Caco-2 proliferation and differentiation via decreasing intracellular cAMP (Sgambati et al. 1996). The impairment in disaccharidase activities and SI complex expression induced by rhesus monkey rotavirus is also cAMP-PKA dependent (Martin-Latil et al. 2004). The present study showed that H-89 may reverse the inhibitory effects of berberine on disaccharidase activities and SI complex mRNA expression, which revealed that the PKA-dependent pathway may be involved in regulation of disaccharidase activities and SI complex mRNA expression by berberine. Several reports demonstrated that berberine did not alter cellular cAMP levels (Zhou et al. 2008; Taylor et al. 1999). The present result also showed that H-89 itself did not inhibit disaccharidase activities neither SI complex mRNA expression in Caco-2 cells. These data indicated that the inhibitory effects of berberine on both disaccharidase activities and SI complex mRNA expression via affecting the PKA-dependent pathway in Caco-2 cells are complex.

Several evidences showed that the expression of SI complex was involved in hepatocyte nuclear factor-1 (HNF-1 α and HNF-1 β), GATA-4, and caudal related homeodomain proteins CDX2 and CDX1 (Boudreau et al. 2002). GATA-4 was reported to be a downstream effector of cAMP/PKA signaling (Tremblay and Viger 2003), but CDX2 expression was specially regulated by cAMP/Epac pathway (Chen et al. 2005). The present study showed that berberine only suppressed SI complex mRNA expression in a PKA-dependent manner, which led to the repression of sucrase and maltase activities. These finding indicated that GATA-4 may participate in the inhibitory effects of berberine on SI complex mRNA expression. The real relationship between the PKA-dependent pathway and the

inhibitory effects of berberine on disaccharidase activities and SI complex mRNA expression is still obscure and requires further investigation. However, this study still provided direct evidences that the inhibitory effects of berberine on disaccharidase activities and SI complex mRNA expression are partly via the PKA-dependent pathway.

Conclusion

The findings in the present study supported the hypothesis that berberine exerts its antidiabetic effects partly via suppressing intestinal disaccharidases activities and SI complex mRNA expression. The inhibitory effect, at least partly, involves the PKA-dependent pathway.

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The authors declare that they have no conflict of interest.

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