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Berberine attenuates intestinal disaccharidases in streptozotocin-induced diabetic rats

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Previous studies demonstrated anti-diabetic effects of berberine. However, the facts that berberine had low bioavailability and poor absorption through the gut wall indicated that berberine might exert its antihyperglycaemic effect in the intestinal tract before absorption. The purpose of this study was to investigate whether berberine attenuates disaccharidase activities and β-glucuronidase activity in the small intestine of streptozotocin (STZ)-induced diabetic rats. Two groups of STZ-induced diabetic rats were treated with protamine zinc insulin (10 U/kg) subcutaneously twice daily and berberine (100 mg/kg) orally once daily for 4 weeks, respectively. Both age-matched normal rats and diabetic control rats received physiological saline only. Fasting blood glucose levels, body weight, intestinal disaccharidase and β-glucuronidase activities in duodenum, jejunum and ileum were assessed for changes. Our findings suggested that berberine treatment significantly decreases the activities of intestinal disaccharidases and β-glucuronidase in STZ-induced diabetic rats. The results demonstrated that the inhibitory effect on intestinal disaccharidases and β-glucuronidase of berberine might be one of the mechanisms for berberine as an antihyperglycaemic agent.

1. Introduction

Carbohydrates are digested by α-glucosidase and disaccharidase in the small intestine. Carbohydrate digestion directly increases the postprandial blood glucose level. Disaccharidases including sucrase, lactase and maltase, located on the brush border membrane, play important roles in the final digestion of carbohydrates. Diabetes mellitus (DM) is often associated with an increased digestion of carbohydrates, protein, fat as well as absorption of glucose, amino acids and fatty acids. Some reports showed hyperplasia and hypertrophy in small intestine of alloxan-induced or streptozotocin-induced rats (Stenling et al. 1984; Debnam et al. 1990; Zoubi et al. 1995) associated with an increase in disaccharidase activity (McAnuff-Harding et al. 2006; Adachi et al. 2003). This is to say that an increased disaccharidase activity may be one of the reasons for high blood glucose levels.

Rhizoma coptidis has been used for treating diabetes mellitus for more than one thousand years in Chinese traditional medicine. Berberine, a quaternary protoberberine-type alkaloid is one of the main constituents in *Rhizoma coptidis* and many other herbs. Previous studies demonstrated anti-diabetic effects of berberine (Leng et al. 2004; Tang et al. 2006; Lee et al. 2006). Berberine had been demonstrated to be beneficial by lowering blood glucose, modulating lipids metabolic and scavenging free radicals in streptozotocin-induced diabetic rats (Tang et al. 2006). However, the facts that berberine showed low bioavailability (Lu et al. 2006; Sheng et al. 1993) and poor absorption through the gut wall (<5%) (Pan et al. 2002) indicated that berberine might exert its antihyperglycaemic effect in the intestinal tract before absorption. Our previous studies using Caco-2-cells showed that berberine might significantly inhibit disaccharidase activity and also showed weak inhibition of glucose absorption (Pan et al. 2002, 2003).

The aim of the present study was to investigate whether berberine reverses disaccharidase activity in the small intestine of streptozotocin (STZ)-induced diabetic rats and to explore its mechanism of action. At the same time, the enzyme β-glucuronidase, which is associated with the reabsorption of bile acid was also observed.

2. Investigations and results

Compared with age-matched normal rats, significantly higher blood glucose levels and lower body weight were found in diabetic rats. Both berberine and insulin treatment significantly decreased blood glucose levels and increase body weight in diabetic rats, although berberine effect was not as good as insulin (Fig. 1). The results further verified antihyperglycaemic effects of berberine.

As is shown in Figs. 2–4, the activities of disaccharidases (lactase, sucrase, maltase) were significantly increased in the duodenum, jejunum and ileum regions of small intestine in diabetic rats when compared with those in the normal control group. And the activities of disac-

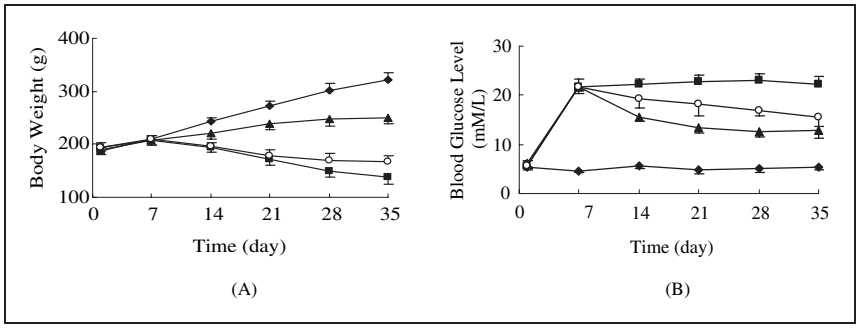

Fig. 1:
Change in the body weight (g) (A) and blood glucose (mM) (B) in STZ-induced diabetic rats after the administration of insulin (10 U/kg/day) and berberine (100 mg/kg/day) for 4 weeks (mean ± S.D., n = 5). Normal rats (◆), diabetic rats (■), insulin treated diabetic rats (▲) and berberine treated diabetic rats (○). Insulin and berberine were treated to diabetic rats from 7th day to 35th day

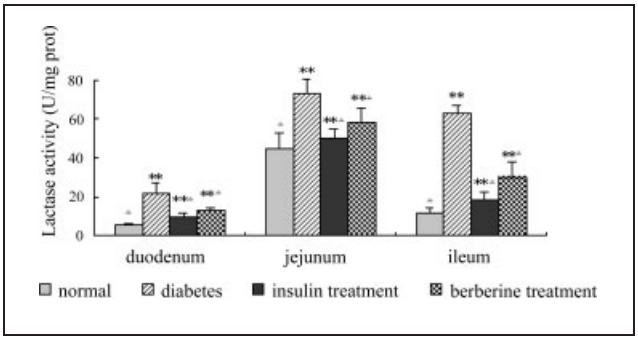

Fig. 2: Lactase activity in the small intestine in different treatment of rats. Each bar represents the mean ± S.D., n = 5, for each region of the intestine. **Denotes statistical difference compared with normal control rats. ($p < 0.01$, by ANOVA). ΔDenotes statistical difference compared with diabetic rats. ($p < 0.01$, by ANOVA)

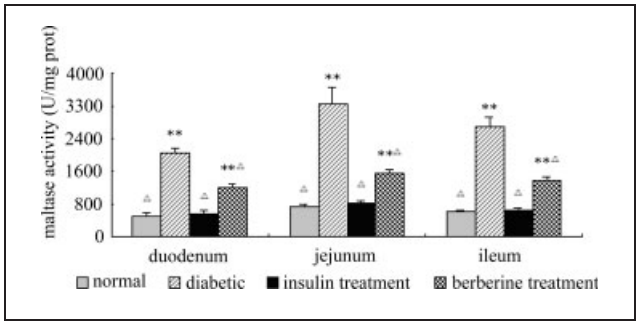

Fig. 4: Maltase activity in the small intestine in different treatment of rats. Each bar represents the mean ± S.D., n = 5, for each region of the intestine. **Denotes statistical difference compared with normal control rats. ($p < 0.01$, by ANOVA). ΔDenotes statistical difference compared with diabetic rats. ($p < 0.01$, by ANOVA)

charidases were always higher in jejunum of diabetic rats than that in duodenum and ileum regions of diabetic rats. The activity of lactase in duodenum, jejunum and ileum regions of diabetic rats was 4.1-fold, 1.6-fold and 5.4-fold that in normal rats, respectively. The activity of sucrase in duodenum, jejunum and ileum regions of diabetic rats was 6.7-fold, 1.6-fold and 1.6-fold of that in normal rats, respectively. The activity of maltase in duodenum, jejunum and ileum regions of diabetic rats was 4.1-fold, 4.4-fold and 4.4-fold of that in normal rats, respectively. Treatment with insulin or berberine resulted in a significant decrease in disaccharidase activities in all three regions of the intestine compared with the diabetic group. Although the decreasing disaccharidase activity effect of berberine was not as good as insulin, the experimental data documented the general normalizing effect of berberine treatment on disaccharidase activities. The intestinal maltase activity of insulin treated diabetic rats had almost recovered to the same level of normal control rats.

Figure 5 shows the changes on intestinal β-glucuronidase activity in four different treated groups of rats. The activity of intestinal β-glucuronidase was significantly increased in all regions in diabetic control rats when compared with that in the normal control group. The activity of β-glucuronidase in duodenum, jejunum and ileum regions of diabetic rats was 1.7-fold, 1.5-fold and 1.9-fold of that in normal rats, respectively. Treatment with insulin or berberine significantly decreased β-glucuronidase activity in all regions of the intestine compared with the diabetic group. The intestinal β-glucuronidase activity of insulin treated diabetic rats had almost recovered to the same level of normal control rats.

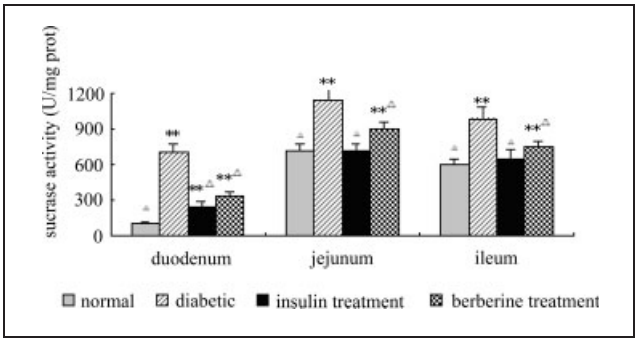

Fig. 3: Sucrase activity in the small intestine in different treatment of rats. Each bar represents the mean ± S.D., n = 5, for each region of the intestine. **Denotes statistical difference compared with normal control rats. ($p < 0.01$, by ANOVA). ΔDenotes statistical difference compared with diabetic rats. ($p < 0.01$, by ANOVA)

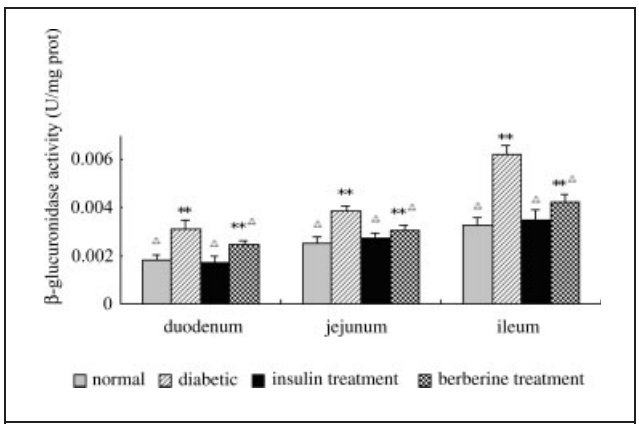

Fig. 5: β-glucuronidase activity in the small intestine in different treatment of rats. Each bar represents the mean ± S.D., n = 5, for each region of the intestine. **Denotes statistical difference compared with normal control rats. ($p < 0.01$, by ANOVA). ΔDenotes statistical difference compared with diabetic rats. ($p < 0.01$, by ANOVA)

3. Discussion

The results from the present study showed a significant increase in the activities of disaccharidases in the intestinal mucosa of diabetic rats leading to the observed elevated glucose level in the blood. The significant increase in activities of disaccharidases observed in diabetic rats in this study was consistent with previous reports (Younoszai and Schedl 1972; Nashiro et al. 1992; Murkami and Ikeda 1998). The increased levels of disaccharidase activity in diabetic rats had been postulated to be a result of hyperglycemia (Murakami and Ikeda 1998) and intestinal hyperplasia (Adachi et al. 2003). Tandon et al. (1975) reported that insulin had an inhibitory effect on disaccharidase activity and thus its deficiency (e.g. in diabetes) might result in an increase in the enzyme activity and glucose transport in diabetics might also stimulate disaccharidase activity. However, some other reports suggested that insulin and gastrointestinal hormones had no effect on disaccharidase activity (Nashiro et al. 1992; Schedl et al. 1983). Our study found that diabetic rats treated with insulin had significantly decreased disaccharidase activity as compared with those in diabetic untreated rats. Moreover, treatment with insulin or berberine significantly reduced disaccharidase activities which were indicative of a lowered level of absorbable glucose being formed from carbohydrate digestion leading to reduced blood glucose levels. This may be beneficial in the amelioration of the diabetic state (Yasuda et al. 2003; Matsuo et al. 1992) and could explain the observed lowered level of blood glucose and increased body weight of diabetic rats.

Keelan et al. (1987) and Ettarh et al. (1997) reported changes in the intestinal morphology of untreated diabetic rats. McAnuff et al. (2003) reported that feeding of bitter yam steroidal sapogenin extract which was a kind of sapogenin to diabetic rats may result in alterations in the intestinal morphology associated with a decrease in disaccharidase activity in small intestine of diabetic rats (McArnuff-Harding et al. 2006). Yasuda et al. (2003) reported that voglibose might improve glucose tolerance by inhibiting the activities of intestinal disaccharidases. The present study shows the effect of berberine as a α-glucosidase inhibitor. It is essential for hyperglycemic patients that the intestinal absorption of dietary carbohydrates is suppressed by inhibiting the metabolic processes implicated in carbohydrate digestion and absorption. It is also generally recognized that carbohydrates are digested into oligosaccharides and then into disaccharides by enzymes secreted by the digestive tract, and that disaccharides like maltose, lactose, sucrose are converted into monosaccharides by α-glucosidase localized in the small intestinal mucosa (Ratner 2001).

Although these results strongly suggested that berberine inhibited blood glucose elevation by inhibiting α-glucosidase activity, it is able to take part any other action mechanisms. It is necessary to investigate the mechanism of action of berberine on glucose transportation and insulin secretion. Pan et al. (2003) reported that berberine did not influence glucose transporters and glucose absorption in Caco-2 cells. Leng et al. (2004) reported that serum insulin level was promoted after berberine administration in the BALB/c mice. However, the inhibitory effect of berberine on disaccharidase activity resulting in reduced postprandial hyperglycemia might be one of the reasons for its use as an antihyperglycaemic agent.

Several papers reported that the mRNA level of the sucrase-isomaltase complex (SI complex), and lactase in the small intestine of rats with type 1 diabetes mellitus and type 2 diabetes mellitus was much higher than that in normal rats (Hoffman et al. 1992; Takenoshita et al. 1998; Dyer et al. 2002). This might result in an abnormal increase in the sucrase, isomaltase and lactase activities in the small intestine of diabetic rats.

Takenoshita et al. (1998) reported that insulin had a suppressive effect on the synthesis of the SI complex, presumably by decreasing the transcriptional level of the gene encoding the complex, in small-intestinal epithelial cells. Our report showed that disaccharidase activity was still decreased 24 h after the last administration of berberine. It was reported that half-life of of berberine after oral adiministration was about 6 h (Lu et al. 2006), while a direct action of berberine to intestinal disaccharidase activity might be negative. This result implied that long term treatment with berberine might down-regulate the gene expression of the disaccharidases. The mechanism is still ambiguous and further profound research is required.

According to a previous report (Staels and Kuipers 2007), disturbed bile acid metabolism in diabetes mellitus suggested a link between bile acids and glucose control. Bile acids are activating ligands of the farnesoid X receptor (FXR), a nuclear receptor with an established role in bile acid and lipid metabolism. Evidence suggests a role for FXR also in maintenance of glucose homeostasis. β-glucuronidase causes deconjugation of bilirubin diglucuronide and bile acid glucuronic conjugates and it was an important factor in gallstone formation (Leung et al. 2001). β-Glucuronidase was primarily produced by intestinal and biliary bacteria (Leung et al. 2001; Ishihara et al. 2002). Moreover, previous documents showed that dysbacteriosis is present under diabetic conditions (Rozanova et al. 2002). Our study found that β-glucuronidase was significantly increased in all regions in diabetic control rats. Therefore, according to our investigation, it might be supposed that dysbacteriosis in the pathological condition of diabetes mellitus might result in some enhanced activity of β-glucuronidase in jejunum and ileum and more β-glucuronidase would be excreted into the duodenum from the bile. Accordingly, the absorption and metabolism of bile acids were altered which might ultimately aggravate diabetic symptom. The antibiotic pharmacological activitity of berberine (Mirska et al. 1972) might inhibit intestinal bacteria to produce β-glucuronidase in jejunum and ileum resulting in lowered β-glucuronidase activitity in berberine treated diabetic rats. However, berberine also decreased β-glucuronidase activitity in duodenum of diabetic rats and the mechanism is still ambiguous and further research is required.

In conclusion, according to our findings, the activities of intestinal disaccharidases and β-glucuronidase were significantly increased in diabetic rats as compared with those in the normal rats. Decrease of small intestinal disaccharidase and β-glucuronidase activities in berberine treated diabetic rats might partly contribute to berberines antihyperglycaemic effect.

4. Experimental

4.1. Materials and reagents

Berberine (>95%) were supplied by Medicinal Chemistry of China Pharmaceutical University. The kits of glucose, proteinum, sucrase, lactase, maltase and β-glucuronidase were purchased from Jiancheng Chemical Factory (Nanjing, China). Streptozotocin (Sigma Chemicals, St. Louis, MO, USA) and protamine zinc insulin (Wanbang Pharmaceutical Factory, Jiangsu, China) were used in the study. Berberine was suspended in 0.5% carboxymethyl cellulose sodium salt (CMC-Na) aqueous solution.

4.2. 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 ± 2 °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 five days, and fasted with free access to water for 12 h prior to each experiment. The studies were approved by the Animal Ethics Committee of China Pharmaceutical University, and every efforts were made to minimize stress to the animals.

Rats were made diabetic by an intraperitoneal administration of 55 mg/kg STZ (dissolved in sodium citrate buffer, pH 4.5). The STZ-induced hyperglycemic state was considered to be an animal model of type 1 DM (Kamei et al. 2005). Age-matched control rats received the vehicle only (sodium citrate buffer, pH 4.5). Fasting blood glucose (FBG) levels were measured with a reagent kit on the day 3 following injection. Rats with a FBG in excess of 11.1 mM (Kamei et al. 2005) 3 days after the induction of DM were considered to be diabetic.

4.3. Drug treatment

On the seventh day following injection of STZ, the diabetic rats were equally randomized into three groups: diabetic control rats, rats treated with berberine and rats treated with insulin. Both age-matched normal rats and diabetic control rats received physiological saline only. Rats treated with insulin subcutaneously received protamine zinc insulin (10 U/kg) twice daily and the rats treated with berberine received 100 mg/kg of berberine orally once daily for 28 days. FBG and weight were measured on days 7, 14, 21 and 28 following treatment.

4.4. Measurement of blood glucose level, disaccharidases and β-glucuronidase activities in the small intestine

Animals fasted over night and blood samples were taken from orbital vein. Serum samples were obtained after centrifuging at 8000 rpm/min for 5 min. FBG levels were measured using a commercially available glucose kit based on the glucose oxidase method (Trinder 1969).

The experimental rats were exsanguinated under ether anesthesia. The small intestine was obtained and was divided into three parts: duodenum, jejunum and ileum. The lumen was flushed out using 3 mL of 0.9% NaCl. The mucosal washing and the scraped mucosa were pooled, homogenized, centrifuged (5000 g) and the supernatant was frozen until required for enzymatic assays. Sucrase, maltase and lactase activities were determined by a modified method of Dahlquist (1968). Briefly, the homogenate supernatants were diluted and added to an equal volume of 0.1 M sodium maleate buffer (PH 6.0) containing 56 mM sucrose, maltose or lactose and were incubated for 1 h at 37 °C. The mixtures were then added to the glucose oxidase-peroxidase reagents containing o-dianisidine as a chromogen, and the absorbance was measured at 420 nm. β-glucuronidase activity was quantified with the substrate p-nitrophenyl β-D-glucuronide based on the method developed by Fishman et al. (1967). The reaction mixture consisted of 0.9 mL substrate solution at 5 mM concentration in 50 mM sodium acetate buffer pH 4.8 mixed with 0.1 mL of sample. The mixture was incubated at 37 °C for 1 h in the dark. Hydrolysis was terminated by the addition of 2 mL of ice cold 500 mM sodium carbonate. The absorbance of the liberated p-nitrophenol was read at 400 nm. A calibration curve was constructed using varying concentrations of p-nitrophenol. The protein concentration of each homogenate sample was determined by the Coomassie Brilliant Blue method. The disaccharidase and β-glucuronidase activities are expressed as U/mg protein. One unit is defined as the amount of enzyme that hydrolyses 1 μmol of sucrose, maltose lactose or p-nitrophenyl β-D-glucuronide in 1 min.

4.5. Data analysis

Analysis of variance (ANOVA) was used to test for differences among the groups. Post hoc analysis was carried out using the Duncan's multiple range test to test for significant difference among the means ($P < 0.05$). All results are expressed as mean ± standard deviation (S.D.).

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