Journal of Ethnopharmacology 142 (2012) 474–480

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Berberine acutely inhibits the digestion of maltose in the intestine

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ARTICLE INFO

Article history:
Received 16 October 2011
Received in revised form
8 May 2012
Accepted 13 May 2012
Available online 22 May 2012

Keywords:
Berberine
Diabetes
Hypoglycemic activity
α-Glucosidase
Caco-2 cell
Fluorescence emission spectrums
Euglycemic clamp

ABSTRACT

Ethnopharmacological relevance: The Chinese Goldthread Rhizome has been used in the Traditional Chinese Medicine as an important ingredient of many formulas for the treatment of diabetes mellitus. Berberine, the main effective composition of Chinese Goldthread Rhizome, is also effective in treating diabetes in today's clinical practice of Traditional Chinese Medicine.

Aim of the study: To evaluate the hypoglycemic activity of berberine which treats acutely on the postprandial blood glucose, and to explore the mechanism of this activity.

Materials and methods: 1. One-dose preprandial intragastric administrations of berberine were given to normal animals (dogs and rats), and the postprandial blood glucose concentration curves were measured. Serum insulin enzyme linked immunosorbent assay (ELISA) was only performed in rats. 2. The euglycemic clamp test was performed to evaluate the effect of one-dose berberine intragastric administration on the blood glucose transformation and utilization rate in rats. 3. In the Caco-2 cell monolayer test, the changes of glucose concentration on the apical and basolateral sides were measured when the maltose solution containing berberine was added to the apical side. 4. The inhibition ratio of berberine against α-glucosidase was measured in vitro. 5. The effect of berberine on the fluorescence emission spectrums of α-glucosidase was studied.

Results: One-dose preprandial intragastric administration of berberine delayed the rise of post-maltose blood glucose, did not affect postprandial blood glucose after glucose meal, and did not affect the insulin level in normal rats; reduced post-maltose blood glucose in normal dogs. 2. The result of euglycemic clamp test showed that one-dose intragastric administration of berberine had no effect on the blood glucose transformation and utilization rate in rats. 3. Berberine added to the maltose solution on the apical side of Caco-2 cell monolayer reduced the glucose concentration on the apical side. Glucose in basolateral side of all groups cannot be detected. 4. Berberine inhibited the activity of α-glucosidase in vitro. 5. Berberine significantly and concentration dependently quenched the fluorescence emission spectrum of α-glucosidase.

Conclusion: Our findings suggest an additional mechanism of the hypoglycemic activity of berberine by demonstrating its ability to acutely inhibit the α-glucosidase, and support the traditional use of berberine and Chinese Goldthread Rhizome for the treatment of diabetes mellitus.

1. Introduction

The diabetes mellitus is called "wasting-thirst" in Traditional Chinese Medicine. It has a long history that the Chinese Goldthread Rhizome is used to treat wasting-thirst in China, as the earliest written descriptions can be found in the Wei and Jin dynasties (Li et al., 2008). Berberine is the major active constituent of the rhizome of coptis (Tang et al., 2006), which is the Chinese Goldthread Rhizome for medicinal using, and berberine administered orally has a similar hypoglycemic activity to the Chinese Goldthread Rhizome in clinical (Ni, 1988; Yin et al., 2008b; Zhang et al., 2008; Zhang et al., 2010). All these indicate that berberine is the important effective constituent contributing to the hypoglycemic activity of Chinese Goldthread Rhizome. Berberine has a certain hypoglycemic activity, but its precise mechanism remains uncertain. The reports on hypoglycemic mechanism of berberine are mainly in two classifications: general actions in postabsorption and local actions in digestive tract. A large number of researches suggested that berberine exerted the hypoglycemic activity via general action, e.g. stimulating

Abbreviations: SGLT, Sodium-Dependent Glucose Cotransporter; GLUT, Glucose Transporter; ATP, Adenosine Triphosphate; ADP, Adenosine Diphosphate; PI, Inorganic Phosphate; ELISA, Enzyme Linked Immunosorbent assay; I.V., Intravenous; I.P., Intraperitoneal; GIR, Glucose Infusion Rate; HPLC-UV, High Performance Liquid Chromatography with Ultraviolet detector; PMP, 3-Methyl-1-Phenyl-2-Pyrazolin-5-one; GSH, Glutathione; PNPG, P-Nitrophenyl α-D-Glucopyranoside; ANOVA, Analysis Of Variance; AUC, Area Under Curve; I.G., Intra Gastrically; IC50, 50% Inhibiting Concentration; CI, Confidence Intervals

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http://dx.doi.org/10.1016/j.jep.2012.05.022

insulin secretion or release (Leng et al., 2004; Lu et al., 2009), and the contrary opinion of lowering hyperglycemia and improving the impaired glucose tolerance without increasing insulin release and synthesis (Chen et al., 2010); protecting pancreatic islets (Chueh and Lin, 2011) and β cells (Zhou et al., 2009); increasing insulin receptor expression (Kong et al., 2009; Zhang et al., 2010); insulin-sensitizing (Wang et al., 2011); stimulating glycolysis (Yin et al., 2008a); promoting the utilization and transformation of glucose in hepar (Liu et al., 2010b; Yan et al., 2008), skeletal muscle (Ma et al., 2010), and adipose tissue (Li et al., 2011; Zhou and Zhou, 2010); up-regulating peroxisome proliferator-activated receptors (Zhou and Zhou, 2010), AMP-activated protein kinase (Hardie, 2011; Hwang et al., 2009; Lee et al., 2006), glucose transporter 1 (Cok et al., 2011), and down-regulating mitochondrial respiratory complex I (Turner et al., 2008). These studies demonstrated some antihyperglycemic mechanisms of berberine, but neglected the fact that berberine had a poor absorption through the digestive tract. Therefore, the studies aimed at the local antihyperglycemic actions of berberine exerting in the intestine might be more valuable, such as the findings of a 5-weeks (Liu et al., 2010a), a 4-weeks (Liu et al., 2008) intragastric administration of berberine in rats, and a 5-days (Liu et al., 2010a), a 3-days (Pan et al., 2003) preincubation with berberine in Caco-2 cell monolayer, inhibited the expression of disaccharidase. In addition, it was found that berberine acutely inhibited the activities of maltase and sucrase in Caco-2 cell lines (Pan et al., 2003). The experiments of multiple dosing berberine's effect on the absorption of saccharide are sufficient, including tests in vivo, in vitro and in cell lines, and the mechanisms of them are relatively clear. However, the researches of acute effect of berberine on absorption of saccharide are rare, and were limited in cell lines. In order to verify the activity of berberine acutely inhibiting the digestion and absorption of saccharide in the intestine and to explore the mechanism, a series of exploratory experiments were performed.

2. Materials and methods

2.1. Animals, cells and drugs

Wistar rats were purchased from the Beijing HFK Bio-Technology Co., Ltd. (Beijing, China). Beagle dogs were purchased from the Shenyang Kangping Laboratory Animal Institutes (Shenyang, China). All experiments and procedures were carried out according to the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of China. All animals were fasted for at least 12 h with free access to water prior to experiment.

Caco-2 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The number of cell passages was 20–30.

Berberine chloride form, D-(+)-maltose monohydrate from potato, acarbose, phlorizin hydrate, metformin hydrochloride, α-glucosidase from *Saccharomyces cerevisiae* were all purchased from the Sigma-Aldrich (Shanghai) Trading Co., Ltd. (Shanghai, China).

2.2. Effect of berberine on post-maltose blood glucose

2.2.1. Test in rats

50 healthy male rats (bodyweight 180–220 g) were divided into 5 balanced groups according to fasting blood glucose. Saline, acarbose 40 mg/kg, and berberine 500, 250 and 125 mg/kg were given respectively to control, positive control, and berberine groups. Then, maltose 2 g/kg was given 1 h later. All the drugs and food were given in the way of intragastric administration. And the concentration of glucose in peripheral blood was measured using the ACCU-CHEK Advantage blood glucose meter (Roche Diagnostics (Shanghai) Ltd.) at 0, 30, 60, 90, and 120 min after giving maltose. Additionally, for control group and berberine groups, the peripheral blood 50 μL per rat was collected at 0 min for insulin enzyme linked immunosorbent assay (ELISA).

2.2.2. Test in dogs

6 healthy male beagle dogs (bodyweight 9–13 kg) were divided into 2 groups randomly with 3 dogs in each. Dogs were restrained during the procedure of experiment. Saline and berberine 80 mg/kg were given respectively to group Iand II in the first experiment, then inversely in the second experiment. Maltose 2.67 g/kg was given at 20 min after giving the drugs. All the drugs and food were given in the way of intragastric administration. And the concentration of glucose in peripheral blood obtained from cephalic vein at forearm was measured using the ACCU-CHEK Advantage blood glucose meter at 0, 20, 40, 60, 80, 100 and 120 min after giving maltose.

2.3. Effect of berberine on postprandial blood glucose after glucose meal

The experimental procedure was similar to Section 2.2.1 with modifications. The main differences included that the positive drug was phlorizin 40 mg/kg, glucose 1 g/kg instead of the maltose 2 g/kg was given as a meal, the time points of blood glucose measurements are at 0, 10, 20, 40 and 60 min, and the serum insulin ELISA was not performed.

2.4. Euglycemic clamp test

30 healthy male rats (bodyweight 180–220 g) were divided into 3 groups randomly with 10 rats in each. The procedure of Euglycemic clamp test was similar to the former method (Tran et al., 2003) with modifications. All the rats were anticoagulated using 1 kU/kg heparin (i.v.), and anaesthetized using 1 g/kg ethylcarbamate (i.p.) before operation. Two catheters were intubated in carotid and jugular vein respectively. Metformin was used as positive control. Metformin 180 mg/kg, berberine 500 mg/kg, and saline were injected intragastrically to corresponding groups at 60 min before starting to infuse insulin and glucose. The insulin and glucose are all infused via jugular vein catheter, the rate of insulin infusion was 10 mU/(kg min), the concentration of glucose injected was 100 g/L. The glucose of blood samples that were taken from rat's carotid catheter was measured using the ACCU-CHEK Advantage blood glucose meter every 10 min. The volume of blood withdrawn was 0.4 ml, and the redundant blood was injected back after consuming about 25 μL for determination of glucose. The glucose infusion rate was regulated according to the blood glucose level after every determination. An invariable glucose infusion rate which had kept the blood glucose in the range of 10 ± 1 mmol/L for 60 min was recorded as a GIR (the initial letters of words "glucose infusion rate") for the current rat.

2.5. Caco-2 cell monolayer test

Caco-2 cells were cultured on the Transwell Permeable Supports (Corning Incorporated, USA) in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acids for 21 days (Sambuy et al., 2005). The transepithelial electric resistance $\geq 330\ \Omega\ cm^2$ was required to obtain a tight monolayer. When began to test, the culture medium in basolateral side was changed to glucose-free Hanks' solution, and acarbose (positive control, final concentration 250 mg/L) or