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Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion¹

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KEY WORDS berberine; diabetes mellitus; glucose tolerance test; insulin; HIT-T15 cells; pancreatic islets; lipids

ABSTRACT

AIM: To explore the anti-diabetic effects of berberine and its influence on insulin secretion. **METHODS:** Impaired glucose tolerance rats induced by iv injection of streptozotocin 30 mg/kg were treated with berberine 187.5 and 562.5 mg/kg while fed with high fat laboratory chow. After rats were treated for 4 weeks, oral glucose tolerance was determined, and for 8 weeks, the fasting blood glucose, insulin, lipid series were determined. In insulin secretion experiments, berberine 93.75, 187.5, and 562.5 mg/kg was administered orally to BALB/c mice at a bolus. The murine serum was collected 2 h after the berberine administration for insulin determination. Insulin released from HIT-T15 cells and pancreatic islets incubated with berberine 1-100 $\mu\text{mol/L}$ for 12 h was determined. **RESULTS:** The levels of fasting blood glucose (7.4 ± 1.5 or 7.3 ± 1.3 vs 9.3 ± 1.3 mmol/L), triglycerides (0.61 ± 0.22 or 0.63 ± 0.17 vs 1.8 ± 0.7 mmol/L), total cholesterol (1.8 ± 0.3 or 1.9 ± 0.3 vs 2.2 ± 0.2 mmol/L), free fatty acid (456 ± 93 or 460 ± 72 vs 550 ± 113 $\mu\text{mol/L}$) and apolipoprotein B (0.37 ± 0.02 or 0.42 ± 0.05 vs 0.46 ± 0.04 g/L) were reduced greatly in berberine-treated groups at doses of 187.5 and 562.5 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, respectively as compared with those in control group ($P<0.05$ or $P<0.01$), whereas high density lipoprotein-cholesterol (1.5 ± 0.3 or 1.4 ± 0.3 vs 1.1 ± 0.1 g/L), apolipoprotein AI (0.80 ± 0.08 or 0.87 ± 0.08 vs 0.71 ± 0.06 g/L) were significantly increased ($P<0.05$ or $P<0.01$), and oral glucose tolerance was improved. *In vitro* experiment showed that berberine 1-10 $\mu\text{mol/L}$ facilitated insulin secretion of HIT-T15 cells and murine pancreatic islets in a dose-dependent manner. Meanwhile murine serum insulin level (27.5 ± 2.7 or 29 ± 4 or 29 ± 4 vs 24.3 ± 2.8 pIU/L) was undoubtedly promoted and blood glucose (4.52 ± 0.31 or 4.45 ± 0.29 or 4.30 ± 0.19 vs 4.87 ± 0.21 mmol/L) was reduced after berberine administration at doses of 93.75, 187.5, and 562.5 mg/kg, respectively in the BALB/c mice. **CONCLUSION:** Berberine possesses anti-diabetic effects, which is related to the property of stimulating insulin secretion and modulating lipids.

INTRODUCTION

Rhizoma coptidis has been used to treat diabetes

mellitus for more than one thousand years in the history of Chinese medicinal remedy. Berberine, one of the main constituents of *Rhizoma coptidis*, is a kind of isoquinoline alkaloid. It is suggested that berberine might be one of the principal anti-diabetic constituents of *Rhizoma coptidis*. Tetrahydroprotoberberine, one of the derivatives of berberine, inhibits lipid peroxidation and scavenges hydroxyl free radicals^[1]. It is therefore raised the question whether berberine possesses lipids

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modulation action or not. According to the reports berberine acts as a kind of α -adrenoceptor antagonist^[2-4]. In addition, some α -adrenoceptor antagonists, such as phentolamine, stimulate insulin release by inhibiting pancreatic β -cell ATP-sensitive potassium channels^[5,6]. Berberine also inhibits cardiac ATP-sensitive potassium channels^[7,8]. Therefore we raised the hypothesis that berberine might possess the property of promoting insulin release and modulating lipids. In order to understand the mechanisms of berberine in treating diabetes, here we investigated the hypoglycemic and lipids modulating effects of berberine using murine diabetic model^[9-12] induced by streptozotocin (STZ) and high fat laboratory chow, and to investigate the action of berberine on insulin secretion of HIT-T15 cells and pancreatic islets *in vitro*. Berberine exerted glucose-lowering effect in hepatocytes in insulin independent way^[13]. As elevating blood glucose is a powerful mediator in modulating insulin release, also the peripheral effect of berberine will counteract its supposed insulin secretion effect *in vivo*. It is very difficult to correctly identify the potency of insulin release of berberine using diabetes model. To reduce the counteracting effect of berberine on insulin secretion by peripheral effect as great as possible, we therefore applied normal mice with the administration of berberine at a bolus to observe the property of berberine in promoting insulin release *in vivo*.

MATERIALS AND METHODS

Drug preparation Berberine hydrochloride (Hengda Pharmaceutical Factory, Chengdu, China), glibenclamide (Pacific Pharmaceuticals Ltd, Tianjin, China), and metformin hydrochloride (Lipha-45402 Semoy-France Pharmaceuticals Ltd, France) suspension used *in vivo* experiments were prepared by dissolving them respectively in PBS buffer (containing NaCl 136 mmol/L, KCl 2.7 mmol/L, Na₂HPO₄ 10 mmol/L and KH₂PO₄ 1.7 mmol/L, pH 7.40) added with 5 % methylcellulose. Berberine (National Institute for the Control of Pharmaceutical and Biological Products, China) and repaglinide (Novo Nordisk, Denmark) used *in vitro* experiments were dissolved in KRBH buffer (containing NaCl 136 mmol/L, KCl 4.8 mmol/L, CaCl₂ 1 mmol/L, MgSO₄ 1.2 mmol/L, KH₂PO₄ 1.2 mmol/L, NaHCO₃ 5 mmol/L, HEPES 25 mmol/L, BSA 0.1 % and indicated concentration of glucose, pH 7.40). BSA and HEPES were purchased from Sigma Chemical Company, the

other chemicals were of analytical reagent grade.

Animal modeling, grouping and treatment

Sixty healthy male Wistar rats (from Experimental Animal Center, Tongji Medical College, HUST, Grade II, No 19-050), weighing 217±19 g, were fed in the standard rat-feeding room. Fifty-one rats of them were randomly selected and were iv injected with STZ (Sigma Chemical Co, MO, USA) 30 mg/kg. Then rats were fed with conventional laboratory chow. Two weeks after the injection, 44 impaired glucose tolerance (IGT) rats determined by oral glucose tolerance test (OGTT) were randomly divided into four groups as the following: low dose berberine group (BerL, *n*=11), ig 187.5 mg·kg⁻¹·d⁻¹ berberine; high dose berberine group (BerH, *n*=11), ig 562.5 mg·kg⁻¹·d⁻¹ berberine; metformin group (MET, *n*=11), ig 187.5 mg·kg⁻¹·d⁻¹ metformin hydrochloride; control group (*n*=11), ig the same volume of PBS added with 5 % methylcellulose. Drugs were performed ig between 8:30-9:30 everyday. Nine normal rats not injected with STZ were taken as normal group. The high fat laboratory chow (sucrose: lard: milk powder: egg: conventional feed=30:20:4:2:63) was given to the rats except for the normal group rats during the whole treatment period. Unless 12 h before OGTT and antemortem, rats had free access to food and water. All rats were kept individually in cages.

OGTT OGTT was performed at the end of the fourth week after the beginning of experimental treatment. Being fasted for 12-15 h, the rats were orally administered with glucose 2.2 g/kg at a bolus. Then blood samples were collected from the tail vein at 0 (just before the glucose load), 30, 60, and 120 min (after the glucose load) for the glucose assay.

Determination of glucose and lipid metabolic parameters Blood glucose was determined by the glucose-oxidase method, total cholesterol (TC) and triglycerides (TG) by enzyme end-point method, high density lipoprotein-cholesterol (HDL-C) by PTA-Mg²⁺ method, apolipoprotein AI (APOAI) and apolipoprotein B (APO B) by immunoturbidimetric method^[14,15]. The reagent kits for measuring the above serum parameters were produced by Wenchow Dong-Ou Bioengineering Company Ltd, China. Free fatty acid (FFA) was measured with reagent kits produced by Nanjing Jiancheng Bioengineering Institute, China. All the above parameters were determined with KP-95 spectrophotometer (Bio-Tech Instruments, Italy). Serum insulin was measured by radioimmunoassay using insulin reagent kit produced by Northern Bioengineering Institute,

China.

Experiments on BALB/c mice Thirty healthy BALB/c mice, 21.8 ± 0.9 g, bought from Center for Hygiene and Epidemic Control of Hubei Province (Grade VPF, No 19-083), were randomly divided into 5 groups, and were orally administrated with PBS added with 5 % methylcellulose, glibenclamide 1.04 mg/kg and different doses of berberine respectively after being fasted for 2 h. Before and 2 h after the drug administration, murine blood glucose were determined by glucometry ONE TOUCH II. The murine retrobulbar blood was taken to collect serum, and insulin was measured by radioimmunoassay.

Cell culture The insulin-secreting cell line HIT-T15 cells (kind gift from Prof ROBERTSON, Pacific Northwest Research Institute, Seattle, USA) were cultured in the incubator at 37 °C under an atmosphere of 5 % CO₂-95 % O₂. The HIT-T15 cells (passages 72-79) were cultured in RPMI-1640 (Sigma Chemical Co, MO, USA) supplemented with 10 % fetal serum, 10 mmol/L HEPES, benzylpenicillin 100 kU/L, streptomycin 100 kU/L (pH 7.40). Pancreatic islets were isolated from adult male Wistar rat using the methods as previously reported^[16]. The Wistar rat was injected with pentobarbital 40 mg/kg. Under the anesthesia condition the murine pancreas was moved out and cut into about 1×1 mm² pieces and dispersed into islet cells by 0.25 % type V collagenase (Sigma Chemical Co, MO, USA) in 37 °C water bath for about 10 min. The product was sifted by a 20- μ m sifter and centrifuged ($50 \times g$) three times to eliminate collagenase, broken cells and fibroblasts. Then the islets were cultured in the same condition as HIT-T15 cells described above. After incubation for 12-24 h, the fibroblast in the isolated islets were further excluded by its property attaching to the flask wall. The purity of islets cells (more than 85 %) was determined by dithizone staining method^[17]. The viable cells (more than 95 %) were determined by Trypan blue exclusive test. For insulin secretion and MTT test, passage 79 HIT-T15 cells or pancreatic islets were seeded one day before the experiments in 96-well microtitre plates at a density of $6-8 \times 10^4$ cells per well.

Insulin secretion test After washed twice with modified glucose free KRBH (pH 7.40), HIT-T15 cells or murine pancreatic islets taken as control groups were incubated in 0, 5, or 10 mmol/L glucose KRBH. All of the repaglinide or berberine treated groups were the same to the control groups except for containing repaglinide 1 μ g/L or indicated concentration of berberine respec-

tively. After an incubation at 37 °C under an atmosphere of 5 % CO₂-95 % O₂ for 12 h, the supernatant of cell culture product was harvested and kept at -20 °C until the insulin assay.

MTT assay The MTT assay was carried out as previously reported^[18]. The formazan crystals formed by the reduction of MTT under the action of mitochondrial dehydrogenase were dissolved with dimethyl sulfoxide (Me₂SO, Sigma Chemical Co, MO, USA) and measured by ELX800 Universal Microplate Reader (Bio-Tek Instruments Inc) at wavelength of 630 nm. The value was considered to reflect the activity of cell metabolism.

Statistical analysis All results are expressed as mean \pm SD. The significance of the difference in the food intake and body weight during the treating period was analyzed by two-way analysis of variance (ANOVA). Other data was analyzed by one-way ANOVA or chi-square. The statistical value of $P < 0.05$ was considered to be significantly different.

RESULTS

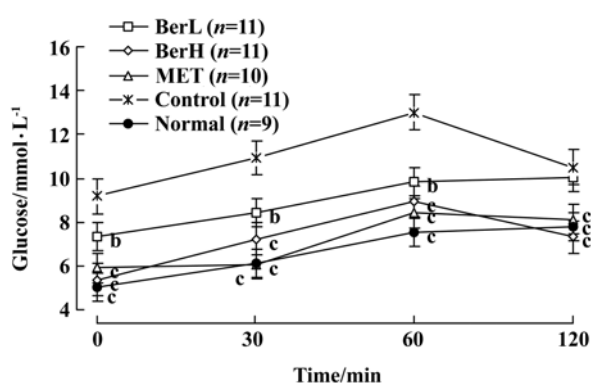
Effect of streptozotocin on oral glucose tolerance in Wistar rats The Wistar rats were injected with STZ 30 mg/kg, then fed with conventional chow for 15 d. After that murine oral glucose tolerance was determined by OGTT, the blood glucose at 30, 60, and 120 min in BerL, BerH, MET, and control groups were all higher than those in normal group ($P < 0.05$ or 0.01), and there was no statistical difference among BerL, BerH, MET and control groups ($P > 0.05$) at any time point before the experimental therapy (Tab 1).

Effect of berberine and metformin on oral glucose tolerance After treated with berberine for 4 weeks, the serum glucose levels at 0, 30, 60, and 120 min in control group were homogeneously higher than those in normal group ($P < 0.01$). The serum glucose levels at 0, 30, 60, 120 min in BerH group and at 0, 30, 60 min in BerL group were lower than those in control group ($P < 0.01$). The murine glucose tolerance in Met group was also ameliorated (Fig 1).

Preventive effects of berberine and metformin on the development of murine diabetes The impaired glucose tolerance rats induced by STZ were fed with high fat chow and meanwhile were treated with berberine 187.5 mg \cdot kg⁻¹ \cdot d⁻¹, 562.5 mg \cdot kg⁻¹ \cdot d⁻¹, and metformin 187.5 mg \cdot kg⁻¹ \cdot d⁻¹. At the end of the fourth week after treatment, the rats were fasted for 12-15 h

Tab 1. Effect of streptozotocin 30 mg/kg on oral glucose tolerance in Wistar rats. Mean±SD. ^b*P*<0.05, ^c*P*<0.01 vs normal group (One-way ANOVA followed by Turkey test).

	Glucose tolerance/mmol·L ⁻¹			
	0 min	30 min	60 min	120 min
BerL (n=11)	3.2±0.5	4.6±0.6 ^b	4.2±0.4 ^c	3.7±0.7 ^b
BerH (n=11)	3.0±0.5	4.7±0.5 ^b	4.3±1.0 ^b	3.4±0.5 ^b
MET (n=11)	3.1±0.6	4.7±0.6 ^b	4.3±0.9 ^c	3.5±0.4 ^b
Control (n=11)	3.0±0.4	4.5±0.5 ^b	4.1±1.0 ^b	3.5±0.5 ^b
Normal (n=9)	3.0±0.7	4.1±0.4	3.4±0.4	3.07±0.19

**Fig 1. Effect of berberine 187.5 mg·kg⁻¹·d⁻¹ (BerL), 562.5 mg·kg⁻¹·d⁻¹ (BerH) and metformin 187.5 mg·kg⁻¹·d⁻¹ (MET) on oral glucose tolerance in impaired glucose tolerance (IGT) rats. Mean±SD. ^b*P*<0.05, ^c*P*<0.01 vs control group (One-way ANOVA followed by Turkey test).**

and OGTT was performed. The diabetic rats were diagnosed according to the modified diabetes diagnosis standard suggested by the America Diabetes Association for human: fast blood glucose (FBG)>7.0 mmol/L

or 2 h postprandial blood glucose >11.1 mmol/L. The number of diabetic rats in BerL, BerH, Met, control, and normal groups was 8, 2, 2, 11, and 0 respectively. The number of diabetic rats in BerL, BerH, Met was smaller than that in control group analyzed by chi-square (*P*<0.01).

Glucose and lipid metabolic parameters eight weeks after berberine treatment The FBG levels in both BerH and BerL were significantly lower than those in control group (*P*<0.01). In BerH and BerL groups, not in Met group, the level of FFA, TC, TG, APOB, and TG levels were obviously reduced compared with those in control group (*P*<0.05). The HDL-C and APOA I levels in BerH, BerL, and Met groups were significantly elevated compared with those in control group (*P*<0.01, Tab 2).

Effects of berberine on the mean food intake and body weight of IGT rats At the beginning of experimental treatment, murine mean food intake per day and mean body weight were similar among all groups. However, the mean food intake per day and mean body weight per rat were reduced in BerH and BerL groups (*P*<0.05) after treatment, as compared to those in control group (Fig 2, 3).

Effects of berberine on insulin secretion and blood glucose in BALB/c mice Before drug administration, blood glucose in all groups were similar. However, 2 h after the drug administration, serum insulin levels in all doses of berberine groups were elevated (*P*<0.05), and their glucose levels were lowered (*P*<0.05) correspondingly in a dose-dependent manner. Compared with berberine groups, glibenclamide showed stronger insulin-promoting and glucose-lowering efficiency (Tab 3).

Effects of berberine on insulin secretion and

Tab 2. Serum parameters in impaired glucose tolerance rats treated for 8 weeks. Mean±SD. ^b*P*<0.05, ^c*P*<0.01 vs control group (One-way ANOVA followed by Turkey test).

Group	BerL (n=10)	BerH (n=10)	Met (n=11)	Control (n=11)	Normal (n=9)
FBG (mmol·L ⁻¹)	7.4±1.5 ^c	7.3±1.3 ^c	7.3±0.7 ^c	9.3±1.3	4.98±1.2 ^c
FINS (nIU·L ⁻¹)	51±16	49±13 ^b	44±15 ^b	62±16	53±17
FFA (μmol·L ⁻¹)	456±93 ^b	460±72 ^b	481±87	550±113	433±44 ^c
TC (mmol·L ⁻¹)	1.8±0.3 ^b	1.9±0.3 ^b	2.1±0.3	2.2±0.2	1.6±0.2 ^c
TG (mmol·L ⁻¹)	0.61±0.22 ^c	0.63±0.17 ^c	1.5±0.5	1.85±0.73	0.43±0.11 ^c
HDL-C (mmol·L ⁻¹)	1.5±0.3 ^c	1.4±0.3 ^c	1.4±0.2 ^c	1.1±0.1	1.5±0.2 ^c
APOA (g·L ⁻¹)	0.80±0.08 ^c	0.87±0.08 ^c	0.62±0.03 ^c	0.71±0.06	1.0±0.02 ^c
APOB (g·L ⁻¹)	0.37±0.02 ^c	0.42±0.05 ^b	0.44±0.05	0.46±0.04	0.37±0.01 ^c

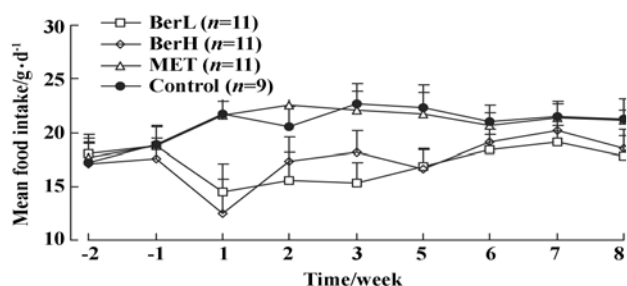


Fig 2. Effects of berberine 187.5 mg·kg⁻¹·d⁻¹ (BerL), 562.5 mg·kg⁻¹·d⁻¹ (BerH) and metformin 187.5 mg·kg⁻¹·d⁻¹ (MET) on the mean food intake in impaired glucose tolerance rats. Mean±SD.

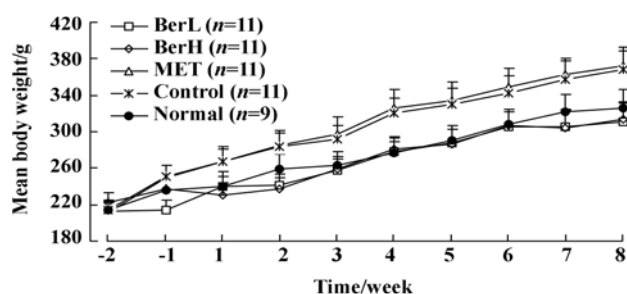


Fig 3. Effects of berberine 187.5 mg·kg⁻¹·d⁻¹ (BerL), 562.5 mg·kg⁻¹·d⁻¹ (BerH) and metformin 187.5 mg·kg⁻¹·d⁻¹ (MET) on the mean body weight of impaired glucose tolerance rats. Mean±SD.

metabolism of HIT-T15 cells and murine pancreatic islets Although HIT-T15 cell line is very sensitive to glucose, it loses insulin responsibility and diminishes the intracellular insulin levels and its mRNA expression greatly if it is continuously cultured in media containing glucose 11.1 mmol/L^[19]. In this study, the cells were cultured at the presence of lower glucose level. It was observed that berberine 1-10 μmol/L enhanced insulin secretion at the presence of glucose 0, 5 and 10 mmol/L

in a dose-dependent manner on HIT-T15 cells ($P<0.05$). Besides, berberine 1-10 μmol/L also promoted insulin secretion on pancreatic islets at the presence of glucose 16.7 mmol/L in a dose-dependent manner. In this study, berberine lowered MTT value of HIT-T15 cells at the concentration of 100 μmol/L, but did not affect pancreatic islets (Fig 4).

DISCUSSION

Streptozotocin injection targets pancreatic β-cells and leads to serum insulin reduction, and high fat chow feeding leads to insulin resistance^[10], which in feed-back elevates serum insulin. The murine model induced by streptozotocin injection plus high fat chow feeding has been recognized as type 2 diabetes model^[9-12]. With this model we dynamically investigated the anti-diabetic effects of berberine. It was demonstrated that berberine at dosages of 187.5 mg·kg⁻¹·d⁻¹ and 562.5 mg·kg⁻¹·d⁻¹ reduced the number of diabetic rats developed from IGT. How to understand this therapeutic effect of berberine on disturbance of murine glucose homeostasis? The administration of berberine to IGT rats could significantly reduce FBG level and ameliorate oral glucose tolerance. Moreover, berberine could also decrease mean food intake per day and body weight in diabetic rats, reduce serum TG, FFA, TC, APO B, and elevate HDL-C, APO AI. In addition, berberine was also potent in lowering plasma FFA, which is considered to be one of the most important factors that cause insulin resistance^[20-22]. In short, berberine could reduce some risk factors of type 2 diabetes.

What is the mechanism of the hypoglycemic action of berberine? We observed that berberine promotes insulin release *in vitro* and *in vivo*. HIT-T15 cell, a kind of insulin secreting cell line, retains the essential features of the insulin releasing response of normal pan-

Tab 3. Effects of berberine on insulin secretion and blood glucose in BALB/c mice. $n=6$. Mean±SD. ^b $P<0.05$, ^c $P<0.01$ vs corresponding control group (one-way ANOVA followed by Turkey test).

Group	Glu/mmol·L ⁻¹ (before treatment)	Glu/mmol·L ⁻¹ (2 h after treatment)	Insulin/pIU·L ⁻¹ (2 h after treatment)
Control	5.28±0.33	4.87±0.21	24.33±2.81
Gli (104 mg/kg)	5.37±0.32	3.12±0.85 ^c	33.39±2.99 ^c
Ber (93.75 mg/kg)	5.32±0.60	4.52±0.31 ^b	27.50±2.69 ^b
Ber (187.5 mg/kg)	5.33±0.67	4.45±0.29 ^b	28.64±4.45 ^b
Ber (562.5 mg/kg)	5.28±0.15	4.30±0.19 ^c	29.17±4.45 ^b

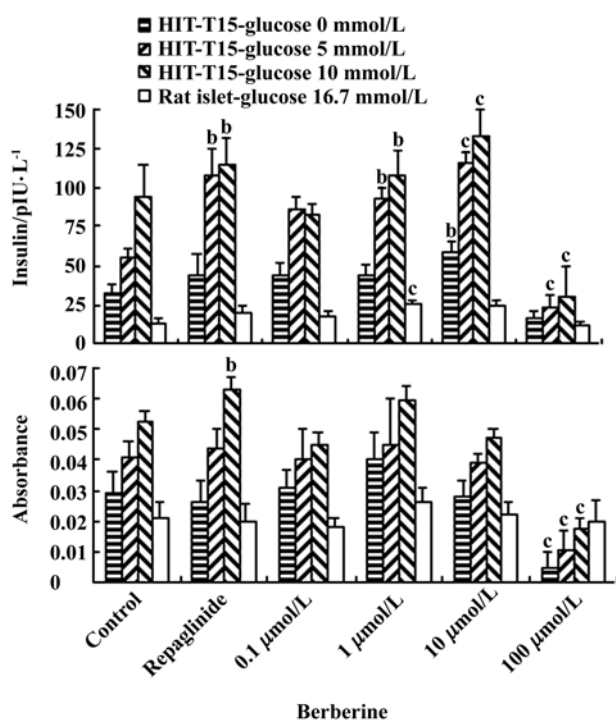


Fig 4. Effects of berberine on insulin secretion (A) and cell metabolism (B) in HIT-T15 cells and murine pancreatic islets. $n=6$. Mean \pm SD. ^b $P<0.05$, ^c $P<0.01$ vs corresponding control group (one-way ANOVA followed by Turkey test).

cretic β cells^[23]. It was shown that berberine 1-10 $\mu\text{mol/L}$ promoted insulin secretion in HIT-T15 cells incubated at the presence of glucose 0, 5, or 10 mmol/L . The similar result was obtained in murine pancreatic islets incubated in the presence of glucose 16.7 mmol/L . *In vivo*, berberine promoted insulin release and reduced blood glucose by administering orally at a bolus to BALB/c mice. It comes to the conclusion that berberine possesses facilitating insulin secretion property. However, why the serum insulin level was not elevated in diabetic rats treated with berberine. According to the recent research^[13], berberine exerted glucose-lowering effect in hepatocyte in insulin independent way. As well known, glucose is powerful in stimulating insulin release. If blood glucose level is lowered through hepatocyte metabolic way, in feedback the insulin release from pancreatic β -cell was reduced, which will counteract the insulin promoting action of berberine. The result in this study was coincided with our speculation.

The exact mechanisms of lipids modulating effects of berberine are still unknown. Berberine might act as a kind of α_1 , α_2 adrenoceptor antagonist^[2,4], which is believed to exert beneficial influence to the lipids metabolism in the body.

In summary, the effects of berberine on lowering blood glucose and modulating lipids in the treatment of diabetes were further confirmed through animal experiments in this study. In the exploration on its therapeutic mechanisms, we identified that berberine possessed the potency of facilitating insulin excretion either in HIT-T15 cell line and pancreatic islets *in vitro* or in BALB/c mice. In addition to the peripheral effect of berberine on improving insulin resistance as previously reported, the findings in this study might be helpful to understand the role of berberine in the clinical treatment of diabetes mellitus.

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