Hypoglycemic effect of *Ganoderma lucidum* polysaccharides[1]

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KEY WORDS *Ganoderma lucidum;* polysaccharides; hypoglycemic agents; glucose; insulin; calcium; pancreas

ABSTRACT

AIM: To investigate the hypoglycemic effect of *Ganoderma lucidum* polysaccharides (*Gl*-PS) in the normal fasted mice and its possible mechanism. **METHODS:** Normal fasted mice were given a single dose of *Gl*-PS 25, 50, and 100 mg/kg by ip and the serum glucose was measured at 0, 3, and 6 h after administration. *Gl*-PS 100 mg/kg were also given by ip and the serum glucose and insulin levels were measured at 0 min, 30 min, 1 h, 3 h, 6 h, and 12 h. Pancreatic islets were isolated and incubated with glucose 5.6 mmol/L and different concentration of *Gl*-PS, the insulin content of islets and insulin release were examined. The islets fluorescent intensity of $[Ca^{2+}]_i$ was also studied with a confocal microscope. Verapamil and egtazic acid were used to testify whether the insulin-releasing effect of *Gl*-PS was mediated by its ability to raise the Ca^{2+} influx. **RESULTS:** *Gl*-PS dose-dependently lowered the serum glucose levels at 3 h and 6 h after administration. *Gl*-PS 100 mg/kg raised the circulating insulin levels at 1 h after administration. *In vitro*, *Gl*-PS had no effect on islets insulin content, but it stimulated the insulin release after incubation with glucose 5.6 mmol/L. Confocal microscope showed that *Gl*-PS 100 mg/L had the capacity to raise the $[Ca^{2+}]_i$. The insulin-releasing effect of *Gl*-PS was inhibited by verapamil/egtazic acid. **CONCLUSION:** *Gl*-PS possesses the hypoglycemic effect on normal mice; one mechanism is through its insulin-releasing activity due to a facilitation of Ca^{2+} inflow to the pancreatic β cells.

INTRODUCTION

Plants have always been utilizable sources of drugs and many of the currently available drugs have been directly or indirectly from plants. In accordance to the recommendations of the WHO Expert Committee on diabetes mellitus, it is important to investigate the hypoglycemic action from plants which were originally used in traditional medicine[1]. The biological active components of the plants with hypoglycemic action include flavonoides, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids, and terpenoids, *etc*[2]. *Ganoderma lucidum* polysaccharides (*Gl*-PS) is one of the main efficacious ingredient of *Ganoderma lucidum* (Leyss ex Fr) Karst, which has been reported to have antitumor, antioxidant and immunomodulatory activities[3-7]. It was reported that *Gl*-PS also processed the hypoglycemic potential by increasing the plasma insulin level in normal mice and rats[8,9]. In the present study, we investigated the hypoglycemic effect of *Gl*-PS in normal mice and its possible hypoglycemic mechanisms.

MATERIALS AND METHODS

Drugs *Ganoderma lucidum* Leyss ex Fr Karst was collected in Fujian province, China. The fruiting body of *Ganoderma lucidum* Leyss ex Fr Karst was authenticated by Prof Xiao-lan MAO, the Institute of

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Microbiology, Chinese Academy of Sciences. *Ganoderma lucidum* polysaccharides (*Gl*-PS) were extracted by hot water from the fruiting body of *Ganoderma lucidum* (Leyss ex Fr) Karst, which were provided by the Fuzhou Institute of Green Valley Bio-Pharm Technology, Fujian province, China. The yield of *Gl*-PS was 0.82 % (w/w) in terms of the fruiting body of *Ganoderma lucidum.* It is a polysaccharide peptide with an average molecular weight of 584 900 and has 17 amino acids. The ratio of polysaccharides to peptides is 93.51 %: 6.49 %. The polysaccharides consist of rhamnose, xylose, fructose, galactose, mannose, and glucose with molar ratios of 0.793:0.964:2.944:0.167: 0.384:7.94 and are linked together by β-glycosidic linkages. It is hazel-colored and water-soluble powder.

Collagenase (type V), verapamil, and ethylene glycol-tetraacetic acid (egtazic acid) were purchased from Sigma. RPMI-1640, DMEM medium were purchased from Gibco BRL, Fluo3/AM was purchased from Biotium.

***In vivo* studies** Fifty male albino Swiss mice weighing 20 g±2 g purchased from Department of Experimental Animals, Peking University, Health Science Center, Beijing, China (Grade II, Certificate No scxk11-00-0004). All procedures were in accordance with the Institute Ethical Committee for Experimental Use of Animals. Mice fasted for 6 h were divided into 5 groups. Group 1: animals were given saline by ip; group 2, 3, 4: animals were given *Gl*-PS by ip at the dose of 25, 50, and 100 mg/kg body weight, respectively; group 5: animals were given by ig glibenclamide (50 mg/kg) as a standard hypoglycemic agent. After a single dose of drug administration, blood samples were collected at 0 h, 3 h, and 6 h from the tail vein. The serum was separated and the glucose levels in the serum samples were measured.

Another experiment was to study the time kinetics of hypoglycemic effect of *Gl*-PS on the 6-h fasted mice. One hundred and twenty mice were divided into control and *Gl*-PS 100 mg/kg-treated group. Each group consisted of 10 mice. After a single ip administration of normal saline or *Gl*-PS 100 mg/kg, blood samples were collected at 0 min, 30 min, 1 h, 3 h, 6 h and 12 h from the tail vein respectively. The serum was separated and the glucose and insulin levels in the serum samples were measured.

Preparation of the pancreatic islets Male Wistar rats weighing 60 g±10 g, purchased from the Institute of Animals, Chinese Academy of Medical Sciences, were used in the *in vitro* studies (Grade II, Certificate No scxk11-00-0006). Pancreatic islets were isolated from the rats by collagenase digestion[10]. About 50 islets/well were placed onto 24-well plate and cultured in RPMI-1640 medium containing glucose 11.1 mmol/L, HEPES 20 mmol/L, benzylpenicillin 100 kU/L, streptomycin 100 mg/L, and 7 % heat-inactived fetal bovine serum at 37 ºC under 95 % O_2 and 5 % CO_2 atmosphere for 20-24 h.

Islet insulin content studies After culture, the islets were incubated at 37 ºC in DMEM culture medium containing 10 % FCS with glucose 5.6 mmol/L and different concentrations of *Gl*-PS for 24 h, the islets insulin contents were extracted by acid alcohol (HCl 0.18 mol/L dissolved in 95 % ethanol) for overnight at 4 ºC and measured by radioimmunoassay.

Insulin release studies After culture, the islets were incubated at 37 ºC in DMEM culture medium containing 10 % FCS with glucose 5.6 mmol/L and different concentrations of *Gl*-PS for 1 h. The aliquot were collected and stored at -20 ºC for the later determination of insulin.

For inhibitor studies, the islets were preincubated in DMEM medium containing 10 % FCS with glucose 5.6 mmol/L and verapamil 50 μmol/L+egtazic acid 0.5 mmol/L for 15 min before addition of different concentrations of *Gl*-PS for 1 h, and the aliquot was collected and stored at -20 ºC for the later determination of insulin.

Biochemical analysis Glucose was determined by the glucose-oxidase method and insulin was measured by radioimmunoassay, using commercially available kits (China Institute of Atomic Energy, Beijing, China) and expressed as mIU/L or mIU·h^{-1} per 50 islets.

Time series scan of cell fluorescence of $[Ca^{2+}]_i$ After isolation, islets were placed onto the 35-mm-petri dishes and cultured in DMEM medium for 48 h. The cells were washed with PBS and loaded with fluorescent probe (Fluo3/AM) at a final concentration of 10 μmol/L in PBS for 45-60 min. Then the cells were washed with PBS for three times and incubated in the Krebs-Ringer bicarbonate buffer for the confocal microscope investigation. *D*-glucose was added to the medium (the final concentration is 5.6 mmol/L) 1 h before *Gl*-PS addition and the image was recorded for the final 5 min to get the baseline intracellular fluorescence of Ca^{2+}, then *Gl*-PS 100 mg/L was added to the medium and the image was recorded for another 15 min with confocal microscope. Islets with glucose 5.6 mmol/L and without *Gl*-PS were as control. Cells were imaged via the epifluorescence mode with a 40×immersion lens

at Exl 488 nm and Eml 506 nm. The cell images were stored on computer and transformed to the cell fluorescence intensity.

Statistical analysis All values, expressed as mean±SD, were subjected to *t*-test and one-way ANOVA employing the computer SPSS statistic package.

RESULTS

Serum glucose levels in normal mice *Gl*-PS treatment (50 mg/kg and 100 mg/kg) caused a significant reduction in the serum glucose levels in normal fasted mice. The reductive rate was 24.5 %, 31.8 % at 3 h and 46.2 %, 41.3 % at 6 h, respectively (Tab 1).

Tab 1. Effect of a single administration of *Ganoderma lucidum* (*Gl*-PS) on the serum glucose level in normal mice. *n*=10. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control group.

Group/mg·kg^{-1}	Fasting serum glucose level/g·L^{-1}		
	0 h	3 h	6 h
Control	1.50±0.30	1.3±0.4	1.22±0.29
Gl-PS 25 mg/kg ip	1.40±0.30	1.4±0.4	1.06±0.23
Gl-PS 50 mg/kg ip	1.47±0.29	1.01±0.17^b	0.65±0.12^b
Gl-PS 100 mg/kg ip	1.49±0.25	0.91±0.25^b	0.71±0.15^b
Glibenclamide 50 mg/kg ig	1.45±0.28	0.57±0.17^c	0.38±0.10^c

Time kinetics on serum glucose levels in normal mice There was a transient increase in the serum glucose level at 30 min after *Gl*-PS administration. The serum glucose level in *Gl*-PS-treated mice decreased significantly after 3 h and 6 h as compared to the untreated mice at the same time point (Fig 1).

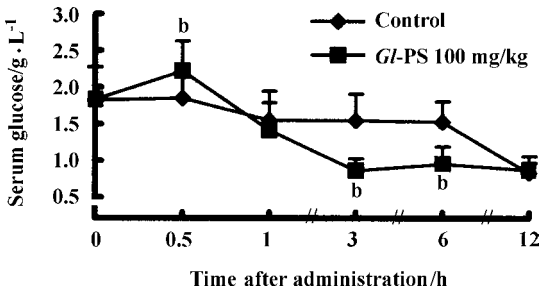

Fig 1. Time kinetics of hypoglycemic effect of *Gl*-PS on serum glucose levels in normal mice at different time points after a single dose of intraperitoneal administration. *n*=10. Mean±SD. $^bP<0.05$ *vs* control at the same time point.

Time kinetics on serum insulin levels in normal mice *Gl*-PS elevated the serum insulin levels significantly at 1 h after ip administration as compared to the untreated mice at the same time point (Fig 2).

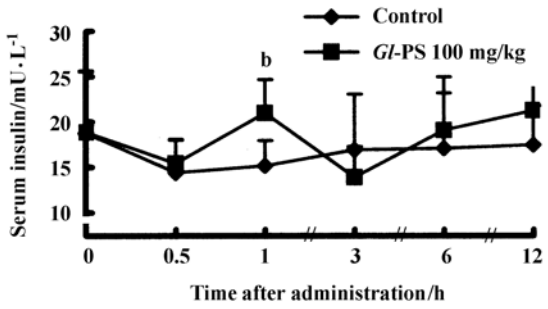

Fig 2. Time kinetics of hypoglycemic effect of *Gl*-PS on serum insulin levels in normal mice at different time points after a single dose of intraperitoneal administration. *n*=10. Mean±SD. $^bP<0.05$ *vs* control at the same time point.

Insulin content of pancreatic islets induced by glucose 5.6 mmol/L When the islets were incubated with 5.6 mmol/L glucose and different concentration of *Gl*-PS for 24 h, *Gl*-PS showed no significant effect on the insulin content of pancreatic islets as compared to the medium control (Fig 3).

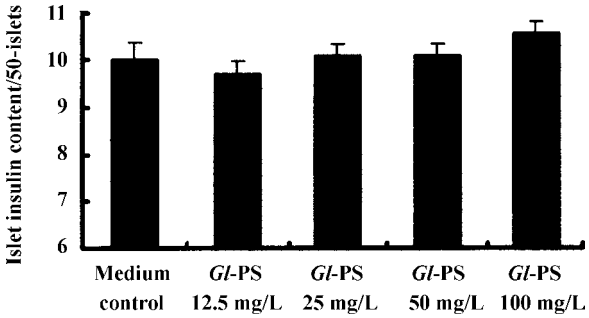

Fig 3. Effect of *Gl*-PS on the pancreatic islet insulin content induced by 5.6 mmol/L glucose. *n*=6. Mean±SD.

Insulin release induced by glucose 5.6 mmol/L in pancreatic islets In the presence of glucose 5.6 mmol/L glucose, *Gl*-PS stimulated the insulin release significantly at the concentration of 100 mg/L as compared to the culture medium group (Fig 4).

Cell fluorescence of $[Ca^{2+}]_i$ After addition of *Gl*-PS 100 mg/L, the intracellular fluorescence of Ca^{2+} began to elevate slowly. Ten minutes later, the fluorescence increased significantly as compared to that of

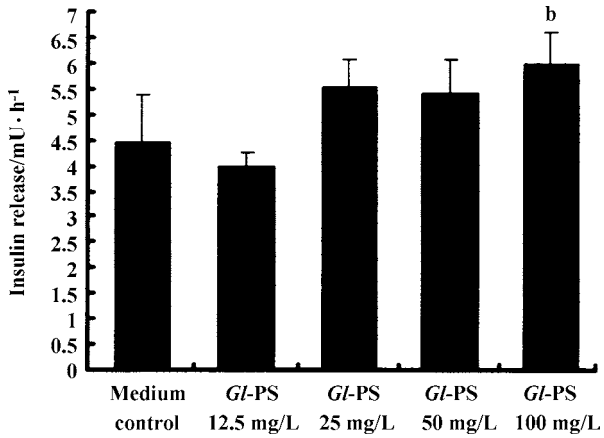

Fig 4. Dose-response effect of *Gl*-PS on the insulin release induced by glucose 5.6 mmol/L in pancreatic islets. Islets were incubated at 37 ºC in DMEM with glucose 5.6 mmol/L and different concentrations of *Gl*-PS for 1 h. Insulin in the aliquot was collected and measured by RIA. *n*=6. $^{b}P<0.05$ *vs* medium control.

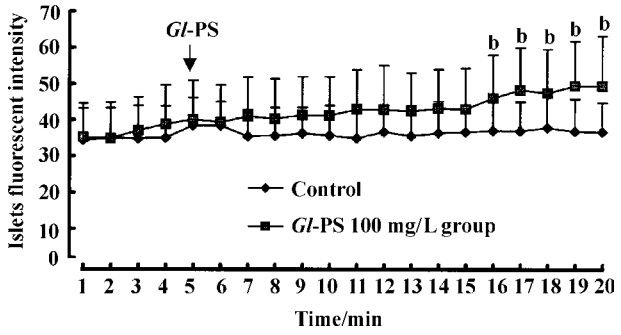

Fig 5. Effect of *Gl*-PS on the cell fluorescence of $[Ca^{2+}]_i$. *n*=15. $^{b}P<0.05$ *vs* control at the same time point.

the control group. *Gl*-PS caused about 40 % increase on the cell fluorescence at 15 min after addition as compared to that of the control group (Fig 5).

Effect of egtazic acid and verapamil on insulin release at glucose 5.6 mmol/L in isolated pancreatic islets Verapamil 50 μmol/L and egtazic acid 0.5 mmol/L inhibited the insulin release of islets under glucose 5.6 mmol/L. The stimulation of insulin release by *Gl*-PS 100 mg/L under glucose 5.6 mmol/L was inhibited by verapamil/egtazic acid (Fig 6).

DISCUSSION

Ganoderma lucidum polysaccharides elicit hypoglycemic potential in normal mice[8,9], but the hypoglycemic mechanism remains unknown. In this study, we found that *Gl*-PS dose-dependently lowered serum

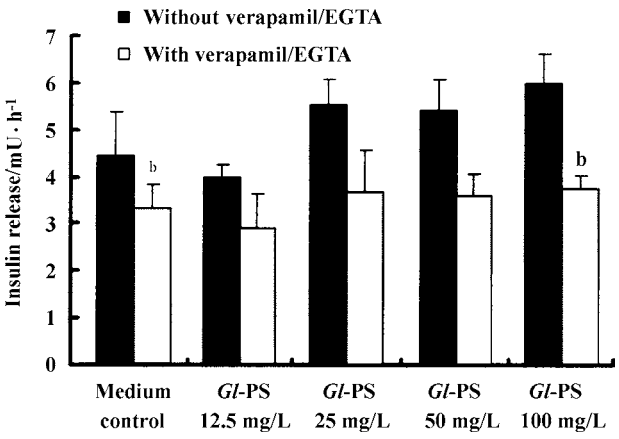

Fig 6. Effect of *Gl*-PS on the insulin release induced by glucose 5.6 mmol/L in isolated pancreatic islets. *n*=6. Mean±SD. $^{b}P<0.05$ *vs* medium control in the without verapamil/egtazic acid group.

glucose at 3 h and 6 h after ip administration. Because the hypoglycemic action was very obvious at the dose of 100 mg/kg, so the effect of *Gl*-PS was investigated at this dose. The results showed that *Gl*-PS 100 mg/kg caused increase of serum insulin level at 60 min and decreased serum glucose levels, the hypoglycemic effect of *Gl*-PS lasted for more than 5 h. In this study, there was a transient increase in the serum glucose levels at 30 min after *Gl*-PS administration, it was thought that this phenomenon maybe due to the rapid decomposition of liver glycogen, because earlier study reported that Ganoderan B affected the metabolism of liver glycogen in a short time and reduced the glycogen content in liver at 3 h after ip administration[9].

In vitro experiment demonstrated that *Gl*-PS could stimulate the insulin release directly at physiological concentration of 5.6 mmol/L glucose, but it showed no significant effect on the islet insulin content. This result suggested that *Gl*-PS could not stimulate the insulin synthesis in 24-h incubation, but it could stimulate the insulin release from the islets directly. This result is in accordance with the view that the concentration of circulating insulin depends mainly on the regulation of secretion, rather than on the rate of biosynthesis of the hormone itself[11].

Insulin is stored in large dense core vesicles in pancreatic β cells and released by exocytosis. Calcium constitutes the major stimulus for exocytosis. Ca^{2+} regulates several steps in exocytosis such as the size of vesicle pools, the fusion event, and the size of the fusion pool[12]. *Ganoderma lucidum* polysaccharides could increase $[Ca^{2+}]_i$ in murine peritoneal macrophages and T lym-

phocytes due to the influx of extracellular Ca^{2+} and intracellular Ca^{2+} release[13,14]. The fluorescent experiment revealed that *Gl*-PS raised the $[Ca^{2+}]_i$ slowly but significantly 10 min after addition. Subsequent to Ca^{2+} influx, synaptic vesicle exocytosis occurs in short time and the insulin release increased.

Pancreatic β cells express N-, P/Q-, and L-type Ca^{2+} channel[15], the major contribution to Ca^{2+}-influx eliciting insulin exocytosis is contributed by L-type channel[16]. So we choosed verapamil, one kind of L-type Ca^{2+} channel blocker, and egtazic acid in this experiment to testify whether the insulin-releasing effect of *Gl*-PS was mediated by its capacity to raise the $[Ca^{2+}]_i$. The results indicated that the stimulation of insulin release by *Gl*-PS under 5.6 mmol/L glucose was inhibited by verapamil/egtazic acid. It suggested that on physiological glucose concentration, the insulin-stimulating effect of *Gl*-PS depended mainly on the extracellular Ca^{2+} influx; it did not promote an intracellular Ca^{2+} redistribution or even if it promoted an intracellular Ca^{2+} redistribution, but this effect was too weak to affect the islets insulin release.

In conclusion, the present findings demonstrate that the administration of *Gl*-PS to normal mice elicited the hypoglycemic action, one mechanism is through its insulin-releasing activity due to a facilitation of Ca^{2+} inflow to the pancreatic β cells.

REFERENCES

1 Alarcon-Aguilara FJ, Rornan-Ramos R, Perez-Gutierrez S. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol 1998; 61 (Pt 2): 101-10.

2 Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2002; 81: 81-100.

3 Lin ZB, editor. Modern research of *Ganoderma*. 2nd ed. Beijing: Beijing Medical University Press; 2001.

4 Miyazaki T, Nishjima M. Studies on fungal polysaccharides. XXVII. Structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum*. Chem Pharm Bull 1981; 29: 3611-6.

5 Zhang QH, Lin ZB. The antitumor activity of *Ganoderma lucidum* (Curt.:Fr.)P.Karst.(Ling Zhi) (Aphyllophoromycetideae) polysaccharides is related to tumor necrosis factor-α and interferon-γ. Int J Med Mushrooms 1999; 1: 207-15.

6 Xia D, Lin ZB. Effects of *Ganoderma* polysaccharides on immune function in mice. J Beijing Med Univ 1989; 21: 533-6.

7 You YH, Lin ZB. Protective effects of *Ganoderma lucidum* polysaccharides peptide on injury of macrophages induced by reactive oxygen species. Acta Pharmacol Sin 2002; 23: 787-91.

8 Hikino H, Konno C, Mirin Y, Hayashi T. Isolation and hypoglycemic activity of Ganoderans A and B, glycans of *Ganoderma lucidum* fruit bodies. Planta Med 1985; 4: 339-40.

9 Hikino H, Ishiyama M, Suzuki Y, Konno C. Mechanisms of hypoglycemic activity of ganoderan B: a glycan of *Ganoderma lucidum* fruit bodies. Planta Med 1989; 55: 423-8.

10 Joshph L, Stephen L, editor. Diabetes research; volume 1. Labortary methods. A Wiley-Interscience Publication; 1985.

11 Lang J. Molecular mechanism and regulation of insulin exocytosis as a paradigm of endocrine secretion. Eur J Biochem 1999; 259: 3-17.

12 Ernandez-Chacon RH, Alvarez de Toledo G. Cytosolic calcium facilitates release of secretory products after exocytotic vesicle fusion. FEBS Lett 1995; 263: 221-5.

13 Li MC, Lei LS, Liang DS. Effect of *Ganoderma* polysaccharide on intracellular free calcium in murine peritoneal macrophage. Chin Pharm J 1999; 34: 805-7.

14 Li MC, Lei LS, Wang QB, Liang DS. Effect of *Ganoderma* polysaccharide on intracellular free calcium and intracellular pH in murine T cells. Chin Pharmacol Bull 2001; 17: 167-70.

15 Rvath A, Szabadkai G, Vamai P, Enyedi P. Voltage dependent calcium channels in adrenal glomerulosa cells and in insulin producing cells. Cell Calcium 1998; 23: 33-42.

16 Ligon B, Boyd AER, Dunlap K. Class A calcium channel variants in pancreatic islets and their role in insulin secretion. J Biol Chem 1998; 273: 13905-11.