Anti-diabetic potential of Pueraria lobata root extract through promoting insulin signaling by PTP1B inhibition

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ABSTRACT

Type 2 diabetes mellitus is a fast-growing epidemic affecting people globally. We initiated the project by searching the possible target of the Pueraria lobata root extract (P. lobata). We conducted the IC₅₀ assays of P. lobata on the four diabetes-related proteins: PTP1B, TCPTP, SHP-2 and DPP-4. Results indicated that P. lobata exhibited high PTP1B inhibitory activity with IC₅₀ of 0.043 mg/ml. Treated insulin-resistant HepG2 cells with 0.0115 mg/ml of P. lobata increased the glucose uptake by two times compared with the negative control. Further, we performed OGTT test on the diabetic C57BL/6 male mice. 20% decreased blood glucose (AUC) was obtained with a dose of 1 g/kg P. lobata compared with the negative control. Herein, we were able to demonstrate the antidiabetic effects of P. lobata might be related to the inhibition of PTP1B and therefore, bettering the insulin signaling pathway.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic illness due to endocrine dysfunction. Uncontrolled diabetes is associated with various acute and chronic comorbidities. According to reports, the incidence of diabetes in China has reached 11.6% (about 114 million people in the country with diabetes), of which 90% are T2DM [1]. To date, the prevention of T2DM in patients primarily focuses on education, diet, and exercise. Lifestyle modifications along with pharmacotherapy and patient education are the mainstay of therapy for patients afflicted with T2DM.

While pharmacological interventions and chemical drugs are coming with the baggage of side effects due to off-target influences, it is more and more desired to switch those chemical compounds with natural, safe and effective anti-diabetic agents.

Pueraria lobata (Wild.) Ohwi. (P. lobata) is a traditional Chinese medicine used widely in clinical over thousands of years. Modern pharmacological studies have reported that P. lobata root and leaf extracts have various therapeutic effects on cardiovascular disorders, osteoporosis, inflammation, liver injury, cancer, as well as hyperglycemic defects [2]. In hyperglycemic studies is reported that P. lobata could markedly reduce the blood glucose levels [3]. However, information about P. lobata potential benefits on STZ-diabetic mice and HepG2 cells has remained vague and insufficient. Thus, it is important to explore how P. lobata affects insulin signaling to better understand its anti-diabetic potential.

In this study, we employed type 2 diabetes-related target proteins (PTP1B, TCPTP, SHP-2, DPP-4), insulin-resistant HepG2 cells and HFD/STZ-diabetic C57BL/6 mice to investigate the anti-diabetic properties of P. lobata.

2. Results

2.1. Protein inhibitory activity test (IC₅₀ assessments)

The table (Table 1) illustrates that P. lobata exhibits different inhibitory effects on the selected proteins. As shown, the most significant inhibitory activity is against PTP1B protein at 0.043 mg/ml then SHP-2 protein at 0.088 mg/ml. The weakest inhibitory activity is demonstrated against DPP-4 protein. These data verified that the major hypoglycemic character of P. lobata might be derived by inhibition of PTP1B.

2.2. Cytotoxicity assay of P. lobata by MTT

Due to the complexity of natural Chinese medicines, we conducted MTT assay to investigate cytotoxicity of P. lobata. In doing so, we...
employed HepG2 cells to measure cytotoxicity of extract. As shown in Fig. 1, *p < 0.05 versus the blank (no drug treated), one-way ANOVA with Dunnett’s post hoc test.

Table 1
IC50 values of P. lobata.

<table>
<thead>
<tr>
<th></th>
<th>PTP1B</th>
<th>TCPTP</th>
<th>SHP-2</th>
<th>DPP-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puerarin</td>
<td>0.046 mg/ml</td>
<td>&gt; 0.1</td>
<td>0.088</td>
<td>0.28</td>
</tr>
<tr>
<td>Na3O4V (pNpp)</td>
<td>0.046 µM</td>
<td>0.014 µM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alogliptin (AP-AFC)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.004 µM</td>
</tr>
</tbody>
</table>

Values are means of three experiments.
* means not examined. And we used Na3O4V and alogliptin as positive control.

Fig. 1. MTT Cell viability assay of P. lobata in HepG2 (n = 3 per conc.), *p < 0.05 versus the blank (no drug treated), one-way ANOVA with Dunnett’s post hoc test.

2.3. Enhanced glucose uptake by P. lobata in HepG2

Next, we evaluated the glucose uptake effects of P. lobata in insulin-resistant HepG2 cells. The cells were treated with different concentrations of P. lobata (0.0115 mg/ml, 0.0058 mg/ml, 0.0029 mg/ml and 0.0014 mg/ml) respectively. As demonstrated in Fig. 2, at different concentrations of P. lobata glucose uptake enhances with a dose-dependent manner.

Compared with control group containing just insulin and fluorescent glucose, the glucose uptake ability of HepG2 cells at indicated concentrations of P. lobata increased about 100%, 88%, 40% and 16% respectively. Our results must note that the glucose uptake ability of the cells treated with 0.0115 mg/ml P. lobata is quite close to the group treated with 3 µM and 5 µM of Pioglitazone (positive control). Inarguably, it is concluded that P. lobata has the ability to increase the insulin-sensitivity in HepG2 cells and enhances the glucose uptake.

2.4. Anti-diabetic activity assay of P. lobata in vivo

Following, to further verify the hypoglycemic effects of P. lobata, we employed HFD/STZ-diabetic C57BL/6 mice model for in vivo examinations. Three groups of diabetic mice including metformin group, P. lobata group and control group underwent OGTT. The assays were initiated by oral administrating of metformin and P. lobata. We waited for 15–20 mins then began the OGTT procedure. Glucose solution (20%) was administered intraperitoneally. Blood glucose levels were recorded at 30, 60, 90, 120 and 240 mins. As shown in Fig. 3, the maximum and minimum points of blood glucose levels in P. lobata group at the dose of 1 g/kg were fairly lower than metformin group and moreover, than those in control group at all doses of extract treatment.

The area under the curve (AUC) of OGTT analysis for P. lobata compared with control group illustrates remarkable decrease of blood glucose levels by 21.0%, 20.0%, 10.0% and 5.7%, respectively, while the AUC of metformin group dropped by 17.4% (Fig. 4). These results solidify that P. lobata from medium and high concentration could control the blood glucose and improve the glucose tolerance significant in mice model.

3. Discussion

With the continuous improvement of people’s living standard, diabetes has become a high incidence disease. The treatment of existing chemical medications usually brings undesired side effects such as hypoglycemia, gastrointestinal reactions, liver damage and lactic acidosis, while the treatment effects are also unsatisfactory [4]. Because of the medicine-food homology of traditional Chinese medicine, its toxicity is relatively weak. Therefore, more and more scholars are turning to traditional Chinese medicine and hope to find natural products with notable anti-diabetic effect. It has been reported that the main components of P. lobata such as puerarin and soy isoflavones have hypoglycemic effects [5,6]. However, there are few studies about hypoglycemic effect of those extracts and underlying mechanisms.

In this study, we utilized T2DM linked target proteins, insulin-resistant HepG2 cells and HFD/STZ-diabetic C57BL/6 male mice to scrutinize the hypoglycemic effects of P. lobata in vitro and in vivo. The results demonstrated that P. lobata could effectively better the glucose uptake in HepG2 cells. Moreover, oral administration of P. lobata gravely lessened blood glucose in type 2 diabetic mice. Even though at the higher doses of application the hypoglycemic effect could excel those of metformin.

Type 2 diabetes is closely related to insulin resistance, whereas the tyrosine phosphatase PTP family protein (PTP1B) alters the insulin receptor’s sensitivity by negatively regulating insulin signaling [7]. This study explored that P. lobata is a potent inhibitor of PTP1B, which could explain the hypoglycemic effect. Conclusively, P. lobata could achieve the purpose of moderating blood glucose levels by markedly boosting glucose uptake in HepG2 human cells and escalating glucose tolerance in diabetic mice.

We prospect since the effective ingredients are not in high concentrations in extract, further research should be done focusing on analysis of the active ingredients of P. lobata to obtain, modify and
optimize major molecules responsible for the therapeutic effects yet reach to a new class of anti-diabetic treatment.

4. Materials and methods

4.1. Plant extraction

Pueraria lobata (Wild.) Ohwi. was purchased from Shanghai Lei Yun Shang Company. According to reference [8], dried P. lobata (50 g) were pulverized and soaked in 75% ethanol (1 l) three times at 40 °C for 2 h. The residue was filtered off and the filtrate was combined and concentrated, then 20 ml butanol was added to the extract. After multiple extractions, concentration and drying, the extract (7.6 g) was sealed and stored in a refrigerator at 4 °C.

4.2. Protein inhibitory activity assay (IC50)

Methods were performed, as described [9], we used p-nitrophenyl phosphate (pNPP, Macklin, Shanghai, China) as substrate and sodium orthovanadate (Na3O4V, Adamas, Shanghai, China) as positive control to test the P. lobata inhibitory activity against protein tyrosine phosphatase-1B (PTP1B, 40 kDa), T-cell protein tyrosine phosphatase (TCPTP, 45 kDa) and protein tyrosine phosphatase-2 (SHP-2, 60 kDa). P. lobata, protease and buffer were added respectively, sodium hydroxide was mixed after 30 mins to stop the reaction. Immediately we measured the absorbance value at 405 nm.

As the Ref. [10], the inhibitory activity of P. lobata on dipeptidyl peptidase-4 (DPP-4, 80 kDa) was determined using AP-AFC as substrate and alogliptin as positive control. After adding different concentrations of extract, protease and buffer respectively, AP-AFC was added. The absorbance was measured after incubation for 10 mins. and read at 405, 505 nm.

4.3. Cell culture and viability assay

4.3.1. Cell culture

Human HepG2 cells were obtained from Chinese Academy of Science and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/ml penicillin, 100 streptomycins μg/ml, and 10% fetal bovine serum (FBS) from Gibco BRL (Grand Island, NY, USA). Then the cells were incubated at 37 °C in a humidified incubator containing 5% CO2.

4.3.2. MTT assay

Cell proliferation and growth was determined using MTT assay according to publications [11]. A total of 5 × 10^4 cells/well were seeded in a 96-well culture plate for 24 h at 37 °C and treated with different concentrations (0.0460 mg/ml ∼ 0.0014 mg/ml) of P. lobata extract at 37 °C with 5% CO2. Non-treated cells served as the control. After 48 h, the MTT solution (Sigma-Aldrich; Merck KGaA) was added to each well and incubated at 37 °C for 4 h. Following the removal of the culture medium, the cells were washed twice with PBS and 100 μl DMSO was added to dissolve the Formosan crystals. Absorbance was recorded at 490 nm using a microplate reader (Thermo Scientific, CA, USA).

4.3.3. Insulin-resistant model establishment

The HepG2 cells were seeded onto 24-well plates at 8 × 10^4 cells/well. After 24 h incubation, cells were then cultured in 10 μM insulin (from bovine pancreas, Sigma) for another 24 h to produce insulin resistant model [12].

4.3.4. Glucose uptake assay

The glucose uptake was measured using fluorescent glucose (2-NDBG) according to published procedure [13]. Briefly, the supernatant was discarded and washed three times with PBS, then serum-free medium and insulin (final concentration 100 nM) were added. After incubation for 10 mins, cells were cultured with fluorescent glucose (final concentration 50 μM) and different concentrations of extract (0.0115 mg/ml, 0.0058 mg/ml, 0.0029 mg/ml) for another 1 h. Cells were washed with PBS three times and transferred to flow cytometry tubes for analysis by flow cytometer (488 nm

Fig. 3. Oral glucose tolerance test in TD2M mice (n = 4).

Fig. 4. AUC of different drugs in T2DM mice (n = 4), *p < 0.05, **p < 0.01 versus the control. One-way ANOVA with Dunnett's post hoc test.
wavelength). The percentage was calculated by this following formula:

\[
\text{The glucose consumption (%)} = \frac{\text{Fluorescence}_{\text{drug}} - \text{Fluorescence}_{\text{control}}}{\text{Fluorescence}_{\text{control}}} \times 100\%
\]

4.4. In vivo experiment

4.4.1. Animal model

The C57BL/6 mice (male, four weeks, 20 ± 2 g) were purchased from Shanghai Slaccas Experimental Animal Company. High fat food and distilled water (pH = 6.5, and chlorine free) were given for 2 weeks. Diabetic mice were prepared by injecting STZ (Sigma, St. Louis, MO) after fasting (85 mg/kg). Mice with plasma glucose concentration of ≥11.1 mM counted as diabetic [14].

4.4.2. OGTT

The mice were randomly selected and divided into six groups. The control group, positive control (metformin, 10 mg/kg) group and P. lobata extract group (2 g/kg, 1 g/kg, 0.5 g/kg, 0.25 g/kg). After 8 h fasting, the fasting blood sugar level was measured. Each group was given the samples by IP and the equal amount of 20% glucose solution after 30 mins. Blood sugar levels were measured every 30 mins. Absorbance at 505 nm was measured by glucose oxidase reduction [15].

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.02.046.

References