Chronic Effects of Berberine on Blood, Liver Glucolipid Metabolism and Liver PPARs Expression in Diabetic Hyperlipidemic Rats

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Berberine is one of the main alkaloids of Rhizoma coptidis which has been used as a folk medicine to treat diabetes mellitus for more than 1400 years in China. To investigate the chronic effect of berberine on diabetic hyperlipidemic rats, fasted rats were intraperitoneally injected 35 mg/kg streptozotocin. Diabetic rats were admitted after 2 weeks and given a high-carbohydrate/high-fat diet to induce hyperlipidemia. The rats were divided into 7 groups at the end of week 16: normal and diabetic rats received no drug, 5 treatment groups were administered with either 75, 150, 300 mg/kg berberine, 100 mg/kg fenofibrate or 4 mg/kg rosiglitazone per day for 16 weeks, respectively. The blood glucose, hemoglobin A1c, total cholesterol, triglyceride, low density lipoprotein-cholesterol, apolipoprotein B and the decreased high density lipoprotein-cholesterol, apolipoprotein A1 levels in diabetic rats to near the control ones. Berberine alleviated the pathological progression of liver and reverted the increased hepatic glycogen and triglyceride to near the control levels. Berberine increased PPARα/δ expression and reduced PPARγ expression in liver of diabetic rat to near the control ones. Berberine improved glucolipid metabolism both in blood and liver in diabetic rats possibly through modulating the metabolic related PPARα/δγ protein expression in liver.

Key words berberine; liver; peroxisome proliferator-activated receptor; type 2 diabetes mellitus; hyperglycemia; hyperlipidemia

Type 2 diabetes mellitus (T2DM) is a serious and growing health threat affecting approximately 5% of the population in the whole world. The medical management of diabetic patients often focuses on plasma glucose levels because elevated glucose levels increase the risk for retinopathy, kidney disease, and peripheral neuropathy. The importance of the tight control of blood glucose in either preventing or delaying the progression of complications is recognized.1,2) Diabetic patients are also at high risk for atherosclerosis and myocardial infarction, and some of this risk can probably be attributed to diabetic hyperlipidemia, which is characterized by high triglyceride (TG), low high density lipoprotein (HDL) levels and is one of the major causes of morbidity and death.3,4) Treatment of hyperglycemia or hyperlipidemia in diabetes involves diet control, exercise and the use of hypoglycemic or lipid-lowering diets and drugs. Management of hyperglycemia or hyperlipidemia with low side effects is still a challenge to the current medical system. Peroxisome proliferator-activated receptors (PPARs) consist of three nuclear receptor isoforms, PPARα, PPARδ and PPARγ, encoded by separate genes.5,6) PPARs are ligand dependent transcription factors that regulate expression of target genes related to lipid and glucose metabolism. The PPARα receptor is expressed primarily in the liver and to a lesser degree in kidney, skeletal muscle, and cardiac muscle. It plays a critical role in the regulation of the cellular uptake, activation, and β-oxidation of fatty acids.7–9) In contrast, the PPARγ receptor is predominantly expressed in adipose tissue where it mediates transcriptional activation of genes involved in the regulation of lipid uptake and lipogenesis.9) The physiologic role of the PPARδ receptor is less well determined. The PPARδ agonists may have therapeutic utility in metabolic complication by increasing fatty acid consumption in skeletal muscle and adipose tissue, but more exact evidences are needed to support a primary role for the human PPARδ receptor as a key modulator of lipid metabolism.5,7)

Both PPARα agonists (fenofibrate) and PPARγ agonists (rosiglitazone) have actions with distinct benefits for T2DM. Currently available pharmacological antidiabetic agents such as rosiglitazone, however, have a number of limitations, for instance, various adverse effects and high rates of secondary failure.5) Due to these factors, there is thus an urgent medical need for the development of antidiabetic drugs that treat not only hyperglycemia but also hyperlipidemias. Diabetic patients and healthcare professions are increasingly considering complimentary and alternative approaches, including by using medicine herbs with combined actions of PPARα and PPARγ agonists. Moreover, there are some traditional Chinese medicines, which have been reported for treatment of diabetes,9–11) but there has been inadequate pharmacological research relating to them until now. Taking these facts into account, an attempt was made to find natural drugs with both hypoglycemic and hypolipidemic activities.

Rhizoma coptidis (root of Coptis chinensis Franch from Ranunculaceae) has been used to treat diabetes mellitus for more than 1400 years in China. Berberine, one of the main alkaloids of Rhizoma coptidis, has been extensively used as a nonprescription drug to treat diarrhea caused by bacteria since the 1950s.12) It is showed that berberine might be one of the principal antidiabetic components of Rhizoma coptidis.13) In the present study, we undertook a longer experimental period to determine the effects of berberine on hyperglycemia, hyperlipidemia, hepatic glycogenic degeneration and steatosis, hepatoprotection and PPARα/δγ protein expression in murine T2DM model induced by a low dose streptozotocin (STZ) and a high-carbohydrate/high-fat diet.

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MATERIALS AND METHODS

Animals Male Wistar rats (200±20 g, Grade II) were provided by Research Institute of Surgery Experimental Animal Center of Third Military Medical University (Chongqing, China). The study was in compliance with Declaration of Helsinki. All animals, maintained in individual cages, had unlimited access to a standard diet and tap water, and adapted to the experimental conditions (temperature: 20±2 °C, humidity: 60±5%, 12-h dark–light cycle) for 1 week.

Experimental Groups After an overnight fast, rats were administered a single i.p. injection of 35 mg/kg STZ (Sigma, Saint Louis, MO, U.S.A.) dissolved in a 0.1-mm citrate–phosphate buffer (pH 4.5) and injected immediately within few minutes to avoid degradation from 8:00 to 11:00. Non-diabetic control rats were injected with citrate-phosphate buffer alone. After injecting, all animals continued on the standard diet for 2 weeks. The development of hyperglycemia in rats was confirmed by fasting blood glucose estimation 2 weeks after STZ injection. Blood glucose was estimated for 12 h fasted rats during the daily visit (8:00—9:00) with a portable glucometer (OneTouch SureStep Meter, LifeScan Company, U.S.A.) using a drop of blood from the tail vein. The animals with fasting blood glucose level of above 16.7 mM were considered diabetic and only uniformly diabetic rats were used in the study.14 After determined glucose level, to induce diabetic hyperlipidemia, diabetic rats were allowed free access to the high-carbohydrate/high-fat diet (70% standard diet, 12% lard, 9% yolk powder, 9% plantation white sugar). Control rats were still given the standard diet. After the second fasting blood glucose estimation at the end of week 16, the STZ and the high-carbohydrate/high-fat diet induced diabetic animals became T2DM rats.14 Then the rats were divided into 7 groups: age-matched non-diabetic control rats (group A) that neither received STZ nor the high-carbohydrate/high-fat diet; diabetic rats without any drug treatment (group B); diabetic rats treated with berberine (Northeast General Pharmaceutical Factory, China) at a dose of 75, 150 or 300 mg/kg every day (group C, group D and group E, respectively), diabetic rats treated with fenofibrate (Kaifeng Pharmaceutical Group Co., Ltd., China) at a dose of 100 mg/kg or rosiglitazone (Beijing Comens Chemical Co., Ltd., China) at a dose of 4 mg/kg every day (group F and group G, both served as positive control; 10 animals in each group). Berberine, fenofibrate or rosiglitazone was mixed daily with a vehicle consisting of the standard diet for 16 weeks. The standard diet or the high-carbohydrate/high-fat diet was given only after the vehicle was completely ingested by the animals. The rats in groups A and B received the same vehicle without any drug. Animal weight was measured every 2 weeks throughout the experiment and the drug dose was accordingly adjusted. Fasting blood glucose levels were also detected after rats were fasted for 12 h at the end of week 20, 24, 28 (during treatment) and 32 (before sacrificed).

Tissue Preparation and Measurement of Lipid Metabolic Parameters After 32 weeks induction of diabetes, the overnight fasted rats were anaesthetized with an overdose of sodium pentobarbital (120 mg/kg, i.p.). After blood samples were collected from the heart (venous pool), half of each group rats were perfused with 200 ml physiology saline through the left ventricle via the ascending aorta at 25 °C, at a flow rate of 50 ml/min for 3—4 min, followed by 500 ml 4°C 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at the same flow rate for 3—4 min and then at a flow rate of 5 ml/min for 1 h. The livers were excised, weighted, dissected and fragments of the right lobe liver were postfixed in 4% paraformaldehyde overnight. The other half animals were directly sacrificed after blood collection. The livers were rapidly excised and washed with saline on ice. After excess water on the surface was removed with filter paper, the livers were weighed. The ratio of liver weight to body weight was calculated. One part of the right lobe liver was cut into slices, frozen in liquid nitrogen and stored at −70 °C for studies of lipid determination and oil red O (Sigma, Saint Louis, MO, U.S.A.) staining. Another part of the right lobe liver was postfixed in 4% paraformaldehyde overnight. Then all the tissues were dehydrated through graded ethanol series, made transparent with xylene, embedded with paraffin, cut into slices (7 mm thickness), and mounted onto 3-aminopropyltriethoxysilane-coated glass slides for periodic acid-schiff (PAS), hematoxylin eosin (HE) and immunohistochemical staining. Total cholesterol (TC), TG, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), apolipoprotein AI (ApoAI), apolipoprotein B (ApoB) and hemoglobin A1c (HbA1c) (Nanjing Jiancheng Bioengineering Institute, China) levels were measured by HITACHI 7170 automatic biochemistry analyzer (HITACHI, Japan), and TC/HDL-C ratio was calculated.

Measurement of Hepatic Glycogen and Lipid Slides were stained by PAS. A digestion step with diastase was used for a negative control in PAS staining. Hepatic glycogen content was estimated by Beckman DU 800 spectrophotometry (Beckman Coulter Inc., Fullerton, CA, U.S.A.) with commercially available hepatic glycogen/muscle glycogen detection kit (Nanjing Jiancheng Bioengineering Institute, China). Cryostat sections, 15 μm thick, were stained with oil red O and counterstained with Mayer hematoxylin. For determination of TG in liver, a portion of the right lobe liver was homogenized and the lipids were extracted with isopropanol (20 ml/g).15 TG content in the supernatant was determined by spectrophotometry with commercial kit (Nanjing Jiancheng Bioengineering Institute, China).

Morphology of the Rat Liver Tissue sections were stained with HE. The sections were also subjected to immunohistochemical staining for PPARα, δ and γ (Santa Cruz Biotechnology, U.S.A.). Briefly, the sections were deparaffinized with xylene, and rehydrated through graded concentrations of ethanol. Tissue sections were treated in a microwave oven at low power for 10 min in 10 mm sodium citrate buffer (pH 6.0). Endogenous peroxidase was inactivated using 3% hydrogen peroxide in methanol for 20 min. The sections were then incubated in protein-blocking agent for 30 min. Subsequently, the sections were incubated overnight at 4 °C with primary polyclonal rabbit anti-PPARα, δ or γ antibody, diluted 1 : 100 in phosphate buffer saline, respectively. The secondary antibody incubation was performed by applying biotinylated goat anti-rabbit IgG (Boster Biotechnology Co., Wuhan, China) for 20 min at room temperature. Sections were then incubated with streptavidin–biotin–alkaline phosphatase complex for 20 min and developed with 3,3-diaminobenzidine (Boster Biotechnology Co., Wuhan, China).
for 1 min. Light Mayer hematoxylin was applied as a counterstain. The slides were then dehydrated in a series of ethanol, in xylene, covered with DPX, and coverslipped. For negative control in the immunohistochemistry procedures performed, phosphate buffer saline replaced the primary PPARα/δ/γ antibodies. After the tissue sections were observed with an inverted microscope (DMIRB, Leica, Germany), images were obtained using a microscopy camera (MPS60, Leica, Germany) at a magnification of ×400, and the integrated optical density (area detected above threshold×mean optical density within the thresholded area) of PPARα/δ/γ expression in the sections was measured by Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, U.S.A.).

Statistical Analysis All quantitative data are expressed as mean±standard deviation (S.D.). Statistical analysis was carried out by means of one-way ANOVA using SPSS 13.0 (SPSS Inc., Chicago, U.S.A.). For all analyses, values of p<0.05 were considered statistically significant.

RESULTS

Effects of Berberine on Body Weight, Liver Weight and Liver to Body Weight Ratio  The changes of rats’ body weight, liver weight and liver to body weight ratio were shown in Table 1. Control rats grew faster than the other group ones and body weight of diabetic rats continued to increase from begin to before drug treatment (data not shown). The initial (week 16) body weight of control rats was significantly higher than that of the control ones. Both final (week 32) body weight and weight gain of control rats were remarkably higher than that of the other groups. At weeks 32, fenofibrate and middle-dose, high-dose berberine (150, 300 mg/kg) treatment obviously reduced diabetic body weight. Administration of middle-dose, high-dose berberine and fenofibrate inhibited weight gain, while neither low-dose berberine (75 mg/kg) nor rosiglitazone affected diabetic body weight and weight gain. Both diabetic liver weight and the ratio of liver weight to body weight were higher than the control ones, middle-dose, high-dose berberine treatment decreased diabetic liver weight and the ratio.

Effects of Berberine on Fasting Blood Glucose and HbA1c Levels  Blood glucose levels during the study in experimental rats were shown in Fig. 1. On weeks 2 and 16, diabetic rats both had high baseline fasting blood glucose levels. Blood glucose level of diabetic rats increased significantly comparing to that of the control ones. From weeks 20 to 32, middle-dose, high-dose berberine and rosiglitazone gradually decreased fasting blood glucose to normal level, while low-dose berberine and fenofibrate had no effect on blood glucose even before sacrificed. HbA1c level of diabetic rats was significantly higher than that of the control ones. Treated with middle-dose, high-dose berberine and rosiglitazone for 16 weeks reverted the increased diabetic HbA1c level to near the control ones, but low-dose berberine and fenofibrate didn’t affect HbA1c level (Table 2).

Effect of Berberine on Lipid Metabolic Parameters  Plasma lipid metabolic values were shown in Table 2. The TC, TG, LDL-C, ApoB levels and TC/HDL-C ratio of diabetic rats were significantly higher than those of the control ones, while HDL-C and ApoAI was remarkably lower than those of the control ones. Treated with middle-dose, high-

### Table 1. Changes of Body Weight, Liver Weight and Liver to Body Weight Ratio in Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Weight gain (g)</th>
<th>Liver weight (wt gain) (g)</th>
<th>Liver to body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>391.3±10.2</td>
<td>473.9±17.9</td>
<td>82.6±17.6</td>
<td>11.57±1.13</td>
</tr>
<tr>
<td>B</td>
<td>305.3±7.2**</td>
<td>348.6±10.6**</td>
<td>43.3±6.1**</td>
<td>17.92±1.01**</td>
</tr>
<tr>
<td>C</td>
<td>301.7±9.8</td>
<td>339.1±12.5</td>
<td>37.4±6.8</td>
<td>17.12±1.11</td>
</tr>
<tr>
<td>D</td>
<td>303.0±0.0</td>
<td>313.1±8.3*</td>
<td>10.1±3.4*</td>
<td>9.88±0.70*</td>
</tr>
<tr>
<td>E</td>
<td>303.0±7.7</td>
<td>311.0±8.3*</td>
<td>8.0±3.2*</td>
<td>9.61±0.57*</td>
</tr>
<tr>
<td>F</td>
<td>300.2±12.7</td>
<td>306.7±14.0**</td>
<td>6.5±2.4**</td>
<td>9.35±0.58**</td>
</tr>
<tr>
<td>G</td>
<td>304.3±10.3</td>
<td>352.1±15.6</td>
<td>47.8±10.8</td>
<td>18.80±1.72</td>
</tr>
</tbody>
</table>

* A vs. B, p<0.01; # A vs. C, p<0.01; ## A vs. D, p<0.01.

### Table 2. Changes of Lipid Metabolic Parameters in Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>TC/HDL-C ratio</th>
<th>ApoAI (mg/dL)</th>
<th>ApoB (mg/dL)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.77±0.28</td>
<td>0.76±0.14</td>
<td>0.51±0.10</td>
<td>0.91±0.10</td>
<td>1.98±0.39</td>
<td>0.45±0.11</td>
<td>0.18±0.04</td>
<td>35.6±9.8</td>
</tr>
<tr>
<td>B</td>
<td>2.53±0.37**</td>
<td>2.24±0.24**</td>
<td>0.77±0.20**</td>
<td>0.72±0.07**</td>
<td>3.55±0.68**</td>
<td>0.26±0.06**</td>
<td>0.31±0.06**</td>
<td>61.8±14.5**</td>
</tr>
<tr>
<td>C</td>
<td>2.45±0.63</td>
<td>2.05±0.22</td>
<td>0.74±0.22</td>
<td>0.77±0.08</td>
<td>3.20±0.88</td>
<td>0.26±0.06</td>
<td>0.30±0.06</td>
<td>59.3±15.0</td>
</tr>
<tr>
<td>D</td>
<td>1.86±0.31**</td>
<td>0.81±0.15**</td>
<td>0.59±0.29**</td>
<td>0.89±0.08**</td>
<td>2.09±0.37**</td>
<td>0.45±0.10**</td>
<td>0.18±0.04**</td>
<td>37.9±11.1**</td>
</tr>
<tr>
<td>E</td>
<td>1.84±0.49**</td>
<td>0.79±0.14**</td>
<td>0.54±0.14**</td>
<td>0.93±0.08**</td>
<td>1.99±0.54**</td>
<td>0.45±0.05**</td>
<td>0.18±0.04**</td>
<td>36.3±10.7**</td>
</tr>
<tr>
<td>F</td>
<td>1.84±0.41**</td>
<td>0.78±0.14**</td>
<td>0.53±0.17**</td>
<td>0.95±0.10**</td>
<td>1.96±0.48**</td>
<td>0.45±0.12**</td>
<td>0.18±0.05**</td>
<td>60.4±15.5</td>
</tr>
<tr>
<td>G</td>
<td>2.49±0.56</td>
<td>2.22±0.34</td>
<td>0.75±0.23</td>
<td>0.72±0.09</td>
<td>3.50±0.84</td>
<td>0.26±0.05</td>
<td>0.30±0.06</td>
<td>36.3±10.9**</td>
</tr>
</tbody>
</table>

* A vs. B, p<0.01; # A vs. C, p<0.01; ## A vs. D, p<0.01.

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dose berberine and fenofibrate for 16 weeks significantly re-
verted TC, TG, LDL-C, HDL-C, ApoAI, ApoB contents and
TC/HDL-C ratio to the control, but low-dose berberine and
rosiglitazone didn’t affect these lipid metabolic parameters.

**Histopathology of the Rat Liver**  The typical HE stain-
ing results obtained upon histological examination were
shown in Fig. 2. Hepatocyte arranged orderly and few mi-
crovesicular vacuolization, hydropic swelling, granular
degeneration and necrosis of hepatocyte appeared in the control
rats (Fig. 2A). However, the most significant alterations in di-

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**Fig. 1. Effects of Berberine, Fenofibrate or Rosiglitazone on Glucose Content in Diabetic Rats**

Group A=Control rats; group B=diabetic rats without drug treatment; group C=diabetic rats treated with berberine (75 mg/kg); group D=diabetic rats treated with berberine (150 mg/kg); group E=diabetic rats treated with berberine (300 mg/kg); group F=diabetic rats treated with fenofibrate (100 mg/kg), and group G=diabetic rats treated with rosigli-
tazone (4 mg/kg). **p<0.01 vs. A, *p<0.05 vs. B, ***p<0.01 vs. B. n=10 for each group. Mean±S.D.

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**Fig. 2. Effect of Drugs on HE Staining in the Liver**

Group A=Control rats; group B=diabetic rats without drug treatment; group C=diabetic rats treated with berberine (75 mg/kg); group D=diabetic rats treated with berberine (150 mg/kg); group E=diabetic rats treated with berberine (300 mg/kg); group F=diabetic rats treated with fenofibrate (100 mg/kg), group G=diabetic rats treated with rosigli-
tazone (4 mg/kg). Magnification ×400.

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**Fig. 3. Effects of Drugs on PAS Staining in the Liver**

Group A=Control rats; group B=diabetic rats without drug treatment; group C=diabetic rats treated with berberine (75 mg/kg); group D=diabetic rats treated with berberine (150 mg/kg); group E=diabetic rats treated with berberine (300 mg/kg); group F=diabetic rats treated with fenofibrate (100 mg/kg), group G=diabetic rats treated with rosigli-
tazone (4 mg/kg), and H=negative control in PAS staining using a digestion step with diastase. Magnification ×400.
abietic livers were disorderly hepatocyte, microvesicular vacuolization, hydropic swelling, granular degeneration and necrosis of hepatocyte (Fig. 2B). These were ameliorated markedly in middle-dose, high-dose berberine and fenofibrate treated groups (Figs. 2D—F), and no significant difference was observed among the 3 treated groups. While low-dose berberine and rosiglitazone could not improve the damaged liver tissue (Figs. 2C, G).

Effect of Berberine on Hepatic Glycogen and Lipid

The mauer glycogenosome of PAS staining was abundant, strong and well-distributed in liver cytoplasm of normal rats (Fig. 3A), but it reduced expression, glycogenosome amount decreased, uneven-distributed, its volume reduced and even disappeared in diabetes mellitus (Fig. 3B). Middle-dose, high-dose berberine and rosiglitazone all decreased glycogenosome expression, amount and distribution (Figs. 3D, E, G). Low-dose berberine and fenofibrate neither had effect on PAS staining (Figs. 3C, F). Hepatic glycogen level in diabetes, low-dose berberine and fenofibrate groups were markedly lower than that of the other four groups by kit detection (Fig. 4). Regardless of groups, small amounts of fat accumulation were observed in the liver as microvesicles of fat within periportal hepatocytes. Livers from control rats showed minimal visible fat in hepatic cells as demonstrated by the sparse distribution of small microvesicles within hepatocytes. By contrast, livers from diabetic rats showed widespread deposition of fat globules of different sizes inside hepatic cells. The fat accumulation in diabetic rat liver fed berberine was found to be similar to that of the control (Fig. 5). TG content in diabetic liver was obviously higher than that of control ones; middle-dose, high-dose berberine and fenofibrate treatment reverted TG level nearly to normal, while low-dose berberine and rosiglitazone had no effect on increased TG of diabetic liver by Kit detection (Fig. 4).

Immunohistochemical Stains for PPARα/δ/γ in the Liver

Immunohistochemical analysis of liver tissue sections revealed that PPARα/δ expression, as brown or yellow granules in cytoplasm and nucleus, was apparently decreased, while PPARγ obviously increased in response to 35 mg/kg STZ and 30-week feeding of the high-carbohydrate/high-fat diet. Middle-dose, high-dose berberine and rosiglitazone significantly reduced PPARγ expression in diabetic rat liver, but low-dose berberine and fenofibrate had no effect on PPARγ expression. While middle-dose, high-dose berberine and fenofibrate obviously increased PPARα/δ expression, low-dose berberine and rosiglitazone could not inhibit PPARα/δ expression (Figs. 6—8, Table 3).

DISCUSSION

Rosiglitazone, a newly and efficiently antidiabetic drug and function as insulin-sensitizing agent, is currently in clinical use for lowering glucose levels in diabetics, but does not decrease lipid levels. In both diabetic and nondiabetic patients, hypertriglyceridermia is often treated with fenofibrate, mainly due to its’ hypolipidemic actions, such as lowering TG and raising HDL levels. Fenofibrate does not improve glucose level, but is associated with better glycemia control in T2DM. Thus, rosiglitazone and fenofibrate were selected for evaluating hypoglycemic and hypolipidemic activities in this diabetic-dyslipidemic model both as positive control drugs. There might be major clinical utility, therefore, for novel therapeutic agents that can correct both hyperglycemia and diabetic hyperlipidemia. It is well known that in uncontrolled T2DM, the rise in blood glucose is accompanied with the increase in TC, TG, LDL-C levels and decrease in HDL-C levels of diabetic rats, which contribute to several complications including coronary artery disease. From this point of view, it is encouraging that berberine brought down the elevated contents of TC, TG, LDL-C, ApoB and increased HDL-C, ApoAI levels in diabetic animals. The increase in HDL-C level indicates that berberine may be beneficial to diabetic individuals with atherosclerosis, because elevated HDL-C level is associated with the lowered risk of the development of atherosclerosis in diabetes mellitus. What’s more, TC/HDL-C ratio is a marker of hyperlipidemia, berberine restored the increased TC/HDL-C ratio in diabetic rats to the control level.

Combined treatments with PPARα and PPARγ agonists may potentially improve insulin resistance and alleviate atherogenic hyperlipidemia, whereas PPARγ properties may prevent the development of overweight which typically accompanies “pure” PPARγ ligands. With extended use, these effects can decrease the risk of long-term cardiovascular complications. PPARα and PPARγ stimulation play complementary roles in the prevention of atherosclerosis. Thus, compounds with PPARα, PPARδ and PPARγ activity appear
Fig. 5. Oil Red O Stains Distribution in Experimental Liver

Group A = Control rats; group B = diabetic rats without drug treatment; group C = diabetic rats treated with berberine (75 mg/kg); group D = diabetic rats treated with berberine (150 mg/kg); group E = diabetic rats treated with berberine (300 mg/kg); group F = diabetic rats treated with fenofibrate (100 mg/kg), and group G = diabetic rats treated with rosiglitazone (4 mg/kg). Brown or yellow stains in Figs. 5A, D, E and F were weaker than that in Figs. 5B, C and F. No brown or yellow stains in Fig. 5H, the negative immunohistochemistry of PPARα. Magnification ×400.

Fig. 6. Effects of Drugs on PPARα Expression in Liver

Group A = Control rats; group B = diabetic rats without drug treatment; group C = diabetic rats treated with berberine (75 mg/kg); group D = diabetic rats treated with berberine (150 mg/kg); group E = diabetic rats treated with berberine (300 mg/kg); group F = diabetic rats treated with fenofibrate (100 mg/kg), and group G = diabetic rats treated with rosiglitazone (4 mg/kg). Brown or yellow stains in Figs. 6A, D, E and F were stronger than that in Figs. 6B, C and G. No brown or yellow stains in Fig. 6H, the negative immunohistochemistry of PPARα. Magnification ×400.

Fig. 7. Effects of Drugs on PPARβ Expression in Liver

Group A = Control rats; group B = diabetic rats without drug treatment; group C = diabetic rats treated with berberine (75 mg/kg); group D = diabetic rats treated with berberine (150 mg/kg); group E = diabetic rats treated with berberine (300 mg/kg); group F = diabetic rats treated with fenofibrate (100 mg/kg), and group G = diabetic rats treated with rosiglitazone (4 mg/kg). Brown or yellow stains in Figs. 7A, D, E and F were stronger than that in Figs. 7B, C and G. No brown or yellow stains in Fig. 7H, the negative immunohistochemistry of PPARβ. Magnification ×400.

Fig. 8. Effects of Drugs on PPARγ Expression in Liver

Group A = Control rats; group B = diabetic rats without drug treatment; group C = diabetic rats treated with berberine (75 mg/kg); group D = diabetic rats treated with berberine (150 mg/kg); group E = diabetic rats treated with berberine (300 mg/kg); group F = diabetic rats treated with fenofibrate (100 mg/kg), and group G = diabetic rats treated with rosiglitazone (4 mg/kg). Brown or yellow stains in Figs. 8A, D, E and G were weaker than that in Figs. 8B, C and F. No brown or yellow stains in Fig. 8H, the negative immunohistochemistry of PPARγ. Magnification ×400.
well-suited for the treatment of diabetic patients with the additional risk factor of hyperlipidemia. PPARγ expression was apparently increased, while PPARα/δ expressions were obviously decreased in diabetic rats. Administration of berberine to diabetic animals tended to bring the PPARα, PPARδ and PPARγ protein to near the control levels. Berberine inhibited PPARγ mRNA and protein levels in 3T3-L1 preadipocytes and even suppressed PPAR target genes involved in adipocyte differentiation, what's more, berberine inhibited the full-length PPARγ and α transcription activities by reporter gene assays. But in this study, berberine obviously increased both PPARα and PPARδ protein levels of diabetic rat liver which contributed to improve lipid metabolism. The reason may lie in that PPARs have different expression and action in different tissues and regulation of PPARs expressions in diabetic tissues were different from that in the related cultured cells. In the renal cortex and medulla of obesity-prone rats, PPARγ mRNA expression was significantly lower compared with that in obesity-resistant rats. PPARγ ligand treatment increased PPARγ expression in obesity-prone rats. But PPARγ ligand treatment caused a significant decrease in PPARγ mRNA and protein expressions in adipose tissue. PPARγ protein expression significantly increased in diabetic mouse placentas as compared with in normal placenta, while, PPARγ ligand attenuated the significantly enhanced PPARγ expression at high glucose condition. But high glucose caused both PPARα mRNA and protein expression reductions in islets or INS (832/13) β-cells. Berberine inhibited lipid synthesis in human hepatocytes not only through elevation the low density lipoprotein receptor expression, but also through the activation of AMP-activated protein kinase (AMPK).

Experimentally induced diabetic animal models showed anatomical and functional alterations in the liver, which accords with liver histopathologic changes in our diabetic rats. Berberine attenuated hepatic histopathology of diabetic rats to near the control ones. It has been reported that the high-carbohydrate/high-fat diet promoted body weight, liver weight and liver to body weight ratio, which is the similar to high-carbohydrate/high-fat diet. This study provides results, which are potentially important for supporting the extension of these findings to clinical trials, to demonstrate the effectiveness of berberine in the treatment of diabetic cardiovascular complications, such as atherosclerosis and coronary artery disease, through modulation of abnormal glucose and lipid metabolism.

REFERENCES


In summary, our findings show that berberine has PPARs activator activities, and improves hyperglycemia, hyperlipidemia and hepatic glycogenic degeneration, steatosis, histopathologic change in T2DM rats, a diabetic animal model induced by 35 mg/kg STZ and the high-carbohydrate/high-fat diet. This study provides results, which are potentially important for supporting the extension of these findings to clinical trials, to demonstrate the effectiveness of berberine in the treatment of diabetic cardiovascular complications, such as atherosclerosis and coronary artery disease, through modulation of abnormal glucose and lipid metabolism.

REFERENCES


