Effects of Berberine on Glucose Metabolism In Vitro

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The action of berberine was compared with metformin and troglitazone (TZD) with regard to the glucose-lowering action in vitro. HepG2 cell line, phenotypically similar to human hepatocytes, was used for glucose consumption (GC) studies. Cell proliferation was measured by methylthiotetrazole (MTT) assay. In moderate high glucose concentration (11.1 mmol/L), GC of HepG2 cells was increased by 32% to 60% ($P < .001$ to $P < .0001$) with 5×10^{-6} mol/L to 1×10^{-4} mol/L berberine, which was comparable to that with 1×10^{-3} mol/L metformin. The glucose-lowering effect of berberine decreased as the glucose concentration increased. The maximal potency was reached in the presence of 5.5 mmol/L glucose, and it was abolished when the glucose concentration increased to 22.2 mmol/L. The effect was not dependent on insulin concentration, which was similar to that of metformin and was different from that of TZD, whose glucose-lowering effect is insulin dependent. TZD had a better antihyperglycemic potency than metformin when insulin was added ($P < .001$). In the meantime, a significant toxicity of the drug to HepG2 cells was also observed. The βTC3 cell line was used for insulin release testing, and no secretogogue effect of berberine was observed. These observations suggest that berberine is able to exert a glucose-lowering effect in hepatocytes, which is insulin independent and similar to that of metformin, but has no effect on insulin secretion.

BERBERINE IS AN antibiotic, which used to be a regular drug in treating gastrointestinal infections.[1] In China in the 1980s, a hypoglycemic effect was accidentally found when berberine was administered to diabetic patients with diarrhea.[2] Since then berberine has often been used as an antihyperglycemic agent by many physicians in China. There have been substantial amounts of clinical and experimental reports about new actions of berberine. For example, berberine was found to have a similar effect to metformin on improving insulin sensitivity in high–fat-fed rats.[3]

Because the liver is the center of glucose metabolism, it synthesizes more glucose when its function is altered in diabetes.[4] HepG2, a human hepatocellular carcinoma line,[5] was used to study whether berberine was able to exert a glucose-lowering effect in hepatocytes. A detailed comparison of how berberine, metformin, and troglitazone (TZD) regulated glucose metabolism was also performed. βTC3, a β-cell line derived from transgenic mice expressing a hybrid insulin gene-oncogene,[6] was also used to determine whether berberine was able to stimulate the secretion of insulin.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, and other culture reagents were obtained from Gibco Life Technologies (Grand Island, NY). The 96-well and 24-well tissue culture plates were purchased from Nalge Nunc International (Roskilde, Denmark). Methylthiotetrazole (MTT) was purchased from Sigma Chemicals (St Louis, MO). Bovine serum albumin (BSA) was purchased from Shanghai Shisheng Cell Biological Technologies (Shanghai, China). Glucose oxidase was purchased from Shanghai Shensuo Reagents (Shanghai, China). The insulin radioimmunoassay kit was purchased from Shanghai Institute of Biological Products (Shanghai, China). Gliclazide was obtained from Shanghai Institute of Pharmaceutical Industry (Shanghai, China). Berberine was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The structure of berberine is given in Fig 1.

Glucose Consumption

HepG2 cell line was purchased from Tsucuba Cell Bank (Tsucuba, Japan). The cells were grown in DMEM (5.5 mmol/L glucose) containing 10% fetal bovine serum. Two days before the experiments, the cells were plated into 96-well tissue culture plates with some wells left blank. After the cells reached confluence, the medium was replaced by DMEM supplemented with 0.2% BSA and glucose at various concentrations. After 12 hours, the medium was removed and the same BSA DMEM containing berberine, metformin or TZD, and/or insulin was added to all wells including the blank wells. Finally, the medium was removed and its glucose concentrations were determined by the glucose oxidase method. The amount of glucose consumption (GC) was calculated by the glucose concentrations of blank wells subtracting the remaining glucose in cell plated wells.

Insulin Release

βTC3 cell line was kindly given by Shimon Efat from the Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel. The cells were grown in RPMI 1640 containing 15% fetal bovine serum and were plated into 24-well cultures before the experiments. On day 3 or 4, the medium was replaced with the new one containing berberine or gliclazide. After a 24-hour incubation, the medium was removed and the insulin concentrations were determined by radioimmunoassay. A total of 100 μL insulin standards, controls, or media was added to the appropriate tubes, and 100 μL insulin antiserum and [I^{125}]insulin reagent were added sequentially. After all tubes had been incubated at 2°C to 8°C for 16 hours, 100 μL precipitating reagent was added, and they were incubated again at room temperature for 30 minutes. All tubes except total count tubes were then centrifuged and decanted. Finally, each tube was counted in a COBRA autogamma counter (Canberra Industries, Meriden, CT) for 1 minute.

MTT Method

MTT was dissolved at a concentration of 5 mg/mL in sterile phosphate-buffered saline (PBS). One vol 5 mg/mL stock solution of MTT was mixed with 9 vol DMEM. It was added to the 96-well or 24-well

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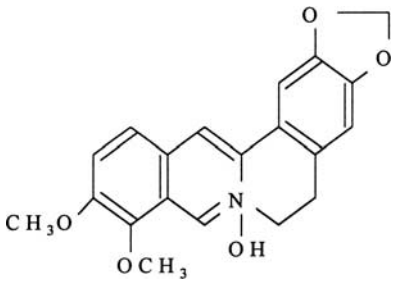

Fig 1. Structure of berberine.

culture plates when the test of GC or the test of insulin release was finished. After a 4-hour incubation at 37°C, the MTT medium was replaced with dimethyl sulfoxide (DMSO). After shaking, the optical densities (OD) at 570 nm were measured using a Labsystems Multiskan MS (Thermo Labsystems, Helsinki, Finland).[7,8]

Statistical Analyses

Results are presented as mean ± SEM. Statistical analysis was performed using Statistical Analysis Software (version 6.04; SAS Institute, Cary, NC). Significance was assessed by analysis of variance (ANOVA) followed by Duncan's or Dunnett's test for the comparisons of multiple means within an experiment. P less than .05 was considered statistically significant.

RESULTS

Effects of Berberine in HepG2 Cells

In moderate hyperglycemia (11.1 mmol/L), a significant glucose-lowering effect of berberine was observed. In doses of 5×10^{-6}, 1×10^{-5}, 2×10^{-5}, 5×10^{-5}, and 1×10^{-4} mol/L, the GC rates were increased by 32% to 60% ($P < .001$ to $P < .0001$) (Fig 2). In the same condition, the GC was elevated by 50% in the presence of 1×10^{-3} mol/L metformin ($P < .0001$) (data not shown). The effect was similar to that of berberine.

The glucose-lowering effect of berberine was not due to an increment of cell number. On the contrary, berberine ($\geq 5 \times 10^{-6}$ mol/L) depressed the growth of HepG2 cells because MTT OD was decreased markedly after berberine treatment ($P < .0001$). At the same time metformin induced no change in MTT OD (data not shown).

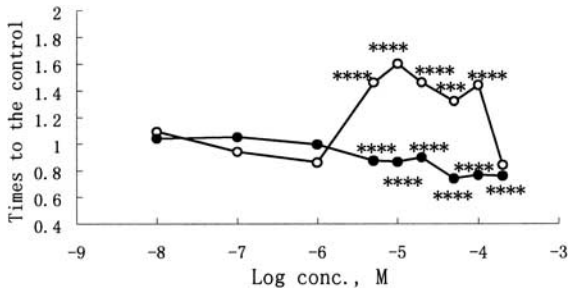

Fig 2. Dose-dependent effects of berberine at 11.1 mmol/L glucose in HepG2 cells. GC (○) and MTT OD (●) were measured after a 24-hour incubation with increasing concentrations of berberine. Data are mean values of berberine over mean values of control; n = 9 in each condition. ***$P < .001$; ****$P < .0001$ berberine *v* control.

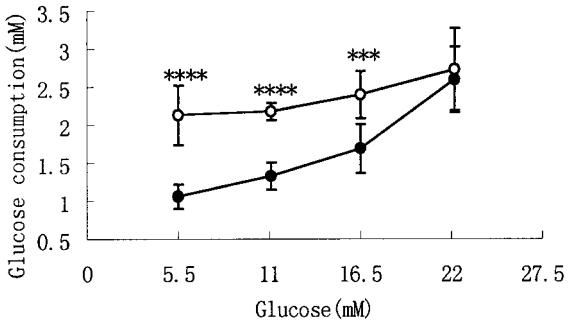

Fig 3. Dose-dependent effects of glucose on berberine's glucose-lowering effect in HepG2 cells. GCs were measured after a 24-hour incubation with 10^{-4} mol/L berberine (○) or control (●). Data are means ± SEM; n = 9 in each condition. ***$P < .001$; ****$P < .0001$ *v* control.

The GC of HepG2 cells was upregulated by the hyperglycemia without any drugs or insulin. When the glucose concentration increased from 5.5 mmol/L to 22.2 mmol/L, the GC was elevated from 1.06 mmol/L to 2.60 mmol/L ($P < .0001$) (Fig 3). The glucose-lowering effect of berberine declined as the glucose concentration was increased. The maximal potency of berberine was reached in the presence of 5.5 mmol/L glucose, and the amount of glucose consumed in 24 hours was enhanced by 1.07 mmol/L (101%, $P < .0001$). When the glucose concentration increased to 11.1 mmol/L or 16.5 mmol/L, the glucose-lowering effects of berberine were weakened, the GC was increased by 0.85 mmol/L (64%, $P < .0001$) and 0.71 mmol/L (42%, $P < .001$) respectively. When the glucose concentration increased further (22.2 mmol/L), the glucose-lowering effect of berberine was abolished. Data in Fig 3 were obtained at 1×10^{-4} mol/L berberine. The same trials were also performed at 5×10^{-5} mol/L berberine, and results were similar to that of 1×10^{-4}mol/L berberine (data not shown).

Insulin added solely to the culture medium had no significant effect on the GC of HepG2 cells. Insulin (10^{-9} mol/L or 10^{-7} mol/L) also had no influence on the glucose-lowering effect of berberine (Fig 4).

Effects of Metformin and TZD in HepG2 Cells

At a high glucose concentration (22.2 mmol/L), 10^{-3} mol/L metformin had an evident glucose-lowering effect, and the GC was increased by 48% ($P < .0001$) (Fig 5A). However, TZD did not perform the same way. The latter depressed markedly the proliferation of HepG2 cells, and the MTT OD was reduced by 23.7% ($P < .0001$). When 10^{-7} mol/L insulin was added, the GC and MTT OD of control and metformin remained unchanged. But a much higher toxicity of TZD was observed. The MTT OD was decreased by 41.6% compared with control ($P < .0001$) (Fig 5B). Because of the remarkable reduction of the cells number, the GC needed to be adjusted by MTT OD. The greater glucose-lowering effect of TZD than that of metformin was observed after the adjustment. The GC of certain cells was increased by 95.8% ($P < .0001$) (Fig 5C).

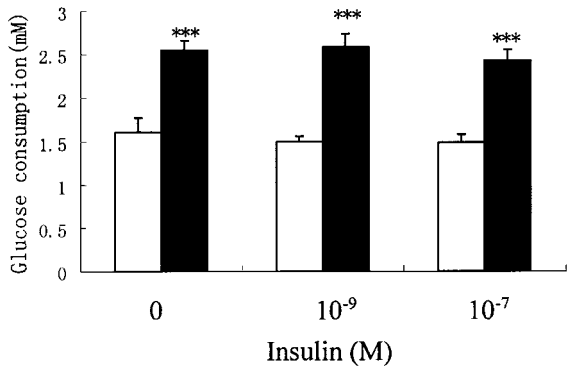

Fig 4. Glucose-lowering effect of berberine in HepG2 cells with insulin. The cells were incubated with 2 × 10^{-5} mol/L berberine (■) or control (□) in the presence or absence of insulin at 11.1 mmol/L glucose for 24 hours. Data are means ± SEM; n = 9 in each condition. ***$P < .001$ *v* control.

Insulin Secretogogue Effect of Gliclazide

Because βTC3 is an insulinoma cell line, we planned to test whether its secretion was regulated by the additional stimulation, such as that caused by sulfonylureas. At first, gliclazide was added to the test to effect on insulin release. βTC3 cells were stimulated to secret much more insulin with gliclazide, which was dose dependent. A 32% increment was induced by 10^{-6}mol/L gliclazide and 51% was induced by 10^{-5} mol/L ($P < .001$), indicating that these β cells cultured were able to secret insulin.

Effects of Berberine in βTC3 Cells

No insulin secretogogue effect of berberine was observed (Fig 6). At the effective concentration, 10^{-6} mol/L, insulin secretion was decreased significantly ($P < .01$). It might, however, be caused by the inhibitory effect of berberine on cell proliferation. The reduction of insulin release disappeared after the insulin concentrations had been adjusted by MTT OD.

DISCUSSION

Liver plays a critical role in glucose metabolism. In diabetes, hyperglycemia is partly contributed to by less glycogen synthesis and more glucogenesis.[9] Recent studies have shown that reduction of hepatic glucose output might be the chief basis for the use of metformin in treating diabetes.[10,11] Treatment of animals with metformin decreased the ability of glucagon to stimulate adenylate cyclase activity.[12] Metformin also inhibits gluconeogenesis in hepatocyte cultures by potentiating the allosteric activation of pyruvate kinase by fructose-1,6-diphosphate.[13] Because its glucose-lowering effect in HepG2 cells was not affected by insulin, our in vitro data indicate that the effect is insulin independent. In contrast to metformin, TZD performed another mode of action. It had no effect without insulin, but better effect than metformin with insulin. TZD requires insulin as a cofactor for its stimulation of glucose metabolism in HepG2 cells. In streptozotocin-treated mice, TZD is ineffective at lowering glucose.[14] These data demonstrate that the glucose-lowering effect of TZD is attributed to enhancement of insulin action. TZD has been found to be a high-affinity ligand for the peroxisome proliferator-activated receptor γ(PPARγ), which belongs to a nuclear receptor superfamily.[15] PPARγand retinoid X receptor form a heterodimer, which in the presence of a ligand, such as TZD, bind to DNA response elements that help regulate expression of target genes.[16] Effects of TZD are due to activation of PPARγ-mediated gene transcription and adipogenesis.[17] Because PPARγis expressed at a high level in fat tissue and at low levels in many tissues including liver, studies on the action of TZD have been focused on fat, muscle, and the cardiovascular system.[18,19] There has been little attention about TZD on the liver.

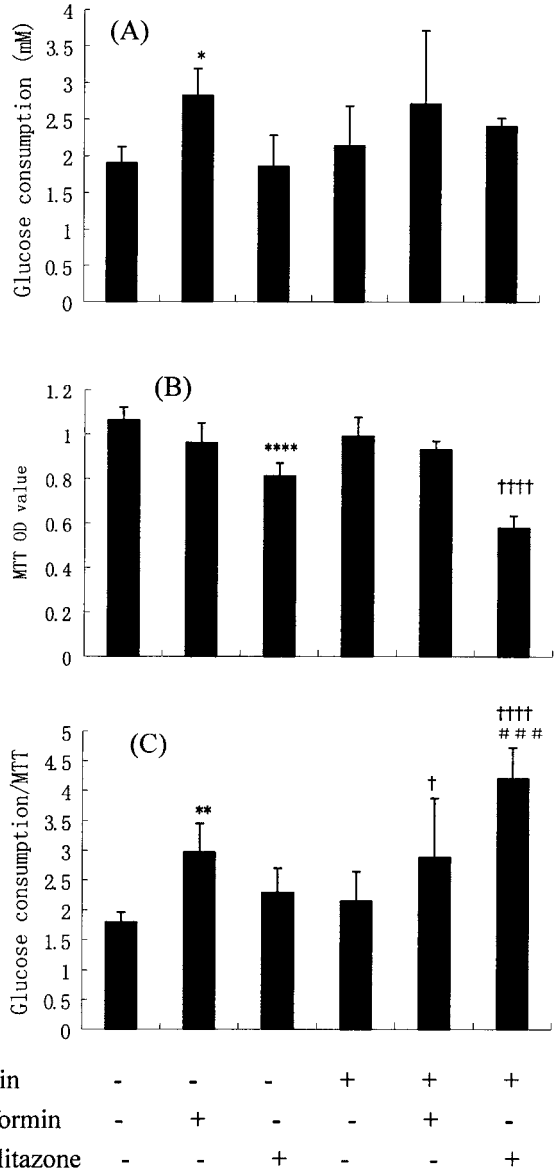

Fig 5. Effects of 10^{-3} mol/L metformin and 10^{-5} mol/L TZD on (A) GC, (B) MTT, and (C) GC/MTT in HepG2 cells. The tests were performed at 22.2 mmol/L glucose in the presence or absence of 10^{-7} mol/L insulin. Data are means ± SEM; n = 9 in each condition. *$P < .05$; **$P < .01$; ****$P < .0001$ *v* control. †$P < .05$; ††††$P < .0001$ *v* insulin. ###$P < .001$ *v* insulin + metformin.

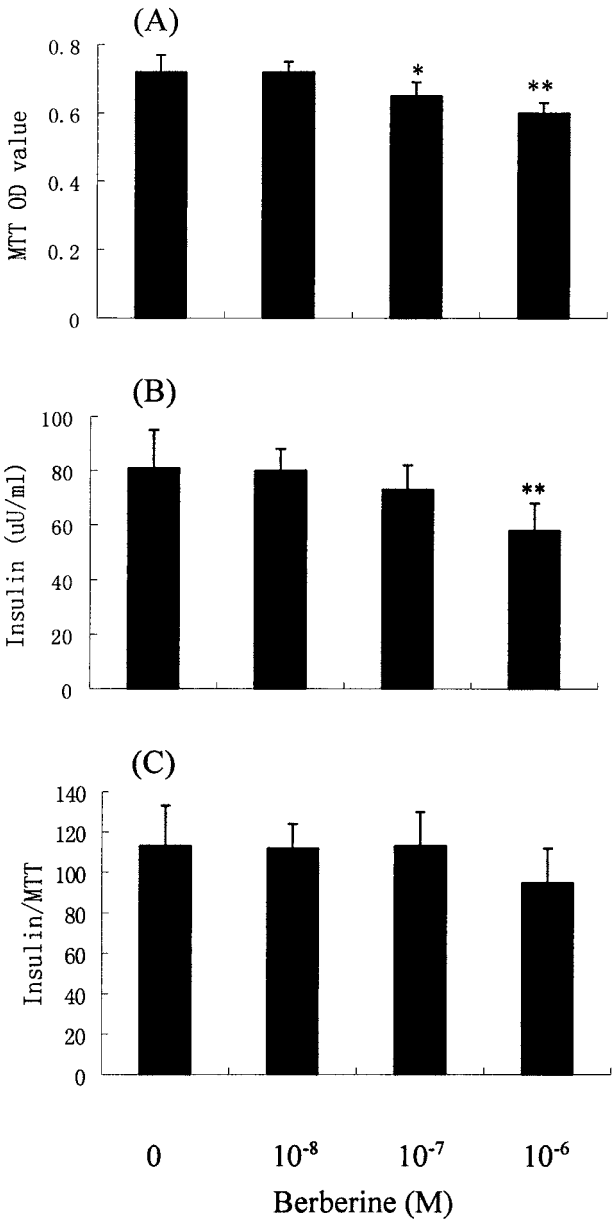

Fig 6. Effects of berberine on (A) insulin release, (B) MTT, and (C) insulin/MTT in βTC3 cells. Data are means ± SEM; n = 6 in each condition. *$P < .05$; **$P < .01$ *v* control.

Our results demonstrated clearly that TZD could still exert a nice antidiabetic effect on hepatocytes in the presence of insulin.

Berberine, its molecular formula, $C_{20}H_{19}NO_5$, with a molecular weight of 353.36, is the main active component of an ancient Chinese herb *Coptis chinensis French*. The drug being used in clinics is extracted from *Berberis amurense Rupr*., *Phellodendron amurense Rupr*., or some other plants. It has many chemical forms, such as berberine hydrochloride, berberine sulfate, berberine citrate, or phosphate.[20] Its most popular form is hydrochloride ($B \cdot HCl \cdot 2H_2O$), which is also our choice. Berberine and related isoquinoline alkaloids are quite different from sulfonylureas, biguanides, or thiazolidinediones. Hence, berberine might belong to a new class of hypoglycemic agents. Due to previous studies, berberine is known to have a wide range of antidiabetes effects, eg, hypoglycemic, insulin sensitizing, and affecting aldose reductase activities.[2,3,21] Our study focused on its hypoglycemic effect, and we intended to investigate whether the underlying mechanisms are similar to metformin or TZD.

A total of 5×10^{-6} mol/L to 1×10^{-4} mol/L berberine could exert a significant glucose-lowering effect, which is present at normal (5.5 mmol/L) or moderate high glucose (11.1 mmol/L and 16.5 mmol/L) and absent in very high glucose (22.2 mmol/L). Insulin could not affect the action of berberine, which is similar to metformin. However, metformin is effective in 22.2 mmol/L glucose, the same as in 11.1 mmol/L glucose. In the clinic, metformin could be used to treat diabetes regardless of the insulin and glucose levels. However, berberine could be used in impaired glucose tolerance, impaired fasting glucose,[22] or diabetes when blood glucose is moderately elevated. The strongest antihyperglycemic effect was observed at normal glucose, thus berberine may be helpful to decrease insulin release in the fasting state and do good to β-cell function. In brief, berberine might be beneficial for islets function recovery in diabetes or in prediabetes. Berberine might not bring about fasting hypoglycemia because no insulin release is stimulated.

The tetrazolium salt (MTT) method involves conversion of MTT to colored formazan by cells serving as indirect measurements of cell growth/cell kill. Because there is a linear relationship between MTT OD and cell numbers, the MTT assay is widely used to monitor cell proliferation.[7,8] In the present study, significant toxicity of TZD and berberine to cells was observed via the MTT method.

TZD has been found to cause significant hepatic toxicity, which has resulted in death or the need for liver transplantation in a small number of patients.[23,24] A previous study demonstrated TZD was toxic to rat hepatocytes at a concentration of 20 μmol/L in vitro.[25] Our study indicates that appreciable toxicity to human hepatocytes is present at a concentration of 10 μmol/L in vitro.

Berberine was also found to cause toxicity in HepG2 and βTC3 cells in our study. However, the hepatotoxicity or pancreotoxicity induced by berberine has never been observed clinically. Berberine is one type of mitotic poison able to inhibit neoplastic cells to synthesis of nucleic acid.[26] MTT OD lowered by berberine might be due to one of its anticancer effects because HepG2 and βTC3 cells are both immortalized cell lines.

REFERENCES

1. Sun D, Abraham SN, Beachey EH: Influence of berberine sulfate on synthesis and expression of Pap fimbrial adhesin in uropathogenic Escherichia coli. Antimicrob Agents Chemother 32:1274-127, 1988

2. Ni YX, Liu AQ, Gao YF, et al: Therapeutic effect of berberine on 60 patients with type II diabetes mellitus and experimental research. Zhong Xi Yi Jie He Za Zhi 8:711-713, 1988

3. Gao CR, Zhang JQ, Huang QL: Experimental study on the insulin-sensitivity-increasing effects of berberine in insulin resistant rat models. Zhong Xi Yi Jie He Za Zhi 17:162-164, 1997

4. McCarty MF: High-dose biotin, an inducer of glucokinase expression, may synergize with chromium picolinate to enable a definitive nutritional therapy for type II diabetes. Med Hypotheses 52:401-406, 1999

5. Wilson DB, Salem HH, Mruk JS, et al: Biosynthesis of coagulation Factor V by a human hepatocellular carcinoma cell line. J Clin Invest 73:654-658, 1984

6. Efrat S, Linde S, Kofod H, et al: Beta-cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogene. Proc Natl Acad Sci USA 85:9037-9041, 1988

7. Hansen MB, Nielsen SE, Berg K: Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 119:203-210, 1989

8. Loosdrecht AA, Nennie E, Ossenkoppele G, et al: Cell mediated cytotoxicity against U 937 cells by human monocytes and macrophages in a modified colorimetric MTT assay. J Immunol Methods 141:15-22, 1991

9. Treadway JL, Mendys P, Hoover DJ: Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus. Expert Opin Investig Drugs 10:439-454, 2001

10. Wiernsperger NF: Membrane physiology as a basis for the cellular effects of metformin in insulin resistance and diabetes. Diabetes Metab 25:110-127, 1999

11. Wiernsperger NF, Bailey CJ: The antihyperglycemic effect of metformin: Therapeutic and cellular mechanisms. Drugs 58:75-82, 1999 (suppl 1)

12. Gawler DJ, Wilson A, Houslay MD: Metformin treatment of lean and obese Zucker rats modulates the ability of glucagon and insulin to regulate hepatocyte adenylate cyclase activity. J Endocrinol 122:207-212, 1989

13. McCarty MF: A proposal for the locus of metformin's clinical action: Potentiation of the activation of pyruvate kinase by fructose-1, 6-diphosphate. Med Hypotheses 52:89-93, 1999

14. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37:1549-1558, 1988

15. Lehmann JM, Moore LB, Smith-Oliver TA, et al: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ(PPAR γ). J Biol Chem 270:12953-12956, 1995

16. Kliewer SA, Umesono K, Noonan DJ, et al: Convergence of 9-cis retinoic anid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. Nature 358:771-774, 1992

17. Berger JP, Bailey C, Biswas CA, et al: Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-γ: Binding and activation correlate with antidiabetic actions in db/db mice. Endocrinology 137:4189-4195, 1996

18. Spiegelman BM: PPAR-γ: Adipogenic regulator and thiazolidinedione receptor. Diabetes 47:507-514, 1998

19. Hsueh WA, Law RE: PPARgamma and atherosclerosis: Effects on cell growth and movement. Arterioscler Thromb Vasc Biol 21:1891-1895, 2001

20. Berberine, in Chinese Materia Medica Information Center of National Medical Management Bureau: Handbook of Effective Compositions in Plants, vol 1. Beijing, China, Hygienic, 1991, pp 12-18

21. Liu CS, Dong YH, Pang lN, et al: The effects of baicalin and berberine on proteinuria and glomerular ultrastructure in alloxan-induced diabetic rats. Zhong Guo Tang Niao Bing Za Zhi 4:163-166, 1996

22. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539-553, 1998

23. Wagenaar LJ, Kuck EM, Hoekstra JB: Troglitazone. Is it all over? Neth J Med 55:4-12, 1999

24. Plosker GL, Faulds D: Troglitazone: A review of its use in the management of type 2 diabetes mellitus. Drugs 57:409-438, 1999

25. Elcock FJ, Lyon JJ, Hitchcock J, et al: Toxicity of troglitazone in cultured rat hepatocytes. Presented at the American Diabetes Association Meeting, San Diego, CA, June 19-22, 1999

26. *Coptis chinensis French*, in Jiangshu New Medical College: Chinese Materia Medica Dictionary, vol 1. Shanghai, China, Academic, 1986, pp 2024-2025