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Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression

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Abstract

Our previous work demonstrated that berberine (BBR) increases insulin receptor (InsR) expression and improves glucose utility both in vitro and in animal models. Here, we study the InsR–up-regulating and glucose-lowering activities of BBR in humans. Our results showed that BBR increased InsR messenger RNA and protein expression in a variety of human cell lines, including CEM, HCT-116, SW1990, HT1080, 293T, and hepatitis B virus–transfected human liver cells. Accordingly, insulin-stimulated phosphorylations of InsR β-subunit and Akt were increased after BBR treatment in cultured cells. In the clinical study, BBR significantly lowered fasting blood glucose (FBG), hemoglobin A_{1c}, triglyceride, and insulin levels in patients with type 2 diabetes mellitus (T2DM). The FBG- and hemoglobin A_{1c}–lowering efficacies of BBR were similar to those of metformin and rosiglitazone. In the BBR-treated patients, the percentages of peripheral blood lymphocytes that express InsR were significantly elevated after therapy. Berberine also lowered FBG effectively in chronic hepatitis B and hepatitis C patients with T2DM or impaired fasting glucose. Liver function was improved greatly in these patients by showing reduction of liver enzymes. Our results confirmed the activity of BBR on InsR in humans and its relationship with the glucose-lowering effect. Together with our previous report, we strongly suggest BBR as an ideal medicine for T2DM with a mechanism different from metformin and rosiglitazone.

1. Introduction

The insulin receptor (InsR) is a membrane-spanning glycoprotein that is essential for the action of insulin. Binding of insulin to InsR in the liver, muscles, or adipose tissues triggers multiple intracellular pathways that cause glycogen synthesis and glucose uptake increase, as well as hepatic/muscle glucose output reduction. The blood glucose level is thus lowered [1,2]. This is one of the major mechanisms for the human body to keep glucose homeostasis. Disruption of the expression of InsR generates a hyperglycemic phenotype in mice [3]. Type 2 diabetes mellitus (T2DM) is a human hyperglycemic state characterized by insulin resistance in peripheral tissues, particularly the liver, muscles, adipocytes, and pancreatic β-cells [1,4,5]. About 92% of the patients with T2DM show insulin resistance [6]. Individuals with insulin resistance have either decreased levels or absence of InsR expression [7-9]. Thus, InsR is considered as a potential target to treat T2DM and insulin resistance, in which the intrinsic tyrosine kinase could be activated for insulin signaling. At the present time, small–molecular weight compounds that mimic insulin

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action are under development aiming for the discovery of hypoglycemic insulin mimetics [8,10,11]. With similar concept, novel InsR up-regulators may also have clinical benefit on improving insulin sensitivity and lowering blood glucose in T2DM. With the adverse effect reports for the thiazolidinediones (TZDs) [12,13], hypoglycemic drugs with new targets are highly desirable.

Berberine (BBR) is a natural compound and has been a nonprescription medicine for diarrhea in China since the 1950s [14-16]. In 2004, we found that BBR is a promising cholesterol-lowering drug that increases the expression of the low-density lipoprotein receptor through an extracellular signal–regulated kinase–dependent mechanism [17-21]. Our recent study revealed that BBR also up-regulates the expression of the InsR gene in the liver and muscle cells [22]. Different from its action on low-density lipoprotein receptor, BBR increases InsR expression at the transcriptional level by stimulating InsR promoter; protein kinase C activation is essential for its activity [22]. Our results support the hypoglycemic effect of BBR observed in patients with T2DM [23]. The present study bridges our mechanism research with the clinical observations.

2. Materials and methods

2.1. Cell culture

CEM, HCT-116, SW1990, HT1080, 293T cells, and the hepatitis B virus (HBV) full genome–transfected human liver cells (HepG2.2.15 cells) were maintained in minimum essential medium (Gibco-Invitrogen, Grand Island, NY) containing 10% fetal bovine serum and antibiotics. Cells were cultured in an atmosphere of 5% CO_2 at 37°C. One day before treatment, cells were trypsinized and allowed to grow to about 80% confluence. Afterward, fresh media supplemented with vehicle (dimethyl sulfoxide [DMSO]) or BBR (Sigma Chemical, St Louis, MO) were added to cells as indicated. Cells were treated for 12 hours. For experiments with insulin stimulation, 0.5 or 10 nmol/L of human insulin (Sigma Chemical) was added to the media; cells were treated for an additional 15 minutes before harvest.

2.2. RNA isolation and real-time reverse transcriptase polymerase chain reaction

Total RNAs were isolated from cells using the TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the protocol. For reverse transcription, 1 μg of total RNA from each sample was used as template in a 20-μL reaction system containing random primers and the avian myeloblastosis virus (AMV) reverse transcriptase (Promega, Madison, WI). The reverse transcription reactions were conducted at 42°C for 20 minutes and then inactivated at 95°C for 5 minutes. Quantitative real-time polymerase chain reaction (PCR) was performed using the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) as

described previously [22]. Each experiment was repeated for at least 3 times. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control; normalized InsR messenger RNA (mRNA) expression levels were plotted as fold of vehicle treatment, which was designated as 1.

2.3. Flow cytometry

After treatment, cells were collected and fixed in 2% paraformaldehyde at room temperature for 30 minutes. After blocking, cells were incubated on ice for 1 hour with a mouse monoclonal antibody against the β-subunit of InsR (Santa Cruz Biotechnology, Santa Cruz, CA) with a dilution of 1:100. Normal mouse immunoglobulin G (IgG) was used as control for background staining. Afterward, cells were washed and stained on ice for 30 minutes with a fluorescein isothiocyanate–conjugated goat anti-mouse IgG (Santa Cruz) with a dilution of 1:200. The fluorescent intensities on cell surface were analyzed with a FACSCalibur system (BD Biosciences, San Jose, CA).

2.4. Western blot

Cells were lysed and proteins were extracted as described previously [17]. Protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Uppsala, Sweden) by a Semi-Dry Transfer Cell (Bio-Rad, Hercules, CA). For detection of InsR protein expression, membrane was probed with a mouse monoclonal antibody against the β-subunit of InsR (Santa Cruz Biotechnology), with GAPDH as internal control. After incubation with appropriate peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), signals were visualized by the Chemiluminescence Kit (Amersham Pharmacia Biotech). The membranes were then stripped; a rabbit monoclonal antibody specific for the phosphorylated Tyr1361 (Cell Signaling Technology, Danvers, MA) was used to examine the insulin-stimulated phosphorylation of InsR β-subunit. Ser473-phosphorylated Akt and total Akt in the cells were detected by specific rabbit polyclonal antibodies (Cell Signaling Technology).

2.5. Subjects and design

Ninety-seven T2DM patients with fasting blood glucose (FBG) of at least 7.0 mmol/L or postprandial blood glucose of at least 11.1 mmol/L were enrolled in this study at the Nanjing First Hospital. These patients were asked to terminate their previous therapies for more than 2 weeks before enrollment in this study. Fifty patients (male/female, 27/23; age, 57 ± 8 years) were randomly assigned for BBR treatment (1 g/d), 26 patients (male/female, 15/11; age, 56 ± 11 years) were for metformin therapy (1.5 g/d), and 21 patients (male/female, 11/10; age, 49 ± 10 years) were in the rosiglitazone group (4 mg/d). The patients were treated orally with BBR, metformin, or rosiglitazone for 2 months. The daily doses of BBR and metformin were divided into
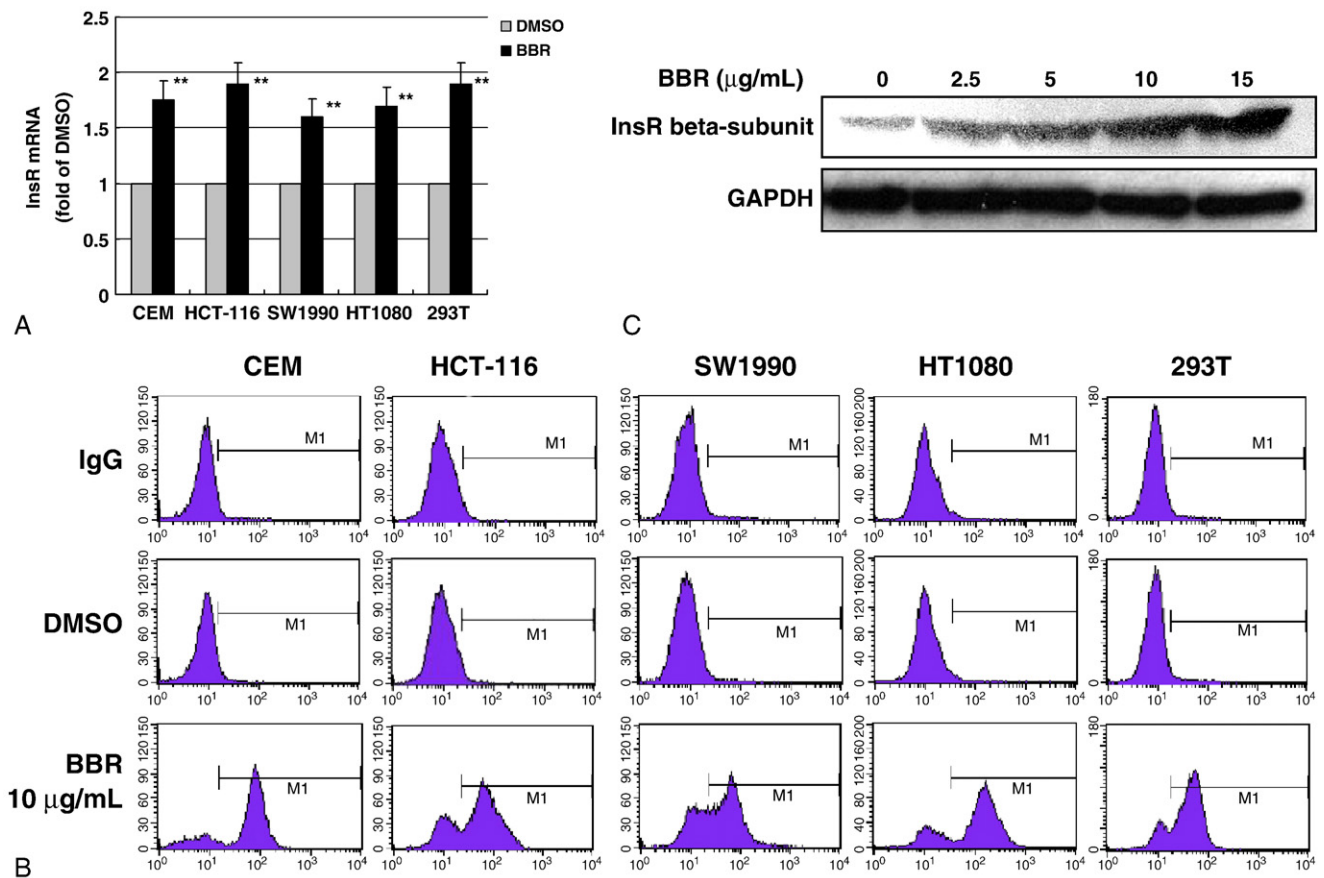
Fig. 1. InsR–up-regulating activity of BBR in human cell lines. A, Insulin receptor mRNA expression levels. The CEM, HCT-116, SW1990, HT1080, and 293T cells were treated with vehicle (DMSO) or 10 μg/mL of BBR for 12 hours; InsR mRNA expression levels were determined by real-time RT-PCR. Insulin receptor mRNA levels were normalized to those of GAPDH and plotted as fold of DMSO treatment, which was designated as 1. Values are mean ± SEM of at least 3 separate experiments; **$P < .01$ compared with that of DMSO by Student *t* test. B, Flow cytometry. After treatment, InsR protein expression on cell surface was determined by flow cytometry using a mouse monoclonal antibody against InsR. Normal mouse IgG was used as control for background fluorescence. C, Western blot. The CEM cells were treated with BBR at different concentrations for 12 hours. Cells were lysed, and proteins were extracted as described in "Materials and methods." The expression level of InsR β-subunit was examined by Western blot using a mouse monoclonal antibody with GAPDH as internal control.

two, and rosiglitazone was taken once a day. Berberine hydrochloride used in this study was from the Nanjing Second Pharmaceutics (Nanjing, China), metformin hydrochloride was from the Double-Crane Pharmaceutical (Beij-

ing, China), and rosiglitazone maleate (Avandia) was from GlaxoSmithKline (Brentford, Middlesex, United Kingdom). Metformin and rosiglitazone were used as references in this study. Blood samples were taken before and 2 months after
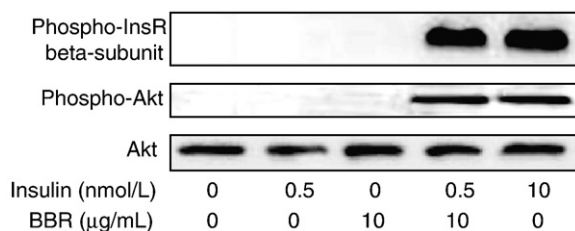

Fig. 2. Effects of BBR on insulin signaling in CEM cells. The CEM cells were left untreated or were treated with 10 μg/mL of BBR for 12 hours, and then 0.5 or 10 nmol/L of insulin was added. Cells were incubated for an additional 15 minutes and harvested. Proteins were extracted and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis; the levels of Akt, Ser473-phosphorylated Akt, and Tyr1361-phosphorylated InsR β-subunit were examined by Western blot using specific antibodies as described in "Materials and methods."

Table 1
Effects of BBR, metformin, and rosiglitazone in T2DM patients

Measurement (reference range)	Treatment (2 mo)	BBR (1 g/d, n = 50)	Metformin (1.5 g/d, n = 26)	Rosiglitazone (4 mg/d, n = 21)
FBG	Before	10.4 ± 0.4	10.9 ± 0.5	9.1 ± 0.8
(3.9-5.6 mmol/L)	After	7.7 ± 0.3[†]	7.6 ± 0.3[†]	7.5 ± 0.6[*]
HbA$_{1c}$	Before	8.3 ± 0.3	9.4 ± 0.5	8.3 ± 0.4
(4.0%-6.0%)	After	6.8 ± 0.2[†]	7.2 ± 0.3[†]	6.8 ± 0.3[*]
TG	Before	1.7 ± 0.1	1.7 ± 0.2	1.9 ± 0.3
(<1.7 mmol/L)	After	1.4 ± 0.1[*]	1.6 ± 0.1	1.6 ± 0.1

Values are mean ± SEM.

[*] $P < .01$ compared with that before treatment by paired *t* test.

[†] $P < .001$ compared with that before treatment by paired *t* test.
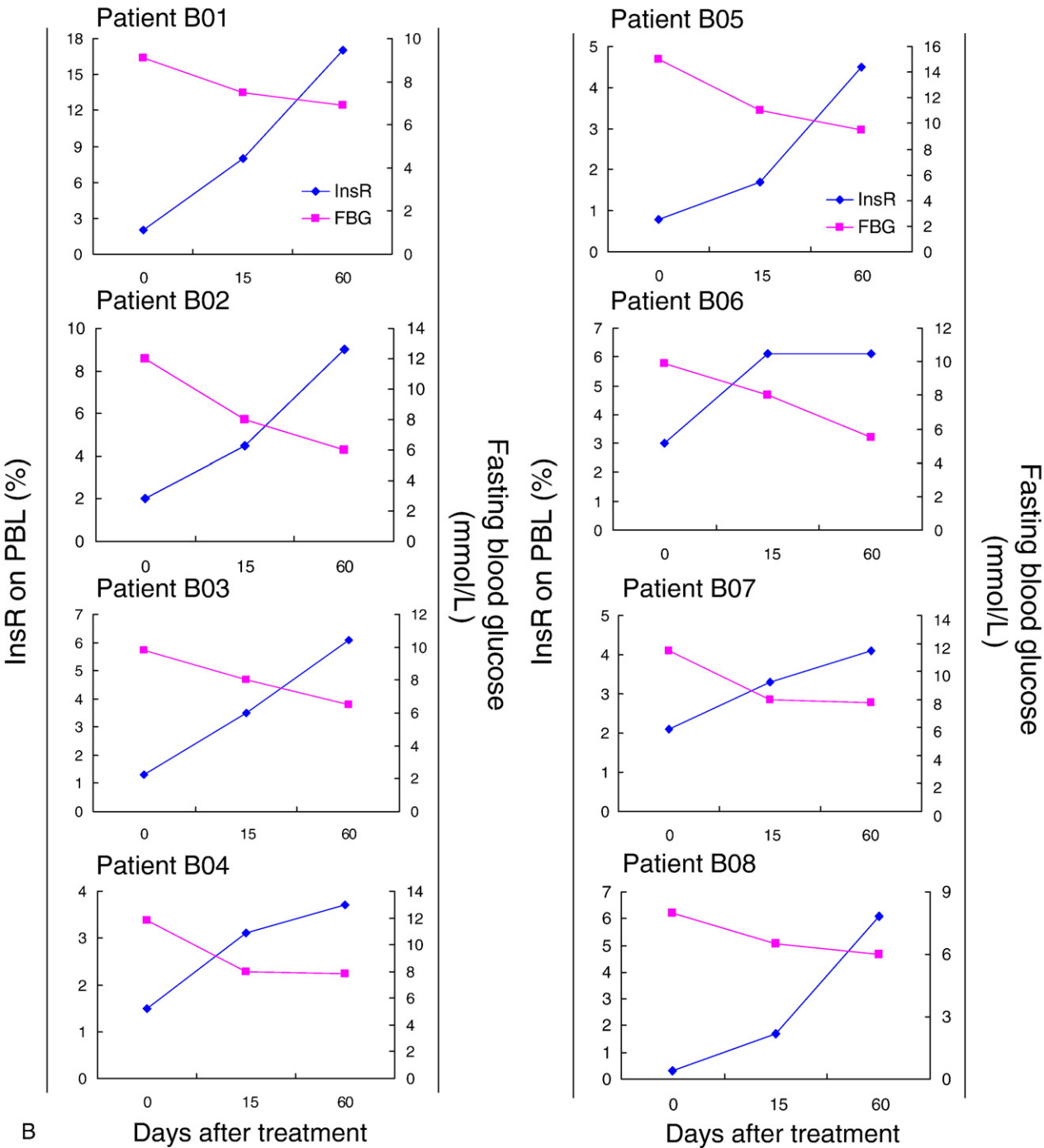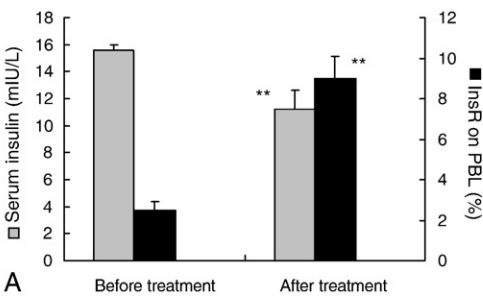
therapy. Fasting blood glucose, hemoglobin A_{1c} (HbA$_{1c}$), triglyceride (TG), and serum insulin levels were measured. Liver and kidney functions were examined in patients treated with BBR. Peripheral blood lymphocytes (PBL) from BBR-treated patients were isolated before and after treatment to examine the InsR protein expression on the surface of PBL using flow cytometry.

In another set of study, 35 patients with chronic hepatitis C virus (HCV) infection (HCV antibody [+] and HCV RNA [+]) or HBV infection (HBsAg [+], HBeAg [+], and HBV DNA [+]) were enrolled in the Nanjing Second Hospital. Among them, 18 patients (male/female, 11/7; age, 52.6 ± 7.8 years) were in the hepatitis C group; and 17 patients (male/female, 14/3; age, 56.7 ± 8.1 years) had chronic hepatitis B. These patients were diagnosed with T2DM or impaired fasting glucose (IFG; FBG range, 5.6~6.9 mmol/L). All of the patients received oral BBR (Nanjing Second Pharmaceutics) therapy at a dose of 1 g/d for 2 months. Blood samples were taken before and after treatment for the measurement of FBG, TG, and liver function.

The clinical studies were approved by the Ethics Committees of the Institute of Medicinal Biotechnology, Nanjing First Hospital, and Nanjing Second Hospital and were conducted in adherence to the guidelines in the Declaration of Helsinki. All participants gave informed consent.

2.6. Statistical analysis

Results of patients before and after treatment were compared for difference using the paired t test. After validation of the test for homogeneity of variance, differences between or among study groups were examined by Student t test or by 1-way analysis of variance followed by the Newman-Keuls test for multiple comparisons.

3. Results

3.1. BBR increases InsR expression and enhances insulin signaling in human cell lines

Our previous study demonstrated that BBR increases InsR mRNA and protein expression in human liver cells in a dose- and time-dependent manner [22]. To determine if BBR can up-regulate InsR in other types of human cells, we used BBR to treat a variety of human cell lines, including CEM T-lymphocytes, HCT-116 colon cancer cells, SW1990 pancreatic cells, HT1080 fibrosarcoma cells, and 293T fibroblast cells. The InsR mRNA expression levels in these cells were assayed by real-time reverse transcriptase

(RT)–PCR. As shown in Fig. 1A, after 12 hours of treatment, 10 μg/mL of BBR increased InsR mRNA expression in all of the cell lines with degrees between 1.6- and 1.9-fold of those of the DMSO solvent control ($P <$ .01). Subsequently, InsR protein expression levels on cell surface were determined with flow cytometry. As shown in Fig. 1B, BBR up-regulated InsR protein expression significantly in all of the study cell lines by showing that a majority of the cells moved toward the right-hand side and formation of new peaks. The increase of InsR protein expression was confirmed by Western blot assay. As shown in Fig. 1C, after treatment for 12 hours, BBR up-regulated InsR protein dose dependently in CEM cells. Similar results were obtained in other cell lines (data not shown).

Next, we determined the effect of BBR on insulin signaling in CEM cells. As shown in Fig. 2, neither BBR alone nor 0.5 nmol/L of insulin had any observed impact on insulin signaling. However, when 0.5 nmol/L of insulin was added to cells that had already been treated with BBR for 12 hours, the insulin signaling pathway was activated. We observed that BBR did not change Akt protein level; 0.5 nmol/L of insulin plus BBR increased the phosphorylations of InsR β-subunit and Akt significantly and close to those of 10 nmol/L of insulin treatment.

3.2. BBR lowers blood glucose and increases InsR expression in T2DM patients

To learn the efficacy of BBR in respect to the known hypoglycemic drugs, clinical study was conducted. Ninety-seven patients with T2DM according to the diagnostic criteria of the American Diabetes Association were enrolled in this study. No placebo group was included to avoid the acceleration of disease progress. Instead, standard treatment of T2DM with metformin and rosiglitazone served as reference controls. Berberine therapy (1 g/d for 2 months) significantly lowered FBG by 25.9% ($P <$ .001), HbA$_{1c}$ by 18.1% ($P <$ .001), and TG by 17.6% ($P <$ .01) as compared with values before treatment (Table 1). The FBG- and HbA$_{1c}$-lowering efficacies of BBR were close to those of metformin and rosiglitazone. After 2 months of treatment, 1.5 g/d of metformin lowered FBG and HbA$_{1c}$ by 30.3% and 23.4% ($P <$ .001); 4 mg/d of rosiglitazone lowered FBG and HbA$_{1c}$ by 17.6% and 18.1% ($P <$ .01), respectively. As compared with BBR, metformin and rosiglitazone had less effect on the serum level of TG. No adverse effects were observed in the BBR-treated patients.

Although the liver enzymes of the patients were within the reference range before and after BBR treatment, their levels declined significantly after BBR therapy. The

Fig. 3. Effects of BBR in T2DM patients. Fifty T2DM patients from Nanjing First Hospital were treated with 1 g/d of BBR for 2 months. Before and after therapy, blood samples were taken to assay serum insulin; PBL were isolated, and InsR protein expression level was analyzed by flow cytometry. A, Serum insulin levels and percentage of PBL that express InsR on the surface. Values are mean ± SEM; **$P <$ .01 compared with that before treatment by paired t test. B, Negative correlation between FBG and PBL InsR expression. Eight patients of the 50 were analyzed for the relationship between their FBG and InsR expression; the increase of InsR expression on the surface of PBL was accompanied by the reduction of blood glucose.
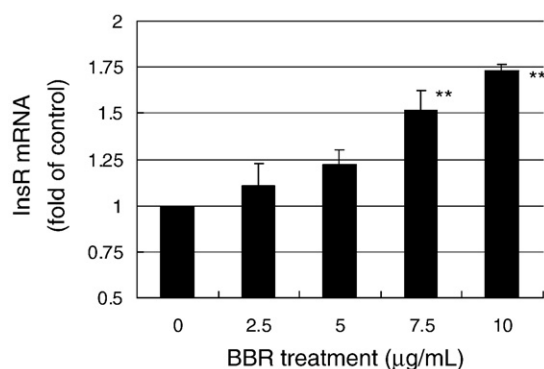
Fig. 4. Berberine up-regulates InsR expression in HepG2.2.15 cells. The HepG2.2.15 cells were treated with different concentrations of BBR as indicated for 12 hours. Insulin receptor mRNA levels were assayed by real-time RT-PCR, normalized to those of GAPDH, and plotted as fold of untreated control, which was designated as 1. Values are mean ± SEM of at least 3 separate experiments; **P < .01 compared with that of untreated control by 1-way analysis of variance and the Newman-Keuls test.

average alanine aminotransferase (ALT) level declined after BBR treatment from 31 ± 19 to 23 ± 16 U/L (P < .01); and γ-glutamate transpeptidase, from 47 ± 26 to 31 ± 23 U/L (P < .01). Kidney function remained stable and in the reference range in BBR treatment. The results of the liver and kidney functions were consistent with those in our previous report [17]. Serum insulin level of the patients (Fig. 3A) declined significantly by 28.2% after BBR therapy (P <.01), indicating an increased insulin sensitivity in the peripheral tissues.

To investigate the InsR–up-regulating effect of BBR in humans, PBL were isolated from the patients in the BBR group, followed by determination of the InsR protein expression on cell surface using flow cytometry. As shown in Fig. 3A, as compared with that before BBR treatment, the mean percentage of PBL that express InsR on the surface was elevated by 3.6-fold (P < .01) after 2 months on BBR therapy, consistent with our finding in the BBR-treated CEM cells (Fig. 1). Out of these 50 patients, 8 were able to come back to the hospital after 2 weeks on BBR therapy, providing

us an opportunity to analyze the relationship between FBG and InsR expression in these patients. As shown in Fig. 3B, all of the 8 patients demonstrated a negative correlation between FBG and InsR expression. The increase of InsR expression on the surface of PBL was accompanied by the reduction of blood glucose.

3.3. BBR is suitable for hyperglycemic patients with liver diseases

Although metformin and TZDs are currently the first-line drugs for T2DM, their liver adverse effects [12,13] cause concerns for hyperglycemic patients with liver diseases, especially in China that has a big population of patients with viral hepatitis. Our previous report proved that BBR was safe and effective in treating hyperlipidemic patients with chronic viral hepatitis or liver cirrhosis [24]; we therefore infer that BBR may also be a good option in treating hyperglycemic patients with liver impairment. To test this hypothesis, we first used BBR to treat HBV (+) human liver cells (HepG2.2.15 cells) to learn whether or not BBR can up-regulate InsR expression in the liver cells with HBV replication. As shown in Fig. 4, InsR mRNA expression level in HepG2.2.15 cells was up-regulated by BBR in a dose-dependent manner. At 10 μg/mL, BBR increased InsR mRNA level by 1.73-fold (P < .01 compared with that of untreated control).

Therefore, we extended the study into the clinic. Thirty-five chronic hepatitis patients with T2DM or IFG (diagnostic criteria according to American Diabetes Association) were enrolled in the Nanjing Second Hospital. Among them, 18 patients were diagnosed with chronic hepatitis C (6 with T2DM, 12 with IFG); and 17 patients had hepatitis B (7 with T2DM, 10 with IFG). All of the patients received BBR treatment at a dose of 1 g/d for 2 months. The results are summarized in Table 2. In the hepatitis C group, BBR therapy lowered FBG by 17.1% and 13.4% and lowered TG by 19% and 17.6% in T2DM and IFG patients, respectively (P < .01 compared with that before treatment). The effects of BBR in hepatitis B patients were similar, with 15.1% and

Table 2
Effects of BBR in chronic hepatitis patients with T2DM or IFG

Measurement (reference range)	Treatment (2 mo)	Hepatitis C		Hepatitis B	
		T2DM (n = 6)	IFG (n = 12)	T2DM (n = 7)	IFG (n = 10)
FBG	Before	7.6 ± 0.5	6.7 ± 0.3	7.3 ± 0.2	6.4 ± 0.5
(3.9-5.6 mmol/L)	After	6.3 ± 0.6*	5.8 ± 0.3*	6.2 ± 0.3*	5.5 ± 0.4*
TG	Before	2.1 ± 0.2	1.7 ± 0.2	2.5 ± 0.2	2.5 ± 0.3
(<1.7 mmol/L)	After	1.7 ± 0.2*	1.4 ± 0.1*	2.1 ± 0.2*	2.1 ± 0.2*
ALT	Before	70.8 ± 10.1	92.6 ± 12.1	99.2 ± 8.7	96.3 ± 11.2
(<40 U/L)	After	40.3 ± 5.4†	46 ± 5.3†	70.8 ± 8.2*	63.3 ± 7.6*
AST	Before	57.8 ± 6.8	92 ± 10.3	89 ± 9.2	75.2 ± 8.9
(<40 U/L)	After	35.3 ± 4.2†	41.1 ± 5.4†	66.1 ± 7.4*	57 ± 6.7*

Values are mean ± SEM. AST indicates aspartate aminotransferase.
  * P < .01 compared with that before treatment by paired t test.
  † P < .001 compared with that before treatment by paired t test.

14.1% reduction of FBG and 16% and 16% reduction of TG in the T2DM and IFG subgroups, respectively ($P < .01$). Berberine is safe in these patients. The elevated ALT and aspartate aminotransferase levels of the patients declined significantly after BBR therapy ($P < .01$ or $P < .001$). These results suggest that BBR is safe and effective in hyperglycemic patients with liver function damage.

4. Discussion

Type 2 diabetes mellitus is a sugar-related metabolic disorder with complicated mechanisms, in which InsR is one of the major factors responsible for the state of insulin resistance [25-27]. Defects in InsR expression or function will cause insulin resistance and diabetes mellitus [26,27]. Our previous studies demonstrated that BBR increases InsR expression both in vitro and in animal models [22]. In the present study, we verify that BBR indeed up-regulates InsR in the peripheral tissues in humans and lowers blood glucose in T2DM patients.

It appears that the principal hypoglycemic mechanism of BBR is its up-regulation of InsR expression. As InsR is widely expressed in all types of tissues [28], we analyzed the effect of BBR on InsR in several types of human cell lines. Our results demonstrated that, in addition to liver and muscle cells, BBR also increased InsR mRNA and protein expression in a variety of other cell lines. Accompanied by the up-regulation of InsR, insulin signaling was significantly enhanced after BBR treatment, confirming the action of BBR as an insulin sensitizer. The enhancement of insulin-stimulated phosphoinositol-3-kinase–Akt pathway here was in agreement with the glucose consumption/uptake experiments in our previous study [22] and in another report [29].

We next analyzed the InsR–up-regulating activity of BBR and its relationship to the glucose-lowering effect in hyperglycemic patients. Inconsistent with the animal experiment results [22], BBR increased InsR expression in vivo in humans. As InsR expression on PBL negatively correlated with hypoglycemic effect of BBR, it could be a future bioassay to evaluate T2DM patients for their sensitivity to BBR treatment.

In addition to FBG, HbA_{1c} was measured in this study because it is a preeminent factor for monitoring glycemia and evaluating the risks of complications in diabetic patients [30]; serum TG level was determined as well because its synthesis is closely associated with glucose metabolism. Berberine effectively lowers both FBG and HbA_{1c} in T2DM patients in this study. The efficacy of BBR is comparable to that of metformin and rosiglitazone. However, they work through different mechanisms. Rosiglitazone reduces insulin resistance by targeting an orphan nuclear receptor, peroxisome proliferator–activated receptor–γ [31,32]. Metformin has been used to treat diabetes for nearly 50 years; it acts on the glucose transporters and stimulates tissue glucose uptake [33].

Contrary to TZDs, several studies revealed that BBR reduces the expression level of peroxisome proliferator–activated receptor–γ, suppresses the differentiation of preadipocytes, and reduces the accumulation of lipid droplets [34-36]. Thus, unlike TZDs, which may lead to weight gain, BBR may be more suitable for insulin-resistant/T2DM patients with obesity. The insulin-sensitizing and glucose-lowering mechanisms of BBR are of great interest in this field. Recent studies also showed that BBR activates adenosine monophosphate–activated protein kinase, which may play a role in reducing insulin resistance [37,38]. These informations as a whole might help us to elucidate the entire signal network of BBR. As compared with metformin and rosiglitazone, BBR showed more activity in reducing serum TG level, representing its advantage of synergistic effect on both sugar and lipid metabolism.

Metformin and rosiglitazone are first-line drugs for T2DM in the clinic; however, they are not recommended for patients with impaired liver function [39,40]. It has become a clinical issue for Chinese physicians because of the increased population of patients with T2DM in China. As BBR effectively increases InsR expression in the HBV-transfected human liver cells, we consider it a potential option for hyperglycemic patients with hepatitis. The clinical study was conducted in hepatitis patients diagnosed with T2DM or IFG, an intermediate state between normal and diabetes mellitus [41]. Our results demonstrated that BBR was safe and lowered blood glucose significantly in these patients. Liver function of the hepatitis patients improved after BBR treatment, agreeing with the observation of BBR in treating hyperlipidemic patients with liver diseases [24].

Taken together, as BBR enhances insulin sensitivity in peripheral tissues through a protein kinase C–dependent InsR up-regulation and always shows convincing safety record in patients, we strongly suggest this natural compound to be an ideal medicine for T2DM.

Acknowledgment

References

[1] Saltiel AR. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell 2001;104:517-29.

[2] Kido Y, Nakae J, Accili D. Clinical review 125: the insulin receptor and its cellular targets. J Clin Endocrinol Metab 2001;86:972-9.

[3] Michael MD, Kulkarni RN, Postic C, et al. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 2000;6:87-97.

[4] Yki-Jarvinen H. Pathogenesis of non–insulin-dependent diabetes mellitus. Lancet 1994;343:91-5.

[5] Ferrannini E. Insulin resistance versus insulin deficiency in non–insulin-dependent diabetes mellitus: problems and prospects. Endocr Rev 1998;19:477-90.

[6] Haffner SM, D'Agostino Jr R, Mykkanen L, et al. Insulin sensitivity in subjects with type 2 diabetes. Relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. Diabetes Care 1999;22:562-8.

[7] Taylor SI, Accili D, Imai Y. Insulin resistance or insulin deficiency. Which is the primary cause of NIDDM? Diabetes 1994;43:735-40.

[8] Zhang B, Salituro G, Szalkowski D, et al. Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. Science 1999;284:974-7.

[9] Foti D, Chiefari E, Fedele M, et al. Lack of the architectural factor HMGA1 causes insulin resistance and diabetes in humans and mice. Nat Med 2005;11:765-73.

[10] Air EL, Strowski MZ, Benoit SC, et al. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nat Med 2002;8:179-83.

[11] Srivastava AK, Mehdi MZ. Insulin-mimetic and anti-diabetic effects of vanadium compounds. Diabet Med 2005;22:2-13.

[12] Masubuchi Y. Metabolic and non-metabolic factors determining troglitazone hepatotoxicity: a review. Drug Metab Pharmacokinet 2006;21:347-56.

[13] Chitturi S, George J. Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihypertensives, antidiabetic agents, anticonvulsants, lipid-lowering agents, psychotropic drugs. Semin Liver Dis 2002;22:169-83.

[14] Luo LJ. Experience of berberine in the treatment of diarrhea. Chin J Med 1955;41:452-5.

[15] Lau CW, Yao XQ, Chen ZY, et al. Cardiovascular actions of berberine. Cardiovasc Drug Rev 2001;19:234-44.

[16] Taylor CE, Greenough III WB. Control of diarrheal diseases. Annu Rev Public Health 1989;10:221-44.

[17] Kong W, Wei J, Abidi P, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 2004;10:1344-51.

[18] Kong WJ, Liu J, Jiang JD. Human low-density lipoprotein receptor gene and its regulation. J Mol Med 2006;84:29-36.

[19] Kong WJ, Wei J, Zuo ZY, et al. Combination of simvastatin with berberine improves the lipid-lowering efficacy. Metabolism 2008;57:1029-37.

[20] Yang P, Song DQ, Li YH, et al. Synthesis and structure-activity relationships of berberine analogues as a novel class of low-density-lipoprotein receptor up-regulators. Bioorg Med Chem Lett 2008;18:4675-9.

[21] Abidi P, Zhou Y, Jiang JD, et al. Extracellular signal-regulated kinase–dependent stabilization of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine. Arterioscler Thromb Vasc Biol 2005;25:2170-6.

[22] Kong WJ, Zhang H, Song DQ, et al. Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. Metabolism 2009;58:109-19.

[23] Ni YX. Therapeutic effect of berberine on 60 patients with type II diabetes mellitus and experimental research. Zhong Xi Yi Jie He Za Zhi 1988;8:711-3.

[24] Zhao W, Xue R, Zhou ZX, et al. Reduction of blood lipid by berberine in hyperlipidemic patients with chronic hepatitis or liver cirrhosis. Biomed Pharmacother 2008;62:730-1.

[25] Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365:1333-46.

[26] Hone J, Accili D, al-Gazali LI, et al. Homozygosity for a new mutation (Ile119–>Met) in the insulin receptor gene in five sibs with familial insulin resistance. J Med Genet 1994;31:715-6.

[27] Foti D, Iuliano R, Chiefari E, et al. A nucleoprotein complex containing Sp1, C/EBP beta, and HMGI-Y controls human insulin receptor gene transcription. Mol Cell Biol 2003;23:2720-32.

[28] Lee J, Pilch PF. The insulin receptor: structure, function, and signaling. Am J Physiol 1994;266:C319-34.

[29] Ko BS, Choi SB, Park SK, et al. Insulin sensitizing and insulinotropic action of berberine from *Cortidis rhizoma*. Biol Pharm Bull 2005;28:1431-7.

[30] Manley S. Haemoglobin A1c—a marker for complications of type 2 diabetes: the experience from the UK Prospective Diabetes Study (UKPDS). Clin Chem Lab Med 2003;41:1182-90.

[31] Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature 2000;405:421-4.

[32] Vasudevan AR, Balasubramanyam A. Thiazolidinediones: a review of their mechanisms of insulin sensitization, therapeutic potential, clinical efficacy, and tolerability. Diabetes Technol Ther 2004;6:850-63.

[33] Klip A, Leiter LA. Cellular mechanism of action of metformin. Diabetes Care 1990;13:696-704.

[34] Liu Y, Lou SY, He YM. Effects of berberine on cell proliferation, peroxisome proliferation activated receptor gamma, CAAT/enhancer binding protein mRNA and protein expression in 3T3-L1 pre-adipocytes. Zhongguo Zhong Xi Yi Jie He Za Zhi 2008;28:1005-9.

[35] Zhou JY, Zhou SW, Zhang KB, et al. Chronic effects of berberine on blood, liver glucolipid metabolism and liver PPARs expression in diabetic hyperlipidemic rats. Biol Pharm Bull 2008;31:1169-76.

[36] Huang C, Zhang Y, Gong Z, et al. Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARgamma pathway. Biochem Biophys Res Commun 2006;348:571-8.

[37] Turner N, Li JY, Gosby A, et al. Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. Diabetes 2008;57:1414-8.

[38] Lee YS, Kim WS, Kim KH, et al. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes 2006;55:2256-64.

[39] Emslie-Smith AM, Boyle DI, Evans JM, et al. Contraindications to metformin therapy in patients with type 2 diabetes—a population-based study of adherence to prescribing guidelines. Diabet Med 2001;18:483-8.

[40] Wagstaff AJ, Goa KL. Rosiglitazone: a review of its use in the management of type 2 diabetes mellitus. Drugs 2002;62:1805-37.

[41] Rao SS, Disraeli P, McGregor T. Impaired glucose tolerance and impaired fasting glucose. Am Fam Physician 2004;69:1961-8.