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REVIEW

Berberine as a therapy for type 2 diabetes and its complications: From mechanism of action to clinical studies[1]

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Abstract: The incidence of type 2 diabetes is increasing rapidly worldwide, and the development of novel anti-diabetic drugs is emerging. However, most anti-diabetic drugs cannot be used in patients with hepatic dysfunction, renal disease, and heart disease, which makes pharmacological therapy of type 2 diabetes complicated. Despite continued introduction of novel agents, the search for an ideal drug that is useful as both a hypoglycemic agent and to reduce diabetes-related complications remains elusive. Berberine is an isoquinoline alkaloid extract that has shown promise as a hypoglycemic agent in the management of diabetes in animal and human studies. Mechanistic studies have revealed beneficial effects of berberine on diabetes-related complications. Although there have been few clinical reports of the anti-diabetic effects of berberine, little documentation of adverse effects in humans positions it as a potential candidate drug to treat type 2 diabetes. In the present review, the anti-diabetic mechanism of berberine, its effect on diabetes-related complications, and its recent use in human clinical studies is highlighted. In addition, we summarize the different treatments for type 2 diabetes in adults and children.

Key words: type 2 diabetes mellitus, adults, children, berberine, complications, hypoglycemic, drug, metabolism, adenosine-5′-monophosphate kinase.

Résumé : L'incidence du diabète de type 2 augmente rapidement à travers le monde et le développement de nouveaux médicaments antidiabétiques est en pleine émergence. Cependant, la plupart des médicaments antidiabétiques ne peuvent être utilisés chez des patients qui présentent une dysfonction hépatique, une maladie rénale ou une maladie cardiaque, ce qui complique la thérapie pharmacologique du diabète de type 2. Malgré l'introduction continuelle de nouveaux agents, la recherche d'un médicament idéal qui serait utile comme agent hypoglycémiant et qui réduirait les complications reliées au diabète demeure hors de portée. La berbérine est une isoquinoline alcaloïde qui s'est montrée prometteuse comme agent hypoglycémiant dans le contrôle du diabète lors d'études chez l'animal et chez l'humain. Des études mécanistiques ont révélé des effets bénéfiques de la berbérine sur les complications reliées au diabète. Même s'il n'existe que peu d'études cliniques portant sur les effets antidiabétiques de la berbérine, la faible documentation d'effets secondaires chez l'humain la place comme médicament potentiel pour traiter le diabète de type 2. Dans cet article de revue, le mécanisme d'action antidiabétique de la berbérine, son effet sur les complications reliées au diabète et son utilisation récente lors d'études cliniques sont mises en évidence. De plus, les auteurs résument les différents traitements du diabète de type 2 chez les adultes et les enfants. [Traduit par la Rédaction]

Mots-clés : diabète sucré de type 2, enfants, berbérine, complications, hypoglycémiant, médicament, métabolisme.

Type 2 diabetes in adults and children

Thirty years ago, type 2 diabetes (T2D) was a fairly rare occurrence in adults and was almost undocumented in children (Arslanian 2002). In 2010, 285 million people with T2D comprised approximately 90% of diabetes worldwide (Guariguata et al. 2014). The global prevalence of diabetes is estimated to rise to 592 million by 2035, and there will be more than 500 million patients with T2D. T2D occurs when insulin secretion is inadequate to meet the increased demand due to insulin resistance. It was previously regarded as a disease of obese adults who led sedentary lifestyles or had genetic predisposition to developing diabetes. However, today this disease has markedly increased in prevalence among children. Currently in the United States, approximately 1 in 3 new cases of T2D is diagnosed in patients younger than 18 years (Pinhas-Hamiel and Zeitler 2007). Diabetes is associated with a high rate of both microvascular and macrovascular complications. In fact, diabetes-related complications are the main factor for death among diabetic patients. The American Diabetes Association reported that the incidence of diabetic related complications was up to 98% of patients who had the disease for 10 years. In addition, evidence suggests that these complications progress more rapidly in youth than in adults.

Type 2 diabetes therapy in adults and children

Although some difference in therapy targets exist in T2D, the treatment strategy for the disease in both adults and children is similar (Kalra 2013; American Diabetes Association 2014) (Table 1). T1
The overall goals for treatment of T2D are: weight loss, increase in exercise capacity, normalization of plasma glucose levels, and control of co-morbidities, including hypertension, cardiomy-

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Table 1. T2D therapy targets in children and adults.

	Youth and adolescents[a,c]			Adults[b,c]
	0–6 years	6–12 years	13–19 years	≥20 years
Glycemia (pre-meal, mg/dL)	100–180	90–180	90–130	≤120
HbA1c (pre-meal)	≤8.5%	≤8.0%	≤7.5%	≤7.0%
Insulin (IU/kg/d)	~0.5	0.7–1.0	~2.0	~1.0

[a]Blood glucose goals should be modified in children with frequent hypoglycemia or hypoglycemia unawareness.

[b]The therapy targets for adults are only recommended for nonpregnant adults.

[c]Values are summarized from Standards of Medical Care in Diabetes ADA 2014, developed by the American Diabetes Association, Guidelines and Protocol-Diabetes Care GPAC 2010, developed by the Guidelines and Protocols Advisory Committee, and Global IDF/ISPAD Guideline for Diabetes in Childhood and Adolescence Rosenbloom et al. 2011, developed by the International Diabetes Foundation.

opathy, nephropathy, and hepatic steatosis (American Diabetes Association 2014). Pharmacological intervention is recommended upon failure of lifestyle modification. Although various drugs are used to treat T2D, including insulin, acarbose, rosiglitazone, metformin, and sulphonylureas, only few of these are approved for use in children (Rosenbloom et al. 2011). Even with drugs that are approved for use in children, insulin and metformin, most youth with T2D do not achieve optimal glycemic control and are at high risk for later health complications (Pulgaron and Delamater 2014). For example, patients using insulin are exposed to a high risk of weight gain and significant hypoglycemia (GPAC 2010). In addition, the present drugs provide suboptimal control of diabetic complications. A recent clinical trial in the United States indicated that mono-therapy with metformin was not sufficient to control co-morbidities in T2D patients, as 33.8% of participants exhibited hypertension, 16.6% microalbuminuria, and 13.7% retinopathy (Narasimhan and Weinstock 2014). Moreover, some of these drugs are limited to use in only certain classes of patients (Kajbaf et al. 2013; Scheen 2014; Strugaru et al. 2013). For example, metformin and rosiglitazone are contra-indicated in patients with renal impairment, hepatic disease, cardiac or respiratory insufficiency. Thus, it is difficult to find an appropriate drug to treat patients with these diseases.

Berberine

Berberine (BBR), an isoquinoline alkaloid originally isolated from the Chinese herb Coptis chinensis (Huanglian), is one of the main components of R. coptidis (Leng et al. 2004). BBR has been used in traditional Chinese, Indian, and middle-eastern folk medicine for more than 400 years. Its chemical structure as a quaternary base is quite different from other commonly used hypoglycemic agents, such as sulfonylureas, biguanides, thiazolidinediones, or acarbose. Recent studies have demonstrated that BBR has remarkable effects as an anti-hyperglycemic and anti-hyperlipidemic, and it reduces weight gain in T2D patients (Yin et al. 2008; Zhang et al. 2010; Zhao et al. 2008). In addition, the beneficial effects of BBR on cardiovascular, liver, and renal disease have been demonstrated in both pre-clinical and clinical research (Cheng et al. 2013; Derosa et al. 2013; Affuso et al. 2010; Marin-Neto et al. 1988; Zhao et al. 2008; Lan et al. 2010). These observations make BBR a potentially promising drug for the management of T2D.

The anti-diabetic mechanism of berberine

The direct mechanism of BBR's anti-diabetic properties is not completely understood. To probe its metabolic function, studies have been conducted at the organ and gene expression levels, primarily in rodent models and in a small number of human clinical studies. The pathways that play a role in the anti-diabetic effects of BBR are summarized below and in Fig. 1.

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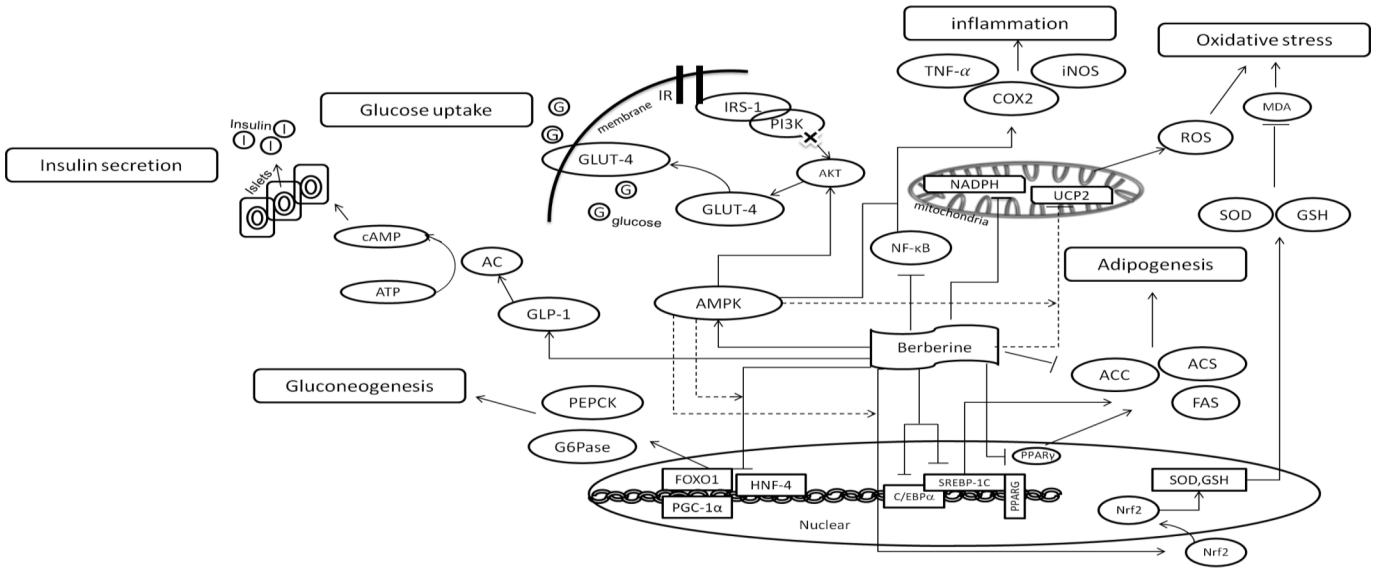
Berberine is an anti-hyperglycemic agent

BBR alters glucose metabolism through the stimulation of glycolysis via increasing glucokinase activity, increasing insulin secretion, and suppressing hepatic gluconeogenesis and adipogenesis (Ko et al. 2005; Chang et al. 2013; Hu and Davies 2010). BBR activation of 5′-adenosine monophosphate kinase (AMPK) appears to be at the center of these effects. We previously demonstrated that BBR increased glucose transporter-4 translocation to the plasma membrane and improved insulin sensitivity in insulin resistant H9c2 cardiomyocytes via activation of AMPK (Chang et al. 2013). In that study, BBR increased glucose consumption in both normal and insulin resistant H9c2 cardiomyocytes. In the state of insulin resistance, which accounts for more than 80% of failure of glucose control in T2D, the protein kinase B (Akt) signaling pathway is attenuated. We observed that BBR treatment increased phosphorylation of Akt, hence activating AKT, in insulin resistant H9c2 cardiomyocytes through activation of AMPK. The beneficial effects of BBR on increasing glucose uptake were attenuated after treatment of these cells with the AMPK inhibitor compound C. These results were consistent with other studies in L6 myotubes (Cheng et al. 2006; Lee et al. 2006). However, the mechanism of BBR's anti-hyperglycemic effects are controversial. BBR activated GLUT1-mediated glucose uptake via the ERK and AMPK pathways in 3T3-L1 adipocytes (Kim et al. 2007). In contrast, another study of 3T3-L1 adipocytes reported that the BBR-induced glucose uptake pathway was distinct from insulin and was not completely inhibited by ERK inhibitor, nor was GLUT-4 or GLUT-1 expression altered (Zhou et al. 2007). In addition, BBR treatment was shown to improve insulin resistance via increased expression of Akt in alloxan-induced diabetic C57BL/6 mice, and it increased insulin receptor substrate-2 mRNA expression in nonalcoholic fatty liver disease rat liver (Xie et al. 2011; Xing et al. 2011). Thus, even though the downstream pathways regulating glucose uptake may be different, it is likely that BBR mediates its anti-hyperglycemic effect through activation of the AMPK pathway.

BBR has been shown to improve glucose metabolism in diabetic rats by inhibition of gluconeogenesis (Zhang et al. 2012). In insulin resistant liver and kidney, BBR inhibited several transcription factors including fork head transcription factor O1, hepatic nuclear factor 4, and peroxisome proliferator-activated receptor-γ coactivator-1α, which in turn suppressed the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, two rate-limiting enzymes in gluconeogenesis. Activated AMPK was also observed in liver of diabetic rats (Kim et al. 2009). The elevated AMPK activity was likely the reason for the observed inhibition of gluconeogenesis in that study.

Other mechanisms have also been postulated to explain the complex effect of BBR on glucose homeostasis. BBR was shown to increase glucose-stimulated insulin secretion and cell proliferation in MIN6 β cells, and thus it can act as an effective insulin sensitizing and insulinotropic agent by enhancing the insulin signaling cascade (Ko et al. 2005). Pharmacological data showed that BBR was poorly absorbed in the intestine, and only nanomolar plasma concentrations may be reached in either humans or animals (Ye et al. 2009). Since BBR inhibits α-glycosidase in the small intestine it may also decrease glucose transport through the intestinal epithelium, suggesting it may in fact exert an anti-hyperglycemic effect prior to absorption (Pan et al. 2003). Glucagon like peptide-1 (GLP-1) receptors are important components involved in islet cell survival. GLP-1 activation of adenylate cyclase increases cyclic AMP levels, which leads to increases in intracellular Ca^{2+} and stimulates migration and exocytosis of insulin granules. BBR was shown to increase insulin secretion in islet cells through elevated GLP-1 levels (Yu et al. 2010; Lu et al. 2009).

Fig. 1. Schematic illustration of the proposed molecular mechanisms and pathways of BBR action related to its anti-diabetic properties. Dashed lines represent the role of AMPK in these pathways and require further validation.



Berberine is an anti-adipogenic and anti-hyperlipidmic agent

The anti-adipogenic activity of BBR has been shown to be associated with down-regulation of several transcription factors, including peroxisome proliferator activated receptor-γ (PPAR-γ,) CCAAT enhancer binding protein α (C/EBPα), and sterol response element binding protein-1c (SREBP-1c) (Rosen and MacDougald 2006). PPAR-γ functions as both a direct regulator of many fat-specific genes and a "master" regulator that may trigger the entire program of adipogenesis. Adipogenic enzymes such as tumor necrosis factor receptor super family member 6, acetyl-CoA carboxylase, and acetyl-CoA synthetase are overexpressed by activated C/EBPα and SREBP-1c. But these factors alone cannot promote differentiation of non-adipogenic fibroblasts unless co-expressed in fibroblasts expressing PPAR-γ. In 3T3-L1 pre-adipocytes and mature adipocytes, BBR treatment suppressed PPAR-γ and SREBP-1, and at the same time down-modulated C/EBPα via up-regulation of the expression of two different sets of C/EBP inhibitors, C/EBP homologous protein and basic helix-loop-helix family, member e41 (Pham et al. 2011). Inhibition of mitochondrial respiration through activated AMPK might also play a role in its inhibitory effect on adipogenesis (Huang et al. 2006; Turner et al. 2008). However, these observations need further confirmation.

The lipid lowering effect of BBR may be due to stabilization of the hepatic low density lipoprotein receptor (LDLR) by increased extracellular signal-regulated kinase-dependent pathway via increased transcriptional activity of LDLR promoter, mediated by the c-Jun N-terminal kinase pathway (Lee et al. 2007*b*). Moreover, BBR reduced the LDLR mRNA 3′-untranslated region binding of heterogenous nuclear ribonucleoprotein I and KH-type splicing regulatory protein, which are key modulators of LDLR mRNA stability in liver cells (Wang et al. 2012).

Berberine is an anti-oxidant agent

Reactive oxygen species (ROS) have been typically viewed as the toxic byproducts of metabolism. Recent studies have demonstrated that ROS generation, associated with insulin resistance, resulted in damage and apoptosis of pancreatic islet β-cells in pathological models of T2D (Evans et al. 2005; Scivittaro et al. 2000; Kaneto et al. 2002). In addition, oxidative stress contributed to the development of chronic complications of diabetes including nephropathy, retinopathy, and neuropathy (Rosen et al. 2001). BBR has been shown to attenuate oxidation, and the molecular mechanisms for its reduction in oxidative stress may be regulated by several pathways. For example, in the diabetic state, BBR treatment upregulated mRNA expression of superoxide dismutase (SOD) and increased the contents of SOD, glutathione (GSH), and GSH-peroxidase in rat liver (Zhou and Zhou 2011; Tang et al. 2006). Increases in the levels of these compounds are known to scavenge excessive free radicals and overcome oxidative stress and decrease malondialdehyde, a marker for oxidative stress-induced cell injury (Del Rio et al. 2005). A major source of ROS production in cells is through the expression level of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Fatty acids, glucose, and glycation end products up-regulate NADPH and result in overproduction of ROS. Regulation of NADPH oxidase is considered to be a new pharmacotherapeutic target for diabetes and its related complications (Li and Shah 2003; Gray et al. 2013; Huynh et al. 2013). BBR suppressed expression of NADPH oxidase 2/4 and subsequent ROS generation in THP-1 monocyte-derived macrophages and partially reduced oxidative stress of vascular endothelium induced by circulating CD31+/CD42-microparticles in humans (Sarna et al. 2010; Cheng et al. 2013). Thus, BBR may have a beneficial effect on diabetic vascular complications through attenuation of ROS production.

The anti-oxidative activity of BBR may also involve regulation of uncoupling protein 2 (UCP2) expression. UCP2 is a mitochondrial inner membrane protein that is negatively associated with ROS production and oxidative stress. BBR treatment reduced atherosclerosis in mice and attenuated non-alcoholic fatty liver disease in rats, and this was associated with an upregulation of UCP2 (Wang et al. 2011; Yang et al. 2011). However, whether BBR regulates UCP2 expression in a beneficial manner in all tissues is controversial. For example, although upregulation of UCP2 reduces ROS production in some tissues, increased UCP2 expression in islet β-cells was associated with a reduction in insulin secretion (Souza et al. 2011).

Induction of the nuclear factor erythroid-2-related factor-2 (Nrf2) pathway may play a role in the anti-oxidative activities of BBR. Nrf2 activates the expression of antioxidant enzymes. BBR treatment induced nuclear translocation of Nrf2, which increased SOD and GSH content in NSC34 motor neuron-like cells with a resultant reduction in ROS production and oxidative stress (Hsu et al. 2012). BBR supplementation in rats reverted mitochondrial

dysfunction induced by high fat diet and hyperglycemia in skeletal muscle, in part due to an increase in mitochondrial biogenesis, and this was mediated by increased expression of sirtuin 1 (SIRT1), a deacetylase with multiple biological activities and antioxidant activity (Gomes et al. 2012). In that study, increased SIRT1 induced deacetylation of the fork head transcription factor O1 and increased the transcription of its target genes, including SOD.

The beneficial effect of BBR on oxidative stress in humans was observed in a small clinical study. Healthy volunteers who received 0.4 g of BBR (tid) for one month showed significantly decreased serum malondialdehyde levels (before, 33.46 ± 4.14 μmol/L; after, 16.44 ± 4.91 μmol/L, $p < 0.05$) (Cheng et al. 2013). In that study, BBR reduced endothelial microparticles-mediated oxidative stress.

Berberine is an anti-inflammatory agent

The role of inflammation in the pathogenesis of T2D and its complications is well documented (Dorota Zozulinska 2006). Pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-6, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX2), play important roles in the pathology of diabetes. Excessive iNOS in cells results in overproduction of nitric oxide, and this is associated with the development of insulin resistance. COX2 is a key enzyme for the synthesis of prostaglandins, which are important mediators for the pathogenesis of diabetes and diabetic nephropathy. Nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) is a nuclear translation factor involved in the production of these pro-inflammatory cytokines. BBR was shown to reduce expression of these pro-inflammatory cytokines by suppressing phosphorylation of IκB kinase-β (IKK-β) serine residue at position 181(ser181), resulting in the stabilization of IκB-α, which in turn inhibited NF-κB (Lee et al. 2007*a*; Jiang et al. 2011). In addition to inhibition of NF-κB through IKK-β, BBR was shown to inhibit NF-κB through suppression of the Rho GTPase signaling pathway (Xie et al. 2013). Moreover, the anti-inflammatory effect of BBR may be mediated through AMPK. Blocking AMPK activity abolished the inhibitory effect of BBR on the production of the proinflammatory cytokines iNOS and COX2 in macrophages (Mo et al. 2014).

Berberine reduces diabetic complications

Endothelial and cardiovascular

Endothelial dysfunction is the basis of many diabetic complications, including cardiovascular disease. BBR treatment was found to improve endothelial function induced by circulating CD31+/CD42- microparticles in humans (Cheng et al. 2013). BBR treatment restored renal function, ameliorated endothelial dysfunction and memory dysfunction, and was anti-arrhythmic in the diabetic state (Wang et al. 2009; Wu et al. 2012; Zhan et al. 2014; Kalalian-Moghaddam et al. 2013). BBR treatment significantly improved endothelial dysfunction induced by hyperglycemia and protected acetylcholine-mediated vasorelaxation in aorta of T2D rats (Wu et al. 2012). The protection of endothelial function was attributed to both the anti-oxidation effects of BBR and through AMPK activation.

The myocardial protective effects of BBR are not simply indirect through modulating lipid metabolism and glucose homeostasis but appear to act directly through modulation of the myocardium and its sympathetic activity. BBR treatment reduced infarction size in rats subjected to ischemia-reperfusion injury and reduced left ventricular myocardium size in rats subjected to experimentally induced cardiac hypertrophy mediated by suprarenal aortic constriction (Chang et al. 2012). Moreover, BBR treatment shortened the prolonged action potential duration and reversed inward rectifying potassium ion channel 2 levels to near normal in T2D diabetic rats (Wang et al. 2014).

Nephropathy

BBR treatment improved the ratio of kidney to body weight, decreased glomerular area, glomerular volume, fasting blood glucose, blood urea nitrogen (BUN), blood creatinine (Cr), and 24 h urinary protein in a streptozotocin-induced diabetic nephropathy rat model (Wu et al. 2012; Wang et al. 2013). In addition, BBR attenuated renal hypertrophy mediated by elevated transforming growth factor beta 1 synthesis and fibronectin accumulation, critical pathological characteristics of diabetic renal fibrosis (Lan et al. 2012; Tang et al. 2011).

Neuropathy

BBR treatment was shown to significantly improve nerve conduction velocity and inhibit glutamate release from cerebral cortex in rats (Lin et al. 2013). A recent report showed that BBR ameliorated cold and mechanical allodynia in a rat model of diabetic neuropathy (Kim and Kim 2013). The anti-neuropathic effects of BBR were likely related to the inhibition of aldose reductase. Activation of this enzyme results in suppression of presynaptic Cav2.1 P/Q voltage-dependent calcium channels and the extracellular signal-regulated kinase/synapsin I signaling cascade, which subsequently leads to the development of diabetic neuropathy.

Beneficial effects of berberine in clinical studies

Although most research on BBR has been performed in animal models, evidence from several clinical studies suggest a beneficial effect of BBR in human diseases. Multiple-complex strategies are recommended for management of T2D patients, especially those who have a poor plasma glucose control. Table 2 summarizes selected representative clinical studies conducted on T2D patients that utilized BBR as a monotherapy, or in a two-drug or three-drug combination. Moreover, metabolic syndrome often co-exists with T2D. Table 2 summarizes studies conducted in T2D patients as an add-on therapy in metabolic syndrome and in hypercholesterolamic patients. The clinical observations were acquired from 2 to 12 months in T2D patients with metabolic syndrome including insulin resistance and hypercholesterolemia, and in those patients that had a long-term (12 months) treatment with BBR (Table 2). The overall results of these studies appear to indicate that BBR treatment as a monotherapy or add-on therapy to standard anti-diabetic treatments produces enhanced anti-diabetic effects. The hypoglycemic and hypolipidemic effects of BBR were similar, compared with the first-line clinical drugs metformin and rosiglitazone. In fact, in one study the lipid lowering effect of BBR was even greater than with metformin. Metformin is the first-choice drug to treat T2D in both adults and children. However, its use is contra-indicated in patients with hepatitis, heart failure, and renal dysfunction. T2D patients with viral hepatitis C treated with BBR for 2 months showed significant reductions in plasma AST and ALT, indicating the potential for BBR to improve simultaneously both hepatic and metabolic dysfunction. Although there have been no clinical reports on BBR's effect on diabetic patients with heart failure or nephropathy, clinical studies in non-diabetic patients with heart failure treated with BBR have shown promise. For example, patients with congestive heart failure showed better left ventricular ejection fraction after BBR administration (Marin-Neto et al. 1988). Moreover, BBR appears to be well tolerated as patients treated with BBR for 3 or 12 months showed no adverse effects or only very mild gastrointestinal effects (Table 2).

If BBR proves to have the same anti-diabetic effects with mild adverse effects in larger clinical trials as in these smaller studies, then its possible use as a candidate drug for treatment of T2D in children might be considered. Currently, there are no clinical reports on the use of BBR as an anti-diabetic in children. In fact, there have been only a few small clinical studies in children using BBR as an anti-diarrhea agent (4–5 day treatment) (Chauhan et al.

Table 2. Clinical trials using BBR as a monotherapy or as an add-on therapy for T2 D, metabolic syndrome, insulin resistance and hypercholesterolemia.

References	Subjects	Therapy targets	Participants (n)	Length of treatment	Daily dose of berberine	Results	Adverse effects report
Li et al. 2013	Female (18–35 years)	Insulin resistance with polycystic ovary syndrome (PCOS)	120	3 months	500 mg, tid	FBG↓, FPI↓, TC↓, TG↓, LDL-C↓, HDL-C↑. BBR showed better effects on dyslipidemia than metformin.	No significant adverse effects were observed in patients.
Di Pierro et al. 2013	Adults (Male and female)	T2 D (Add-on therapy)	31	4 months	1000 mg/day	FBG↓, HbA1c↓, TG↓, TC↓.	15% reported a mild transient abdominal discomfort. None of the patients experienced any musculoskeletal disorders, such as myopathy, or showed clinical signs of liver toxicity. No patients reported any serious adverse events.
Perez-Rubio et al. 2013	Adults (Male and female)	Metabolic syndrome	24	3 months	500 mg, tid	Body weight↓, SBP↓, TG↓, AUC of glucose↓, AUC of insulin, Matsuda index↑.	No significant adverse effects were observed in patients.
Marazzi et al. 2011	Elderly (>75 years)	Hypercholesterolemic patients previously intolerant to statins (Add-on therapy)	40	12 months	500 mg/day	TC↓, LDL-C↓.	No significant adverse effects were observed in patients.
Zhang et al. 2010	Adults (Male and female)	T2 D	50	2 months	1000 mg/day	FBG↓, HbA1c↓, and TG↓. The FBG and HbA1c-lowering efficacies of BBR were close to those of metformin and rosiglitazone. BBR had better effect on the serum level of TG compared to metformin and rosiglitazone.	No adverse effects were observed in the BBR-treated patients.
		T2 D with chronic hepatitis C virus (HCV) infection poorly controlled	35	2 months	1000 mg/day	FBG↓, HbA1c↓, and TG↓ at the same time ALT↓ and AST↓ in patients with hepatitis C and B.	No adverse effects observed in the BBR-treated patients.
Yin et al. 2008	Adults (Male and female)	T2 D patients (Add-on therapy)	48	3 months	500 mg, tid	HbA1c↓, FBG↓, PPG↓, FPI↓.	No significant changes in plasma ALT, γ-GT, and creatinine observed. 34.5% patients suffered transient GI adverse effects during the trail.
	Adults (Male and female)	T2 D	36	3 months	500 mg, tid	HbA1c↓, FBG↓, PPG↓ and TG↓. Hypoglycemic effect of BBR was similar to that of metformin.	No significant changes of plasma ALT, γ-GT, and creatinine were observed during 13 weeks of BBR treatment. 34.5% of patients suffered transient GI adverse effects during the trail.
Zhang et al. 2008	Adults (Male and female)	T2 D and dyslipidemia	116	3 months	1000 mg, bid	FBG↓, PPG↓, HbA1c↓, TG↓, TC↓, LDL-C↓. Body weight↓, SBP↓, DBP↓.	No serious adverse events occurred. Safety parameters including renal and hepatic function, serum electrolytes, blood counts, and urinary analysis were assessed.

Note: FBG: fast blood glucose, HbA1c: (%), TG: triacylglycerol, TC: total cholesterol, PPG: post-load plasma glucose, FGI: fasting plasma insulin, SBP: Systolic blood pressure, DBP: diastolic blood pressure, AST: aspartate transaminase, ALT: alanine transaminase, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol.

1970; Sharda 1970) or as an anti-trachoma agent (3 month treatment) (Khosla et al. 1992; Mahajan and Mohan 1985; Mohan et al. 1982). In both of these studies, good therapeutic results were achieved with no adverse effects.

Safety and tolerability of berberine

Generally, BBR has been shown to be safe in the majority of laboratory and clinical trials. The IC_{50} of BBR is 48 ug/mL and 41 μg/mL, respectively, in HepG2 cells and 3T3-L1 adipocytes (Yi et al. 2013). This is in the order of 120–140 μmol/L. In addition, low μmol/L concentrations of BBR resulted in rapid mitochondria-dependent toxicity in primary neurons (Kysenius et al. 2014). However, no mortality has been observed in rats treated with BBR at 521 $mg \cdot kg^{-1} \cdot day^{-1}$ for 3 months (Yi et al. 2013). The effective antihyperglycemic dose of BBR is generally no more than 100 $mg \cdot kg^{-1} \cdot day^{-1}$ in rats (Chang et al. 2012). Hence, this standard dose of BBR appears to be safely tolerated in animals. In human clinical trials, only a small percentage of patients reported nausea, vomiting, diarrhea, or constipation with BBR treatment, and no severe side effects were observed with standard doses (Sabir and Bhide 1971; Zhang et al. 2010). High doses of BBR resulted in rare adverse effects including headache, skin irritation, and bradycardia (Cannillo et al. 2013). BBR is excreted mainly through the hepatobiliary system, and no adverse effect has been observed in hepatic function. In fact, BBR appears to have beneficial effects on patients with hepatitis (Di Pierro et al. 2012).

The main safety issue of BBR may likely involve the risk of pharmacological interaction. An earlier study indicated that BBR should not be used by pregnant or breast-feeding women due to the fact it displaces bilirubin from albumin (Chan 1993). However, this was not observed in a more recent clinical trial (Linn et al. 2012). BBR may increase gastric emptying time by inhibiting P-glycoprotein in the gut wall and may also increase blood cyclosporine by inhibiting CYP3A4. Thus, cyclosporine levels should be monitored in renal transplant patients using BBR. In addition, BBR also was reported to inhibit CYP1A1 in vitro (Xin et al. 2006). Thus, BBR may potentially alter bioavailability of drugs metabolized by the CYP1A1 system.

Limitations of berberine as an anti-diabetic

As indicated in Table 2, BBR has a similar hypoglycemic and hypolipidemic effect as metformin with little adverse effects. In addition, the clinical studies performed to date indicate that BBR may have a potential beneficial effect on diabetic complications, such as nephropathy, neuropathy, and cardiomyopathy and can be used in diabetic patients with hepatitis C. However, these clinical studies in humans have not been comprehensive. More rigid trials with defined clinical endpoints are needed, especially in Caucasians and in children. In addition, longer trials will be required to better evaluate its safety profile.

Poor oral bioavailability has been another limitation of BBR. This is mediated by the presence in enterocytes of P-glycoprotein, an active adenosine triphosphate consuming efflux protein that extrudes BBR into the intestinal lumen, thus limiting its absorption. Several studies have recently addressed this issue with encouraging results. These include loading BBR onto nanoparticles (Xue et al. 2013), dispersing BBR with absorption enhancers such as sodium caprate (Meng et al. 2014), or simply oral co-administration of BBR with the P-glycoprotein antagonist silymarin (Di Pierro et al. 2012). In all cases oral bioavailability was improved to various degrees. These therapeutic approaches may have the dual advantage of both reducing the dose of BBR required for treatment as well as attenuating its reported adverse gastrointestinal effects.

Conclusion

BBR is a potential new class of anti-diabetic pharmacotherapy. Although there still are no multicenter, well controlled, long-term clinical trials to evaluate its efficacy and safety, it does exhibit promise as an oral medication for reducing plasma glucose and lipid control and has beneficial effects toward diabetic complications. If it proves to be safe and well-tolerated, BBR may represent a good treatment option before initiating insulin therapy in T2D diabetic patients with suboptimal glycemic control.

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