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Review article

Berberine, a plant alkaloid with lipid- and glucose-lowering properties: From *in vitro* evidence to clinical studies

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ABSTRACT

Berberine (BBR) is an isoquinoline plant alkaloid endowed with several pharmacological activities, including anti-microbial, glucose- and cholesterol-lowering, anti-tumoral and immunomodulatory properties. The main mechanism by which BBR exerts a protective role in atherosclerosis relates to its cholesterol-lowering activity. BBR significantly increases hepatic low density lipoprotein receptor (LDLR) expression and reduces the expression and secretion of the LDLR modulator proprotein convertase subtilisin/kexin type 9 (PCSK9). In addition to this, several other atheroprotective effects have been ascribed to BBR, including anti-inflammatory and anti-oxidant properties, inhibition of vascular smooth muscle cell proliferation and improvement of endothelial dysfunction. BBR also increases glucose utilization in adipocytes and myocytes, while decreases glucose absorption in intestinal cells, resulting in a net hypoglycemic effect. In hypercholesterolemic animals, BBR significantly decreases LDL-C and total cholesterol (TC) levels and reduces aortic lesions, an effect similar to that of statins. In diabetic animals, BBR significantly reduces glucose levels, improves glucose tolerance, reduces body weight gain and adipose tissue mass. Several clinical studies have also tested the efficacy of BBR in humans. In hypercholesterolemic subjects, BBR induces a significant reduction of TC, triglycerides and LDL-C levels and a significant increase of HDL-C levels, without major adverse effects. BBR also reduces glycemia and plasma cholesterol in diabetic patients, improves lipid and glucose profile and decreases body mass index and waist circumference in subjects with metabolic syndrome. These findings, together with the good tolerability, suggest that BBR administration might be considered a potential therapeutic approach for the treatment of hypercholesterolemia or diabetes. Given the level of evidence available to date well-designed randomized controlled trials to test safety and efficacy of BBR are warranted.

1. Introduction

Berberine (BBR) is an isoquinoline plant alkaloid belonging to the class of protoberberines present in several plants such as *Berberis vulgaris*, *Coptis chinensis*, *Berberis aristata* [1]. BBR has several pharmacological properties, including anti-microbial, glucose- and cholesterol-lowering, anti-tumoral and immune-modulatory activities [1]. Several studies have shown that BBR has beneficial effects on the cardiovascular system, due to vaso-relaxant and hypotensive effects and to the ability to prevent congestive heart failure, cardiac hypertrophy, and arrhythmia [1].

1.1. Berberine absorption and metabolism

Four major metabolites of BBR have been identified, namely berberrubine (M1), thalifendine (M2), demethyleneberberine (M3) and jatrorrhizine (M4) (Fig. 1) [2]. Biotransformation of BBR is strictly dependent on BBR dosing route; after an oral intake, the first-pass elimination of BBR takes place mainly in the small intestine, while liver is the main accumulating organ, followed by kidney, muscle, heart and pancreas [3,4]. Oxidative demethylation (generating M1) and the subsequent glucuronidation are the main pathways after the oral intake [3], while oxidative demethylenation (generating M3) and glucuronidation of M3 are prevalent after intravenous dosing [5].

Absorption rate of BBR in the small intestine is very low (<5%) [6]; in fact, BBR is a substrate of some ATP-binding cassette (ABC)

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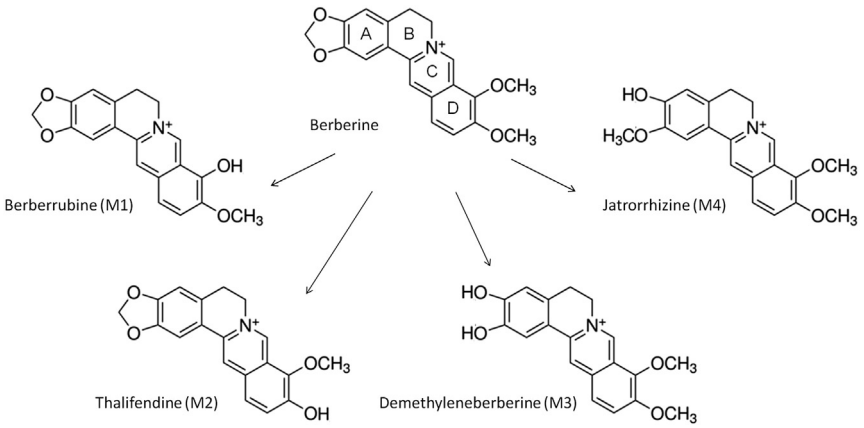

Fig. 1. Structure of berberine and its metabolites.

transporters including P-glycoprotein (P-gp) and multidrug resistance-associated protein-1 (MRP1), which transport BBR out of cells thus reducing BBR absorption [6,7]. In addition, BBR upregulates P-gp expression and function *in vitro* in intestinal cells [6], suggesting that BBR might reduce its own absorption as well as the absorption of other P-gp substrates; on the contrary, the co-administration of BBR with a P-gp inhibitor results in an increased absorption of BBR [8]. BBR is also a high-affinity substrate for organic cation transporters 1 (OCT1) and 2 (OCT2), responsible for the transport of metformin, the most used anti-diabetic drug [9]. This might translate into a possible drug–drug interaction in the case of co-administration of BBR with metformin: in rats, BBR increases the plasma concentration and area under the curve (AUC) of metformin and reduces its systemic clearance and volume of distribution [9].

The cytochrome P450 (CYP) superfamily plays a crucial role in the metabolism of endogenous compounds, drugs, hormones and xenobiotics; thus, modulation of cytochrome P450 enzymes by specific natural compounds increases the chance of metabolic interaction with other drugs administered simultaneously and may have clinical consequences. BBR modulates the expression/activity of some CYP isoenzymes both *in vitro* and *in vivo* [10,11], and this may translate into clinically relevant interactions with some drugs (Table 1): in renal-transplant recipients, BBR markedly increases the blood concentration of cyclosporine A, due to the inhibition of CYP3A4 and P-gp [12]. An interaction between tacrolimus and BBR, both substrates of CYP3A4-A5, has been reported, resulting in a rapid increase of tacrolimus blood concentration [13]. These observations suggest that potential interactions should be taken into account when BBR is administered concurrently with other drugs, and dose adjustment based on drug monitoring is recommended.

Table 1
Mechanisms of BBR-drug interactions.

Drug	CYPs/transporters involved
Metformin	OCT1/OCT2
Tacrolimus	CYP3A4
Cyclosporine A	P-gp/CYP3A4
HIV protease inhibitors	P-gp
Verapamil	P-gp
Tolbutamide	CYP2C9
Midazolam	CYP3A4
Dextromethorphan	CYP2D6
Losartan	CYP2C9
Phenacetin	CYP1A2
Digoxin	P-gp
Quinidine	OCT/P-gp

1.2. *In vitro effects of BBR*

A high number of studies have identified the mechanisms of action of BBR at cellular level, particularly in hepatic cells, cells of the arterial wall, pancreatic β-cells, adipocytes and myocytes (Table 2, Fig. 2). Among the pathways through which BBR modulates cellular processes, AMP-activated protein kinase (AMPK) plays a central role [14]. AMPK is a cellular energy sensor that, upon activation, stimulates catabolic processes (such as fatty acid oxidation, glucose uptake, lipolysis) while inhibits anabolic processes (such as gluconeogenesis, fatty acid synthesis, cholesterol synthesis) [14].

1.3. *Hepatic cells*

The main mechanism by which BBR exerts its protective role in atherosclerosis is by acting as a cholesterol-lowering drug [15]. In fact, BBR significantly increases hepatic low density lipoprotein receptor (LDLR) expression, due to increased LDLR mRNA stability, thus resulting in a higher LDL uptake in BBR-treated cells [15]. BBR upregulates LDLR receptor expression through the activation of the signaling cascade AMPK/Raf-1/MEK/ERK [15,16], although JNK pathway plays a role as well [17].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease mainly expressed in hepatocytes and enterocytes; after secretion, circulating PCSK9 binds the extracellular domain of LDLR to induce its internalization and degradation, resulting in increased plasma LDL-C levels [18]. Statins, the leading cholesterol-lowering drugs, act by increasing the expression of hepatic LDLR; however, they also increase PCSK9 expression [19], suggesting that the statin therapeutic effect might be reduced by their effect on PCSK9. On the contrary, BBR reduces PCSK9 mRNA expression and the amount of PCSK9 secreted by HepG2 cells [20]; this effect does not appear to be due to changes in the PCSK9 mRNA stability but rather to a reduced cellular abundance of two critical trans-activators, hepatocytes nuclear factor 1α (HNF1α) and sterol regulatory element-binding protein 2 (SREBP2), resulting in PCSK9 transcriptional repression [21]. When added to cells in combination with a statin, BBR counteracts the inducing effects of statin on PCSK9 transcription [21]. Although SREBP2 is essential for LDLR transcription, its suppression by BBR does not result in LDLR transcription repression [21], suggesting that the overall effect of BBR is in favor of LDLR

Table 2
In vitro effects of berberine.

Cell type	Effect	Ref.
Hepatic cells	• ↑LDLR	[15]
	• ↓PCSK9	[20,21]
	• ↓lipid synthesis	[22,23]
	• ↑PON1	[24]
Macrophages	• ↓Macrophage migration	[25]
	• ↓Pro-inflammatory mediators	[26]
	• ↑Anti-oxidant enzymes	[26]
	• ↓Oxidative stress	[27]
	• ↓MMP expression	[31,32]
	• ↓LOX-1 expression	[33]
	• ↑ABCA1 expression and cholesterol efflux	[34]
Smooth muscle cells	• ↓Proliferation and migration	[36,37]
Endothelial cells	• ↓Pro-inflammatory mediators	[39]
	• ↓Leukocyte adhesion	[39,40]
	• ↑eNOS activation	[41,42]
	• ↓ROS production	[41,42]
	• ↓EMPs and EMP-induced eNOS downregulation	[26]
	• ↑EPC number, mobilization and function	[45–47]
β-cells	• ↑Anti-inflammatory	[26]
	• ↓Oxidative stress	[48]
	• ↑Insulin secretion	[48]
	• ↑InsR	[49]
	• ↓Insulin gene transcription	[50]
Adipocytes	• ↑Glucose consumption and uptake	[51]
	• ↓Pro-inflammatory genes	[55]
	• ↓Adipocyte differentiation	[57–60]
Myocytes	• ↑Glucose consumption and uptake	[51]
Cardiomyocytes	• ↓Insulin-induced hypertrophy	[61]
	• ↑Glucose consumption and uptake	[62]
	• ↓Ischemia/reperfusion-induced apoptosis	[63,64]
Mesangial cells	• ↓Proliferation	[65,66]
	• ↓Oxidative stress	[65,66]
	• ↓Matrix accumulation	[65,66]
Bone cells	• ↓Osteoclast differentiation and activity	[68]
	• ↑Osteoblast differentiation and activity	[69]

Abbreviations: LDLR, low density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9; PON1, paraoxonase 1; MMP, matrix metalloproteinase; LOX-1, lectine-like oxidized low density lipoprotein receptor 1; OxLDL, oxidized low density lipoprotein; ABCA1, ATP-binding cassette transporter 1; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; EMP, endothelial microparticles; EPC, endothelial progenitor cells; InsR, insulin receptor.

expression.

BBR also inhibits cholesterol and triglyceride (TG) synthesis and secretion in HepG2 through the activation of AMPK, resulting in reduced lipid and fatty acid content in the liver of fat-fed hamsters [22]. Additionally, BBR and its metabolites increase lipolysis gene expression while reducing lipogenesis genes through the activation of AMPK, thus resulting in a reduction of lipid synthesis and increase of fatty acid oxidation [23]. These findings indicate that BBR acts as lipid-lowering drug through several mechanisms.

Paraoxonase 1 (PON1) is an enzyme synthesized mainly in the liver, carried by plasma HDL and known to play an anti-atherogenic role: PON1 protects lipoproteins from oxidation and its activity is lower in subjects with atherosclerosis-related diseases, while its overexpression in animal models results in reduced atherosclerotic lesions [24]. BBR significantly increases PON1 mRNA and protein expression in HepG2 cells through the activation of JNK and c-Jun signaling pathway [24]; this finding suggests that the induction of PON1 by BBR treatment could be one of the multiple mechanisms by which this compound exerts its cardioprotective effects.

1.4. Macrophages

Abnormal macrophage recruitment by activated endothelium followed by the release of pro-inflammatory cytokines represent key processes in atherosclerosis; BBR is able to control macrophage trafficking as well as the expression and secretion of pro-inflammatory cytokines and the increase of oxidative stress in activated macrophages [25–27] through the activation of several signaling pathways [28–30].

BBR influences also several other processes in which macrophages are involved. As an example, BBR might contribute to atherosclerotic plaque stabilization; in fact, the pretreatment of macrophages with BBR before the exposure to stimuli that upregulate the expression of matrix metalloproteinases (MMPs), enzymes that have a causative role in plaque vulnerability, leads to a reduced expression of MMPs and extracellular MMP inducer (EMMPRIN) [31,32]. BBR significantly affects the expression of scavenger receptors in activated macrophages: it decreases lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) expression and decreases scavenger receptor class B type I (SR-BI) [33]. Furthermore, BBR increases ABCA1 expression by activating liver X receptor α (LXRα), thus resulting in an increased cholesterol efflux [34]. BBR also inhibits macropinocytosis, a receptor-independent process of LDL internalization, thus inhibiting cholesterol accumulation and negative responses induced by excess cholesterol [35].

1.5. Vascular smooth muscle cells

Vascular smooth muscle cell (VSMC) proliferation and migration initiate the intimal thickening in atherosclerosis and restenosis; *in vitro,* BBR inhibits VSMC proliferation and migration by interfering with several cellular pathways, including Akt and ERK pathways and by reducing intracellular reactive oxygen species

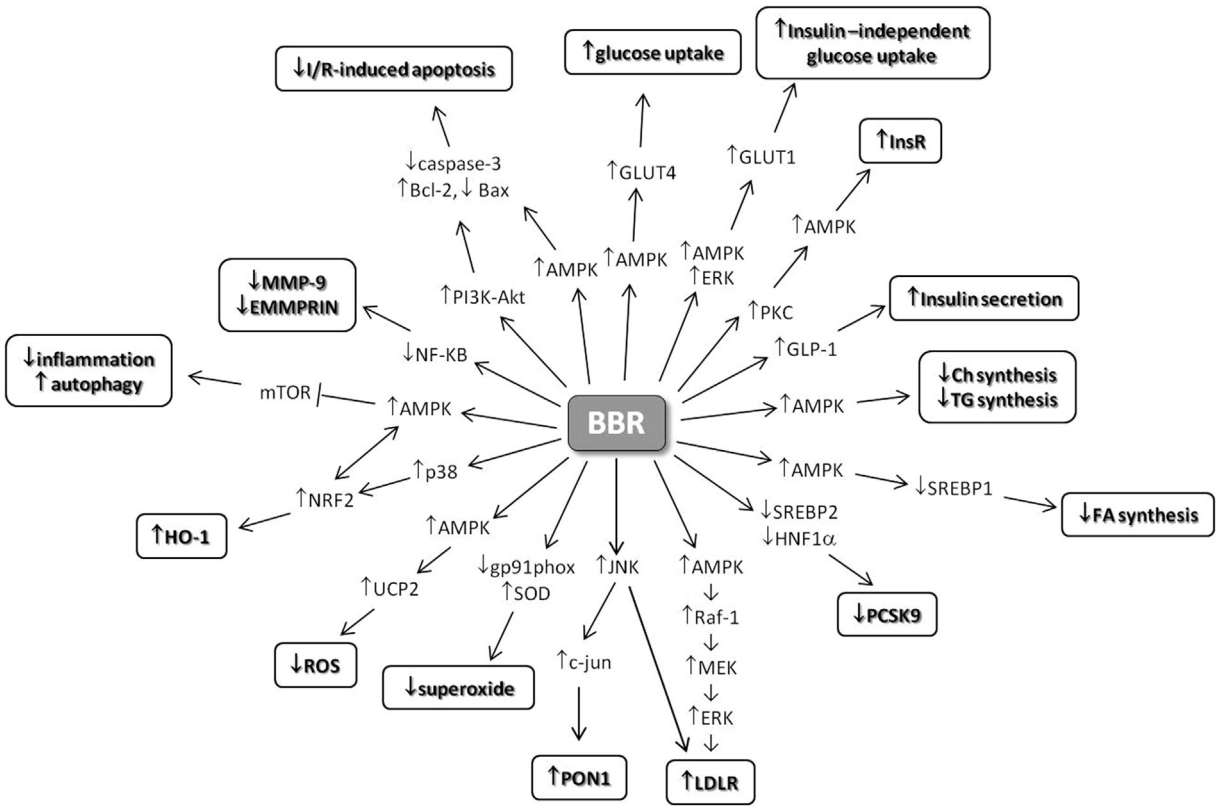

Fig. 2. Main mechanisms of action of berberine.

(ROS) [36,37]. *In vivo*, chronic BBR treatment improves neointimal formation in a rat carotid artery injury model [37]. Altogether these findings suggest that BBR might be able to control restenosis.

1.6. Endothelial cells

Endothelial dysfunction is one of the early events in the atherosclerotic process; thus, restoring or correcting the functionality of endothelium may have several beneficial effects [38]. The exposure of dysfunctional endothelial cells (ECs) to BBR results in an improved cell function: BBR inhibits the expression and secretion of MCP-1 and vascular cell adhesion molecule-1 (VCAM-1) in activated ECs and reduces MCP-1 receptor expression in monocytes, resulting in a reduced adhesion of monocytes to ECs [39,40].

High oxidative stress and reduced nitric oxide (NO) availability contribute to the onset of endothelial dysfunction. These two conditions may be induced by several factors, including high glucose levels and free fatty acids [41]. BBR reduces ROS production, increases endothelial nitric oxide synthase (eNOS) expression and activation, resulting in increased NO generation and reduces monocyte adhesion in ECs exposed to palmitate or high glucose [41,42]. The physiological relevance of these findings is supported by the *ex vivo* observation that BBR improves vasodilatation in isolated aorta rings exposed to high glucose [42]. In diabetic subjects, retinopathy represents one of the most severe complications, due to increased oxidative stress and inflammation in the retina; some observations have suggested that leukocytes play a key role in the development of diabetic retinopathy, by inducing apoptosis following the direct contact with retinal ECs [43]. *In vitro,* BBR inhibits leukocyte adhesion and, as a consequence, leukocyte-induced apoptosis of human retinal endothelial cells cultured in high glucose, and leukocytes from diabetic patients treated with BBR have a reduced ability to induce EC apoptosis [43]. These observations suggest a therapeutic potential for BBR against diabetes-related vascular complications.

High levels of endothelial-derived microparticles ($CD31^+/CD42^-$ EMPs) represent a marker of endothelial dysfunction, as they are strictly related to vascular dysfunction in subjects with cardiovascular disorders [44]. In ECs exposed to microparticles, BBR inhibits microparticle-induced eNOS downregulation and maintains NO formation, while reducing oxidative stress [26]. In healthy subjects treated for 1 month with BBR, circulating $CD31^+/CD42^-$ EMPs are reduced compared with controls and endothelium-dependent vasodilatation is improved, while endothelium-independent is not [26], suggesting that the effect of BBR is largely related to an improved endothelial function.

Circulating endothelial progenitor cells (EPCs) play a relevant role in the maintenance of normal endothelial function; BBR administration for 1 month increases the number of EPCs in healthy subjects and improves small artery elasticity index, a marker of endothelial function, with a positive correlation between these two parameters [45]. This suggests that BBR may increase EPC mobilization, as well as the number and function of circulating EPCs and the levels of plasma NO, with a linear regression relationship between these two parameters [46]. Furthermore, in the presence of high levels of inflammatory cytokines, EPCs may reduce their proliferative capacity, and BBR improves the proliferation of EPCs exposed to an inflammatory stimulus [47].

1.7. Other cell types

1.7.1. Pancreatic β-cells

Inflammation and apoptosis contribute to β-cell failure in diabetes; *in vitro*, the exposure of β-cells to LPS results in an

inflammatory response that is attenuated by BBR through a TLR4-independent JNK/NF-kB pathway [26]. In addition, BBR inhibits oxidative stress and restores insulin secretion in β-cells exposed to high glucose by stimulating AMPK activity, that in turn upregulates the expression of uncoupling protein-2 (UCP2), a modulator of mitochondria-derived ROS [48]. BBR increases insulin receptor (InsR) expression and enhances insulin signaling in several human cell lines; accordingly, a 2-month therapy with BBR lowers blood glucose and insulin levels in type 2 diabetes patients, due to an increased expression of InsR in peripheral cells [49]. BBR also inhibits insulin protein expression *in vitro* in a mouse β-cell line, reduces insulin content in islet of high-fat fed mice and improves insulin resistance and glucose intolerance [50].

1.7.2. Adipocytes and myocytes

BBR increases glucose consumption and uptake in adipocytes and myocytes [51]; the increased glucose uptake is due to an enhanced expression and activity of glucose transporters (GLUT) 1 and 4 through the activation of AMPK and ERK pathways [52,53]. In the presence of insulin, glucose consumption is further increased by BBR, suggesting an additive action of BBR and insulin [51]. The enhanced glucose metabolism is due to the stimulation of glycolysis [51]. On the other hand, BBR decreases glucose absorption in intestinal cells by inhibiting α-glucosidases, the enzymes involved in the digestion of dietary carbohydrates, and by decreasing glucose transport through the intestinal epithelium [54].

BBR reduces the expression of pro-inflammatory genes in the white adipose tissue of obese mice as well as in LPS- and TNFα-treated adipocytes, similarly to metformin or rosiglitazone [55]. In addition, conditioned media from BBR-treated macrophages improves the insulin response of adipocytes; this might be due to a BBR-induced secretion of cytokines and chemokines from macrophages that can improve the response of surrounding cells [55]. BBR exerts an anti-lipolytic effect in adipocytes independently of AMPK pathway, contrarily to the effect described in hepatic cells [56].

Another relevant effect of BBR is its anti-adipogenic effect, due to its ability to modulate the expression of transcription factors and other genes involved in adipogenesis, resulting in the inhibition of adipocyte differentiation and contributing to the proposed anti-obesity activity of BBR [57–60].

1.7.3. Cardiomyocytes

High glucose and insulin levels may induce cardiomyocyte hypertrophy in diabetes; BBR prevents this effect and restores NOS activity and NO levels through the activation of PPARα signaling pathway [61]. In addition, BBR increases glucose consumption and uptake in cardiomyocytes, and attenuates the reduction of these two processes in insulin-resistant cardiomyocytes through the activation of AMPK [62]. Myocardial apoptosis is a process that occurs following an ischemia/reperfusion injury and may lead to cardiac injury and heart failure; BBR reduces the apoptosis of cardiomyocytes induced by hypoxia/reoxygenation by modulating several processes [63,64].

1.7.4. Mesangial cells

Dysfunction of mesangial cells is a hallmark of diabetic nephropathy and includes cell proliferation, increased production of extracellular matrix and inflammation. BBR inhibits glucose-induced mesangial cell proliferation, oxidative stress as well as the expression and activity of aldose reductase, a key enzyme in the development of diabetic nephropathy and reduces collagen and fibronectin accumulation [65,66], leading to the hypothesis that BBR may improve diabetes-induced renal dysfunction.

1.7.5. Bone cells

Diabetic osteopathy is a diabetes complication that results in reduced bone mineral density and bone formation due to increased osteoclastogenesis and reduced osteoblastogenesis [67]. BBR reduces osteoclast differentiation and activity [68] and increases osteoblast differentiation and activity [69], suggesting a potential role in the treatment of this pathological condition, likely through the activation of AMPK signaling pathway [70].

2. BBR effects in animal models

2.1. Hypercholesterolemic animals

The first *in vivo* demonstration that BBR reduces LDL-C levels by increasing LDLR expression has been obtained in hamsters fed a high-fat high-cholesterol diet: the oral administration of BBR for 10 days reduced total cholesterol (TC) and LDL-C levels [15] (Table 3); this effect was due to a 3.5-fold increase of hepatic LDLR mRNA and 2.6-fold increase of LDLR protein [15]. Since then, several other studies have been performed in animal models showing beneficial effects of BBR (Table 3). Chronic treatment with BBR significantly reduces aortic lesions in apoE$^{-/-}$ mice fed a pro-atherogenic diet, reduces oxidative stress and vascular inflammation through an AMPK-dependent mechanism [71] (Table 3). In hypercholesterolemic rats, BBR decreases blood cholesterol levels also by reducing intestinal cholesterol absorption [72], suggesting multiple mechanisms of action for this compound. Besides, BBR shows positive effects also on other cardiovascular risk factors (Table 3), including serum homocysteine levels and inflammation [73,74].

When BBR was compared with simvastatin in high-fat high-cholesterol (HFHC) fed rats, similar LDL-C reductions were observed (−26.8% and −28.3%, respectively), and the combination of the two drugs resulted in a greater reduction of LDL-C levels (−46.2%) [75], suggesting that BBR and simvastatin act in an additive manner. Similar results have been observed for TC and hepatic LDLR mRNA expression [75]. Both BBR and simvastatin reduce steatosis induced by HFHC diet, due to reduction of hepatic TC and TG content, and improves liver tissue morphology and hepatic enzyme levels; the improvement was even higher with the combination therapy [75].

BBR also ameliorates kidney injury in hypercholesterolemic rats by reducing blood pressure, LDL-C levels, urinary albumin as well as inflammation and the oxidative stress status by inhibiting the NF-kB signaling pathway [76], suggesting that BBR, beside its significant effect on hypercholesterolemia, may also protect against the worsening of atherosclerosis-induced renal dysfunction (Table 3).

2.2. Type 1 diabetes

Type 1 diabetes (T1D), an autoimmune disease, is caused by T-lymphocyte-induced injury and disruption of insulin secreting β-cells [77]. Th1 and Th17 lymphocytes play a role in the development of T1D [26]. In non-obese diabetic (NOD) mice, that spontaneously develop T1D, the administration of BBR for 14 weeks significantly reduces fasting serum glucose levels [78], improves serum lipids and insulin levels, and increases islet cell number, suggesting a protective effect on pancreatic islets [79] (Table 3). One mechanism proposed to explain the beneficial effect of BBR on T1D is through its ability to reduce Th17 and Th1 cytokine secretion, thus inhibiting the differentiation of Th17 and Th1 lymphocytes [26]. The observed effects on these T cell subpopulations also provide new information on the anti-inflammatory properties of BBR.

Table 3
Effects of berberine in animal models.

Animal model	Effect	Ref.
Hypercholesterolemia		
Hamsters	• ↓TC, LDL-C	[15]
Mice	• ↓Aortic lesions	[71]
	• ↓Oxidative stress	[71]
	• ↓Vascular inflammation	[71]
Rats	• ↓TC, LDL-C, TG, non-HDL-C	[72,73,75,76]
	• ↓Fractional dietary cholesterol absorption	[72]
	• ↓Hepatic cholesterol and steatosis	[75]
	• Improvement of liver tissue morphology	[75]
	• ↓Blood pressure	[76]
	• ↓Urinary albumin	[76]
	• ↓Homocysteine	[73]
	• ↓Inflammation and oxidative stress	[76]
Type 1 diabetes		
Non-obese diabetic mice	• ↓Fasting glucose levels	[78]
	• Improvement of serum lipid and insulin levels	[79]
	• ↑Islet cell number	[79]
	• ↓Th1 and Th17 cytokine secretion	[26]
Type 2 diabetes		
Mice	• ↓Fasting blood glucose; ↑glucose tolerance	[85]
	• ↓Body weight	[85]
	• ↓Adipose tissue mass; ↓ adipocyte size	[85]
	• ↑Energy expenditure	[86]
	• Improvement of cold tolerance	[86]
	• ↑Brown adipose tissue activity	[86]
	• ↑Liver function	[88]
	• Prevention of hyperglycemia-induced renal dysfunction	[91]
Rats	• ↓Blood glucose levels, ↑glucose tolerance	[80,81,83,84]
	• ↑Insulin secretion; ↓insulin resistance	[80,85,87]
	• ↓Lipid (cholesterol, TG, FFA) levels; ↑HDL and apoA-I	[80,81,85,87,90,91]
	• ↓Body weight	[85,87]
	• ↓Onset of diabetes	[80]
	• ↑Myocardial antioxidant enzyme activity	[26]
	• Improvement of cardiac function	[63,80,89]
	• Restored the injured pancreatic tissue	[26]
	• Restored renal functional parameters	[90,91]
	• Prevented renal structural alterations	[90,91]
Hamsters	• ↓TC, TG, FFA, LDL-C	[81]
	• ↓TBARS and 8-isoprostane	[81]
	• ↑Superoxide dismutase activity	[81]
	• ↑Glucose tolerance	[81]
Obesity		
Mice	• ↓TC, TG, glucose	[53,93]
	• ↑Glucose tolerance	[93]
	• ↓Fasting serum insulin and HOMA-IR	[53]
	• ↓Pro-inflammatory genes	[55]
Rats	• Prevented body weight increase	[94,96]
	• Prevented changes of IR, IRS-1 and glucagon expression	[94]
	• Changes in gut microbiota composition	[96,97]

TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides; non-HDL-C: non-high density lipoprotein cholesterol; Hcy: homocysteine; TBARS: thiobarbituric acid reactive substances; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; FFA: free fatty acids; I/R: ischemia/reperfusion; HOMA-IR: homeostasis model assessment-estimated insulin resistance; IR: insulin receptor; IRS-1: insulin receptor substrate 1.

2.3. Type 2 diabetes

Berberine efficacy has been evaluated in several models of diabetes (Table 3). In streptozotocin-treated, high-fat fed rats, a 4-week treatment with BBR significantly decreases blood glucose levels after oral glucose tolerance test and reduces the onset of diabetes [80]. In addition, BBR reduces fasting levels of glucose, cholesterol, TG and free fatty acids, while increases HDL and apoA-I [80]. In alloxan-induced diabetic Wistar rats fed a high-cholesterol diet, BBR improves glucose and lipid levels, increases the levels of antioxidant enzymes in heart tissue and restores the injured pancreatic tissues [26]. In high glucose and high fat diet-induced diabetic hamsters, BBR significantly reduces TC, TG, FFA, LDL-C plasma levels as well as TBARS and 8-isoprostane levels, while increases plasma superoxide dismutase activity [81]. In addition, BBR modulates glucose tolerance in diabetic animals [81]. One mechanism by which BBR exerts its effect on glucose metabolism might be through the induction of glucagon-like peptide 1 (GLP-1), a gut-derived hormone secreted from intestinal cells in response to glucose load and involved in the glucose-dependent stimulation of insulin secretion and β-cell proliferation [82]. BBR lowers blood glucose in type 2 diabetes rats by increasing liver and muscle InsR expression [83]. BBR also improves glucose metabolism through an insulin-independent pathway by inhibiting gluconeogenic genes [84]. BBR may also favor insulin secretion, as shown both *in vitro*, in a model of insulin-secreting cell line cultured in the presence of glucose, and *in vivo* in BALB/c mice [80].

In *db/db* mice, that exhibit marked obesity, dyslipidemia and glucose intolerance, BBR, beside the reduction of fasting blood glucose levels and the improvement of glucose tolerance [85], also

lowers body weight and adipose tissue mass by reducing the size of adipocytes [85]. BBR also increases energy expenditure, limits weight gain, improves cold tolerance and increases brown adipose tissue activity in mice, suggesting a possible role for BBR in the treatment of obesity [86]. In agreement with these findings, BBR reduces body weight, plasma TG levels and insulin resistance in diabetic rats [85,87]. These effects are, at least in part, mediated by the activation of AMPK in several tissues, including adipose tissue and muscle [85,86]. BBR also stimulates AMPK activity in the liver of obese mice, increases fatty acid oxidation and modulates the expression of genes involved in lipid metabolism, thus resulting in improved liver function, and decreases the expression of hepatic pro-inflammatory genes that may play a role in the development of steatohepatitis [88].

BBR improves the oxidative stress status in diabetic animals [26,81], and may positively affect some complications of diabetes. For example, BBR improves myocardial dysfunction induced by hyperglycemia/hypercholesterolemia by increasing the expression of cardiac fatty acid transport proteins, fatty acid β-oxidase, GLUT-4 and PPARγ, while decreasing PPARα expression [89], and treatment of diabetic rats with BBR improves the recovery of cardiac systolic/diastolic function and myocardial apoptosis after myocardial ischemia/reperfusion [63]. Due to its various pharmacological actions, BBR is also believed to have a potential therapeutic action on diabetic nephropathy through several mechanisms [90]. BBR improves fasting blood glucose and lipid metabolism, restores renal functional parameters, prevents structural alterations of kidney tissues and increases the levels of E prostanoid receptor 4 (EP4), reduces inflammation and the deposition of extracellular matrix in diabetic rats [90,91]. BBR also prevents hyperglycemia-induced kidney dysfunction, including glomerulosclerosis formation, blood urea nitrogen increase, creatinine clearance decrease, urinary albumin excretion increase and oxidative stress in diabetic mice through AMPK activation [91].

2.4. Obesity

Obesity, characterized by a prolonged imbalance between energy intake and expenditure, can lead to insulin resistance and impaired glucose control, thus resulting in type 2 diabetes [92]. BBR has positive effects on several factors involved in obesity and insulin resistance (Table 3): it reduces weight gain and food intake in diet-induced obese mice and lowers glucose, TG and TC levels and controls adipogenesis by modulating the expression of adipogenic transcription factors [93]; similarly, BBR prevents body weight increase and changes in the expression of InsR, insulin receptor substrate-1 (IRS-1) and glucagon in rats fed a high-fat diet [94], suggesting a possible role of BBR in the control of insulin resistance.

In KK-Ay mice, a model of diabetes with obesity, BBR treatment reduces fasting blood glucose, fasting serum insulin levels and HOMA-IR, improves glucose tolerance and reduces TC and TG by modulating several pathways [53]. In addition, BBR inhibits the expression of pro-inflammatory genes in the adipose tissue of obese mice, indicating that BBR may attenuate both the acute inflammatory response and the low-grade inflammatory status typical of obesity [55].

Another mechanism by which BBR may inhibit the development of diabetes might be related to its anti-microbial properties and to the ability to modulate gut microbiota (whose composition may impact the predisposition to diabetes) [95]. BBR is poorly adsorbed and thus acts topically in the gastrointestinal tract, resulting in the modification of gut microbiota [95]. Thus, the treatment of obese rats with BBR prevents the body weight increase observed in non-treated animals and reduces the adiposity index, prevents the development of insulin resistance and reduces systemic inflammation [96]. The analysis of gut microbiota reveals that BBR markedly changes its composition, with several intestinal microbes that are eliminated or inhibited and other selectively enriched [96,97], leading to the alleviation of systemic inflammation and contributing to the beneficial effects of BBR against insulin resistance, obesity and diabetes.

3. Clinical studies

3.1. BBR in the treatment of hypercholesterolemia

The first study that evaluated the effect of BBR in Chinese hypercholesterolemic subjects (TC > 201 mg/dL) reported a significant cholesterol lowering-property accompanied by a TG-lowering effect (Table 4) [15]. These effects are even more marked in subjects not taking other medications before or during BBR treatment [15]. A recent meta-analysis of randomized controlled trials shows that treatment with BBR induces a significant reduction of TC, TG and LDL-C levels and a significant increase of HDL-C levels, without serious adverse effects [98]. When BBR has been evaluated in a Caucasian hypercholesterolemic population at low cardiovascular risk, significant decreases of lipid parameters have been observed with a relevant increase of HDL-C (Table 4) [99]. No significant adverse reactions have been reported during the study [99].

BBR effects have also been compared with those of simvastatin: a 2-month therapy using BBR, simvastatin or both in hypercholesterolemic patients results in significant reductions of LDL-C, TC and TG [75]. In particular, a significant beneficial effect has been obtained with the combined therapy on all lipid parameters; BBR and simvastatin in fact seem to reduce LDL-C levels in an additive manner; moreover, while simvastatin alone induces only a moderate TG decrease, when administered in combination with BBR results in a significant decrease of TG levels (Table 4) [75], suggesting that this combination may be beneficial for the treatment of hypercholesterolemic subjects with high TG levels.

A nutraceutical combination containing BBR and other cholesterol-lowering compounds such as policosanol and red yeast rice (RYR) has been tested in hypercholesterolemic patients in a double blind, placebo-controlled study [100] (Table 4). TC and LDL-C decrease and endothelial-mediated flow dilation significantly increases after treatment [100]. In addition, in patients with insulin resistance at baseline, the treatment with the nutraceutical combination reduces HOMA and increases insulin sensitivity [100]. The nutraceutical combination is effective also in elderly (>75 years) hypercholesterolemic patients intolerant to statins, with a high tolerability and compliance [101]. When compared with ezetimibe in hypercholesterolemic subjects intolerant to or refusing statin therapy, the nutraceutical combination is more effective in reducing TC (−24.2% *vs* −19.0% $p < 0.001$), LDL-C (−31.7% *vs* −25.4%, $p < 0.001$) and non-HDL-C (−30.3% *vs* −24.2%, $p < 0.001$), with a tendency to lower TG more than ezetimibe (−19.5% *vs* −14.9%) [102]. The reduction obtained with this combination therapy is similar to that obtained with statin therapy (simvastatin 20, atorvastatin 10, rosuvastatin 5 mg/day) [103], and thus may represent a safe alternative for patients intolerant to statins. In patients with heterozygous familial hypercholesterolemia on stable treatment with statins or statin + ezetimibe, adding BBR leads to a further reduction of LDL-C (−42.6% on statin or statin + ezetimibe vs −53.2% after BBR/P/RYR addition, $p < 0.001$) [102].

As postmenopausal women present an increased cardiometabolic risk, a nutraceutical combination containing BBR and isoflavones has been tested versus placebo in healthy, mildly dyslipidemic postmenopausal women, showing an improvement of plasma lipid levels and serum levels of metalloproteinases [104].

Table 4
Effects of berberine in clinical studies.

	BBR treatment	Number of subjects	BBR effects
Hypercholesterolemia [Ref.]			
Kong et al., 2004 [15]	0.5 g twice a day, 3 months	63 BBR 28 Placebo	• ↓TC (−18%), LDL-C (−20%), TG (−28%)
Derosa et al., 2013 [99]	0.5 g twice a day, 3 months	71 BBR 70 Placebo	• ↓TC (−11.6%), LDL-C (−16.4%), TG (−21.2%) • ↑HDL-C (+9.1%)
Kong et al., 2008 [75]	BBR 1 g/day, 2 months Simva 20 mg/day BBR + Simva	24 BBR 16 Simvastatin 23 BBR + Simvastatin	• ↓TC (−21.8%), LDL-C (−23.8%), TG (−22.1%) • ↓TC (−9.1%), LDL-C (−14.3%), TG (−11.4%) • ↓TC (−29.1%), LDL-C (−31.8%), TG (−38.9%)
Affuso et al., 2010 [100]	0.5 g/day BBR (in combination with RYR and policosanol) 6 weeks	25 BBR + RYR + Pol 25 Placebo	• ↓TC (−17.4%),LDL-C (−23.3%) • ↑FMD (+3%)
Diabetes/MetS			
Yin et al., 2008 [105]	0.5 g thrice a day, 3 months	15 BBR 16 Metformin	• ↓Fasting glucose levels (−35.5%) • ↓Postprandial glucose levels (−44%) • ↓TC (−13%), TG (−21.2%) • ↓HbA1c (−2%)
Dong et al., 2012 [106]	0.5–1.5 g/day, 8–12 weeks	138 BBR 133 Placebo	• ↓Fasting glucose levels (−12%) • ↓Postprandial glucose levels (−16%) • ↓HbA1c (−0.9%) • ↓TC (−11%), LDL-C (−16%), TG (−21%) • ↑HDL-C (+5%)
Perez-Rubio et al., 2013 [107]	0.5 g thrice a day, 3 months	12 BBR 12 Placebo	• ↓ 36% MetS • ↓Waist circumference (−2.8%) • ↓Systolic blood pressure (−6.5%) • ↓TG (−42%) • ↓Glucose levels (−10%) • ↓Insulin secretion (−27%) • ↑Insulin sensitivity (+48%)
Yang et al., 2012 [108]	0.3 g thrice a day, 3 months	37 BBR	• ↓TC (−14%), LDL-C (−22%), TG (−39%) • ↓BMI (−13%) • ↓Leptin levels (−36%) • ↓Leptin/adiponectin ratio (−24%) • ↓HOMA-IR (−41%)
Affuso et al., 2012 [109]	0.5 g/day (in combination with RYR and policosanol) 18 weeks	29 BBR + RYR + Pol 30 Placebo	• ↓HOMA-IR (−19%) • ↓TC (−15%), LDL-C (−23%)

TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; FMD, flow-mediated dilation; HOMA-IR: homeostasis model assessment-estimated insulin resistance; HbA1c: glycated albumin; MetS: metabolic syndrome; BMI: body mass index.

3.2. BBR in the treatment of diabetes and metabolic syndrome

BBR has a proved hypoglycemic activity; it appears that the main hypoglycemic mechanism of BBR is through the upregulation of InsR, thus resulting in the increase of glucose uptake and the glucose-lowering effect [49]. However, additional mechanisms have been established, including the anti-microbial activity of BBR that may contribute to modulate the gut microbiota composition [26], whose alterations have been associated with obesity and diabetes. In a pilot study assessing the efficacy of BBR as anti-diabetic in humans, newly diagnosed subjects treated with BBR showed reduced levels of fasting and postprandial glucose, HbA1c, TG and TC [105]; the reductions of parameters involved in glucose metabolism are comparable to that induced by metformin, but BBR has also effects on lipid levels [105] (Table 4).

A meta-analysis confirms the hypoglycemic efficacy of BBR [106] (Table 4). When BBR treatment is combined with lifestyle modification, fasting and postprandial glucose levels, HbA1c and plasma lipids show a higher improvement compared with simple lifestyle modification [106]. When compared with oral hypoglycemics, a similar efficacy in reducing fasting plasma glucose and HbA1c is observed, but BBR also improves lipid profile [106]. The combination of BBR with oral hypoglycemics significantly improves fasting and postprandial glucose levels as well as HbA1c levels compared with the oral hypoglycemics alone [106]. No significant differences in the incidence of adverse effects are observed between BBR group and controls, and no serious adverse events are reported [106].

In subjects with metabolic syndrome, BBR administration induces a 36% remission of the presence of metabolic syndrome, decreases waist circumference, systolic blood pressure, TG, glucose levels and insulin secretion, and increases insulin sensitivity [107]. BBR also reduces BMI, leptin levels and leptin/adiponectin ratio (Table 4), suggesting an adjustment of adipokine profile, as indicated by the *in vitro* finding that treatment of pre-adipocytes with BBR inhibits the differentiation to mature adipocytes as well as leptin and adiponectin expression and secretion [108]. BBR in combination with policosanol and RYR for 18 weeks significantly reduces HOMA-IR, TC and LDL-C, while no significant effects on TG and HDL-C are observed [109].

3.3. Clinical studies limitations

Despite the positive results of a large number of clinical trials evaluating the effects of BBR in the treatment of human dyslipidemia or diabetes, limitations of these studies must be acknowledged. First, most of the studies have been performed in Chinese subjects [15,75,105,106,108], raising the possibility of a high risk of selection bias and the need to confirm the validity of these results also in subjects of other ethnic origins. The few studies performed in Caucasians are, however, in agreement with the results reported in those studies [99,100,109]. Second, these trials are usually short-term studies; on one hand this may led to an underestimation of the possible effects of BBR on some metabolic pathways that may require a longer intervention to show beneficial effects, as well as to establish a possible beneficial effect on clinical outcomes. On the other hand, they may overestimate the safety and tolerability of BBR. Third, these studies have been performed in low number of subjects, thus suggesting the need to replicate the findings in larger

populations. Finally, additional limitations have been reported, such as the heterogeneity of baseline characteristics of the participants or the inadequate clinical design [106]. Altogether, these observations suggest that long-term studies in large populations are warranted to establish the possible clinical beneficial effects of BBR as well as its long-term safety.

3.4. Approaches to improve the bioavailability of BBR

The maximum concentration (Cmax) of BBR in plasma is 4 ng/ml after an oral administration of 100 mg/kg BBR in rats [5], and a Cmax of 0.4 ng/ml after a single oral dose of 400 mg of BBR has been reported in humans [110]. Due to its low bioavailability (<5%) [6], in humans BBR is generally used at high dose (0.9–1.5 g/day) that, despite a good safety profile, may cause gastrointestinal side effects [98,105,106,111], thus limiting its clinical application. For this reason, several approaches have been evaluated to improve the bioavailability of BBR. The two main approaches are the co-administration of BBR with an absorption enhancer and the generation of BBR derivatives or analogues retaining the drug properties but with improved bioavailability.

3.5. Co-administration with absorption enhancers

BBR is a substrate of P-gp and its bioavailability is greatly limited by the activity of this membrane transporter [6,7]. Thus, the absorption of BBR in the presence of P-gp inhibitors significantly increases both in *in vivo* and *in vitro* models, resulting in a markedly increased hypoglycemic effect [26]. To increase BBR bioavailability in humans, a combination of BBR with *Silybum marianum* extract, a P-gp inhibitor, has been tested in dyslipidemic patients, resulting in an improved lipid profile and insulin secretion (Table 5) [112]. Similar effects have been observed in overweight dyslipidemic patients at low cardiovascular risk, as well as in diabetic or hypercholesterolemic patients intolerant to statins either as monotherapy or add-on therapy to ezetimibe or low-dose statins [111,113,114] (Table 5). When compared with BBR alone, both treatments similarly reduce fasting glucose, TC, LDL-C, TG and hepatic enzymes, but the combination BBR/silymarin lowers HbA1c to a greater extent than BBR alone [115], suggesting that blocking P-gp function may increase BBR bioavailability and its hypoglycemic effect.

Sodium caprate is a medium chain fatty acid that promotes the absorption of poorly absorbable drugs by increasing the permeability of intestinal epithelium and by inhibiting the function of P-gp [116]. In the presence of sodium caprate, the absorption rate of BBR in the small intestine increases, leading to higher plasma levels of BBR and to a stronger hypoglycemic effect in diabetic rats (Table 5) [116,117]. An amorphous solid dispersion of BBR with sodium caprate increases BBR bioavailability and improves glucose and lipid metabolism compared with BBR or metformin in diabetic rats [118]. These findings suggest that sodium caprate may be potentially effective in increasing BBR bioavailability (Table 5), thus reducing the BBR dose required for a therapeutic effect.

Chitosan is a permeability enhancer that, due to its high molecular weight, is not absorbed and does not induce systemic side effects and improves the absorption profile of BBR in rats [119]. Similarly, D-α-tocopheryl polyethylene glycol 100 succinate (TPGS), a water-soluble form of vitamin E that inhibits the biological activity of P-gp, increases BBR absorption in rats [120]. A nanosuspension composed of BBR and TPGS shows a superior capacity to reduce glucose, TC and body weight compared with an equivalent dose of metformin or BBR in diabetic C57BL/6 mice [121]. The co-administration of BBR with lysergol, a natural bioenhancer, increases BBR oral bioavailability in rats [122], and complexation with β-cyclodextrin improves the intestinal absorption of BBR, due to the reduction of P-gp activity and expression [123] (Table 5). These findings suggest that several compounds might be considered if an enhancement of BBR absorption is requested.

To improve BBR absorption, alternative delivery systems to traditional oral formulations have also been tested (Table 5). For example, an oral microemulsion formulation of BBR shows a higher bioavailability compared with BBR tablet suspension in rats [124]. Similarly, an anhydrous reverse micelle delivery system increases bioavailability of BBR and results in a higher reduction of blood glucose levels in diabetic mice compared with free BBR solution [125], and a self-microemulsifying drug delivery system of BBR significantly increases its plasma concentration compared with the commercial tablets in rats [126]. Finally, BBR solid lipid nanoparticles show increased bioavailability and improved anti-diabetic effects compared with an equivalent dose of BBR in diabetic mice [127]. Altogether these observations suggest that alternative delivery systems may be a promising strategy to increase BBR bioavailability and its therapeutic efficacy.

3.6. Development of BBR analogues or derivatives with increased bioavailability

Several BBR analogues or derivatives have been developed and tested for their bioavailability and cholesterol- and glucose-lowering activities in comparison with BBR (Table 5). In a study, 19 BBR analogues have been obtained with substituents on the benzene ring D; among them, pseudoberberine (pseudoBBR) is an isomer of BBR with an increased capacity to upregulate LDLR mRNA and protein in HepG2 cells compared with BBR, while retaining the ability to increase InsR expression and to activate AMPK [128,129]. PseudoBBR has a lower affinity for P-gp compared with BBR resulting in an increased retention within cells, and upregulates P-gp expression to a lesser extent [129]. The higher bioavailability of pseudoBBR translates into a higher lipid- and glucose-lowering efficacy compared with BBR [128,129]. As this compound does not show toxicity *in vivo* [128], it merits further investigation as a promising cholesterol- and glucose-lowering drug candidate.

Another interesting derivative of BBR is 8-hydroxy-dihydroBBR (Hdber) that shows a significantly greater intestinal absorption compared with BBR [130]. Both compounds inhibit the intestinal absorption of glucose and sucrose, but Hdber shows a stronger activity [130]. In hyperlipidemic rats, Hdber significantly reduces the serum levels of TC, LDL-C, TG, free fatty acids and apoB, increases the expression of LDLR and decreases the expression of SREBP2 and PCSK9 in the liver [131]. All these effects are equivalent to those obtained by the treatment with BBR, but with a dose that was only a quarter of the original dose of BBR used [131]; the mechanism of action seems to be the same and due to an increased expression of LDLR and a decreased expression of PCSK9.

Dihydroberberine (dhBBR) is another BBR derivative with increased bioavailability and improved *in vivo* efficacy compared with BBR [132]. In L6 cells, dhBBR stimulates AMPK activation and glucose uptake similarly to BBR; however, in mice fed a high-fat diet, dhBBR significantly reduces adiposity and improves glucose tolerance at a dose (100 mg/kg per day) at which BBR has no effect [132]. The pharmacokinetic analysis shows a rapid appearance of dhBBR in the plasma compared with BBR [132]; once absorbed, dhBBR is converted back to BBR [132], as suggested by the presence of BBR in the plasma of animals treated with dhBBR, suggesting that BBR is likely the active moiety and that dhBBR might be considered as a mean to efficiently deliver BBR to the circulation, thus allowing the reduction of the oral dose required for a beneficial effect.

8,8-dimethyldihydro-berberine (Di-MeBBR) has an improved aqueous solubility and acid stability, a higher bioavailability and is

Table 5
Strategies to increase BBR bioavailability.

Co-administration with absorption enhancers	Observed effects [Ref.]
Silymarin	• ↓TC, TG, LDL-C [111,112,114] • ↑HDL-C [112] • ↑Insulin release [112] • ↓Fasting plasma insulin [111,113] • ↓HOMA-IR [111,113] • ↓Fasting blood glucose [114] • ↓Glycosylated haemoglobin [111,114]
Sodium caprate	• ↑BBR intestinal absorption, bioavailability and AUC [116,118] • ↑Hypoglycemic effect of BBR [116–118] • Improvement of lipid metabolism [118]
Chitosan; TPGS; Lysergol; β-cyclodextrin	• ↑BBR absorption [119,120,122,123] • ↓Glucose, TC, body weight [121]
Alternative delivery systems	
Microemulsion	• ↑BBR bioavailability [124]
Anhydrous reverse micelle	• ↓Blood glucose levels (*vs* free BBR solution) [125]
Self-microemulsifying drug delivery system	• ↑BBR plasma concentration [126]
BBR-loaded solid lipid nanoparticles	• ↑BBR bioavailability [127] • ↑Fasting blood insulin (*vs* BBR) [127] • ↓Fasting blood glucose [127]
BBR analogues or derivatives	
PseudoBBR	• ↑LDLR mRNA and protein (*vs* BBR) [128] • ↓TC and LDL-C (*vs* BBR) in hyperlipidemic rats and mice [128] • ↓Blood glucose levels (*vs* BBR) [129]
Hdber	• ↑Intestinal absorption [130] • ↓TC, LDL-C, TG, FFA (*vs* BBR) in hyperlipidemic rats [131] • ↑LDLR, ↓PCSK9 (*vs* BBR) [131]
dhBBR	• ↑Bioavailability [132] • ↓Adiposity [132] • ↑Glucose tolerance (*vs* BBR) [132] • ↓Inflammation [134] • ↓Atherosclerotic plaque size and vulnerability (vs BBR) [134]
Di-MeBBR	• ↑Bioavailability [133] • ↓Plasma and liver TG [133] • ↓Subcutaneous fat proportion [133] • ↓Plasma insulin levels [133] • ↓Inflammation [134] • ↓Atherosclerotic plaque size and vulnerability (vs BBR) [134]

PseudoBBR: pseudoberberine; Hdber: 8-hydroxy-dihydroBBR; dhBBR: dihydroberberine; Di-MeBBR: 8,8-dimethyldihydro-berberine.

not converted back to BBR *in vivo* [133]. Di-MeBBR activates AMPK and enhances glucose uptake in L6 myotubes [133]; in diet-induced obese mice, Di-MeBBR exhibits a greater efficacy compared with dhBBR in reducing plasma and liver TG, subcutaneous fat proportion and plasma insulin level [133]; in *db/db* mice, Di-MeBBR significantly reduces fasting blood glucose, improves glucose tolerance and reduces plasma TG and insulin [133]; these effects are more marked compared with those observed with the same dose of dhBBR, and the dose is much lower than that of BBR previously used in *db/db* mice (50 mg/kg *vs* 560 mg/kg) [133].

DhBBR and Di-MeBBR have been shown to be superior to BBR in inhibiting inflammatory processes *in vitro* and reducing plaque size and vulnerability in apoE$^{-/-}$ mice [134]. In addition, they reduce several inflammatory markers within atherosclerotic plaque areas, and while Di-MeBBR increases collagen deposition, dhBBR increases the content of α-smooth muscle actin and the thickness of fibrous cap, whereas BBR has no effect on these markers [134]. Thus, the higher bioavailability of BBR derivatives seems to translate into a higher benefit.

4. Conclusions

BBR is a promising hypocholesterolemic and hypoglycemic agent, as established by several studies conducted in animal models and in humans. BBR inhibits PCSK9 transcription, which makes BBR an attractive candidate for enhancing statin efficacy owing the fact that statins increase PCSK9 plasma levels; similarly, BBR shows beneficial effects in the treatment of diabetic patients and its efficacy seems to be comparable to that of conventional antidiabetic drugs, suggesting also the possibility of combination therapy to obtain a better control of diabetes. However, due to the affinity of BBR for the same transporters involved in metformin pharmacokinetics, and to the possibility that metformin and its metabolites may alter the pharmacokinetic of BBR, a potentially harmful drug–drug interaction must be carefully evaluated to design the best pharmacological approach. It is reasonable that the development of BBR analogues or derivatives with improved bioavailability may allow the use of lower doses with the same pharmacological efficacy but with reduced chance of pharmacokinetic interactions with other co-existing therapies. To date, no serious adverse effects have been reported for BBR, apart from gastrointestinal side effects; however, the long-term safety of this compound remains to be addressed. As well, clinical studies on the real efficacy of BBR in the prevention of cardiovascular events are still lacking.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1] M. Imanshahidi, H. Hosseinzadeh, Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine, Phytother. Res. 22 (2008) 999–1012.
[2] F. Qiu, Z. Zhu, N. Kang, S. Piao, G. Qin, X. Yao, Isolation and identification of urinary metabolites of berberine in rats and humans, Drug Metab. Dispos. 36

(2008) 2159–2165.
[3] Y.T. Liu, H.P. Hao, H.G. Xie, L. Lai, Q. Wang, C.X. Liu, G.J. Wang, Extensive intestinal first-pass elimination and predominant hepatic distribution of berberine explain its low plasma levels in rats, Drug Metab. Dispos. 38 (2010) 1779–1784.
[4] X.S. Tan, J.Y. Ma, R. Feng, C. Ma, W.J. Chen, Y.P. Sun, J. Fu, M. Huang, C.Y. He, J.W. Shou, W.Y. He, Y. Wang, J.D. Jiang, Tissue distribution of berberine and its metabolites after oral administration in rats, PLoS One 8 (2013) e77969.
[5] Y. Liu, H. Hao, H. Xie, H. Lv, C. Liu, G. Wang, Oxidative demethylenation and subsequent glucuronidation are the major metabolic pathways of berberine in rats, J. Pharm. Sci. 98 (2009) 4391–4401.
[6] H.J. Maeng, H.J. Yoo, I.W. Kim, I.S. Song, S.J. Chung, C.K. Shim, P-glycoprotein-mediated transport of berberine across Caco-2 cell monolayers, J. Pharm. Sci. 91 (2002) 2614–2621.
[7] N. Shitan, M. Tanaka, K. Terai, K. Ueda, K. Yazaki, Human MDR1 and MRP1 recognize berberine as their transport substrate, Biosci. Biotechnol. Biochem. 71 (2007) 242–245.
[8] G.Y. Pan, G.J. Wang, X.D. Liu, J.P. Fawcett, Y.Y. Xie, The involvement of P-glycoprotein in berberine absorption, Pharmacol. Toxicol. 91 (2002) 193–197.
[9] M. Kwon, Y.A. Choi, M.K. Choi, I.S. Song, Organic cation transporter-mediated drug-drug interaction potential between berberine and metformin, Arch. Pharm. Res. 38 (2015) 849–856.
[10] H.M. Cui, Q.Y. Zhang, J.L. Wang, J.L. Chen, Y.L. Zhang, X.L. Tong, In vitro studies of berberine metabolism and its effect of enzyme induction on HepG2 cells, J. Ethnopharmacol. 158 (Pt A) (2014) 388–396.
[11] Y. Guo, Y. Chen, Z.R. Tan, C.D. Klaassen, H.H. Zhou, Repeated administration of berberine inhibits cytochromes P450 in humans, Eur. J. Clin. Pharmacol. 68 (2011) 213–217.
[12] D. Colombo, L. Lunardon, G. Bellia, Cyclosporine and herbal supplement interactions, J. Toxicol. 2014 (2014) 145325.
[13] Q. Hou, W. Han, X. Fu, Pharmacokinetic interaction between tacrolimus and berberine in a child with idiopathic nephrotic syndrome, Eur. J. Clin. Pharmacol. 69 (2013) 1861–1862.
[14] S. Krishan, D.R. Richardson, S. Sahni, Adenosine monophosphate-activated kinase and its key role in catabolism: structure, regulation, biological activity, and pharmacological activation, Mol. Pharmacol. 87 (2015) 363–377.
[15] W. Kong, J. Wei, P. Abidi, M. Lin, S. Inaba, C. Li, Y. Wang, Z. Wang, S. Si, H. Pan, S. Wang, J. Wu, Z. Li, J. Liu, J.D. Jiang, Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins, Nat. Med. 10 (2004) 1344–1351.
[16] Z. Li, J.D. Jiang, W.J. Kong, Berberine up-regulates hepatic low-density lipoprotein receptor through Ras-independent but AMP-activated protein kinase-dependent Raf-1 activation, Biol. Pharm. Bull. 37 (2014) 1766–1775.
[17] S. Lee, H.J. Lim, J.H. Park, K.S. Lee, Y. Jang, H.Y. Park, Berberine-induced LDLR up-regulation involves JNK pathway, Biochem. Biophys. Res. Commun. 362 (2007) 853–857.
[18] N.G. Seidah, Z. Awan, M. Chretien, M. Mbikay, PCSK9: a key modulator of cardiovascular health, Circ. Res. 114 (2014) 1022–1036.
[19] A. Sahebkar, L.E. Simental-Mendia, F. Guerrero-Romero, J. Golledge, G.F. Watts, Effect of statin therapy on plasma PCSK9 concentrations: a systematic review and meta-analysis of clinical trials, Diabetes Obes. Metab. (2015), http://dx.doi.org/10.1111/dom.12536.
[20] J. Cameron, T. Ranheim, M.A. Kulseth, T.P. Leren, K.E. Berge, Berberine decreases PCSK9 expression in HepG2 cells, Atherosclerosis 201 (2008) 266–273.
[21] H. Li, B. Dong, S.W. Park, H.S. Lee, W. Chen, J. Liu, Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine, J. Biol. Chem. 284 (2009) 28885–28895.
[22] J.M. Brusq, N. Ancellin, P. Grondin, R. Guillard, S. Martin, Y. Saintillan, M. Issandou, Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine, J. Lipid Res. 47 (2006) 1281–1288.
[23] S. Cao, Y. Zhou, P. Xu, Y. Wang, J. Yan, W. Bin, F. Qiu, N. Kang, Berberine metabolites exhibit triglyceride-lowering effects via activation of AMP-activated protein kinase in Hep G2 cells, J. Ethnopharmacol. 149 (2013) 576–582.
[24] C.C. Cheng, C.M. Hsueh, K.W. Liang, C.T. Ting, C.L. Wen, S.L. Hsu, Role of JNK and c-Jun signaling pathway in regulation of human serum paraoxonase 1 gene transcription by berberine in human HepG2 cells, Eur. J. Pharmacol. 650 (2011) 519–525.
[25] W.E. Cheng, M. Ying Chang, J.Y. Wei, Y.J. Chen, M.C. Maa, T.H. Leu, Berberine reduces Toll-like receptor-mediated macrophage migration by suppression of Src enhancement, Eur. J. Pharmacol. 757 (2015) 1–10.
[26] Z. Li, Y.N. Geng, J.D. Jiang, W.J. Kong, Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus, Evid. Based Complement. Altern. Med. 2014 (2014) 289264.
[27] L.K. Sarna, N. Wu, S.Y. Hwang, Y.L. Siow, K. O, Berberine inhibits NADPH oxidase mediated superoxide anion production in macrophages, Can. J. Physiol. Pharmacol. 88 (2010) 369–378.
[28] D. Lee, J. Bae, Y.K. Kim, M. Gil, J.Y. Lee, C.S. Park, K.J. Lee, Inhibitory effects of berberine on lipopolysaccharide-induced inducible nitric oxide synthase and the high-mobility group box 1 release in macrophages, Biochem. Biophys. Res. Commun. 431 (2013) 506–511.
[29] C. Mo, L. Wang, J. Zhang, S. Numazawa, H. Tang, X. Tang, X. Han, J. Li, M. Yang, Z. Wang, D. Wei, H. Xiao, The crosstalk between Nrf2 and AMPK signal pathways is important for the anti-inflammatory effect of berberine in LPS-stimulated macrophages and endotoxin-shocked mice, Antioxid. Redox. Signal 20 (2014) 574–588.
[30] X. Fan, J. Wang, J. Hou, C. Lin, A. Bensoussan, D. Chang, J. Liu, B. Wang, Berberine alleviates ox-LDL induced inflammatory factors by up-regulation of autophagy via AMPK/mTOR signaling pathway, J. Transl. Med. 13 (2015) 450.
[31] Z. Huang, S. Meng, L. Wang, Y. Wang, T. Chen, C. Wang, Suppression of oxLDL-induced MMP-9 and EMMPRIN expression by berberine via inhibition of NF-kappaB activation in human THP-1 macrophages, Anat. Rec. Hob. 295 (2012) 78–86.
[32] Z. Huang, L. Wang, S. Meng, Y. Wang, T. Chen, C. Wang, Berberine reduces both MMP-9 and EMMPRIN expression through prevention of p38 pathway activation in PMA-induced macrophages, Int. J. Cardiol. 146 (2011) 153–158.
[33] S. Guan, B. Wang, W. Li, J. Guan, X. Fang, Effects of berberine on expression of LOX-1 and SR-BI in human macrophage-derived foam cells induced by ox-LDL, Am. J. Chin. Med. 38 (2010) 1161–1169.
[34] T.S. Lee, C.C. Pan, C.C. Peng, Y.R. Kou, C.Y. Chen, L.C. Ching, T.H. Tsai, S.F. Chen, P.C. Lyu, S.K. Shyue, Anti-atherogenic effect of berberine on LXRalpha-ABCA1-dependent cholesterol efflux in macrophages, J. Cell Biochem. 111 (2010) 104–110.
[35] F. Zimetti, M.P. Adorni, N. Ronda, R. Gatti, F. Bernini, E. Favari, The natural compound berberine positively affects macrophage functions involved in atherogenesis, Nutr. Metab. Cardiovasc Dis. 25 (2015) 195–201.
[36] B.J. Cho, E.K. Im, J.H. Kwon, K.H. Lee, H.J. Shin, J. Oh, S.M. Kang, J.H. Chung, Y. Jang, Berberine inhibits the production of lysophosphatidylcholine-induced reactive oxygen species and the ERK1/2 pathway in vascular smooth muscle cells, Mol. Cells 20 (2005) 429–434.
[37] S. Lee, H.J. Lim, H.Y. Park, K.S. Lee, J.H. Park, Y. Jang, Berberine inhibits rat vascular smooth muscle cell proliferation and migration in vitro and improves neointima formation after balloon injury in vivo. Berberine improves neointima formation in a rat model, Atherosclerosis 186 (2006) 29–37.
[38] I. Mordi, N. Tzemos, Is reversal of endothelial dysfunction still an attractive target in modern cardiology? World J. Cardiol. 6 (2014) 824–835.
[39] Y.J. Ko, J.S. Lee, B.C. Park, H.M. Shin, J.A. Kim, Inhibitory effects of zoagumhwan water extract and berberine on angiotensin II-induced monocyte chemoattractant protein (MCP)-1 expression and monocyte adhesion to endothelial cells, Vasc. Pharmacol. 47 (2007) 189–196.
[40] Y.H. Wu, S.Y. Chuang, W.C. Hong, Y.J. Lai, G.J. Chang, J.H. Pang, Berberine reduces leukocyte adhesion to LPS-stimulated endothelial cells and VCAM-1 expression both in vivo and in vitro, Int. J. Immunopathol. Pharmacol. 25 (2012) 741–750.
[41] M. Zhang, C.M. Wang, J. Li, Z.J. Meng, S.N. Wei, R. Bucala, Y.L. Li, L. Chen, Berberine protects against palmitate-induced endothelial dysfunction: involvements of upregulation of AMPK and eNOS and downregulation of NOX4, Mediat. Inflamm. 2013 (2013) 260464.
[42] Y. Wang, Y. Huang, K.S. Lam, Y. Li, W.T. Wong, H. Ye, C.W. Lau, P.M. Vanhoutte, A. Xu, Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilatation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase, Cardiovasc Res. 82 (2009) 484–492.
[43] P. Tian, H. Ge, H. Liu, T.S. Kern, L. Du, L. Guan, S. Su, P. Liu, Leukocytes from diabetic patients kill retinal endothelial cells: effects of berberine, Mol. Vis. 19 (2013) 2092–2105.
[44] M. Pirro, G. Schillaci, R. Paltriccia, F. Bagaglia, C. Menecali, M.R. Mannarino, M. Capanni, A. Velardi, E. Mannarino, Increased ratio of CD31+/CD42- microparticles to endothelial progenitors as a novel marker of atherosclerosis in hypercholesterolemia, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 2530–2535.
[45] M.G. Xu, J.M. Wang, L. Chen, Y. Wang, Z. Yang, J. Tao, Berberine-induced mobilization of circulating endothelial progenitor cells improves human small artery elasticity, J. Hum. Hypertens. 22 (2008) 389–393.
[46] M.G. Xu, J.M. Wang, L. Chen, Y. Wang, Z. Yang, J. Tao, Berberine-induced upregulation of circulating endothelial progenitor cells is related to nitric oxide production in healthy subjects, Cardiology 112 (2009) 279–286.
[47] M. Xiao, L.N. Men, M.G. Xu, G.B. Wang, H.T. Lv, C. Liu, Berberine protects endothelial progenitor cell from damage of TNF-alpha via the PI3K/AKT/eNOS signaling pathway, Eur. J. Pharmacol. 743 (2014) 11–16.
[48] L. Liu, J. Liu, Y. Gao, X. Yu, G. Xu, Y. Huang, Uncoupling protein-2 mediates the protective action of berberine against oxidative stress in rat insulinoma INS-1E cells and in diabetic mouse islets, Br. J. Pharmacol. 171 (2014) 3246–3254.
[49] H. Zhang, J. Wei, R. Xue, J.D. Wu, W. Zhao, Z.Z. Wang, S.K. Wang, Z.X. Zhou, D.Q. Song, Y.M. Wang, H.N. Pan, W.J. Kong, J.D. Jiang, Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression, Metabolism 59 (2010) 285–292.
[50] N. Shen, Y. Huan, Z.F. Shen, Berberine inhibits mouse insulin gene promoter through activation of AMP activated protein kinase and may exert beneficial effect on pancreatic beta-cell, Eur. J. Pharmacol. 694 (2012) 120–126.
[51] J. Yin, Z. Gao, D. Liu, Z. Liu, J. Ye, Berberine improves glucose metabolism through induction of glycolysis, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E148–E156.
[52] S.H. Kim, E.J. Shin, E.D. Kim, T. Bayaraa, S.C. Frost, C.K. Hyun, Berberine

activates GLUT1-mediated glucose uptake in 3T3-L1 adipocytes, Biol. Pharm. Bull. 30 (2007) 2120–2125.
[53] Q. Zhang, X. Xiao, K. Feng, T. Wang, W. Li, T. Yuan, X. Sun, Q. Sun, H. Xiang, H. Wang, Berberine moderates glucose and lipid metabolism through multipathway mechanism, Evid. Based Complement. Altern. Med. 2011 (2011).
[54] G.Y. Pan, Z.J. Huang, G.J. Wang, J.P. Fawcett, X.D. Liu, X.C. Zhao, J.G. Sun, Y.Y. Xie, The antihyperglycaemic activity of berberine arises from a decrease of glucose absorption, Planta Med. 69 (2003) 632–636.
[55] H.W. Jeong, K.C. Hsu, J.W. Lee, M. Ham, J.Y. Huh, H.J. Shin, W.S. Kim, J.B. Kim, Berberine suppresses proinflammatory responses through AMPK activation in macrophages, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E955–E964.
[56] L. Zhou, X. Wang, Y. Yang, L. Wu, F. Li, R. Zhang, G. Yuan, N. Wang, M. Chen, G. Ning, Berberine attenuates cAMP-induced lipolysis via reducing the inhibition of phosphodiesterase in 3T3-L1 adipocytes, Biochim. Biophys. Acta 1812 (2011) 527–535.
[57] J. Zhang, H. Tang, R. Deng, N. Wang, Y. Zhang, Y. Wang, Y. Liu, F. Li, X. Wang, L. Zhou, Berberine suppresses adipocyte differentiation via decreasing CREB transcriptional activity, PLoS One 10 (2015) e0125667.
[58] Y. Hu, G.E. Davies, Berberine increases expression of GATA-2 and GATA-3 during inhibition of adipocyte differentiation, Phytomedicine 16 (2009) 864–873.
[59] C. Huang, Y. Zhang, Z. Gong, X. Sheng, Z. Li, W. Zhang, Y. Qin, Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARgamma pathway, Biochem. Biophys. Res. Commun. 348 (2006) 571–578.
[60] B.H. Choi, I.S. Ahn, Y.H. Kim, J.W. Park, S.Y. Lee, C.K. Hyun, M.S. Do, Berberine reduces the expression of adipogenic enzymes and inflammatory molecules of 3T3-L1 adipocyte, Exp. Mol. Med. 38 (2006) 599–605.
[61] M. Wang, J. Wang, R. Tan, Q. Wu, H. Qiu, J. Yang, Q. Jiang, Effect of berberine on PPAR alpha/NO activation in high glucose- and insulin-induced cardiomyocyte hypertrophy, Evid. Based Complement. Altern. Med. 2013 (2013) 285489.
[62] W. Chang, M. Zhang, J. Li, Z. Meng, S. Wei, H. Du, L. Chen, G.M. Hatch, Berberine improves insulin resistance in cardiomyocytes via activation of 5′-adenosine monophosphate-activated protein kinase, Metabolism 62 (2013) 1159–1167.
[63] K. Chen, G. Li, F. Geng, Z. Zhang, J. Li, M. Yang, L. Dong, F. Gao, Berberine reduces ischemia/reperfusion-induced myocardial apoptosis via activating AMPK and PI3K-Akt signaling in diabetic rats, Apoptosis 19 (2014) 946–957.
[64] L. Yu, F. Li, G. Zhao, Y. Yang, Z. Jin, M. Zhai, W. Yu, L. Zhao, W. Chen, W. Duan, S. Yu, Protective effect of berberine against myocardial ischemia reperfusion injury: role of Notch1/Hes1-PTEN/Akt signaling, Apoptosis 20 (2015) 796–810.
[65] W. Liu, F. Tang, Y. Deng, X. Li, T. Lan, X. Zhang, H. Huang, P. Liu, Berberine reduces fibronectin and collagen accumulation in rat glomerular mesangial cells cultured under high glucose condition, Mol. Cell Biochem. 325 (2009) 99–105.
[66] W. Liu, P. Liu, S. Tao, Y. Deng, X. Li, T. Lan, X. Zhang, F. Guo, W. Huang, F. Chen, H. Huang, S.F. Zhou, Berberine inhibits aldose reductase and oxidative stress in rat mesangial cells cultured under high glucose, Arch. Biochem. Biophys. 475 (2008) 128–134.
[67] A.B. Rahigude, S.V. Kaulaskar, P.S. Bhutada, Possible therapeutic potential of berberine in diabetic osteopathy, Med. Hypotheses 79 (2012) 440–444.
[68] P. Wei, L. Jiao, L.P. Qin, F. Yan, T. Han, Q.Y. Zhang, Effects of berberine on differentiation and bone resorption of osteoclasts derived from rat bone marrow cells, Zhong Xi Yi Jie He Xue Bao 7 (2009) 342–348.
[69] H.W. Lee, J.H. Suh, H.N. Kim, A.Y. Kim, S.Y. Park, C.S. Shin, J.Y. Choi, J.B. Kim, Berberine promotes osteoblast differentiation by Runx2 activation with p38 MAPK, J. Bone Min. Res. 23 (2008) 1227–1237.
[70] J. Jeyabalan, M. Shah, B. Viollet, C. Chenu, AMP-activated protein kinase pathway and bone metabolism, J. Endocrinol. 212 (2012) 277–290.
[71] Q. Wang, M. Zhang, B. Liang, N. Shirwany, Y. Zhu, M.H. Zou, Activation of AMP-activated protein kinase is required for berberine-induced reduction of atherosclerosis in mice: the role of uncoupling protein 2, PLoS One 6 (2011) e25436.
[72] Y. Wang, X. Yi, K. Ghanam, S. Zhang, T. Zhao, X. Zhu, Berberine decreases cholesterol levels in rats through multiple mechanisms, including inhibition of cholesterol absorption, Metabolism 63 (2014) 1167–1177.
[73] X.X. Chang, H.M. Yan, Q. Xu, M.F. Xia, H. Bian, T.F. Zhu, X. Gao, The effects of berberine on hyperhomocysteinemia and hyperlipidemia in rats fed with a long-term high-fat diet, Lipids Health Dis. 11 (2012) 86.
[74] H.B. Xiao, Z.L. Sun, H.B. Zhang, D.S. Zhang, Berberine inhibits dyslipidemia in C57BL/6 mice with lipopolysaccharide induced inflammation, Pharmacol. Rep. 64 (2012) 889–895.
[75] W.J. Kong, J. Wei, Z.Y. Zuo, Y.M. Wang, D.Q. Song, X.F. You, L.X. Zhao, H.N. Pan, J.D. Jiang, Combination of simvastatin with berberine improves the lipid-lowering efficacy, Metabolism 57 (2008) 1029–1037.
[76] X. Wan, X. Chen, L. Liu, Y. Zhao, W.J. Huang, Q. Zhang, G.G. Miao, W. Chen, H.G. Xie, C.C. Cao, Berberine ameliorates chronic kidney injury caused by atherosclerotic renovascular disease through the suppression of NFkappaB signaling pathway in rats, PLoS One 8 (2013) e59794.
[77] R. Tisch, H. McDevitt, Insulin-dependent diabetes mellitus, Cell 85 (1996) 291–297.
[78] W.H. Chueh, J.Y. Lin, Protective effect of berberine on serum glucose levels in non-obese diabetic mice, Int. Immunopharmacol. 12 (2012) 534–538.
[79] W.H. Chueh, J.Y. Lin, Berberine, an isoquinoline alkaloid in herbal plants, protects pancreatic islets and serum lipids in nonobese diabetic mice, J. Agric. Food Chem. 59 (2011) 8021–8027.
[80] S.H. Leng, F.E. Lu, L.J. Xu, Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion, Acta Pharmacol. Sin. 25 (2004) 496–502.
[81] C. Liu, Z. Wang, Y. Song, D. Wu, X. Zheng, P. Li, J. Jin, N. Xu, L. Li, Effects of berberine on amelioration of hyperglycemia and oxidative stress in high glucose and high fat diet-induced diabetic hamsters in vivo, Biomed. Res. Int. 2015 (2015) 313808.
[82] S.S. Lu, Y.L. Yu, H.J. Zhu, X.D. Liu, L. Liu, Y.W. Liu, P. Wang, L. Xie, G.J. Wang, Berberine promotes glucagon-like peptide-1 (7-36) amide secretion in streptozotocin-induced diabetic rats, J. Endocrinol. 200 (2009) 159–165.
[83] W.J. Kong, H. Zhang, D.Q. Song, R. Xue, W. Zhao, J. Wei, Y.M. Wang, N. Shan, Z.X. Zhou, P. Yang, X.F. You, Z.R. Li, S.Y. Si, L.X. Zhao, H.N. Pan, J.D. Jiang, Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression, Metabolism 58 (2009) 109–119.
[84] X. Xia, J. Yan, Y. Shen, K. Tang, J. Yin, Y. Zhang, D. Yang, H. Liang, J. Ye, J. Weng, Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis, PLoS One 6 (2011) e16556.
[85] Y.S. Lee, W.S. Kim, K.H. Kim, M.J. Yoon, H.J. Cho, Y. Shen, J.M. Ye, C.H. Lee, W.K. Oh, C.T. Kim, C. Hohnen-Behrens, A. Gosby, E.W. Kraegen, D.E. James, J.B. Kim, Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states, Diabetes 55 (2006) 2256–2264.
[86] Z. Zhang, H. Zhang, B. Li, X. Meng, J. Wang, Y. Zhang, S. Yao, Q. Ma, L. Jin, J. Yang, W. Wang, G. Ning, Berberine activates thermogenesis in white and brown adipose tissue, Nat. Commun. 5 (2014) 5493.
[87] H.L. Zhao, Y. Sui, C.F. Qiao, K.Y. Yip, R.K. Leung, S.K. Tsui, H.M. Lee, H.K. Wong, X. Zhu, J.J. Siu, L. He, J. Guan, L.Z. Liu, H.X. Xu, P.C. Tong, J.C. Chan, Sustained antidiabetic effects of a berberine-containing Chinese herbal medicine through regulation of hepatic gene expression, Diabetes 61 (2012) 933–943.
[88] W.S. Kim, Y.S. Lee, S.H. Cha, H.W. Jeong, S.S. Choe, M.R. Lee, G.T. Oh, H.S. Park, K.U. Lee, M.D. Lane, J.B. Kim, Berberine improves lipid dysregulation in obesity by controlling central and peripheral AMPK activity, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E812–E819.
[89] S.F. Dong, Y. Hong, M. Liu, Y.Z. Hao, H.S. Yu, Y. Liu, J.N. Sun, Berberine attenuates cardiac dysfunction in hyperglycemic and hypercholesterolemic rats, Eur. J. Pharmacol. 660 (2011) 368–374.
[90] W.J. Ni, H.H. Ding, L.Q. Tang, Berberine as a promising anti-diabetic nephropathy drug: an analysis of its effects and mechanisms, Eur. J. Pharmacol. 760 (2015) 103–112.
[91] W.J. Ni, H.H. Ding, H. Zhou, Y.Y. Qiu, L.Q. Tang, Renoprotective effects of berberine through regulation of the MMPs/TIMPs system in streptozocin-induced diabetic nephropathy in rats, Eur. J. Pharmacol. 764 (2015) 448–456.
[92] D. Gupta, C.B. Krueger, G. Lastra, Over-nutrition, obesity and insulin resistance in the development of beta-cell dysfunction, Curr. Diabetes Rev. 8 (2012) 76–83.
[93] Y. Hu, G.E. Davies, Berberine inhibits adipogenesis in high-fat diet-induced obesity mice, Fitoterapia 81 (2010) 358–366.
[94] J.J. Gu, F.Y. Gao, T.Y. Zhao, A preliminary investigation of the mechanisms underlying the effect of berberine in preventing high-fat diet-induced insulin resistance in rats, J. Physiol. Pharmacol. 63 (2012) 505–513.
[95] J. Han, H. Lin, W. Huang, Modulating gut microbiota as an anti-diabetic mechanism of berberine, Med. Sci. Monit. 17 (2011) RA164–RA167.
[96] X. Zhang, Y. Zhao, M. Zhang, X. Pang, J. Xu, C. Kang, M. Li, C. Zhang, Z. Zhang, Y. Zhang, X. Li, G. Ning, L. Zhao, Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats, PLoS One 7 (2012) e42529.
[97] W. Xie, D. Gu, J. Li, K. Cui, Y. Zhang, Effects and action mechanisms of berberine and Rhizoma coptidis on gut microbes and obesity in high-fat diet-fed C57BL/6J mice, PLoS One 6 (2011) e24520.
[98] H. Dong, Y. Zhao, L. Zhao, F. Lu, The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials, Planta Med. 79 (2013) 437–446.
[99] G. Derosa, A. D'Angelo, A. Bonaventura, L. Bianchi, D. Romano, P. Maffioli, Effects of berberine on lipid profile in subjects with low cardiovascular risk, Expert Opin. Biol. Ther. 13 (2013) 475–482.
[100] F. Affuso, A. Ruvolo, F. Micillo, L. Sacca, S. Fazio, Effects of a nutraceutical combination (berberine, red yeast rice and policosanols) on lipid levels and endothelial function randomized, double-blind, placebo-controlled study, Nutr. Metab. Cardiovasc Dis. 20 (2010) 656–661.
[101] G. Marazzi, L. Cacciotti, F. Pelliccia, L. Iaia, M. Volterrani, G. Caminiti, B. Sposato, R. Massaro, F. Grieco, G. Rosano, Long-term effects of nutraceuticals (berberine, red yeast rice, policosanol) in elderly hypercholesterolemic patients, Adv. Ther. 28 (2011) 1105–1113.
[102] L. Pisciotta, A. Bellocchio, S. Bertolini, Nutraceutical pill containing berberine versus ezetimibe on plasma lipid pattern in hypercholesterolemic subjects and its additive effect in patients with familial hypercholesterolemia on stable cholesterol-lowering treatment, Lipids Health Dis. 11 (2012) 123.
[103] T.C. Weng, Y.H. Yang, S.J. Lin, S.H. Tai, A systematic review and meta-analysis on the therapeutic equivalence of statins, J. Clin. Pharm. Ther. 35 (2010) 139–151.
[104] A.F. Cicero, E. Tartagni, A. Ferroni, V. De Sando, E. Grandi, C. Borghi,

Combined nutraceutical approach to postmenopausal syndrome and vascular remodeling biomarkers, J. Altern. Complement. Med. 19 (2013) 582–587.
[105] J. Yin, H. Xing, J. Ye, Efficacy of berberine in patients with type 2 diabetes mellitus, Metabolism 57 (2008) 712–717.
[106] H. Dong, N. Wang, L. Zhao, F. Lu, Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis, Evid. Based Complement. Altern. Med. 2012 (2012) 591654.
[107] K.G. Perez-Rubio, M. Gonzalez-Ortiz, E. Martinez-Abundis, J.A. Robles-Cervantes, M.C. Espinel-Bermudez, Effect of berberine administration on metabolic syndrome, insulin sensitivity, and insulin secretion, Metab. Syndr. Relat. Disord. 11 (2013) 366–369.
[108] J. Yang, J. Yin, H. Gao, L. Xu, Y. Wang, M. Li, Berberine improves insulin sensitivity by inhibiting fat store and adjusting adipokines profile in human preadipocytes and metabolic syndrome patients, Evid. Based Complement. Altern. Med. 2012 (2012) 363845.
[109] F. Affuso, V. Mercurio, A. Ruvolo, C. Pirozzi, F. Micillo, G. Carlomagno, F. Grieco, S. Fazio, A nutraceutical combination improves insulin sensitivity in patients with metabolic syndrome, World J. Cardiol. 4 (2012) 77–83.
[110] W. Hua, L. Ding, Y. Chen, B. Gong, J. He, G. Xu, Determination of berberine in human plasma by liquid chromatography-electrospray ionization-mass spectrometry, J. Pharm. Biomed. Anal. 44 (2007) 931–937.
[111] F. Di Pierro, N. Villanova, F. Agostini, R. Marzocchi, V. Soverini, G. Marchesini, Pilot study on the additive effects of berberine and oral type 2 diabetes agents for patients with suboptimal glycemic control, Diabetes Metab. Syndr. Obes. 5 (2012) 213–217.
[112] G. Derosa, A. Bonaventura, L. Bianchi, D. Romano, A. D'Angelo, E. Fogari, P. Maffioli, Berberis aristata/Silybum marianum fixed combination on lipid profile and insulin secretion in dyslipidemic patients, Expert Opin. Biol. Ther. 13 (2013) 1495–1506.
[113] G. Derosa, A. Bonaventura, L. Bianchi, D. Romano, A. D Angelo, E. Fogari, P. Maffioli, Effects of Berberis aristata/Silybum marianum association on metabolic parameters and adipocytokines in overweight dyslipidemic patients, J. Biol. Regul. Homeost. Agents 27 (2013) 717–728.
[114] F. Di Pierro, I. Bellone, G. Rapacioli, P. Putignano, Clinical role of a fixed combination of standardized Berberis aristata and Silybum marianum extracts in diabetic and hypercholesterolemic patients intolerant to statins, Diabetes Metab. Syndr. Obes. 8 (2015) 89–96.
[115] F. Di Pierro, P. Putignano, N. Villanova, L. Montesi, S. Moscatiello, G. Marchesini, Preliminary study about the possible glycemic clinical advantage in using a fixed combination of Berberis aristata and Silybum marianum standardized extracts versus only Berberis aristata in patients with type 2 diabetes, Clin. Pharmacol. 5 (2013) 167–174.
[116] X.Y. Lv, J. Li, M. Zhang, C.M. Wang, Z. Fan, C.Y. Wang, L. Chen, Enhancement of sodium caprate on intestine absorption and antidiabetic action of berberine, AAPS Pharm. Sci. Tech. 11 (2010) 372–382.
[117] M. Zhang, X. Lv, J. Li, Z. Meng, Q. Wang, W. Chang, W. Li, L. Chen, Y. Liu, Sodium caprate augments the hypoglycemic effect of berberine via AMPK in inhibiting hepatic gluconeogenesis, Mol. Cell Endocrinol. 363 (2012) 122–130.
[118] M. Zhaojie, Z. Ming, W. Shengnan, B. Xiaojia, G.M. Hatch, G. Jingkai, C. Li, Amorphous solid dispersion of berberine with absorption enhancer demonstrates a remarkable hypoglycemic effect via improving its bioavailability, Int. J. Pharm. 467 (2014) 50–59.
[119] W. Chen, D. Fan, L. Meng, Y. Miao, S. Yang, Y. Weng, H. He, X. Tang, Enhancing effects of chitosan and chitosan hydrochloride on intestinal absorption of berberine in rats, Drug Dev. Ind. Pharm. 38 (2012) 104–110.
[120] W. Chen, Y.Q. Miao, D.J. Fan, S.S. Yang, X. Lin, L.K. Meng, X. Tang, Bioavailability study of berberine and the enhancing effects of TPGS on intestinal absorption in rats, AAPS Pharm. Sci. Tech. 12 (2011) 705–711.
[121] Z. Wang, J. Wu, Q. Zhou, Y. Wang, T. Chen, Berberine nanosuspension enhances hypoglycemic efficacy on streptozotocin induced diabetic C57BL/6 mice, Evid. Based Complement. Altern. Med. 2015 (2015) 239749.
[122] S. Patil, R.P. Dash, S. Anandjiwala, M. Nivsarkar, Simultaneous quantification of berberine and lysergol by HPLC-UV: evidence that lysergol enhances the oral bioavailability of berberine in rats, Biomed. Chromatogr. 26 (2011) 1170–1175.
[123] Y. Zhang, Y.L. Cui, L.N. Gao, H.L. Jiang, Effects of beta-cyclodextrin on the intestinal absorption of berberine hydrochloride, a P-glycoprotein substrate, Int. J. Biol. Macromol. 59 (2013) 363–371.
[124] S.Y. Gui, L. Wu, D.Y. Peng, Q.Y. Liu, B.P. Yin, J.Z. Shen, Preparation and evaluation of a microemulsion for oral delivery of berberine, Pharmazie 63 (2008) 516–519.
[125] T. Wang, N. Wang, H. Song, X. Xi, J. Wang, A. Hao, T. Li, Preparation of an anhydrous reverse micelle delivery system to enhance oral bioavailability and anti-diabetic efficacy of berberine, Eur. J. Pharm. Sci. 44 (2011) 127–135.
[126] J.X. Zhu, D. Tang, L. Feng, Z.G. Zheng, R.S. Wang, A.G. Wu, T.T. Duan, B. He, Q. Zhu, Development of self-microemulsifying drug delivery system for oral bioavailability enhancement of berberine hydrochloride, Drug Dev. Ind. Pharm. 39 (2013) 499–506.
[127] M. Xue, M.X. Yang, W. Zhang, X.M. Li, D.H. Gao, Z.M. Ou, Z.P. Li, S.H. Liu, X.J. Li, S.Y. Yang, Characterization, pharmacokinetics, and hypoglycemic effect of berberine loaded solid lipid nanoparticles, Int. J. Nanomed. 8 (2013) 4677–4687.
[128] Y.H. Li, P. Yang, W.J. Kong, Y.X. Wang, C.Q. Hu, Z.Y. Zuo, Y.M. Wang, H. Gao, L.M. Gao, Y.C. Feng, N.N. Du, Y. Liu, D.Q. Song, J.D. Jiang, Berberine analogues as a novel class of the low-density-lipoprotein receptor up-regulators: synthesis, structure-activity relationships, and cholesterol-lowering efficacy, J. Med. Chem. 52 (2009) 492–501.
[129] Y.Q. Shan, G. Ren, Y.X. Wang, J. Pang, Z.Y. Zhao, J. Yao, X.F. You, S.Y. Si, D.Q. Song, W.J. Kong, J.D. Jiang, Berberine analogue IMB-Y53 improves glucose-lowering efficacy by averting cellular efflux especially P-glycoprotein efflux, Metabolism 62 (2013) 446–456.
[130] S.C. Wei, S. Dong, L.J. Xu, C.Y. Zhang, Intestinal absorption of berberine and 8-hydroxy dihydroberberine and their effects on sugar absorption in rat small intestine, J. Huazhong Univ. Sci. Technol. Med. Sci. 34 (2014) 186–189.
[131] D.L. Liu, L.J. Xu, H. Dong, G. Chen, Z.Y. Huang, X. Zou, K.F. Wang, Y.H. Luo, F.E. Lu, Inhibition of proprotein convertase subtilisin/kexin type 9: a novel mechanism of berberine and 8-hydroxy dihydroberberine against hyperlipidemia, Chin. J. Integr. Med. 21 (2015) 132–138.
[132] N. Turner, J.Y. Li, A. Gosby, S.W. To, Z. Cheng, H. Miyoshi, M.M. Taketo, G.J. Cooney, E.W. Kraegen, D.E. James, L.H. Hu, J. Li, J.M. Ye, 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 57 (2008) 1414–1418.
[133] Z. Cheng, A.F. Chen, F. Wu, L. Sheng, H.K. Zhang, M. Gu, Y.Y. Li, L.N. Zhang, L.H. Hu, J.Y. Li, J. Li, 8,8-Dimethyldihydroberberine with improved bioavailability and oral efficacy on obese and diabetic mouse models, Bioorg. Med. Chem. 18 (2010) 5915–5924.
[134] J. Chen, J. Cao, L. Fang, B. Liu, Q. Zhou, Y. Sun, Y. Wang, Y. Li, S. Meng, Berberine derivatives reduce atherosclerotic plaque size and vulnerability in apoE(−/−) mice, J. Transl. Med. 12 (2014) 326.