Curr Pharmacol Rep (2016) 2:281–292
DOI 10.1007/s40495-016-0070-1

Therapeutic Potential and Mechanisms of Berberine in Cardiovascular Disease

Ming Zhang[1] · **Linjing Feng**[1] · **Junnan Li**[1] · **Li Chen**[1]

Published online: 15 September 2016

Abstract Berberine is originally isolated from extracts of the Chinese herb *Coptis chinensis*. It was initially used to treat diarrhea. Over the past two decades, berberine has been shown to be effective for improving glycemic and lipid profiles. Interest in its protective effects against diabetes and cardiovascular risk is beginning to emerge, and research investigating the pharmacological activity of berberine is developing rapidly and being reported internationally. Some clinical evidence has demonstrated the ability of berberine to prevent endothelial dysfunction, myocardial infarction, and arrhythmia in patients with cardiovascular risks. Numerous molecular targets of berberine have been explored, including adenosine monophosphate-activated protein kinase (AMPK), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and NADPH oxidase (NOX). The aim of this review is to focus on the therapeutic potential of berberine in cardiovascular disease and to describe its underlying mechanisms in the pathogenesis of artherosclerosis, hypertension, heart failure, ischemia reperfusion heart injury, stroke, and arrhythmias.

Keywords Berberine · Artherosclerosis · Hypertension · Heart failure · Ischemia reperfusion heart injury · Stroke · Arrhythmias

This article is part of the Topical Collection on *Cardiovascular Pharmacology*

✉ Li Chen
chen_lab@163.com

[1] Department of Pharmacology, College of Basic Medical Sciences, School of Nursing, Jilin University, 126 Xin Min Street, Changchun, Jilin 130021, China

Introduction

Cardiovascular diseases are the leading cause of death globally and are classified in various ways. They are primarily classified based on the anatomical location as diseases of the heart and diseases of the vessels. Another classification is based on the primary involvement of atherosclerosis, such as cardiovascular diseases due to atherosclerosis and other cardiovascular disorders. In fact, atherosclerosis is responsible for more than 75 % of all deaths due to cardiovascular diseases. As noted previously, there are many risk factors associated with the development of cardiovascular diseases. These risk factors include tobacco use, hypertension, high cholesterol, obesity, physical inactivity, and an unhealthy diet. These risk factors are all closely related to the development of atherosclerosis. The growing problem of cardiovascular diseases worldwide has inspired various organizations or government agencies to take steps to control these diseases. Essentially, the key to controlling the global cardiovascular epidemic is to take actions to control the major adaptable risk factors of cardiovascular diseases. Meanwhile, many drugs have been investigated and clinically applied to prevent cardiovascular disease. However, intensive exploration of new drugs for the treatment of cardiovascular disease is ongoing, especially those drugs with multiple functions to manage the risk factors of cardiovascular disease.

Berberine (BBR) is an isoquinoline alkaloid that was originally isolated from extracts of the Chinese herb *Coptis chinensis* (Huang lian). Since the late 1980s, numerous studies have reported multiple pharmacological properties of berberine, including anti-diarrheal, anti-arrhythmia, anti-hypertension, glucose- and cholesterol-lowering, and anti-tumor and immunemodulatory activities [12, 26, 27, 30, 54, 70, 83, 94, 105]. In 1989, the effects of berberine on platelet aggregation and release were reported [105]. Subsequent reports about the

pharmacology and clinical application of berberine for its anti-arrhythmic effects, as well as clinical investigations of berberine for the treatment of hypertension, were published in 1993 and 1992 [54, 70]. These studies supported the benefits of berberine for cardiovascular diseases. After the 1990s, an increasing number of papers were published to explore the therapeutic effect of berberine on cardiovascular diseases, including atherosclerosis, hypertension, myocardial infarction, cardiomyopathy, heart failure, stroke, and arrhythmias [12, 16, 50, 52, 53].

Effect of Berberine on Atherosclerosis

Atherosclerosis is asymptomatic for decades. Signs and symptoms occur only after severe narrowing or obstruction of the blood flow to vital organs that are sufficient to induce symptoms. In general, patients discover their atherosclerosis only when they experience cardiovascular disorders such as stroke or heart attack. To date, the development of atherosclerosis is not fully understood. It predominantly affects the intimal layer of the arterial vessel wall characterized by the deposition of extracellular lipids, the proliferation and migration of local smooth muscle cells, and chronic inflammation. It leads to luminal narrowing and/or thrombus formation, resulting in clinical events such as coronary artery disease, peripheral arterial disease, or stroke. It has been confirmed that atherosclerosis is initiated by inflammatory progression in the endothelial cells associated with the collected low-density lipoprotein (LDL) particles [80]. Therefore, improving endothelial dysfunction, recuperating dyslipidemia, and blocking the inflammatory process are effective methods for blocking the progression of atherosclerosis. Since 2002, numerous studies have described the anti-atherosclerotic effect conferred by berberine in multiple phases, indicating the multiple mechanisms involved. However, anti-atherosclerotic research investigating BBR is still in the experimental stage.

Berberine Improves Endothelial Dysfunction

Endothelial dysfunction is one of the primary events in the development of atherosclerosis [59]. The correction of endothelial function is essential for the treatment of atherosclerosis. In 2002, the effects of berberine on angiotensin-converting enzyme and the NO/cGMP system in vessels were investigated. The authors reported that the treatment of intact aortic rings with berberine increased NO and cGMP production. Berberine induced relaxation in aortic rings in a dose-dependent manner [36]. Thereafter, more intense studies were conducted. Current evidence indicates that berberine can protect endothelial function against hypertension, type 2 diabetes [52, 53, 82]. It has been broadly accepted that high levels of oxidative stress and reduced nitric oxide (NO) production, as two key contributors to the onset of endothelial dysfunction, may be induced by high glucose levels, inflammatory factors, and free fatty acids [93]. Our group investigated the effect of berberine on the endothelial dysfunction induced by type 2 diabetes and palmitate. We found that berberine reduced ROS production, increased endothelial nitric oxide synthase (eNOS) expression and activation, and down-regulated NOX4 expression, resulting in the generation of NO in endothelial cells exposed to palmitate [69, 93]. Wang et al. studied the effect of berberine in both cultured endothelial cells and blood vessels isolated from rat aorta. They reported that berberine enhanced the phosphorylation of eNOS in a dose-dependent manner. Berberine also attenuated high glucose-induced generation of reactive oxygen species, cellular apoptosis, nuclear factor-kappa B activation, and the expression of adhesion molecules, thus suppressing monocyte attachment to endothelial cells. Our studies indicated that the effect of berberine on the phosphorylation of eNOS might be due to the activation of adenosine monophosphate-activated protein kinase (AMPK). The authors demonstrated that pharmacological inhibition of AMPK or adenovirus-mediated overexpression of a dominant-negative version of AMPK could abolish the beneficial effects of berberine on the endothelium [75, 93]. AMPK activation induced by berberine might be the key to improving endothelial function under diabetes conditions. Furthermore, it has been reported that berberine can improve endothelial cell functions by inhibiting the expression and secretion of monocyte chemoattractant protein (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) and by reducing MCP-1 receptor expression in monocytes, resulting in reduced adhesion of monocytes to endothelial cells [41, 81].

In addition to endothelial cells themselves, circulating endothelial progenitor cells (EPCs) also play an important role in the maintenance of normal endothelial cell function. Xu et al. reported that berberine increased the number of EPCs in healthy subjects after administration for 1 month; it also improved the small artery elasticity index, which is a marker of endothelial function [83]. Furthermore, Xiao et al. reported that berberine could increase the protein expression of PI3K, p-Akt, eNOS, and p-eNOS, thus improving the proliferation of TNF-α-inhibited EPCs. This beneficial effect of berberine could be attenuated by the blockade of PI3K and eNOS. This study indicated that the proliferation of EPCs may be reduced in response to high levels of inflammatory cytokine stimulation and that berberine improves the proliferation of EPCs exposed to an inflammatory stimulus [82].

Berberine Inhibits Dyslipidemia

Collected LDL particles are the other critical pathogenic factors related to atherosclerosis. This accumulation may be a cause, an effect, or both of inflammatory enlargement. Normally, LDL particles carry 3000 to 6000 fat molecules, including cholesterol, phospholipids, cholesteryl esters,

triglycerides, and all other fats [80]. Lipoprotein has been proposed to be central to atherogenesis. Once inside the vessel wall, LDL particles can become more prone to oxidation. Pathophysiological oxidation occurs after the accumulation of lipoproteins in vessels. After lipid oxidation, endothelial cells attract monocytes and induce their transformation into macrophages. The macrophages ingest the oxidized LDL particles, triggering a cascade of immune responses. The macrophages absorb the oxidized LDL particles and develop into specialized foam cells. If these foam cells are not recruiting HDL particles to remove fats, they will grow and ultimately rupture, releasing cellular membrane fragments, oxidized substances, and fats, attracting more white blood cells and resulting in a vicious cycle [59, 80]. Current evidence suggests that improving the lipid profile in the development of atherosclerosis could be of interest due to the involvement of lipids specifically LDLs.

Berberine exerts a protective role in atherosclerosis that is related to its cholesterol-lowering activity. It has been shown to have lipid-lowering properties in animals and in hyperlipidemic subjects. In hypercholesterolemic subjects in China, BBR significantly reduced the serum level of TC, triglycerides, and LDL-C and significantly increased the HDL-C levels [42]. Another study reported that oral administration of berberine hydrochloride (0.5 g b.i.d.) for 3 months reduced LDL cholesterol without any effect on high-density lipoprotein cholesterol in hypercholesterolemic subjects in Australia [17]. The beneficial effects of berberine on cholesterol reduction were also observed in hypercholesterolemic European patients [3]. A meta-analysis conducted in 2013 confirmed a significant reduction in total cholesterol, triglycerides, and LDL cholesterol levels and a small but significant increase in HDL cholesterol after berberine treatment in 11 randomized controlled studies involving 874 Chinese participants affected by hyperlipidemia, T2DM, or both diseases [18]. Another meta-analysis based on 27 randomized controlled clinical trials including 2569 patients reported that berberine with lifestyle intervention was better than lifestyle intervention alone and that berberine with oral lipid-lowering drugs was better than lipid-lowering drugs alone in reducing the level of TC and LDL-C and raising the level of HDL-C [43]. Studies examining the cholesterol-lowering effect of berberine in animal models have further examined the mechanism underlying this effect. Notably, the mechanism was shown to differ from that of statins.

The LDL receptor (LDLR) is a cell-surface receptor that can be pinched off to form clathrin-coated vesicles. When LDLR binds to LDL-cholesterol, it is internalized via endocytosis and prevents LDL from diffusing around the membrane surface. This endocytosis occurs mainly in the liver to remove more than 70 % of the LDL from the circulation [57]. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is critically important in the regulation of LDL receptor-mediated metabolism of cholesterol. Inhibitors of PCSK9 have been extensively studied. The effectiveness of the PCSK9 inhibitor in reducing levels of LDL-cholesterol and the primary indication of PCSK9 inhibitor in cardiovascular disease intervention suggest that the PCSK9 inhibitor can be used in patients with hypercholesterolemia with statin intolerance. It has been recommended that PCSK9 inhibitors be used for patients who do not respond to statins [40, 56]. A previous study reported that treatment of hyperlipidemic hamsters with BBR reduced serum cholesterol and LDL-cholesterol and increased mRNA and protein levels of hepatic LDLR [42]. Furthermore, a study conducted by Li's group has demonstrated that berberine upregulates LDLR expression via a post-transcriptional mechanism of mRNA stabilization [1, 2, 45]. Interestingly, Cameron et al. reported that BBR also exerts inhibitory effects on the expression of PCSK9 protein and mRNA in HepG2 cells [8]. Current evidence indicates that berberine could have dual actions on LDLR metabolism by prolonging its mRNA half-life and directly increasing protein abundance through the blockade of PCSK9-mediated degradation. Therefore, Li's group further explored the molecular mechanisms of this interesting discovery. They described the first example of SREBP pairing with HNF1 to control an important regulatory pathway in cholesterol homeostasis. They also demonstrated that a coordinated modest reduction of HNF1α and nuclear SREBP2 by BBR might be the reason for strong suppression of PCSK9 transcription [46]. However, another study reported that berberine enhanced circulating PCSK9 concentrations through the SREBP-2 pathway in high fat diet-fed rats [35]. Therefore, a detailed investigation of the molecular mechanism of berberine in the treatment of dyslipidemia is still needed. Furthermore, accumulating evidence indicated that gut microbiota impacted the adipose tissue and liver fat storage, fat liver metabolism, atherosclerosis, and cardiovascular diseases (CVD) [6]. Berberine, as an oral drug to treat gastroenteritis and secretary diarrhea, was poorly absorbed. It has been pointed out that the multiple therapeutic effect of berberine might be closely correlated to its modulatory effect on gut microbiota [20, 96, 97]. Zhang et al. reported that the prevention of obesity and insulin resistance by berberine in HFD-fed rats is partially mediated by structural modulation of the gut microbiota [96, 97]. Although, many mechanisms of berberine on regulation the lipid homestasis have been reported, more effort is still needed for using berberine as an antidyslipidemia drug in clinic.

Other Effects of Berberine on Atherosclerosis

In addition to improving endothelial dysfunction and correcting the lipid profile, several other atheroprotective effects of berberine have been described, including anti-inflammatory and antioxidant properties, as well as the inhibition of vascular smooth muscle cell proliferation. Berberine

has been shown to inhibit angiotensin II and heparin-binding epidermal growth factor-induced vascular smooth muscle cell proliferation and migration [44]. Moreover, berberine induces endothelium-dependent vasorelaxation and enhances endothelium-independent vascular smooth muscle cell (VSMC) dilatation through a partial reduction of oxidative stress [75]. The anti-inflammatory effects of berberine are currently being extensively investigated. Because inflammation is one of the key factors in the development of atherosclerosis, the anti-inflammatory effect of berberine has been observed under atherosclerotic conditions. Zimetti et al. demonstrated a double protective effect of berberine on cholesterol homeostasis underlying foam cell formation and on the inflammatory phenotype in mouse and human macrophages [108]. Matrix metalloproteinases (MMPs) and extracellular matrix metalloproteinase inducer (EMMPRIN) overproduced by monocytes/macrophages can degrade the extracellular matrix, in turn leading to atherosclerotic plaque rupture. Berberine reduces MMP-9 and EMMPRIN expression by suppressing activation of the p38 pathway in PMA-induced macrophages, supporting a potential role of berberine in stabilizing atherosclerotic plaques [33]. Furthermore, as the typical target of berberine in cells, AMPK is also involved in the molecular mechanism underlying the anti-inflammatory effects of berberine. It has been reported that berberine represses proinflammatory responses through AMPK activation in macrophages. Berberine treatment significantly decreases the expression of TNF-α, IL-1β, IL-6, iNOS, and MCP-1 in adipose tissue of obese db/db mice [34]. Meanwhile, BBR also inhibits LPS-induced expression of proinflammatory genes, including IL-1beta, IL-6, iNOS, MCP-1, COX-2, and matrix metalloprotease-9, in peritoneal macrophages and RAW 264.7 cells [34]. In response to various proinflammatory signals, including LPS, free fatty acids, and hydrogen peroxide, BBR suppresses the phosphorylation of MAPKs, such as p38, ERK, and JNK, as well as the level of reactive oxygen species in macrophages. Moreover, these inhibitory effects of BBR on proinflammatory responses are abolished by AMPK inhibition via either compound C, an AMPK inhibitor, or dominant-negative AMPK, implying that BBR would down-regulate proinflammatory responses in macrophages via AMPK stimulation [34]. In another study, apoE$^{(-/-)}$ and apoE$^{(-/-)}$/AMPK α2$^{(-/-)}$ mice were used. The authors found that administration of berberine significantly reduced aortic lesions, markedly reduced oxidative stress and expression of adhesion molecules in the aorta, and increased UCP2 levels in apoE$^{(-/-)}$ mice. In contrast, in apoE$^{(-/-)}$/AMPK alpha 2$^{(-/-)}$ mice, berberine had a reduced effect on these parameters. These findings indicated that berberine could reduce oxidative stress and vascular inflammation, ultimately suppressing atherogenesis through stimulation of AMPK-dependent UCP2 expression [74]. Although most evidence supports the anti-atherosclerotic effect of berberine, another study reported that berberine induced in vivo foam cell formation and promoted atherosclerosis development in apoE$^{(-/-)}$ mice. This promotion of foam cell formation might counterbalance the beneficial effect of berberine on serum cholesterol [48]. Considering all of this evidence, berberine administration might be a potential therapeutic approach for the treatment of atherosclerosis. Well-designed randomized controlled trials to test the safety and efficacy of BBR are still needed.

Effect of Berberine on Hypertension

Hypertension is the most common chronic medical problem and the most important preventable risk factor for premature death worldwide. Hypertension increases the risk of ischemic heart disease, strokes, peripheral vascular disease, heart failure, and other cardiovascular diseases if not detected early and treated appropriately. Lowering blood pressure significantly reduces the risk of death due to heart diseases and stroke. In 1993, the first clinical study demonstrated the beneficial effect of berberine on the treatment of hypertension in 42 cases [70]. Later, numerous clinical and preclinical studies were conducted to explore the anti-hypertensive effects and mechanisms of berberine [19, 32, 43, 47, 51, 52, 64, 70, 71, 100]. A current clinical study described the renoprotective effects of berberine for hypertensive patients with type 2 diabetes mellitus based on alteration of biochemical markers and color Doppler ultrasonography [32]. Meanwhile, a recent meta-analysis emphasized the role of berberine in the treatment of hypertension. The efficacy and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipidemia, and hypertension were assessed by conducting a meta-analysis of the available clinical data. The authors calculated the data from 27 randomized controlled clinical trials. They stated that berberine combined with lifestyle intervention tended to lower blood pressure more than lifestyle intervention alone, and the same occurred when berberine was combined with oral anti-hypertensive drugs [43].

Although clinical and experimental studies have suggested anti-hypertensive properties of berberine and its derivatives, the mechanism underlying the modulatory effects of berberine on hypertension is still under intense investigation. Activation of endoplasmic reticulum (ER) stress in endothelial cells provokes oxidative stress and results in cell death, which has been shown to be involved in the pathogenesis of hypertension. Liu et al. have reported that berberine reduces endothelium-dependent contractions by activating AMPK, thus inhibiting ER stress and subsequently scavenging ROS and leading to COX-2 down-regulation in SHR carotid arteries [52]. Guo et al. observed the effects of berberine on the renin-angiotensin system (RAS) and proinflammatory cytokines, as well as its effects on blood pressure and renal damage in spontaneously hypertensive rats. The authors showed that

berberine delayed the onset of hypertension and attenuated the severity of hypertension, and it also inhibited the pathophysiology of hypertension by activating RAS and decreasing the production of the proinflammatory cytokines IL-6, IL-17, and IL-23 [19].

It is broadly acknowledged that vascular stiffness is closely associated with an elevated vascular tone. VSMC contraction is the main cause of vascular stiffness, which is predominantly regulated by myosin light chain (MLC). The Ca^{2+} influx triggered by cell depolarization increases the cytoplasmic free Ca^{2+} concentration, which promotes the phosphorylation of MLC. Therefore, calcium channel blocker is a first-line treatment for hypertension. Transient receptor potential vanilloid 4 (TRPV4) is a Ca^{2+}-permeable cation channel, which was initially recognized as a transducer of hypotonic stimuli [68]. A recent study indicated that berberine induces direct vasorelaxation to lower blood pressure and also reduces vascular stiffness in aged mice [71]. The report provided the first proof that administration of berberine in vivo lowers high BP in deoxycorticosterone acetate-induced hypertensive mice and reduces vascular stiffness in aged ApoE-KO mice. These effects of berberine are instinctively induced by the suppression of TRPV4, decreased intracellular calcium levels, decreased MLC phosphorylation, and consequent relaxation of VSMCs. Berberine might function as a calcium channel blocker to decrease high blood pressure and vascular stiffness [71]. Meanwhile, in addition to the calcium channel, the potassium channel has been shown to contribute to the vasodilation effect of berberine because K^+ channel blockers significantly attenuate berberine-induced vasodilatation in endothelium-denuded arteries [78]. This evidence might provide clues to exploring the effects of berberine on the K^+ channel in VSMCs in future studies.

Effect of Berberine on Heart Failure

Heart failure is a common clinical syndrome representing the end stage of different cardiac diseases. It is a serious condition and there is currently no cure. Any structural or functional cardiac disorder could result in impairment of the ability of the ventricle to fill with or eject blood.

As early as 1988, a clinical study reported the protective effect of berberine in patients with severe congestive heart failure. Twelve patients with refractory congestive heart failure were studied before and during berberine intravenous infusion. The authors reported that berberine intravenous infusion at dosages of 0.2 mg/kg per min decreased systemic and pulmonary vascular resistance, as well as right atrium and left ventricular end-diastolic pressures. It also increased the cardiac index, stroke index, and LV ejection fraction measured by contrast angiography. Furthermore, this treatment led to increases in hemodynamic and echocardiographic indices of LV performance: peak measured velocity of shortening, peak shortening velocity at zero load, percent fractional shortening, and the mean velocity of circumferential fiber shortening. Finally, it led to decreases in arteriovenous oxygen differences without changes in total body oxygen uptake, arterial oxygen tension, or hemoglobin dissociation properties [55].

In 2003, a randomized clinical trial assessed the efficacy and safety of berberine in 156 patients with chronic congestive heart failure. Seventy-nine patients were also given berberine at a rate of 1.2 to 2.0 g/day. The remaining 77 patients were given placebo. The berberine-treated group showed significantly greater increases in the left ventricular ejection fraction and exercise capacity, as well as significant improvements on the dyspnea-fatigue index and decreased rates of ventricular premature complexes. Additionally, mortality was significantly decreased during long-term follow-up. Neither proarrhythmia nor apparent side effects were observed [92], confirming the results of a previous study showing that berberine improved quality of life and decreased mortality in patients with congestive heart failure.

Cardiac hypertrophy is a maladaptive change in response to pressure overload and is considered a critical intermediate step toward heart failure. Many signaling pathways have been confirmed to be involved in the induction and progression of pathological hypertrophy. P21-activated kinase-1 (Pak1) is a member of a serine/threonine protein kinase family, which is essential for adaptive physiological cardiac remodeling. Pak1 is critical in the development of adrenergic and hypertrophic cardiac problems; its roles in maintaining ventricular Ca^{2+} homeostasis and electrophysiological stability under physiological, β-adrenergic and hypertrophic stress conditions have also been explored [37]. F-box only protein 32 (FBXO32) acts as a cardiac ubiquitin ligase. A recent study indicated that FBXO32 is a candidate gene for recessive dilated cardiomyopathy. Mutated FBXO32 can perturb the degradation of target proteins in the UPS; impairment of the UPS has been observed in cardiomyopathy [4]. A recent study reported that Smad3 and FBXO32 might be novel downstream components of the Pak1-dependent signaling pathway for the suppression of hypertrophy, and berberine ameliorated hypertrophic remodeling in cardiac-deficient Pak1 mice under pressure overload via upregulation of FBXO32 [67]. Furthermore, another study investigated the beneficial effect of berberine on the development of diastolic heart failure by incomplete ligation of the abdominal aorta. Berberine administered at 63 mg/kg/d can decrease LEVDP, improve ($-dp/dt_{max}$), decurtate left ventricular relax time constant quantity and decrease $[Ca^{2+}]_i$ levels in a dose-dependent manner [98]. Additionally, in transverse aortic contraction (TAC) surgery-induced cardiac hypertrophy, berberine could attenuate left ventricular remodeling and cardiomyocyte apoptosis through an autophagy-dependent mechanism in a rat model of cardiac hypertrophy, which is at least partially associated with enhanced autophagy,

through inhibition of mTOR, p38, and ERK1/2 MAPK signaling pathways.

Based on current evidence, it can be concluded that berberine has beneficial effects on heart failure and that multiple pharmacological activities are involved. The mechanisms underlying these beneficial effects still need to be elucidated. Such findings are essential for managing the clinical application of berberine in the future.

Effect of Berberine on Myocardial Ischemia Reperfusion Injury

Ischemic heart disease is the most common cause of death in developed and developing countries. Coronary heart disease is the main cause of ischemic heart disease, which mostly includes acute myocardial infarction (MI) and ischemia-reperfusion injury (IR). Myocardial reperfusion is the restoration of coronary blood flow after a period of coronary occlusion. Reperfusion is important and necessary to salvage ischemic myocardium. However, it can also cause myocardial injury. This phenomenon is known as reperfusion injury (IR) [22]. It has been demonstrated that oxidative stress, apoptosis, and inflammation are the most important mechanisms initiated during ischemia, and they continue for several hours into reperfusion [23]. The most common initial event of ischemic heart disease is the disturbance of an atherosclerotic plaque in the coronary artery, resulting in occlusion of the artery. The beneficial effect of berberine on atherosclerosis has been extensively investigated. Moreover, BBR exerts a potential protective effect against myocardial ischemia/reperfusion (MI/R) injury.

In a study published in 2003, the authors isolated cardiac myocytes from neonatal SD rats and developed hypoxia and reoxygenation cell models. It was reported that berberine could decrease the level of LDH and MDA in the supernatant and attenuate apoptosis in a dose-dependent manner [103]. Investigations then began exploring the mechanisms behind the therapeutic effect of berberine on ischemia reperfusion. It was reported that berberine could protect the heart from ischemia reperfusion via suppressing autophagy activation [32], attenuating mitochondrial dysfunction [76], anti-oxidative and anti-inflammatory activity [60, 91], and by attenuating ER stress-induced apoptosis [99]. Numerous signaling pathways are involved in these processes. Huang et al. reported that berberine decreased the expression of autophagy-related proteins such as SIRT1, BNIP3, and Beclin-1, which might be correlated with diminished levels of p-AMPK and p-mTORC2 (Ser2481) in H9c2 myocytes exposed to H/R by berberine treatment [32]. Furthermore, typical mitochondrial-dependent apoptosis was observed; Wang et al. reported that berberine attenuated myocardial apoptosis and improved mitochondrial dysfunction by decreasing the expression of Bcl-2, Bax, and cytochrome c [76]. Another study investigated the effect of berberine on ER stress-induced apoptosis during the myocardial ischemia reperfusion process. BBR suppressed the phosphorylation levels of myocardial PERK and eIF2α and the expression of ATF4 and CHOP in a heart subjected to ischemia reperfusion. Pretreatment with BBR also activated the JAK2/STAT3 signaling pathway in heart tissues, and a specific JAK2/STAT3 inhibitor blocked the protective effects of berberine and the inhibition of IR-induced ER stress by berberine [99]. Moreover, it has also been reported that BBR modulates SIRT1 signaling to prevent myocardial apoptosis induced by myocardial ischemia reperfusion injury [90, 91]. Interestingly, our group found that the beneficial effect of berberine on ischemia reperfusion heart injury might occur via regulation of AMPK activity in both non-ischemic areas and risk areas of the heart. We found that berberine treatment significantly decreased the AMPK protein concentration and the ratio of ADP/ATP and AMP/ATP in areas of myocardial risk. In contrast, berberine treatment significantly increased the AMPK protein concentration and the ratio of ADP/ATP and AMP/ATP in non-ischemic areas compared with the controls [11].

It has been reported that diabetes increases the risk of cardiovascular diseases. Some studies also investigated the cardioprotective effect of BBR against ischemia/reperfusion (I/R) injury under diabetic conditions, and the underlying mechanisms. In diabetic rat models, BBR protects the heart against I/R injury, improves cardiac function, and reduces myocardial apoptosis by activating AMPK and PI3K/Akt and eNOS signaling, AMPK/AKT and GSK 3β [13]. Our group found that pretreatment with berberine increased AMPK activation and AKT phosphorylation, and also reduced glycogen synthase kinase 3β (GSK3beta) activity in non-ischemic areas compared with untreated diabetic controls [10].

Effect of Berberine on Stroke

Stroke, also defined as a cerebrovascular accident, is one of the major causes of mortality, morbidity, and long-term disability, and it is induced by either inadequate focal blood flow or hemorrhage into the brain tissue or the surrounding subarachnoid space. Like ischemic heart disease, blood flow reentry into the ischemic area is the initial intent for the treatment of cerebral ischemic injury. However, this reentry blood flow also induced remarkably damage in the brain. The current therapeutic options for the prevention and treatment of stroke comprise five fields of management, including primary prevention, recanalization and thrombolysis, neuroprotection, secondary prevention, and neurorepair.

The beneficial effect of berberine on the treatment of stroke was primarily reported in 2008. In a middle cerebral artery

occlusion (MCAO) model, BBR improved neurological outcomes and reduced I/R-induced cerebral infarction 48 h after MCAO mode. The protective effect of BBR was confirmed in injured oxygen-glucose deprivation (OGD)-induced PC12 cells [106]. After the 2008 study, a series of other studies were conducted to investigate the mechanism underlying the beneficial action of berberine in the treatment of cerebral ischemia. Benasissa et al. confirmed a previous study by reporting that berberine reduced brain ischemic-hypoxic injury dose-dependently in rat pups [5]. Studies on the mechanism of the beneficial effect of berberine on stroke mostly focused on its neuroprotective actions. It was reported that berberine prevents apoptosis induced by cerebral ischemia via increased phosphorylation of Akt, leading to phosphorylation of Bad and decreased cleavage of caspase-3. Additionally, the action of berberine is specific to PI3K rather than the upstream receptor tyrosine kinase [25]. Another study indicated that HIF-1 and the associated apoptotic pathway are involved in the neuroprotective effects of berberine [72]. Additionally, berberine could prevent neuron damage by up-regulating the expression of p-Akt, p-GSK 3β, p-CREB, and claudin-5, and by decreasing the nuclear accumulation of NF-kB in an ischemic brain [95]. Moreover, similar to the myocardial protective effect of berberine on ischemia reperfusion heart injury, the PI3K/AKT pathway and AMPK pathway were also involved in the neuroprotective effect of berberine on cerebral ischemia reperfusion injury [9, 15, 39, 63].

Effect of Berberine on Arrhythmias

The human heart is an electrical organ. Cardiac arrhythmia is an abnormal rhythm of the heart caused by abnormal performance of the conduction system. The anti-arrhythmia effect of berberine was reported in 1989. The study proved that berberine prevents tachycardias and total ventricular premature beats induced by ligating the left anterior descending (LAD) coronary artery of a canine [31]. The same year, Xu et al. reported that berberine may be effective for preventing the onset of reentrant ventricular tachyarrhythmias and sudden coronary death after myocardial ischemic damage in dogs [61, 84]. The anti-arrhythmia effect of berberine has also been observed in human patients [29]. Following these studies, the mechanism underlying the anti-arrhythmia action of berberine was investigated. It was reported that the mechanisms underlying the anti-arrhythmic action of berberine may be suppression of delayed after-depolarization, partially by decreasing Na^+ influx [77]. The effect of berberine on inhibition of K_{ATP} channel activation and subsequent shortening of the action potential duration and the effective refractory period might be related to its protective effect on ischemia-induced arrhythmias [78].

The anti-arrhythmias effect of berberine has also been reported in diabetic patients subjected to ischemia heart injury. Berberine could recover arrhythmia scores and resting membrane potential by recovering the I(K1) current and current density, which was markedly decreased in the presence of myocardial infarction with or without diabetes [73]. In addition, berberine significantly shortened the prolonged QTc interval in ischemic diabetic rats, and also restored the diminished I(to) and I(Ca) current densities [72].

Clinical Efficacy and Safety of BBR in Cardiovascular Disease

Berberine has low solubility and poor membrane permeability leading to the low bioavailability, which limited its application in clinic. We found that berberine was a very slightly soluble compound with the aqueous solubility approximately 1 g/L [101]. Others reported that berberine had extremely low absolute bioavailability less than 5 % [14]. All the evidence indicated the solubility of berberine was really poor belong to low solubility compound [7]. Therefore, currently, for the treatment of chronic disease such as cardiovascular disease and diabetes, the dose of berberine was recommended at doses of 0.2~1.0 g/day clinically [58]. Dose forms include tablets and capsules [88].

Numeriours clinical studies conducted to explore the efficacy of berberine in cardiovascular disease. It has been reported that berberine 1.2 to 2.0 g/day was given to 79 patients combined with conventional therapy of congestive heart failure. Quality of life was assessed after 8 weeks of treatment and during a mean 24-month follow-up. They found that berberine improved quality of life and decreased VPCs and mortality in patients with CHF [92]. Another study reported that low dose berberine (0.4 g/day) effectively improved the life quality of chronic heart failure patient combined with regular anti heart failure medications [85].

Most recent met analysis report indicated that berberine combined with oral lipid-lowering drugs was reducing the level of TC and LDL-C, and raising the level of HDL-C and better than lipid-lowering drugs alone. And berberine treatment alone shows better effect in lowering the level of TG and raising the level of HDL-C compared to lipid-lowering drug [24, 65, 79, 89, 102, 107]. As for the treatment of hypertension, berberine combined with oral anti-hypertension drug tended to lower the level of blood pressure more than oral anti-hypertension drug alone. Notably, no serious adverse reaction was reported in the ten randomized controlled clinical trials they calculated in the met analysis [21, 28, 43, 66, 104]. Among these studies, berberine 0.9–1.2 g/day was used. Meanwhile, 1.2 g/day berberine was also used to treat stroke induced by acute macro artery atherosclerosis combined with atorvastatin calcium

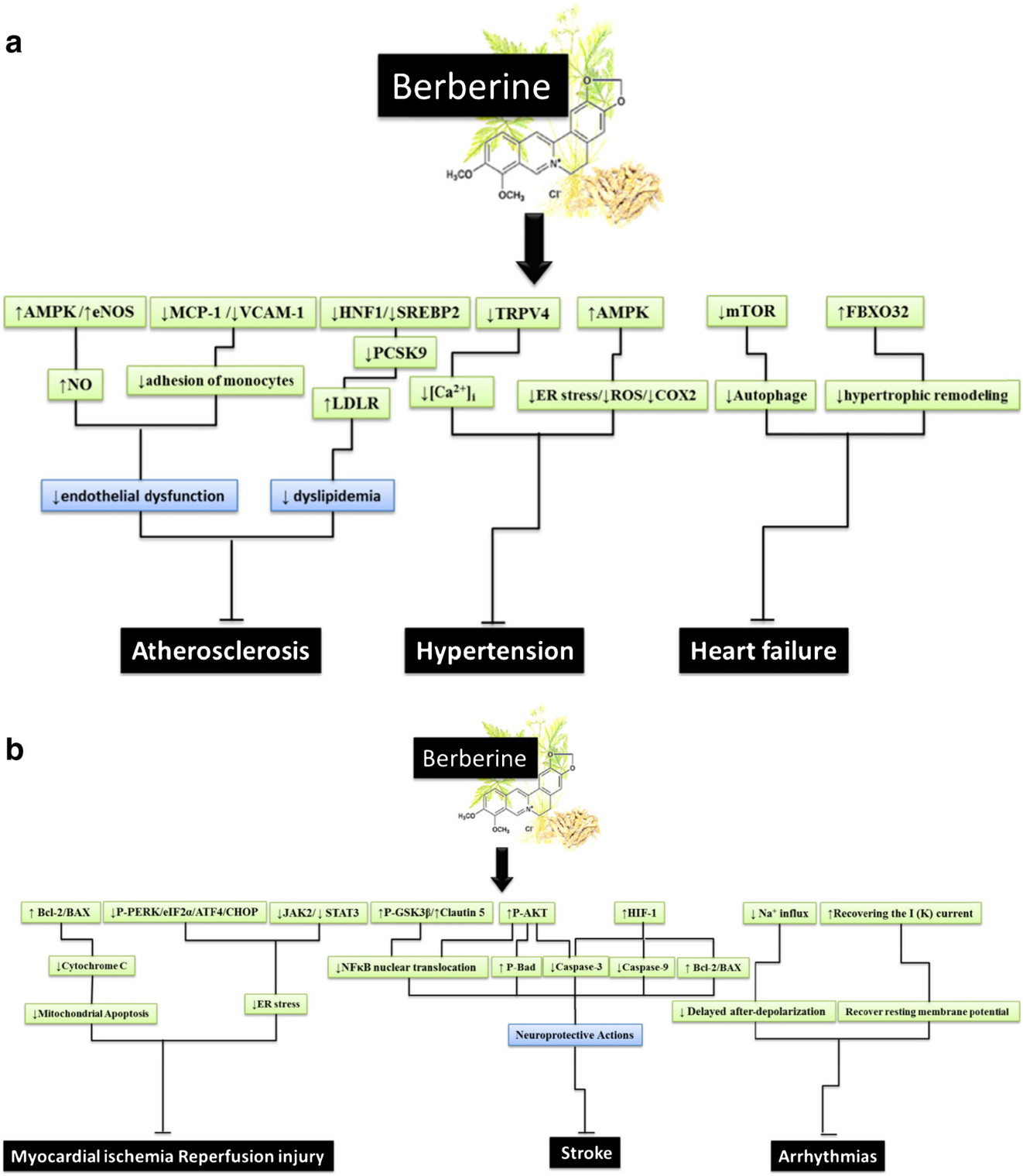

Fig. 1 The potential targets and mechanisms of berberine in the treatment of cardiovascular disease. **a** The potential targets and mechanisms of berberine in the treatment of atherosclerosis, heart failure, and hypertension. **b** The potential targets and mechanisms of berberine in the treatment of myocardial ischemia reperfusion injury, stroke, and arrythmias

treatment [49]. Another clinical trial on 86 diabetes mellitus combined with cerebral stroke patients reported that 0.4 g/day berberine combined with 40 mg/day atorvastatin and insulin treatment could improve patients' symptoms and reduce blood glucose, improve blood lipid disorder, and reduce the injury of nerve function [62].

Furthermore, understanding the safety of berberine was also really necessary due to the higher dose and long course of berberine application in chronic diseases. It has been reported that a shortage of glucose 6 phosphate dehydrogenase (G6PD) could be caused after a pregnant woman or a newborn baby takes BBR, which may lead to hemolytic jaundice of the newborn baby [86]. Another study reported that the administrated method was a significant factor affecting the acute toxicity of berberine, they reported that intragastric administration caused no LD50 [38]. Moreover, in the sub-chronic toxicity study, no mortality and morbidity were observed which could be related toberberine [7, 58, 87]. These above studies proved that orally administration of berberine is relatively safe. All the clinical and experimental studies indicate the efficiency and safety of berberine on the treatment of cardiovascular disease and diabetes under recommended dosage. However, the application of berberine in pregnant patient and newborn baby may not be recommended. Several limitations still existed in the evaluation of efficiency and safety of berberine. Well-designed, large-scale multicenter random clinical trials are needed, and long-term outcomes are also needed to be evaluated, especially the safety profile and the effects on organ damages.

Conclusion

The multiple pharmacological actions of berberine support its numerous potential clinical applications for cardiovascular disease. We have reviewed the beneficial effects of berberine on cardiovascular disease (Fig. 1a, b). Many clinical and preclinical studies have been conducted, including studies on atherosclerosis, hypertension, heart failure, ischemia reperfusion heart disease, stroke, and arrhythmias. However, further analyses of the mechanisms of berberine are needed for its proper application in clinical settings.

Acknowledgments This work was conducted in Preclinical Pharmacology R&D Center of Jilin Province and Key Lab of Traditional Medicine for Diabetes of Jilin Province. This work was supported by Young Scholars Program of Norman Bethune Health Science Center of JiLin University (2013201011), Jilin Science & Technology Development Plan (20140203011YY,20150311013YY, 20140414052GH).

Compliance with Ethical Standards

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Abidi P, Chen W, Kraemer FB, Li H, Liu J. The medicinal plant goldenseal is a natural LDL-lowering agent with multiple bioactive components and new action mechanisms. J Lipid Res. 2006;47(10):2134–47. doi:10.1194/jlr.M600195-JLR200.
2. Abidi P, Zhou Y, Jiang JD, Liu J. Extracellular signal-regulated kinase-dependent stabilization of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine. Arterioscler Thromb Vasc Biol. 2005;25(10):2170–6. doi:10.1161/01.ATV.0000181761.16341.2b.
3. Affuso F, Ruvolo A, Micillo F, Sacca L, Fazio S. 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. 2010;20(9):656–61. doi:10.1016/j.numecd.2009.05.017.
4. Al-Hassnan ZN, Shinwari ZM, Wakil SM, Tulbah S, Mohammed S, Rahbeeni Z, et al. A substitution mutation in cardiac ubiquitin ligase, FBXO32, is associated with an autosomal recessive form of dilated cardiomyopathy. BMC Med Genet. 2016;17:3. doi:10.1186/s12881-016-0267-5.
5. Benaissa F, Mohseni-Rad H, Rahimi-Moghaddam P, Mahmoudian M. Berberine reduces the hypoxic-ischemic insult in rat pup brain. Acta Physiol Hung. 2009;96(2):213–20. doi:10.1556/APhysiol.96.2009.2.6.
6. Burcelin R, Serino M, Chabo C, Blasco-Baque V, Amar J. Gut microbiota and diabetes: from pathogenesis to therapeutic perspective. Acta Diabetol. 2011;48(4):257–73. doi:10.1007/s00592-011-0333-6.
7. Caliceti C, Franco P, Spinozzi S, Roda A, Cicero AF. Berberine: new insights from pharmacological aspects to clinical evidences in the management of metabolic disorders. Curr Med Chem. 2016;23(14):1460–76.
8. Cameron J, Ranheim T, Kulseth MA, Leren TP, Berge KE. Berberine decreases PCSK9 expression in HepG2 cells. Atherosclerosis. 2008;201(2):266–73. doi:10.1016/j.atherosclerosis.2008.02.004.
9. Chai YS, Hu J, Lei F, Wang YG, Yuan ZY, Lu X, et al. Effect of berberine on cell cycle arrest and cell survival during cerebral ischemia and reperfusion and correlations with p53/cyclin D1 and PI3K/Akt. Eur J Pharmacol. 2013;708(1–3):44–55. doi:10.1016/j.ejphar.2013.02.041.
10. Chang W, Li K, Guan F, Yao F, Yu Y, Zhang M, et al. Berberine pretreatment confers cardioprotection against ischemia-reperfusion injury in a rat model of type 2 diabetes. J Cardiovasc Pharmacol Ther. 2016. doi:10.1177/1074248415627873.
11. Chang W, Zhang M, Li J, Meng Z, Xiao D, Wei S, et al. Berberine attenuates ischemia-reperfusion injury via regulation of adenosine-5'-monophosphate kinase activity in both non-ischemic and ischemic areas of the rat heart. Cardiovasc Drugs Ther. 2012;26(6):467–78. doi:10.1007/s10557-012-6422-0.
12. Chang XX, Yan HM, Xu Q, Xia MF, Bian H, Zhu TF, et al. The effects of berberine on hyperhomocysteinemia and hyperlipidemia in rats fed with a long-term high-fat diet. Lipids Health Dis. 2012;11:86. doi:10.1186/1476-511X-11-86.
13. Chen K, Li G, Geng F, Zhang Z, Li J, Yang M, et al. Berberine reduces ischemia/reperfusion-induced myocardial apoptosis via activating AMPK and PI3K-Akt signaling in diabetic rats. Apoptosis. 2014;19(6):946–57. doi:10.1007/s10495-014-0977-0.
14. Chen W, Miao YQ, Fan DJ, Yang SS, Lin X, Meng LK, et al. Bioavailability study of berberine and the enhancing effects of TPGS on intestinal absorption in rats. AAPS PharmSciTech. 2011;12(2):705–11. doi:10.1208/s12249-011-9632-z.
15. Chen W, Wei S, Yu Y, Xue H, Yao F, Zhang M, et al. Pretreatment of rats with increased bioavailable berberine attenuates cerebral

ischemia-reperfusion injury via down regulation of adenosine-5'monophosphate kinase activity. Eur J Pharmacol. 2016;779:80–90. doi:10.1016/j.ejphar.2016.03.015.
16. Cicero AF, Colletti A. Combinations of phytomedicines with different lipid lowering activity for dyslipidemia management: the available clinical data. Phytomedicine. 2015. doi:10.1016/j.phymed.2015.10.011.
17. Doggrell SA. Berberine—a novel approach to cholesterol lowering. Expert Opin Investig Drugs. 2005;14(5):683–5. doi:10.1517/13543784.14.5.683.
18. Dong H, Zhao Y, Zhao L, Lu F. The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. Planta Med. 2013;79(6):437–46. doi:10.1055/s-0032-1328321.
19. Guo Z, Sun H, Zhang H, Zhang Y. Anti-hypertensive and renoprotective effects of berberine in spontaneously hypertensive rats. Clin Exp Hypertens. 2015;37(4):332–9. doi:10.3109/10641963.2014.972560.
20. Han J, Lin H, Huang W. Modulating gut microbiota as an anti-diabetic mechanism of berberine. Med Sci Monit. 2011;17(7):RA164–7.
21. Han ZX, Huang JX, Zhang ZS, Ji SJ, Xu J. Elderly hypertensive disease observation of Betaloc treated with berberine. Med Res Commun. 1999;28:34.
22. Hashmi S, Al-Salam S. Acute myocardial infarction and myocardial ischemia-reperfusion injury: a comparison. Int J Clin Exp Pathol. 2015;8(8):8786–96.
23. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J Clin Invest. 2013;123(1):92–100. doi:10.1172/JCI62874.
24. He JJ, Xu XG, Zheng X. Effect of simvastatin and berberine in treating hyperlipidemia and carotid atherosclerosis. Tianjin Med. 2007;35:539–41.
25. Hu J, Chai Y, Wang Y, Kheir MM, Li H, Yuan Z, et al. PI3K p55gamma promoter activity enhancement is involved in the anti-apoptotic effect of berberine against cerebral ischemia-reperfusion. Eur J Pharmacol. 2012;674(2–3):132–42. doi:10.1016/j.ejphar.2011.11.014.
26. Huang CG, Chu ZL, Wei SJ, Jiang H, Jiao BH. Effect of berberine on arachidonic acid metabolism in rabbit platelets and endothelial cells. Thromb Res. 2002;106(4–5):223–7.
27. Huang CG, Chu ZL, Yang ZM. The progress in pharmacological researches on berberine. Commun Inform Pharm. 1991;9(4):10–2.
28. Huang GL. Therapeutic evaluation in the treatment of Amlodipine combined with berberine on light, moderate hypertension complicated with gout. China Pharm. 2013;22:32–3.
29. Huang W. Ventricular tachyarrhythmias treated with berberine. Zhonghua Xin Xue Guan Bing Za Zhi. 1990;18(3):155–6, 90.
30. Huang WM, Li BZ, Yian XJ. Clinical and basic research on anti-platelet aggregation effect of berberine. Chin J Hematol. 1989;10:228–31.
31. Huang WM, Wu ZD, Gan YQ. [Effects of berberine on ischemic ventricular arrhythmia]. Zhonghua Xin Xue Guan Bing Za Zhi. 1989;17(5):300–1, 19.
32. Huang Z, Han Z, Ye B, Dai Z, Shan P, Lu Z, et al. Berberine alleviates cardiac ischemia/reperfusion injury by inhibiting excessive autophagy in cardiomyocytes. Eur J Pharmacol. 2015;762:1–10. doi:10.1016/j.ejphar.2015.05.028.
33. Huang Z, Wang L, Meng S, Wang Y, Chen T, Wang C. Berberine reduces both MMP-9 and EMMPRIN expression through prevention of p38 pathway activation in PMA-induced macrophages. Int J Cardiol. 2011;146(2):153–8. doi:10.1016/j.ijcard.2009.06.023.
34. Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, et al. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. Am J Physiol Endocrinol Metab. 2009;296(4):E955–64. doi:10.1152/ajpendo.90599.2008.
35. Jia YJ, Xu RX, Sun J, Tang Y, Li JJ. Enhanced circulating PCSK9 concentration by berberine through SREBP-2 pathway in high fat diet-fed rats. J Transl Med. 2014;12:103. doi:10.1186/1479-5876-12-103.
36. Kang DG, Sohn EJ, Kwon EK, Han JH, Oh H, Lee HS. Effects of berberine on angiotensin-converting enzyme and NO/cGMP system in vessels. Vasc Pharmacol. 2002;39(6):281–6.
37. Ke Y, Wang X, Jin XY, Solaro RJ, Lei M. PAK1 is a novel cardiac protective signaling molecule. Front Med. 2014;8(4):399–403. doi:10.1007/s11684-014-0380-9.
38. Kheir MM, Wang Y, Hua L, Hu J, Li L, Lei F, et al. Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol. 2010;48(4):1105–10. doi:10.1016/j.fct.2010.01.033.
39. Kim M, Shin MS, Lee JM, Cho HS, Kim CJ, Kim YJ, et al. Inhibitory effects of isoquinoline alkaloid berberine on ischemia-induced apoptosis via activation of phosphoinositide 3-kinase/protein kinase B signaling pathway. Int Neurourol J. 2014;18(3):115–25. doi:10.5213/inj.2014.18.3.115.
40. Klose G. Introduction of PCSK9 inhibitors: new perspectives in treatment and practical implementation. Herz. 2016. doi:10.1007/s00059-016-4428-2.
41. Ko YJ, Lee JS, Park BC, Shin HM, Kim JA. 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. 2007;47(2–3):189–96. doi:10.1016/j.vph.2007.06.004.
42. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med. 2004;10(12):1344–51. doi:10.1038/nm1135.
43. Lan J, Zhao Y, Dong F, Yan Z, Zheng W, Fan J, et al. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. J Ethnopharmacol. 2015;161:69–81. doi:10.1016/j.jep.2014.09.049.
44. Lee S, Lim HJ, Park HY, Lee KS, Park JH, Jang Y. 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. 2006;186(1):29–37. doi:10.1016/j.atherosclerosis.2005.06.048.
45. Li H, Chen W, Zhou Y, Abidi P, Sharpe O, Robinson WH, et al. Identification of mRNA binding proteins that regulate the stability of LDL receptor mRNA through AU-rich elements. J Lipid Res. 2009;50(5):820–31. doi:10.1194/jlr.M800375-JLR200.
46. Li H, Dong B, Park SW, Lee HS, Chen W, Liu J. Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. J Biol Chem. 2009;284(42):28885–95. doi:10.1074/jbc.M109.052407.
47. Li HB, Cai Y, Qi CL, Lv JH. Effects and mechanism of berberine on the hypertensive renal injury rats induced by enriched high fat-salt-fructose diet. Zhong Yao Cai. 2011;34(3):412–5.
48. Li K, Yao W, Zheng X, Liao K. Berberine promotes the development of atherosclerosis and foam cell formation by inducing scavenger receptor A expression in macrophage. Cell Res. 2009;19(8):1006–17. doi:10.1038/cr.2009.76.
49. Li XMZF, Liu JX, Chen L, Zhu JH. Clinical trial of berberine combined with atorvastatin calcium on the treatment of acute macroartery atherosclerosis Stroke MES. China Pract Med. 2015;36:192–3.
50. Li Z, Geng YN, Jiang JD, Kong WJ. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. Evid Based Complement Alternat Med. 2014;2014:289264. doi:10.1155/2014/289264.

51. Liu JC, Chan P, Chen YJ, Tomlinson B, Hong SH, Cheng JT. The antihypertensive effect of the berberine derivative 6-protoberberine in spontaneously hypertensive rats. Pharmacology. 1999;59(6):283–9.
52. Liu L, Liu J, Huang Z, Yu X, Zhang X, Dou D, et al. Berberine improves endothelial function by inhibiting endoplasmic reticulum stress in the carotid arteries of spontaneously hypertensive rats. Biochem Biophys Res Commun. 2015;458(4):796–801. doi:10.1016/j.bbrc.2015.02.028.
53. Liu SJ, Yin CX, Ding MC, Wang YZ, Wang H. Berberine inhibits tumor necrosis factor-alpha-induced expression of inflammatory molecules and activation of nuclear factor-kappaB via the activation of AMPK in vascular endothelial cells. Mol Med Rep. 2015;12(4):5580–6. doi:10.3892/mmr.2015.4061.
54. Liu YC, Zhang J. The pharmacology and clinical application of the antiarrhythmic effect of berberine. Pharm J Chin Hosp. 1992;12(11):499–500.
55. Marin-Neto JA, Maciel BC, Secches AL, Gallo Junior L. Cardiovascular effects of berberine in patients with severe congestive heart failure. Clin Cardiol. 1988;11(4):253–60.
56. Mayor S. NICE recommends PCSK9 inhibitors for patients not responding to statins. BMJ. 2016;353:i2609. doi:10.1136/bmj.i2609.
57. Nykjaer A, Willnow TE. The low-density lipoprotein receptor gene family: a cellular Swiss army knife? Trends Cell Biol. 2002;12(6):273–80.
58. Pang B, Zhao LH, Zhou Q, Zhao TY, Wang H, Gu CJ, et al. Application of berberine on treating type 2 diabetes mellitus. Int J Endocrinol. 2015;2015:905749. doi:10.1155/2015/905749.
59. Pirillo A, Catapano AL. Berberine, a plant alkaloid with lipid- and glucose-lowering properties: from in vitro evidence to clinical studies. Atherosclerosis. 2015;243(2):449–61. doi:10.1016/j.atherosclerosis.2015.09.032.
60. Qin-Wei Z, Yong-Guang LI. Berberine attenuates myocardial ischemia reperfusion injury by suppressing the activation of PI3K/AKT signaling. Exp Ther Med. 2016;11(3):978–84. doi:10.3892/etm.2016.3018.
61. Riccioppo NF. Electropharmacological effects of berberine on canine cardiac Purkinje fibres and ventricular muscle and atrial muscle of the rabbit. Br J Pharmacol. 1993;108(2):534–7.
62. Shen JH, Yeh SW. The clinical effect of combination of atorvastatin and berberine on diabetes mellitus combined with cerebral stroke. J Med Theor Pract. 2016;29:1121–3.
63. Simoes Pires EN, Frozza RL, Hoppe JB, Menezes Bde M, Salbego CG. Berberine was neuroprotective against an in vitro model of brain ischemia: survival and apoptosis pathways involved. Brain Res. 2014;1557:26–33. doi:10.1016/j.brainres.2014.02.021.
64. Singer DR, Kite A. Management of hypertension in peripheral arterial disease: does the choice of drugs matter? Eur J Vasc Endovasc Surg. 2008;35(6):701–8. doi:10.1016/j.ejvs.2008.01.007.
65. Su XY, Xing JW, Ju J, Qian CF. Sixty cases of treatment of simvastatin combined with berberine in hyperlipidemia. Chin Med Mod Distance Educ China. 2012;10:41–2.
66. Sun SP, Huang ZS, Ruan WJ. Effect observation of combination therapy in the treatment of elderly hypertensive patients with gout. Forefront Med. 2013;35:394–5.
67. Tsui H, Zi M, Wang S, Chowdhury SK, Prehar S, Liang Q, et al. Smad3 couples Pak1 with the antihypertrophic pathway through the E3 ubiquitin ligase, Fbxo32. Hypertension. 2015;66(6):1176–83. doi:10.1161/HYPERTENSIONAHA.115.06068.
68. Ueda T, Shikano M, Kamiya T, Joh T, Ugawa S. The TRPV4 channel is a novel regulator of intracellular Ca^{2+} in human esophageal epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2011;301(1):G138–47. doi:10.1152/ajpgi.00511.2010.
69. Wang C, Li J, Lv X, Zhang M, Song Y, Chen L, et al. Ameliorative effect of berberine on endothelial dysfunction in diabetic rats induced by high-fat diet and streptozotocin. Eur J Pharmacol. 2009;620(1–3):131–7. doi:10.1016/j.ejphar.2009.07.027.
70. Wang CL, He SX, Wei F. A clinical investigation of berberine on treatment of 42 cases with hypertension. Med J Intern Mong. 1993;13(2):54.
71. Wang J, Guo T, Peng QS, Yue SW, Wang SX. Berberine via suppression of transient receptor potential vanilloid 4 channel improves vascular stiffness in mice. J Cell Mol Med. 2015;19(11):2607–16. doi:10.1111/jcmm.12645.
72. Wang LH, Li XL, Li Q, Fu Y, Yu HJ, Sun YQ, et al. Berberine alleviates ischemic arrhythmias via recovering depressed I(to) and I(Ca) currents in diabetic rats. Phytomedicine. 2012;19(3–4):206–10. doi:10.1016/j.phymed.2011.11.002.
73. Wang LH, Yu CH, Fu Y, Li Q, Sun YQ. Berberine elicits antiarrhythmic effects via IK1/Kir2.1 in the rat type 2 diabetic myocardial infarction model. Phytother Res. 2011;25(1):33–7. doi:10.1002/ptr.3097.
74. Wang Q, Zhang M, Liang B, Shirwany N, Zhu Y, Zou MH. Activation of AMP-activated protein kinase is required for berberine-induced reduction of atherosclerosis in mice: the role of uncoupling protein 2. PLoS One. 2011;6(9):e25436. doi:10.1371/journal.pone.0025436.
75. Wang Y, Huang Y, Lam KS, Li Y, Wong WT, Ye H, et al. Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilatation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase. Cardiovasc Res. 2009;82(3):484–92. doi:10.1093/cvr/cvp078.
76. Wang Y, Liu J, Ma A, Chen Y. Cardioprotective effect of berberine against myocardial ischemia/reperfusion injury via attenuating mitochondrial dysfunction and apoptosis. Int J Clin Exp Med. 2015;8(8):14513–9.
77. Wang YX, Yao XJ, Tan YH. Effects of berberine on delayed afterdepolarizations in ventricular muscles in vitro and in vivo. J Cardiovasc Pharmacol. 1994;23(5):716–22.
78. Wang YX, Zheng YM, Zhou XB. Inhibitory effects of berberine on ATP-sensitive K+ channels in cardiac myocytes. Eur J Pharmacol. 1996;316(2–3):307–15.
79. Wei J, Jiang JD, Wu JD, Wang SK, Wang ZZ, Pan WN, et al. Study of lipid regulating effects of berberine hydrochloride. Chin J Diabetes. 2003;13:49–51.
80. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol. 1995;15(5):551–61.
81. Wu YH, Chuang SY, Hong WC, Lai YJ, Chang GJ, Pang JH. Berberine reduces leukocyte adhesion to LPS-stimulated endothelial cells and VCAM-1 expression both in vivo and in vitro. Int J Immunopathol Pharmacol. 2012;25(3):741–50.
82. Xiao M, Men LN, Xu MG, Wang GB, Lv HT, Liu C. Berberine protects endothelial progenitor cell from damage of TNF-alpha via the PI3K/AKT/eNOS signaling pathway. Eur J Pharmacol. 2014;743:11–6. doi:10.1016/j.ejphar.2014.09.024.
83. Xu MG, Wang JM, Chen L, Wang Y, Yang Z, Tao J. Berberine-induced mobilization of circulating endothelial progenitor cells improves human small artery elasticity. J Hum Hypertens. 2008;22(6):389–93. doi:10.1038/sj.jhh.1002311.
84. Xu Z, Cao HY, Li Q. Protective effects of berberine on spontaneous ventricular fibrillation in dogs after myocardial infarction. Zhongguo Yao Li Xue Bao. 1989;10(4):320–4.
85. Yan XM, Yan JH. Clinical trial of low dose berberine for the treatment of chronic heart failure. Jiangxi Med J. 2012;47:976–7.
86. Yang SY. Discussion between Chinese herbal researches on skin infections of newborn infants and 'jaundice of the newborn baby caused by Rhizoma Coptidis'. Yi Xue Yan Jiu. 2008;37:95.

87. Yi J, Ye X, Wang D, He K, Yang Y, Liu X, et al. Safety evaluation of main alkaloids from Rhizoma Coptidis. J Ethnopharmacol. 2013;145(1):303–10. doi:10.1016/j.jep.2012.10.062.
88. Yin J, Zhang H, Ye J. Traditional chinese medicine in treatment of metabolic syndrome. Endocr Metab Immune Disord Drug Targets. 2008;8(2):99–111.
89. Yu JY, Li Z, Wang QZ. The combination therapy of small dose simvastatin and berberine in hyperlipidemia of fifty patients. Shandong Med. 2007;47:39.
90. Yu L, Li F, Zhao G, Yang Y, Jin Z, Zhai M, et al. Protective effect of berberine against myocardial ischemia reperfusion injury: role of Notch1/Hes1-PTEN/Akt signaling. Apoptosis. 2015;20(6):796–810. doi:10.1007/s10495-015-1122-4.
91. Yu L, Li Q, Yu B, Yang Y, Jin Z, Duan W, et al. Berberine attenuates myocardial ischemia/reperfusion injury by reducing oxidative stress and inflammation response: role of silent information regulator 1. Oxidative Med Cell Longev. 2016;2016:1689602. doi:10.1155/2016/1689602.
92. Zeng XH, Zeng XJ, Li YY. Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol. 2003;92(2):173–6.
93. Zhang M, Wang CM, Li J, Meng ZJ, Wei SN, Li J, et al. Berberine protects against palmitate-induced endothelial dysfunction: involvements of upregulation of AMPK and eNOS and downregulation of NOX4. Mediat Inflamm. 2013;2013:260464. doi:10.1155/2013/260464.
94. Zhang MF, Shen YQ. The antidiarrheal and antiinflammatory effects of berberine. Acta Pharmacol Sin. 1989;10(2):174–6.
95. Zhang X, Zhang X, Wang C, Li Y, Dong L, Cui L, et al. Neuroprotection of early and short-time applying berberine in the acute phase of cerebral ischemia: up-regulated pAkt, pGSK and pCREB, down-regulated NF-kappaB expression, ameliorated BBB permeability. Brain Res. 2012;1459:61–70. doi:10.1016/j.brainres.2012.03.065.
96. Zhang X, Zhao Y, Xu J, Xue Z, Zhang M, Pang X, et al. Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. Sci Rep. 2015;5:14405. doi:10.1038/srep14405.
97. Zhang X, Zhao Y, Zhang M, Pang X, Xu J, Kang C, et al. Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats. PLoS One. 2012;7(8):e42529. doi:10.1371/journal.pone.0042529.
98. Zhang XD, Ren HM, Liu L. Effects of different dose berberine on hemodynamic parameters and $[Ca^{2+}]_i$ of cardiac myocytes of diastolic heart failure rat model. Zhongguo Zhong Yao Za Zhi. 2008;33(7):818–21.
99. Zhao GL, Yu LM, Gao WL, Duan WX, Jiang B, Liu XD, et al. Berberine protects rat heart from ischemia/reperfusion injury via activating JAK2/STAT3 signaling and attenuating endoplasmic reticulum stress. Acta Pharmacol Sin. 2016;37(3):354–67. doi:10.1038/aps.2015.136.
100. Zhao HP, Hong Y, Xie JD, Xie XR, Wang J, Fan JB. Effect of berberine on left ventricular remodeling in renovascular hypertensive rats. Yao Xue Xue Bao. 2007;42(3):336–41.
101. Zhaojie M, Ming Z, Shengnan W, Xiaojia B, Hatch GM, Jingkai G. Amorphous solid dispersion of berberine with absorption enhancer demonstrates a remarkable hypoglycemic effect via improving its bioavailability. Int J Pharm. 2014;467(1–2):50–9. doi:10.1016/j.ijpharm.2014.03.017.
102. Zheng BB, Wei J, Jiang JD, Gong HH, Zhang RS. Evaluate the efficacy of statins combined with berberine for the treatment of hyperlipidemia. Acta Universitis Medicinalis Nanjing (Nat Sci). 2009;29:1493–7.
103. Zheng L, Zhou Z, Tao D, Lan T. Protective effect of berberine on cardiac myocyte injured by ischemia-reperfusion. Sichuan Da Xue Xue Bao Yi Xue Ban. 2003;34(3):452–4.
104. Zhong SL, Zhou YS, Chen HM, Yang GS. Clinical observation on 96 cases of elderly patients with hypertension of berberine treatment. Chin J Gerontol. 1997;17:349–50.
105. Zhonglu C. Effects of berberine on platelet aggregation and release. Acad J Sec Mil Med Coll. 1989;10:323–7.
106. Zhou XQ, Zeng XN, Kong H, Sun XL. Neuroprotective effects of berberine on stroke models in vitro and in vivo. Neurosci Lett. 2008;447(1):31–6. doi:10.1016/j.neulet.2008.09.064.
107. Zhou YF, Huang SJ. Clinical observation on hyperlipidemia of sixty cases of berberine treatment. Chin J Clin Rational Drug Use. 2011;4:76–7.
108. Zimetti F, Adorni MP, Ronda N, Gatti R, Bernini F, Favari E. The natural compound berberine positively affects macrophage functions involved in atherogenesis. Nutr Metab Cardiovasc Dis. 2015;25(2):195–201. doi:10.1016/j.numecd.2014.08.004.