Review

Berberine in Cardiovascular and Metabolic Diseases: From Mechanisms to Therapeutics

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Abstract

Cardiovascular and metabolic diseases (CVMD) are the leading causes of death worldwide, underscoring the urgent necessity to develop new pharmacotherapies. Berberine (BBR) is an eminent component of traditional Chinese and Ayurvedic medicine for more than 2000 years. Recently, BBR has attracted much interest for its pharmacological actions in treating and/or managing CVMD. Recent discoveries of basic, translational and clinical studies have identified many novel molecular targets of BBR (such as AMPK, SIRT1, LDLR, PCSK9, and PTP1B) and provided novel evidences supporting the promising therapeutic potential of BBR to combat CVMD. Thus, this review provides a timely overview of the pharmacological properties and therapeutic application of BBR in CVMD, and underlines recent pharmacological advances which validate BBR as a promising lead drug against CVMD.

Key words: berberine, cardiovascular diseases, metabolic diseases, targets, therapeutics

Introduction

Cardiovascular diseases (including atherosclerosis, myocardial infarction, hypertension, cardiac hypertrophy and heart failure), and metabolic diseases (including diabesity, obesity, and non-alcoholic fatty liver disease), are the leading causes of death worldwide [1-8]. These diseases are caused by the combined effects of multiple pathological factors, and their pathogenesis has not been fully clarified yet [1-8]. Although the prevention and treatment of cardiovascular diseases and metabolic diseases (CVMD) have made great progress in the past 20 years, the morbidity and mortality arising from CVMD are still very high [1-8]. Western medicine is still the mainstream therapy of CVMD [9-12]. For example, hypoglycemic agents, statins, anticoagulants, beta receptor blockers, nitrates, and anti-

thrombotic drugs are widely used in patients with CVMD [9-11, 13, 14]. Despite widespread evidences showing that these drugs are effective in a treatment of CVMD, potentially serious adverse consequences remain key challenges [9-14]. Therefore, there is an urgent need to identify alternative and complementary therapies to better manage CVMD. Berberine (BBR) is widely used in Asian countries (mainly in China) due to its good clinical and safety profile [15-17]. With the advances of pharmacological research, BBR is considered to be one of the most promising natural product-derived drug for the treatment of CVMD (Figure 1). To this end, we provide a timely and insightful overview of the therapeutic potential and molecular targets of BBR in treating CVMD.

BBR – basic characteristics and history of use

BBR is the principal bioactive ingredient of *Rhizoma coptidis* (also named 'Huang Lian' in Chinese), a common traditional Chinese medicinal herb used for the therapy of inflammatory disorders and diabetes mellitus (DM) [18, 19]. The earliest record of the use of *Rhizoma coptidis* as a medication is dated in A.D. 200 in the book of 'Shen Nong Ben Cao Jing' [18, 19]. For the first time, the anti-diabetic effect of *Rhizoma coptidis* was recorded in the book *Note of Elite Physicians* in about 1500 years ago by Hongjing Tao [20].

BBR ($C_{20}H_{18}NO_4$) is a quaternary ammonium salt derived from isoquinoline alkaloid, with a molar weight of 336.36 g/mol [21, 22]. BBR is a yellow powder that is odorless and has characteristic alkaloid bitterness [21]. It is sparingly soluble in water, slightly soluble in ethanol or methanol; however, the salt form is relatively water-soluble [21, 22]. BBR can be easily obtained from medicinal plants or through total synthesis [23, 24].

Bioavailability and metabolism of BBR

Chloride or sulfate of BBR are commonly used

for clinical purposes [15, 17]. Nevertheless, pharmacokinetic data in rodents and humans have revealed poor absorption from the gut and rapid metabolism in the body that caused its low oral bioavailability [21]. For example, BBR is converted to ionic form under physiological conditions and self-aggregates at low pH values [25-27]. Self-aggregation of BBR reduces its solubility in the gastrointestinal tract and its ability to permeate the gut wall [26, 27]. P-glycoprotein (P-gp) is located in the epithelial cell membrane and can efflux many drugs (including BBR), thereby limiting their oral bioavailability [26]. P-gp inhibitors, including D-tocopheryl polyethylene glycol 1000 succinate, are common adjuvants to increase the oral bioavailability of BBR [25]. In addition, penetration enhancers and lipid particle delivery systems can also increase the bioavailability of BBR [27]. BBR is metabolized by oxidative demethylation and glucuronidation to berberrubine, thalifendine, demethyleneberberine and jatrorrhizine and their corresponding glucuronides in the liver [21, 28] (Figure 2). CYP2D6 is the major cytochrome P450 (CYPs) for BBR metabolism, followed by CYP1A2, 3A4, 2E1 and CYP2C19. Finally, BBR metabolites are excreted through bile, feces, and urine [21, 28].

Although plasma concentration of BBR is low, the tissue concentrations of BBR and its metabolites are high [29]. BBR and its metabolites are widely distributed in the liver, kidney, muscle, lung, brain, heart, pancreas and adipose tissue [29-31]. BBR can also penetrate the blood-brain barrier [32]. Specifically, the rapid clearance of BBR from plasma as compared to the hippocampus indicates that BBR may have an important effect on hippocampal neurons [31]. Moreover, infusion of BBR (2 µg/h, 28d) by bilateral hypothalamic paraventricular nucleus (PVN) through an osmotic minipump can reduce hypertension and sympathoexcitation in two-kidney, one-clip (2K1C) renovascular hypertensive rats by ROS/ERK1/2 (extracellular-signal regulated kinase 1/2)/ inducible nitric oxide (iNOS) pathway [33].
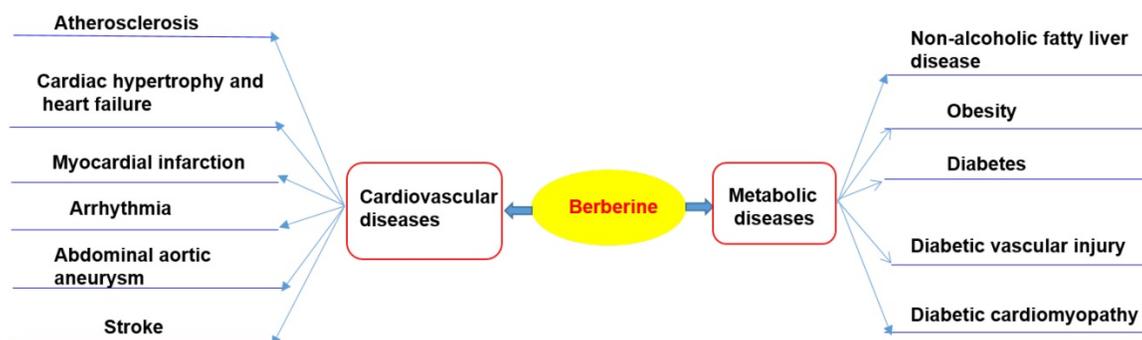
Figure 1. Therapeutic potential of BBR in cardiometabolic diseases. Current researches support that BBR may play a therapeutic role in the treatment of cardiovascular disease (including atherosclerosis, heart failure, myocardial infarction, arrhythmia, abdominal aortic aneurysm, stroke) and metabolic diseases (including nonalcoholic fatty liver, obesity, diabetes and its cardiovascular complications).
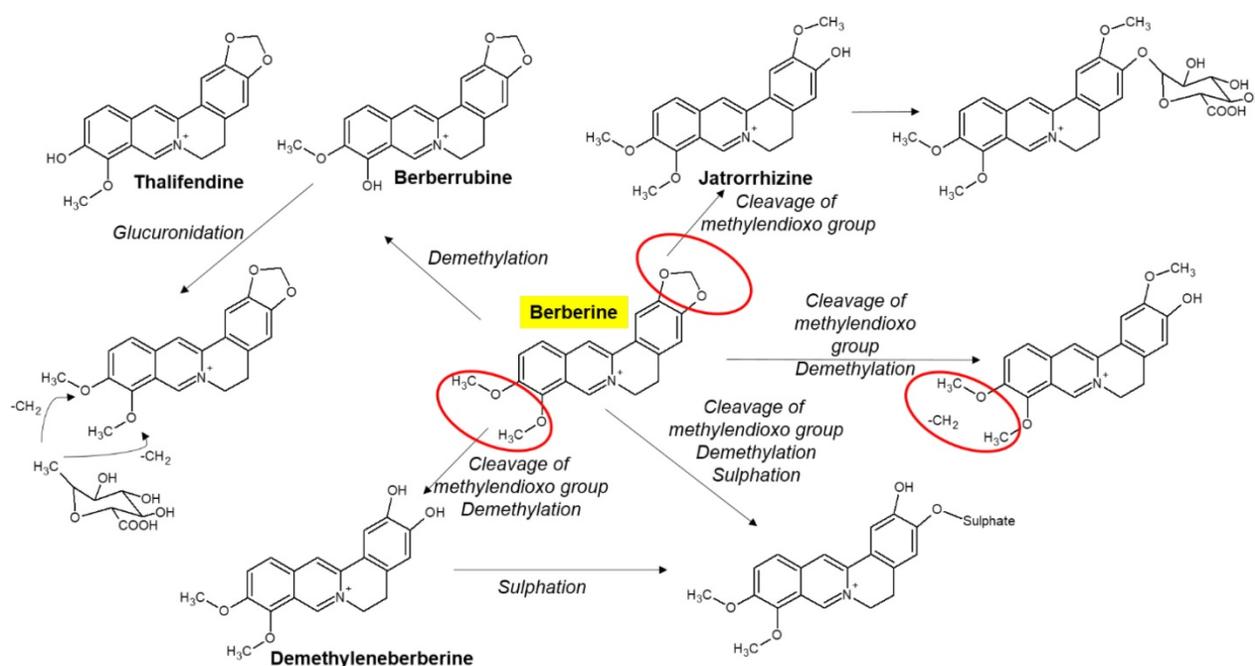
Figure 2. Selected metabolites of BBR in human. BBR is metabolized in the body by metabolic pathways (such as demethylation, glucuronidation etc) to thalifendin, berberrubine, jatrorrhizin, demethyleneberberin.

Emerging studies have shown that BBR is almost safe at conventional doses, with a relatively low incidence of adverse reactions, such as gastrointestinal discomfort, and transient increases in plasma bilirubin levels [27, 34]. Although the safety of BBR is relatively high, it should be taken carefully to avoid adverse reactions in specific cases. For example, BBR replaces bilirubin in binding to albumin (in nearly 10 times greater effect compared to phenylbutazone), so any BBR containing herbs should be avoided in jaundice in pregnant women and infants [35]. BBR interacts with macrolides and it may lead to potentially dangerous arrhythmias [36]. BBR in combination with statins increases cardiotoxicity by inhibiting CYP3A4 and human ether-a-go-go related genes (hERG) potassium channels [37]. On the other hand, BBR can prevent toxic reactions in different tissues caused by antitumor drugs such as cisplatin [38], cyclophosphamide [39], doxorubicin [40] and bleomycin [41] as well as side effects of analgesics (*e.g.* acetaminophen [42]).

BBR in the treatment of cardiovascular diseases

Atherosclerosis

Atherosclerosis is mainly a lipid metabolic disorder which underlies multiple cardio- and cerebro-vascular diseases [43-46]. Atherosclerosis commences with endothelial dysfunction, followed by neointima formation, lipid accumulation, foam cell formation, and plaque rupture [43, 44, 46-48]. BBR exerts protective effects against atherosclerosis by modulating various pro-atherogenic cellular events (Figure 3).

Mechanism of BBR in protecting against atherosclerosis *in vitro*

Normalizing endothelial function

Endothelial dysfunction and its related complications are typically specified by reduced local bioavailability of nitric oxide (NO) and excessive oxidative stress; the increased NADH/NADPH and xanthine-oxidase activity are important factors for oxidative stress in endothelial cells [49-51]. Enhanced adhesion of leukocytes to the endothelium plays an initiating role in inflammation [3, 7, 52, 53]. Studies have proven the ameliorative effects of BBR in endothelial dysfunction *via* regulating reactive oxygen species (ROS)/NO balance [49, 52, 54]. BBR treatment is capable to reduce oxidized low density lipoprotein (oxLDL)-stimulated production of ROS in human umbilical vein endothelial cells (HUVECs) [54]. BBR could inhibit oxLDL-stimulated monocyte adhesion to HUVECs *via* the mechanism associated with the suppression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [52]. BBR has been shown to reduce the tumor necrosis factor α (TNFα)-stimulated expression of the pro-inflammatory monocyte chemotactic protein-1 (MCP-1) and ICAM-1 in human amniotic epithelial cells (HAECs) and to suppress the

activation of nuclear factor kappa B (NF-κB) in HAECs involving AMP protein kinase (AMPK)-dependent pathway [55]. The exposure of HUVECs to oxLDL or TNFα significantly increased the expression of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) and the production of intracellular ROS [56-58]. Treatment with BBR reduced ERK1/2 signaling pathway, the expression of nicotinamide adenine dinucleotide phosphate-oxidase 2 (NOX2) and the expression of VCAM-1 and ICAM-1 [56]. BBR protected against palmitate-induced endothelial dysfunction *via* increasing NO level, and endothelial nitric oxide synthase (eNOS) expression and down-regulating the expression of NOX4 in HUVECs [59]. BBR also contributed to the inhibition of the vascular inflammation *via* preventing angiotensin II (Ang II)-induced adhesion of monocytes to endothelial cells and reduced ROS and MCP-1 expression in HUVECs [60]. Berberrubine, the active metabolite of BBR, has recently been shown to decrease xanthine oxidase activity and reduce TNFα-induced ICAM-1 expression in HUVECs [61].

BBR also dose-dependently inhibited the proliferation of HUVECs induced by oxLDL; its anti-proliferative and anti-inflammatory properties are exerted through decreasing the expression of NF-κB, LOX-1, proliferating cell nuclear antigen (PCNA) and inhibiting the phosphorylation of the phosphoinositide 3-kinase (PI3K)/Akt, ERK1/2, p38

mitogen activated protein kinases (MAPKs) signaling pathways [62]. In HUVECs, BBR treatment showed protective effects also against oxLDL-induced endothelial injury and apoptosis through cytochrome c-mediated caspase activation pathway [54].

Inhibiting migration and proliferation of vascular smooth muscle cells (VSMCs)

The enhancement of VSMCs proliferation and migration is an important factor for causing of neointimal hyperplasia and luminal stenosis after vascular injury [63]. Components of extracellular matrix, cytokines, shear stress, ROS, and other factors can significantly reduce the expression of markers of the differentiation of VSMCs, increase VSMCs proliferation and migration, and also a capacity for the synthesis of the extracellular matrix and promotion of neointimal formation [64]. BBR had a beneficial effect on vascular remodeling [65, 66]. BBR inhibited fetal bovine serum-induced proliferation of VSMCs by up-regulation of peroxisome proliferator-activated receptor α (PPARα) expression and NO concentration [67]. BBR inhibited the migration of human aortic VSMCs by down-regulating matrix metalloproteinase (MMP)-2 and MMP-9, as well as urokinase-type plasminogen activator (u-PA) [68]. Similarly, BBR attenuated *Chlamydia pneumoniae* infection-induced VSMCs migration by inhibiting PI3K and down-regulating the expression of MMP-3 and MMP-9 [69].
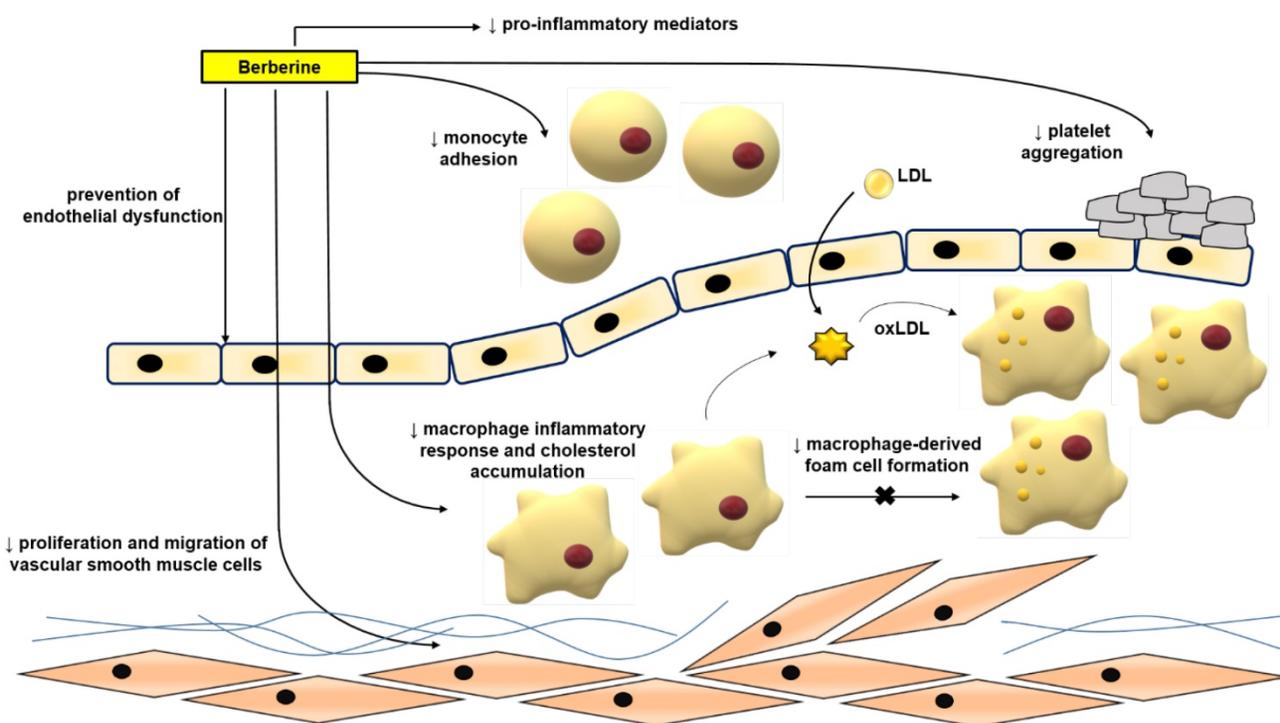
Figure 3. Anti-atherosclerotic effects of BBR. The role of BBR in inhibiting atherosclerosis includes improving endothelial dysfunction; inhibiting smooth muscle cell proliferation and migration; reducing monocyte adhesion, macrophage inflammation and cholesterol aggregation, foam cell formation, and platelet aggregation. **Abbreviations**: low density lipoprotein (LDL); oxidized LDL (oxLDL).

In addition to its effect on the extracellular matrix, BBR reduced platelet-derived growth factor (PDGF)-induced proliferation of VSMCs by activating AMPK/p53/p21 signaling, while simultaneously inactivating Ras/Rac1/Cyclin D/Cdks, and attenuated PDGF-induced migration by inhibiting Rac1 and Cdc42 [70]. BBR limited the proliferation and migration of lysophosphatidylcholine (lysoPC)-stimulated VSMCs by decreasing ROS level and subsequent activation of the ERK1/2 pathway [71]. BBR also significantly decreased the proliferation and migration of VSMCs stimulated by Ang II and heparin binding epidermal growth factor (HB-EGF) by inhibiting the activation of Akt pathway [65]. In norepinephrine (NE)-induced pulmonary arterial VSMC proliferation and migration models, BBR can reverse the effect of NE by increasing protein phosphatase 2A (PP2A) signaling, suggesting that BBR may have a therapeutic effect in pulmonary hypertension [72]. Furthermore, BBR induced the cell cycle arrest of A7r5 cells by disrupting the combination of p27, p21 and Skp2, inhibited the proliferation of A7r5 induced by PDGF-BB and enhanced the anti-proliferative effect of adrenal steroid dehydroepiandrosterone sulfate on A7r5 [73]. BBR blocked the cell cycle progression of VSMCs at G(1) phase by down-regulating the expression of cyclin D1, without affecting G(2)/M phase [74].

Inhibiting of macrophage-derived foam cell formation, inflammation, and inflammasome activation

The uptake of oxLDL into macrophages is mediated by diverse scavenger receptors (SRs), such as scavenger receptor A (SR-A)I/II, scavenger receptor class B type I (SR-BI), LOX-1, and the cluster of differentiation 36 (CD36)[75]. When oxLDL is endocytosed into macrophages, they develop into foam cells that form the core of atherosclerotic lesion [76, 77]. BBR reduced oxLDL uptake by inhibiting CD36 and LOX-1, and promoted cholesterol efflux by suppressing the expression of adipocyte enhancer-binding protein 1 (AEBP1) in macrophages [78]. BBR also inhibited the effect of oxLDL on macrophage-derived foam cell formation by inhibiting LOX-1, while up-regulating SR-BI expression [79]. Moreover, it has been reported that BBR minimized the accumulation of lipids in human macrophages exposed to hypercholesterolemic serum by reducing the process of micropinocytosis and also decreased the secretion of MCP-1 [80]. Furthermore, BBR eliminated foam cells by enhancing liver X receptor α (LXRα) and ATP-binding membrane cassette transport protein A1 (ABCA1)-stimulated cholesterol efflux [81]. The combination of atorvastatin and BBR suppressed the foam cells formation more effectively

than atorvastatin alone [82]. BBR suppressed the formation of foam cells from THP-1-derived macrophages by increasing the expression of AMPK and silent information regulator 1 (SIRT1), by decreasing the expression of PPARγ, and reducing the uptake of oxLDL [82]. In addition, BBR combined with atorvastatin diminished the levels of serum triglyceride (TG), TC, and low-density lipoprotein cholesterol (LDL-C) and inflammation and oxidative stress markers [83]. BBR combined with atorvastatin down-regulated the expression of LOX-1 through endothelin 1 receptor (ET-1R) in monocytes/macrophages [83], and in macrophages-derived foam cells, BBR-mediated sonodynamic therapy enhanced cholesterol efflux by promoting the production of ROS, and enhanced autophagy through the PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway [84]. However, it was shown that BBR induced the foam cell formation and promoted atherosclerosis by inducing the expression of SR-A in macrophages and increasing the uptake of modified low-density lipoprotein (LDL) [85]. Although the vast majority of above mentioned studies show that BBR can suppress foam cell formation and the progression of atherosclerosis, this conclusion is still controversial.

BBR also exerted anti-inflammatory effects in macrophages stimulated with oxLDL. BBR down-regulated lipopolysaccharide (LPS)-induced IL-1β, IL-6, iNOS, MCP-1, cyclooxygenase (COX)-2 and MMP-9 expression, and p-MAPKs (including p38, c-Jun N-terminal kinase (JNK), and ERK) by activating AMPK [86]. BBR reduced the production of inflammatory markers and induced autophagy in oxLDL-exposed J774A.1 cells [87]. The mechanism of effect was associated with the activation of AMPK/mTOR signaling pathway [87]. Furthermore, BBR inhibited the activation of the Nod-like receptor family pyrin domain containing-3 (NLRP3) inflammasome: BBR exposure reduced ROS-dependent activation of NLRP3 inflammasome in macrophages and inhibited the expression and release of IL-1β by inhibition of NF-κB [88]. BBR can also reduce NLRP3 inflammatory expression in PMA-stimulated macrophages by inhibiting Toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88)/NF-κB signaling pathway [89]. BBR decreased sodium uric acid crystal induced inflammasome activation by suppressing the expression of NLRP3 and IL-1β [90]. In addition, in adipose tissue-derived macrophages, BBR suppressed NLRP3 inflammasome activation and IL-1β production stimulated by saturated fatty acid (palmitate), by activating AMPK-dependent autophagy [91]. BBR also inhibited the activation of the NLRP3 inflammasome pathway, and then improved liver injury in mice by inhibiting the

purinoreceptor P2X7 [92]. TNFα and LPS stimulated the expression of miR-23a in RAW 264.7 macrophages, resulting in TLR4/TNF receptor-associated factor 2 (TRAF2)-mediated activation of apoptosis-signaling kinase 1 (ASK1) and phosphorylation of p38, which mediate inflammatory reaction [93]. BBR treatment inhibited LPS and TNFα-induced inflammatory responses by up-regulating miR-23a, ameliorating TLR4, TRAF2, TNFα, IL-6 and IL-23 gene expression, and inhibiting TLR4/TRAF2-mediated ASK1/p38 phosphorylation [93]. After percutaneous coronary intervention, macrophage activation plays an essential role in neovascular atherosclerosis and in-stent restenosis [94]. Galectin-3, which was mainly expressed on macrophages, is an important mediator of inflammation. BBR down-regulated oxLDL-induced macrophage activation by down-regulating Galectin-3 *via* NF-κB and AMPK signaling pathways [94].

Inhibiting platelet activation

BBR has been shown to effectively prevent thrombus formation in arteries [95], and assisted thrombolysis induced by the plasminogen activators urokinase and streptokinase [96]. The anti-platelet aggregation effects of BBR have been demonstrated in platelets isolated from healthy subjects, patients with platelet hyperaggregability, and also patients with atherosclerotic cerebral infarction [95]. Chu *et al*. well reviewed the effect of BBR on platelets in 1996 [97]. BBR *in vitro* could inhibit platelet aggregation stimulated by adenosine diphosphate (ADP), collagen, adrenaline, arachidonic acid (AA), and calcium ionophore A23187 [97]. It also had inhibitory effect on clot retraction induced by ADP [98]. BBR inhibited platelet activation by modulating the effect on cAMP level in platelets, antagonistic activity against Ca^{2+}, partial and competitive binding with α_2-adreno-receptors on platelet membrane, affecting AA metabolism and ADP release from platelets [97].

BBR chloride decreased plasmatic levels of thromboxane B2 and P-selectin in mice with dextran sulphate sodium-stimulated colitis [99]. BBR chloride also inhibited platelet activation *via* down-regulation of P-selectin expression and inhibiting fibrinogen binding to platelet GP IIb/IIIa integrin receptor on the surface of platelets [100]. Shah *et al*. [101] reported that BBR inhibited collagen-induced platelet aggregation without affecting the platelet responses to the other platelet agonists including platelet-activating factor (PAF), AA and adrenaline. Its anti-platelet activity has been suggested to be exerted *via* suppressing with the collagen-induced adhesion process without any effect on cAMP, or A23187-induced platelet aggregation [101]. While in one animal study investigating the effect of BBR on GPVI (glycoprotein receptor for collagen, expressed in platelets) BBR could not statistically inhibit the expression of GPVI [102], another study has proven the protective effects of BBR in combination with ligustrazine (alkylpyrazine from *Ligusticum chuanxiong*) in a rat model of coronary microembolization [103]. In this study, ADP-induced platelet activation, von Willebrand factor, ET-1, and levels of TNFα, IL-1β, and ICAM-1 in serum and heart tissues were decreased by BBR/ligustrazine combination [103]. Tissue factor (TF) is closely related to coagulation; its expression by vascular cells surrounding blood vessels is stimulated by pro-inflammatory substances such as LPS or TNFα [104]. In this regard, BBR attenuated LPS-stimulated TF protein expression by suppressing NF-κB, Akt and MAPK signaling pathways in THP-1 cells [105]. However, another study revealed that BBR could increase TNFα-stimulated TF expression in HAECs, and could decrease the expression of TF pathway inhibitor in HAECs and in arteries of apolipoprotein E-deficient mice (ApoE$^{-/-}$) mice, what might promote thrombosis [104]. BBR therefore might act bidirectionally on TF expression in different cell types [105].

Anti-atherosclerotic effects of BBR *in vivo*

Diverse investigations have reported the therapeutic potential of BBR to inhibit atherosclerosis in mouse models of atherosclerosis. For example, BBR treatment (150 mg/kg/d, *p.o.*, 12wk) obviously reduced the atherosclerotic plaque area and displayed suppression of inflammatory and oxidative markers [106]. Specifically, BBR diminished the serum levels of IL-1β and TNFα, and decreased the expression of ICAM-1, iNOS, and IL-6 in the aorta. Furthermore, the treatment with BBR reduced the translocation of NF-κB to the nucleus [106]. In homocysteine thiolactone (HTL)-fed ApoE$^{-/-}$ mice, BBR (1.0 g/kg/d, *p.o.*, 8wk) increased atherosclerotic plaque stability in the carotid artery and normalized the redox state. These protective effects on vascular function might be ascribed to an activation of PPARγ, which leads to a down-regulation of oxidative stress [107]. BBR also abrogated the HTL–induced oxidative stress in HUVECs by activating PPARγ [107].

Visfatin is a pro-inflammatory adipokine, which is expressed in visceral fat and induces endothelial dysfunction by promoting the production of inflammatory and adhesion molecules [108]. Oral administration of BBR (5 mg/kg/d, *p.o.*, 12wk) in ApoE$^{-/-}$ mice lowered the serum expression of visfatin and inflammatory cytokines (TNFα and IL-6), as well as it reduced the distribution of visfatin in the atherosclerotic plaques [108]. BBR pretreatment also reversed the visfatin-induced increase of IL-6 and

TNFα in HUVECs [108]. The amelioration of visfatin-induced endothelial dysfunction was associated with the inhibition of MAPK signaling pathway (p38 and JNK) [108]. Moreover, BBR suppressed the increase of p-p38 MAPK, p-JNK and Bcl2- associated X protein (Bax) in visfatin-induced endothelial dysfunction in HUVECs [108]. Furthermore, it was suggested that the protective effects of BBR (in drinking water (0.5 g/L), 14wk) in ApoE$^{-/-}$ mice can be related to the modulation of gut microbiota, specifically an up-regulation in *Akkermansia* spp. abundance which has been known to regulate inflammation, metabolic endotoxemia, and gut barrier integrity [109]. A recent study showed that BBR (50 mg/kg, *p.o.*, twice weekly, 12wk) inhibition of HFD-induced atherosclerosis in ApoE$^{-/-}$ mice is associated with changes in gut microbiota composition, such as BBR treatment altering an occurrence of *Firmicutes* and *Verrucomicrobia* [110].

Increased circulating endothelial microparticles in the peripheral circulation show the state of endothelial damage and endothelial cell activation, apoptosis and injury [111]. BBR treatment (1.2 g/d, *p.o.*) in healthy humans led to a decrease in serum malondialdehyde and circulating CD31$^+$/CD4$^-$ microparticles (as markers of endothelial injury) and the improvement of flow-mediated vasodilation. BBR contributed to an improvement of endothelial function *via* up-regulating eNOS expression and down-regulating NOX4 expression, and ROS production in HUVECs [112-114].

The MyD88 dependent TLR4 signaling pathway plays a critical role in hypertension-induce endothelial damage. TLR4 activation could activate NF-κB, leading to endothelial cell injury *via* an increased production of inflammatory enzymes and mediators (including COX-2, IL-1, and IL-6) [115]. An increased level of TNFα initiates apoptosis and endothelial cell turnover, resulting in the formation of atherosclerotic plaques [115]. BBR treatment inhibited the apoptosis, decreased the expression of NF-κB as well as the levels of TNFα and IL-6 in aortic endothelial cells isolated from spontaneously hypertensive rats (SHR) by decreasing the expression of TLR4 and MyD88 [116]. Endothelium-dependent contractions (EDCs) are also involved in endothelial dysfunction, and EDCs are caused by contracting factors such as endothelial COX-produced prostanoids or augmented level of ROS [117]. EDCs of the carotid arteries of SHR were reduced by BBR administration [118]. This effect has been reported to be caused by triggering of activated AMPK, inhibition of endoplasmic reticulum (ER) stress, reduction of ROS and expression of COX-2 [118]. BBR (5, 10 mg/kg, *i.p.*) has also been reported to inhibit NF-κB activity and decrease VCAM-1 production in lung of LPS-stimulated rats [119].

Cardiac hypertrophy and heart failure

Cardiac remodeling is associated with progressive cardiac hypertrophy, fibrosis, and the eventual occurrence of heart failure (HF) [120-123]. Interventions in cardiac remodeling are important strategies for the treatment of HF [124]. The effect of BBR on HF is shown in Figure 4.

In animal models, BBR can reduce the degree of HF. For example, in ischemic left ventricular heart failure dogs, intravenous injection of BBR (1 mg/kg, within 3 min) and then repeated infusion (0.2 mg/kg/min, 30 min) for 10 consecutive days, increased the cardiac output and peak rate of rise of left ventricular pressure (+dp/dt) while left ventricular end-diastolic pressure (LVEDP), diastolic blood pressure (DBP), and systemic vascular resistance were decreased[125]. The total saponins of *Panax ginseng* (20 mg/kg/d) combined with BBR (20 mg/kg/d), and captopril were administered for 12 consecutive days to rats in a model of HF induced by injecting of high dose of isoproterenol (*s.c.*), and a similar anti-HF effect was observed in both groups [126]. BBR (63 mg/kg/d, *p.o.*, 4wk) further proved to be a potential drug for improving HF symptoms by inhibiting the Ca^{2+} overload in cardiomyocytes [127].

Pathological cardiac hypertrophy is a critical intermediate step toward HF and other cardiac diseases [128]. BBR (10 mg/kg/d, *p.o.*, 8wk) prevented the development of left ventricular hypertrophy in rats caused by pressure overload; +dp/dt increased, while the weight of the whole heart and left ventricle decreased, and the increase of left ventricular myocardial cell size was significantly reduced [129, 130]. Mechanistic studies have shown that BBR can up-regulate F-Box protein 32 (FBXO32), an E3 ubiquitin ligase, and down-regulated mTOR, ERK1/2, as well as p38 phosphorylation, which enhances autophagy and thus inhibits cardiac hypertrophy [128, 131]. Cardiac fibrosis is an important pathological change in the development of HF, arrhythmia and cardiac arrest [132]. BBR can restrict cardiac fibrosis by up-regulating the expression of relaxin *in vitro* [133]. BBR administration (5, 10 mg/kg/d, *p.o.*, 4wk) also obviously reduced cardiac fibrosis in two-kidney, two-clip (2K2C) rats *via* increased levels of NO and cAMP in left ventricular tissue[134].

Abdominal aortic aneurysm

Abdominal aortic aneurysm is characterized by dilatation of the abdominal aorta with an etiology not completely understood [135]. The presence of aneurysm leads to the weakening of the aortic wall, followed by a progressive dilatation and potential risk

of aortic rupture [136]. To date, only very limited studies investigating the effects of BBR against abdominal aortic aneurysm were published. One of the potential causes of formation of aneurysms is the increase in the ratio of collagen to elastin [137]. It has been evidenced that BBR significantly reduced collagen synthesis in cardiac fibroblasts subjected to Ang II [138]. The activation of MMPs is also a mechanism that participates in the etiology of aneurysms since it weakens the aortic media [139]. In this sense, it was reported that BBR obviously decreased the activity and expression of MMP-9 as well as the expression of extracellular MMP inducer (EMMPRIN) in stimulated macrophages [140]. In addition, BBR treatment ameliorated vascular remodeling in a rat model of metabolic syndrome by reducing the aortic levels of MMP-2 [141].

It is well established that the formation of abdominal aortic aneurysm is related to abnormal wall stiffness [142]. A clinical study investigated the effects of the two months consumption of 200 mg red yeast rice (RYR), 500 mg BBR and 10 mg policosanols in 70 hypercholesterolemic patients [143]. The intervention significantly improved aortic stiffness by reducing lipid levels and increasing aortic pulse wave velocity [143]. Furthermore, in aged mice, it has been evidenced that BBR induced the relaxation of VSMCs with decreasing of blood pressure and also reduced vascular stiffness *via* suppression of the activity of Ca^{2+} channel transient receptor potential vanilloid 4 (TRPV4) [144]. The effect of BBR on abdominal aortic aneurysm was summarized in Figure 4.

Ischemia-reperfusion injury

Ischemic heart disease (ISHD) is primarily caused by coronary atherosclerosis and its complications [145, 146]. Myocardial infarction is the most severe ISHD with high mortality [145]. Reperfusion is beneficial for ischemia, but it can also cause severe cardiac damage [145]. Thus, studying the underlying mechanisms of myocardial ischemia-reperfusion (I/R), contributes to the treatment of I/R injury in myocardial tissue [145, 146]. The effect of BBR on myocardial ischemia was shown in Figure 4.

Experiments on isolated cardiomyocytes in hypoxia-reoxygenation model showed that pretreatment with BBR resulted in reduction in lactate dehydrogenase (LDH) and malondialdehyde (MDA) release, which are biomarkers of the extent of I/R injury [147]. BBR inhibited I/R injury by the following mechanisms: i) direct and/or indirect antioxidant activity [148]; ii) post-ischemic anti-inflammatory activity [149]; iii) vasodilation of coronary arteries [150]; iv) anti-apoptotic activity on cardiomyocytes [151]; v) suppressing an activation of autophagy

during I/R[152]; vi) promoting angiogenesis in heart after I/R injury[153].

Antioxidant activity of BBR

BBR showed direct antioxidant activity in several cell-free systems, such as DPPH radical scavenging assay [154, 155]. Moreover, antioxidant activity was observed in cultured cells subject to oxidative stress [148, 156]. BBR inhibited oxidative stress by increasing super oxide dismutase (SOD), uncoupling protein 2 (UCP2) and decreasing NOX expression, *via* SIRT1/FoxO (forkhead box O) or AMPK signaling pathways [157].

Anti-inflammatory effects of BBR

BBR is anti-inflammatory active by decreasing the secretion of an array of pro-inflammatory cytokines/mediators (IL-6, IL-1β, and TNFα) in myocardial tissue and serum by suppressing PI3K/Akt signaling pathway [157-159]. BBR in cardiomyocytes in the mouse model of I/R injury inhibited inflammatory responses through the suppression of NF-κB signaling pathway [149]. Another study showed that BBR can also activate SIRT1 signaling, and thus further reduce myocardial inflammatory response [160].

Hypotensive effects of BBR

BBR has hypotensive effects by functioning as a vasodilator in isolated blood vessels [161, 162]. Vasodilation of coronary arteries and thus coronary flow is of key importance during the I/R injury of the heart [163]. In this context, BBR enhanced coronary blood flow in isolated guinea pig hearts with ventricular fibrillation [150]. Moreover, BBR can stimulate cardiac contractility (positive inotropic activity) by increasing intracellular calcium levels in addition to lowering peripheral vascular resistance and blood pressure [127]. Several mechanisms are proposed for vasodilation and/or hypotensive effect of BBR: i) the antagonism on α1-adrenoreceptors on VSMCs [164, 165]; ii) the enhancement of the hypotensive effect of acetylcholine (ACh) on *nervus vagus*, and thus the inhibition of carotid sinus pressor reflex [166]; iii) the endothelium-dependent release of NO [167, 168]; iv) *via* angiotensin-converting enzyme (ACE) inhibition of the NO-cGMP axis [169]; v) the direct activation of K^+ channels in arterial VSMCs leading to hyperpolarization, thereby the inhibition of calcium influx leading to smooth muscle relaxation [168, 170]; vi) the inhibition of L- and T-type voltage-gated Ca^{2+} currents in ventricular myocytes [171].

Anti-apoptotic effects of BBR

One of the main mechanisms of BBR in

protecting against I/R injury is the inhibition of apoptosis of cardiomyocytes. For example, in a mouse model of myocardial I/R, BBR inhibited the activation of caspase-3, caspase-9 and apoptotic protease-activating factor 1 (Apaf-1) in cardiomyocytes, while increased the expression of Bcl-2-like protein 1 and p53 [149]. In the rat hearts subjected to I/R injury, BBR treatment decreased AMPK and Bcl-2-like protein 1 in the myocardial risk areas, while increased AMPK in the non-ischemia areas compared to the control hearts [172]. In neonatal rat cardiomyocytes, BBR decreased hypoxia-reoxygenation-stimulated cardiomyocytes apoptosis, increased Bcl-2/Bax ratio, activated PI3K-Akt, AMPK and eNOS phosphorylation, while decreased caspase-3 expression [173, 174]. Recent studies on hypoxia-reoxygenation injury on H9C2 rat cardiac myoblasts proposed that BBR prevents apoptosis by modulating Notch1/Hes1-PTEN (phosphatase and tensin homolog)/Akt signaling pathway [175], as well as Smad7 (Mothers against decapentaplegic homolog 7) activation [151]. BBR-mediated activation of Smad7 pathway resulted in the suppression of caspase-3 expression and activity. Indeed, the Smad7 knockdown confirmed this hypothesis [151]. Importantly, BBR also activated the Janus kinase 2 (JAK2)/ signal transducer and activator of transcription 3 (STAT3) pathway, and thus decreased the endoplasmic reticulum (ER) stress [176].

Effect of BBR on autophagy and angiogenesis

Inhibition of activation of autophagy is an additional mechanism underlying the protective effects of BBR [152]. Incubation of the cells with BBR or treatment with BBR increased survival of cells, decreased the infarct zone in mice hearts, and suppressed autophagy, which was confirmed by the decreased expression levels of autophagy-related proteins such as SIRT1, BNIP3, and Beclin-1 [152]. BBR can also be useful after I/R injury, since BBR can promote angiogenesis of the small vascular beds in coronary arteries [153]. Post-ischemic application of BBR for 14 days reduced the infract zone, improved the heart function, as well as up-regulated miR-29b expression, and enhanced cell proliferation and migration in endothelial cells from left anterior descending (LAD) coronary artery [153].

Importantly, similar protective activities of BBR against I/R injury were observed also in non-cardiac tissues [177]. In neuronal cells during ischemia, BBR reduced p53 and cyclin D1, enhanced phosphorylation of Bad and decreased cleavage of caspase-3, thereby inducing cell cycle arrest and inhibiting apoptosis [177]. During the reperfusion phase, BBR protected from cerebral ischemia injury *via* activating the PI3K/Akt signaling pathway [177]. A pretreatment with BBR significantly preserved cells in response to a strong oxidant (CoCl$_2$) [178]. BBR injections were given prior to I/R surgery in rats and led to increased survival of neurons due to anti-apoptotic effects mediated by PI3K/Akt signaling pathway [179]. Moreover, in a mouse model of cerebral ischemia, BBR decreased the neuronal apoptosis *via* activating PI3K/Akt signaling pathway [180].

Protective activity was not only BBR-specific but it can be related to the other members of structural class of isoquinoline alkaloids, as both coptisine [181] and palmatine [182] showed similar protective effects against I/R injury on hearts of rats.
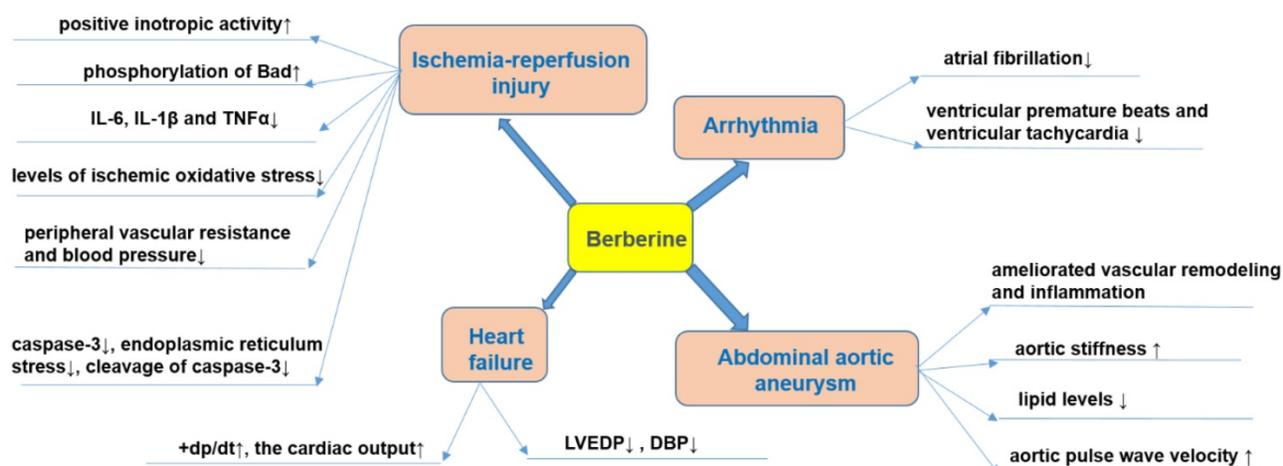
Figure 4. Effects of BBR on heart failure, arrhythmia, myocardial ischemia and abdominal aortic aneurysm. Specifically, BBR prevents ischemia/reperfusion injury via its positive inotropic activity, increased phosphorylation of Bad, decreased production of pro-inflammatory mediators (IL-6, IL-1β, and TNFα), reducing oxidative stress, blood-pressure lowering, anti-apoptotic effects and protective effects against endoplasmic reticulum stress. BBR prevents heart failure by increasing cardiac output, and decreasing LVEDP and DBP. BBR prevents arrhythmia by reducing ventricular premature beats and tachycardia. BBR prevents abdominal aortic aneurysm by reducing vascular remodeling and pressure, reducing aortic stiffness, modulating lipid level, and increase aortic pulse wave velocity. ↑indicates increase or activation, and ↓indicates decrease or suppression. **Abbreviations**: Bad, Bcl-2-associated death promoter; diastolic blood pressure (DBP), interleukin-1β (IL-1β), interleukin-6 (IL-6), left ventricular end-diastolic pressure (LVEDP) , peak rate of rise of left ventricular pressure (+dp/dt), tumor necrosis factor α (TNFα).

Furthermore, administration of BBR (100 mg/kg/d, *p.o.,* 14d) in rats prior to I/R injury (a surgical occlusion of LAD coronary artery) resulted in a reduced size of infarction and protection from arrhythmias [172]. BBR prevented the occurrence and duration of isolated premature beats, ventricular fibrillation and ventricular tachycardia [172]. In another model of acute myocardial infarction (induction by isoproterenol injection), BBR (30, 60 mg/kg/d, *p.o.,* 14d) decreased the ST elevation stimulated by acute myocardial ischemia, and also down-regulated serum levels of ischemic biomarkers muscle/brain (MB) isoenzyme of creatine kinase (CK-MB), IL-6, TNFα and LDH [183]. Moreover, *ex vivo* experiments evaluating I/R on isolated hearts showed that hearts pre-treated with BBR before ischemia had improved preservation of left ventricular developed pressure and LVEDP when compared to the non-treated group [172]. Importantly, BBR had no influence on these parameters *per se* (on hearts without I/R injury) [172]. In addition, a solid dispersion of BBR and sodium citrate (HGSD) improved BBR bioavailability and membrane permeability, and HGSD treatment (12.5, 25, 50 mg/kg/d, *p.o.,* 7d) protected the rat heart from I/R injury by attenuating NF-κB and JNK signaling pathways [184].

Stroke

Similar to myocardial infarction, stroke also results from vascular or microvascular diseases which cause an interruption of cerebral blood supply and consequently brain dysfunction [185]. To date, the reperfusion is the merely approved treatment for acute ischemic stroke [186]. Consistently, thrombolytic/anti-platelet agents and surgery are the only available options [187]. However, due to the fact that multiple mechanisms are involved in stroke progression, the therapeutic effect of current regimens is limited and agents that possess multiple pharmacologic actions have attracted much attention. Inflammation, apoptosis, and oxidation are three major mechanisms for the pathogenic proceeding of stroke [188].

In addition to the routine treatment, the administration of BBR 300 mg (*t.i.d., p.o.*) significantly reduced serum levels of macrophage migration inhibitory factor (MIF) and IL-6 in patients with acute cerebral ischemic stroke and to a certain extent decreased the carotid atherosclerosis and neurological deficit [189].

The right carotid artery of rats was ligated (ischemic injury) and BBR solution (0.2–2 mg/kg) was intraperitoneally injected. After 30 min, the rats were subjected to hypoxic conditions by breathing in air with 10% oxygen and 90% nitrogen (a model of hypoxic damage). BBR dose-dependently reduced

cerebral ischemia-hypoxic injury [190]. BBR (0.2, 0.5, 1 or 2 mg/kg, *i.p.* or 0.2, 0.02, 0.002 mg/kg, *i.v.*) inhibited ischemia-induced apoptosis *via* enhancing the PI3K/Akt signaling pathway and exerted its neuroprotective effects *in vivo* and *in vitro* [179, 191]. Additional mechanisms of BBR in preventing ischemic brain damage include reducing intracellular ROS levels and subsequently inhibiting of the mitochondrial apoptotic pathway [192]. Moreover, BBR (10, 40 mg/kg) can reduce the ischemic brain injury in rats by promoting the activation of Akt/GSK3β (glycogen synthase kinase 3β) signaling pathway and claudin-5, while down-regulating the NF-κB expression [32]. A similar study showed that BBR (25, 50 mg/kg/d, *p.o.,* 14d) pretreatment dose-dependently reduced infarct size, neurological deficits, and cerebral edema in model of cortical tissue ischemia performed by transient middle cerebral artery occlusion [193]. Its mechanism may depend on its anti-inflammatory effects, including an inhibition of nuclear translocation of high-mobility group box1 (HMGB1) and NF-κB, as well as TLR4 expression [193]. In addition, BBR can promote angiogenesis in rats suffering from cerebral ischemia-reperfusion injury, which may be related to increased expression of hypoxia-inducible factor 1α (HIF-1α) and its downstream gene-vascular endothelial growth factor (VEGF) [194].

BBR can protect neurons during focal cerebral ischemia by up-regulating anti-inflammatory cytokines and down-regulating pro-inflammatory cytokines [195]. The combination of BBR and other drugs has been tested to improve cerebral ischemia. For example, rats were reperfused after bilateral common carotid occlusion to induce a transient global cerebral ischemia model. Co-administration of BBR (5, 10, 20 mg/kg/d, 19d) and verapamil (2mg/kg/d, 19d) enhanced brain uptake of BBR and improved brain functions in rats [196]. *Berberis koreana* extract (containing 7.39±0.78 mg/g of BBR) significantly ameliorated nerve injury induced by ischemic stroke in gerbils, by inhibiting COX-2 expression and PGE$_2$ production [197]. The main components of Huang-Lian-Jie-Du-Decoction (HLJDD) are BBR, baicalin and jasminoidin, which are commonly used in traditional Chinese medicine to treat ischemic stroke [198]. HLJDD administration ameliorated ischemic stroke in rats by improving abnormal metabolism and regulating oxidative stress, neuronal autophagy, and inflammatory responses [198]. A study evaluating the Yi-qi-jie-du formula (YJ) with the main constituents ginsenosides (G), BBR and jasminoidin (J) (in a ratio 3 (G): 2 (BBR): 0.5 (J)) showed the improvement of state in the focal cerebral ischemia in rats [199].

Arrhythmia

The anti-arrhythmic effect of BBR was firstly reported by Huang *et al.* in 1989 [200]. The authors induced ischemic ventricular arrhythmias in canines through occluding the LAD coronary artery and showed that BBR can prevent total ventricular premature beats and ventricular tachycardia [200]. Recently, BBR (2 mg/kg, *i.v.*) hindered ACh-induced atrial fibrillation in rabbits *via* extension of action potential (AP) and effective refractory period in atrial myocytes [201]. In addition, BBR (at a concentration of 300 mmol/L) prevented stretch-induced arrhythmia in isolated myocardial infracted hearts of Wistar rats [202].

Early studies proposed that BBR has class III anti-arrhythmic effects (by blocking K$^+$ channel) [203]. However, further studies revealed that BBR targets several types of channels such as the cardiac slow (I_{Ks}) and rapid (I_{Kr}) delayed rectifier K$^+$ channels, ATP-sensitive K$^+$ channel (K$_{ATP}$), inwardly-rectifying K$^+$ channel (I_{Kl}), L-type Ca^{2+} (I_{Ca}) [166] and human hyperpolarization-activated cyclic nucleotide-gated 4 (hHCN4) channels [204]. BBR (10, 20 mg/kg, *p.o.*) suppressed expression of I_{Kr} channel in rat ventricular tissues [205]. Moreover, BBR inhibited the expression of hERG channel in hERG-transfected HEK293 cells [205]. HERG channel encoded by hERG is an important subunit of I_{kr} channel [206]. It crucially contributes to cardiac repolarization and its defect leads to long QT syndrome with manifestations such as delayed repolarization and prolonged QT interval [206]. BBR (27.1 mg/kg, *p.o.*) induced extension of the AP duration and the QT interval in guinea pigs [207]. BBR caused mature hERG reduction through inducing defect in channel trafficking, which led to an immature hERG response (unfolded protein response), and defective hERG underwent degradation in lysosomes and proteasomes [207]. Fexofenadine and resveratrol, as two drugs preventing hERG trafficking defect, can reverse BBR-induced prolongation of AP duration [208]. BBR can also block hERG channel directly *via* interacting with residues V625, Y652, and F656, respectively, in the central cavity of the channel [209]. The effect of BBR on arrhythmia was summarized in Figure 4.

BBR in treating metabolic diseases

BBR in diabetes mellitus and its cardiovascular complications

Diabetes mellitus (DM) is linked to chronic hyperglycemia and abnormal metabolism of carbohydrates, lipids, and lipoproteins, which are caused by an inadequate production of insulin and/or insulin action [210, 211]. DM is increasingly being recognized as a significant cause of mortality and morbidity, and profoundly contributes to the global health burden and death toll [210, 211]. Based on the latest disease statistical report (The International Diabetes Federation, https://www.idf.org/): approximately 425 million people were diabetics in 2017 and this number is anticipated to increase to 629 million by 2045. There are two categories of DM: Type 1 diabetes (T1DM) and Type 2 diabetes (T2DM) [212]. T1DM is characterized by the absolute deficiency of insulin secretion, while T2DM, the most common one, has features of insulin resistance (IR) and an insufficient compensatory insulin secretory response [212]. Therapeutic effects of BBR on diabetes mellitus and its cardiovascular complications were summarized in Figure 5.

Effects of BBR on T1DM and T2DM

Physiologically, blood glucose level is regulated by the liver (gluconeogenesis and glycogenolysis) and glucose utilization by the peripheral tissues. An increase in the hepatic glucose production due to an inadequate insulin secretion/action is the major cause of hyperglycemia in DM patients [210, 211]. DM can lead to many complications such as hyperlipidemia, hypertension, atherosclerosis, hyperinsulinemia, retinopathy, nephropathy and peripheral neuropathy [213, 214]. Approved oral hyperglycemic drugs such as derivatives of sulfonylurea, metformin, and thiazolidinediones are reported to induce some side effects [9-11]. In addition to the adverse effects, the conventional medicines are expensive and not always satisfactory in maintaining normal level of blood glucose [215]. Many herbal medicines with potential of anti-diabetic effect have been widely used for treating DM in various traditional systems of medicine worldwide since the immemorial time [216]. Nowadays, the market for natural anti-diabetic drugs is booming as they are preferred for their effectiveness, lower occurrence of side effects and relative low costs [216].

Recently, a plethora of investigations has shown that BBR is a promising hypoglycemic, and anti-hyperlipidemic compound by modulating various signaling pathways [217-221]. It diminished body mass in DM patients [217-219]. The exact mechanism of action underlying the hypoglycemic effect of BBR is not fully elucidated. *In vitro* and *in vivo* studies have highlighted that BBR contributed to its therapeutic activities *via* variety of mechanisms and signaling pathways, including improving IR, activating AMPK, modulating gut microbiota, reducing gluconeogenesis in liver and enhancing glycolysis in peripheral tissues [217, 222-224]. Although many studies have shown that BBR is an AMPK activator [30, 225, 226], in

HepG2 and C2C12 cells BBR promoted glucose metabolism by stimulating glycolysis, and this effect may be independent of AMPK activity [227]. A recent study has shown that BBR (150 mg/kg/d, *p.o.*, 7d) promoted glucose uptake and restrict gluconeogenesis in rats by inhibiting SIRT3 and promoting ubiquitination of phosphoenol pyruvate carboxykinase 1 (PEPCK1) [228]. The entry of pyruvate into the mitochondria *via* the mitochondrial pyruvate carrier (MPC) is a central step in hepatic gluconeogenesis [229]. BBR limited the gluconeogenesis of mitochondrial pyruvate by inhibiting the deacetylation of MPC1 by SIRT3 [229]. This can be a therapeutic strategy to prevent excessive hepatic glucose production [229]. The hypoglycemic effect of BBR was also partially mediated by an anti-inflammatory and antioxidative mechanism [157, 230, 231]. It is challenging to justify the clinical efficacy of BBR due to its low oral bioavailability, although it is clinically active, and it exerted therapeutic effects through different mechanisms [232, 233].

Effects of BBR on insulin resistance (IR)

BBR has been shown to ameliorate IR, which is the main metabolic abnormality culminating not only to T2DM, but also to metabolic syndrome [234]. It can be defined as a state in which normal or increased insulin level produces an attenuated biological response [235]. Several researchers have reported that BBR is effective in mitigating the IR through different pathways. Kong *et al.* [236] have investigated the molecular mechanism of BBR against IR and BBR was found to reduce the fasting blood glucose (FBG) and fasting serum insulin (FSI), increase insulin receptor (InsR) mRNA and insulin sensitivity as well as protein kinase C (PKC) activity both *in vitro* and in T2DM rats. Mahmoud *et al.* [237] have demonstrated that treating rats, with IR syndrome, with 50 mg/kg/day of BBR for 2 weeks was effective against IR syndrome by improving IR, lipid profile, antioxidant enzymes, pro-inflammatory cytokines, and IFN-γ. Liu *et al.* [238, 239] have also revealed the effectiveness of BBR against IR. Increased branched-chain amino acids (BCAAs) are involved in obesity and IR as Yue *et al.* [240] have reported that BBR (200 mg/kg/d, *p.o.*, 10wk) can improve IR in HFD-fed mice and diabetic patients by altering the intestinal microbiota of BCAAs biosynthesis and BCAAs catabolism in liver and adipose tissue.
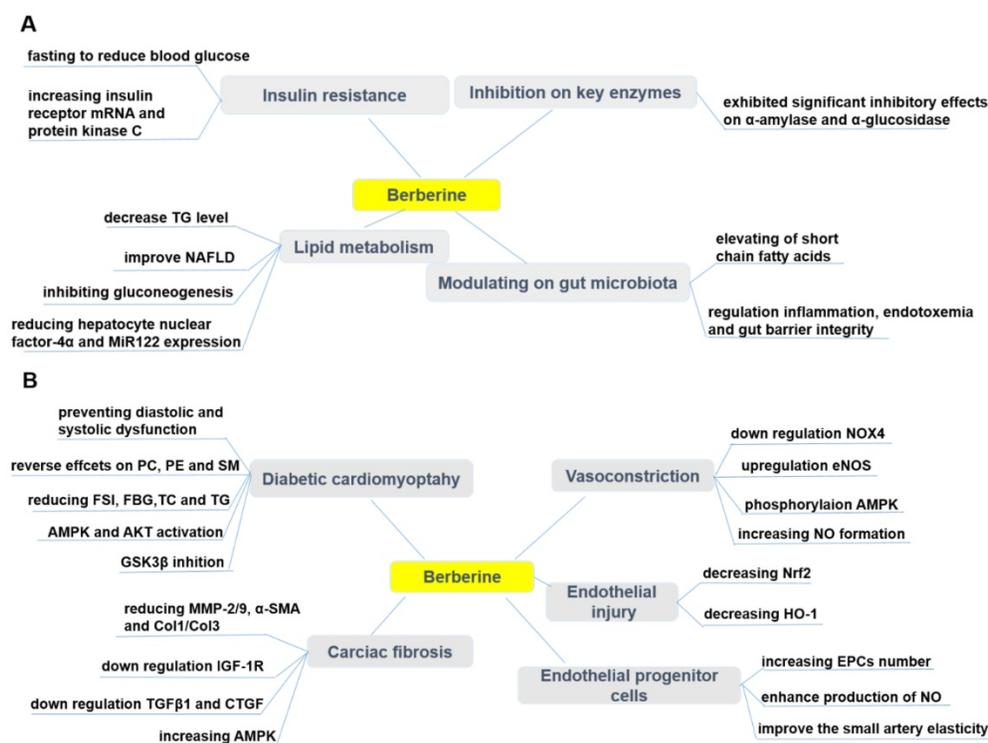
Figure 5. Pharmacological effects of berberine in treating diabetes (A) and its cardiovascular complications (B). BBR exerts protective effects in diabetes by ameliorating insulin resistance, modulating lipid metabolism and gut microbiota, inhibiting α-amylase and α-glucosidase activity. BBR also prevents cardiovascular complications associated with diabetes, such as diabetic cardiomyopathy, cardiac fibrosis, endothelial injury, endothelial progenitor cell dysfunction, and vasoconstriction. ↑indicates increase or activation, and ↓indicates decrease or suppression. **Abbreviations**: AMP protein kinase (AMPK), α-smooth muscle actin (α-SMA), cholesterol (TC), collagen (Col), connective tissue growth factor (CTGF), dinucleotide phosphate-oxidase (NOX), endothelial nitric oxide synthase (eNOS), endothelial progenitor cells (EPCs), fasting blood glucose (FBG), fasting serum insulin (FSI), glycogen synthase kinase 3β (GSK3β), heme oxygenase-1 (HO-1), insulin-like growth factor-1 receptor (IGF-1R), matrix metalloproteinase (MMP), non-alcoholic fatty liver disease (NAFLD), nitric oxide (NO), nuclear factor erythroid 2-related factor 2 (Nrf2), phosphatidylcholine (PC), phosphatidylethanolamine (PE), protein kinase B (Akt), sphingolipid (SM), triglyceride (TG), transforming growth factor β1 (TGFβ1).

Effects of BBR on lipid metabolism

BBR is well-known to improve glucose and lipid metabolic disorders. BBR was an approved component of nutraceuticals for treatment of hyperlipidemia in many countries [241]. Kong *et al.* [23] have described BBR as "a new lipid-lowering drug" after they observed the efficacy of BBR in lowering lipid level *in vitro* and *in vivo*, which was comparable to that of statins. Zhao *et al.* [242] have reported that BBR (150 mg/kg/d, *p.o.*, 16wk) improved NAFLD, a critical hepatic manifestation of metabolic syndrome, by inhibiting gluconeogenesis and regulating lipid metabolism. BBR (40, 60 mg/kg/d, *p.o.*, 4wk) regulated hepatic gluconeogenesis and lipid metabolism in T2DM mice *via* reducing the expression of hepatocyte nuclear factor-4α and miR122 [243]. The main metabolite of BBR *in vivo*, berberrubine (M3), showed the most potential hypolipidemic effect by up-regulating LDLR expression in HepG2 cells [244]. Nine berberrubine analogs modified at the C9 position were tested for their lipid-lowering activity. The results showed that berberrubine and hydroxypropyl-berberine can regulate the expression of liver LDLR and PCSK9 through ERK signaling pathway. Hydroxypropyl-berberine showing a greater effect, which may indicate it as a candidate drug for anti-hyperlipidemia [244].

Effect of BBR on modulating gut microbiota

Human gut microbiota plays a vital role in mediating obesity-related metabolic dysfunction, including T2DM [245]. For example, Fei *et al.* [246] have revealed that an endotoxin-producing *Enterobacter cloacae* B29, obtained from the gut of obese subjects, induced IR and obesity in germ-free mice. Another study has shown that endotoxin produced by the pathogen in the gut, such as *Escherichia coli*, caused obesity and IR when it was subcutaneously administered into mice [247]. As BBR has been known for treating intestinal infection-related diarrhea, Han *et al.* [248] hypothesized that gut microbiota regulation can be one of the anti-diabetic mechanisms of BBR. Recently, Zhu *et al.* [109] have reported that BBR (in drinking water (0.5 g/L), 14wk) treatment significantly reduced atherosclerosis in HFD treated mice by up-regulating the population of *Akkermansia* spp. which was confirmed to regulate inflammation, endotoxemia and gut barrier integrity. The study carried by Zhang *et al.* [233] showed that BBR (100 mg/kg/d, *p.o.*, 18wk) modulated gut microbiota, and it is particularly important that the observed elevating of short chain fatty acids (SCFAs) levels in the intestine, may contribute to its therapeutic effect against DM, obesity and other metabolic diseases.

BBR as inhibitors of α-amylase and α-glucosidase

Inhibition of key carbohydrate hydrolyzing enzymes, in clinic relevant α-amylase and α-glucosidase, has been acknowledged as one of the most effective approaches for managing of DM and delaying postprandial hyperglycemia [249, 250]. Enzymes α-amylase and α-glucosidase are present in the small intestine and its brush border and are responsible for hydrolytic breakdown of complex oligosaccharides and disaccharides into glucose and further monosaccharides suitable for absorption [249, 250]. Inhibition of these enzymes delayed carbohydrate digestion, resulting in a down-regulation in the rate of intestinal glucose absorption and subsequent reduction of the post-prandial glucose levels [249, 250]. Interestingly, BBR has been found to exhibit significant inhibitory potency against both α-amylase and α-glucosidase [223, 249, 251, 252].

Effect of BBR on DM-induced cardiovascular disease and complications

Chronic exposure to hyperglycemic conditions (accompanying DM), can cause damages to various tissues, as well as induces vascular injury and cardiomyopathy [253, 254]. High glucose level plays an important role for endothelial injury. BBR (100 mg/kg/d, *p.o.*, 8wk) ameliorated impaired endothelium-dependent vasorelaxation of aorta in T2DM rats by down-regulating NOX4 expression and up-regulating eNOS expression [255]. Normal vascular tone is sustained by various dilatory and constrictory agents where NO is the major vasodilator. A main feature of endothelial dysfunction is impaired endothelium-dependent relaxation [256]. An effect of BBR on vascular tone has been reported in different studies [168, 169, 257]. BBR caused vasorelaxation in isolated rat aorta cells *via* phosphorylation of AMPK and eNOS [257]. BBR has been evidenced to exert its protective effects on vascular endothelium *via* increasing NO formation [168, 169]. In addition, it has been shown that BBR (50, 100, 200 mg/kg/d, *p.o.*, 4wk) might contribute to the protection against endothelial injuries in retinal tissue of DM rats *via* decreasing the expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) [258]. Both the endothelium and the underlying VSMCs could be affected by BBR to induce relaxation [168]. BBR, as a sensitizer of the insulin signal in HUVECs, also improved insulin-mediated vasodilatation in diabetic rats involving PI3K/Akt and AMPK activation *via* the up-regulation of the InsR [259]. The vasodilator effect of BBR (200 mg/kg/d, *p.o.*, 4wk) also improved

ACh-induced mesenteric artery vasodilation in diabetic rats [259]. There are findings indicating that the decrease in UCP2 expression and mitochondrial ROS accumulation are related to vascular damage [260]. UCP2 has been found to reduce high glucose-induced apoptosis in HUVECs by increasing Bcl-2 while decreasing caspase-3 and cytochrome c [261]. Concurrently, BBR promoted the mitochondrial biogenesis and enhance UCP2 mRNA and protein expression in cultured HUVECs [262]. BBR exerted this pharmacological effect through AMPK-dependent manner, which led to reduction in oxidative stress and vascular inflammation [262]. Increased atherothrombotic events due to platelet activation and apoptosis are the leading cause of high mortality and morbidity during DM [263]. Platelet hyperresponsiveness and apoptosis during DM are ROS accumulation caused by activation of aldose reductase (AR) and NOX. BBR protects platelets by suppressing AR and NOX activity in high glucose treated platelets [263].

Endothelial progenitor cells (EPCs) play a significant role in endothelial function [264]. The reduced number and impaired function of circulating EPCs are markers of vascular diseases, and contribute to impaired arterial elasticity and endothelial dysfunction, particularly in cardiovascular diseases [264]. BBR (1.2 g/d, *p.o.*, 30d) has been confirmed to increase the number of circulating EPCs [265, 266], enhanced the production of NO[265] and improved the small artery elasticity [266] in healthy volunteers. However, in one randomized trial there were no changes in circulating EPCs in individuals with dyslipidemia after treatment with a nutraceutical formulation containing BBR [267]. Oppositely, another trial concluded the improvement of endothelial function after treatment by combination of BBR, RYR and policosanols, through increasing the endothelial-dependent flow-mediated dilation (FMD) in patients with hypercholesterolemia [268].

Diabetic cardiomyopathy (DCM) is considered to be a clinical pathology independent of concomitant vascular diseases and can be primarily caused by disturbances in the energy substrate [269]. In the high-sucrose and HFD/streptozotocin (STZ)-induced rat DCM model, BBR (10, 30 mg/kg/d, *p.o.,* 16wk) significantly prevented diastolic and systolic dysfunction, and cardiac hypertrophy [269]. Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingolipid (SM) are potential biomarkers of DCM, and the protective effect of BBR on DCM can be through reversal of PC, PE and SM metabolic disturbances [269]. BBR treatment (100 mg/kg/d, *p.o.,* 16wk) of diabetic rats can partially improve cardiac function and significantly reduce FSI, FBG, total TC and TG levels. The underlying mechanism can be that

BBR activated cardiac AMPK and Akt in diabetic rats and inhibited GSK3β activity [270]. Similarly, in the palmitate-induced H9C2 cell hypertrophy model, BBR up-regulated alpha-myosin heavy chain (α-MHC) expression, down-regulated beta-myosin heavy chain (β-MHC) expression and thus inhibited H9C2 cell hypertrophy [270]. Moreover, BBR also enhanced AMPK and Akt activation in H9C2 cells and inhibited GSK3β activity [270, 271]. Diabetic hearts are more sensitive to I/R injury, and BBR treatment protected the heart of diabetic rats from I/R damage [272]. This protective effect of BBR was achieved by activating AMPK and Akt activity, while inhibiting GSK3β activity in non-ischemic regions of the diabetic rat hearts [272].

Diabetic cardiac fibrosis causes ventricular stiffness and leads to diastolic dysfunction [273]. In a diabetic rat model, BBR treatment (100 mg/kg/d, *p.o.,* 4wk) improved cardiac fibrosis and dysfunction by down-regulating insulin-like growth factor-1 receptor (IGF-1R) expression in cardiac fibroblasts, specifically by reducing expression of MMP-2/9, α-smooth muscle actin (α-SMA) and type 1 collagen (Col1) in diabetic hearts. BBR also exerted a similar effect in cardiac fibroblasts exposed to high levels of glucose [273]. Furthermore, BBR (50, 100, 150 mg/kg/d, *p.o.,* 12wk) reduced cardiac fibrosis in DM rats by down-regulating the expression of transforming growth factor β1 (TGFβ1) and connective tissue growth factor (CTGF), reducing the expression of Col1 and Col3 [274]. In addition, BBR treatment promoted glucose depletion and glucose uptake as well as improved IR in H9C2 cells, at least partially by increasing AMPK activity [222]. Further, BBR (100 mg/kg/day, *p.o.,* 7d) ameliorated arrhythmias induced by ischemia (LAD ligature) in diabetic rats [275]. Researchers have shown that BBR reversed the down-regulation of the transient outward K^+ current (I_{to}) and I_{Ca} [275]. In a similar study, BBR (60 mg/kg/day*, p.o.,* 14d) led to the restoration of diminished inwardly rectifying potassium channel Kir2.1 to nearly normal levels [276]. The effect of BBR on Kir2.1, the main subunit of I_{K1}, was associated with recovering of resting membrane potential and alleviating of ischemia-induced arrhythmias in diabetic rats [276].

In conclusion, BBR is a promising candidate for therapy of T2DM and metabolic syndrome. However, further investigations of the mechanisms of BBR are required for its appropriate application in clinical settings.

Obesity

Obesity is a complex metabolic disease occurring in both developed and developing countries [277].

Obesity contributes to the development of several other health problems, such as cardiovascular diseases, DM, cancer, chronic obstructive pulmonary disease, and depression [277]. Etiology of obesity is multi-factorial and is associated with the energy input exceeding energy output [278]. Most commonly, it is induced by excessive food intake, imbalanced nutrition, physical inactivity, and genetic predispose-itions. Other causes of obesity include endocrine dysfunction, mental illnesses, or iatrogenic etiologies [278].

BBR reduced food intake in the mice fed with HFD, and decreased their weight gain [279]. Such an effect led to the question as to how BBR affects the appetite. BBR can improve lipid dysregulation by controlling both peripheral and central AMPK activity [280]. Although BBR cannot cross the cerebrovascular barrier, studies have shown that an application of BBR intraperitoneally caused a 47% up-regulation in serotonin levels in the brain [281]. The expression of glucagon-like peptide 1 receptor (GLP-1R), orexin A and neuropeptide Y in brain of tested animals were found to be up-regulated [282]. Together with changes observed in hypothalamus, this can be a factor causing the decrease of body weight, enhanced lipolysis, and decrease of IR in obese experimental animals and even humans.

It is clear, that anti-obesity effects of BBR are associated with its anti-diabetic activity [283]. Many studies identified BBR as an activator of AMPK, which is, besides other effects, responsible for triggering of the glucose uptake and fatty acid oxidation in skeletal muscles, and regulation of insulin secretion by pancreatic β-secretory cells [30, 225, 226]. Effects of BBR on an increase of glucagon-like peptide-1 (GLP-1) *via* enhancing GLP-1 secretion and GLP-1 biosynthesis in enteroendocrine L-cells and some neurons were also observed, and BBR stimulated GLP-1 secretion can trigger the feeling of satiety and decrease the food intake [284]. Mitochondrial stress responses (*e.g.*, mitochondrial swelling and membrane rupture) and GLP-1 expression were decreased in the colon of diet-induced obese mice [285]. Administration of BBR (100 mg/kg/d, *p.o.*, 8wk) up-regulated GLP-1 expression in mice and prevented mitochondrial stress response [285]. All these pharmacological effects of BBR can translate to improved outcomes in DM and obesity.

Several studies have shown altered intestinal architecture as a result of a shift in gut microbiota composition to the phyla *Bacteroidetes* and *Firmicutes* [286]. The pivotal barrier-protecting function conferred by BBR was mediated by elevated levels of SCFAs [233]. Gene profiling analysis showed that BBR can regulate gut microbiota diversity. In the group of BBR-treated rats (100 mg/kg/d, *p.o.,* 18wk), the shift to bacteria producing SCFAs was shown to be significant [233]. On the other hand, fasting-induced adipose factor (FIAF), which inhibits circulating lipoprotein lipase (LPL), is a regulator as a putative mediator of microbial modulation of energy storage, and fat mobilization [287]. BBR (200 mg/kg/d, *p.o.,* 6wk) up-regulated FIAF expression in adipose tissues and intestinal and also changed the abundance of fecal *Bacteroidetes* and *Firmicutes* in total bacteria in the gut of mice fed with HFD [288]. Additionally, Chae *et al.*[289] showed modulation of growth of gut microbiota, especially *Lactobacillus* and *Bifidobacterium* by BBR.

Non-alcoholic fatty liver disease (NAFLD)

NAFLD is manifested as an accumulation of TG in liver [290]. NAFLD is closely related to the obesity and related metabolic risk factors (T2DM and dyslipidemia). Untreated NAFLD can progress to non-alcoholic steatohepatitis (NASH), characterized by persistent hepatocyte inflammation and injury with or without fibrosis. NASH can progress to cirrhosis and liver cancer [291, 292].

According to the main characteristics of NAFLD, the first therapeutic option is the restoration of dysregulated lipid metabolism, which leads to the fat reduction and weight loss [290]. BBR (5 mg/kg/d, *p.o.,* 3wk) improved fatty liver in obese mice by regulating neural signaling from the central nervous system and peripheral AMPK signaling [293]. Sterol regulatory element binding protein 1c (SREBP1c) can be regulated by AMPK *via* direct phosphorylation [294]. AMPK activation suppresses SREBP-1c activity and lowers hepatic and plasma levels of TG and TC [294]. BBR acted as a potent inhibitor of SREBP-1c, possibly through AMPK pathway [295, 296]. BBR (100 mg/kg) can down-regulate SREBP-1c and ameliorate lipid profile in rats [297]. The combination of BBR (50 mg/kg/d, *p.o.,* 8wk) and curcumin (50 mg/kg/d, *p.o.,* 8wk) is more effective than lovastatin (100 mg/kg/d, *p.o.,* 8wk) [298]. BBR can reverse the disorder of lipid metabolism in 3T3-L1 cells induced by olanzapine, a second-generation antipsychotic drug, by inhibiting SREBP and activating AMPKα [299]. Overexpression of SREBP-1c leads to the down-regulation of insulin receptor substrate 2 (IRS-2) mRNA levels which is closely linked to the hepatic IR [300]. BBR reduced liver TC levels by down-regulation of sterol carrier protein 2 expression and inhibiting COX-2 induced prostaglandin production[301]. The improvement of IR by increasing IRS-2 mRNA expression is one of the possible mechanisms in the treatment of NAFLD with BBR [302].

Another transcription factor carbohydrate-responsive element binding protein (ChREBP) also participated in the modulation of lipogenic genes (*e.g.* L-PK) as it has been demonstrated in the liver of carbohydrate-fed rats [303]. BBR (380 mg/kg/d, *p.o.*, 5wk) was able to suppress the expression of ChREBP and ameliorated fatty acid synthesis in the liver [304]. BBR has been reported as a potential PPARα activator in diabetic hamsters [305], although this finding is inconsistent with the results of the study conducted previously [306], where BBR did not influence the expression of PPARα, but directly up-regulated the expression of microsomal triglyceride transfer protein (MTTP) through reduction of methylation of MTTP promoter. MTTP regulated the assembly and secretion of ApoB-containing lipoproteins (including LDL and VLDL) [306]. Similarly, BBR (200 mg/kg/d, *p.o.*, 16wk) can ameliorate the symptoms of NAFLD in rats by increasing the expression of LXRα/FAS [307], UCP2 [308] and MTTP [306] in hepatocytes. In addition, BBR (50mg/kg/d, *p.o.*, 8wk) inhibited HFD-induced down-regulation of carnitine-palmitoyltransferase 1A (CPT 1A) and PPARα expression in fish [309]. BBR obviously reduced TC and LDL-C in hyperlipidemic rats while up-regulated high-density lipoprotein-cholesterol (HDL-C) and CPT 1A expression [310]. BBR (5 mg/kg/d, *p.o.*, 4wk) maintained the energy balance and reduced hepatic steatosis in mice by enhancing autophagy and activating fibroblast growth factor 21 (FGF21), and this effect was dependent on SIRT1 [311], which is the inductor of the expression of PPARα. The depletion of SIRT1 attenuated PPARα signaling and fatty acid β-oxidation, and it can lead to the development of ER stress, hepatic inflammation, and hepatic steatosis [312]. Thus, BBR may be effective in reversing steatohepatitis and fibrosis similarly as fibrates and other PPARα agonists in mice, although there was no significant improvement in histological findings in human subjects [313]. C-C motif ligand 19 (CCL19) was highly expressed in patients with NAFLD [314]. In a model of HFD-induced rat NAFLD, metformin and BBR improved NAFLD by activating AMPK signaling, and metformin and BBR significantly reduced high expression of CCL19 in NAFLD rats. It is postulated that inhibition of CCL19 may be an effective treatment for NAFLD [314].

Oxidative stress, lipid peroxidation, and inflammatory cytokines lead to the development of hepatic steatosis that can progress to devastating NASH, potentially leading to fibrosis and cirrhosis [315]. An increased inflammatory response (*e.g.* TNFα, IL-6 up-regulation) has been observed in patients with NAFLD [316]. TNFα is recognized as a critical factor in NAFLD and related liver diseases and an inhibition of pro-inflammatory cytokines production could be an important strategy to treat NAFLD [316]. BBR obviously reduced the p-JNK1 in mouse primary hepatocytes, whereas the level of p-AMPK was not significantly changed. These results showed, that the inhibition of inflammation by BBR could have AMPK-independent mechanisms [317], although Jeong *et al.* [86] have reported that BBR (5 mg/kg/d, *i.p.*, 3wk) down-regulated the production of pro-inflammatory cytokines (IL-6, IL-1β and TNFα) in adipose tissue of obese db/db mice through AMPK activation. Additional mechanisms of attenuation of hepatic steatosis can be the modulation of gut microbiota [318]. HFD could cause the overgrowth of Gram negative bacteria in the intestine and subsequently the alternation of composition of intestinal microbiota, which leads to the increase of the intestinal permeability and inflammation [319]. BBR (150 mg/kg/d, *p.o.*, 4 wk) could decrease the level of *Faecalibacterium prausnitzii* in rats [320] as well as the number of *Firmicutes* and *Bacteroidetes* in the feces of mice fed a HFD [288].

Taken together, BBR represents a very promising therapeutic drug in various metabolic diseases *via* its pleiotropic effects, including the regulation of lipid and glucose metabolism, oxidative stress, and inflammatory response. Since most studies are performed in pre-clinical animal models, more randomized large-scale clinical trials are needed to evaluate the metabolic effects of BBR in human patients.
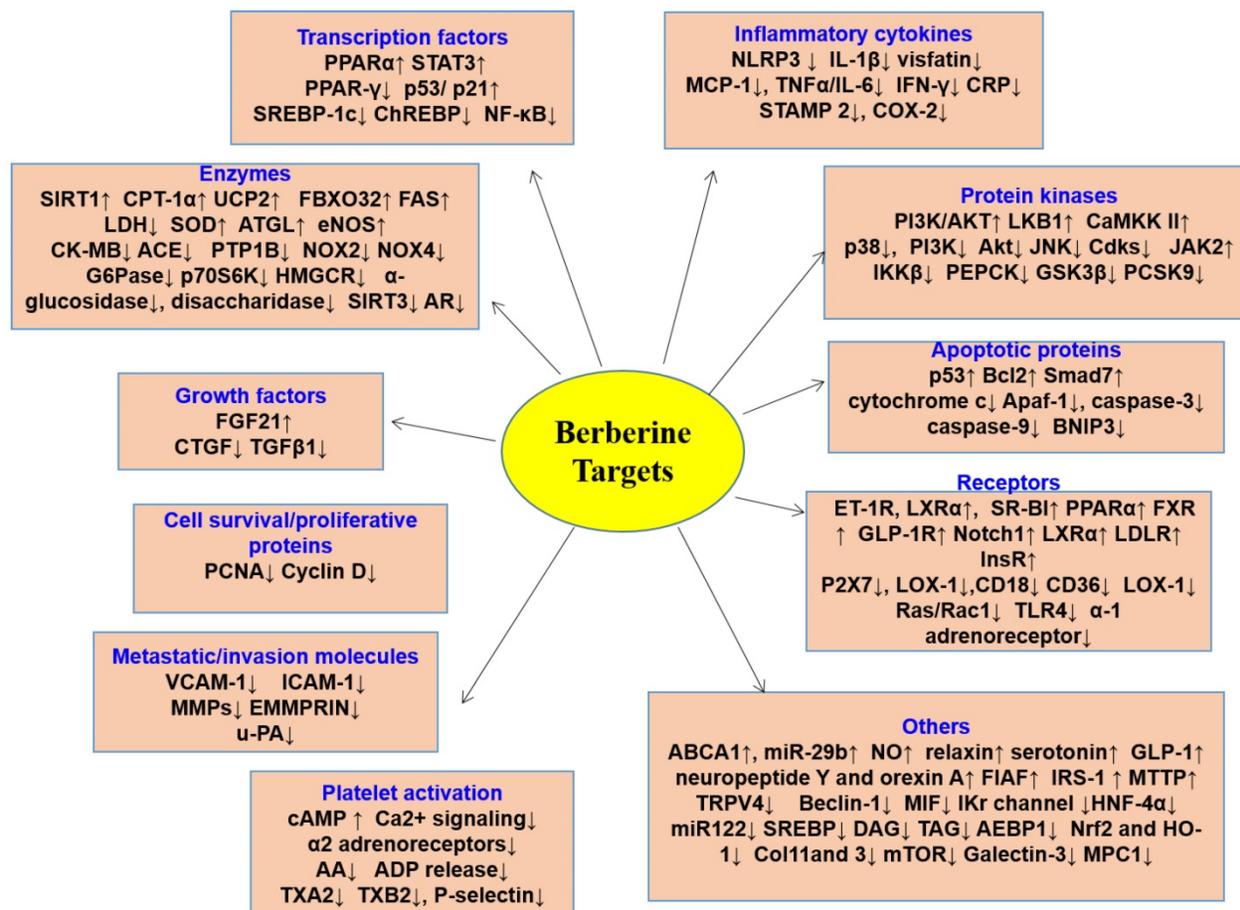
Molecular targets of BBR

BBR modulates multiple cellular events involved in cardiometabolic diseases by modulating multiple disease-relevant targets (Figure 6). The major molecular targets involved in the cardioprotective and metabolic effects are AMPK, PPAR, LDL receptor (LDLR), hepatocyte nuclear factor 1α (HNF1α), IκB kinase β (IKKβ), silent information regulator (SIRT1), and gut microbiota. In this section, a detailed account on the molecular targets of BBR in various CVMD will be summarized.

Increased ratio of AMP/ATP promotes the activation of AMPK, which is a decisive regulator of energy metabolism and is responsible for controlling several features of cellular resistance [321, 322]. BBR was found to decrease ROS production by either up-regulation of SOD expression [323, 324] or negative regulation of NOXs, probably through AMPK activation [30, 225]. Pharmacological actions of BBR are similar to that of metformin, which acts through regulation of different effectors in lipid and energy metabolism, such as PKC, AMPK, and MAPK [236, 325]. The enhancement of adipose triglyceride

lipase (ATGL) expression, which was mediated by AMPK, was mainly considered to be responsible for the long term body weight losing effect of BBR and this effect is also attributed to the enhancement of basal lipolysis of TG present in the adipocytes which is directly correlated with dyslipidemia and obesity [326]. However, the proliferation of preadipocytes was inhibited by BBR, *via* C/EBPα and PPARγ pathways [327, 328]. One of the risk factor of CVD, LDL-C, was also lowered by BBR and this effect was *via* increasing LDLR expression, which also upsurges the LDLR-mediated liver clearance and BBR-caused stabilization of LDLR mRNA was regulated through the ERK signaling pathway revealed the mechanism behind cholesterol lowering effect of BBR [23, 329]. The modulation of LDLR takes place at a post translational level where BBR degraded and ubiquitinized HNF1α [330-332]. Moreover, the up-regulation of LDLR expression is dependent on the ERK activation [23].

Figure 6. Molecular targets of BBR. BBR exerts its pharmacological effects by regulating the expression of genes/proteins responsible for transcription factors, enzymes, growth factors, cell survival/proliferative proteins, metastatic/invasion molecules, platelet activation, inflammatory cytokines, protein kinases, apoptotic proteins, receptors, and the others. By targeting these molecules, BBR prevents the development of multiple cardiovascular and metabolic diseases. ↑indicates increase or activation, and ↓indicates decrease or suppression. **Abbreviations**: arachidonic acid (AA), ATP-binding membrane cassette transport protein A1 (ABCA1), angiotensin-converting enzyme (ACE), adenosine diphosphate (ADP), adipocyte enhancer-binding protein 1 (AEBP1), apoptotic protease-activating factor 1 (Apaf-1), adipose triglyceride lipase (ATGL), aldose reductase (AR), calmodulin-dependent kinase II (CaMKK II), C-C motif ligand 19 (CCL19), the cluster of differentiation 18 (CD18), the cluster of differentiation 36 (CD36), carbohydrate responsive element binding protein (ChREBP), MB isoenzyme of creatine kinase (CK-MB), type 1 collagen (Col1), C-reactive protein (CPR), connective tissue growth factor (CTGF), 1,2-diacyl-sn-glycerol (DAG), endoplasmic reticulum (ER), extracellular MMP inducer (EMMPRIN), endothelin 1 receptor(ET-1R), fatty acid synthase (FAS), fibroblast growth factor 21 (FGF21), fasting-induced adipose factor (FIAF), farnesoid X receptor (FXR), glucose-6-phosphatase (G6Pase), glucagon-like peptide 1 receptor (GLP-1R), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), hepatocyte nuclear factor-4α (HNF-4α), intercellular adhesion molecule-1 (ICAM-1), IκB kinase β (IKKβ), interleukin-1β (IL-1β), interleukin-6 (IL-6), insulin receptor (InsR), insulin receptor substrate 2 (IRS-2), N-terminal kinase (JNK), lactate dehydrogenase (LDH), LDL receptor (LDLR), lectin-like oxidized low density lipoprotein receptor-1 (LOX-1), monocyte chemotactic protein-1 (MCP-1), migration inhibitory factor (MIF), matrix metalloproteinases (MMPs), mitochondrial pyruvate carrier (MPC), microsomal triglyceride transfer protein (MTTP), nuclear factor-kappaB (NF-κB), Nod-like receptor family pyrin domain containing3 (NLRP3), nitric oxide (NO), nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2), nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4), nuclear factor erythroid-2-related factor-2 (Nrf2), protein S6 kinase (p70S6K), proprotein convertase subtilisin/kexin type 9 (PCSK9), ribosomal proliferating cell nuclear antigen (PCNA), P-glycoprotein (P-gp), phosphoenol pyruvate carboxykinase (PEPCK), phosphatidylinositol 3 kinase (PI3K), peroxisome proliferator-activated receptor α (PPARα), peroxisome proliferator-activated receptor γ (PPARγ), protein tyrosine phosphatase1B (PTP1B), activate silent information regulator 1 (SIRT1), super oxide dismutase (SOD), scavenger receptor class B type I (SR-BI), sterol regulatory element binding protein (SREBP), 1,2,3-triacyl-sn-glycerol (TAG), tissue growth factor-β1(TGFβ1), Toll-like receptor 4 (TLR4), tumor necrosis factor α (TNFα), TNF receptor-associated factor 2 (TRAF2), transient receptor potential vanilloid 4 (TRPV4), uncoupling protein 2 (UCP2), urokinase-type plasminogen activator (u-PA), vascular cell adhesion molecule-1 (VCAM-1).

The up-regulation of AMP level and consequent activation of AMPK by BBR was also related to the inhibition of mitochondrial respiratory complex 1 which represents a prominent target for the compounds which improve entire body insulin sensitivity [333]. BBR blocked complex 1 and led to the enhancement of lactate release and glucose consumption. This process is independent of AMPK activation [331, 334]. Modulation of glycolipid metabolism by BBR occurred through enhancing PPARα/δ expression and down-regulated PPARγ expression in liver [335]. Lipid and glucose SOD homeostasis is also regulated by AMPK [336]. Zhao *et al.* [337] have demonstrated that in normal and Mac-CM-damaged adipocytes, nandinine, a BBR derivative, and BBR attenuated IR *via* the reduction of inflammation mediated by an effect on AMPK and also by targeting IKKβ. Apparent suppression of pro-inflammatory responses by AMPK activation in macrophages by BBR has also been reported [86, 337] Thus, the authors recommend the use of BBR and nandinine as dietary supplement in obesity.

The principal lipid-lowering mechanism of BBR was hepatic LDLR stabilization, which was mediated *via* intensification of the signal regulated kinase dependent pathway, along with the enhanced LDLR promoter transcriptional activity responsible for its cardioprotective effect against I/R injury [272]. A recent study has reported that BBR was beneficial for the maintenance of energy supply to diabetic hearts during I/R injury [272]. BBR inhibited Col1 and Col2 synthesis, promoted IL-10 secretion both in Ang II stimulated heart fibroblasts and suppressed TGFβ1 secretion [138]. The same study also revealed that AMPK phosphorylation was increased by BBR [138]. After Ang II stimulation in cardiac fibroblasts, protein S6 kinase (p70S6K) and mTOR phosphorylation was down-regulated by BBR. Moreover, myofibroblasts transformation could also be inhibited by BBR *via* decreasing the α-SMA expression [138]. BBR also exhibited inhibitory effects on protein tyrosine phosphatase 1B (PTP1B) which functions as a mediator of insulin signaling and catalyzes protein dephosphorylation by interacting InsR, and showed insulin mimicking effects in myocytes and adipocytes which suggested that BBR is a representative of a different class of anti-hyperglycemic agent [338].

Apart from the effect on AMPK, an effect of BBR on human health was plausible due to the stimulation of the SIRT1, which is also responsible for the potential effects against consequences of oxidative stress [339]. A recent study has shown that BBR (5 mg/kg/day, *i.p.*, 3 wk) attenuated oxidative stress in diabetic mice *via* miR-106b/SIRT1 signaling pathway[340]. BBR also affected the islet function and thus was beneficial in plummeting cardiovascular risk factors in DM [340]. Another study has revealed that BBR has the potential not only to suppress formation of foam cells, but also the ability to adjust lipid accumulation [82]. This effect was mediated through activation of AMPK-SIRT1-PPARγ pathway [82]. Additionally, the same study has shown that the foam cell inhibitory effect was increased when both BBR and atorvastatin were used in combination rather than atorvastatin alone [82]. Another study performed in doxorubicin treated H9C2 cardiomyoblasts showed that pre-treatment with BBR up-regulated the protein levels of SIRT1 and SIRT3 in the presence of doxorubicin [341]. BBR also repressed the activation of caspase-3 and caspase-9 induced by doxorubicin. In doxorubicin- treated H9C2 cardiomyoblasts, BBR showed an autophagy modulating effect and increased mitochondrial biogenesis markers and hence BBR can be used as a modulator in decreasing doxorubicin induced cardiotoxicity [341]. The SIRT1/ER stress pathway represents a vital mechanism for the neuroprotective effects of BBR in diabetic encephalopathy [342]. BBR has been found to up-regulate SIRT1 protein expression and down-regulate the expression of proteins associated with ER stress, such as protein disulfide isomerase (PDI), inositol-requiring enzyme (IRE)1α, eukaryotic translation initiation factor 2 (eIF2), C/EBP homologous protein (CHOP) and PKR-like endoplasmic reticulum kinase (PERK) [342].

The regulation of gut microbiota is another important target of BBR in hyperglycemia, hyperlipidemia, IR, DM, and atherosclerosis [109, 233, 343-346]. Gut microbiota converted BBR to an absorbable form of dihydroberberine, which can be further oxidized to BBR after intestinal absorption [347]. The increase of gut *Akkermansia* may contribute to atheroprotective and metabolic protective effects of BBR [109]. BBR increases the amount of gut microbiota that produces SCFAs, contributing to the beneficial effects on the host [343]. Similar conclusions have appeared from several studies [233, 344-346]. Supplementation of BBR in broiler diet promoted intestinal colonization of healthy microbiota at high stocking densities [348]. By normalizing gut microbiota, BBR alleviated NASH and its predisposing factors in mice [349]. BBR also regulated energy metabolism as it could reduce lipids by regulating the turnover of bile acids, and subsequent ileal FXR signaling pathway [350]. Therefore, BBR acts through numerous signaling pathways which clearly suggests that it does not act *via* a ubiquitous mechanism and it acts through distinct mechanisms to combat cardiovascular and metabolic diseases.

Clinical trials of BBR in cardiometabolic diseases

Several clinical trials have been performed to study the effects of BBR in the therapy of cardiometabolic diseases and/or conditions related to them. These ongoing or completed clinical trials are summarized in Supplementary Table 1 (in supplemental material) [23, 112, 325, 351-364]. Trials testing the effects of BBR-based nutraceuticals combination were excluded, because the observed effects are not specifically attributable to BBR, but, probably, to the synergistic effects of all bioactive components. Generally, BBR is tested in dosage ranged from 0.5 to 2.0 g/d as well as treatment period ranged from 4 to 24 weeks. In these trials, BBR has been demonstrated to improve blood lipid profile [23, 112, 325, 351-360], glucose level [112, 325, 351, 357-361], blood pressure [112, 357, 360], IR [358, 360], endothelial function [112], body weight [357, 362], and systemic inflammation [356].

Selected clinical trials have been carried out on subjects with hypercholesterolemia [23, 112, 352, 353, 355], T2DM [325, 354, 360, 361, 363], obesity [362], congestive heart failure (CHF) [364], acute coronary syndrome (ACS) [356], metabolic syndrome [357], NAFLD [351, 359], and polycystic ovary syndrome (PCOS) [358]. Only one study was conducted on healthy subjects [112]. Particularly, 25 subjects were randomized into two groups: 1.2 g/d BBR treatment group and a control group without any treatment. After 4 weeks of treatment, TC, LDL-C, fasting blood glucose (FBG) and blood pressure were obviously decreased. In addition, improved endothelial function, and a reduction of levels of CD31+/CD42- microparticles were observed [112].

Clinical trial of metabolic diseases

The effect of BBR in patients with T2DM

In patients with T2DM, BBR ameliorated glucose metabolism and improved insulin sensitivity [361]. A pilot study was conducted in diabetic patients to investigate the efficacy of BBR. 36 diabetic patients were randomized into two groups: BBR or metformin (both 1.5 g/d, 12wk) [361]. After the treatment, in both groups, a statistically significant down-regulation in levels of glycosylated HbA1c (p<0.01), FPG and postprandial blood glucose (PBG) (p<0.01) were observed, suggesting that BBR exerted a hypoglycemic effect comparable to that of metformin. Further, BBR significantly reduced the levels of TG and TC [361]. Zhang *et al.* [325] have compared the effects of BBR (1.0 g/d) and metformin (1.5 g/d) or rosiglitazone (4.0 g/d) for 8 weeks in patients with T2DM. After the treatment period, BBR lowered the level of FPG and HbA1c. The effects of BBR were comparable to that of metformin and rosiglitazone. To evaluate the potential effects and mechanism of BBR in IR, the expression levels of InsR were assessed on peripheral blood lymphocytes (PBL) isolated from subjects before and after BBR treatment [325]. A significant up-regulation of InsR-expressing PBL was observed (p<0.01), suggesting that BBR can be effective in treating IR by up-regulation of InsR expression [325].

The effect of BBR in patients with T2DM and dyslipidemia

Zhang *et al.* [360] conducted a randomized, placebo-controlled, double-blinded study on 106 subjects with T2DM and dyslipidemia. After 12 weeks of treatment with BBR (1.0 g/d), a obvious improvement was observed in levels of FBG, post-load blood glucose (P-LBG), HbA1c, TG, TC, and LDL-C and insulin-sensitivity [360]. In addition, BBR also reduced systolic (SBP, p = 0.001) and DBP [360]. Similarly, Gu *et al.* [363] have shown that BBR (1.0 g/d, 12wk) improved lipid profile and insulin sensitivity. In addition, BBR treatment reduced serum concentration of 13 free fatty acids (FFAs), suggesting that BBR was effective in the T2DM-related pathological conditions reducing the level of FFAs [363].

The effect of BBR in patients with dyslipidemia

Kong *et al.* [23] have reported that BBR (1.0 g/d, 12wk) in a clinical study reduced the lipids (TC, TG and LDL-C) and increased LDLR expression. The proprotein convertase subtilisin/kexin type 9 (PCSK9) negatively regulates LDLR and has become a potential target for hypercholesterolemia [330, 365], and it was shown, that BBR can significantly improve dyslipidemia and LPS-induced inflammatory response in mice by down-regulation of the expression of PCSK9 in the liver and then up-regulating LDLR expression [365]. The specific mechanism by which BBR inhibited PCSK9 expression may be through a decrease in HNF1α expression [366] and attenuation of HNF1α and SREBP2 transcription of PCSK9 [332]. This increased LDLR mRNA is independent on HMGCR activity, suggesting that BBR is able to lower lipid level by a mechanism different from statins [23]. A follow-up study evaluated the hypolipidemic effects of BBR (1.0 g/d) and simvastatin (0.2 g/d) alone or in combination for 8 weeks, in subjects with hypercholesterolemia [355]. In the combination therapy, the serum levels of LDL-C, TC and TG were obviously reduced after the treatment. Interestingly, BBR monotherapy was more effective that simvastatin in reducing LDL-C, TC and TG [355].

Clinical trial of cardiovascular diseases

BBR also exerted beneficial effects in patients with CVD [356, 364]. In 12 patients with refractory CHF, an infusion (0.02 or 0.2 mg/kg/min, 30 min) of high doses of BBR could reduce systemic vascular resistance and enhanced cardiac index, while in the lower dosed group only reduced the patients' heart rate [367]. Reaching the plasma concentration of BBR in patients with CHF greater than 0.11 mg/L, significantly reduced the frequency and complexity of ventricular premature beats (VPBs) while left ventricular ejection fraction (LVEF) was increased [368]. 79 patients with CHF received BBR 1.2 to 2.0 g/day and routine anti-HF treatment for 8 consecutive weeks. BBR significantly increased exercise capacity and the dyspnea-fatigue index, while decreased frequency and complexity of VPBs [364]. Meng *et al.* [356] have evaluated the effects of BBR in 130 patients with ACS. Subjects were randomized into a treatment group (BBR 0.9 g/d as add-on therapy for 4 weeks) and a control group (only standard therapy). After treatment period, BBR reduced TC and LDL-C levels as well as markers of inflammation (including hypersensitive CRP, IL-6, MMP-9, VCAM-1, ICAM-1and MCP-1), suggesting that BBR is effective in the global management of CVD [356]. A recent clinical study evaluated the effect of an oral nutraceutical combination comprised of RYR and BBR on subclinical inflammation, lipid profile, PCSK9, and arterial stiffness in antiretroviral therapy treated HIV-infected patients [369]. The results of this study showed positive effect and reported that the nutraceutical combination substantially reduced PCSK9 and plasma cholesterol levels and improved arterial stiffness [369].

New development of BBR derivatives in treating cardiometabolic diseases

Many derivatives of BBR were synthesized with an aim to improve its relatively low bioavailability and to decrease the dosage necessary to trigger the desired therapeutic effects. The main positions of the BBR skeleton are depicted in Figure 7. Especially positions 7, 8, 9, 12 and 13 were targets of BBR modifications [370]. It has been observed that alkylation and acylation of BBR on the ring D can change bioavailability [370]. As example, pseudoberberine (10,11-dimethoxyderivative) IMB-Y53 *in vivo* increased the glucose lowering properties, probably due to diminished affinity to P-gp [371]. BBR has relatively low oral bioavailability due to extensive P-gp mediated drug efflux in gastrointestinal tract [372]. To address this issue, IMB-Y53 was prepared with the increased gastrointestinal uptake and thus further anti-diabetic effects [371]. The potential of IMB-Y53 in CVMD remains to be investigated in future.

Similarly, dihydroberberine and its analog 8,8-dimethyldihydroberberine were synthesized and tested. Dihydroberberine and 8,8-dimethyldihydroberberine were more potent than BBR and appeared to have similar pharmacodynamic effects in treatment of type 2 diabetes at lower doses [373] . A study by Turner *et al.* [333] indicated that 560 mg/kg of BBR in a model of rats fed HFD had similar effects compared to 100 mg/kg of dihydroberberine in slowing the adiposity, tissue TG accumulation and IR. It represents a novel therapeutic agent for the therapy of T2DM [333]. Furthermore, BBR metabolites were studied, their activity was explored, and they were also used for synthesis of derivatives with more pronounced activity. As examples, we can mention derivatives of 12-aminomethyl berberrubine (*e.g.* 12-(*N*-methyl-piperazine-4-methyl)-berberrubine), which were synthetized as potential anti-diabetic substances, and tested on 3T3-L1 adipocytes and L6 myotubes to evaluate their effects on the insulin-resistant reversal activity and glucose transportation. Their effect was comparable to rosiglitazone [374]. Further *in vitro* assay analyzed effect of berberrubine derivatives (*e.g.* [2-oxo-2-[2-[(2,3,4-trihydroxyphenyl)methylene]hydrazinyl]ethoxy]-9-berberrubine) on the α-glucosidase, showing their ability to inhibit this enzyme and to be a potential anti-diabetic drug [370].
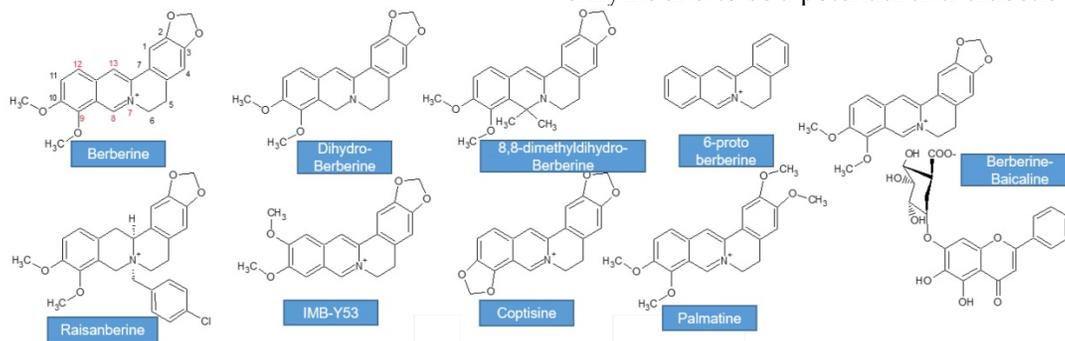
Figure 7. Derivatives of BBR in treating cardiovascular and metabolic diseases. Currently, there are several derivatives being developed based on BBR structural skeleton. These derivatives, such as dihydroberberine, 6-protoberberine, Raisanberine, IMB-Y53, and berberine-baicaline hybrids etc have therapeutic potential in treating cardiovascular and metabolic diseases.

BBR is an isoquinoline alkaloid that, and due to its quaternary ammonium ion, can form compounds with other molecules with negative charge, such as BBR-baicalin, BBR-wogonoside, and BBR-glycyrrhizin [375]. These complexes have been shown to possess improved bioavailability due to increased lipophilicity in comparison to parent drug BBR [375]. A good example of such as complex compound is berberrubine-magnolol, which was synthesized as anti-diabetic compound. When assayed, this derivative showed low toxicity, and improved absorption, metabolism time, and even greater effect on decreasing glycemia [376].

6-Protoberberine showed hypotensive activity, namely it decreased SBP and heart rate by a central sympatholytic effect [377]. Tetrahydroprotoberberine is a competitive antagonist at α1-adrenoceptors in rat aorta, and showed no endothelium-dependent vasorelaxatory activity [378]. Some protoberberine complexes, *e.g.* tetrahydroprotoberberine-aporphine dimer, were described in nature [379]. Novel tetrahydroprotoberberine derivatives are recently being developed as selective inhibitors of dopamine D1 receptor [380].

Raisanberine (*p*-chlorobenzyltetrahydroberberine chloride), named also CPU 86017, is a novel cardioprotective drug for the therapy of pulmonary arterial hypertension (PAH), HF and arrhythmia [381-384]. In a rat model of PAH, CPU 86017 reduced ET-1 in lung tissues, reversed an increase of iNOS mRNA level [381]. CPU 86017 also improved heart function and hemodynamic parameters in rats subject to development of artificial myocardial infarction [382]. The main mechanism of protective effects of CPU 86017 were attributed to the inhibitory effects on calcium influx, and stabilized expression of PLB, FKBP12.6, and SERCA2a [382].

Analysis of pharmacological activity in LPS-stimulated RAW264.7 macrophages and acute inflammation mouse models indicate that BBR and its natural derivatives have anti-inflammatory effects, with the order of oxyberberine (OBB) > BBR> reduced derivatives (two hydroquinone, DHBB) [385]. The mechanisms of BBR and its derivatives involve the inhibition of the NF-κB signaling pathway [385]. Further elucidation of the mechanism and therapeutic potential of BBR derivatives in cardiometabolic diseases are warranted.

Concluding remarks and future perspectives

Emerging studies over the past decade have convincingly shown that BBR has diverse therapeutic effects on various cardiometabolic diseases, including cardiac hypertrophy, HF, atherosclerosis, stroke, DM, and NAFLD. The efficacy of BBR for treating multiple diseases is mediated by its multi-target pharmacological profile, including amelioration of AMPK, PTP1B, SIRT1, PCSK9, LDLR, PPAR, NF-κB, and modulation of gut microbiota.

The safety of BBR may be based on the poor oral absorption. High doses of BBR can inhibit certain types of CYPs, such as CYP3A11 and CYP3A25 [386, 387] and repeated oral administration of BBR (0.3 g three times daily) can reduce the activity of CYP2D6, CYP2C9 and CYP3A4 in healthy subjects [388]. This may affect the metabolism of other drugs and could cause some herb-drug interactions; however, this should be further studied and supported by clinical investigations.

As mentioned, the low bioavailability of BBR due its poor oral absorption and extensive metabolism are main obstacles for practical application of BBR. Modern oral dosage forms offer a number of benefits including reduced frequency of dosing, better therapeutic control, and fewer side effects. Advances in polymer materials, particle engineering and nanotechnology could solve some of the problems described for BBR. Different types of nanocarriers (polymers, magnetic mesoporous silica, lipids, graphene, gold and silver nanoparticles) encapsulating BBR can also overcome its deficiencies [389]. In addition. BBR has been recently recommended by several groups of experts as an effective lipid-lowering nutraceutical both in low-risk patients and in statin-intolerant ones [390, 391].

Plants with content of BBR are commonly part of preparations used in Traditional Chinese Medicine and other traditional medicinal systems. Besides of this alkaloid, they contain other related compounds and derivatives of BBR. Preparations of TCM are commonly complex and also contain other, structurally unrelated plant secondary metabolites. This inspired research on combinations of BBR and other substances. As example, the oligomeric proanthocyanidins (OPCs) are the main anti-diabetic components in Cinnamon water extracts [392]. The combination of OPCs and BBR can significantly improve the pharmacokinetics of BBR in diabetic mice (by inhibiting the expression of P-gp in Caco-2 intestinal cells and thereby reducing the efflux of BBR) and hypoglycemic efficacy [392]. The combination of BBR and resveratrol has an enhanced hypolipidemic effect in mice fed with HFD [393]. Mechanistic studies have shown that the combination of BBR and resveratrol significantly increased the expression of LDLR in HepG2 cells to approximately one-fold compared to the two drugs alone [393].

Collectively, BBR represents a promising naturally-occurring lead compound for treating CVMD. Further structural modification of BBR may

improve the oral bioavailability, efficacy, and reduces potential toxicity of BBR. However, study outcomes of BBR mixture with other nutraceuticals or compounds have to be analyzed carefully since not pure BBR was used for these studies. Furthermore, well-designed, large-scale, high-quality, and multi-center clinical trials are required to assess the safety, toxicology profile, clinical utility of BBR (as compared to traditional treatment regimens) on CVMD in human patients.

Acknowledgements

Supplementary Material

Supplementary table 1.
http://www.thno.org/v09p1923s1.pdf

Competing Interests

The authors have declared that no competing interest exists.

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