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Promising Anti-atherosclerotic Effect of Berberine: Evidence from In Vitro, In Vivo, and Clinical Studies

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Abstract Elevated levels of plasma cholesterol, impaired vascular wall, and presence of inflammatory macrophages are important atherogenic risk factors contributing to atherosclerotic plaque formation and progression. The interventions modulating these risk factors have been found to protect against atherosclerosis development and to decrease atherosclerosis-related cardiovascular disorders. Nutritional approaches involving supplements followed by improving dietary habits and lifestyle have become growingly attractive and acceptable methods used to control atherosclerosis risk factors, mainly high levels of plasma cholesterol. There are a large number of studies that show berberine, a plant bioactive compound, could ameliorate atherosclerosis-related risk factors. In the present literature review, we put together this studies and provide integrated evidence that exhibits berberine has the potential atheroprotective effect through reducing increased levels of plasma cholesterol, particularly low-density lipoprotein (LDL) cholesterol (LDL-C) via LDL receptor (LDLR)-dependent and LDL receptor-independent mechanisms, inhibiting migration and inflammatory activity of macrophages, improving the functionality of endothelial cells via anti-oxidant activities, and suppressing proliferation of vascular smooth muscle cells. In conclusion, berberine can exert inhibitory effects on the atherosclerotic plaque development mainly through LDL-lowering activity and suppressing atherogenic functions of mentioned cells. As the second achievement of this review, among the signaling pathways through which berberine regulates intracellular processes, AMP-activated protein kinase (AMPK) has a central and critical role, showing that enhancing activity of AMPK pathway can be considered as a promising therapeutic approach for atherosclerosis treatment.

Keywords AMP-activated protein kinase · Atherosclerosis · Berberine · Cholesterol · Endothelial cells · LDL-C · Macrophage dysfunction · Vascular smooth muscle cell

Abbreviations

3′-UTR	3′-untranslated region
ABCA1	ATP-binding membrane cassette transport protein A1
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
EMMPRIN	Exhibited a decreased expression of MMPs and extracellular MMP inducer
eNOS	endothelial nitric oxide synthase
ERK	Extracellular receptor-activated kinase
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A

HNF1α	hepatocytes nuclear factor 1α
HUVECs	Human umbilical vein endothelial cells
IL-6	Interleukin 6
JNK	c-Jun N-terminal kinase
LDL	Low-density lipoprotein
LDL-C	LDL cholesterol
LDLR	LDL receptor
LOX 1	Low-density lipoprotein receptor 1
LXRα	Liver X receptor α
lysoPC	Lysophosphatidylcholine
MAP	Mitogen-activated protein
MCP-1	Monocyte chemoattractant protein-1
MIP-1α	Macrophage inflammatory Protein 1 alpha
MMPs	Matrix metalloproteinases
NADPH	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcohol fatty liver disease
NOD	Nonobese diabetic
PCSK9	Proprotein convertase subtilisin kexin 9
PCSK9	Proprotein convertase subtilisin/kexin type 9
ROS	Reactive oxygen species
SR-BI	Scavenger receptor class B type I
SREBP2	sterol regulatory element-binding protein 2
TG	Triglyceride
VCAM-1	Vascular cell adhesion molecule-1
VSMCs	Vascular smooth muscle cells

1 Introduction

Berberine is an isoquinoline alkaloid presented as the principal bioactive ingredient in stem, bark, rhizome, and roots of several plants, including barberry (*Berberis vulgaris*), Coptis (*Coptis chinensis)*, goldenseal (*Hydrastis canadensis*), tree turmeric (*Berberis aristata*), and Oregon grape (*Berberis aquifolium*) (Singh and Mahajan 2013; Srivastava et al. 2015). Evidence from traditional and modern medicine shows that berberine exerts polytrophic pharmacological effects, including lipid-lowering, anti-diabetic, anti-tumor, anti-inflammatory, anti-diarrheal, and antimicrobial activities (Ayati et al. 2017; Imanshahidi and Hosseinzadeh 2008; Li et al. 2014; Singh et al. 2010; Zhang et al. 2019).

A growing body of research confirms the anti-atherogenic properties of berberine, although there is no enough report showing its direct effect on atherosclerotic plaque formation and progression. Herewith, to exhibit the possible protective effect of berberine on atherosclerotic plaque progression, the present review was aimed to gather underlying mechanisms ascribed to the anti-atherogenic effects of berberine.

As discussed in the following sections, there are documented findings demonstrating that berberine has potential anti-atherosclerotic effects through protecting or lowering hypercholesterolemia, modulating the atherogenic activity of inflammatory macrophages, and improving abnormal functions of vascular smooth muscle and endothelial cells.

2 Berberine Lowers Atherogenic Lipids

Atherogenic lipids, especially low-density lipoprotein (LDL) cholesterol (LDL-C), have a casual effect on atherosclerotic plaque development and progression (Ference et al. 2017). Various LDL-lowering medications, such as statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, have been found to reduce the risk of atherosclerosis-related cardiovascular disorders proportional to the absolute reduction of LDL-C in numerous randomized trials (Ference et al. 2017; Ridker et al. 2017; Sabatine et al. 2017; Sahebkar and Watts 2013). Hence, an LDL-lowering agent may have the potential to exert protective and therapeutic effects against atherosclerotic plaque progression. Although statin therapy is the most commonly used approach to treat hypercholesterolemia, there might be a relatively large number of patients who are statin-resistant or statin-intolerant and unable to achieve optimal LDL-C levels despite intensive statin therapy (Toth et al. 2018; Ward et al. 2019). Therefore, exploring complementary or alternative LDL-lowering agents to prevent or treat atherosclerotic lesions is important. There are several in vivo, clinical, and mechanistic studies that show berberine can efficiently reduce increased levels of atherogenic lipids, mainly LDL-C, suggesting that this natural compound has the strong potential to protect against atherosclerotic plaque development.

2.1 In Vivo Evidence

A large number of experimental studies on rodent models of diet-induced hypercholesterolemia have been conducted to determine the cholesterol-lowering effects of the various doses and administration routes of berberine (Table 1). Since an early report introduced berberine as a new cholesterol-lowering agent (Kong et al. 2004), a growing body of experimental studies has further investigated and confirmed the therapeutic potential of berberine on hypercholesterolemia in recent years (Briand et al. 2013; Brusq et al. 2006; Chang et al. 2012; He et al. 2016; Hu and Davies 2010; Hu et al. 2012; Jia et al. 2008; Kong et al. 2004; Li et al. 2011; Wang et al. 2013, 2014; Xiao et al. 2012). As reviewed elsewhere, berberine can modulate the dysregulated lipid profile through reducing plasma levels of TC, LDL-C, and TG as well as increasing plasma HDL-C in various rodent models of hyperlipidemia (Wang and Zidichouski 2018). These results are supported by the recent animal

Table 1 In vivo lipid-lowering effect of berberine in various animal models

Animal model	Berberine dose	Route and time of administration	Effect on plasma lipid profile	Ref.
Male ApoE$^{-/-}$ mice	50 mg/kg/day 100 mg/kg/day	Daily gavage for 13 weeks	Decreasing TC and LDL-C Increasing HDL-C	Wu et al. (2020)
Male C57BL/6 mice	200 mg/kg/day	Daily gavage for 2 weeks	TC (−28%) LDL-C (51%)	Singh and Liu (2019)
Sprague-Dawley male rats	100 mg/kg/day	Daily gavage for 4 weeks	Significantly decreasing TC and LDL-C Significantly increasing HDL-C	Kim et al. (2019)
Female C57BL/6 J mice	30 mg/kg/day	Daily gavage for 4 weeks	TC (−10.5) LDL-C (−9.8%)	Zhu et al. (2018)
Sprague-Dawley male rats	50, 100, 150 mg/kg/day	Daily gavage for 8 weeks	TC (−29%, −33%, −33%), non-HDL-C (−31%, −41%, −38%), at 50, 100, and 150 mg/kg/day, respectively	Wang et al. (2014)
Sprague-Dawley male rats	100 mg/kg/day	Daily gavage for 6 weeks	No effect on both TC and non-HDL-C. but (−31%) plasma TG levels	Jia et al. (2008)
Sprague-Dawley male rats	200 mg/kg/day	Daily gavage for 16 weeks	TC (−28%) and HDL-C (−41%)	Chang et al. (2012)
Male hamsters	100 mg/kg/day	Orally twice a day for 10 days	Inhibited both cholesterol and TG synthesis	Brusq et al. (2006)
Male hamsters	150 mg/kg/day	Daily injection for 2 weeks	LDL-C (−35%), TG (−34%)	Briand et al. (2013)
Female C57BL/6 J mice	10 and 30 mg/kg/day	Daily gavage for 4 weeks	TC (−42%, −56%) and TG (−37%, −47%) at 10 and 30 mg/kg/day, respectively	Xiao et al. (2012)
Sprague-Dawley rat	500 mg/kg	3 times a day gavage for 12 weeks	T-C (−9%) and TG (−34.7%)	Hu et al. (2012)
Female hamsters	50 and 100 mg/kg/day	Daily gavage for 10 days	LDL-C (−26%, −42%), at 50 and100 mg/kg/day, respectively	Kong et al. (2004)
Hamsters	46.7 mg/kg/day	Daily gavage for 140 days	TC (−19%) and HDL-C (−11.34%)	He et al. (2016)
Male hamsters	1.8 mg/kg/day	Daily gavage for 24 days	TC (−30%), TG (−34%) and LDL-C (−9.2%)	Abidi et al. (2006)
Male rats	7, 60, and 300 mg/kg/day	Daily gavage for 12 weeks	Decreased TC and LDL-C, but increased HDL-C	Wang et al. (2011)

(continued)

Table 1 (continued)

Animal model	Berberine dose	Route and time of administration	Effect on plasma lipid profile	Ref.
Rats	200 mg/kg/day	Daily gavage for 16 weeks	T-C (−29%) and HDL-C (−41%)	Chang et al. (2010)
Male hamsters	50 and 100 mg/kg/day	Daily gavage for 10 days	TC (−44%, −70%,), TG (−34%, −51%), and LDL-C (−47%, −71%) at 50 and100 mg/kg/day, respectively	Li et al. (2008)

study that showed the oral administration of berberine (100 mg/kg/day) via gavage feeding or dietary supplementation can ameliorate severe hypercholesterolemia in hyperlipidemic rats (Kim et al. 2019). It is consistent with another study that demonstrated the oral gavage of berberine at both high dose (100 mg/kg/day) and low dose (50 mg/kg/day) could effectively and dose-dependently reduce elevated levels of plasma TC and LDL-C in ApoE$^{-/-}$ mice fed with a high-fat diet, which the effect of high dose was stronger than that of the low dose (Wu et al. 2020). Further study revealed that berberine treatment (200 mg/kg/day) could decrease plasma levels of TC and LDL-C by 28% and 51% in wild-type mice fed a high cholesterol diet (Singh and Liu 2019). It was also found that intraperitoneal (i.p.) injection of berberine (1.5–5 mg/kg/day) could decrease plasma cholesterol to a similar amount as compared to oral administration; however, the therapeutic dose was decreased by 10 to 100-folds in i.p. route (Abidi et al. 2006; Hu and Davies 2010; Kim et al. 2009; Wu et al. 2010). Although i.p. injection of berberine shows similar cholesterol-lowering effects at a lower dose, it is not an acceptable route for administration in humans because of its inconvenience and invasiveness.

The cholesterol-lowering potential of berberine is further supported by the study that showed oral administration of berberine (90 mg/kg/day) and simvastatin (6 mg/kg/day) exerted similar reductions of LDL-C (−27% and −28%, respectively) in rats fed with a high-cholesterol diet. Of note, a combination of berberine with simvastatin decreased plasma LDL-C by 46%, which was significantly higher than that of either berberine or simvastatin monotherapy, resulting in the reduction of simvastatin dose by 50% that still exerts similar lipid-lowering effect (Kong et al. 2008). It was also reported that combination therapy of berberine (orally, 30 mg/kg/day) with resveratrol (orally, 20 mg/kg/day) in hyperlipidemic mice decreased plasma levels of total cholesterol by 27% and LDL-C by 31.6%, which was significantly greater than that of berberine (10.5% and 9.8%) or the resveratrol (8.4% and 6.6%) monotherapy (Zhu et al. 2018). Therefore, berberine can be considered as an add-on therapy to improve lipid-lowering effects and decrease the therapeutic dosage.

Besides, the cholesterol-lowering effect of berberine is also documented by other studies where it has been shown that administration of berberine markedly reduced the plasma level of LDL-C in animal models of type 2 diabetes mellitus fed with high-fat diet (Zhang et al. 2008, 2011, 2014). Interestingly, in nonobese diabetic (NOD) mice, berberine supplementation (50, 150, and 500 mg/kg/day) significantly

decreased the ratio of LDL-C/TC in a dose-dependent manner (Chueh and Lin 2011). Moreover, studies carried out in animal models of hyperlipidemia and non-alcohol fatty liver disease (NAFLD) have investigated lipid-modulating effects of berberine. In a good agreement with the previous findings, the results of these studies have also confirmed that berberine (alone or in combination with other natural compounds such as curcumin) can facilitate the treatment of hyperlipidemia and NAFLD through its LDL-lowering effects (Feng et al. 2018; Kou et al. 2016; Zhou et al. 2017).

To sum up, berberine in combination with lipid-lowering drugs can efficiently ameliorate hypercholesterolemia, whereby it can reduce therapeutic doses of such drugs, like statins, and thereby might improve treatment of patients who are statin intolerance or statin resistance even at high-dose therapy.

2.2 Clinical Evidence

The beneficial cholesterol-lowering effect of berberine has been verified in numerous clinical trials conducted on patients with mild to moderate hypercholesterolemia in various populations (Table 2). Recently, a systematic review and meta-analysis of 16 randomized clinical trials with a total of 2,147 participants was directed to evaluate the efficacy and safety of berberine in patients with dyslipidemia. The results indicate that berberine can improve plasma lipid profile in dyslipidemia with satisfactory safety, in which significant reduction of TC and LDL-C as well as no significant incidence of adverse events is evident (Ju et al. 2018). A placebo-controlled clinical trial investigating the cholesterol-lowering efficacy of berberine in patients with mild hyperlipidemia showed that oral administration of berberine capsule (900 mg/day, for 3 months) effectively improved the plasma levels of TC and LDL-C (Wang et al. 2016b). Comparably, a clinical trial conducted on Chinese population indicated that oral administration of berberine (1 g/day, for 3 months) decreased the plasma levels of TC by 29% and LDL-C by 35% in patients with hypercholesterolemia suffering from TC levels more than 200 mg/dL (Doggrell 2005). Similarly, another clinical trial conducted on the Caucasian population has demonstrated that berberine treatment (1 g/day, for 3 months) decreased TC by 11% and LDL-C by 16%, without any adverse effects, in hypercholesterolemic patients with low cardiovascular risk (Derosa et al. 2013). Moreover, it was shown that berberine treatment could lower LDL-C (20–30%) (Johnston et al. 2017; Koppen et al. 2017) at a percentage range close to those achieved by statin therapy (30–50%) (Dong et al. 2013).

Cholesterol-lowering effects of berberine have been also indicated by the other studies that investigated the effects of berberine in combination with lipid-lowering nutraceuticals and/or drugs in hypercholesterolemic patients. Of note, berberine has poor intestinal absorption and low oral bioavailability, which limits its therapeutic efficacy. Silymarin, which is a flavonolignan-rich complex extracted from the milk thistle *Silybum marianum* (L.), has been found to possess hepato- and cardio-

Table 2 Clinical trials evaluating cholesterol-lowering effect of berberine in hypercholesterolemic patients

Subject	Treatment group	Does, frequency, duration	Effect on plasma lipid profile	Ref.
Prediabetic patients	Berberine-containing polyherbal dietary supplement, $n = 40$	500 mg/day, once daily, 12 weeks	Decreasing TC and LDL-C	Feinberg et al. (2019)
Patients with mild-to-moderate hypercholesterolemia	Berberine-containing food supplement, $n = 90$; placebo, $n = 90$	500 mg/day, once daily, 4 weeks	LDL-C (−26%)	D'Addato et al. (2017)
Hyperlipidemic patients	Berberine-containing nutraceuticals[a], n = 30 (15/15, M/F); placebo, n = 9 (3/6, M/F)	0.2 g/d, once daily, 12 weeks	Non-HDL-C (−15%), LDL-C (−19%)	Spigoni et al. (2017)
Moderately hypercholesterolemic patients	AP-1[b], n = 51 (18/33, M/F); placebo, n = 51 (14/37, M/F)	500 mg/d, once a day, 12 weeks	TC (−5%), LDL-C (−7.8%)	Sola et al. (2014)
Caucasians with low cardiovascular risk	Berberine, n = 71 (35/36, M/F); placebo, n = 70 (35/35, M/F)	1 g/d, twice daily, 3 months	T-C (−11.6%), LDL-C (−16.4%), HDL-C (+9.1%)	Derosa et al. (2013)
Patients with metabolic syndrome	AP-1, n = 29 (20/9, M/F); placebo, n = 30 (18/12, M/F)	500 mg/d, once daily, 18 weeks	T-C (−15%), LDL-C (−23%)	Affuso et al. (2012)
Hypercholesterolemic patients	AP-1, n = 152 (62/90, M/F); compared to baseline	500 mg/d, once daily, 6 months	TC (−24%), LDL-C (−32%), non-HDL-C (−30%), TG (−20%)	Pisciotta et al. (2012)
Menopausal women with moderate dyslipidemia	Berberine + isoflavones, n = 60; compared to baseline	Berberine and isoflavones combination, 12 weeks	T-C (−14%), LDL-C (−12%), TG (−19%)	Cianci et al. (2012)
Dyslipidemic patients	AP-1, n = 933 (416/518, M/F); placebo, n = 818 (384/434, M/F)	500 mg/d, once daily, 16 weeks	T-C (−10%), LDL-C (−13%), TG (−7%), HDL-C (+8%)	Trimarco et al. (2011)
Elderly (>75 years) hypercholesterolemic patients	AP-1, n = 40 (21/19, M/F); placebo, n = 40 (20/20, M/F)	500 mg/d, once daily, 12 months	T-C (−20%), LDL-C (−31%)	Marazzi et al. (2011)
Hypercholesterolemic patients	AP-1, n = 25 (13/12, M/F); placebo, n = 25 (13/12, M/F)	500 mg/d, once daily, 6 weeks	T-C (−17%), LDL-C (−23%)	Affuso et al. (2010)

(continued)

Table 2 (continued)

Subject	Treatment group	Does, frequency, duration	Effect on plasma lipid profile	Ref.
Hypercholesterolemic patients	Berberine, n = 24; compared to baseline (no sex ratio provided)	1 g/d, twice daily, 2 months	TC (−21.8%), LDL-C (−23.8%), TG (−22.1%)	Kong et al. (2008)
Moderate dyslipidemic subjects	Berberine, n = 20 (8/12, M/F); AP-1, n = 20 (8/12, M/F)	500 mg/d, once daily, 4 weeks	T-C (−16%), LDL-C (−20%), TG (−22%), HDL-C (+7%); berberine and AP-1 did not differ	Cicero et al. (2007)
Hypercholesterolemic patients	Berberine, n = 63 (35/28, M/F); placebo, n = 28 (17/11, M/F)	1 g/d, twice daily, 3 months	T-C (−29%), LDL-C (−25%), TG (−35%)	Kong et al. (2004)

[a]Berberine-containing nutraceutical: berberine 200 mg, chitosan 10 mg, monacolin K 3 mg, and CoQ_{10} 10 mg

[b]AP-1: 1 tablet contains berberine 500 mg, red yeast rice extract 200 mg (equivalent to 3 mg monacolins), policosanol 10 mg, CoQ_{10} 2 mg, folic acid 0.2 mg, and astaxanthin 0.5 mg

protective activities and optimize intestinal berberine absorption (Guarino et al. 2017). A meta-analysis of 19 controlled and cross-sectional trials showed that a combination of berberine with silymarin could significantly improve the cholesterol-lowering effect of berberine (Bertuccioli et al. 2019).

Results from a randomized double-blind, placebo-controlled clinical trial on patients with moderate hypercholesterolemia (LDL-C up to 130–190 mg/dL) without cardiovascular disease demonstrated that, after 2 months treatment, berberine combined with a dry extract of artichoke, a lipid-lowering agent, could exert a significant reduction in plasma TC by 19%, LDL-C by 16%, and non-HDL-C by 19%, with no significant side effect (Cicero et al. 2019).

Furthermore, it has been shown that the oral administration of berberine alone (500 mg/day) or with a combination of lipid-lowering nutraceuticals (consisting policosanols, red yeast extract, folic acid, coenzyme Q_{10}, and astaxanthin) for 4 weeks effectively reduced the plasma levels of LDL-C (by 20% and 25%, respectively) and total cholesterol (by 16% and 20%, respectively) in 40 subjects with moderate dyslipidemia (Cicero et al. 2007). A multicenter, randomized, double-blind, placebo-controlled trial on patients with mild-to-moderate hypercholesterolemia showed that 4 weeks treatment with a daily oral dose of berberine-continuing nutraceutical supplement (red yeast rice, coenzyme Q10, and hydroxytyrosol) had a favorable efficacy and safety profile, wherein LDL-C was decreased by 26%

(D'Addato et al. 2017). Another double-blind placebo-controlled study showed that 6 weeks oral administration of the mentioned combination of berberine and nutraceuticals could decrease plasma levels of LDL-C by 23% and TC by 19% without adverse effects in 25 hypercholesterolemic patients (Affuso et al. 2010). Moreover, 12-month treatment with this combination was found to reduce the plasma levels of TC by 20% and LDL-C by 31% in elderly hypercholesterolemic patients (more than 75 years) who were statin intolerance (Marazzi et al. 2011). The combination containing berberine was also compared with ezetimibe drug in hypercholesterolemic subjects who were intolerant or refusing to take statin; the combination therapy was more effective in decreasing LDL-C (−32% vs − 25%) and TC (−24% vs − 19%) (Pisciotta et al. 2012).

Several clinical trials have also investigated the effects of berberine combined with statins in hypercholesterolemic patients. A recent comprehensive meta-analysis indicates that berberine treatment could synergistically increase cholesterol-lowering potential of statins, while the incidence of statin-related adverse reactions, such as elevation of transaminase and muscle aches, was markedly decreased (Zhang et al. 2019).

Cholesterol-lowering efficacy of berberine is further verified by the other clinical trials in dyslipidemic subjects with various disease conditions, such as prediabetic (Feinberg et al. 2019) and diabetic patients (Bertuccioli et al. 2019; Yin et al. 2008; Zhang et al. 2010), patients with metabolic syndrome (Affuso et al. 2012; Pérez-Rubio et al. 2013), and post-menopause subjects (Affuso et al. 2012).

These findings confirm that berberine improves plasma levels of cholesterol in dyslipidemic conditions, and since dyslipidemia, particularly high level of plasma LDL-C, is an independent risk factor for promotion and progression of atherosclerotic lesions, this natural compound possesses potential preventive and/or therapeutic effects for treating atherosclerosis. Hence, given that atherosclerosis is a chronic disorder, further rigorous clinical studies are needed to better determine the long-term efficacy of berberine and its effects on the progression of atherosclerotic lesions.

2.3 Mechanisms Underlying the Cholesterol-Lowering Effect of Berberine

Berberine is known to reduce plasma cholesterol through modulating cholesterol metabolism and hemostasis. Berberine has been shown to suppress cholesterol biosynthesis through inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase via activating AMP-activated protein kinase (AMPK) in hepatocytes. The activated AMPK phosphorylates the rate-limiting enzyme HMG-CoA reductase and leads to the inactivation of cholesterol biosynthesis (Brusq et al. 2006).

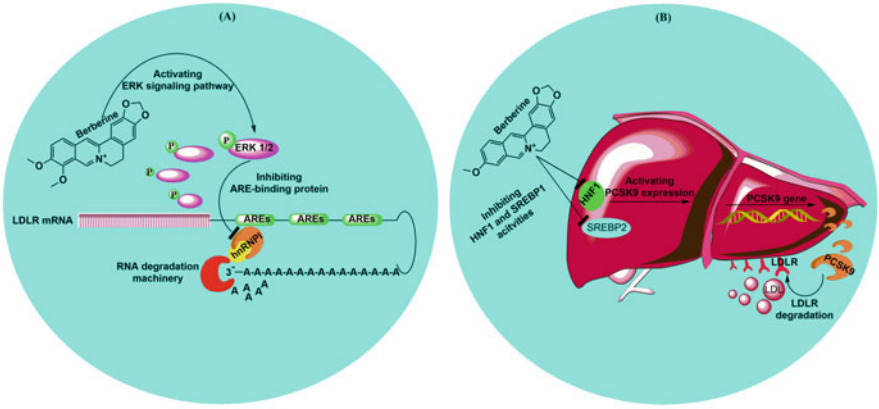

Fig. 1 Schematic view of mechanisms underlying LDLR-dependent cholesterol lowering effects of berberine. (**a**) Berberine can enhance LDLR expression through elevating LDLR mRNA stability via inducing ERK signaling activity that suppresses binding of heterogeneous nuclear ribonucleoprotein 1 (hnRNP I) to AREs, leading to inhibition of RNA degradation machinery. (**b**) Berberine can also induce LDLR expression through inducing the degradation of two cellular trans-activators, hepatocytes nuclear factor 1α (HNF1α) and sterol regulatory element-binding protein 2 (SREBP2)

Moreover, intestinal absorption of dietary cholesterol has an important role in cholesterol hemostasis, and berberine has been shown to inhibit various processes involved in intestinal absorption, including intraluminal cholesterol micellization, cholesterol uptake by enterocytes, and cholesterol esterification in the enterocytes via reducing expression of acyl-coenzyme A cholesterol acyltransferase-2 (Wang et al. 2014).

Plasma cholesterol is mainly removed from the blood circulation by the liver LDL receptor (LDLR). LDLR deficiency increases plasma cholesterol and accelerates atherosclerosis (Umans-Eckenhausen et al. 2002; Xu and Weng 2020). Recently, an in vivo study showed that berberine treatment failed to decrease the plasma levels of cholesterol in hypercholesterolemic LDLR-deficient mice (*Ldlr*$^{-/-}$), providing direct evidence that supports cholesterol-lowering effects of berberine might be mediated, at least in part, by regulating the liver LDLR (Singh and Liu 2019). Further mechanistic studies (Abidi et al. 2005, 2006; Dong et al. 2015; Kong et al. 2006; Li et al. 2009a; Momtazi et al. 2017) indicate that berberine can increase stability of LDLR at both mRNA (Fig. 1a) and protein (Fig. 1b) levels and, thereby, upregulate the expression of hepatic LDLR. The 3′-untranslated region (3′-UTR) of human LDLR mRNA contains three AU-rich elements (AREs) responsible for rapid mRNA turnover, which mediates berberine-induced mRNA stabilization. Berberine was found to extend LDLR mRNA half-life entirely through 3′ UTR in an extracellular receptor-activated kinase (ERK) cascade-dependent manner, in which inhibit interactions of *cis*-regulatory sequences of 3′ UTR and mRNA binding proteins that are downstream effectors of this signaling pathway (Abidi et al. 2005, 2006; Kong et al. 2006). AREs are the well-known RNA cis-regulatory elements that play a

central role in the mRNA stability (Zhang et al. 2002). Further studies highlighted that berberine can inhibit binding of heterogeneous nuclear ribonucleoprotein 1 (hnRNP I), a destabilizing ARE binding protein, to the LDLR mRNA 3′UTR, whereby enhances LDLR mRNA stability resulting in increased LDLR expression and improved plasma LDL-C clearance (Li et al. 2009b; Singh et al. 2014).

Besides, berberine also can increase the stability of LDLR protein on the surface of hepatocytes through post-translational regulation via modulatory effects on PCSK9/LDLR pathway (Cameron et al. 2008; Momtazi et al. 2017; Xiao et al. 2012). PCSK9 is mainly produced by hepatocytes and secreted to the bloodstream where it binds the extracellular domain of hepatic LDLR and targets it to lysosomal degradation, leading to insufficient hepatic LDLR to trap plasma circulating LDL-C (Qian et al. 2007), causing hypercholesterolemia and atherosclerosis-related cardiovascular disease (Abifadel et al. 2003). Mechanistically, berberine can decrease expression of PCSK9 through accelerating the degradation of two cellular transactivators, hepatocytes nuclear factor 1α (HNF1α) and sterol regulatory element-binding protein 2 (SREBP2) that are essential for PCSK9 expression (Dong et al. 2015; Li et al. 2009a; Momtazi et al. 2017). To sum up, berberine can increase the liver LDLR at both mRNA and protein levels through two distinct mechanisms including inhibition of hnRNPI activity and reduction of PCSK9 expression, respectively.

Cholesterol is finally eliminated from the liver via the conversion into bile acids and/or excretion as free cholesterol into bile, resulting in more cholesterol uptake by the liver from the bloodstream. Berberine was found to increase cholesterol secretion from the liver into bile (Guo et al. 2016; Li et al. 2015) and also elevate hepatic expression of enzymes regulating the synthesis of bile acid from cholesterol, such as mitochondrial sterol 27-hydroxylase, which was associated with a significant reduction of plasma cholesterol (Wang et al. 2010).

In conclusion, berberine can regulate plasma cholesterol through inhibiting cholesterol biosynthesis by the liver together with intestinal absorption of dietary cholesterol, elevating liver LDLR stability, and enhancing cholesterol excretion via increasing biosynthesis of bile acid and secretion of free cholesterol.

3 Berberine's Effects on Atherosclerosis Lesion Progression: In Vitro Evidence

3.1 Berberine Improves Endothelial Cell Function

Vascular endothelial dysfunction is known to be a primary event promoting initiation and progression of atherosclerotic lesions, thus improving or repairing endothelium functionality that can exert beneficial effects on the atherosclerotic process (Mordi and Tzemos 2014). Endothelial dysfunction is characterized by elevated oxidative stress and decreased nitric oxide (NO) synthesis/availability, leading to

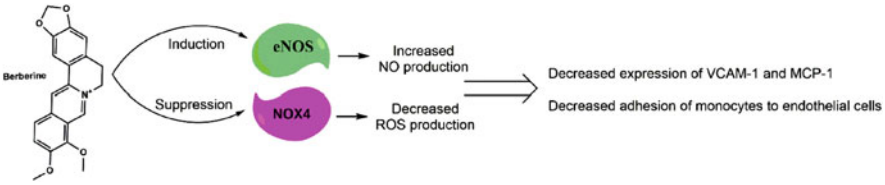

Fig. 2 Improved functions of berberine-treated endothelial cells

proinflammatory and proliferative processes that assist all steps of atherogenesis progression (Yuyun et al. 2018).

Berberine was found to protect against endothelial dysfunction in both the cultured human umbilical vein endothelial cells (HUVECs) and blood vessels isolated from rat aorta. This protective effect of berberine is through activating AMPK signaling that upregulates the expression of endothelial nitric oxide synthase (eNOS) and downregulates the expression of NADPH oxidase (NOX4) resulting in decreased generation of reactive oxygen species (ROS) and increased production of NO (Wang et al. 2009; Zhang et al. 2013).

The activated endothelial cells express adhesion molecules, such as E- and P-selectins, intercellular adhesion molecules (ICAM) like ICAM-1 and vascular cell adhesion molecules (VCAM) like VCAM-1, which act as a receptor for recruitment of the inflammatory monocytes (Collins et al. 2000; Dong et al. 1998; Shih et al. 1999). The improved functionality of injured endothelial cells by berberine treatment can be further supported by other studies showing berberine suppressed the production of ICAM-1 and VCAM-1, leading to a decrease adhesion of monocytes to endothelial cells (Chen et al. 2014; Ko et al. 2007; Wu et al. 2012).

To sum up, berberine can improve endothelial cell function through balancing NO and ROS production via modulating AMPK/eNOS/NOX4 signaling pathway together with reducing the adhesion capacity to monocytes (Fig. 2).

3.2 Berberine Modulates Atherogenic Activities of Macrophages

Macrophages, derived from recruited monocytes, play a central role in atherosclerotic plaque formation and progression. After adhesion, monocytes subsequently infiltrate into the subendothelial layer and differentiate into macrophages. When these cells transmigrate across the endothelial monolayer into the intima, they uptake ox-LDL-C and form the so-called foam cells (Moore et al. 2013; Moore and Tabas 2011).

Berberine was shown to decrease the expression of the infiltration marker CD68, revealing decreased macrophage infiltration into aortic plaques (Chen et al. 2014). Mechanistically, berberine can suppress transendothelial migration through reducing the toll-like receptor (TLR)-mediated macrophage migration ability via inhibiting

the enzymatic activity of Src that is an inducible tyrosine kinase having a pivotal role in cell movement (Cheng et al. 2015). It is further approved by the other study that showed berberine treatment could significantly and highly reduce macrophages contents in mice's atherosclerotic plaques (Yang et al. 2020).

The impaired cholesterol efflux and the excessive internalization of LDL-C lead to cholesterol accumulation in the infiltrated macrophages, causing foam cell formation and atherosclerosis lesion progression (Khera et al. 2011). Reverse cholesterol transporters, such as class B scavenger receptor type I (SR-BI) and ATP-binding cassette transporter (ABC)A1 and G1 (ABCG1), play crucial roles in the efflux of intracellular cholesterol in foam cells (Ohashi et al. 2005). Upon macrophage cholesterol loading, autophagy enhances the lysosomal hydrolysis of stored cholesterol droplets and generates free cholesterol mainly for ABCA1-mediated efflux, whereby it facilitates cholesterol efflux (Ouimet et al. 2011; Razani et al. 2012; Wang et al. 2016a). During atherogenic states, the autophagy process is imparted in macrophages, and berberine treatment was found to enhance autophagy in macrophages, prevent autophagy resistance in the foam cells, and consequently induce cholesterol efflux in both treated cells. Berberine could promote autophagy-dependent cholesterol efflux through suppression of the PI3K/AKT/mTOR pathway resulting in ABCA1-dependent cholesterol efflux in macrophage-derived foam cells (Kou et al. 2017). The positive effect of berberine on cholesterol efflux is further confirmed by other studies that show berberine can increase expression of SR-BI (Chi et al. 2014) and ABCA1/G1 via activating the liver X receptor α (LXRα) transcription factor (Lee et al. 2010; Yang et al. 2020).

Meanwhile, autophagy dysfunction and consequent intracellular lipid accumulation induces macrophage-mediated inflammation and thus exacerbates atherosclerotic development (Razani et al. 2012). Berberine was shown to inhibit oxLDL-induced inflammatory factors, such as macrophage inflammatory protein 1 alpha (MIP-1α) and RANTES in macrophages, through inducing autophagy that was mediated by activation of the AMPK/mTOR signaling pathway (Fan et al. 2015). Berberine was also found to decrease macrophage inflammation through reducing the expression and secretion of tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and Interleukin 6 (IL-6), in vitro (Chen et al. 2008). Potential inhibitory effect of berberine on atherosclerotic plaque progression can be supported by other studies that show therapeutic approaches enhancing autophagy in macrophages potentially prevent or treat atherosclerosis progression (Maiuri et al. 2013; Schrijvers et al. 2011).

Besides, berberine was also demonstrated to inhibit cholesterol accumulation and foam cell formation promoted by the influx of either native or oxLDL-C in macrophages. These cells can internalize native LDL-C through a receptor-independent route called macropinocytosis that has been demonstrated to promote the formation of foam cells and suggests a new therapeutic target to decrease cholesterol accumulation in atherosclerotic plaques (Kruth 2013). Of note, berberine was found to inhibit macropinocytosis and, thereby, decrease cholesterol accumulation and corresponding negative feedbacks promoted by foam cell formation (Zimetti et al. 2015). Macrophages also internalize oxLDL-C using scavenger receptors class A SR

(SR-A), CD36, and lectin-like oxidized LDL receptor-1 (LOX-1), and berberine treatment could reduce the elevated expression of the mentioned receptors and, thereby, decrease cholesterol influx and lipid content in oxLDL-exposed macrophages/foam cells (Chi et al. 2014; Guan et al. 2010; Yang et al. 2020).

Importantly, a plaque with a large lipid core and covered by a thin fibrous cap is at a higher risk for rupture (Schaar et al. 2004). Since the atherosclerotic plaque rupture followed by thrombus formation causes myocardial infarction, stroke, and death (Carr et al. 1996), suppressing the rupture of unstable plaques are crucial to prevent emergency medical conditions. The ruptured fibrous cap is found to be rich in macrophage and foam cells generating matrix metalloproteinases (MMPs) and extracellular MMP inducer (EMMPRIN), which digest extracellular matrix proteins and weaken the fibrous cap, resulting in the vulnerability of atherosclerotic plaques (Gough et al. 2006; Ha et al. 2020; Perrucci et al. 2020; Schmidt et al. 2006). Berberine treatment was indicted to decrease elevated expression of MMPs and EMMPRIN in oxLDL-exposed macrophages (Chen et al. 2014; Huang et al. 2012; Huang et al. 2011), suggesting that berberine can stabilize vulnerable plaques.

To sum up, berberine has potential to prevent or treat atherosclerotic plaque formation and progression, at least in part, through suppressing macrophage migration into the intima, inhibiting foam cell formation and inflammation via suppressing intracellular cholesterol accumulation through enhancing autophagy and cholesterol efflux and reducing cholesterol influx, as well as increasing plaque stability (Fig. 3).

3.3 Berberine Blocks Proliferation and Migration of Vascular Smooth Muscle Cells

Vascular smooth muscle cells (VSMCs) are a major cell type contributing to atherosclerotic lesion progression. Proliferation and migration of VSMCs from media to intima layers of vascular wall and formation of muscle-origin foam cells after lipid ingestion promote the intimal thickening and, thereby, exert an important role in atherosclerosis pathology (Basatemur et al. 2019; Bennett et al. 2016). Therefore, inhibiting VSMCs proliferation and migration can beneficially affect atherosclerotic plaque initiation and progression.

Arterial injury is known to promote cell cycle re-entry in VSMCs, following a wave of immediate early gene expression. After the injury, activation of extracellular signal-regulated kinase (ERK) is among the earliest of biochemical changes and is strongly associated with subsequent expression of early response transcription factors and growth factors (Kim et al. 1998; Koyama et al. 1998; Roostalu and Wong 2018).

ERK is a key transducer of extracellular signals that induce cell growth and movement, which are essential for the promotion and development of vascular lesions (Lu et al. 2020). ERK transmits mitogenic signals by translocation to the nucleus where activates many of its substrates, such as early growth response factor

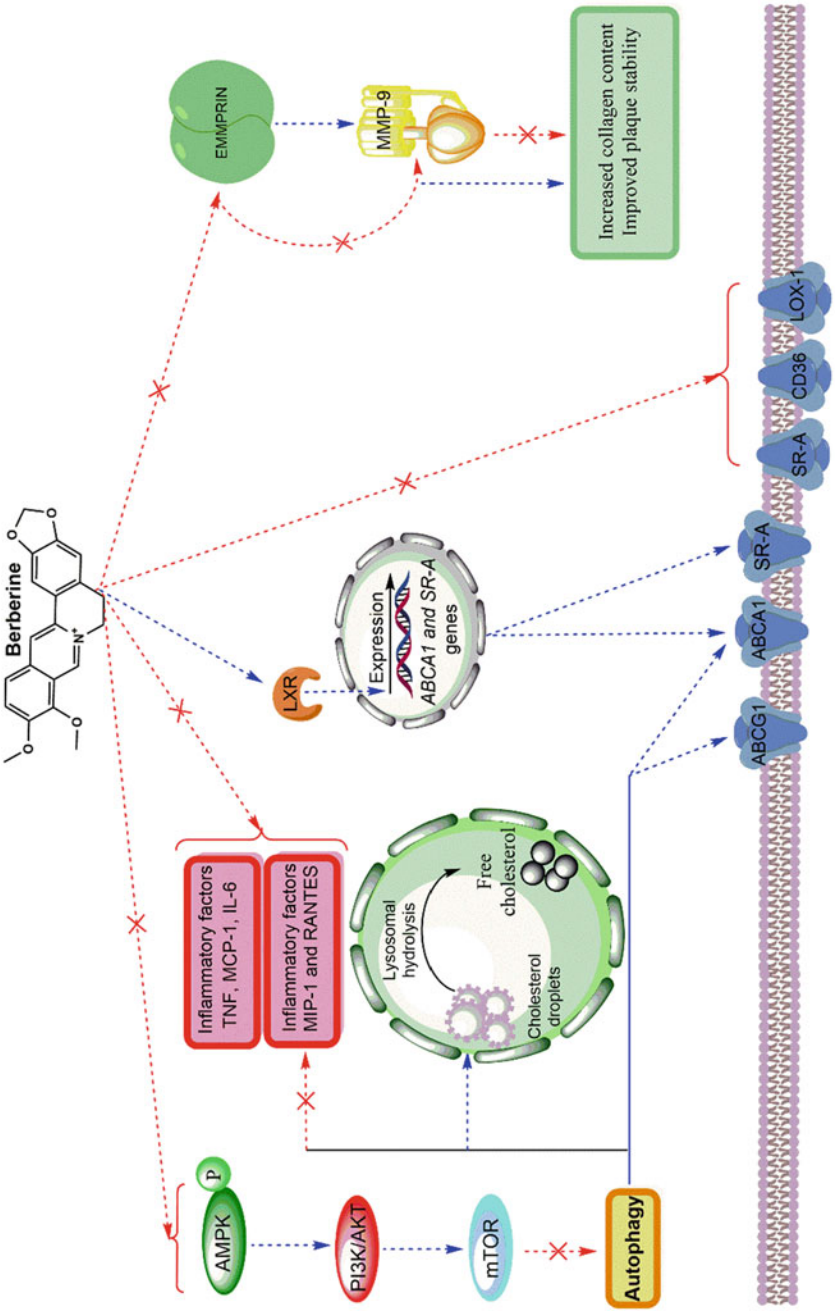

Fig. 3 Alleviating effects of berberine on atherogenic function of macrophages. Berberine can inhibit intracellular cholesterol accumulation and foam cell formation through inducing autophagy via suppressing AMPK/PI3K/AKT/mTOR signaling pathway. The autophagy activation leads to the lysosomal

hydrolysis of cholesterol droplets and the increased ATP-binding cassette transporter A1 (ABCA1) and ABCG1 activity, resulting in the enhanced cholesterol efflux activity. Activated autophagy can also suppress oxLDL-induced inflammatory factors, such as macrophage inflammatory Protein 1 alpha (MIP-1α) and RANTES in macrophages. Berberine can also increase expression of ABCA1 and class B scavenger receptor type I (SR-BI) through inducing the activity of LXR-α transcription factor. Besides, berberine can also reduce intracellular cholesterol accumulation through reducing the expression of cholesterol influx receptors, including class A scavenger receptor (SR-A), CD38, and LOX-1. Berberine mediates stabilization of atherosclerotic plaques through reducing the macrophage-produced matrix metalloproteinase-9 (MMP-9) via inhibiting activity of extracellular MMP inducer (EMMPRIN)

1 (Egr-1) (Gille et al. 1995; Goetze et al. 1999). Egr-1 is a zinc finger transcription factor that is lowly expressed in the normal vessel wall but is rapidly and transiently expressed in VSMCs in response to arterial injury (Khachigian et al. 1996; Kim et al. 1995). The activated Egr-1 regulates the expression of several genes participating in cell growth and differentiation, such as platelet-derived growth factor (PDGF) (Havis and Duprez 2020; Khachigian et al. 1996; Khachigian et al. 1995; Silverman et al. 1997) and Cyclins (Guillemot et al. 2001; Havis and Duprez 2020; Yan et al. 1997), which are known to be implicated in the pathogenesis of atherosclerosis (Guillemot et al. 2001; Havis and Duprez 2020; Khachigian and Collins 1997; Yan et al. 1997). Berberine has been found to abolish injury-induced VSMC regrowth through the inactivation of ERK and inhibiting the expression of Egr-1 and downstream production of growth mediators Cyclin D1 and PDGF-A, thereby preventing early signaling related with cell cycle re-entry induced by injury. Of note, the inhibitory effect of berberine was identified to be by suppressing the activity of mitogen-activated protein kinase 1/2 (MEK1/2) that mediates activation of ERK/Egr-1 signaling by injury (Liang et al. 2006).

Moreover, PDGF is an important growth factor released after vascular injury and is related to VSMC proliferation and migration. Berberine was shown to inhibit PDGF-stimulated VSMC proliferation through activating AMPK/p53/$p21^{Cip1}$ signaling and inactivation the Rac1/Cyclin D/Cyclin-dependent kinase (Cdk) leading to G1 arrest (Liang et al. 2008). AMPK is a serine/threonine protein kinase that is known to suppress PDGF-induced proliferation in human aortic VSMC (Igata et al. 2005). The activated AMPK can induce a cell cycle G1 arrest via AMPK-dependent phosphorylation of p53 in human VSMCs (Igata et al. 2005). Inhibition of PDGF-induced VSMC proliferation by berberine was indicated to be mediated in part through increasing the activity of AMPK, which led to the activation of p53 phosphorylation and up-regulation of the Cdk inhibitor $p21^{Cip1}$ (Liang et al. 2008). Furthermore, Rac1 is a signaling GTPase that regulates a wide variety of cellular activities, including cell proliferation, migration, and apoptosis. Rac1 is known to mediate PDGF-induced VSMC proliferation through activating the production of cell cycle regulatory molecules Cyclins and Cdks that are responsible for G1/S cell cycle transition (Liang et al. 2008). Of note, berberine was shown to inhibit PDGF-induced Rac1 activation and upregulation of Cyclin D1, Cyclin D3, Cdk2, and Cdk4, whereby it induces G1-phase arrest in VSMCs (Liang et al. 2008).

In addition to promoting cell proliferation, PDGF can also stimulate migration in VSMCs, as PDGF is the most potent reported chemoattractant for VSMCs (Zhang et al. 2018). Many reports show PDGF increases both Rac1 activity and cell migration (Al-Koussa et al. 2020). Herewith, berberine was also found to PDGF-induced VSMCs migration through inhibiting activation of Rac1 and another GTPase, Cdc42, that mediate the migratory function of PDGF through VSMCs injury (Liang et al. 2008).

Regarding the mechanism of berberine on the inhibition of Rac1 and Cdc42, there has been evidence that AMPK activation could lead to suppression of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis (Liang et al. 2008). Suppression of HMG-CoA reductase decreases cholesterol synthesis as well as

some critical isoprenoids downstream of mevalonate such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are essential for membrane translocation and activation of Rac1 and Cdc42 (Liang et al. 2008). Therefore, AMPK activation by berberine can drive suppression of HMG-CoA reductase and reduce downstream isoprenoids that are required for Rac1 and Cdc42. Hence, berberine can indirectly inhibit activation of Rac1 and Cdc42 and prevent cell proliferation and migration induced by PDGF in VSMCs.

In conclusion, berberine-elicited anti-proliferative and anti-migratory effects in the injured VSMCs are related to a multifaceted attack on multiple signaling targets that critically participated in growth suppression. Given that abnormal proliferation and migration of VSMCs is a hallmark event in atherosclerotic lesions development, such findings can support the preventive effect of berberine on initiation and progression of atherosclerosis lesions.

4 Berberine's Effects on Atherosclerosis Lesion Progression: In Vivo Evidence

There are several in vivo studies on mouse models of atherosclerosis that support the abovementioned in vitro studies showing potential anti-atherogenic effects of berberine. ApoE$^{-/-}$ mouse fed with the atherogenic diet is the most common model of human atherosclerosis that is widely used to evaluate the preventive or therapeutic effects of drugs on atherosclerosis lesion progression.

It was found that oral gavage of berberine at a low dose (50 mg/kg/day) and a high dose (100 mg/kg/day) after 13 weeks could significantly decrease atherosclerotic plaque area and lesion size in ApoE$^{-/-}$ mice bearing atherosclerosis, in which the high dose was found to be more effective (Wu et al. 2020). Further study reveals that berberine treatment (8 weeks) could decrease vascular inflammation and oxidative stress via an AMPK-dependent mechanism, leading to a significant reduction of atherosclerotic plaque progression in mouse aorta (Wang et al. 2011). However, another in vivo study indicated that berberine treatment at a lower dose (orally 10 mg/kg/day, for 16 weeks) failed to exert a significant effect on atherosclerosis plaque progression in hypercholesterolemic ApoE$^{-/-}$ mice, while the same dose of its derivatives, dihydroberberine and 8,8-dimethyldi-hydroberberine possessing higher bioavailability, were found to at the same dose effectively decrease atherosclerotic plaque size and improve plaque stability (Chen et al. 2014). This finding was suggested to be due to poor intestinal absorption of berberine (Chen et al. 2014). Thus, oral administrating of berberine derivatives with improved intestinal absorption or its administration via alternative routes can enhance therapeutic potential. It can be further supported by a recent in vivo study that showed berberine (10 mg/kg/day, for 14 weeks) via intraperitoneal injection in hypercholesterolemic ApoE$^{-/-}$ mice could decrease the size of atherosclerotic plaque in aortic sinus by 45% (Yang et al. 2020).

Cellular and molecular examination of atherosclerosis lesions isolated from berberine-treated mice reveals that berberine can suppress atherosclerosis plaque progression through mechanisms consistent with those found by in vitro studies discussed in previous sections. Of note, berberine has been indicated to reduce macrophage content in mice's atherosclerotic plaques through reducing macrophage adhesion and infiltration to the vascular endothelium via inhibiting the expression of two critical adhesion molecules ICAM-1 and VCAM-1 (Chen et al. 2014; Yang et al. 2020). Moreover, berberine treatment was shown to reduce lipids contents in aortic macrophages through increasing expression of ABCA1/G1-mediated cholesterol efflux and reducing expression of SR-A/CD36-mediated cholesterol influx in hypercholesterolemic ApoE$^{-/-}$ mice (Yang et al. 2020). Likewise, immunohistochemical assays of mice's aortic plaque showed that berberine treatment decreased protein expression of MMP-9 and EMMPRIN and whereby increased collagen content and, consequently, improved fibrous cap thickness and plaque stability (Chen et al. 2014).

Overall, these results indicate that berberine inherently has the potential to protect and ameliorate the progression of atherosclerotic plaques. Since there is no clinically approved lipid-lowering agent that can target atherosclerotic plaques and locally inhibit the function of cellular and molecular mediators in lesion progression, these findings underscore the therapeutic importance of berberine for preventing and treating atherosclerosis. However, low bioavailability of berberine can influence such beneficial effects, and, therefore, further preclinical studies are required to determine its effectual derivatives or administration routes, such as intravenous injection, for providing an efficient anti-atherosclerotic therapeutic tool.

5 Conclusion

Berberine is found to be a promising anti-atherogenic agent protecting against atherosclerosis progression, as it can modulate cholesterol metabolism and hemostasis and the other important atherogenic factors directly involved in atherosclerotic lesion formation. The majority of findings consistently demonstrate that the lipid-lowering effect of berberine is mainly in term of reducing TC and LDL-C through several LDLR-dependent and LDLR -independent mechanisms. Berberine decreases plasma levels of LDL-C through upregulating the mRNA and protein expression of the hepatic LDLR via two distinct mechanisms, including evaluation of LDLR mRNA stability and inhibition of PCSK9, a main negative regulator of LDLR protein. Besides the LDLR-mediated mechanism, berberine also can impact cholesterol hemostasis through regulating the other important factors, including liver biosynthesis of cholesterol, intestinal absorption of dietary cholesterol, as well as bile acid synthesis and secretion, and secretion of free cholesterol. Berberine has been also found to have anti-inflammatory and anti-oxidant abilities, whereby it can modulate function and proliferation of inflammatory macrophages, VSMC, and endothelial cells that directly collaborate in atherosclerotic lesion formation.

Among the signaling pathways through which berberine regulates intracellular processes, AMPK has a central and critical role. Interestingly, activated AMPK contributes to various mechanisms involved in the atheroprotective effect of berberine, including suppressing cholesterol biosynthesis, improving endothelial dysfunction via upregulating the expression of eNOS and downregulating the expression of NOX4, as well as blocking injury-stimulated VSMCs regrowth and proliferation.

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References

Abidi P, Zhou Y, Jiang J-D, Liu J (2005) Extracellular signal-regulated kinase–dependent stabilization of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine. Arterioscler Thromb Vasc Biol 25(10):2170–2176

Abidi P, Chen W, Kraemer FB, Li H, Liu J (2006) The medicinal plant goldenseal is a natural LDL-lowering agent with multiple bioactive components and new action mechanisms. J Lipid Res 47(10):2134–2147

Abifadel M, Varret M, Rabès J-P, Allard D, Ouguerram K, Devillers M et al (2003) Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 34(2):154–156

Affuso F, Ruvolo A, Micillo F, Saccà L, Fazio S (2010) Effects of a nutraceutical combination (berberine, red yeast rice and policosanols) on lipid levels and endothelial function randomized, double-blind, placebo-controlled study. Nutr Metab Cardiovasc Dis 20(9):656–661

Affuso F, Mercurio V, Ruvolo A, Pirozzi C, Micillo F, Carlomagno G et al (2012) A nutraceutical combination improves insulin sensitivity in patients with metabolic syndrome. World J Cardiol 4(3):77

Al-Koussa H, El Atat O, Jaafar L, Tashjian H, El-Sibai M (2020) The role of Rho GTPases in motility and invasion of glioblastoma cells. Anal Cell Pathol 2020

Ayati SH, Fazeli B, Momtazi-Borojeni AA, Cicero AFG, Pirro M, Sahebkar A (2017) Regulatory effects of berberine on microRNome in cancer and other conditions. Crit Rev Oncol Hematol 116:147–158

Basatemur GL, Jørgensen HF, Clarke MCH, Bennett MR, Mallat Z (2019) Vascular smooth muscle cells in atherosclerosis. Nat Rev Cardiol 1:18

Bennett MR, Sinha S, Owens GK (2016) Vascular smooth muscle cells in atherosclerosis. Circ Res 118(4):692–702

Bertuccioli A, Moricoli S, Amatori S, Rocchi MBL, Vici G, Sisti D (2019) Berberine and dyslipidemia: different applications and biopharmaceutical formulations without statin-like molecules—a meta-analysis. J Med Food 23(2):101–113

Briand F, Thieblemont Q, Muzotte E, Sulpice T (2013) Upregulating reverse cholesterol transport with cholesteryl ester transfer protein inhibition requires combination with the LDL-lowering drug berberine in dyslipidemic hamsters. Arterioscler Thromb Vasc Biol 33(1):13–23

Brusq J-M, Ancellin N, Grondin P, Guillard R, Martin S, Saintillan Y, Issandou M (2006) Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine. J Lipid Res 47(6):1281–1288

Cameron J, Ranheim T, Kulseth MA, Leren TP, Berge KE (2008) Berberine decreases PCSK9 expression in HepG2 cells. Atherosclerosis 201(2):266–273

Carr S, Farb A, Pearce WH, Virmani R, Yao JST (1996) Atherosclerotic plaque rupture in symptomatic carotid artery stenosis. J Vasc Surg 23(5):755–766

Chang XX, Yan HM, Fei J, Jiang MH, Zhu HG, Lu DR, Gao X (2010) Berberine reduces methylation of the MTTP promoter and alleviates fatty liver induced by a high-fat diet in rats. J Lipid Res 51(9):2504–2515

Chang X-x, Yan H-m, Xu Q, Xia M-f, Bian H, Zhu T-f, Gao X (2012) The effects of berberine on hyperhomocysteinemia and hyperlipidemia in rats fed with a long-term high-fat diet. Lipids Health Dis 11(1):86

Chen FL, Yang ZH, Liu Y, Li LX, Liang WC, Wang XC et al (2008) Berberine inhibits the expression of TNFα, MCP-1, and IL-6 in AcLDL-stimulated macrophages through PPARγ pathway. Endocrine 33(3):331–337

Chen J, Cao J, Fang L, Liu B, Zhou Q, Sun Y et al (2014) Berberine derivatives reduce atherosclerotic plaque size and vulnerability in apoE−/− mice. J Transl Med 12(1):326

Cheng WE, Chang MY, Wei JY, Chen YJ, Maa MC, Leu TH (2015) Berberine reduces toll-like receptor-mediated macrophage migration by suppression of Src enhancement. Eur J Pharmacol 757:1–10. https://doi.org/10.1016/j.ejphar.2015.03.013

Chi L, Peng L, Hu X, Pan N, Zhang Y (2014) Berberine combined with atorvastatin downregulates LOX1 expression through the ET1 receptor in monocyte/macrophages. Int J Mol Med 34 (1):283–290. https://doi.org/10.3892/ijmm.2014.1748

Chueh W-H, Lin J-Y (2011) Berberine, an isoquinoline alkaloid in herbal plants, protects pancreatic islets and serum lipids in nonobese diabetic mice. J Agric Food Chem 59(14):8021–8027

Cianci A, Cicero AFG, Colacurci N, Matarazzo MG, Leo D, Vincenzo (2012) Activity of isoflavones and berberine on vasomotor symptoms and lipid profile in menopausal women. Gynecol Endocrinol 28(9):699–702

Cicero AFG, Rovati LC, Setnikar I (2007) Eulipidemic effects of berberine administered alone or in combination with other natural cholesterol-lowering agents. Arzneimittelforschung 57 (01):26–30

Cicero AF, Fogacci F, Bove M, Giovannini M, Veronesi M, Borghi C (2019) Short-term effects of dry extracts of artichokeand berberis in hypercholesterolemic patients without cardiovascular disease. Am J Cardiol 123(4):588–591

Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, Beaudet AL (2000) P-selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E–deficient mice. J Exp Med 191(1):189–194

D'Addato S, Scandiani L, Mombelli G, Focanti F, Pelacchi F, Salvatori E et al (2017) Effect of a food supplement containing berberine, monacolin K, hydroxytyrosol and coenzyme Q10 on lipid levels: a randomized, double-blind, placebo controlled study. Drug Des Devel Ther 11:1585

Derosa G, D'Angelo A, Bonaventura A, Bianchi L, Romano D, Maffioli P (2013) Effects of berberine on lipid profile in subjects with low cardiovascular risk. Expert Opin Biol Ther 13 (4):475–482

Doggrell SA (2005) Berberine--a novel approach to cholesterol lowering. Expert Opin Investig Drugs 14(5):683–685. https://doi.org/10.1517/13543784.14.5.683

Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD (1998) The combined role of P-and E-selectins in atherosclerosis. J Clin Invest 102(1):145–152

Dong H, Zhao Y, Zhao L, Lu F (2013) The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. Planta Med 79(06):437–446

Dong B, Li H, Singh AB, Cao A, Liu J (2015) Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1alpha protein expression through the ubiquitin-proteasome degradation pathway. J Biol Chem 290(7):4047–4058. https://doi.org/10.1074/jbc.M114.597229

Fan X, Wang J, Hou J, Lin C, Bensoussan A, Chang D et al (2015) Berberine alleviates ox-LDL induced inflammatory factors by up-regulation of autophagy via AMPK/mTOR signaling pathway. J Transl Med 13:92–92. https://doi.org/10.1186/s12967-015-0450-z

Feinberg T, Wieland LS, Miller LE, Munir K, Pollin TI, Shuldiner AR et al (2019) Polyherbal dietary supplementation for prediabetic adults: study protocol for a randomized controlled trial. Trials 20(1):1–13

Feng W-w, Kuang S-y, Tu C, Ma Z-j, Pang J-y, Wang Y-h et al (2018) Natural products berberine and curcumin exhibited better ameliorative effects on rats with non-alcohol fatty liver disease than lovastatin. Biomed Pharmacother 99:325–333

Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E et al (2017) Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 38(32):2459–2472

Gille H, Kortenjann M, Thomae O, Moomaw C, Slaughter C, Cobb MH, Shaw PE (1995) ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation. EMBO J 14(5):951–962

Goetze S, Xi X-P, Graf K, Fleck E, Hsueh WA, Law RE (1999) Troglitazone inhibits angiotensin II-induced extracellular signal-regulated kinase 1/2 nuclear translocation and activation in vascular smooth muscle cells. FEBS Lett 452(3):277–282

Gough PJ, Gomez IG, Wille PT, Raines EW (2006) Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice. J Clin Invest 116(1):59–69

Guan S, Wang B, Li W, Guan J, Fang X (2010) Effects of berberine on expression of LOX-1 and SR-BI in human macrophage-derived foam cells induced by ox-LDL. Am J Chin Med 38 (06):1161–1169

Guarino G, Strollo F, Carbone L, Della TC, Letizia M, Marino G, Gentile S (2017) Bioimpedance analysis, metabolic effects and safety of the association Berberis aristata/Bilybum marianum: a 52-week double-blind, placebo-controlled study in obese patients with type 2 diabetes. J Biol Regul Homeost Agents 31(2):495–502

Guillemot L, Levy A, Raymondjean M, Rothhut B (2001) Angiotensin II-induced transcriptional activation of the cyclin D1 gene is mediated by Egr-1 in CHO-AT1A cells. J Biol Chem 276 (42):39394–39403

Guo Y, Zhang Y, Huang W, Selwyn FP, Klaassen CD (2016) Dose-response effect of berberine on bile acid profile and gut microbiota in mice. BMC Complement Altern Med 16(1):394. https://doi.org/10.1186/s12906-016-1367-7

Ha S-H, Choi H, Park J-Y, Abekura F, Lee Y-C, Kim J-R, Kim C-H (2020) Mycobacterium tuberculosis–secreted protein, ESAT-6, inhibits lipopolysaccharide-induced MMP-9 expression and inflammation through NF-κB and MAPK signaling in RAW 264.7 macrophage cells. Inflammation 43(1):54–65

Havis E, Duprez D (2020) Egr1 transcription factor is a multifaceted regulator of matrix production in tendons and other connective tissues. Int J Mol Sci 21(5):1664

He K, Kou S, Zou Z, Hu Y, Feng M, Han B et al (2016) Hypolipidemic effects of alkaloids from Rhizoma Coptidis in diet-induced hyperlipidemic hamsters. Planta Med 82(08):690–697

Hu Y, Davies GE (2010) Berberine inhibits adipogenesis in high-fat diet-induced obesity mice. Fitoterapia 81(5):358–366

Hu Y, Ehli EA, Kittelsrud J, Ronan PJ, Munger K, Downey T et al (2012) Lipid-lowering effect of berberine in human subjects and rats. Phytomedicine 19(10):861–867

Huang Z, Wang L, Meng S, Wang Y, Chen T, Wang C (2011) Berberine reduces both MMP-9 and EMMPRIN expression through prevention of p38 pathway activation in PMA-induced macrophages. Int J Cardiol 146(2):153–158

Huang Z, Meng S, Wang L, Wang Y, Chen T, Wang C (2012) Suppression of oxLDL-induced MMP-9 and EMMPRIN expression by berberine via inhibition of NF-kappaB activation in human THP-1 macrophages. Anat Rec 295(1):78–86. https://doi.org/10.1002/ar.21489

Igata M, Motoshima H, Tsuruzoe K, Kojima K, Matsumura T, Kondo T et al (2005) Adenosine monophosphate-activated protein kinase suppresses vascular smooth muscle cell proliferation through the inhibition of cell cycle progression. Circ Res 97(8):837–844

Imanshahidi M, Hosseinzadeh H (2008) Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother Res 22(8):999–1012
Jia X, Chen Y, Zidichouski J, Zhang J, Sun C, Wang Y (2008) Co-administration of berberine and plant stanols synergistically reduces plasma cholesterol in rats. Atherosclerosis 201(1):101–107
Johnston TP, Korolenko TA, Pirro M, Sahebkar A (2017) Preventing cardiovascular heart disease: promising nutraceutical and non-nutraceutical treatments for cholesterol management. Pharmacol Res 120:219–225
Ju J, Li J, Lin Q, Xu H (2018) Efficacy and safety of berberine for dyslipidaemias: a systematic review and meta-analysis of randomized clinical trials. Phytomedicine 50:25–34
Khachigian LM, Williams AJ, Collins T (1995) Interplay of Sp1 and Egr-1 in the proximal platelet-derived growth factor A-chain promoter in cultured vascular endothelial cells. J Biol Chem 270 (46):27679–27686
Khachigian LM, Lindner V, Williams AJ, Collins T (1996) Egr-1-induced endothelial gene expression: a common theme in vascular injury. Science 271(5254):1427–1431
Khachigian LM, Collins T (1997) Inducible expression of Egr-1–dependent genes: a paradigm of transcriptional activation in vascular endothelium. Circ Res 81(4):457–461
Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K et al (2011) Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 364(2):127–135
Kim S, Kawamura M, Wanibuchi H, Ohta K, Hamaguchi A, Omura T et al (1995) Angiotensin II type 1 receptor blockade inhibits the expression of immediate-early genes and fibronectin in rat injured artery. Circulation 92(1):88–95
Kim S, Izumi Y, Yano M, Hamaguchi A, Miura K, Yamanaka S et al (1998) Angiotensin blockade inhibits activation of mitogen-activated protein kinases in rat balloon-injured artery. Circulation 97(17):1731–1737
Kim WS, Lee YS, Cha SH, Jeong HW, Choe SS, Lee M-R et al (2009) Berberine improves lipid dysregulation in obesity by controlling central and peripheral AMPK activity. Am J Physiol Endocrinol Metab 296(4):E812–E819
Kim M, Kim T-W, Kim C-J, Shin M-S, Hong M, Park H-S, Park S-S (2019) Berberine ameliorates brain inflammation in poloxamer 407-induced hyperlipidemic rats. Int Neurourol J 23(Suppl 2): S102
Ko YJ, Lee J-S, Park BC, Shin HM, Kim J-A (2007) Inhibitory effects of Zoagumhwan water extract and berberine on angiotensin II-induced monocyte chemoattractant protein (MCP)-1 expression and monocyte adhesion to endothelial cells. Vasc Pharmacol 47(2–3):189–196
Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C et al (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 10(12):1344–1351
Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C et al (2006) Berberine is a promising novel cholesterol-lowering drug working through a unique mechanism distinct from statins. In: Paper presented at the Atherosclerosis Supplements
Kong W-J, Wei J, Zuo Z-Y, Wang Y-M, Song D-Q, You X-F et al (2008) Combination of simvastatin with berberine improves the lipid-lowering efficacy. Metabolism 57(8):1029–1037
Koppen LM, Whitaker A, Rosene A, Beckett RD (2017) Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: a systematic review. J Evid Based Complementary Altern Med 22(4):956–968
Kou S, Han B, Wang Y, Huang T, He K, Han Y et al (2016) Synergetic cholesterol-lowering effects of main alkaloids from Rhizoma Coptidis in HepG2 cells and hypercholesterolemia hamsters. Life Sci 151:50–60
Kou JY, Li Y, Zhong ZY, Jiang YQ, Li XS, Han XB et al (2017) Berberine-sonodynamic therapy induces autophagy and lipid unloading in macrophage. Cell Death Dis 8(1):e2558–e2558. https://doi.org/10.1038/cddis.2016.354
Koyama H, Olson NE, Dastvan FF, Reidy MA (1998) Cell replication in the arterial wall: activation of signaling pathway following in vivo injury. Circ Res 82(6):713–721

Kruth HS (2013) Fluid-phase pinocytosis of LDL by macrophages: a novel target to reduce macrophage cholesterol accumulation in atherosclerotic lesions. Curr Pharm Des 19 (33):5865–5872

Lee TS, Pan CC, Peng CC, Kou YR, Chen CY, Ching LC et al (2010) Anti-atherogenic effect of berberine on LXRalpha-ABCA1-dependent cholesterol efflux in macrophages. J Cell Biochem 111(1):104–110. https://doi.org/10.1002/jcb.22667

Li Y-H, Yang P, Kong W-J, Wang Y-X, Hu C-Q, Zuo Z-Y et al (2008) Berberine Analogues as a novel class of the low-density-lipoprotein receptor up-regulators: synthesis, structure− activity relationships, and cholesterol-lowering efficacy. J Med Chem 52(2):492–501

Li H, Chen W, Zhou Y, Abidi P, Sharpe O, Robinson WH et al (2009a) Identification of mRNA binding proteins that regulate the stability of LDL receptor mRNA through AU-rich elements. J Lipid Res 50(5):820–831. https://doi.org/10.1194/jlr.M800375-JLR200

Li H, Dong B, Park SW, Lee HS, Chen W, Liu J (2009b) Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. J Biol Chem 284(42):28885–28895. https://doi.org/10.1074/jbc.M109.052407

Li G-S, Liu X-H, Zhu H, Huang L, Liu Y-L, Ma C-M, Qin C (2011) Berberine-improved visceral white adipose tissue insulin resistance associated with altered sterol regulatory element-binding proteins, liver X receptors, and peroxisome proliferator-activated receptors transcriptional programs in diabetic hamsters. Biol Pharm Bull 34(5):644–654

Li Z, Geng Y-N, Jiang J-D, Kong W-J (2014) Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. Evid Based Complement Alternat Med 2014:289264

Li X-Y, Zhao Z-X, Huang M, Feng R, He C-Y, Ma C et al (2015) Effect of Berberine on promoting the excretion of cholesterol in high-fat diet-induced hyperlipidemic hamsters. J Transl Med 13 (1):278

Liang K-W, Ting C-T, Yin S-C, Chen Y-T, Lin S-J, Liao JK, Hsu S-L (2006) Berberine suppresses MEK/ERK-dependent Egr-1 signaling pathway and inhibits vascular smooth muscle cell regrowth after in vitro mechanical injury. Biochem Pharmacol 71(6):806–817. https://doi.org/10.1016/j.bcp.2005.12.028

Liang K-W, Yin S-C, Ting C-T, Lin S-J, Hsueh C-M, Chen C-Y, Hsu S-L (2008) Berberine inhibits platelet-derived growth factor-induced growth and migration partly through an AMPK-dependent pathway in vascular smooth muscle cells. Eur J Pharmacol 590(1):343–354. https://doi.org/10.1016/j.ejphar.2008.06.034

Lu Y, Sun X, Peng L, Jiang W, Li W, Yuan H, Cai J (2020) Angiotensin II-Induced vascular remodeling and hypertension involves cathepsin L/V-MEK/ERK mediated mechanism. Int J Cardiol 298:98–106

Maiuri MC, Grassia G, Platt AM, Carnuccio R, Ialenti A, Maffia P (2013) Macrophage autophagy in atherosclerosis. Mediat Inflamm 2013:584715

Marazzi G, Cacciotti L, Pelliccia F, Iaia L, Volterrani M, Caminiti G et al (2011) Long-term effects of nutraceuticals (berberine, red yeast rice, policosanol) in elderly hypercholesterolemic patients. Adv Ther 28(12):1105–1113

Momtazi AA, Banach M, Pirro M, Katsiki N, Sahebkar A (2017) Regulation of PCSK9 by nutraceuticals. Pharmacol Res 120:157–169

Moore KJ, Tabas I (2011) Macrophages in the pathogenesis of atherosclerosis. Cell 145 (3):341–355

Moore KJ, Sheedy FJ, Fisher EA (2013) Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 13(10):709

Mordi I, Tzemos N (2014) Is reversal of endothelial dysfunction still an attractive target in modern cardiology? World J Cardiol 6(8):824

Ohashi R, Mu H, Wang X, Yao Q, Chen C (2005) Reverse cholesterol transport and cholesterol efflux in atherosclerosis. QJM 98(12):845–856

Ouimet M, Franklin V, Mak E, Liao X, Tabas I, Marcel YL (2011) Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase. Cell Metab 13(6):655–667

Pérez-Rubio KG, González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA, Espinel-Bermúdez MC (2013) Effect of berberine administration on metabolic syndrome, insulin sensitivity, and insulin secretion. Metab Syndr Relat Disord 11(5):366–369

Perrucci GL, Rurali E, Corlianò M, Balzo M, Piccoli M, Moschetta D et al (2020) Cyclophilin A/EMMPRIN axis is involved in pro-fibrotic processes associated with thoracic aortic aneurysm of marfan syndrome patients. Cell 9(1):154

Pisciotta L, Bellocchio A, Bertolini S (2012) Nutraceutical pill containing berberine versus ezetimibe on plasma lipid pattern in hypercholesterolemic subjects and its additive effect in patients with familial hypercholesterolemia on stable cholesterol-lowering treatment. Lipids Health Dis 11(1):123

Qian Y-W, Schmidt RJ, Zhang Y, Chu S, Lin A, Wang H et al (2007) Secreted PCSK9 downregulates low density lipoprotein receptor through receptor-mediated endocytosis. J Lipid Res 48(7):1488–1498

Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S et al (2012) Autophagy links inflammasomes to atherosclerotic progression. Cell Metab 15(4):534–544

Ridker PM, Revkin J, Amarenco P, Brunell R, Curto M, Civeira F et al (2017) Cardiovascular efficacy and safety of bococizumab in high-risk patients. N Engl J Med 376(16):1527–1539

Roostalu U, Wong JKF (2018) Arterial smooth muscle dynamics in development and repair. Dev Biol 435(2):109–121

Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA et al (2017) FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 376(18):1713–1722

Sahebkar A, Watts GF (2013) New therapies targeting apoB metabolism for high-risk patients with inherited dyslipidaemias: what can the clinician expect? Cardiovasc Drugs Ther 27(6):559–567. https://doi.org/10.1007/s10557-013-6479-4

Schaar JA, Muller JE, Falk E, Virmani R, Fuster V, Serruys PW et al (2004) Terminology for high-risk and vulnerable coronary artery plaques. Eur Heart J 25(12):1077–1082

Schmidt R, Redecke V, Breitfeld Y, Wantia N, Miethke T, Massberg S et al (2006) EMMPRIN (CD 147) is a central activator of extracellular matrix degradation by Chlamydia pneumoniae-infected monocytes. Thromb Haemost 95(01):151–158

Schrijvers DM, Meyer D, Guido RY, Martinet W (2011) Autophagy in atherosclerosis: a potential drug target for plaque stabilization. Arterioscler Thromb Vasc Biol 31(12):2787–2791

Shih PT, Brennan M-L, Vora DK, Territo MC, Strahl D, Elices MJ et al (1999) Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet. Circ Res 84(3):345–351

Silverman ES, Khachigian LM, Lindner V, Williams AJ, Collins T (1997) Inducible PDGF A-chain transcription in smooth muscle cells is mediated by Egr-1 displacement of Sp1 and Sp3. Am J Phys Heart Circ Phys 273(3):H1415–H1426

Singh AB, Liu J (2019) Berberine decreases plasma triglyceride levels and upregulates hepatic TRIB1 in LDLR wild type mice and in LDLR deficient mice. Sci Rep 9(1):1–14

Singh IP, Mahajan S (2013) Berberine and its derivatives: a patent review (2009–2012). Expert Opin Ther Pat 23(2):215–231

Singh A, Duggal S, Kaur N, Singh J (2010) Berberine: alkaloid with wide spectrum of pharmacological activities. J Nat Prod 3:64–75

Singh AB, Li H, Kan CF, Dong B, Nicolls MR, Liu J (2014) The critical role of mRNA destabilizing protein heterogeneous nuclear ribonucleoprotein d in 3′ untranslated region-mediated decay of low-density lipoprotein receptor mRNA in liver tissue. Arterioscler Thromb Vasc Biol 34(1):8–16. https://doi.org/10.1161/atvbaha.112.301131

Sola R, Valls R-M, Puzo J, Calabuig J-R, Brea A, Pedret A et al (2014) Effects of poly-bioactive compounds on lipid profile and body weight in a moderately hypercholesterolemic population with low cardiovascular disease risk: a multicenter randomized trial. PLoS One 9(8):e101978

Spigoni V, Aldigeri R, Antonini M, Micheli M, Fantuzzi F, Fratter A et al (2017) Effects of a new nutraceutical formulation (berberine, red yeast rice and chitosan) on non-HDL cholesterol levels in individuals with dyslipidemia: results from a randomized, double blind, placebo-controlled study. Int J Mol Sci 18(7):1498

Srivastava S, Srivastava M, Misra A, Pandey G, Rawat AKS (2015) A review on biological and chemical diversity in Berberis (Berberidaceae). EXCLI J 14:247

Trimarco B, Benvenuti C, Rozza F, Cimmino CS, Giudice R, Crispo S (2011) Clinical evidence of efficacy of red yeast rice and berberine in a large controlled study versus diet. Mediterr J Nutr Metab 4(2):133–139

Toth PP, Patti AM, Giglio RV, Nikolic D, Castellino G, Rizzo M, Banach M (2018) Management of statin intolerance in 2018: still more questions than answers. Am J Cardiovasc Drugs 18 (3):157–173

Umans-Eckenhausen MAW, Sijbrands EJG, Kastelein JJP, Defesche JC (2002) Low-density lipoprotein receptor gene mutations and cardiovascular risk in a large genetic cascade screening population. Circulation 106(24):3031–3036

Wang Y, Zidichouski JA (2018) Update on the benefits and mechanisms of action of the bioactive vegetal alkaloid berberine on lipid metabolism and homeostasis. Cholesterol 2018:7173920

Wang Y, Huang Y, Lam KSL, Li Y, Wong WT, Ye H et al (2009) Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilatation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase. Cardiovasc Res 82(3):484–492

Wang Y, Jia X, Ghanam K, Beaurepaire C, Zidichouski J, Miller L (2010) Berberine and plant stanols synergistically inhibit cholesterol absorption in hamsters. Atherosclerosis 209 (1):111–117. https://doi.org/10.1016/j.atherosclerosis.2009.08.050

Wang H, Shi L, Yin H, Zhou Q (2011) Study on effect of berberine on modulating lipid and CPT IA gene expression. Zhongguo Zhong Yao Za Zhi 36(19):2715–2718

Wang FL, Tang LQ, Yang F, Zhu LN, Cai M, Wei W (2013) Renoprotective effects of berberine and its possible molecular mechanisms in combination of high-fat diet and low-dose streptozotocin-induced diabetic rats. Mol Biol Rep 40(3):2405–2418

Wang Y, Yi X, Ghanam K, Zhang S, Zhao T, Zhu X (2014) Berberine decreases cholesterol levels in rats through multiple mechanisms, including inhibition of cholesterol absorption. Metabolism 63(9):1167–1177

Wang L, Jiang Y, Song X, Guo C, Zhu F, Wang X et al (2016a) Pdcd4 deficiency enhances macrophage lipoautophagy and attenuates foam cell formation and atherosclerosis in mice. Cell Death Dis 7(1):e2055–e2055

Wang L, Peng LY, Wei GH, Ge H (2016b) Therapeutic effects of berberine capsule on patients with mild hyperlipidemia. Zhongguo Zhong Xi Yi Jie He Za Zhi 36(6):681–684

Ward NC, Watts GF, Eckel RH (2019) Statin toxicity: mechanistic insights and clinical implications. Circ Res 124(2):328–350

Wu N, Sarna LK, Siow YL, Karmin O (2010) Regulation of hepatic cholesterol biosynthesis by berberine during hyperhomocysteinemia. Am J Phys Regul Integr Comp Phys 300(3):R635–R643

Wu YH, Chuang SY, Hong WC, Lai YJ, Chang GJ, Pang JHS (2012) Berberine reduces leukocyte adhesion to LPS-stimulated endothelial cells and VCAM-1 expression both in vivo and in vitro. Int J Immunopathol Pharmacol 25(3):741–750

Wu M, Yang S, Wang S, Cao Y, Zhao R, Li X et al (2020) Effect of berberine on atherosclerosis and gut microbiota modulation and their correlation in high-fat diet-fed ApoE−/− mice. Front Pharmacol 11:223

Xiao H-B, Sun Z-L, Zhang H-B, Zhang D-S (2012) Berberine inhibits dyslipidemia in C57BL/6 mice with lipopolysaccharide induced inflammation. Pharmacol Rep 64(4):889–895

Xu S, Weng J (2020) Familial hypercholesterolemia and atherosclerosis: animal models and therapeutic advances. Trends Endocrinol Metab 31(5):331–333

Yan Y-X, Nakagawa H, Lee M-H, Rustgi AK (1997) Transforming growth factor-α enhances cyclin D1 transcription through the binding of early growth response protein to a cis-regulatory element in the cyclin D1 promoter. J Biol Chem 272(52):33181–33190

Yang X-J, Liu F, Feng N, Ding X-S, Chen Y, Zhu S-X et al (2020) Berberine attenuates cholesterol accumulation in macrophage foam cells by suppressing AP-1 activity and activation of the Nrf2/HO-1 pathway. J Cardiovasc Pharmacol 75(1):45–53

Yin J, Xing H, Ye J (2008) Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 57(5):712–717

Yuyun MF, Ng LL, Ng GA (2018) Endothelial dysfunction, endothelial nitric oxide bioavailability, tetrahydrobiopterin, and 5-methyltetrahydrofolate in cardiovascular disease. Where are we with therapy? Microvasc Res 119:7–12. https://doi.org/10.1016/j.mvr.2018.03.012

Zhang T, Kruys V, Huez G, Gueydan C (2002) AU-rich element-mediated translational control: complexity and multiple activities of trans-activating factors. Portland Press, London

Zhang W, Xu Y-c, Guo F-j, Meng Y, Li M-l (2008) Anti-diabetic effects of cinnamaldehyde and berberine and their impacts on retinol-binding protein 4 expression in rats with type 2 diabetes mellitus. Chin Med J 121(21):2124

Zhang H, Wei J, Xue R, Wu J-D, Zhao W, Wang Z-Z et al (2010) Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 59 (2):285–292

Zhang Q, Xiao X, Feng K, Wang T, Li W, Yuan T et al (2011) Berberine moderates glucose and lipid metabolism through multipathway mechanism. Evid Based Complement Alternat Med 2011:924851

Zhang M, Wang CM, Li J, Meng ZJ, Wei SN, Li J et al (2013) Berberine protects against palmitate-induced endothelial dysfunction: involvements of upregulation of AMPK and eNOS and downregulation of NOX4. Mediat Inflamm 2013:260464. https://doi.org/10.1155/2013/260464

Zhang X-J, Deng Y-X, Shi Q-Z, He M-Y, Chen B, Qiu X-M (2014) Hypolipidemic effect of the Chinese polyherbal Huanglian Jiedu decoction in type 2 diabetic rats and its possible mechanism. Phytomedicine 21(5):615–623

Zhang L-S, Zhang J-H, Feng R, Jin X-Y, Yang F-W, Ji Z-C et al (2019) Efficacy and safety of berberine alone or combined with statins for the treatment of hyperlipidemia: a systematic review and meta-analysis of randomized controlled clinical trials. Am J Chin Med 47 (04):751–767

Zhang L, Shao J, Zhou Y, Chen H, Qi H, Wang Y et al (2018) Inhibition of PDGF-BB-induced proliferation and migration in VSMCs by proanthocyanidin A2: involvement of KDR and Jak-2/STAT-3/cPLA2 signaling pathways. Biomed Pharmacother 98:847–855

Zhou X, Ren F, Wei H, Liu L, Shen T, Xu S et al (2017) Combination of berberine and evodiamine inhibits intestinal cholesterol absorption in high fat diet induced hyperlipidemic rats. Lipids Health Dis 16(1):239

Zhu X, Yang J, Zhu W, Yin X, Yang B, Wei Y, Guo X (2018) Combination of berberine with resveratrol improves the lipid-lowering efficacy. Int J Mol Sci 19(12):3903

Zimetti F, Adorni MP, Ronda N, Gatti R, Bernini F, Favari E (2015) The natural compound berberine positively affects macrophage functions involved in atherogenesis. Nutr Metab Cardiovasc Dis 25(2):195–201. https://doi.org/10.1016/j.numecd.2014.08.004