Berberine: new insights from pharmacological aspects to clinical evidences in the management of metabolic disorders

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Abstract

Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids found in such plants as gender *Berberis*. Berberine is recognised to improve glucose and lipid metabolism disorders and preliminary clinical evidences suggest the ability of berberine to reduce endothelial inflammation improving vascular health, even in patients already affected by cardiovascular diseases, suggesting a possible interesting role of berberine and its metabolites in clinical practice. However, its physicochemical properties, pharmacokinetic, and metabolism are not fully elucidated and contradictory data have been reported.

This review provides a summary regarding the pharmacological and biological features of berberine, with a focus on berberine as well as their pharmacologically active metabolites and the different mechanisms underlying their activities in order to clarify the correct use of berberine supplementation, alone or in association with other nutraceuticals, for the management of metabolic disorders associated to increased cardiovascular disease risk. A particular attention has been given also to the available clinical trials assessing its short- and middle- term use tolerability, safety and efficacy in various condition, such as dyslipidaemia, impaired fasting glucose, metabolic syndrome and type 2 diabetes.

Key words: berberine, physico-chemical properties, metabolites, bioavailability, safety, hyperglicemia, lipid disorders, cardiovascular disease

1. Introduction

Despite the efforts towards primary prevention, cardiovascular diseases are yet the most common causes of death and one of the first causes of disability in industrialized countries [1]. The most cost-effective preventive approach still remain diet and physical activity [2], also in people without a history of cardiovascular disease [3]. However, lifestyle programs are often difficult to follow for long periods and changes in dietary habits and physical activity sometimes are not enough to reduce risk parameters, such as hypercholesterolemia [4]. On the other hand, a relatively large number of dietary supplements, nutraceuticals and functional foods have been studied for their supposed or demonstrated ability to improve blood lipid profile in humans [5]. The scientific community has recognized their effectiveness since in 2001, during the third National Cholesterol Educational Program, it has been suggested to integrate dietary supplements such as soluble fibers, omega-3 polyunsaturated fatty acids (PUFA), plant sterols and soy protein in the diet in order to achieve an optimal Low Density Lipoprotein-Cholesterol (LDL-C) level [6]. These suggestions have also been supported by the recent new European guidelines of the management of dyslipidemias [7] that also added some nutraceuticals as potentially useful lipid-lowering substances. Since the prevention of cardiovascular diseases need an everyday approach, both the tolerability and safety of dietary supplements rather than nutraceuticals used to control plasma cholesterol levels has to be adequately defined as well as the risk/benefit ratio of their assumption. A large number of reviews recently focused on the mechanism of action and the efficacy of the different nutraceuticals and botanicals with lipid-lowering effects [8, 9].

Among them berberine (BBR), a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids found in different plants as *Berberis* gender [e.g. *Berberis aristata* (tree turmeric), *Berberis aquifolium* (Oregon grape), *Hydrastis canadiensis* (goldenseal), *Xanthorhiza simplicissima* (yellowroot), *Phellodendron amurense* (Amur cork tree), *Coptis chinensis* (Chinese goldthread), *Tinospora cordifolia*, *Arcangelisia flava*, *Cortex rhellodendri Argemone mexicana* (prickly poppy), *Eschscholzia californica* (Californian poppy) and *Berberis vulgaris* (barberry)] has

multiple pharmacological effects including lipid-lowering effects rather than antimicrobial activity against a large number of microorganisms, intestinal ion secretion and smooth muscle contraction inhibition, ventricular tachyarrhythmia inhibition, stimulation of bile secretion linked to bilirubin discharge and reduction of inflammation [10, 11].

BBR is utilized in clinical practice as luminally acting agents with different antidiarrhoeal mechanisms, including bactericidal activity against *V. cholera* and entertoxigenic *E. coli* as well as protozoacidal activity against *Giardia lamblia*. BBR has also been used to treat radiation-induced diarrhoea and it was found to considerably reduce the incidence and severity of radiation-induced acute intestinal symptoms in patients who had received abdominal or whole-pelvic radiation [12].

Recent reports demonstrated the effectiveness of BBR supplementation in lipid disorders and hyperglycemia [13], even if the physico-chemical properties as well as the bioavailability of BBR and its metabolites, are not yet fully elucidated. The aim of this review is to critically summarize the available literature data on the chemical, biochemical, and pharmacological characteristics of BBR and its metabolites, and the relationship of these characteristics with their clinical effects. A brief paragraph that treats the recent advances in the development of BBR derivatives has been included, to demonstrate the multiple translational applications related to BBR.

To achieve our objectives a bibliographic literature search was conducted in different scientific databases including Scopus, Google Scholar, Pubmed and Web of science for peer-reviewed studies by focusing on BBR, its metabolites and derivatives of which it has been studied the physico-chemical properties, the pharmacokinetic, the bioavailability, the tolerability, the safety as well as a scientifically evaluation *in vitro* and *in vivo* (both in animals and in clinical trials) to prove the potential effects for the treatment of cardiovascular disease linked to lipid dysregulation and of hyperglicemia (almost all papers have been published from 2000 to 2016).

2. Physico-chemical properties of Berberine

BBR is a quaternary ammonium salt from the group of isoquinoline alkaloids (2,3–methylenedioxy-

9,10-dimethoxyprotoberberine chloride; $C_{20}H_{18}NO_4^+$), highly concentrated in the roots, rhizomes and stem bark of various plants. BBR is strongly yellow coloured, which explains the fact that, in the past, berberis species were used to dye wool, leather and wood [14].

Physico-chemical properties as pKa, solubility and lipophilicity are directly correlated to the extent of intestinal absorption of BBR and to its distribution in different organs and body fluids; therefore, their knowledge helps to understand the mechanism of action of BBR in a target disease.

There are contradictory data in literature regarding the acid/base character of BBR, with two different pKa values reported, 2.47 [15] and 15.7 [16] respectively. As reported by Spinozzi and colleagues, BBR is a positively charged molecule at physiological pH, due to the presence of the iminium cation and the absence of any proton donor or acceptor groups [17]. Consequently, the thermodynamic pKa value should be closer to 15.7 rather than 2.47, as the latter would indicate strongly acidic behaviour.

Water solubility of BBR depends on temperature and buffer composition, while it is independent from pH, being permanently charged compound having not ionizable groups in its structure. Water solubility of BBR chloride slightly increases with temperature (at 25 and 37 °C solubility is 5.27 ± 0.29 and 8.50 ± 0.40 mM, respectively) [18]. The authors showed that the maximum solubility of BBR chloride is obtained in phosphate buffer (4.05 ± 0.09 and 9.69 ± 0.37 mM at 25 and 37 °C, respectively) and observed that utilizing different buffers, such as phthalate or borate buffer, there is a twenty-fold decrease in the solubility [18].

The poor intestinal absorption and bioavailability of BBR is the main drawback when orally administered, suggesting that such poor absorption was related to its low solubility in aqueous medium and poor lipophilicity [19].

Lipophilicity has been evaluated using the octanol–water partition coefficient (log P), percent hydrophilic surface area (%HSA), topological polar surface area (tPSA) [18]. All these parameters (log P value of -1.51, %HSA value of 25.97% and tPSA value of 40.8 A) highlight a strong hydrophilicity of the molecule. The tPSA parameter can be correlated to the membrane transport characteristics, as previously reported [20].

3. Pharmacokinetic and Bioavailability of Berberine

Previous studies showed that the phase I metabolism of BBR takes place in the liver by cytochrome P450 isoenzymes (CYP450) where oxidative demethylation occurs at positions 2, 3, 9, and 10. Phase I metabolites are successively conjugated with a molecule of glucuronic acid or sulphuric acid in Phase II [21]. In humans and rats, the main primary metabolites of BBR are berberrubine (M1), thalifendine (M2), demethyleneberberine (M3), and jatrorrhizine (M4), whose structures are reported in Figure 1. As other alkaloids contained in *H. canadensis* extracts (*i.e.* hydrastine and canadine), BBR can inhibit CYP450 2E1 (CYP2E1) [22] and 1A2 (CYP1A2) [23]. Intestinal bacterial microflora plays a pivotal role in enterohepatic circulation of BBR and its conjugated metabolites: recent reports demonstrated that an intestinal microbiota of healthy subjects is essential to convert BBR in its absorbable forms [24]. Pharmacokinetic studies [25] performed on rats and humans after chronic BBR administration demonstrated the presence of BBR, M1, M2, M3, and M4 in bile, urine and faeces as well as their sulphate and glucuronide conjugates. These findings showed that BBR undergoes similar biotransformation in rats and humans through similar metabolic pathways [26].

Pharmacokinetic profile of BBR and its metabolites, deeply studied both in animal models [27] and in humans [28], demonstrated analogies between the two models, mainly as regards the poor oral bioavailability that requires relatively high dosage (0.5-1 g/die) in clinical practice. Chen and colleagues studied the pharmacokinetic profile of BBR in rabbits after an intravenous administration of 2 mg/kg of BBR sulphate, obtaining the following kinetic parameters [29]: $t_{1/2}$ (ka): 2.32 ± 1.18 minutes, $t_{1/2}$ (ke): 5.28 ± 1.00 h, total plasma clearance (CL): 5.46 ± 1.62 l/h, elimination rate constant (K_{10}): 1.75 ± 1.17 h^{-1} and the area under the concentration–time curve (AUC): 0.84 ± 0.27 μg h/ml.

Spinozzi and colleagues [17] reported that, after a single oral administration of BBR chloride in healthy human subjects (500 mg), plasma levels of BBR, M3 and M4 are very low (0.07 ± 0.01, 0.14 ± 0.01, and 0.13 ± 0.02 nM, respectively), with a very similar pharmacokinetic profile: a plateau was reached after one hour for BBR and M3, and after 2 hours for M4, and persisted up to 24 hours. In

contrast, M1 concentration in plasma reached 10 times higher levels after 4 hours, *i.e.*, 1.4 ± 0.3 nM, slowly decreasing to a concentration of 0.15 ± 0.02 nM after 24 h.

Otherwise, the same authors reported that after chronic administration of 15 mg/kg body weight/day for three months in hypercholesterolemic patients, it was observed a plasma bioaccumulation of BBR and its primary metabolites (maximum steady-state concentrations were 4.0 ± 2.0, 6.7 ± 3.0, 1.7 ± 0.3, and 5.6 ± 2.0 nM for BBR, M1, M3, and M4, respectively). Even in this case, M1 was the most abundant compound present in plasma [17]. Although low BBR plasma levels and low bioavailability, its metabolites maintained higher concentration in plasma, behaving like the pharmacologically active forms of BBR [30, 31]. Moreover, as it is reported by Tan and colleagues, after oral intake BBR is rapidly distributed in the body with maximum concentration in liver, followed by kidneys, muscle, lungs, brain, heart, pancreas and fat [32]. Indeed, after oral administration of BBR chloride, plasma levels are very low, as it is rapidly transferred to the liver and then excreted by bile (mainly as M2), urine (mainly as M2 and M1) and faeces (mainly as such) [25, 29]. In rats, after a single oral administration of BBR (200 mg/kg) the total recovery of BBR and its metabolites in feces, urine and bile is 22.83%, with 22.74% in feces, 0.0939% in urine and $0.9x10^{-6}$ % in bile [28]. As other authors, Hong-Mei Yan and colleagues reported that, in rats treated with a high fat diet -induced Non-Alcoholic Fatty Liver Disease (NAFLD), after a single dose of BBR (200 mg/kg), BBR as well as its metabolites M1, M2, M3 and M4 are preferentially located in liver with a concentration 50 times higher than that in plasma. The first peak of BBR (886.80 ± 174.55 ng/g) in the liver occurred at 4 hours after oral administration of the drug and the second peak at 24 hours (724.44 ± 51.89 ng/g), followed by a significant decline. Its metabolites exhibited a similar time-concentration relationship [33]. The same authors [33] focused also on BBR therapeutic effects on NAFLD patients treated for 16 weeks with 1.5 g/die. Results showed that BBR treatment reduced hepatic fat content in NAFLD patients, with metabolic benefits such as reduced body weight and improved glucose and lipid profiles. Overall, the data indicated that after an oral intake of BBR, this compound and its metabolites are rapidly distributed in the organism with maximum concentration in liver, at least in part explaining

its beneficial effects on glucose and lipid metabolisms.

New BBR formulations were developed, in order to enhance its absorption after oral administration. For instance, a new formulation based on a self-nanoemulsifying drug delivery system that ensured a faster release of the active principle (90% of BBR release in 20 minutes) respect to the traditional tablets (90% of BBR release in 2 hours) [34].

Godugu and colleagues [35] prepared a formulation of BBR spray dried (SD) mucoadhesive microparticle using a dual channel spray gun technology to increase its bioavailability. Pharmacokinetics studies conducted *in vivo* in rats showed that the use of BBR as SD formulation improved the bioavailability of BBR compared with BBR treated group, with an increase in the plasma Cmax (3.90 and 3.46 fold, respectively) and in the AUC values (7.41 and 6.98 fold, respectively).

4. Physico-chemical properties of Berberine Metabolites

BBR metabolites are present in plasma at higher concentration than the parent compound; therefore is reasonable to speculate that they could be the active molecules responsible for the biological activity [30]. The knowledge of their physico-chemical properties is very important for a complete biochemical characterization of these compounds. Physico-chemical properties of BBR metabolites are largely unknown in literature. Spinozzi and colleagues experimentally determined the main physico-chemical properties for all the primary metabolites and correlated such properties to the plasma levels after *in vivo* pharmacokinetic studies performed on human subjects [17].

The presence of both iminium group and the hydroxyl groups in BBR metabolites, differently from BBR that has only the iminium cation, leads to multiple species in solution at different pH values. In silico calculations of the pKa demonstrated that metabolites M3 and M4 have similar pKa values (≈ 9.5). As consequence, at physiological pH they are present mainly as iminium cations, similarly to BBR. Otherwise, M1 has a pKa value of ≈5-6 and, for this reason, in aqueous solution it takes part to a keto-enolic equilibrium (Figure 2). The authors demonstrated the presence of the keto-enolic

equilibrium of M1 by NMR experiments that showed how the prevalence of the enol rather than the quinoid form is modulated by the pH of the solution.

The presence of different ionization forms for the metabolites affects also the lipophilicity, as the log P can be modified changing the pH conditions. Log P values vary from -1.1 to -0.5 and from -1.5 to 0.1 for M3 and M4, respectively, in phosphate buffer 0.1 M at pH ranging from 4.5 to 8.5.

The M1 lipophilicity modifications, as a function of pH, are correlated to specific forms in respect with another; specifically, the positively charged enolic form is predominant at acidic pH (log P of -0.02 at pH 4.5) while the lipophilic quinoid form at basic pH (log P of 1.6 at pH 8.5). M1, in its quinoid form, passively diffuses better across the membranes, resulting in higher plasma concentrations compared to BBR and the other metabolites.

An increase in pH causes an increase in the solubility of M3 and M4, but a decrease of M1 solubility. A bathochromic effect, characteristic of M1, was observed when, increasing the pH, the solution colour turned from yellow (prevalence of the enolic form) to red (prevalence of the quinoid form)[17].

5. Berberine effects on lipid metabolism and vascular health

It's very well known that one of the major risk factors for endothelial dysfunction and its progression in atherosclerosis is due to the presence of high levels of LDL-C and their oxidized counterpart, oxidized LDL (oxLDL), into the blood vessels [36]. Inactivity of LDL receptor (LDLR) or its low-level expression induces accumulation of LDL-C in blood vessels [37].

BBR increases expression of the hepatic LDLR both at post translational and post transcriptional levels. BBR upregulates LDLR by the inhibition of the Pro-protein-convertase-subtilisin-kexin-9 (PCSK9) [38] and by the activation of c-jun N-terminal kinase (JNK)/c-jun pathway [39]. In liver tissues, PCSK9 synthesis is controlled at the gene transcriptional level by hepatocyte nuclear factor 1 (HNF1), a dimeric transcriptional activator containing homeodomain [40] and Dong and colleagues reported that BBR reduces PCSK9 by ubiquitin-induced proteasomal degradation of HNF1α [41].

Through a post-transcriptional mechanism, BBR stabilizes the LDLR mRNA by adenosine

monophosphate-activated protein kinase (AMPK)-dependent Raf-1 activation [42] that leads to Extracellular Signal-Regulated Kinase (ERK) activation [43] and subsequent specific reduction of mRNA decay-promoting factor heterogeneous nuclear ribonucleoprotein D (hnRNP D) [44] (Figure 3). These mechanisms are distinct from that recognized by statins therapy: statins decrease cellular cholesterol biosynthesis through HMG-CoA reductase inhibition that leads to a depletion of intracellular cholesterol level and activation of the Sterol Regulatory Element 1- sterol regulatory element binding proteins (SRE-1–SREBPs) pathway resulting in upregulation of the hepatic LDLR and subsequent lowering of the LDL-C in blood [45]. In accordance with previous cited studies, it has been showed that, in rats, the treatment of BBR with simvastatin increased the hepatic LDLR gene expression to a level significantly higher than that in monotherapies [46].

Zhou and colleagues recently demonstrated that BBR metabolites M1, M3 and M4 increase signalling in human hepatoma cells inhibiting cellular lipid accumulation [31].

Pro-inflammatory and oxidative stimuli related to atherogenesis [47] such as Tumor Necrosis Factor α (TNFα) or Interferon γ promote the oxLDL formation which in turn develops a vicious cycle by the activation of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) linked to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) to promote transcription of pro-inflammatory molecules [48]. In arterial walls oxidative stress and inflammation are closely linked; LOX-1 is undetectable in healthy vessels but overexpressed in atherosclerotic lesions and in acute coronary syndromes [49].

In human macrophage-derived foam cells treated with oxLDL, BBR inhibits the expression of LOX-1 [50, 51] as well as the oxLDL uptake of macrophages and reduces foam cell formation in a dose - dependent manner [51] by activating the AMPK – sirtuin 1 - peroxisome proliferator-activated receptor λ (AMPK-SIRT1-PPARγ) pathway [52]. Chi and colleagues [53] demonstrated that BBR combined with atorvastatin is more effective in diminishing LOX-1 expression than atorvastatin alone in monocyte-derived macrophages both *in vitro* and *in vivo* in rats through modulation of endothelin-1 receptor [52]. BBR improves also the survival of TNFα- treated endothelial progenitor cells (EPCs)

via the activation of Phosphoinositide 3-kinase – AKT - endothelial nitric oxide synthase (PI3K/AKT/eNOS) signalling pathway [54] possibly through AMPK induction. Both *in vitro* and *in vivo* studies, BBR reduces the leukocyte-endothelium adhesion and vascular cell adhesion molecule-1 (VCAM-1) expression induced by lipopolysaccharide (LPS). BBR also inhibits the nuclear translocation and DNA binding activity of LPS-activated NF-κB signalling pathway [55].

BBR was reported to protect against ROS- induced LDL oxidation in macrophages and endothelial cells upon stimulation with inflammatory stimuli at least in part through downregulation of NAD(P)H oxidases 2, 4 (NOX) and consequent decrease of ROS production in macrophages and endothelial cells [56, 57] [58]. NOX could be negatively regulated by AMPK activation [59, 60]; in fact, AMPK activators, such as metformin, may exert their cardiovascular protective function through NOX inhibition [61]. AMPK pathway is activated by BBR [62] and it seems to play a pivotal role in mediating its antioxidant activity [63, 64].

AMPK plays also an important role in regulating nitric oxide (NO) synthesis in endothelial cells: Zhang and colleagues observed that BBR ameliorates palmitate-induced endothelial dysfunction by upregulating eNOS and downregulating NOX4 through the activation of AMPK [65]. In both cultured endothelial cells and blood vessels isolated from rat aorta it has been showed that BBR promotes eNOS phosphorylation and reduces high glucose-induced generation of ROS, cellular apoptosis, NF-κB activation, and expression of adhesion molecules through AMPK signalling cascade activation, a key event in preventing oxidative and inflammatory signalling induction [66].

AMPK activation has been linked to upregulation of the antioxidant enzyme superoxide dismutase (SOD) [67, 68], which promotes the dismutation of anion superoxide in hydrogen peroxide. It was observed an increased SOD expression in BBR treated diabetic mice [69, 70]. Glutathione (GSH) is another antioxidant enzyme which helps to maintain the balance of redox state in organisms and it is a substrate of glutathione peroxidase (GSH-Px) in the clearance of peroxides [71]. BBR treatment promotes a GSH-Px and SOD hyperactivation in the liver of mice [72] and in adipocytes [73]; moreover BBR attenuates H_2O_2-induced ROS production and increases the expression of detoxifying

enzymes GSH-Px and SOD in NSC34 motor neuron-like cells [74]. Besides its modulatory effects on antioxidant mechanisms, AMPK signalling pathway activation promotes the abrogation of cardiac fibroblasts transformation into myofibroblasts: Ai and colleagues [75] showed that BBR induced AMPK and downregulated mammalian target of rapamycin - p70S6K (mTOR/p70S6K) signalling pathway in cardiac fibroblasts, suppressing cardiac fibrotic remodeling which can lead to heart failure.

AMPK is a key regulatory factor not only for cholesterol but also to fatty acids and triglycerides (TG) biosynthesis: Cao and colleagues reported that BBR and its metabolites M1, M3 and M4 have strong TG lowering effects in hepatoma cells through AMPK activation [30]. Dong and colleagues reported that BBR markedly inhibited fat accumulation and lipid droplets in 3T3-L1 adipocytes and decreased triglyceride content [76] (Figure 3).

The lipid-lowering activity of BBR, alone or in association with other nutraceuticals, has been clearly confirmed in a relatively large number of randomized clinical trials, often associated to other lipid-lowering nutraceuticals (mainly red yeast rice) or drugs (statins, ezetimibe). In table 1, we summarized the main results obtained in trials where BBR was tested as single dietary supplement. In a recent meta-analysis of randomized clinical trials [77] the administration of BBR produced a significant reduction in total cholesterol (mean difference -0.61 mmol/L; 95% confidence interval [CI] -0.83 to -0.39), TG (mean difference -0.50 mmol/L; 95% CI -0.69 to -0.31), and LDL-C (mean difference -0.65 mmol/L; 95% CI -0.76 to -0.54) levels, with a remarkable increase in high-density lipoprotein - cholesterol (HDL-C) (mean difference 0.05 mmol/L; 95% CI 0.02 to 0.09). Moreover, beyond the effect on lipids, in patients with acute coronary syndrome following percutaneous coronary intervention, the supplementation with BBR on top of the standard therapy determined significant improvement in the serum level of inflammatory markers as matrix metallopeptidase 9 (MMP-9), ICAM-1 and VCAM-1 compared to the control group [78].

The most studied combination of lipid lowering-nutraceuticals is a property product containing 500 mg of BBR and red yeast rice extract with 3 mg of monacolin K per daily dose (Armolipid Plus®).

Red yeast rice extract (*Monascus purpureus*) contains monacolins that compete structurally at HMG-CoA reductase level with HMG-CoA, precursor of mevalonate, thus reducing plasma cholesterol [79]. So, it was supposed and tested that the combination of BBR and red yeast rice could have an additive or synergistic lipid-lowering effect in humans. After a preliminary trial [80], many others confirmed that this hypothesis was true.

Among others, in a large study carried out in the setting of every day clinical practice on dyslipidemic patients, the use of the tested association was linked to a persistent and significant improvement of different lipid parameters with a reduction of −19.1 % for TC (p<0.001), −23.5% for LDL-C (p < 0.001), +11.6% for HDL-C (p<0.001), −17.9 % for TG (p<0.001) after 16 weeks [81].

In another 8-week randomized, double-blind cross-over clinical trial, carried out in the setting of a lipid clinic, the same combination was able to increase HDL-C (+4.8%) and to reduce total cholesterol (−12.8%) and LDL-C (−21.1%) in patients with moderate dyslipidemia and metabolic syndrome with results similar to pravastatin (−16% and −22.6%, respectively) [82].

The treatment is usually well tolerated, even when employed by elderly hypercholesteraemic patients who were previously statin-intolerant [5].

The BBR-red yeast rice association has also shown to improve endothelial function [83] and pulse wave velocity [84] in dyslipidaemic patients.

The association of BBR with silymarin, a complex mixture of seven major flavonolignans and one flavonoid, has also been investigated. It significantly reduces total cholesterol, TG and LDL-C and increases HDL-C after 3 months of treatment in dyslipidemic patients [85].The efficacy and tolerability of this nutraceutical combination has been evaluated in type 2 diabetic hypercholesterolemic patients previously intolerant to statins, in which the improvement of lipid pattern was also associated to a parallel improvement in glucose metabolism [86].

BBR effects have also been studied when associated with chlorogenic acids and tocotrienols.

Chlorogenic acids represent a group of phenolic secondary metabolites, present in particular in green coffee, that inhibits the intestinal absorption and hepatic biosynthesis of cholesterol by inhibiting the

HMG-CoA reductase [87].

Tocotrienols represent a class of vitamin E which are mainly localized in cereal grains (oat, barley, rye) and in certain vegetable oils (palm oil and rice bran oil); they are known to modulate several mechanisms associated with cardioprotection, in particular, they reduce the atherogenic apolipoprotein and lipoprotein plasma levels [88].

The combination of BBR with chlorogenic acids and tocotrienols has been evaluated in a double blind, cross-over clinical trial, controlled with placebo, on overweight subjects with hyperlipidaemia and has demonstrated that it decreased total cholesterol (-19,5%, $p<0.001$) and LDL-C (-15,4%, $p<0.001$), TG (-21,4%, $p<0.001$), non-HDL-C (-19,7%, $p<0.001$), homeostasis model assessment-estimated insulin resistance (HOMA-IR) index (-0,3 %, $p<0.001$), GPT (-7,8%, p= 0,027) and Lipid Accumulation Product (-10,4 %, $p= 0.002$) in the short term [89].

6. Berberine effects on glucose metabolism

A large preclinical evidences support the potential role of BBR as antidiabetic agent, mainly due to its action on AMPK signalling pathway with subsequent induction of glycolysis [90].

In H9c2 myoblast cell line treated with insulin to induce insulin resistance, BBR attenuated the reduction in glucose consumption and glucose uptake at least in part via stimulation of AMPK activity [91]. BBR could activate AMPK and induced glycolysis in skeletal muscle L6, myoblast C2C12, and adipocyte 3T3-L1 cell lines [90]. In insulin-resistant myotubes, BBR enhances acute insulin-mediated glucose transporter type 4 (GLUT4) translocation and glucose transport through activation of AMPK and PI3K pathway [91, 92]. In diabetic animals, BBR partially improves cardiac function and restores fasting blood insulin, fasting blood glucose (FBG), total cholesterol, and TG levels to that of control, through different mechanisms among which AMPK and AKT activation and reduced glycogen synthase kinase 3 beta (GSK3β) activation. The mechanism was confirmed in palmitate-induced hypertrophy H9c2 myoblast cell line [93].

Besides the role in AMPK signalling, BBR increases Insulin Receptor (InsR) expression in a variety

of human cell lines and hepatitis B virus – transfected human liver cells [94]. Zhou and colleagues found that BBR stimulates glucose transport through a mechanism distinct from insulin in 3T3-L1 adipocytes, demonstrating an additive effect even at the maximal effective concentrations of both components [95].

In diabetic rats as well as in normal rats, BBR increased plasma GLP-1 after an oral glucose supplementation as well as the expression of *Glp 1r* gene in the ileum [96, 97]. GLP-1 is a gut derived hormone secreted in response to glucose from intestinal L cells and it exerts important effects on the regulation of glucose metabolism, stimulating glucose dependent insulin secretion by activation of adenylate cyclase and subsequent elevation of cytosolic free calcium as well as promoting β cell proliferation. At the same time, GLP-1 inhibits glucagon release, gastric emptying and food intake [98]. Retinol binding protein 4 (RBP4), a cytokine secreted from adipocytes, was recently found to be inversely correlated with expression of GLUT4 in insulin resistance; in glucose consuming tissues, such as adipose tissue, liver or skeletal muscle, BBR affects both GLUT4 and RBP4 in favour of glucose uptake into cells [99], and inhibits adipogenesis through downregulation of the adipocyte marker peroxisome proliferator-activated receptor λ (PPARλ) [100], finally resulting in reduced insulin resistance.

BBR exhibited similar hypoglycemic efficacy as glibenclamide,an anti-Type 2 Diabetes Mellitus (T2DM) drug that stimulates the release of insulin, to lower area under the curve of FBG in the kidney, liver and brain of mice with T2DM [69]. The main mechanisms of BBR in hypoglycemic effect are summarized in Figure 4.

From a clinical point of view, a recent meta-analysis of randomized clinical trials discussed that BBR chloride supplementation was more effective in reducing blood glucose and glycated haemoglobin levels compared to life-style modification only [FBG: MD =−0.86 mmol/L, 95% CI (−1.14−0.57), P<0.00001; PPG: MD= −1.91 mmol/L, 95% CI (−2.45−1.36), P<0.00001; haemoglobin A1c (HbA_{1c}): −0.71%, 95% CI (−0.94−0.49), P<0.00001]. When added to glucose lowering agents, BBR group can be more effective in lowering blood glucose levels than in the control group [FBG: MD=

−0.67 mmol/L, 95% CI (−0.85, −0.49), *P*<0.00001; PPG: MD= −0.98 mmol/L, 95% CI (−1.54, −0.42), *P*=0.0006; HbA_{1c}: MD= −0.58%, 95% CI (−0.96, −0.21), *P*=0.002]. Finally, when compared to other insulin-sensitizing agents (metformin, rosiglitazone), no significant difference between treatment groups has been observed. Overall, no hypoglycaemia episodes have been registered during the considered trials [101].

Of particular interest is a trial where BBR significantly lowered FBG, HbA_{1c}, TG, and insulin levels in patients with T2DM as well as metformin and rosiglitazone (a combination commonly used for the T2DM therapy); the percentages of peripheral blood lymphocytes expressing InsR were significantly elevated after therapy [102].

The dose-dependent antidiabetic properties of BBR have been clearly confirmed in a relatively large number of randomized clinical trials [103] (table 1).

T2DM in humans is also associated with compositional changes in gut microbiota; the proportions of Firmicutes and Clostridia were significantly reduced, while the relative abundance of Bacteroidetes and Betaproteobacteria was increased in the diabetic patients. BBR is poorly absorbed so it acts as an efficient antibacterial agent topically in the gastrointestinal tract without systemic anti-infective activity. Presumably the modulation of gut microbiota may be one mechanism of the antidiabetic effect of BBR [104].

Thus, based on the available evidences, we can reasonably conclude that BBR seems to be a promising agent for the management of insulin resistance [105].

7. Berberine tolerability and safety

BBR chloride and BBR sulphate are the most diffused salt forms of BBR utilized in clinical practice. The chloride form is commercially available as tablets and/or capsules. Its daily dosage is variable, but usually it is approximately of 400-1000 mg/day (in some clinical trial the dosage of 1500 mg/day has also been used) [106].

The Lethal Dose 50 (LD50) of BBR sulphate is 25 mg/kg in mice, while using *Berberis vulgare* is

moderately high (LD50= 2.6±0.22 g/kg in mice) [50, 105], supporting the use of highly purified and concentrated BBR formulation only.

Standard doses of BBR are usually well tolerated and adverse events are rare and mild. The most studied side effects are those on the gastrointestinal system since BBR and its derivatives could produce gastric lesions [105, 107]. BBR associated to abdominal discomfort could be related to the modifying action of BBR on human microbiota [104]. On the other side, this effect could be also related to part of the positive metabolic effects and antidiarrheal ones of BBR [108].

The main safety issue of BBR involves the risk of pharmacological interactions since BBR displaces bilirubin from albumin about ten-fold more than phenylbutazone, thus any herb containing large amounts of BBR should be avoided in jaundiced infants and pregnant women [109]. BBR displaces warfarin, thiopental and tolbutamide from their protein binding sites, increasing their plasma levels [110]. A combined treatment with BBR reduces the cylcosporine A metabolism due to the inhibition of CYP3A4 and P-glycoprotein in liver and gut wall respectively, and to the increase in emptying time of stomach and small intestine transit time, thus rising cyclosporine A plasma levels as well as its bioavailability [111]. In renal transplant receivers who take cyclosporine A 3 mg/kg twice daily, the co-administration with BBR (0.2 g/day for 3 times a day for 3 months) increases the mean cyclosporine A of 34.5% and its mean half-life of 2.7 hours [112].

Moreover, the phase I metabolism of BBR is partially reduced by SKF-525A (proadifen, a CYPP450 inhibitor) treatment, but the phase II glucuronidation of BBR is not affected by probenecid (a glucuronidation inhibitor) [113].

Even if the main mechanism of BBR involves the pharmacological interaction with CYP3A4 and intestinal P-glycoprotein, it also inhibits CYP1A1, potentially interacting with drugs metabolized by this cytochrome as well. The impact of this observation in clinical practise has to be evaluated since the drugs metabolized by CYP1A1 are relatively rare [114].

8. Berberine semisynthetic derivatives as new perspectives in clinical practice

The synthesis of potent biological active compounds starting from natural molecules is a well-consolidated strategy in medicinal chemistry for the discovery of novel therapeutic agents. In a recent work [115] the biological effects of the derivatization of the BBR isoquinoline moiety using enamines derived from formaldehyde, morpholine, piperidine, carbazole and six substituted of piperazines were studied *in vitro*. Piperazine derivatives bearing a heterocyclic nitrogen substituent (a pyridyl or a pyrimidyl ring) showed the best antioxidant and anticancer activities, while carbazole moiety derivatives demonstrated a potent inhibitory effects on the growth of tumors. Considering the potential applications of these new derivatives, various studies [116, 117] tried to establish the structure-activity relationships among a large number of BBR- related compounds. Cheng and colleagues evaluated a series of isoquinoline alkaloids including tetrahydroprotoberberines, N-methyl tetrahydroprotoberberines and protoberberines for their antibacterial activities against four pathogenic bacteria *Escherichia coli*, *Staphyloccocus aureus*, *Staphylococcus gallinarum* and *Salmonella choleraesuis*. Experimental results indicated that protoberberines were the most active compounds to the target bacteria among the tested alkaloids. It was suggested that planar molecule with high aromatization level (*e.g.* coptisine 5 and berberine 6) or a positive charge of the molecules (*e.g.* N-methyl tetrahydroprotoberberines 11 and 12) have a positive influence on the antibacterial effects [116]. Yang and colleagues [117] performed a systematic investigation among nineteen synthetic derivatives in order to evaluate the changes on cholesterol-lowering activity as consequence of different structural modifications, such as the introduction of methoxyl or ethoxyl groups at different positions of the terminal benzene moiety. The analysis of the structure–activity relationship indicated that the two ortho-distributed methoxyl groups on this benzene ring are fundamental for its activity. Among the nineteen analogues, the compound bearing methoxyl groups at 10- and 11-position showed a markedly increased of LDLR expression in respect with BBR treatment in human hepatoma cells (8,5 ± 0,9 and 5,3 ± 0,4 fold increase, respectively). A similar study [118] dealed with the synthesis as well as the hypoglicemic activity evaluation of various protoberberine derivatives on

a model of alloxan-induced diabetes mice. Results showed that the quaternary positively charged nitrogen atom in the aromatic C ring of all these derivatives plays an important role in glucose lowering activity; tetrahydroberberine is not active because of the presence of the tertiary nitrogen atom but its N-alkylation to form quaternary ammonium salts determines an increase in glucose lowering activity and, in particular, the N-benzyl derivative reached the highest hypoglycemic effect. Shan and colleagues [119] investigated the glucose lowering efficacy and the pharmacokinetic profile of the BBR derivative Y53, a BBR isomer with a demonstrated lipid-lowering activity. Results showed that Y53 posses a better glucose-lowering activity compared with BBR, as it was able to decrease blood glucose by 35.7% in respect with a 19.9% reduction observed in the BBR treated group *in vivo* in diabetic mice. Pharmacokinetic studies of Y53 at a single oral dose of 200 mg/kg showed also an higher bioavailability with a maximum plasma concentration (Cmax) and AUC of, respectively, 1.61 and 2.27-fold increase in respect with BBR, presumably due to its low affinity to P- glycoprotein [119].

9. Conclusion

Management of lipid disorders and hyperglycemia with limited side effects remains a challenge to the medical practice as they can cause long -term complications such as cardiovascular diseases as well as retinopathy, neuropathy and nephropathy. Oral administration of BBR has been used in the treatment of diabetes, obesity and hypercholesterolemia with unclear therapeutic efficacy. However, BBR has a low bioavailability, which is attributed to its poor aqueous solubility so the comprehension of physico-chemical proprieties as well as the biological effects of its metabolites will be fundamental not only to better understand the adequate clinical dosage of BBR but also to discover new potentially more pharmacologically active and safety molecules derived from BBR to use in clinical practice alone or in association with other nutraceuticals to ameliorate the beneficial effects.

Conflict of interest disclosure

The authors declare no competing financial interests.

Table 1. The main effects of berberine observed in clinical trials (published in english language), with a minimal duration of four weeks, a minimal sample size per treatment of 15 subjects and a berberine dosage range from 500 to 2000 mg per day.

Author	Condition	N. of patients	Dose	Duration	Effects (Berberine Arm)	Adverse events
Zeng (2003) [120]	Chronic congestive heart failure	156	1200-2000 mg/day	24 months	Improvement of left ventricular ejection function, exercise capacity, dyspnea-fatigue index, frequency and complexity of ventricular premature complexes, decrease in mortality in the berberine-treated patients during long-term follow-up	No one reported
Kong (2004) [45]	Hypercholesterolemia	91	1000 mg/day	12 weeks	Reduction of TC by 18%, LDL-C by 20%, TG by 28%	No one reported
Cicero (2007) [80]	Hypercholesterolemia	40	500 mg	4 weeks	Reduction of TC by 16% LDL-C by 20%, ApoB by 15%, TG by 22%, increase in HDL-C by 6.6%.	No one reported
Zhang (2008) [121]	Type 2 diabetes	116	1000 mg/day	12 weeks	Reduction of BMI by 0.9 kg/m^2, FBG by 20%, PPG by 26%, HbA1c by 0.9%, HOMA-IR by 38%, TC by 19%, LDL-C by 22%, TG by 36%	Mild to moderate constipation in 5 patients
Yin (2008) [122]	Naive Type 2 diabetes	36	1500 mg/day	13 weeks	Decreases in HbA1c by 2%, FBG by 36%, PPG by 44%, TC by 14%, TG by 23%	Transient gastrointestinal adverse effects (34.5% of patients)
	Treated Type 2 diabetes	48			Decreases in WC by 2 cm, HbA1c by 0.8%, FBG by 21%, PPG by 33%, , FPI by 29%, HOMA-IR by 45%, TC= by 12%, LDL-C= 13%	
Zhang (2010) [102]	Type 2 diabetes	97	1000 mg/day	8 weeks	Decrease in FBG by 25.9%, FPI by 28%, HbA_{1c} by 1.5%, TG by 18%	No one reported
	Impaired fasting glucose plus viral hepatitis	52			Decrease in FBG by 15%, TG by 16%, improvement in liver transaminases and gamma-GT	
Wei (2012) [123]	Polycystic ovary syndrome	89	1500 mg/day	12 weeks	Decrease in WC by 8 cm, FBG by 12%, FPI by 30%, TG by 17%, TC by 16%, LDL-C by 14%. HDL-C increased by 9%	No one reported
Meng (2012) [78]	Coronary Artery Disease in atorvastatin treatment	130	900 mg/day	30 days	Decrease in TC by 16%, LDL-C by 7%, TG by 13%, improvement in inflammatory markers	Abdominal pain, rash and constipation in 3 patients
Derosa (2013) [124]	Hypercholesterolemia	144	1000 mg/day	12 weeks	Reduction of TC by 17% LDL-C by 21%, TG by 20%, increase	No one reported

					in HDL-C by 4%.	
An (2014) [125]	Polycystic ovary syndrome undergoing in vitro fertilization	150	1500 mg/day	12 weeks	Reduction of BMI by 1.8 kg/m^2, waist circumference by 4.6 cm, FBG by 14%, FPI by 50%, HOMA-IR by 47%, TC by 223%, LDL-C by 19%, total FSH requirement, increase in live birth rate	10 patients with gastrointestinal discomfort
Yan (2015) [33]	Non-Alcoholic Fatty Liver Disease	184	1500 mg/day	16 weeks	Reduction of BMI by 1.5 kg/m2, WC by 4.8 cm, TC by 9%, TG by 20%, ApoB by 11%, improvement in liver transaminases	Anorexia and upset stomach (30.95% of BBR-related AEs), diarrhea (26.19%) and constipation (14.29%)
Chen (2015) [108]	Irritable bowel syndrome	132	800 mg/day	8 weeks	Reduction of diarrhea frequency, abdominal pain frequency and urgent need for defecation frequency	No one reported

BMI= body mass index, WC= waist circumference, FBG= fasting blood glucose, PPG= postprandial blood glucose, FPI= fasting plasma insulin, HOMA-IR= homeostasis assessment index – insulin resistance, HbA1c= glycated haemoglobin, TC= Total cholesterol, TG= triglycerides, LDL-C= low density lipoprotein cholesterol, HDL-C= high density lipoprotein cholesterol, ApoB= apolipoprotein B100

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Figure legends

Figure 1. Berberine and its primary metabolites

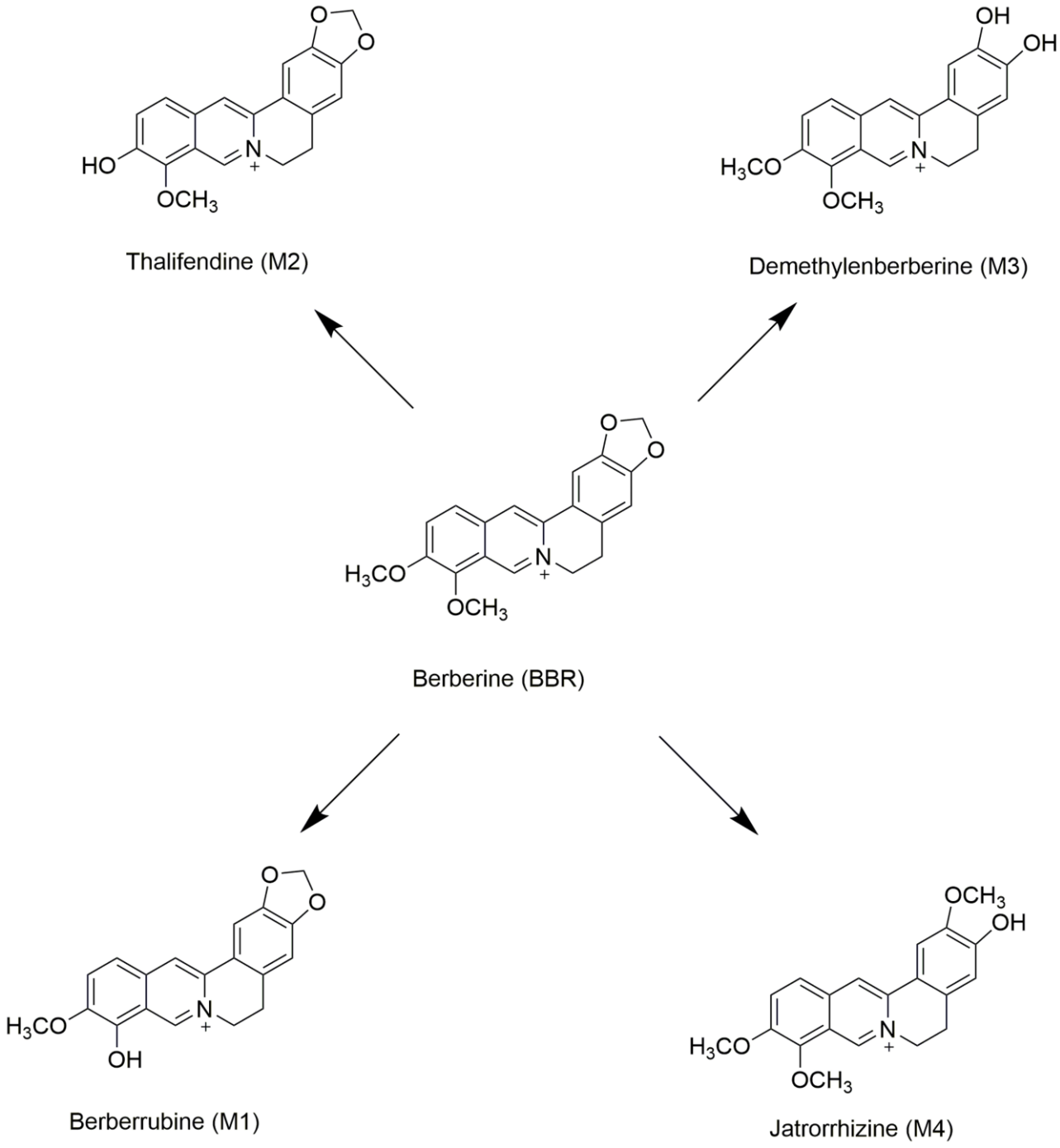


Figure 2. Keto-enolic aequilibrium of Berberrubine (M1)

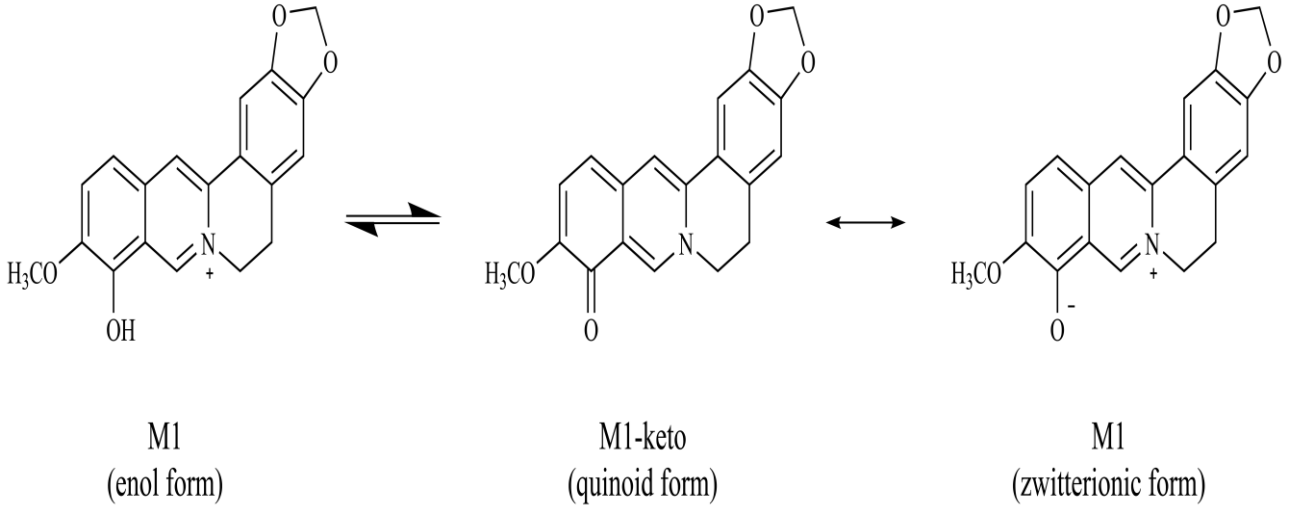
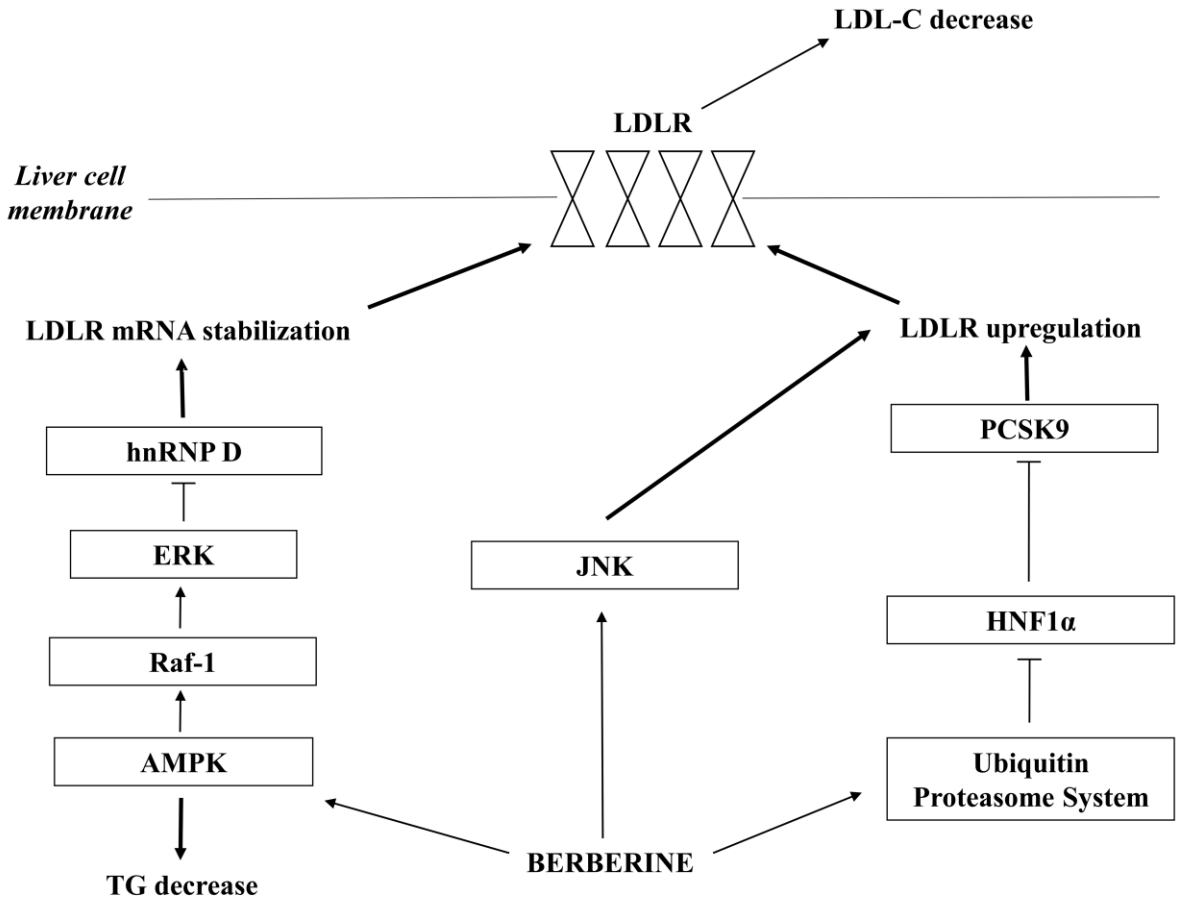
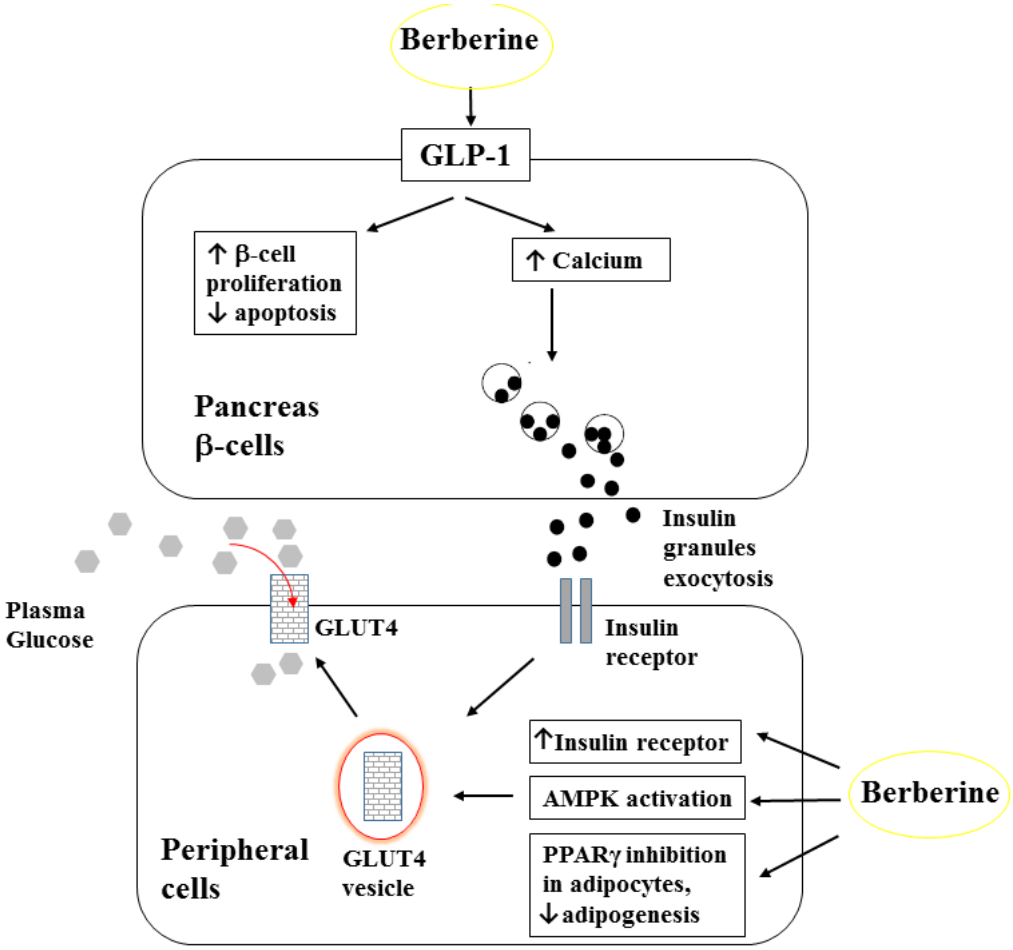


Figure 3. Main lipid-lowering effects of berberine in the human liver cells.



The hepatic LDLR regulates human plasma LDL-C homeostasis: BBR increases the expression of hepatic LDLR both at post translational and post transcriptional levels. BBR up-regulates LDLR by the inhibition of PCSK9 through the ubiquitin-induced proteasomal degradation of HNF1α and by the activation of JNK pathway. Through a post-transcriptional mechanism, BBR stabilizes the LDLR mRNA by AMPK-dependent Raf-1 activation that leads to ERK activation and subsequent specific reduction of mRNA decay-promoting factor hnRNP D. BBR has also strong TG lowering effects through AMPK activation.

Figure 4. Main glucose-lowering effects of berberine in the human cells.



BBR increases plasma GLP-1 as well as the expression of *Glp 1r* gene in ileal L cells. GLP-1 exerts important effects on the regulation of glucose metabolism in pancreatic β cells, stimulating insulin secretion in plasma and subsequent elevation of cytosolic free calcium as well as promoting proliferation and inhibiting apoptosis of β cells. Insulin promotes the activation of InsR in peripheral cells such as adipose, cardiac, skeletal muscle and liver cells, and the consequent translocation of GLUT4 from cytosolic vesicles to plasma membrane, inducing plasma glucose uptake. BBR can directly stimulate glucose transport from plasma into cells through AMPK activation, increased InsR expression and the subsequent GLUT4 translocation as well as it can inhibit adipogenesis through downregulation of the PPARλ, finally resulting in reduced insulin resistance.