Bioavailability of berberine: challenges and solutions

Asha Thomas[1] , Seema Kamble[1] , Sanjeevani Deshkar[2] , Lata Kothapalli[1] , Sohan Chitlange[2]

[1]Patil Institute of Pharmaceutical Sciences and Research, Department of Pharmaceutical Chemistry, Pimpri, Pune, MS, India
[2]Patil Institute of Pharmaceutical Sciences and Research Department of Pharmaceutics, Pimpri, Pune, MS, India

ORCID IDs of the authors: A.T. 0000-0003-1058-8779; S.K. 0000-0003-0967-1225; S.D. 0000-0002-3393-717X;
L.K. 0000-0002-7412-5805; S.C. 0000-0002-9355-3303

ABSTRACT

Berberine is a quaternary benzylisoquinoline alkaloid with multiple pharmacological effects used for the treatment of hypertension, tumors, bacterial infections, inflammation, HIV, and cardiac diseases. In phase I clinical trials, berberine is safe at excessive doses but manifests poor bioavailability. Major challenges like poor absorption, rapid metabolism, and rapid systemic elimination are responsible for low plasma and tissue levels of berberine. Various strategies have been undertaken by several researchers to overcome this issue and enhance the bioavailability of berberine. This includes the design of new formulation strategies; novel drug delivery systems (NDDS) like liposomes, nanosized dosage forms, phospholipid complexes, mucoadhesive microparticles, and micoemulsions; the use of adjuvants; and the design of structural analogous of berberine. This review focuses on the occurrence of berberine in numerous plants and its pharmacological activities as evidenced through numerous preclinical and clinical studies. The later part of this review highlights the bioavailability issue of berberine which arises due to its poor absorption, elevated rate and extent of metabolism, and quick elimination and clearance from the body. A systematic effort has beenmade to analyze the various formulation strategies, including the design of newer berberine analogues and derivatives. These strategies can be further explored to increase the bioavailability, medicinal value, and application of this promising molecule.

Keywords: Berberine, bioavailability improvement, metabolism, pharmacokinetics

INTRODUCTION

Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium) (Figure 1) is an isoquinoline alkaloid with a molar mass of 336.3612 g/mol. It is present as a non-basic and quaternary protoberberine in several herbs, including European barberry, goldenseal, goldthread, Oregon grape, phellodendron, and tree turmeric (Neag et al., 2018). The reported uses of berberine-containing species are summarized in Table 1. Berberine (BBR) is a yellowish crystalline powder with a faint characteristic odor and sour taste. It is moderately soluble in methanol, with marginally solubility in ethanol and water (Battu et al., 2010). Berberine is reactive to light and heat; when exposed to high temperature and light, it undergoes degradation and thus affects its stability (Neag et al., 2018). Several pharmacological effects of BBR are reported in literature, which includes anti-depressant (Sun et al., 2014), anti-atherosclerosis (Sarna, Wu, Hwang, Siow, & O, 2010), anti-diarrheal (Zhang et al., 2012b), anti-osteoporosis (Lee et al., 2008), anti-hypercholesterolemia (Kong et al., 2008), and neuroprotective effects (Zhang et al., 2012a). Reports on the extraction of different BBR-employing techniques (classical and conventional methods) using different solvents like methanol, ethanol, and aqueous or acidified methanol/ethanol are available in literature. Conventional methods enhance the extraction efficiency andreduce extraction times and solvent volumes used in the extraction process when compared to the classical methods. BBR in plant extracts and in biological fluids have been quantified using analytical techniques like Ultra Violet spectroscopy (UV), High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Thin Layer Chromatography (TLC), Capillary
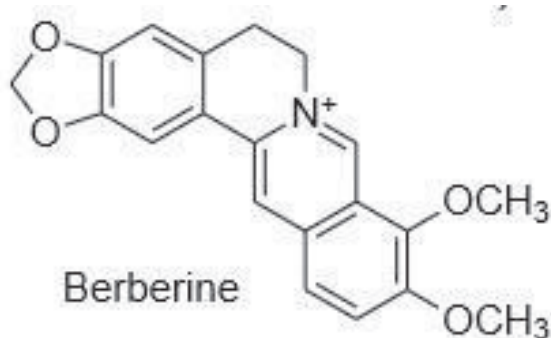
Figure 1. Structure of Berberine.

Electrophoresis (CE), and Liquid Chromatography coupled with Mass Spectrometry (LC-MS) (Neaget al., 2018).

BBR is present in several plants and is also reported to be responsible for numerous biological activities. Table 1 summarizes the plant species, content of berberine in plant parts, and their reported biological activities. BBR is also present in several marketed herbal preparations, either in pure chemical form as berberine or as plant extracts (ex. berberine in raw herb *Daruharidra (Berberis aristata* DC) (Neag et al., 2018). However the major drawback of BBR is its low bioavailability and poor pharmacokinetics, which is majorly responsible for its reduced efficacy. The foremost objective of this review is to discuss in detail the bioavailability, factors controlling bioavailability, and means to improve the bioavailability of BBR.

Method of data collection

A methodical collection of scientific literature, reports, experiments, case studies, and examination of work reported was performed with the help of data available from various sources, using several electronic literature databases such as Google Scholar, PubMed, Medline and Science direct databases. The information was searched up to April 2020. The search phrases used included: physicochemical, phytochemical and pharmacological properties, pharmacokinetics, metabolism, preclinical & clinical studies, bioavailability, strategies reported for bioavailability enhancement of berberine, and preparations containing berberine.

Selection criteria

The data collected was further sorted on the basis of specific principles: articles based on the pharmacological screening of berberine for various activities; articles based on preclinical and clinical studies written with emphasis on the dose requirement of berberine and the bioavailability issues thereof; articles on problems of bioavailability of berberine; and papers on pharmacokinetics, tissue distribution, metabolism, and half-life were all included in the study. This review elaborates the varied strategies that were undertaken till date, with the objective to improve the bioavailability of berberine thereby enhancing its efficacy.

Preclinical studies

Preclinical case studies have reported that BBR is safe at higher doses but exhibits poor bioavailability. Deng et al., in their study, demonstrated that BBR was rapidly absorbed but eliminated very slowly in rat bodies after oral administration of

combinations of Coptis (*Coptidis rhizoma*) and Evodia (*Evodiae fructus*) (1.086 g/kg). The maximum plasma drug concentration achieved was 121.1±11.9 ng/ml, appearing at 90min of the study (Deng et al., 2008). In another study, BBR showed the maximum plasma concentration of 2.31 µg/ml at 0.5h with slow elimination (5h) from rat bodies after administration of the Xiexin-Tang decoction at a dose of 20ml/kg (Yi, Jian-Ping, Xu, & Lixin, 2006). The pharmacokinetics of BBR (200 mg/kg) and lysergol (20 mg/kg) in plasma after oral administration in rats was compared and reported by Patil et al. (2012). The findings of this study indicated that the pharmacokinetic profile of lysergol (C_{max}-316.54±26.83 ng/ml, T_{max}-2.25h, $t_{1/2}$-21.3±1.75h) was higher than BBR (C_{max}-112.30±12.07 ng/ml, T_{max}-2.5h, $t_{1/2}$-5.23±0.41h). However, the oral bioavailability of BBR was enhanced on concomitant administration with lysergol. Following oral administration of 90 mg/kg/d of BBR to rats, improved lipid-lowering efficacy (46.2%) was observed in comparison to BBRmonotherapy (26.8%) (Kong et al., 2008).

A study provided the first preclinical evidence of effect of BBR loaded into chitosan nanoparticle (NP), and their surface modification induced neurodegenerative changes and hepatotoxicity in rats. BBR-NPs formulation (4 mg/kg, intraperitoneal, i.p.) was administered for 14 days, and on the 15th-day lipopolysaccharide (LPS), BBR- NPs showed significant implication on the neuroprotective and hepatoprotective effects (Soudi et al., 2019). Another study demonstrated the pharmacokinetic parameters of BBR, following intragastric administration at a dose of 25 mg/kg in normal and diseased rats (post inflammatory irritable bowel syndrome). The findings showed that the C_{max} in the diseased animal group (18.53±0.61 ng/ml at 15 min), when compared to the normal control group (16.74±4.47 ng/ml at 15 min) was increased significantly while CL/F was decreased (Gong et al., 2014a).

The mean plasma concentration vs. time profile after intragastric (i.g.) administration of *Rhizoma coptidis* extract (96 mg/kg) containing 22 mg/kg berberine was also studied. It was observed that BBR was absorbed quickly in the body within 15 min of the study in both the normal control and the diseased groups (post inflammatory irritable bowel syndrome). It was noteworthy that the plasma drug concentration of BBR remarkably increased in diseased model group (39.2±7.9 ng/ml at 15 min) compared to the normal control group (20.0±12.1 ng/ml at 15 min). However, compared to the normal control group (198.6±75.4 L/h/kg), the diseased model group (124.3±27.4 L/h/kg) demonstrated slower elimination of BBR (Gong et al., 2014a). The pharmacokinetics of BBR following oral administration (20 mg/kg) to normal and Type-2 diabetes mellitus (T2DM)-induced rats (model group) exhibited increase in C_{max} and T_{max} in T2DM group (34.41±4.25 µg/ml at 2.24h) when compared to the normal group (17.35±3.24 µg/ml at 2.04h) (Jia, Xu and Xu, 2017).

Clinical studies

A clinical study, carried out in 50 patients with hypercholesterolemia on short-term administration of oral nutraceutical combination (NC) consisting of 500 mg BBR, 200 mg red yeast rice, and 10 mg policosanols daily for 6 weeks, decreased

Table 1. Uses of Berberine containing species.

Biological name	Berberine content (%)	Activity	Reference
Berberis aristata	Root - 4%	Antimicrobial activity Anti-osteoporotic activity Antidiarrheal activity Antihyperglycemic activity Cytotoxic activity	Singh, Srivastava and Rawat, 2007; Yogesh et al., 2011; Joshi, Shirkhedkar,Prakash, & Maheshwari, 2011; Semwal, Gupta,Singh, Kumar, & Giri, 2009; Das, Das, Mazumder, Dasand Basu, 2009
Berberis asiatica	Root - 2.09% Steam- 1.29%	Antimicrobial activity Antibacterial activity Anti-diabetic activity Anticancer activity	Bhandari, Nath, Ray, & Tewari, 2000; Singh, Vishnoi, Dhingra, & Kishor, 2012; Singh,& Jain,2010
B. chitria Buch-Ham. ex. Lindl	5.0 - 4.2%	Antimicrobial activity Wound healing activity	Eto, Inoue, & Kadowaki, 2012; Biswas & Mukherjee, 2003
B. lycium Royle	2.3%	Antimicrobial activity Antihyperglycemic activity Anti-diabetic activity Wound healing activity	Hussain, Khan, Habib, & Hussain, 2011; Jigneshkumar, 2011; Gulfraz, 2008; Pirbalouti, Azizi, Koohpayeh, & Hamedi, 2010
B.vulgaris L.	5%	Antibacterial activity Antioxidant activity Hepatoprotective activity Antimicrobial activity	Villinski et al.,2003; Tomosaka et al., 2008; Agarwal,Srivastava, Saxena, & Kumar, 2006; Owokotomo & Owoeye, 2011
Coscinium fenestratum (Goetgh.) Colebr.	Stem 3.5%	Antidiabetic activity Antibacterial effect Antihyperglycemic activity	Shirwaikar, Rajendran and I. S.R. Punitha, 2005a; Nair, Narasimhan, Shiburaj, & Abraham, 2005; Shirwaikar, Rajendran and I. S.R. Punitha, 2005b.
Mahonia napaulensis DC.	Root 0.5%	Antifungal activity Anti-inflammatory activity	Bajpai and Vankar, 2007; Andreicut et al., 2018
Thalictrum foliolosum DC.	Rhizome 0.35%	Anti-gastric ulcer activity Antiplasmodial activity	Malairajan, Gopalakrishnan, Narasimhan, & Veni, 2008; Das, Rabha, Talukdar, Goswami, & Dhiman, 2016;
Berberis aquifolium	Rhizome 8-9%	Antimicrobial activity Antiradical and antioxidant	Cernakovaand Košťálová, 2002; Rackova, Majekova, Kost, & Stefek, 2004
Berberis thunbergii	Rhizome 8-9%	Antioxidant and Anti-Inflamantory activity	Zhang, Schutzki and Nair, 2013
Coptis teeta	Rhizome 8-9%	Antibacterial activity Inhibitors of topoisomerase	Kong et al., 2009; Kobayashi et al., 1995
B.lyceum	Steam - 4%	Antimicrobial activity	Ali, Uddin and Jalal, 2015
Palmatine	0.30%	Antifungal activity Inhibition of tyrosine hydroxylase	Li et al., 2015; Lee, Zhang and Kim, 1996
Goldenseal	0.5 – 6%	Antibacterial activity Inhibits human CYP3A activity Human cytochrome p450 inhibition	Ettefagh, Burns, Junio, Kaatz,& Cech, 2011; Gurley et al., 2008; Chatterjee and Franklin, 2003,
Goldthread	8-12%	Hair growth–promoting activity Inhibit prostate cancer cell proliferation and NF-Kbsignaling	Zhao, Collier, Huang, & Whelan, 2014

the atherogenic profile and lipid levels in the hypercholester-olemic patients (Affuso, Ruvolo, Micillo, Saccà, & Fazio, 2010). Another study showed that 40 hyperlipidemic patients on administration of BBR alone (500 mg/day) against BBR (500mg/day) combined with 10 mg policosanol and 3 mg/day red yeast extract for 4 weeks exhibited decreased TG (triglyceride) and LDL-C (low density lipoprotein cholesterol) in the combination group by 26% and 25% respectively when compared to BBR alone (22% and 20% respectively) (Cicero, Rovati and Set-nikar, 2007). Another clinical study in 97 T2DM patients (type-2 diabetes mellitus) suggested the insulin receptor (InsR)-up-regulating and glucose-lowering activities of BBR (1g/d for 2 months). In these patients, the FBG (fasting blood glucose), HbA1c (Hemoglobin A1c), and TG (triglyceride) levels were significantly reduced by 25.9%, 18.1%, and 17.6%, respectively, as compared to their levels prior to the treatment. In patients affected with chronic hepatitis B and C, the FBG and TG levels were significantly lowered after treatment with berberine (Zhang et al., 2010). In another study, which observed the effect of either placebo or a proprietary nutraceutical combination consisting of BBR, policosanol, and red yeast rice in 64 patients with metabolic syndrome after 18 weeks of treatment, a significant reduction in the homeostasis model assessment of insulin resistance (HOMA-IR) index and low density lipoprotein-cholesterol (LDL-C) in the nutraceutical combination group compared with placebo was observed (HOMA: -0.6±1.2vs 0.4±1.9; p<0.05 and LDL-C: -0.82±0.68vs -0.13±0.55 mmol/L; p<0.0001). In addition, significant reduction in blood glucose and insulin levels and increase in endothelial- dependent FMD (flow mediated vasodilation) (1.9±4.2; p<0.05) were also observed (Affuso et al., 2012). A trial carried out on women affected by polycystic ovary syndrome showed that, in the treatment with BBR when compared to metformin treatment, similar decrease in waist circumference and waist-to-hip ratio (p<0.01), FPG (fasting plasma glucose) and FPI (fasting plasma insulin), insulin area under the curve, and HOMA-IR (p<0.05) were recorded (Wei et al., 2012). Table 2 shows the comparative plasma and tissue levels of berberine in animals and humans through different routes of administration.

Challenges in the bioavailability of berberine

The bioavailability of any agent within the body is foreshortened owing to its short inherent activity, poor absorption, elevated rate of metabolism, the formation of metabolic products, and/or the quick elimination and clearance from the body. Studies to date have suggested the utility of BBR as a therapeutic agent for the treatment of various ailments. However, several previous studies related to the absorption, distribution, metabolism, and excretion of BBR indicate poor absorption with rapid metabolism of BBR that significantly affects its bioavailability. Studies of BBR in rats have reported low oral bioavailability of <1% (0.68% and 0.36%) (Chen et al., 2011; Liu et al., 2010) due to extensive intestinal first-pass elimination, interaction with P-glycoprotein (Pg-P) efflux pumps, and the high distribution in the liver. In this section, problems of BBR bioavailability such as low plasma levels, limited tissue distribution, apparent rapid metabolism, and short half-life are described in detail.

Table 2. Plasma and Tissue levels of berberine in animals and human through different routes of administration.

Species	Route	Dose	Plasma/Tissue	Time	Conc. level	Ref.
Rat	Intragastric	96 mg/kg	Plasma	15 min	20.04±12.14 ng/mL	Gong et al., 2014b
Rat	Intravenous	3 mg/kg	Hippocampus	3.6 h	272 ±12 ng/g	Wang et al., 2005b
Rat	Oral	200 mg/kg	Tissue	1.33 h	25.85±7.34 µg/mL	Tan et al., 2013
			Liver	8 h	68.19 ng/g	
			Kidney	12 h	13.92 ng/g	
			Lung	4 h	11 ng/g	
			Muscle	12 h	5 ng/g	
			Brain	12 h	8 ng/g	
			Heart	24 h	9.5 ng/g	
			Pancreas	2 h	9.8 ng/g	
			Fat	2 h	10 ng/g	
Rat	Oral	4 mg/kg	Plasma	0.083 h	1205.3±270.9 ng/mL	Liu, et al., Hao, Xie, Lv, Liu, & Wang, 2009
Rat	Oral	200 mg/kg	Plasma	2.5 h	112.30±12.07 ng/mL	Wang et al., 2005a
Human	Oral	400 mg	Plasma	9.8±6.6 h	0.4356±0.2792 ng/mL	Inbaraj, kukielczak, Bilski, Sandvik, & Chignell, 2001
Mice	Intravenous	2.5 mg	Plasma	0.083 h	0.712 µg/mL	Wang et al., 2011
Rabbits	Intragastric	50 mg/kg	Plasma	1.09±0.37 h	129.49±24.54 ng/mL	Hu et al., 2013
Rat	Intragastric	25 mg/kg	Plasma	15.0 min	18.53±0.61 ng/mL	Yu et al., 2017

Plasma concentration

One of the major problems associated with BBR is its extremely low plasma levels. The low plasma concentration of BBR is mainly related to the following pharmacokinetic causes: 1. extensive metabolism in the gut and/or liver, 2. marked excretion to intestinal lumen, bile, and urine, as well as enterohepatic circulation, 3. poor absorption, 4. predominant tissue distribution.

When BBR was given orally at a dose of 20 mL/kg to rats, a maximum plasma concentration of 2.31 µg/mL was observed at 0.5 h of the study and high concentration until 5 h; BBR was slowly eliminated from the rat bodies. Following intravenous administration of 3 mg/kg of BBR to rats, the concentration of BBR in plasma and hippocampus was found to be 38.5 ng/mL at 4 h and 30.7 ng/mL at 48 h, respectively, with fast elimination (1.13 h) from the body (Wang et al., 2005). The elimination phase of BBR in the liver was significantly slower than that in blood. When BBR at a dose of 20 mg/kg was administered via femoral vein by i.v. bolus injection, it was detected in the liver up to 80 min post administration. BBR in bile was detected for up to 6 h; the disposition of BBR in bile is higher as compared to that of the liver and blood (Tsai & Tsai, 2004).

Tissue distribution

Uptake and distribution of BBR in body tissues is very important for its biological activity, yet only a limited number of studies have been addressed this issue. Although BBR is present at a very low level in blood and its bioavailability was reported to be less than 1%, the pharmacological effect of BBR can be correlated with its high tissue distribution. BBR at a dose of 200 mg/kg via oral route showed a maximum amount in the liver (68.19 ng/g) 8h after dosing. A maximum concentration of BBR was observed at 24 h in the heart; 12 h in the kidneys, muscles, and brain; 4h in the lungs; and 2 h in the pancreas (Tan et al., 2013). BBR can easily penetrate the blood-brain barrier, with rapid accumulation in the hippocampus region after intravenous administration, followed by slow elimination (Tsai & Tsai, 2004). Tissue distribution of BBR in rats after oral administration of 6 g/kg for 1 week was determined by a HPLC method. Repeated administration of BBR dose increased its concentration in the heart, liver, and kidneys, with lower concentration in the spleen and lungs (Xiao et al., 2018). When BBR and 8-cetylberberine (8-BBR-C16) was given orally at a dose of 80 mg/kg to rats, the plasma concentration increased 2.8 times. Tissue distribution was determined by RP-HPLC; a maximum concentration (3731.82 ng/g)was found in the lungs (Hu, Chen, Zou, Li, & Ye, 2014).

Metabolites

Numerous studies have assessed the metabolism of berberine in rats and in humans. Once absorbed, BBR is subjected to conjugations like sulfation and glucuronidation at various tissue sites. BBR was reported to undergo extensive metabolism when administered orally to rats. The liver was indicated as the major organ responsible for the metabolism of BBR. Following oral administration of BBR to rats (100 mg/kg) and humans (300 mg/kg), the urinary metabolites analyzed indicated that BBR undergoes similar bio-transformations in both rats and humans. Nine metabolites were isolated, demethyleneberberine-2-sulfate (HM1 and HM3), jatrorrhizine-3-O-sulfate (HM5), thalifendine

(RM5), jatrorrhizine-3-O-β-D-glucuronide (HM2), thalifendine-10-O-β-D- glucuronide (HM3), berberrubine-9-O-β-D- glucuronide (HM4 and HM2), 3,10-demethylpalmatine-10-O-sulfate HM6 and RM4), columbamin-2-O-β-D-glucuronide (HM7), and demethyleneberberine-2,3-di-O-β-D-glucuronide (RM1), by HPLC (Qiu et al., 2008). After the oral administration of BBR (40 mg/kg), the rat liver tissues and bile samples collected and evaluated showed the presence of four main metabolites (berberrubine, thalifendine, demethyleneberberine, and jatrorrhizine), detected in liver after 0.5 h and in bile 1h after the study (Wang, Feng, Chai, Cao, & Qiu, 2017). Liquid chromatography coupled to ion trap time-of-flight mass spectrometry (LC/MS-IT-TOF) was used for the identification of major metabolites of BBR, thalifendine (M1), berberrrubine (M2), and jatrorrhizine (M4), in the liver and kidney, respectively. The level of AUC (area under the concentration-time curve) for metabolites of BBR in the liver was 40 times higher and in kidney seven times higher than that in plasma (Tan et al., 2013). The LC/MS analysis of the plasma samples of BBR identified berberrubine, demethylene-berberine, and their glucuronide analogous as the important metabolites seen after oral administration (4 mg/kg, berberine) to rats (Liu et al., 2009). After oral administration of BBR (200 mg/kg) to rats, a total of 16 berberine related metabolites including 10 phase-I metabolites and six phase-II metabolites were identified and clarified by LC-IT-TOF/MS system (Ma et al., 2013). Figure 2 represents the *in vivo* phase I metabolites of berberine.

Half-life

The systemic elimination or clearance of BBR from the body is also an important factor which determines its relative biological activity. With the lower dose of BBR (3 mg/kg) administered intravenously to rats, the elimination half-life in thalamus was found to be 14.6 h, which indicates that BBR quickly distributes itself in the thalamus and can pass through the blood-brain barrier (Wang et al., 2005). The kinetic characteristics of BBR are different in the hippocampus and plasma. In the *in vivo* study, after 3 mg/kg i.v. doses were given to rats, BBR was eliminated from plasma in 1.13 h and from the hippocampus in 12 h (Wang et al., 2005). Another study reported that, with the higher dose of BBR (150 mg/kg) by oral gavage in groups i.e. G1 (untreated), G2 (Biofield treated berberine), and G3 (Biofieldtreated animals who received biofielduntreated berberine) groups, plasma clearance of BBR decreased to 81% (G2) and 50% (G3) as compared to the control group (Inbaraj, Kukielczak, Bilski, Sandvik, & Chignell, 2001). After oral administration of BBR at a dose of 200 mg/kg in rats, 83% of BBR was excreted mainly as thalifendine from bile (24 h) and as thalifendine and berberrubine (78%) on urinary excretion (48 h) (Ma et al., 2013).

Solutions to improve the bioavailability of berberine

The low oral bioavailability of berberine is one of the prime hurdles for market approval of BBR. The absorption, biodistribution, metabolism, and elimination studies of BBR have unfortunately shown poor absorption, rapid metabolism, and elimination of BBR as major reasons for its poor bioavailability. One of the approaches to overcome the low gastrointestinal absorption due to extensive intestinal first-pass effect resulting in low plasma levels of BBR involves effective modulation of the P-gp mediated efflux of BBR. Studies have focused on
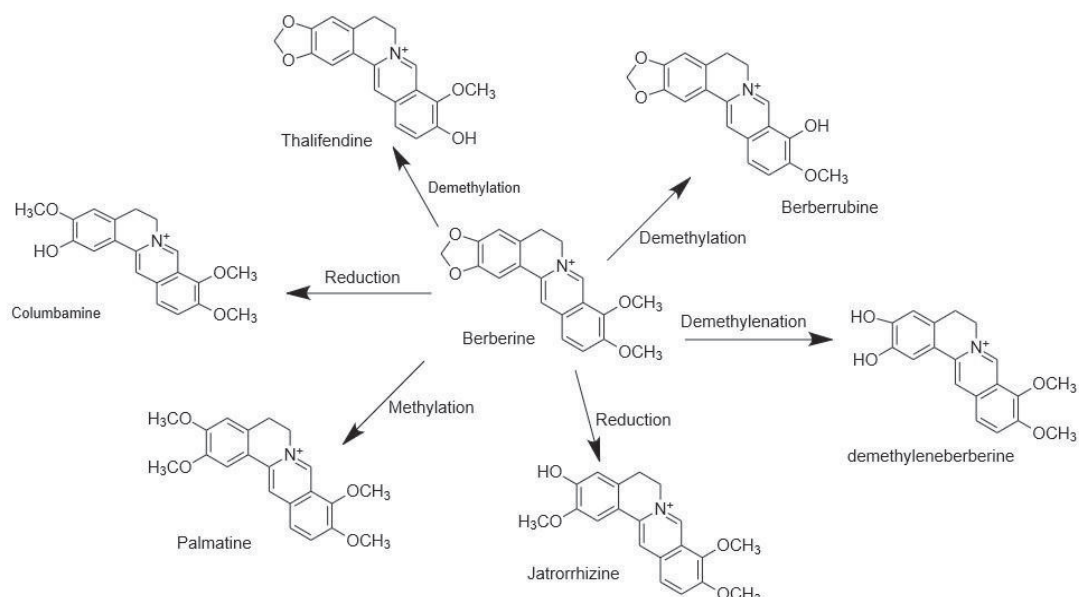
Figure 2. Phase -1 metabolites of berberine *in vivo*.

the effective utilization of pharmaceutical excipients that have P-gp inhibitory effects. Several novel dosage forms like nano-sized dosage forms, liposomes, micelles, and phospholipid complexes are optimistic formulations which overcome the problems of BBR by imparting longer circulation, better permeability, and resistance to metabolic processes (Chen et al., 2011). The strategies to improve the bioavailability of BBR are summarized in Table 3.

Nano-sized dosage forms

Presently, nano-sized technology is an important solution for bioavailability enhancement of therapeutic agents. BBR has been incorporated in nanoparticle-based delivery systems to circumvent the pitfalls of its poor bioavailability. The synthesis, physicochemical characterization, and antimicrobial activity of nanoparticles of BBR with sizes less than 110 nm has been reported. Antimicrobial activity of berberine nanoparticles prepared by evaporative precipitation of nanosuspension (EPN) and anti-solvent precipitation with a syringe pump (APSP) method have shown two-to-four-fold increase in activity against gram-negative bacteria (Sahibzada et al., 2018). Berberine-loaded solid lipid nanoparticles (BBR-SLNs) for topical application were developed and characterized as having a 76.8 nm particle size. Single doses of BBR-SLNs (50 mg/kg) compared to BBR alone showed substantial enhancement in the peak plasma concentration, area under the curve, and mean residence time after oral administration to rats. BBR-SLNs at a high dose (100 mg/kg) showed more significant effect when compared to an equivalent dose of BBR (Xue et al., 2013). Polymer-lipid hybrid nanoparticles (PEG-lipid-PLGA NPs) loaded with BBR phospholipid complex and BBR-soybean phosphatidylcholine complex (BBR–SPC) enhanced the oral BBR efficiency and can be used to enhance the liposolubility of BBR and improve the affinity with the biodegradable polymer in order to increase the drug-loading capacity and controlled/sustained release. The PEG-lipid-PLGA NPs/BBR-SPC enhanced the oral relative bioavailability (approximately 343%) when compared with the free BBR suspension group after administration at a dose of 50 mg/

kg via intragastric gavage in rats (Yu et al., 2017). The selenium-coated nanostructured lipid carriers (SeNLCs),160 nm in particle size, were formulated, which enhanced the oral bioavailability with an entrapment efficacy (EE) of 90% of BBR. An *in vivo* study in rats (50 mg/kg, BB-SeNLCs) by oral route demonstrated enhanced oral bioavailability (approximately 6.63 times) when compared to a BBR solution. The plasma concentration of BBR was quantified by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS) (Yin, Hou, Yin, & Song, 2017). Development of novel nano-systems shelled with heparin for BBR delivery increased the suppressive effect of BBR on *H. pylori* growth while efficiently reducing the cytotoxic effects in *H. pylori*-infected cells. In this research, the nanosystem was prepared by the simple ionic gelation method (Chang et al., 2011).

Nanocrystals dosage form

Nanocrystals are a major alternative for enhancing the poor oral bioavailability of water-soluble drugs as compared to other nanocarrier drug delivery systems. Formation of nanocrystals enhances the solubility and dissolution rate of poorly soluble drugs (Liu, Tu, Cheng,Feng, & Jin, 2020). Semi-crystalline nanoparticles (NPs) prepared by evaporative precipitation of nanosuspension (EPN) and anti-solvent precipitation with a syringe pump (APSP) were prepared to overcome the poor solubility of BBR and improve the dissolution rate and bioavailability of BBR. NPs prepared by the EPN method showed better results than those prepared by the APSP method in terms of solubility and dissolution rate (Sahibzada et al.,2018). Another study showed improved oral bioavailability using Brij-S20 (BS20)-modified nanocrystal formulation (BBR-BS20-NCs). A significant improvement in the maximum concentration (C_{max}) and area under the curve (AUC_{0-t}) in the pharmacokinetic studies were observed (Xiong et al., 2018).

Liposomes, micelles, and phospholipid complexes

Liposome is an excellent drug delivery system as it can be used to incorporate both hydrophilic and hydrophobic mol-

Table 3. Formulation development strategies for bioavailability enhancement of berberine.

Type of dosage form	Methodology used	Improved PK/Bioavailability parameters	Reference
Nano sized dosage form	Berberine loaded solid lipid nanoparticles (BBR-SLNs)	Substantial enhancement in the peak plasma concentration, area under the curve, and mean residence time.	Xue et al., 2013
	Polymer-lipid hybrid nanoparticles loaded with BBR phospholipid complex (PEG-lipid-PLGA NPs/BBR-SPC)	Enhanced the oral relative bioavailability	Yu et al.,2017
	Selenium-coated nanostructured lipid carriers (SeNLCs)	Enhanced the oral bioavailability with an entrapment efficacy (EE) of 90%.	Yin, Hou, Yin,& Song, 2017
Nanocrystals dosage form	Berberine -Brij-S20 -modified nanocrystal formulation (BBR-BS20-NCs)	Significant improvement in the maximum concentration (C_{max}) and area under the curve (AUC_{0-t})	Xiong et al., 2018
Liposomes	Berberine-loaded solid proliposomes	Higher blood plasma concentration (227.08 µg/ml). *In vitro* drug release study indicated sustained release of berberine-proliposomes.	Jia et al., 2019
	Chitosan-coated nano-liposome	*In vitro* study showed improved stability and delayed BH release in the simulated gastrointestinal (GI) environment	Nguyen et al., 2014
Micelles	Berberine loaded anhydrous reverse micelle (ARMs) formulation	Significantly increased plasma level of active BBR in rats	Wang et al., 2011
	Berberine-mixed1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)- 2000] (PEG-PE) and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) micelle system (mMic)	Intestinal absorption enhancement effect of approximately 3-5 fold (maximum plasma concentration of 1.22 mg/kg)	Shen, Kim, Yao, & Elbayoumi, 2016
Microemulsion	Berberine-loaded microemulsion formulation	Improved the bioavailability 6.47 times greater than that of the berberine tablet	Gui et al., 2008
	Berberine hydrochloric nanometer microemulsion	Improved the efficiency of antibacterial activity	Wu, Zhu, & Zhu, 2009
	Self-microemulsifying drug delivery system (SMEDDS)	*In vitro* study showed that the release of BBR from SMEDDS was faster (5h) than the commercial tablet. In the *in vivo* study plasma concentration was 163.4% higher than the commercial tablet.	Zhu et al., 2013
	Cremophore- integrated chylomicron "cremochylomicrons" loaded with berberine	Inhibited the BBR absorption (43%) and confirmed the lymphatic targeting ability of cremochylomicrons.	Elsheikh, Elnaggar, Hamdy, & Abdallah, 2018
	Berberine-loaded chylomicron	10.5-fold enhancement in intestinal permeability over free berberine	Godugu, Patel, Doddapaneni, Somagoni, & Singh, 2014

Table 3. Continue.

Type of dosage form	Methodology used	Improved PK/Bioavailability parameters	Reference
Mucoadhesive Microparticle	Novel duodenum-specific drug delivery system for duodenal application, based on thiolated chitosan and hydroxypropyl methylcellulose acetate maleate (HPMCAM)	90% of the drug released within 6h	Liu et al., 2011
	Mucoadhesive microparticle formulation prepared using the dual channel spray gun technology	Increased plasma concentration by 6.98 folds and decreased the tumor volume by 49.8-53.4% when compared to untreated control groups	Godugu, Patel, Doddapaneni, Somagoni, & Singh, 2014
Adjuvant	Nanosystem-based adjuvants with Baicalin or berberine loaded ultradeformable vesicles	*In vitro* study showed antioxidant effect (baicalin vesicle) and photoprotective effects (berberine vesicle)	Mir-Palomo et al., 2019
	Coptidis rhizome and berberine	*In vitro* study, showed correlation of *Coptidis rhizome* and berberine with the ECC line which decreased the S phase and accumulation of cells in the G0/G1 phase in esophageal cancer.	Iizuka et al., 2000
	BBR and *palmatine*	*In vitro* study showed that a combination of BBR and palmatine inhibits the acetylcholinesterases using the recombinant human acetylcholinesterases.	Mak et al., 2014

ecules. Polymerized, microencapsulated, and polymer-coated liposome systems have been employed to increase the potential of oral drug delivery. A recent study reported the preparation of BBR-loaded solid proliposomes using water as the solution enhanced dispersion by using supercritical CO_2 (SEDS) with an 80 nm size and high entrapment efficacy of 90.3% of BBR. *In vivo* study results indicated that the oral bioavailability of BBR-proliposomes was significantly improved following oral administration of a 150 mg/kg dose to rats. It was observed that the BBR-proliposomes resulted in higher blood plasma concentration (227.08 µg/mL) than that of the plain BBR (23.50 µg/mL). The *in vitro* drug release study indicated sustained release of BBR-proliposomes (Jia et al., 2019). *In vitro* and *in vivo* study for the chitosan-coated nano-liposome for the oral delivery of berberine hydrochloride (BH) carried out in rabbits demonstrated a marked improvement in the pharmacokinetic parameters after oral administration when compared to the uncoated nano-liposome. Also, the *in vitro* study showed improved stability and delayed BH release in the simulated gastrointestinal (GI) environment (Nguyen et al., 2014). 3^{2-} full factorial design was employed for the preparation of liposome. It was observed that the formulated liposome system increased the lipid concentration with the highest entrapment efficiency (78.43%). By increasing the plasma concentration levels and lowering the kinetics of elimination, micelles and phospholipid complexes are reported to enhance the gastrointestinal absorption of natural drugs thereby improving their bioavailability (Sailor, Seth, Parmar, Chauhan, & Javia,

2015). A significant improvement in BBR bioavailability was reported due to berberine-loaded anhydrous reverse micelle (ARMs) formulation. In this study, BBR (100 mg/kg) was administered orally to diabetic rats, and berberine anhydrous reverse micelle (ARM) exhibited a maximum plasma concentration of 0.628 µg/mL at 4 h after oral administration as compared to that of berberine solution(a maximum plasma concentration of 0.298 µg/mL at 2 h after oral dosing). About a 2.4-fold increase in oral bioavailability of berberine from the BBR-loaded ARMs in rats was observed over the free BBR. The results of the study indicated that the BBR-loaded ARMs can significantly increase the plasma level of active BBR in rats (Wang et al., 2011). The *in vitro* and *in vivo* anticancer activity of polymeric phospholipid micelles containing BBR was studied. In the *in vitro* study, faster release of berberine-mixed 1,2-disteroyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (PEG-PE) mixed with D-α-tocopherylpolyethylene glycol 1000 succinate (TPGS) was observed (61% berberine release after 24 h). In the *in vivo* part of this study, the effect of berberine-mixed 1,2-disteroyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethyleneglycol)- 2000] (PEG-PE) and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) micelle system (mMic) was evaluated in comparison to free berberine;enhancement of the intestinal absorption of approximately 3-to-5-fold (maximum plasma concentration of 1.22 mg/kg) was observed after a 300 mg/kg oral dose (Shen, Kim, Yao, & Elbayoumi, 2016).

Microemulsion

As a potentially excellent vehicle candidate, microemulsions can increase the solubility of bioactive materials and can also improve the absorption through human gut membrane because of their extremely low surface tension. Drugs can directly contact the GI epithelium cell; consequently, the bioavailability can be significantly improved (Swenson & Curatolo, 1992; O'Driscoll, 2002). Gui et al. have prepared and evaluated the oral microemulsion formulation of BBR (24.0 nm size) which was found to be stable at room temperature. An *in vivo* study with male Sprague-Dawley rats (50 mg/kg oral dose) demonstrated improved bioavailability of the oral berberine-loaded microemulsion formulation, which was 6.47 times greater than that of the berberine tablet suspension at 7.3 h of the study (Gui et al., 2008). Berberine hydrochloric microemulsion for antibacterial activity was developed and characterized by Sun and Ouyang (2007). The formulated microemulsion (56.8 nm size) was found to be stable under high temperatures and strong light conditions and gave a prolonged *in vitro* release of berberine. The *in vivo* study in rats resulted in improved antibacterial activity employing the microemulsion when compared to the other dosage forms. Zhu et al. developed self-microemulsifying drug delivery system (SMEDDS) to improve the oral bioavailability of BH. The *in vitro* study indicated faster release of BBR from SMEDDS (5h) as compared to the commercial tablet formulation, with improved stability. In the *in vivo* study in rats, the effect of SMEDDS was evaluated in comparison to commercial tablet formulation; the plasma concentration was found to be 163.4% higher than the commercial tablet following the administration of a 25 mg/kg oral dose (Zhu et al., 2013). A previous study demonstrated that the cremophore-integrated chylomicron, "cremochylomicrons," loaded with BBR inhibited the BBR absorption (43%) and confirmed the lymphatic targeting ability of the cremochylomicrons. In the *in vivo* part of this study, the effect of the formulated cremochylomicron was evaluated in comparison to free BBR in rat model. The comparison of the effect of cremochylomicron with free BBR gave a significant enhancement (>2 fold) in rate and extent of absorption of BBR (Elsheikh, Elnaggar, Hamdy, & Abdallah, 2018). Another study showed that the berberine-loaded chylomicron increased the intracellular fluorescence 2-fold, using confocal microscopy analysis with a 10.5-fold enhancement in intestinal permeability over free BBR. Therefore, berberine-loaded chylomicron is considered as an attractive bioactive-nanocarrier for berberine lymphatic targeting and bioavailability improvement (Godugu, Patel, Doddapaneni, Somagoni, & Singh, 2014).

Mucoadhesive microparticles

Chen et al. developed and optimized a novel mucoadhesive drug delivery system (drug-resin complex core loaded with BHberberine hydrochloride) which influences the gastric retention of berberine (85% at 300 min of the study) (Chen et al., 2008). A novel duodenum-specific drug delivery system for duodenal application, based on thiolated chitosan and hydroxy-propyl methylcellulose acetate maleate (HPMC-AM), which is used for the coating of thiolated chitosan microspheres,was also reported. The *in vitro* study showed that 90% of the BBR was released within 6h after transferring into simulated patho-logical duodenal fluid (SPDF) (Liu et al., 2011). Mucoadhesive microparticle formulation prepared using the dual channel spray gun technology significantly increased the oral bioavailability and anticancer effects of BBR. The *in vivo* study in Sprague Dawley rats demonstrated increased plasma concentration of BBR by 6.98-fold and decreased the tumor volume by 49.8-53.4% when compared to the untreated control group (Godugu et al., 2014).

Adjuvant

SilviaA previous study formulated and characterized nano-system-based adjuvants with baicalin/berberine-loaded ultradeformable vesicles for the treatment of vitiligo. The This *in vitro* study showed the antioxidant effect (baicalin vesicle) and photoprotective effects (berberine vesicle) through the stimulation of melanin production and tyrosinase activity in melanocytes (Mir-Palomo et al., 2019). *Coptidis rhizome* and BBR, a known inhibitor of the proliferation of human esoph-ageal cancer cell (ECCs) lines, were administered in human volunteers. The *in vitro* study showed a good correlation of the treatment with *Coptidis rhizome* and BBR on the ECC line, which decreased the S phase and the accumulation of cells in the G0/G1 phase, proving to be useful alternative therapies for oesophageal cancer (Iizuka et al., 2000). BBR and palma-tine, when used in combination, exhibited synergistic effect. The *in vitro* study showed that the combination of BBR and palmatine inhibits the acetylcholinesterases, using the re-combinant human acetylcholinesterases. The drug-reducing index of BBR and palmatine were found to be 2.98 and 2.66, respectively, which might be beneficial in the treatment of patients with Alzheimer disease (Mak et al., 2014). The anti-microbial activity of BBR against Methicillin-resistant *Staphy-lococcus aureus* (MRSA) (90%), the synergistic effect between BBR and oxacillin against MRSA, and the additive effect be-tween BBR and ampicillin were observed. BBR (1-50 μg/mL), compared with the vehicle-treated control group, decreased MRSA adhesion and intracellular invasion in human gingival fibroblasts (Yu et al., 2005). Thus, overall, these studies indi-cated that the activity of BBR can be modulated both at the cellular level and at the organismic level, and we can expect surprising types of regulations when different agents are used simultaneously with BBR.

Derivatives and analogues

The chemical structure of BBR plays a crucial role in its biologi-cal activity. For example, isomerization has proved to have a significant influence on the antioxidant activity of BBR. Thus, several researchers are working on the effective structural modification of BBR with the aim to achieve a better biologi-cal response. Numerous studies dealing with the improved biological activity of BBR derivatives and/or analogues can be found in the literature. Shan et al., in their study, designed the BBR analogue, designated IMB-Y53, which was reported to display improved glucose-lowering efficacy both in the *in vitro* and *in vivo* studies in comparison to BBR. In this study, the maximum tolerable dose of IMB-Y53 following oral administra-tion to male wistar rats was 200 mg/kg. IMB-Y53 absorption was rapid after oral administration. The maximum plasma con-centration of nearly 5.84 ng/ml was detected 2h after the oral

dose. In the *in vitro* study, when IMB-Y53 (40 µM) was orally administered, in the C2C12 cells, IMB-Y53 up-regulated InsR mRNA by 2.3-fold and protein expression levels by 1.9-fold. IMB-Y53 (20 µM-40 µM) stimulated the glucose consumption by 1.5-1.7 fold in the muscle cells. Another strategy to improve the biological activity of BBR involved the synthesis of new 13-diphenylalkyl analogues of BBR. The presence of the diphenylalkyl chain at the 13-position of BBR significantly improved the binding ability to the DNA (Shan et al., 2013). BBR is a novel cholesterol-lowering agent acting through a mechanism different from that of statins; the two methoxyl groups in an ortho-distribution (10- and 11-position) on the benzene ring increased the low-density-lipoprotein receptor (LDLR) up-regulatory activity (Kawanishi et al., 2006). Another group of researchers also designed, synthesized, and evaluated new BBR analogues that can up-regulate the low-density-lipoprotein receptor (LDLR). Accordingly, BBR analogues with the methylene dioxy group at the 2-and 3-position were reported to be the essential requirements for the activity. The *in vivo* study in hyperlipidemicrats (100mg/kg/day) decreased the blood cholesterol by 42.6% and LDL-c by 49.4% (Li et al., 2009). Although many BBR analogues are found to show improved biological activity over BBR, specific evaluation of structural analogues and/or derivatives of BBR demonstrated no significant improvement in the tissue and plasma distribution. However, the promising biological effects of the modified BBR derivatives/analogues compared to BBR throws light onto the possibility of modulating the bioavailability of BBR through such structural modifications.

CONCLUSIONS

Berberine is a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids which has been used for centuries as a remedy for many ailments. Extensive scientific research over the past decade has shown the ability of this compound to modulate multiple cellular targets and hence display favorable preventive and therapeutic action for the treatment of a wide variety of diseases. BBR acts on a diverse range of molecular targets like transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation and apoptosis, and inflammatory factors. As detailed in this review, tissue levels of BBR, irrespective of the route of administration, exhibited rapid metabolism and elimination, which are the major factors limiting its bioavailability. Effective modulation of the route and the medium of BBR administration, the blocking of metabolic pathways by the concomitant administration with other agents, and structural modifications are the main strategies that are now being explored to improve the bioavailability of BBR. Attempts to enhance the *in vitro* and *in vivo* efficacy of BBR through structural modifications of the molecule and/or new formulations have been recently reported and explored. This review outlines the challenges in the bioavailability improvement of BBR and the various solutions being researched to tackle this issue. Varied novel delivery strategies, including those of nanosized dosage forms, liposomes, and phospholipid complexes, offer significant promise and are worthy of further exploration in attempts to enhance the bioavailability,

medicinal value, and application of this interesting molecule from Mother Nature.

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