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Abstract	<p>Berberine is a yellow alkaloid naturally produced by a diversity of plants. It is extracted from the roots, rhizome, and stem bark of several plants. This natural product has been used for centuries in the Chinese medicinal herb. Berberine presents a wide range of pharmacological applications such as antidiabetic, hypolipidemic, antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, antidiarrheal, hepatoprotective, and, above all, antimicrobial. It has been tested clinically in the treatment of oriental sore, diarrhea, trachoma, diabetes mellitus type 2, hypercholesterolemia, and congestive cardiac failure. This alkaloid is also used in the treatment of gastric and inflammatory diseases and more recently is being investigated for its application in neurology, cardiology, and oncology.</p>
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Pharmacological/Biological Effects of Berberine

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Abstract

Berberine is a yellow alkaloid naturally produced by a diversity of plants. It is extracted from the roots, rhizome, and stem bark of several plants. This natural product has been used for centuries in the Chinese medicinal herb. Berberine presents a wide range of pharmacological applications such as antidiabetic, hypolipidemic, antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, antidiarrheal, hepatoprotective, and, above all, antimicrobial. It has been tested clinically in the treatment of oriental sore, diarrhea, trachoma, diabetes mellitus type 2, hypercholesterolemia, and congestive cardiac failure. This alkaloid is also used in the treatment of gastric and inflammatory diseases and more recently is being investigated for its application in neurology, cardiology, and oncology.

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Keywords

Alkaloid • berberine • bioavailability • medicine

Abbreviations

AChE	Acetylcholinesterase enzyme
AML	Acute myeloid leukemia
AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
AD	Alzheimer's disease
A β peptide	Amyloid β -peptide
AR	Androgen receptor
ACE	Angiotensin-converting enzyme
ATM	Ataxia-telangiectasia mutated
Bcl-2	B cell lymphoma 2
COX2	Cyclooxygenase-2
ER	Endoplasmic reticulum
ERK1/2	Extracellular signal-regulated kinase1/2
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
iNOS	Inducible nitric oxide synthase
IC50	Inhibition concentration 50%
LD50	Lethal dose 50
LXR	Liver X receptor α

64	LDLR	Low-density lipoprotein receptor
65	MMP	Matrix metalloproteinase
66	MDR	Multidrug resistance
67	NF- κ B	Nuclear factor- κ B
68	PPAR	Peroxisome proliferator-activated receptor α
69	PI	Protease inhibitor
70	SREBPs	Sterol regulatory element-binding protein ()

71 1 Introduction

72 Berberine (Fig. 182.1) is an isoquinoline plant alkaloid with a bright yellow color
 73 that under ultraviolet light provides a strong yellow fluorescence. It belongs to the
 74 protoberberine structural class and represents one of the most natural alkaloids
 75 studied [1]. It is extracted from the plant *Coptidis rhizoma*, which has been used for
 76 centuries as a traditional Chinese medicinal herb where it was found to have
 77 curative properties. Berberine appears in the roots, flowers, shoots, and bark of
 78 a number of important medicinal plants like *Berberis* (*Berberis aquifolium*,
 79 *Berberis vulgaris*, *Berberis aristata*), *Hydrastis canadensis* (goldenseal), *Coptis*
 80 *chinensis*, *Tinospora cordifolia*, and *Eschscholzia californica* and has been medic-
 81 inally used in many traditional medical cultures, including Ayurvedic herbal and
 82 Chinese herbal medicine. Berberine is an important traditional medicinal herb,
 83 which has been effectively used in the treatment of dysentery, diarrhea, stomatitis,
 84 throat infections, and hepatitis as folk medicine for a long time in China and other
 85 countries [2].

86 This compound has a wide range of pharmacologic effects, including protective
 87 effects on some gastric ulcer [3], treatment of inflammatory [3], cardiovascular or
 88 lipid, and glucose-related [4–11] diseases. In addition, berberine possesses antimicro-
 89 bial activity against some bacterial [12, 13] or fungal infections [14]. Recent
 90 evidence has indicated that berberine has also anticancer properties and is currently
 91 being used to treat a variety of different diseases or medical disorders like diarrhea,
 92 high cholesterol levels, diabetes, and microbial infections such a fungal (*Candida*)
 93 or bacterial (*Salmonella*).

94 2 Pharmacological Applications and Mechanism of Action

95 2.1 Diabetes

96 The hypoglycemic effect of berberine was accidentally found in the 1980s in China
 97 when it was administered to diabetic patients with diarrhea [15].

98 A large number of studies reported berberine to have antidiabetic properties *in vivo* in
 99 animal models as well as during human clinical trials [16–21], although its mechanism of

Fig. 182.1 Berberine cation

action has not yet been completely elucidated. There are many studies that describe and propose different mechanisms of action of berberine related to the antidiabetic properties. Berberine is implicated in protection of β -cells (cells responsible for insulin secretion) in pancreas as well as contributing to their regeneration [22, 23]. In addition, it protects liver cells from ER stress, suggesting that berberine is able to exert a glucose-lowering effect in hepatocytes [24]. It regulates insulin secretion from β -cells acting as an agonist of the fatty acid receptor GPR40 [25]. Some works *in-vitro* and *in-vivo* [26, 27] show that berberine treatment results in increased AMPK activity in 3T3-L1 adipocytes and L6 myotubes. The consequence of this regulation is increased translocation of GLUT4 in L6 cells and reduced accumulation of lipid in 3T3-L1 adipocytes. These findings suggest that berberine displays beneficial effects in the treatment of diabetes and obesity at least in part via stimulation of AMPK activity.

On the other hand, berberine is also able to induce the expression of insulin receptors (at least in liver) in a dose-dependent manner, through upregulation of insulin receptor mRNA [28, 29]. This effect was dependent on the presence of insulin. Berberine has also been linked to improving post-receptor signal transduction [24].

The “lipotoxicity” hypothesis indicates that fat-induced hepatic insulin resistance (FIHIR) may play a major role in the pathogenesis of type 2 diabetes, the most common form of diabetes mellitus. It has been suggested that berberine exerts a therapeutic effect on FIHIR in a model of type 2 diabetes in hamsters by modulating the hepatic SREBPs, LXR α , and PPAR α transcriptional programs [30].

One study has analyzed the hypoglycemic activity of berberine in comparison with current hypoglycemic drugs like metformin [5]. This study indicated that berberine might be beneficial for the recovery of β -cell function during diabetes or prediabetes.

Some of the studies mentioned above were found in *in-vitro* models and should be interpreted with caution. As described later, berberine has low bioavailability and shows poor absorption, supporting the hypothesis that berberine may exert its antihyperglycemic effect in the intestinal tract before absorption before reaching other organs like pancreas or liver [2]. Recent evidences suggest that the gut microbiota composition is associated with obesity and type 2 diabetes [31], which are closely associated with a low-grade inflammatory state. This study hypothesizes that gut microbiota modulation may be one mechanism of the antidiabetic effect of berberine although the mechanism has not been yet explained.

133 2.2 Hyperlipidemias

134 Berberine presents promising lipid-lowering capabilities. It was found to reduce
135 cholesterol in serum of human volunteers by a mechanism different to that of statins
136 [4]. They showed in a rat model that berberine upregulated the LDLR in the liver
137 [4, 32]. Recently Abidi et al. have shown that the root extract of goldenseal, an
138 herbal supplement that contains the alkaloids hydrastine, berberine, berberastine,
139 hydrastinine, tetrahydroberberastine, canadine, and canalidine, had a similar effect
140 in a hamster model [11]. In both studies, the effect of this increase in LDLR was the
141 reduction of LDL-associated cholesterol in blood.

142 Although there is striking evidence that berberine lowers cholesterol through
143 mechanisms independent of statins, its ability to regulate cholesterol biosynthesis
144 has yet to be considered.

145 A few studies have demonstrated that berberine increases bile formation and
146 secretion [33–35]. It has also been shown that berberine is able to interact with
147 micelles through hydrophilic and hydrophobic binding sites to form alkaloid–bile
148 salt agglomerates [36, 37]. This process is thought to decrease the capacity of
149 micelles to solubilize cholesterol and thus affects cholesterol absorption. However,
150 the effect of berberine on cholesterol absorption has not been directly studied.

151 The hypothesis that berberine lowered plasma cholesterol and non-HDL choles-
152 terol levels through a combination of inhibition of cholesterol absorption and
153 stimulation of bile acid synthesis was recently analyzed. This study shows that
154 berberine used in combination with other natural products, plant stanols, which
155 lower cholesterol through a different mechanism, improved the cholesterol-
156 lowering efficacy through a synergistic inhibition on cholesterol absorption.

157 Although the ~~in vitro~~ and ~~in vivo~~ evidences indicate that berberine possesses
158 cholesterol-lowering potential, a recent work suggested that promotion of foam cell
159 formation by berberine could counterbalance its beneficial effects [38]. By using
160 the model of atherosclerosis in apolipoprotein E-deficient mice, they showed that
161 berberine induced ~~in vivo~~ foam cell formation and promoted atherosclerosis devel-
162 opment. Thus, this effect could counterbalance the beneficial effect of this
163 cholesterol-lowering compound on atherosclerosis.

164 2.3 Inflammatory Disorders

165 The anti-inflammatory properties of berberine have been used worldwide for the
166 treatment of several inflammatory diseases including rheumatism and lumbago
167 [39, 40]. In some traditional medicines, the use of berberine to reduce fever is also
168 described [39, 41]. Although many studies have shown the anti-inflammatory
169 activity of berberine, the exact mechanism is unknown. However, it seems to be
170 related to the ability of berberine to inhibit prostaglandin biosynthesis ~~in vivo~~
171 [2, 42]. This effect seems to be mediated by the inhibition of COX2 expression
172 and activity [40, 42]. COX2 activity was not directly inhibited by berberine but
173 through the inhibition of COX2 regulatory proteins.

It has also been suggested that berberine reduces inflammation by inhibiting TNF- α secretion from differentiating adipocytes [40]. Since cyclooxygenases have been implicated in inflammation and carcinogenesis, those *in-vivo* observations may help to explain the anti-inflammatory and anticancer (see below) activity of berberine.

The beneficial effects of berberine as anti-inflammatory agent have also been shown *in-vivo* during endotoxin-induced sepsis. Berberine treatment reduces mortality [43] and prevents heart and lung injury after endotoxin administration in mice [43–45]. The mechanism by which berberine exerts this function seems to be related to the inhibition of TNF- α production and phospholipase A2 activity [45].

2.4 Cardiovascular Disease

Berberine has been used in the treatment of several heart diseases including hypertension and arrhythmia in folk medicine [46, 47]. Several pharmacological studies have shown the beneficial effects of berberine on diverse cardiovascular disorders in animal and human, including prevention of ischemia-induced ventricular tachyarrhythmia, improvement of cardiac contractile function, and reduction of peripheral vascular resistance and blood pressure [48, 49]. Berberine has also positive inotropic, negative chronotropic, antiarrhythmic, vasodilator, and anti-atherosclerosis properties [50].

Data from *in-vivo* studies in animals [51–53] and humans [49, 54] suggest berberine may be useful for patients with severe congestive heart failure.

Berberine is a known vasorelaxant in animals [46, 48] and has been used with success in humans [55], suggesting its possible clinical usefulness in the treatment of arrhythmias. Although the mechanism for berberine's antiarrhythmic effect is not clear, results from a rabbit model indicated it may be related to suppression of delayed afterdepolarization in the ventricular muscle [56]. Another work in rats suggested that, in addition to affecting several other parameters of cardiac performance, berberine has a vasodilatory/hypotensive effect attributable to its potentiation of acetylcholine [48]. The cardiovascular effects of berberine have been attributed to the blockade of delayed rectifier and ATP-sensitive potassium channels and stimulation of Na⁺–Ca²⁺ exchanger. This alkaloid is also able to prolong the duration of ventricular action potential. Diverse cellular mechanisms regulated by berberine have been proposed to explain its vasodilator activity. It appears to work on both the endothelium itself and underlying smooth muscle [57]. Other mechanisms proposed involve the angiotensin-converting enzyme and the NO–cGMP system [58] or blockade of the α 1-adrenoceptor [59, 60].

Berberine is also able to interfere with platelet function through diverse mechanisms like inhibition of thromboxane synthesis [61], enhancement of thrombolysis (breaking of clots) [2], interaction with α 2-adrenoceptor on platelets [62], and inhibition of calcium influx [63].

Finally, berberine has been shown to protect myocytes in a model of ischemia reperfusion [64].

2.5 Gastrointestinal Disorders

In the case of gastric ulcers, berberine has also been shown to have beneficial effects [3]. It was shown that berberine could significantly protect gastric mucosa from damage by ethanol in rats. This effect may be due to reduced expression of iNOS. Thus, berberine may affect nitric oxide production and associated damage by inhibiting iNOS expression in damaged gastric tissue and, ultimately, improve in the healing of ulcers.

Several *in-vitro* works conclude that berberine can ameliorate intestinal epithelial tight junction damage induced by the release of proinflammatory cytokines [65, 66]. This effect has been attributed to the downregulation of the NF- κ B and myosin light chain kinase pathway. These findings suggest that this compound may help to restore barrier function in injured intestinal mucosa [65, 66].

Berberine is currently being used to treat gastrointestinal-related medical disorders due to infections including bacterial diarrhea caused by *Vibrio cholerae* and *Escherichia coli* and intestinal parasites. The mechanism of the antidiarrheal activity of berberine has not been described yet. According to the study performed by Eaker et al., berberine significantly inhibits myoelectric activity and transit of the small intestine that appears to be partially mediated by opioid and α -adrenergic receptors, concluding that the antidiarrheal properties of berberine may be mediated, at least in part, by its ability to delay small intestinal transit [67].

2.6 Neurological Disorders

Berberine seems to have antidepressant and neuroprotective effects in several neurodegenerative and neuropsychiatric disorders. It has been reported to modulate neurotransmitters and their receptor systems in the brain. Moreover, some works indicate a beneficial effect of berberine in the treatment of central nervous system-related disorders including Alzheimer's disease, cerebral ischemia, schizophrenia, anxiety, and mental depression [68–71]. Supporting the observed effect of berberine on the central nervous system, Wang et al. showed that berberine can cross the blood–brain barrier and could be transported into the neurons in a concentration- and time-dependent manner [72].

2.7 Cerebral Ischemia

Stroke, known as a cerebral vascular accident (CVA), is the third leading cause of death in industrialized countries and the leading medical cause of acquired adult disability [73], but few effective therapeutic drugs have been used in clinical practice.

Recently, it was reported that berberine protects neurons from ischemic injury in gerbils and mice by reducing N-methyl-D-aspartate (NMDA) receptor 1 activity [69] and by suppressing apoptotic processes [74]. Studies *in-vitro* suggested that this effect was due to inhibition of ROS production and subsequent blockade of the

mitochondrial apoptotic pathway [74]. MMPs are responsible for maintaining and remodeling the extracellular matrix, and in addition to these physiological roles, abnormal MMP expression and activity may be involved in the pathophysiology of cerebral disease. However, it is not much known about the effect of berberine on MMPs, which are closely related to ischemic cerebral injury. By using a mouse model of brain ischemia, Hong et al. concluded that berberine may attenuate the delayed hippocampal neuronal cell injury [75] that normally follows transient global cerebral ischemia. Their results suggest that suppression of MMP-9 activity contributes to the protective effect of berberine.

2.8 Alzheimer's Disease

AD is the most common form of dementia in elderly people, becoming one of the most threatening diseases in this population. However, there is no efficient therapeutic agent to combat it. It is not easy to find a single target to treat AD, since its pathogenesis is very complex [76]. Diverse factors seem to be involved in the development of AD including oxidative stress, acetylcholinesterase enzyme (AChE), butyrylcholinesterase (BChE), monoamine oxidase (MAO), and A β peptide aggregation [77–82].

New experimental results suggest berberine may have a potential for inhibition and prevention of AD due to the multiple activities that berberine possesses including antioxidant activity, AChE and BChE inhibitory activity, MAO inhibitory activity, and its abilities to reduce A β peptide level and to lower cholesterol. Many studies have proved AChE-inhibiting property of berberine [83–85]. AChE is mainly present in the central nervous system and its major role is to catalyze the hydrolysis of the neurotransmitter acetylcholine to choline. This process can return an activated cholinergic neuron to its resting state. The pathogenesis of AD is linked to acetylcholine deficiency in the brain.

The effect of berberine *in vivo* has been studied in a rat model of AD concluding that intraperitoneal administration of berberine increased the spatial memory of animals by increasing IL1 β and iNOS expression in hippocampus [68].

It has also been shown *in vitro* that berberine reduces the secretion of A β peptide by human neuroglioma cells [86]. Since accumulation and aggregation of A β peptide seems to be the crucial event in the pathogenesis of AD [87, 88], inhibition of A β peptide generation is a promising therapeutic strategy to treat AD. According to Zu et al., berberine decreases the production of A β peptide by inhibiting the expression of BACE via activation of the ERK1/2 pathway. However, the effect of berberine on A β peptide accumulation was not shown *in vivo* until recently. By using a well-established model of AD, the TgCRND8 transgenic mouse, Durairajan et al. showed that berberine ameliorated neuropathology and cognitive impairment in these mice [89]. The mechanism responsible for this effect was studied *in vitro* and seems to be dependent of the modulation of the PKB/glycogen synthase kinase 3 (gsk3) signaling pathway.

2.9 Anxiety

Peng et al. showed using two different models of experimental anxiety that high doses of berberine (100, 500 mg/kg) have anxiolytic effect, which was similar to that observed with 1 mg/kg diazepam and 2 mg/kg buspirone. They concluded that this effect might be related to the enhancement of turnover rates of monoamines in the brain stem and the reduction of serotonergic system activity. Moreover, berberine was able to reduce serotonergic system activity by regulating the activity of somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} and 5-HT₂ receptors [71].

2.10 Schizophrenia

Prolyl oligopeptidase (POP) is a cytosolic serine peptidase that is known to hydrolyze proline-containing peptides at the carboxy terminus [90]. This enzyme has been associated with schizophrenia, bipolar affective disorder, memory loss, depression, and related neuropsychiatric disorders and therefore may be a relevant therapeutic target to treat these disorders. It is known that the activity of POP enzyme is decreased in depression and increased in psychotic conditions, such as mania and schizophrenia. However, current antipsychotic drugs do not affect the function of this enzyme [91]. According to Tarrago et al., berberine inhibits POP in a dose-dependent manner. As berberine is a natural compound that has been safely administered to humans, it is hypothesized that berberine might be used in the treatment of schizophrenia, opening up new perspectives for the treatment of neuropsychiatric diseases. Moreover, berberine also has D₂ dopamine receptor antagonistic and D₁ dopamine receptor agonistic activity [92], which may also play a role in its action.

2.11 Mental Depression

Depression is a common mental disorder. *In-vivo* experimental evidences suggest that berberine possesses central nervous system activities, particularly the ability to inhibit monoamine oxidase-A, an enzyme involved in the degradation of norepinephrine and serotonin (5-HT) [70]. In this study, the effect of berberine was attributed to the nitric oxide pathway and/or sigma receptors.

During a mouse *in-vivo* study, berberine was found to enhance the inhibitory neural pathway and decrease morphine dependence [93]. In addition, it modulated locomotor activity and induced hypnosis.

2.12 Infectious Diseases

Berberine possesses antimicrobial activity against bacterial [13, 94–96], fungal [97], protozoan [98, 99], and viral [100–102] infections. *In-vitro* studies have shown that berberine is effective against *Entamoeba histolytica*, *Giardia lamblia*,

Trichomonas vaginalis [98, 103], *Candida* spp. [97, 104], *Plasmodium falciparum* [99], *Staphylococcus aureus* [95, 105, 106], influenza virus [100], human immunodeficiency virus (HIV) [107], human cytomegalovirus [101], herpes simplex virus, [102] *Chlamydia trachomatis* [105], *Helicobacter pylori* [96], and *Leishmania donovani* [99].

However, few studies have analyzed the *in-vivo* relevance of these findings. Overall, most of the data suggest that berberine is a weak antimicrobial per se, but its activity is strongly synergized by other drugs.

One of the major problems in treating bacterial and fungal infections is the acquisition of the so-called MDR. The weak antimicrobial activity of berberine is mostly due to the MDR pump present in the membrane of the microorganisms, and combination with MDR inhibitors highly enhances the antimicrobial activity of berberine. Ball et al. showed that covalently linking berberine to INF-55, an inhibitor of major facilitator MDRs, results in a highly effective antimicrobial compound that readily accumulates in bacteria [108]. In addition, it has been shown that its activity is strongly synergized by 5'-methoxyhydnocarpin, an inhibitor of the bacterial multidrug resistance pump (MDR). Since gram-positive bacteria are the main targets of plant-derived MDR inhibitors, this may explain why so few of these bacteria are plant pathogens. Plants produce compounds structurally similar to berberine that intercalate into the DNA helix. Since berberine also binds to DNA irrespective of its sequence [109–113], this compound fulfills the requirements for a highly effective antibacterial agent since it is unaffected by mutations.

There are several other examples in which berberine is combined with other substances to potentiate its antibacterial activity. Flavones, chrysosplenol-D, and chrysosplenetin from *Artemisia annua* L. (*Asteraceae*), which possess very weak antibacterial action by themselves, produce potent combinations with berberine resulting in very effective inhibition of *Staphylococcus aureus* growth. The combination of amphotericin B and berberine can reduce by approximately 75 % the amphotericin B dose in the treatment of candidiasis in mice, implying that berberine indeed has synergy with amphotericin B against *C. albicans* [104]. Another example is methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria, which are responsible for substantial morbidity and mortality in hospitals. According to Yu et al., berberine is able to restore the effectiveness of β -lactam antibiotics against MDR strains of *Staphylococcus aureus* (MRSA) and inhibit the MRSA adhesion and intracellular invasion in HGFs (human gingival fibroblasts) [95]. However, the *in-vivo* relevance of this finding is not clear. In chloroquine-resistant malaria, the combination of pyrimethamine and berberine gives the best results in clearing the parasite and is more effective than both tetracycline and co-trimoxazole [114]. Berberine also inhibits *T. vaginalis* and its effect is comparable to metronidazole as regards potency [103]. In the mechanistic aspect, one study has shown that berberine has potent inhibitory activity against sortase A (SrtA) and sortase B. The inhibition of sortase enzymes results in a marked reduction in the virulence and infection potential of *S. aureus*, so it may be an important mechanism in the antibacterial activity of berberine [106].

A clinical study also showed the beneficial effects of the administration of aqueous berberine for the treatment of *Chlamydia trachomatis* infection [115]. It was found that the conjunctival scrapings of patients receiving the berberine chloride eye drops were negative for *C. trachomatis* and there were no relapses, even 1 year after treatment. The results suggested that berberine chloride had no direct antichlamydial properties, but it seemed to cure the infection by stimulating some protective mechanism in the host.

Several studies have analyzed the efficacy of berberine analogs and/or derivatives as antimicrobial agents [116–118]. These studies suggest that the presence of lipophilic substituents with moderate sizes contribute to the optimal antimicrobial activity [116]. In addition, the replacement of methoxyl groups at different positions of the ring by a methylenedioxy group results in increased antibacterial activity [117]. Finally, it was found that alkyl-substituted analogs of berberine are more active than berberine against two strains of *Staphylococcus aureus* and fungal infections [118].

There are some studies where the ability of berberine to inhibit the HIV-I has been evaluated [107, 119]. Zha et al. identified a key cellular mechanism underlying the protective effect of berberine on HIV PI-induced inflammatory response in macrophages. Modulation of the endoplasmic reticulum (ER) stress response represents a potential therapeutic target for various inflammatory diseases and metabolic syndromes, including HIV PI-associated atherosclerosis. However, *in-vivo* and clinical studies are needed to further clarify the putative application of berberine in anti-HIV therapy.

2.13 Anticancer Properties

Different chemotherapeutic drugs currently used to treat cancer patients are alkaloids like vincristine and vinblastine or taxanes paclitaxel and docetaxel. Although berberine is still not used in a clinical setting, it also possesses antitumoral activity against a number of tumor types. Indeed, a study using 60 tumor cell lines showed that the IC₅₀ value for berberine was similar to that of other alkaloids used in chemotherapy like paclitaxel or vinblastine [120]. Moreover, in contrast to other antitumoral drugs, the secondary toxicity of berberine is very low as explained in next sections. Cancer cells sensitive to berberine include hematological malignancies or solid carcinomas as explained below in more detail [121]. Most of the antitumor activity is based on the *in-vitro* evidence on the ability of berberine to affect several steps of tumor development including cell proliferation, cell death, and invasiveness [122, 123]. However, there are increasing evidences suggesting that berberine is also able to inhibit tumor growth *in-vivo* [122]. In addition, berberine enhances the efficiency of chemotherapy or radiotherapy effects. During the next sections, we will summarize the ability of berberine alone or in combination with other treatments to induce tumor cell death or to inhibit tumor cell invasiveness.

2.14 Regulation of Cell Death and Proliferation

Cell death induced by berberine *in vitro* mostly exhibits a typical apoptotic phenotype, including the activation of a family of proteases, the caspases, and cytochrome c release from mitochondria. Berberine has been shown to activate the two major proapoptotic mechanisms known as extrinsic and intrinsic pathways. Intrinsic (also known as mitochondrial) pathway is regulated by the members of the Bcl-2 family. This family comprises proapoptotic (Bak, Bax, Bid, Bim, Puma, etc.) or antiapoptotic (Bcl-2, Bcl-XL, Mcl-1, A1, etc.) proteins [124]. Activation and/or inhibition of proapoptotic or antiapoptotic members, respectively, induces the permeabilization of the mitochondrial outer membrane and, subsequently, the release of apoptogenic factors like cytochrome c, serine protease Htr/Omi, endonuclease G, SMAC/Diablo, or apoptosis-inducing factor (AIF). Cytochrome c together with Apaf-1 and caspase 9 forms a complex known as apoptosome that activates caspase 3 by proteolysis. Active caspase 3 is the ultimate effector molecule, responsible for cell dismantling including chromatin condensation and DNA fragmentation. The intrinsic pathway is activated at different levels like cell cycle arrest, disruption of antiapoptotic/proapoptotic ratio of Bcl-2 family members, DNA damage, and subsequent activation of the p53 repairing factor or proteasome inhibition. The extrinsic (also known as death receptor) pathway is activated after a specific ligand (death ligand, i.e., FasL) binds to its receptor (death receptor, i.e., Fas) on the cell membrane [125]. This binding activates a proteolytic signaling pathway initiated by caspase-8 that induces the direct or mitochondrial-mediated caspase 3 activation and the subsequent apoptotic cell death.

Berberine activates *in vitro* the intrinsic cell death pathway in several mouse and human tumor cell lines. Most of the studies suggest that berberine-induced apoptosis is initiated after DNA damage by cell cycle arrest in G0/G1 mediated by the ATM/p53 pathway [122, 126–129]. However, it has been recently showed that cell cycle arrest in G2M induced by berberine is mediated by the ATM/Chk1 p53-independent pathway in prostate cancer cells [130] (Fig. 182.1). Indeed, berberine is a cationic molecule able to interact with DNA in cell-free systems as well as in intact cells (Fig. 182.1) [110, 112–114]. Although apoptosis induced by berberine seems to be caspase dependent in most cell types [127, 131–133], the molecular mechanism/s that links cell death after DNA damage and cell cycle arrest with caspase activation, overall the role of the different members of the Bcl-2 family, is not known. Indeed, all evidences suggesting the involvement of Bcl-2 family members during cell death induced by berberine are indirect, and it has not been shown yet how overexpression or inhibition of those proteins affects this process. Thus, a formal proof of the implication of these proteins in cell death induced by berberine is still pending.

Two studies have proposed that berberine induces cell death by the extrinsic pathway in human colon carcinoma [134] and human hepatoma [131] cell lines; however, direct evidences of this mechanism were lacking. In neither of those studies, the role of Fas or FasL was tested if blocking the ligand or receptor could prevent cell death induction by berberine.

2.15 Solid Carcinomas

Berberine is active against diverse types of solid carcinomas when tested *in-vitro*, including tongue, liver, lung, thyroid, prostate, breast, brain, skin, esophageal, oral, or colon carcinomas [133–143].

Most interestingly, berberine also prevents the development of solid carcinomas *in-vivo* in both tumor xeno- or syngeneic transplants and carcinogen-induced tumorigenesis. Regarding models of tumor xenotransplants in immunocompromised mice, it has been shown that berberine inhibits the growth of different human lung [127] as well as human prostate cancer cell lines [144]. In both cases, p53-positive cell lines were more sensitive to berberine than p53-negative cell lines, suggesting a crucial role of this protein in the antitumoral effect of this alkaloid *in-vivo*. Concerning human prostate cancer cells, it was later shown that berberine reduced the expression of the androgen receptor in tumor cells *in-vivo*, indicating that it could interfere with pro-survival function of this receptor during the different stages of prostate cancer [145]. Finally, it was also showed that administration of berberine inhibited the growth of human tongue cancer SCC-4 cells, suggesting a beneficial effect of berberine-containing plants in the prevention of this type of neoplasm [135].

Strikingly, although berberine inhibited the growth of human Lewis lung carcinoma implanted subcutaneously in nude mice [127], it did not affect the growth of a mouse Lewis lung carcinoma after orthotopic implantation in lung parenchyma [141]. These apparent contradictory results could be due to the usage of different lung cell lines, the site of implantation, or the usage of xeno- versus syngeneic transplant models. Thus, the interpretation of results coming from human tumor xenotransplant models must be made with caution.

A beneficial effect of berberine has been also described for chemical-induced carcinomas. In a mouse model of two-stage chemical-induced carcinogenesis, it was found that berberine inhibited the enhancer effect of teleocidin in skin tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) [146]. This effect was attributed to the ability of berberine to inhibit 32Pi incorporation into phospholipids of cell membrane and hexose transport mediated by teleocidin. Supporting the efficacy of berberine against DMBA-induced tumorigenesis, using another tumor model, an independent group showed that berberine also prevented oral carcinogenesis induced by this chemical in mice [147]. Berberine was also active against methylcholanthrene- or N-nitrosodiethylamine-induced carcinogenesis in mice and rats [148].

2.16 Hematologic Malignancies

As well as in solid carcinomas, berberine is also able to kill diverse types of hematologic tumor cell lines *in-vitro*, such as acute myeloid leukemias, acute lymphocytic leukemias, and some types of non-Hodgkin lymphomas [111–114, 122, 129, 139, 149]. Despite that the first analyses about the antitumoral activity

of berberine were conducted in leukemic cells [112, 114], at present there are much fewer studies about the efficacy of berberine against hematologic neoplasias than against solid carcinomas. Specifically, only one study has analyzed the activity of berberine against cells from patients with AML [150]. The results showed that berberine was able to inhibit migration of AML cells by reducing the levels of stromal-derived factor-1 secreted by the bone marrow stromal cells from AML patients. The same effect was observed against cancer stem cells isolated from those patients.

A number of studies have analyzed the efficacy of berberine *in-vivo* against transplanted hematologic tumor cell lines as well as during virus-induced leukemia. Specifically, it has been showed that berberine has antitumoral potential *in-vivo* against transplanted mouse acute lymphocytic leukemia P388 [112] and acute myeloid leukemia WEHI-3 [151]. In addition, this alkaloid was able to inhibit the progression of erythroleukemia induced by mouse retrovirus, Friend, in immunocompetent mice [152].

2.17 Combined Therapies

One of the main problems during cancer management is the presence and/or apparition of resistant tumor cells due to mutations in the molecule/pathway targeted by the selected therapy [153]. During the last years, the combination of two or more treatment modalities (i.e., combination of different drugs or combination of drugs with radiotherapy) has emerged as a promising tool to overcome tumor resistance. Berberine has also been shown to enhance the antitumoral effect of chemo- and radiotherapy [122, 154].

Esophageal cancer has a very bad prognosis due to the low efficacy of current treatments. It was recently found that berberine enhances the sensitivity of human esophageal squamous cell carcinoma lines to radiotherapy [140]. This effect was related to the downregulation of the repair protein RAD51. Although this protein was present in specimens from patients with esophageal carcinoma, its role in disease pathogenesis is not clear. Other tumor cell lines that show enhanced susceptibility to radiotherapy in the presence of berberine are lung carcinoma [155], hepatoma [156], and glioma cells [157].

Berberine also enhances the cytotoxicity of chemotherapeutic drugs against tumor cells, including cisplatin [158], evodiamine [159], arsenic trioxide [160], 5-fluorouracil [154], doxorubicin [154], TNF- α [154], TRAIL [161], or nitrosourea [143]. It has been suggested that this effect was due to the suppression of antiapoptotic gene products by inhibiting the activity of the transcription factor NF- κ B [154]. This transcription factor that is constitutively expressed in several tumor cell types is regulated by the upstream I κ B (inhibitory subunit of NF- κ B) kinase (IKK). IKK phosphorylates I κ B, promoting nuclear translocation of active NF- κ B and transcription of target genes. Berberine was able to inhibit IKK and, thus, prevented I κ B phosphorylation and NF- κ B activation.

2.18 Inhibition of Tumor Invasiveness

Apart from its cytotoxic potential, berberine is able to inhibit tumor cell motility and invasiveness *in-vitro* at noncytotoxic doses in a number of human tumor cell lines like lung, melanoma, liver, breast, gastric, tongue, or nasopharyngeal carcinoma cell lines [123, 162–167]. In most cell types, inhibition of cell invasiveness by berberine was dependent on the downregulation of matrix metalloproteinase expression by interfering with diverse signaling pathways like those regulated by ERK, PI3K, or Rho GTPases [163–167]. Moreover, the finding that berberine is able to directly inhibit the activity of the transcription factor NF- κ B [154] suggests that this alkaloid may reduce the expression of matrix metalloproteinases bypassing the above-mentioned upstream signaling pathways.

Concerning the ability of berberine to interfere with tumor cell invasiveness *in-vivo*, it was shown that this alkaloid inhibited the formation of lung metastasis after inoculation of mouse melanoma B16 cells [167]. In addition, it was found that berberine inhibited the apparition of mediastinal lymph node metastasis after orthotopic implantation of mouse Lewis lung carcinoma cells in lung parenchyma [141]. At the berberine dose tested, it was not observed to have any effect on the growth of the tumor at the site of implantation. However, the combination of both camptothecin-11 and berberine was able to inhibit both tumor growth and metastasis. This promising result suggests the usage of berberine as antimetastatic drug in combination with other cytotoxic antitumoral agents.

Au4

2.19 Other Applications

Berberine has also been employed as an analgesic for the treatment of neurological conditions. Berberine has been shown to ameliorate tolerance inducement to some narcotics like morphine [168].

Berberine has been shown to suppress lipid synthesis from sebaceous glands, and thus, it has potential to be used as a topical agent in skin-related infections [169]. In addition, it has surfactant activity, enhancing skin permeability to polar drugs [170]. However, it should be noted that when used on skin, berberine has the potential to form free radicals induced by sunlight [171].

3 Bioavailability and Metabolism

It is widely accepted in the pharmaceutical industry that the distribution, metabolism, and efficacy of many drugs can be modulated depending on their affinity to serum albumin [172]. Hu et al. [173] investigated the association of human serum albumin (HSA) with berberine. It was found that an interaction occurred at the IIA domain of HSA. Since other alkaloid drugs may share with berberine binding sites in HSA, this finding may help to design therapeutic drugs with reduced binding constant to HSA and, thus, more efficient than current ones.

As described above, berberine has a wide spectrum of pharmacological actions, but the poor bioavailability may limit its clinical use. According to Pan et al., berberine is hardly absorbed in the intestine (less than 5 % in 2.5 h) [174]. Some studies [175, 176] suggest that the poor intestinal absorption is due to the negative effect of intestinal P-glycoprotein on this process. If true, P-glycoprotein inhibitors could be of therapeutic value by improving berberine bioavailability. Some works have been directed to improve berberine bioavailability showing that the use of a water-soluble form of vitamin E, called “D- α -tocopheryl polyethylene glycol 1,000 succinate” (TPGS), enhances the bioavailability of berberine [177–179]. For example, 2.5 % TPGS content of the solution enhanced berberine absorption by 4.9-fold [178]. This effect was attributed to the inhibition of P-glycoprotein efflux [179]. Another study concludes that sodium caprate could significantly promote the absorption of berberine in intestine and enhance its antidiabetic effect without any serious mucosal damage [180].

Au5

4 Secondary Toxicity

The most promising finding concerning the use of berberine as a therapeutic agent, especially in cancer, is its low secondary toxicity. One of the major problems of drugs used in chemotherapy is the secondary toxic effect against healthy tissues and/or cells. This is due in part to the low number of specific targets in transformed cells. Indeed, most of the drugs target processes in cancer cells, which are still ongoing in normal cells and tissues.

In vitro and *in vivo* evidences suggest that berberine is more targeted than other chemotherapeutic drugs. It has been reported that naive or activated T cells are resistant to berberine [181]. Furthermore, it has been shown that berberine is able to protect thymocytes (precursors of T cells) from cell death induced by dexamethasone, etoposide, or camptothecin [182]. Finally, it has been reported that macrophages are not only resistant to berberine-induced cell death but also able to get activated and produce immune-stimulatory cytokines like IFN γ and IL12 *in vitro* [183] and *in vivo* [184].

Concerning other organs and/or cell types, recently it was reported that berberine killed hepatoma cells without affecting the viability of normal hepatocytes. In addition, berberine shows much higher specificity for oral-derived tumor cells than for normal oral tissue [185]. Finally, it has been showed that berberine inhibits pathogenic forms of the androgen receptor in prostate cancer cells without affecting its function in normal cells and the morphology of normal prostates [186].

Apart from the low toxicity of berberine against normal tissues, it has also been showed that this molecule exerts a protective effect on lung tissue during irradiation [187].

Several groups have studied the pharmacokinetics and toxicity of berberine in mouse and rat models *in vivo*. Berberine toxicity is low in rat and mouse models when tested by histopathological criteria and with relatively high LD50 (dose at which 50 % of animals die) values [123]. The results show that the lethal dose 50 (LD50, amount of a compound required to kill 50 % of the treated animals) in rats is 205 mg/kg when given

t1.1 **Table 182.1** Clinical trial

t1.2	Study	Status	Condition	Interventions
t1.3	Polycystic Ovary Syndrome (PCOS): Effect Of Letrozole and Berberine	Recruiting	Polycystic ovary syndrome	Drug: letrozole–berberine Drug: letrozole Drug: berberine
t1.4	Role of Pioglitazone and Berberine in Treatment of Non-Alcoholic Fatty Liver Disease	Completed	Nonalcoholic fatty liver disease	Lifestyle intervention Drug: pioglitazone Drug: berberine
t1.5	Effect of Berberine on Hormonal and Metabolic Features in Obese Women With Polycystic Ovary Syndrome (PCOS)	Active, not recruiting	Polycystic ovary syndrome Obesity	Drug: berberine Drug: placebo
t1.6	Efficacy and Safety of Berberine in the Treatment of Diabetes With Dyslipidemia	Completed	Diabetes mellitus, type 2 Metabolic syndrome	Drug: berberine
t1.7	Therapeutic Effects of Berberine in Patients With Type 2 Diabetes	Completed	Type 2 diabetes	Drug: berberine Drug: metformin
t1.8	Armolidip Plus and Metabolic Syndrome	Completed	Metabolic syndrome	Dietary supplement: Armolidip Plus
t1.9	The Role of a Combination of Nutraceuticals in the Control of Cardiovascular Risk	Active, not recruiting	Metabolic syndrome	Drug: Armolidip Prev
t1.10	Combined Effects of Bioactive Compounds in Lipid Profile	Recruiting	Hyperlipidemia low-density-lipoprotein-type elevated triglycerides	Dietary supplement: Armolidip Plus Dietary supplement: placebo
t1.11	Effects of Armolidip Plus on Cholesterol Levels and Endothelial Function	Completed	Hyperlipidemia endothelial dysfunction	Dietary supplement: mixture of berberine, policosanol, red yeast, placebo
t1.12	Ezetimibe Versus Nutraceuticals in Statin-intolerant Patients	Not yet recruiting	Coronary artery disease	Drug: ezetimibe Drug: nutraceuticals

623 intraperitoneally and that it was well tolerated in doses of 100 mg/kg when given orally
624 to rats [188]. An independent study found that the LD50 of berberine in mice after
625 intravenous or intraperitoneal administration was 9 mg/kg and 57 mg/kg, respectively
626 [189]. This group did not find any LD50 for berberine after oral intragastric administra-
627 tion. However, as indicated in the previous section, low amounts of berberine enter the
628 circulation by this way. Of note, during a 3-month trial with patients suffering from type
629 2 diabetes, it was found that therapeutic doses of berberine did not affect liver or kidney
630 and only produced transient gastrointestinal effects on around 35 % of the patients [190].
631 Janhke et al. published in 2006 for first time the developmental toxicity evaluation of
632 berberine in rats and mice and no fetal adverse effects were noted [191].

633 Although berberine has not been reported to be mutagenic, this compound is able
634 to intercalate into the DNA double helix [192, 193]. Thus, it is possible that this
635 binding may lead to mutations in the genome of exposed cells. Some studies have
636 shown than non-transformed cells take up much lower levels of berberine than do

tumor cells [194]. However, at present the basis of this phenomenon is not understood and needs to be investigated.

The most common side effects include constipation, laxative, anaphylaxis, and other skin allergies such as dermatitis and rashes, and high dosages may cause respiratory and circulatory system problems [123]. Furthermore, berberine could displace bilirubin from serum-binding proteins, causing jaundice, kernicterus, and brain damage in infants [195–197]. Therefore, exposure of pregnant women to berberine is not recommended because it may lead to jaundice and kernicterus in the fetus [196].

5 Clinical Trials

Clinical trials have been conducted using berberine suggesting that this compound may be beneficial in the treatment of trachomas (eye infections), bacterial diarrhea, and leishmaniasis (parasitic disease). Berberine has been shown to be safe in the majority of clinical trials. However, there is a potential for interaction between berberine and many prescription medications, and berberine should not be used by pregnant or breastfeeding women, due to potential for adverse effects (Table 182.1).

Conclusions

Berberine, a natural alkaloid used for centuries in folk medicine, shows proven as well as promising therapeutic effects against a number of disorders including infection, cancer, inflammation, diabetes, or cardiovascular diseases. The versatility of this alkaloid to treat this great variety of apparently unrelated affections may be due to the ability of berberine to target key molecular pathways including NF- κ B, ROS, iNOS, COX, or AMPK. The low toxicity of berberine as well as its synergistic and protective effects in combination with other drugs becomes a promising molecule for the treatment of diseases with serious drug-associated sequelae like cancer. A weakness of berberine is its low bioavailability during *in vivo* administration. However, several efforts have been developed during last years in order to solve these problems and ensure a targeted therapeutic activity of berberine.

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