Review article

Potential cytotoxic and anti-metastatic effects of berberine on gynaecological cancers with drug-associated resistance

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Abstract

Gynaecological disorders, such as cervical, ovarian, and endometrial cancers are the second most prevalent cancer types in women worldwide. Therapeutic approaches for gynaecological cancers involve chemotherapy, radiation, and surgery. However, lifespan is not improved, and novel medications are required. Among various phytochemicals, berberine, a well-known natural product, has been shown to be a promising cancer chemopreventive agent. Pharmacokinetics, safety, and efficacy of berberine have been investigated in the several experiments against numerous diseases. Here, we aimed to provide a literature review from available published investigations showing the anticancer effects of berberine and its various synthetic analogues against gynaecological disorders, including cervical, ovarian, and endometrial cancers. In conclusion, berberine has been found to efficiently inhibit viability, proliferation, and migration of cancer cells, mainly, via induction of apoptosis by both mitochondrial dependent and independent pathways. Additionally, structural modification of berberine showed that berberine analogues can improve its antitumor effects against gynaecological cancers.

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1. Introduction

Berberine is a yellowish phytochemical isoquinoline alkaloid ingredient (Fig. 1) belonging to protoberbine group presented in various plant families such as Berberidaceae, Papaveraceae, and Ranunculaceae [1]. It is mainly extracted from stem, bark, rhizome, and roots of barberry (Berberis vulgaris), tree turmeric (Berberis aristata), Coptis (Coptis chinensis), goldenseal (Hydrastis canadensis), and Oregon grape (Berberis aquifolium) [1,2]. Berberine had a long history of medicinal application in traditional Chinese medicine, and had been shown to exert various pharmacological and biological effects with potential benefits to a variety of complex diseases, including cancer [3–5].

1.1. Pharmacokinetic of berberine

Bioavailability of an element in the body is determined via its pharmacokinetic profile encompassing absorption, metabolism, biodistribution, and excretion. Importantly, berberine have the low aqueous solubility that leads to its poor intestinal absorption. The low aqueous solubility also can result in high binding of plasma proteins to berberine and, consequently, negligible unbound fraction of berberine remains to reach and penetrate into target tissues [6–9].

As indicated by experimental studies, berberine is rapidly metabolized in the liver and excreted in the feces. Berberine is found to metabolize in the body through two main phases. Four major metabolites, including berberrubine (M1), thalifendine (M2), demethylberberine (M3) and jatrorrhizine (M4) are produced upon phase I metabolism (Fig. 1) [10]. The phase I metabolism of berberine is mainly conducted by cytochrome P450 enzymes in the liver. Berberine is demethylated to M1 and M2 metabolites via CYP3A1/2 and CYP2B enzymes. The phase II metabolites are created through conjugation of Phase I metabolites with sulfuric acid or glucuronic acid through enzymatic activity of UDP-glucuronosyltransferases (UGT) isoforms (Fig. 2). Biodistribution of berberine has been also been investigated; while levels of berberine metabolites are very low in bloodstream, higher levels were detected in the liver, followed by muscle, pancreas lungs, brain, heart, kidneys, with least distribution in fatty tissues [11–13]. Berberine was found to excrete from urine, feces, and bile by the rates of 0.0939%, 22.74%, and 22.83%, respectively. Thalifendine is the major metabolite excreting from the bile (83%) [14].

To sum up, the poor water solubility and extensive first-pass intestinal and hepatic metabolism are attributable to the low bioavailability of berberine (less than 5%) [6–9].

1.2. Enhancing bioavailability of berberine

To improve berberine aqueous solubility and oral bioavailability, several approaches including altering the structure of berberine or co-administering berberine with an absorption enhancer, as well as nano-based delivery systems have been developed [15,16]. For example, dihydroberberine is a structurally modified derivate of berberine that exhibits high absorption. In the intestine, berberine can be reduced to dihydroberberine through an enzymatic reaction carried out by the bacterial nitroreductase of the gut microbiota. Conversely, dihydroberberine can oxidate to the berberine through a non-enzymatic reaction in intestine tissue [17,18]. Thus, as a suggestion, suitable modification of the gastrointestinal microbiota through co-administration of probiotics could enhance the berberine bioavailability. On the other hand, the oral bioavailability of berberine can also be improved by using absorption enhancers such as sodium caprate that acts through elevating the cellular permeability via transient enlarging the tight junctions [19].
Additionally, a number of nanoparticle carriers has been also employed to improve solubility and bioavailability of berberine. Instance, lipid-based nanoparticulate carriers, including nano-liposomes [20–23], micelles [24], SLNs (solid lipid nanoparticles) [7,25,26], and NLCs (nanostructured lipid carriers) [27,28], have been found to improve solubility, oral bioavailability, and therapeutic efficacy through encapsulating berberine. Polymeric-based nanoparticulate carriers, including chitosan [29–32], modified dextran [33], PLGA (poly [lactic-co-glycolic acid]) [34,35], alginate nanoparticles [36,37], and nanogels [38], as well as the other types of nanoformulations containing berberine, including mesoporous silica-based nanoparticles [39,40], dendrimers [41], graphene nanoparticles [41], gold-based nanoplatforms [42,43], and carrier-free nano-systems such as fluorescent berberine microrods [44] and berberin-nanosuspension [45,46], have been also employed to enhance pharmacokinetic of berberine.

1.3. Safety of berberine

Oral administration of highly purified berberine sulfate has the safety window at a LD50 (lethal dose for the 50% of treated animals) of 25 mg/kg [47]. Although there have been no reports showing its genotoxic, cytotoxic and mutagenic activity, the middle-term use of berberine may result in dose-dependent gastrointestinal complications [48]. Berberine can bind to macrolides and potentially leads to dangerous arrhythmias [49,50]. Since berberine efficiently substitutes bilirubin for albumin [51], it should not be administrated in pregnant women and jaundiced neonates [52]. Berberine at low doses (<1 gr/day) can be metabolized easily by the liver enzymes, whereas at higher doses it can decrease the function of the liver enzymes such as cytochrome P450 [53]. However, several meta-analyses of randomized controlled trials recruiting patients with hypercholesterolemia and type 2 diabetes revealed safety and efficacy of berberine [54–57].

1.4. Pharmacological effects of berberine

The experimental and clinical studies have approved pharmacological activities of berberine. Berberine as a widely used natural product remedy has shown several pharmacological effects in modern and traditional medicine [58–60]. The documented evidence shows polytrophic medicinal effects of berberine and berberine-enriched plants, including antitumor [3], anti-diabetic [7], anti-inflammatory [61], anti-diarrheal [62], antimicrobial [63], antiviral [64] activities. Interestingly, berberine was also found to have cardiovascular benefits through improving cardiac contractility as well as reducing the blood pressure and the peripheral vascular resistance [65]. Of note, berberine can exert LDL-lowering effect in the dyslipidemia, mechanistically, through suppressing proprotein convertase subtilisin/kexin type 9 (PCSK9), an important suppressor of LDL receptor (LDLR), therefore elevating the frequency of LDLR on the surface of the hepatocytes, causing the increase clearance of LDL from blood circulation [66,67]. Additionally, berberine can indirectly suppress the liver synthesis of cholesterol through hampering hydroxymethylglutaryl CoA-reductase (HMG-CoAR) [68,69]. Berberine was also reported to have anti-diabetic effect and improve glycomic indices in type 2 diabetic patients through suppressing protein-tyrosine phosphatase1B (PTP8) and activating adenosine monophosphate-activated protein kinase (AMPK) [70–72]. Besides these metabolic effects, berberine has also shown anxiolytic and antidepressant effects in preclinical experiments, through elevating brain biogenic amines,
including serotonin, dopamine, and norepinephrine [47,73]. These pharmacological effects of berberine stem from its wide biological activities, including antioxidant, anti-inflammatory, and pro-apoptotic activity, as well as regulating cellular signaling pathways, interacting with DNA, and cell cycle arrest.

1.5. Anticancer effects of berberine

During the last decade, the anticancer effects of berberine have been an attractive issue in experimental research. Although there is a little evidence showing clinical effects of berberine in cancer patients, numerous in vivo studies have indicated inhibitory effects of berberine in a variety of cancers. A recent systematic review and meta-analyses of 26 animal studies from 2000 to 2018 investigating impacts of berberine on various cancer types, including breast cancer, liver cancer, colorectal cancer, nasopharyngeal carcinoma, lung cancer, gastric cancer, neuroepithelial cancer, endometrial carcinoma, esophageal cancer, tongue cancer, cholangio carcinoma, and sarcoma, exhibited that berberine inhibited growth of a variety of cancers, particularly breast and lung cancer. Overall, berberine was found to significantly reduce the volume and weight of tumors in a linear dose-response relationship. Importantly, it was also shown that berberine suppressed angiogenesis in tumor tissues, but, no significant effect was found on the body weight of experimental animals [4]. Berberine can exert these broad anticancer effects through targeting various oncogenic pathways and targets. Molecular mechanisms underlying anticancer of berberine were comprehensively explored in a most recent systematic review using systems pharmacology-based approaches, as an emerging interdiscipline that combines experimental assays and computational tools to understand the therapeutic mechanisms. Generally, mechanisms of anti-cancer effects of berberine was systematically summarized into following directions: proliferation (like cell cycle arrest and apoptosis), intracellular oxidative stress, inflammation, angiogenesis, migration, transcription factors, as well as radio- and chemosensitivity [5].

1.6. Molecular mechanism underlying anticancer effect of berberine

The major cellular signaling cascades involving in apoptosis are reactive oxygen species (ROS)-dependent apoptosis, Fas-dependent apoptosis, as well as p53-dependent apoptosis. ROS can directly or indirectly triggers the release of cytochrome C from mitochondria that promotes activation of caspases leading to induction of apoptosis [74,75]. Berberine can promote apoptosis by elevating the level of ROS and some ROS-associated signals, such as JNK/p38 signaling molecules [75]. Accumulated ROS was found to activate JNK/p38 cascade that down-regulates antiapoptotic protein Bcl-XL, causing the release of cytochrome C and activation of caspases [75]. Berberine could also promote ROS-mediated apoptosis through caspase-independent route via apoptosis-inducing factor (AIF) that, in response to different stimuli such as ROS, is moved from mitochondria to the nucleus and promotes condensation of chromatin and fragmentation of nucleosomal DNA [Fig. 3, left part] [76,77].

Besides ROS-dependent pathway, berberine has also been reported to impressively promote apoptosis through inducing Fas-dependent pathway [78,79] and activation of certain caspases, including caspase-8, -9 and -3 [80–82]. Fasl can induce signals through trimerization of FasR, which promotes cell death via apoptosis. Caspases belong to pro-apoptotic enzymes that, after activation by pro-apoptotic factors such as FasR/Fasl, can target and proteolyze cellular enzymes and thereby govern apoptosis process. Berberine can modulate expression and/or function of main mediators of Fas-dependent apoptosis, including Fasl/FasR, caspase-8, BID, Bax, Bcl-2/Bcl-XL, XIAP, as well as caspase-9 and caspase-3.

Berberine up-regulates both Fas and Fasl [78,79] and activates procaspase-8 in cancer cells [80]. Caspase-8 promotes activity of pro-apoptotic BID protein that interacts with Bax protein. Berberine elevates both BID and Bax proteins that are inserted into the outer membrane of mitochondria and forming oligomeric pores. These pores permeabilize mitochondrial membrane and lead to the release of pro-apoptotic factors like Apaf1 and cytochrome C, resulting in caspase activation [78–82]. Anti-apoptotic proteins Bcl-2/Bcl-XL can bind BID/Bax and suppress pore formation, and berberine was found to down-regulate gene expression of these anti-apoptotic proteins, leading to the release of apoptotic factors and subsequent activation of caspases. Additionally, berberine can promote caspase activation through suppressing the main caspase inhibitors such as XIAP and c-IAP1, whereby activates caspase-9 and caspase-3 and induces apoptosis in cancer cells [Fig. 3, right part] [79,83].

In addition to the aforementioned mediators, p53 (tumor suppressor p53) is known to activate gene expression of many proteins that mediate apoptosis and cell cycle arrest. Berberine was reported to induce both expression and activity of p53, causing the apoptosis and cell cycle arrest in malignant cells [81,84,85]. p53 can mediate pro-apoptotic effect of berberine through up-regulating some proteins that activate apoptosis, such as FasR, apoptotic protease activating factor 1 (Apaf1), Noxa, Bax, and Puma [86,87], and down-regulating pro-apoptotic proteins like survivin, Bcl-xL, and Bcl-2 [88]. Berberine can inhibit G1 phase of cancerous cells through up-regulating p53 and Kip1/p27, leading to cell cycle arrest [89,90]. Cyclin-dependent kinase (CDK) inhibitors like Kip1/p27 and Cip1/p21 activated by p53 arrest cell cycle through suppressing G1-cyclin cascades, such as cyclin D1/cdk4 or cyclin D1/cdk6 [89,91]. Studies on p53-mutated and p53 non-mutated cancer cells revealed that berberine can also inhibit cell cycle in G2 step through a p53-independent route [Fig. 3, bottom part] [92]. It has also been shown that berberine can induce apoptosis in hepatocellular carcinoma through suppressing arachidonic acid (AA) metabolic pathway, mechanistically, through elevating the ratio of AA to prostaglandin E2 (PGE2), inhibiting AA pathway, and reducing expression of phospholipase A2 (cPLA2) and COX-2 genes [93]. The chemosensitizing is the other suggested anticancer mechanism of berberine. For example, berberine was found to induce apoptosis and resensitize MCF-7/hypoxia breast cancer cells to doxorubicin through down-regulating the protein expressions of AMPK and hypoxia-inducible factor-1 alpha (HIF-1α) involved in the proliferation, apoptosis, angiogenesis, and hypoxia-induced multidrug resistance in human cancer cells [94,95].

2. Potential therapeutic effects of berberine on gynaecological cancers

Gynecologic cancers involve an unrestricted growth of abnormal cells that develop in a woman’s reproductive system, including cervical, ovarian, uterine/endometrial, gestational trophoblastic disease, primary peritoneal, vaginal and vulvar cancers. The treatment of gynaecological cancers has been known to remain an important challenge. When the cancer is diagnosed in the early stages, surgery is the initial and most effective therapeutic approach. Among gynaecological disorders, both cervical and endometrial cancers are found to be diagnosed in the early stages, while ovarian cancer, known as the “silent killer”, is often diagnosed with delay in an advanced stage in which its treatment is difficult [96,97]. There are several studies that have shown promising therapeutic effect of berberine on gynaecological disorders, including cervical, ovarian, and endometrial cancers. The present study was aimed to represent a literature review from available published investigations reporting effect of berberine on theses cancer cells.
2.1. Berberine and cervical cancer

2.1.1. General concepts of cervical cancer

Cervical cancer is the third leading cause of cancer-related death in women worldwide [98]. An indispensable prerequisite for the cervical cancer expansion is continuous infection with high-risk human papillomavirus (HR-HPV), especially types 16 and 18, with almost 90% of cervical cancer [99,100]. Although mortality rate of cervical cancer is decreased owing to screening the cervical cytology, it is still a challenge to manage the pre-invasive and invasive cervical lesions [101]. Interactions between the viral oncoproteins and the host proteins result in dysregulation of cellular processes, signaling pathways, and cell cycle control via p53 tumor suppressor protein [102], whereby cause development of cervical cancer [103,104]. Conventional treatments, such as using topical medications, direct administration of injectable interferon into the malignant lesions, laser therapy, cryotherapy, excisional surgery may assist to locally eliminate the warts or lesions, but cannot weaken the virus [105]. Therefore, virus transmission and lesion-relapsing are the most important issues in the cervical cancer treatment. The important HR-HPV oncoproteins are E6 and E7 that are responsible for cellular transformation and therefore

Fig. 3. Mechanisms underlying the anticancer effects of berberine. Berberine inhibits cell proliferation and induces cell death via promoting apoptosis and cell cycle arrest mainly through reactive oxygen species (ROS)-dependent apoptosis (Left figure), Fas-dependent apoptosis (Right figure), and p53-mediated apoptosis and cell cycle arrest (Bottom figure). Berberine can induce ROS-dependent apoptosis through elevating ROS production and activation of JNK/p38 cascade resulting in caspase activation and apoptosis induction. Berberine could also promote ROS-mediated apoptosis through caspase-independent route via ROS-dependent apoptosis inducing factor (AIF) which, in response to different stimuli such as ROS, is moved from mitochondria to the nucleus and promotes condensation of chromatin and fragmentation of nucleosomal DNA (Left figure). Berberine can enhance expression of both Fas ligand (Fasl) and Fas receptor (FasR) that are key apoptosis signal transducers. After attachment of FasR and Fasl, apoptosis is triggered by caspase-8 activation, resulting in activation of pro-apoptotic Bax/BID and in turn deactivation of anti-apoptotic Bcl-2/Bcl-xl. Berberine can also enhance this stage through up-regulation of caspase-8 and Bax/BID, and down-regulation of Bcl-2/Bcl-xl. Berberine promotes remaining steps followed by Bax-mediated pore formation in the outer membrane of mitochondria, which permit release of Apaf-1 and cytochrome c from the mitochondria and subsequent activation of a sequential caspase cascade (caspase-9 and caspase-3), leading to cellular apoptosis (Right figure). Additionally, berberine can induce apoptosis and cell cycle arrest through inducing expression of p53 in cancer cells. p53 mediated pro-apoptotic effect of berberine through up-regulating some proteins activating apoptosis such as Fasl, Apaf-1, Noxa, Bax, and Puma and down-regulating pro-apoptotic proteins like survivin, Bcl-xl, and Bcl-2. p53-mediated cell cycle arrest can be enhanced by berberine through up-regulating p53 and Kip1/p27 that suppress G1-cyclin cascades such as cyclin D1/cdk4 or cyclin D1/cdk6 (Bottom figure).
display proper objects for development of anti-HPV therapeutics [106]. The most orthodox chemotherapeutic drugs produce acute toxicity that causes detrimental side-effects. Therefore, it has necessitated investigating alternative medicines from the domain of natural products. Herbal derivatives with minimal or no systemic toxicity can be used as an antiviral method for HPV transcriptional deactivation. This reveals a favorable choice to inhibit HPV infection, especially, in an initial step of cervical cancer progression.

2.1.2. Protective effect of berberine against HPV-mediated cervical cancer

Cervical cancer is known to occur through infection of two specific types of HR-HPVs, HPV types 16 and 18. During tumor progression, the HPV integrates into the genome of the host cell that results in up-regulation of E6 and E7 oncoproteins, which suppress p53 and Rb proteins, respectively, and cause the dysregulated cell growth [107]. Activator protein-1 (AP-1) is a transcription factor constitutively over-expressed in cervical cancer, and was found to have a key role in HPV-mediated carcinogenesis through transcriptional regulation of HPV oncoproteins E6/E7 [108]. Berberine was reported to dose- and time-dependently inhibit the activated AP-1 and downregulate expression of E6/E7 oncogenes in HPV16-infected human cervical cancer cells, SiHa. Berberine-promoted suppression of E6 and E7 expression was identified to be associated with the concurrent elevation of p53 and Rb expression in both HPV16-infected SiHa cells and HPV18-infected HeLa cells. It was also found that berberine could inhibit growth of cervical cancer cells through obstructing DNA replication and/or interrupting genomic DNA by suppressing the transcription of hTERT (human telomerase reverse transcriptase). Notably, the higher dose of berberine has been shown to decrease the cell viability through inducing apoptosis via mitochondria-dependent pathway and activating caspase-3 [109]. Berberine-mediated inhibition of HPV oncoproteins was further supported by molecular docking studies that demonstrated berberine changed epigenetic modifications, disrupted microtubule cytoskeleton, and inhibited HPV-18 E6/E7 oncoproteins through modulating p53 activity in HeLa cells [110]. To sum up, berberine can potentially affect cervical cancer growth through inhibition of HPV oncoproteins (Fig. 4).

2.1.3. In vitro cytotoxicity and pro-apoptotic effects of berberine against cervical cancer cells

An in vitro study showed that berberine could exert dose-dependent selective anticancer effect against HeLa cells with IC50 of 12.08 μg/mL, whereas implied low toxicity (IC50: 71.14 μg/mL) on normal Vero cells [111]. Berberine was found to dose-dependently inhibit the proliferation of HeLa229 cells; maximum cytotoxicity was exhibited following treatment with 215 μM berberine hydrochloride and IC50 value was 42.93 μM following 72 h treatment. Additionally, berberine could dose- and time-dependently provoke apoptosis in HeLa229 cells. Pro-apoptotic effect of berberine was mediated by increasing the expression of p53 and decreasing the expression of Bcl-2 and COX-2. In conclusion, berberine suppressed proliferation and promoted apoptosis in HeLa229 cells, mechanistically through modulating expression of the pro-apoptotic protein p53 and the anti-apoptotic Bcl-2 and COX-2 proteins [112]. Similar results were reported in the other in vitro study, in which HeLa cells were treated by berberine at the dose of 20–40 mg/L and showed time- and dose-dependent inhibitory effect of berberine on treated cells. After exposing HeLa cells for 48 h to 20 and 40 mg/L berberine, the apoptosis rate reached (16.7 ± 2.8) % and (29.6 ± 4.4) %, respectively. Mechanistically, berberine could dose-dependently induce apoptosis via increasing production of pro-apoptotic Bax protein and inhibiting production of anti-apoptotic Bcl-2 protein [113]. Inhibitory effect of berberine on cervical cancer cells was also confirmed in another in vitro study that showed berberine dose- and time-dependently decreased the viability of CaSkI cells. Berberine exerted pro-apoptotic effect on CaSkI cells, mechanistically, through triggering the expression of p53 and Bax proteins and suppressing the expression of Bcl-2 protein, increasing levels of ROS and Ca2+, interrupting the mitochondrial membrane potential, and promoting activity of caspase-3 [114]. Importantly, radiation therapy can result in radiation-induced acute intestinal symptoms (RdAIs), and oral administration of 300-mg berberine three times

- Fig. 4. Inhibitory effect of berberine on HPV-mediated carcinogenesis in cervical cancer. Integration of HPV into the host genome results in over-expression of viral oncogenes E6 and E7. Activator protein-1 (AP-1) transcription factor is up-regulated in cervical cancer cells. E6 and E7 are transcriptionally regulated by AP-1. E6 can promote tumorigenesis through induction of p53 degradation, leading to loss of p53-mediated tumor suppression. E7 inhibits tumor suppressor retinoblastoma (Rb) protein whereby E2F transcription factor is activated and provokes activation of cell cycle and proliferation. Berberine was shown to suppress AP-1 and thereby inhibit E6 and E7 expression, which leads to upregulation of p53 and Rb resulting in inhibition of HPV-mediated tumorigenesis in cervical cancer cells.
2.1.4. Inhibitory effect of berberine on cervical metastasis

The epithelial-to-mesenchymal transition (EMT) is a multistep intricate pathway that involves in tumorigenic processes controlling proliferation and metastasis of cancer cells. Bereberine was reported to inhibit the angiogenesis and invasion of the cervical cancer cells [116]. While tumor is progressed, mesenchymal markers, such as vimentin, fibronectin and N-cadherin, are up-regulated and epithelial markers, such as α-catenin and E-cadherin are lost, eventually causing EMT phenomenon. These alterations lead to migration and invasion of tumor cells into distant sites where they are abnormally proliferated [117]. Berberine (20 μM) was reported to inhibit the migration and invasion of cervical cancer cells through revoking transforming growth factor-β1 (TGF-β1)-promoted EMT, up-regulating epithelial markers such as E-cadherin, and suppressing mesenchymal markers such as snail-1 and N-cadherin in SiHa cells [116]. During cancer metastasis, invasive cancer cells secrete high amount of extracellular proteases [118]. Of them, matrix metalloproteinase-2 (MMP-2) is an extracellular matrix (ECM)-degrading enzyme that can degrade supportive scaffolds of normal and tumor cells and, thereby, exerts an important effect on the angiogenesis and metastasis of malignant cells [119]. Additionally, urokinase-type plasminogen activator (u-PA) is a serine protease, which is significantly produced by cervical cancer cells and found to play critical role in degradation of the ECM through promoting a sequential of proteolytic steps [119,120]. Molecular studies revealed that berberine (20 μM) can exert the anti-metastatic effect through a mostly complete suppression on invasion of metastatic SiHa cells via inhibition of MMP-2 and u-PA [116]. Furthermore, angiogenesis is essential for cancer metastasis, since an elevation in vascular density can make facile access of cancer cells to bloodstream, and provides a valuable target for therapeutic medications. Berberine was exhibited to reduce in vitro and in vivo tumor-promoted angiogenesis. It was demonstrated that berberine (20 μM) can reduce angiogenic potential of SiHa cells through suppressing proliferation, tumor (SiHa cell)-promoted tube formation and invasion on HUVEC cells via down-regulating VEGF expression [116]. Of note, an in vivo study showed that oral gavage of berberine (20 mg/kg) could significantly diminish tumor growth and lung metastasis in BALB/c nude mice bearing cervical cancer SiHa cells [116].

2.1.5. Improved anticancer effect of berberine analogues against cervical cancer cells

Recently, several berberine derivatives have been synthesized and reported. Anticancer effect of berberine–linked piperazine derivatives bearing different functional groups was evaluated and exhibited that berberine analogues were more effective against cervical cancer CaSkI and HeLa cell lines, when compared with berberine compound. Importantly, berberine derivatives showed selective cytotoxicity on the cervical cancer cells in comparison with the normal cells. A berberine derivate with 4-methylpiperazine substituent was found to have the most significant antitumor effect with the IC50 values of 5.5 μg/mL (HeLa) and 6.7 μg/mL (CaSkI), followed by berberine derivate bearing meta-chloropiperazine rings with the IC50 values of 7.5 μg/mL (HeLa) and 6.6 μg/mL (CaSkI) [121]. Furthermore, N-Mannich bases of berberine, a berberine analogue bearing a methoxy functional group, acid functionality, and a cyano group was found to exert significant cytotoxicity against cervical cancer HeLa cells that are resistant to several cytotoxic drugs, showing potential antitumor effect of the berberine analogue against drug-resistant cervical cells [122]. Another study showed that N-Mannich bases of berberine linking piperazine moieties can exert cytotoxic effect on Hela and Caski cell lines, with IC50 values below 6 μg/mL [123]. It is further confirmed by the other study that revealed piperazin based N-Mannich bases of berberine bearing substituted functional groups are effective suppressors of HeLa and CaSki cancer cells with IC50 values ranging 4.346–6.321 μg/mL and 3.408–6.081 μg/mL, with decreased cytotoxic activity against normal cells [124]. Synthetic derivatives of berberine containing benzothiazole moieties with substituted functional groups exhibited the highest potency against HeLa and CaSkI cells with IC50 levels of 5.474 and 5.311 μg/mL, respectively [125]. Notably, Mistry et al. synthesized a series of 9-O-3-(1-piperazinyl/morpholino/piperidinyl) pentyl-berberines and showed that naphthal, benzhydryl, benzoyl, furfuryl and heterocyclic rings were functional groups responsible for expected cytotoxic effects against cervical cancer, HeLa and CaSiK cell lines. Interestingly, these berberine derivates revealed low toxicity against normal Madin-Darby canine kidney (MDCK) cell line, although showed high cytotoxicity on cancer cells [126]. In conclusion, aforementioned findings show that berberine derivates can significantly improve suppressor effect of berberine against cervical cancer cells.

2.2. Berberine and ovarian cancer

2.2.1. Berberine can suppress growth of ovarian cancer cells

The most common cause of death among women with gynaecologic cancers is ovarian malignancy [127–129]. The combination of surgery and chemotherapy is the traditional therapeutic strategy for ovarian cancer [130,131]. However, ovarian cancer is progressively transformed to a chemotherapy-resistant disease, and more than 70% of patients with ovarian cancer usually have the high rate of cancer recurrence [132]. Therefore, new treatment approaches, particularly against relapse after chemotherapy, are crucially required [133]. Although the caspase 3 activation is a hallmark of chemotherapy-induced apoptosis in the targeted cancer cells [134], it also has been found to promote tissue repopulation and regeneration by inducing cell proliferation and signal transduction in adjacent, non-apoptotic cells [135]. The caspase 3 can trigger activation of cytoplasmic calcium-independent phospholipase A2 (iPLA2) [136] and consequently activate COX-2. COX-2 is a determinant enzyme in the metabolic pathway of AA which converts AA into prostanooids, such as PGE2. Activating the cascade reaction of AA metabolic pathway causes abnormal alterations of PGE2 and AA levels in the tumor microenvironment. PGE2 was found to promote tumor growth and proliferation [137,138] through enhancing the phosphorylation of focal adhesion kinase (FAK) [139], a cytoplasmic protein tyrosine kinase, which regulates various cellular signaling pathways involved in the cell proliferation [140,141]. Berberine was reported to block chemotherapy-induced activation of caspase 3-iPLA2-AA-COX-2-PGE2 pathway through suppressing the expression of iPLA2 and COX-2, and reverse the elevated phosphorylation of FAK caused by excessive level of PGE2 and therefore reverse the repopulation of ovarian cancer cells after chemotherapy [142]. Hence, berberine can be considered as a specific inhibitor of iPLA2 and COX-2. Berberine was also reported to promote apoptosis and decrease tumor growth in ovarian cancer cells through inducing oxidative stress and DNA damage and down-regulating homologous recombination repair (HRR). Of note, HRR-defective cells are highly sensitive to inhibition of PARP1, and berberine was found to sensitize ovarian cancer cells to PARP1 inhibitor niraparib, whereby combination of berberine and niraparib have a synthetic lethal effect on ovarian cancer cells [143].
2.2.2. Effect of berberine on cisplatin-resistant human ovarian cancer cells

Ovarian cancer is known to become frequently resistant to systemic therapies [144]. A combination of cisplatin and paclitaxel is the standard first-line approach for treating ovarian carcinoma [145], however, the impression of resistant cells in the tumor restricts their therapeutic potential [146]. Acquired resistance to cisplatin occurs through a multi-factorial process including several mechanisms, among these intensified DNA repair and synthesis is the most common character of resistance in targeted cells. In this regard, the up-regulation of folate cycle enzymes dihydrofolate reductase (DHFR) and thymidylate synthase (TS) have been found to play the key role in the survival of cisplatin-resistant human ovarian cancer cell lines [147], and berberine was reported to inhibit the growth of cisplatin-resistant cells through down-regulating TS and DHFR enzymes [148]. Additionally, polyamines are important factors in cell growth and differentiation and present increased levels in malignant cells, therefore the pathway of polyamine metabolism is an interesting target in development of the anticancer drugs [149,150]. Of note, berberine was also demonstrated to suppress cellular growth in cisplatin-resistant ovarian cancer cells by interfering polyamine metabolism, specifically through the up-regulating the key catabolic enzyme, spermidine/spermine N1-acetyltransferase (SSAT) [148]. An in vitro study revealed that berberine can sensitize ovarian cancer cells (A2780) to cisplatin through inhibiting expression and function of miR-39 and consequent activation of PTEN/AKT signaling pathway [151]. Another in vitro study showed that berberine could enhance the sensitivity of SKOV3 ovarian cancer cells to cisplatin and thereby increase cisplatin-induced apoptosis via suppressing expression and function of miR-21 which subsequently led to enhancement of its target PDCD4, a key tumor suppressor in ovarian cancer cells [152]. Synergistic effect of berberine on anticancer effect of cisplatin was also reported by the other in vitro study that revealed combination treatment of berberine and cisplatin can strongly elevate cell killing effect of cisplatin on SKOV3 cancer cells [153]. Berberine could dose- and time-dependently promote apoptosis and suppress proliferation in ovarian cancer cells through reducing the expression of anti-apoptotic genes BCL-2 and survivin, and increasing the expression of pro-apoptotic gene BAX [153].

2.3. Berberine can alleviate adenomyosis

Adenomyosis is a main type of endometriosis and a common chronic gynaecological disorder, in which inner layer of uterus, ectopic endometrium penetrates into surrounding muscle layer of the uterus, myometrium [154–156]. Although adenomyosis is a non-malignant disorder, in some cases it shows some tumor-like behaviors, such as irregular proliferation, invasion and migration [157,158]. Traditional medications such as Mirena or GnRHa have been found to exert certain clinical benefits for the treatment of adenomyosis, however there are still some patients who eventually have to endure hysterectomy because of persistent exceptional complications [157,159]. Growing evidence indicated that this disease may be attributed to the production of inflammatory mediators and the promotion of an immune response [160–162]. Liu et al. studied the effects of berberine on LPS-induced ectopic endometrial stromal cells (EESCs) derived from patients with adenomyosis. Results showed that berberine could dose- and time-dependently suppress the LPS-induced proliferation of EESCs, mechanistically, through inducing apoptosis and arresting cell cycle in G0/G1 phase [163]. It was also found that berberine could inhibit inflammation in LPS-induced EESCs via suppressing the production of interleukin (IL)-6 and –8, epithelial growth factor, vascular
endothelial growth factor, TGF-β, and MMP-2 [163]. Further studies also revealed similar results, in which berberine was found to markedly inhibit the proliferation and survival of EESCs through inducing apoptosis and cell cycle arrest and suppressing the production of inflammatory mediators, while partially impacted the proliferation of normal endothelial stromal cells [164]. The aforementioned findings suggest that berberine can provide a valuable therapeutic approach for the clinical treatment of adenomyosis.

3. Conclusion

Berberine can inhibit growth of cervical, ovarian, and endometrial cancer cells through inducing apoptosis via modulating expression and function of pro-apoptotic proteins p53, Bax and caspase-3, and anti-apoptotic Bcl-2 protein, accompanied with ROS-induced GADD153 activity, as well as suppression of HPV oncoproteins E6/E7. Berberine can also prevent cervical tumor cell invasion and metastasis through revoking TGF-β1-promoted EMT, increasing the expression of epithelial markers such as E-Cadherin, and inhibiting expression of mesenchymal markers such as N-cadherin and snail-1, and suppression of MMP-2 and u-PA. Berberine is also able to reduce angiogenic potential of cervical cancer cells through suppressing proliferation, tumor-promoted tube formation and invasion via down-regulating VEGF expression. Importantly, structurally altered berberine analogues can significantly enhance anti-cancer potency of berberine against cervical cancer cells (Table 1). These findings suggest that berberine and/or its synthetic derivates could potentially be used as an anticancer drug in gynaecological cancers. Overall, further proof-of-concept studies are warranted to clarify the therapeutic role of berberine in an adjuvant to chemotherapy in patients with gynaecological cancers.

Declaration of competing interest

The authors declare that there are no conflicts of interest and financial support for the present review article.

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**Abbreviations**

- **UCG**: UDP-glucuronosyltransferases
- **LD**: Lethal dose
- **PCKS5**: Propionate convertase subtilisin/kexin type 9
- **HMG-CoA**: 3-hydroxy-3-methylglutaryl CoA-reductase
- **AMPK**: Adenosine monophosphate-activated protein kinase
- **PTP**: Protein-tyrosine phosphatase
- **ROS**: Reactive oxygen species
- **AIF**: Apoptosis-inducing factor
- **CDK**: Cyclin dependent kinase
- **HR-HPV**: High-risk human papillomavirus
- **COX-2**: Cyclooxygenase-2
- **RAS**: Radiation-induced acute intestinal symptoms
- **EMT**: Epithelial-to-mesenchymal transition
- **MMP**: Matrix metalloproteinase
- **ECM**: Extracellular matrix
- **u-PH**: urokinase-type plasminogen activator
- **MDCK**: Madin-Darby canine kidney
- **iPLA2**: independent phospholipase A2
- **PGE2**: Prostaglandin E2
- **FAK**: Focal adhesion kinase
- **HRR**: Homologous recombination repair
- **DHFR**: Dihydrofolate reductase
- **SSAT**: Spermidine/spermine N1-acetyltransferase
- **ECSC**: Ectopic endometrial stromal cells
- **IL**: Interleukin