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BERBERINE, COPTIDIS RHIZOMA AND DIOSPYRIN AS NOVEL ANTINEOPLASTIC AGENTS: A REVIEW ON TRADITIONAL USE AND BIOMEDICAL INVESTIGATIONS

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ABSTRACT

Natural products have played a vital role in drug discovery and development process for cancer. Coptidis Rhizoma (Huanglian) and its major component, berberine, have drawn extensive attention toward their antineoplastic effects in the recent years The antineoplastic effects are related to the Chinese Medicine (CM) properties of Huangliang in treating diseases by removing damp-heat and purging fire and counteracting toxicity. Diospyrin, a plant based bisnaphthoquinonoid, has been used as a lead molecule in an effort to develop anti-cancer drugs. The aim of the review is to trace the long history of the traditional use of Coptidis rhizome (Huanglian) from folk medicines, especially from Chinese medicine, to recent pharmacological studies of Huanglian and its major component berberine and diospyrin with an emphasis on their antineoplastic effects and the promise as novel antineoplastic agents.

Key words: Diospyrin, anti-cancer, Reactive oxygen species, Chinese medicine, Copitidis rhizome, Huanglian, Berberine.

INTRODUCTION

Diospyrin

Natural products have traditionally been a significant resource for drug development. The importance of natural products in drug discovery has been discussed in several reviews and report (Balunas, 2005; Jones *et al*., 2006; Koehn *et al*., 2005), with about 200,000 natural compounds having been already identified (Tulp *et al*., 2005), in the area of anti-cancer drugs, out of the total of 155 small molecules discovered since the 1940s, 47% (73/155) are natural products or their derivatives (Newman *et al*., 2005). The chemical diversity of natural compounds and the large number of chiral centers that they contain provide a basis for their use in further drug development.

Currently, drug discovery from plants has relied mainly on bioactivity-screening methods and isolation of the bioactive molecules (Kinghorn *et al*., 1994). Compounds isolated from plants, marine species, and microorganisms often serve as "lead" molecules which can further be optimized for better activity, reduced toxicity, or improved pharmokinetics to maximize their therapeutic potential. A number of compounds, such as podophyllotoxin, camptothecin, vincristine, vinblastine, paclitaxel, docetaxel, etc., containing a Quinone pharmacophore, have been isolated from plant sources and are considered important "leads" for chemotherapeutic treatment of many cancers.

Quinone compounds are the second largest class of anti-cancer agents currently in use (Sanchez-Cruz, 2009). They are widely distributed in nature, usually occurring as ubiquitous secondary metabolites, but also sometimes playing a vital role in the electron transport chain and the biochemistry of energy production (Powis, 1989). Several plant-derived naphthoquinonoids, such as plumbagin, shikonin, b-lapachone, doxorubicin, etc. are known for their anti-proliferative activity against cancer cell lines (Chen *et al*., 2003). These compounds have been shown to kill cancer cells via apoptosis (Huang *et al*.,

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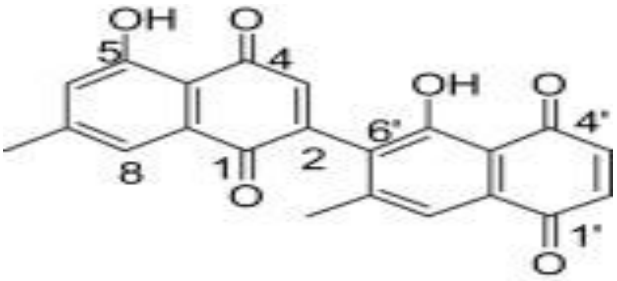
1999). Two main mechanisms have been proposed for the cytostatic and anti-proliferative activities of such compounds; redox cycling and reductive alkylation (Seung *et al*., 1998). A number of natural quinonoids, by virtue of their facile redox cycling capacity, appear to induce apoptosis through generation of intracellular reactive oxygen species (ROS) resulting in their anti-cancer activities.

Diospyrin a bisnaphthoquinone, which is present in the heartwood of many species of Diospyros plants, is well known for its anti-cancer activity (Sagar & Green, 2009). Diospyrin was first isolated in 1961 by Kapil and Dhar (Kapil & Dhar, 1961), as an orange-red constituent of Diospyros Montana Roxb (Ebenaceae). The structure of diospyrin was first proposed by Ganguly and Govindhachari in 1966 as a dimer of 7-methyljuglone linked between C-2 and C-3 (Masao & Kenji, 2000). The final correct structure of diospyrin was proposed by Sidhu and Pardhasaradhi with a linkage between C-2 and C-6 (Sidhu *et al*., 1967). Isolation of diospyrin from plants of other Ebenaceae family members has also been reported (Adeniyi *et al*., 2000). Crystallographic analysis by Harrison and Musgrave has confirmed the structure of diospyrin to be 2, 60 -bis (5-hydroxy-7-methyl-1, 4-naphthoquinone) (Harrison *et al*., 2004).



1: Diospyrin

Coptidis rhizoma (huanglian) and berberine

Coptidis Rhizoma (Huanglian in Chinese) has been used for more than two thousand years by CM physicians for treatment of damp-heat syndromes. Berberine (Fig. 1) is an isoquinoline alkaloid commonly used in both China and other countries as a botanic drug which is also a major active compound in Huanglian. It is widely present in the plant kingdom: Berberidaceae Ranunculaceae and Papaveraceae (Felter *et al*., 2009). In this review, we focused on Huanglian (Coptis spp., Ranunculaceae), linking the traditional uses and ethnopharmacological relevance of Huanglian to biomedical investigations of Huanglian and berberine. As specified in the Chinese Pharmacopoeia, Huanglian may contain three Coptis species: Coptis chinensis Franch. (Weilian in Chinese), Coptis deltoidea C. Y. Cheng et Hsiao. (Yalian in Chinese) and Coptis teetoides C. Y. Cheng (or Coptis teeta Wall., Yunlian in Chinese) (Family Ranunculaceae). There are a few other native Coptis species distributed in other areas of the world which are also used as Huanglian, e.g., Coptis japonica Makino. In Japan and Coptis trifolia Salisb. (Or Goldthread) which were used as domestic medicines in North America (Lloyd *et al*., 2009). In China, Weilian has been extensively cultivated in eastern Sichuan and western Hubei provinces under good agriculture practice (GAP) for Chinese Crude Drugs. It is worthwhile to note that the wild Yunlian has been remarked as vulnerable with the conservation status ranking No. 2 in China. Nevertheless, its status as an herbal drug would not be greatly influenced due to a similar extensive cultivation to Weilian in the southwestern part of China. Berberine is an alkaloid component in Huanglian. The recent studies confirmed that Huanglian is a good producer of berberine. Berberine constitutes the most abundant alkaloid in the dried herb (5.20–7.69%, w/w). High production of berberine can also be achieved via modifications of metabolic pathways in plants by genetic engineering.

The main pharmacodynamic properties of berberine and Huanglian have long been recognized on the treatment of intestinal infections including acute gastroenteritis, cholera and bacillary dysentery which can be linked to their antibacterial, antiviral and anti-inflammatory effects. The purification of berberine from Huanglian has greatly facilitated investigation studies into the therapeutic applications of Huanglian.

The development of Huanglian and berberine in clinical applications can be broadly divided into several stages:

Early clinical use of Huanglian in CM began at about 2000 years ago in the treatment of diarrhea, eye inflammation and women's abdomen ailments caused by damp-heat, which is still a common use of Huanglian today in CM (Xu & Wang, 2004).

Early medical uses and development of berberine-containing botanic drugs in North American folk medicine in the treatment of sub-acute and chronic inflammations including gastric disorders, respiratory affections and cancer during the 1800s; but gradually lost their application status in western medicine (Lloyd *et al*., 2009).

The pre-clinical and clinical studies and development of berberine as a natural antibiotic to treat various bacteria-associated diarrheas in the late 1900s.

It is worth highlighting that the antineoplastic activities of berberine have been reported intermittently. The initial positive results from these studies together with the information from CM led to reconsideration of the use of berberine and Huanglian in the treatment of cancer diseases. After 1990s, there were more studies on the antineoplastic effects of berberine and Huanglian in diverse cancer cell types, and the underlying mechanisms of the antineoplastic action were also investigated

especially on those of berberine. Meanwhile, many other pharmacological benefits have also been reported including lowering of blood cholesterol (Xu & Wang, 2004), anti-inflammatory effects (Kuo *et al*., 2004), therapies of experimental colitis and treatment of diabetes (Zhou *et al*., 2007), for berberine, hepatoprotective and anticachectic effects for Huanglian.

MECHANISMS OF ACTION

Diospyrin

Quinones are potent reducing agents, thus acting as oxidizing or dehydrogenating agents. Several mechanisms have been proposed to rationalize the anti-tumor properties of naphthoquinones (Pinto *et al*., 2006). Diospyrin is a naphthoquinoid also hypothesized to possess similar properties and its derivatives and analogues may cause anti-tumor effects via similar mechanisms.

The well-studied of these mechanisms are redox reactions involving oxidation and reduction. Two types of redox mechanisms are commonly associated with quinones; one-electron and two-electron reduction. One-electron reduction produces a semi-quinone which in turn generates ROS, whereas a hydroquinone is produced during two-electron reduction.

Quinones undergo both enzymatic as well as non-enzymatic reduction. In one-electron enzymatic reduction, several flavoenzymes have been found to catalyze the reduction of quinones to semi quinones. Examples are NADPH-cytochrome P450 reductase, NADH-cytochrome b5 reductase, NADPH ubiquinone oxido-reductase, and ferredoxin NADP reductase (Lyanagi *et al*., 1969). Under aerobic conditions, a semiquinone radical auto-oxidizes by transferring an electron to molecular oxygen (O2) thus leading to generation of a superoxide anion radical (O2). In a downstream reaction, this superoxide anion radical which has a short lifetime in aqueous solutions, is further converted to hydrogen peroxide (H2O2) via a reaction with a protonated superoxide radical, catalyzed by superoxide dismutase (SOD). This step is followed by reduction of peroxide, forming hydroxyl radical (OH) via the iron-catalyzed Haber Weiss reaction (Fig. 2). The hydroxyl radical generated during the reactions, rather than the superoxide anion radical or hydrogen peroxide, is thought to be responsible for cytotoxicity in cells. The highly reactive free radicals generated during one-electron reduction ultimately affect cells by directly binding to DNA, proteins and lipids (Fernandez *et al*., 1964), and causing oxidative damage to DNA and/or other cellular constituents(Giulivi *et al*., 1994).

Enzymatic two-electron reduction is catalyzed mainly by DT-diaphorase (NADPH quinone oxidoreductase) resulting in the formation of hydroquinone which undergoes auto-oxidation in aerobic conditions to give H2O2, which in turn further reacts with Fe (II) to generate reactive oxygen species (OH) via the Fenton reaction (Fig. 2). The two-electron reduction of quinone to hydroquinone results in anti-cancer activity (Lin *et al*., 1973). This is due to elimination of the leaving group which generates ROS, quinine methides that are capable of alkylating DNA or other biological molecules. Chemical evidence has been presented for the existence of quinine methides generated from 2, 3-dimethyl-5, 6-bis (acetoxy-methyl)-1, 4-benzoquinone that are involved in its capacity to alkylate morphine or aniline (Lin *et al*., 1973). A similar role for anaerobic reduction of hydroquinone could lead to bioreductive alkylation involving bioreduction of parent quinone to hydroquinone, which in turn could generate the reactive quinine methides. These compounds alkylate DNA and other biological components. The enzymes xanthine oxidase, NADH:lipoamide oxidoreductase and xanthine dehydrogenase, simultaneously catalyze one and two-electron reduction. Non-enzymatic reduction involves either electron-transfer from a semiquinone or a superoxide anion radical, or reaction of a cellular nucleophile such as glutathione with a reduced pyridine nucleotide.

In the cell, the enzyme DT-diaphorase (NAD (P) H-quinone oxidoreductase) catalyzes and reduces toxic, reactive and unstable quinines (Ross *et al*., 2004). This leads to preferential formation of hydroquinones by blocking formation of toxic semi-quinone radicals and ROS. Hydroquinones, being less reactive than semiquinone radicals, protect cells from the cytotoxic effects of quinone because after conversion to hydroquinone there is less quinone available for reduction. In the literature, three types (Cadenas *et al*., 1995), of hydroquinones have been identified: (i) redox-stable hydroquinones, (ii) redox-labile hydroquinones, and (iii) alkylating hydroquinones (Taguchi *et al*., 2007). Redox-stable hydroquinones conjugate with sulphate or glucuronate leading to their detoxification, while redox-labile hydroquinones are auto-oxidized to go through redox cycling. This auto-oxidation can be stimulated or inhibited by SOD via two mechanisms: a) generation of H2O2, and/or b) stabilizing unstable hydroquinones, respectively (Cadenas *et al*., 1995). The third type, alkylating hydroquinones, undergo bioreductive alkylation leading to toxicity. The balance between detoxification and activation of oxidative stress via ROS generation depends on functional group chemistry, physicochemical properties of the hydroquinone generated (Giulivi *et al*., 1994), and the rate of auto-oxidation of the formed hydroquinone (Munday *et al*., 2007). The rapid oxidation of hydroquinone leads to production of more free radicals leading to enhanced toxicity (Munday, 2000). Das Sarma also provided evidence against the hypothesis that electrochemical parameters of quinonoids correlate with their cytotoxic

properties. Other factors such as membrane permeability, lipophilicity, site-specific binding, etc. should be evaluated to establish a correlation between structure modulation and cytotoxicity.

Quinones affect proteins via either direct arylation of essential protein thiols and/or indirect oxidation of essential thiols by ROS. ROS are products of electron-transfer reactions of quinones and lead to cell damage by directly reacting with DNA, lipids and proteins (Brunmark & Cadenas, 1989). ROS cause oxidative stress in cells which contribute to the cytotoxicity induced by quinone compounds (Dickancaite *et al*., 1997). Studies have shown that derivatives of diospyrin could induce apoptosis via an ROS-mediated pathway, causing changes in mitochondrial transmembrane potential (Hazra *et al*., 2007), illustrating the potential role of oxidative stress through ROS formation via the one-electron reduction (Leland & Michael, 1996). In recent years, mitochondria have emerged as a key locus for initiation of intrinsic apoptotic pathways involving an increase of outer mitochondrial membrane permeability. This change also affect several processes in and around mitochondria, including changes in membrane potential, mitochondrial swelling and rupturing of outer mitochondrial membranes, and release of various proteins (cytochrome c, Smac/Diablo, etc.) into the cytoplasm. This process is called mitochondrial permeability transition (MPT) (Tsujimoto & Shimizu, 2007). The permeability of the mito- chondrial outer membrane is regulated by the Bcl-2 family of proteins involved in apoptosis. One of the key regulators of MPT is cyclophilin D (Cyp D) that has prolyl-cis, trans-isomerase activity and is important for protein folding (Galat *et al*., 1995). Cyclosporin A (CsA), an inhibitor of the cyclophilin protein family, blocks MPT (Broekemeier 1989), confirming a role of Cyp D for MPT to occur during apoptosis. Another mechanism of CsA mediated apoptosis occurs without mitochondrial swelling, but is accompanied by increased inner membrane permeability that leads to efflux of small matrix solutes such as glutathione (GSH) and calcium. Mitochondrial GSH is critical for cell survival and loss of GSH is directly related to decreased cell viability (Thomas & Reed, 1988). Bcl-2 family members are the key decision-makers for cell death (Kiblee & Gilmore, 2007), and also inhibit MPT by binding directly with pro-apoptotic members of this family (Norhanam & Hazra, 1997).

Berberine

Recent advances in the study of cell-cycle checkpoint controls and DNA repair have identified key small-molecule inhibitors of the cell-cycle relevant to understanding the mechanisms of cancer chemotherapeutic agents (Leland & Michael, 1996). Berberine can induce DNA topoisomerase I poisoning and apoptotic cell death. Many more recent findings, stapled into the antineoplastic story of berberine, are based on the knowledge of cell-cycle regulation and the downstream effects or phenotypes related to cell-cycle inhibition, particularly the caspase(s)-dependent apoptosis and associated cell signaling pathways. The intrinsic apoptotic pathways are vital for many normal physiological processes and initiated by a series of apoptotic regulatory and effector proteins such as caspases. Aberrant or dysregulated apoptotic response contributes to the development of cancer (Reed, 2002). There are two main pathways for cell death by triggering the activation of the caspase(s) cascade. One pathway induces apoptosis via interaction of the Fas/FasL system (the extrinsic cell death pathway). Fas is a member of the tumour necrosis factor (TNF) death receptor family, which plays a key role in linking drug-induced damage to apoptotic cell processes. Another one is the activation of the mitochondrial pathway (the intrinsic pathway). Studies on treatment of cancer by berberine indicated that apoptosis is involved in the many cancer cell lines examined. The underlying mechanisms for apoptosis induction by berberine, in more or less degree of diversity, are summarized below. More details can be referred to in Fig. 3.

(a) Cell-cycle arrests at G0/G1, G1 and/or G2/M, and coordinated suppression of cyclin-dependent kinases (e.g., CDK 2, 4, 6) and cyclins (e.g., cyclin B, D, E). Our current studies on human nasopharyngeal carcinoma cells (HONE1 cells), suggest that berberine could induce apoptosis via G2 arrest which demonstrated by increased levels of cleaved-PARP, cleaved caspase 3 and cleaved caspase 9 (Tsang *et al*., 2009). In some cases, the induction of cell-cycle arrest was relied on p53 status, for example, G1 arrest was induced in HCO and U2OS cells (p53 functional), but G2 /M arrest in Saos-2 cells (p53-deficient) (Liu *et al*., 2009), in others, the cell-cycle arrest in G0 /G1 phase was much higher in p53 expressing SK–N–SH cells than those in p53-deficient SK–N–MC cells (Choi *et al*., 2008), and also the case in p53 positive LNCaP prostate cells than those in p53 negative PC-3 cells.

(b) The mitochondria/caspase-dependent and/or Fas/FasL signal pathways, evidenced by the altered ratio of anti-apoptotic (Bcl-2 proper, Bcl-XL) and pro-apoptotic (Bax, Bid) members of the Bcl-2 family proteins, loss of mitochondria membrane potential and/or release of cytochrome C (Lin *et al*., 2008), induction of reactive oxygen species (ROS) and/or Ca2+ production (Jantova *et al*., 2007), activation of Fas or FasL as well as activation of a series of caspases, especially the down- stream executioner, caspase-3.

(c) Many other cell signaling pathways have also been found to be associated with the anticancer effects of

berberine have reported a new mechanism for berberine-induced apoptosis in SW620 human colonic carcinoma cells by targeting the redos/ROS pathway and JNK/p38 pathway. Another study on the same cancer type showed that berberine could induce cell growth arrest and apoptosis of HCT-116 and SW480 cells by targeting two pro-apoptotic proteins ATF3 and NAG-1, in which the p53-dependent pathway and the PKC, ERK, and GSK-3b pathways were involved, respectively (Rojsanga *et al*., 2007). Recent research further unraveled a vital role of p53 related pathway in the berberine-mediated tumour suppression. Berberine could induce apoptosis of both A549 (p53+/+) and H1299 (p53−/−) human lung cancer cells, which effect was more pronounced in the A549 in both in vitro and in vivo studies (Katiyar *et al*., 2009). Similarly, the cells with functional p53 were more susceptible to berberine than the p53-deficient cells, such as in SK–N–SH than SK–N–MC and in LNCaP than PC-3 cells (Choi *et al*., 2008).

(d) Berberine induces apoptosis via positive or negative regulation of various cytokines functioned in cellular network. These include up-regulation of GADD153, inhibition of cyclooxygenase-2 (COX-2) and Mcl-1 and down-regulation of nucleolar phosphoprotein nucleophosmin/B23 and telomerase (Wu *et al*., 1999). A recent study demonstrated that nucleophosmin plays an important role in the regulation of apoptosis as one of Bax chaperons. Also, this chaperon was found to be crucial and critical in regulation of the tumour suppressor p53 (Maiguel *et al*., 2004). GADD153, as a leucine zipper transcription factor, was found to mediate berberine-induced apoptosis in human cervical cancer Ca Ski cells, shown by elevated ratio of p53 and Bax/Bcl-2 proteins and increased levels of ROS and Ca2+ release from endoplasmic reticulum (ER). The proposed mechanism is that enforced expression of GADD153 sensitizes the cells to ER stress, which involves down-regulation of Bcl-2 and enhanced oxidant injury.

In addition to direct apoptosis induction, a large number of studies demonstrated that berberine may exert indirect effects against cancers by involving the pro-inflammatory or NF-B pathway (Lee *et al*., 2007), antioxidant defense system (Thirupurasundari *et al*., 2009), anti-metastasis pathway as well as synergistic effects with estrogen receptor antagonists and radiotherapy. Therefore, mentioned berberine-induced ROS generation, may play a key role in modulating inflammatory responses through the inhibition of transcription factors such as NF-B, which mediates expression of many pro-inflammatory mediators. One recent study dissected the possible role of berberine in the NF-B signaling pathway. Some key components and downstream gene products in this pathway were mediated for berberine-induced apoptotic and anti-inflammatory activities. The mechanism in part, was ascribed to the direct modification of cysteine residue 179 in IKK, resulting in the stabilization of I-B and suppression of phosphorylation and nuclear translocation of p65, and finally inhibition of NF-B reporter activity. Moreover, NF-B -regulated gene products were also suppressed by berberine including those functional in anti-apoptosis (Bcl-XL, Survivin, IAP1, IAP2, and cFLIP), proliferation (cyclin D1), inflammation (COX-2), and invasion (MMP-9). These results established a strong link of enhanced apoptosis induced by TNF-and chemotherapeutic agents and inhibition of TNF-induced cellular invasion with targeted NF-B signaling pathway by berbeine.

Berberine could inhibit motility and invasion ability of the highly metastatic lung cancer cell line, A549 cells, under non-cytotoxic concentrations via down-regulation of nuclear transcription factors e.g., c-fos, c-jun and NF-KB (Peng *et al*., 2006). Other explorations for the anti-metastasis function indicated that berberine could inhibit TNF-induced MMP-9 expression and cell invasion via mediating AP-1 DNA binding activity in MDA-MB-231 human breast cancer, induce down-regulation of MMP-1, -2, and - 9 and related gene expressions in human SNU-5 gastric cancer cells, and down-regulate u-PA, MMP-2 and -9 expressions through MAPK and NF-B signaling pathways in human SCC-4 tongue squamous carcinoma cells. We found that anti-invasion of berberine on HONE1 cell is a different mechanism which through inhibition of RhoA signaling pathway. As regards the control of hematopoietic tumour cell, berberine could inhibit the migration of HL-60 cells, primary acute myeloid leukemia (AML) cells and leukemic stem cells (LSCs) induced by chemokine stromal cell-derived factor-1 (SDF-1), showing to be promising for prevention of leukemia. Berberine could also inhibit AP-1 (a complex of c-FOS and c-jun) activation in human hepatoma cells, oral cancer cells and breast cancer cells, as well as COX-2 expression in colon cancer cells.

Several studies showed that berberine could confer synergistic effects against cancer in combination with ER antagonists, irradiation and other chemotherapeutic agents. A recent study by demonstrated that the combined treatment of ER antagonists with berberine enhanced the growth inhibitory effect on MCF-7 cells (ER+) but not on MDA-MB-231 cells (ER−) (Peng *et al*., 2006), found that 5 and 10M concentrations of berberine combined with irradiation significantly strengthened the radio-sensitivity on A549 cells, which molecular basis involved the induction of autophagic cell death as well as cell-cycle G2/M arrest (only at 5 M berberine). Arsenic trioxide (As2O3) is usually indicated for the treatment of acute promyelocytic leukemia in vivo, and also induces apoptosis in various tumour cells in vitro investigated the co-effects of berberine on As2O3-mediated inhibition of cancer cell metastasis using rat and human glioma cell lines. As a result, berberine at 10 M

enhanced the As2O3 (5M) mediated reduction in motility and invasion of glioma cells, which synergistic effects were achieved by the inhibition of PKC-activity and downstream transcriptional factors, myc and Jun, and MT1-MMP and MMP-2 in the PKC-mediated signaling pathway involved in cancer cell migration. The synergistic anticancer effect using both As2O3 and berberine was also found in human neuroblastoma SH-SY5Y cells. The mechanism was based on the mitochondria/caspase-3 dependent signaling pathway. The synergy researches promote largely the development of multimodality or adjuvant chemotherapy in cancer treatment. However, the idea can be further derived from the principle of CM practice, in which different combination of the Chinese herbs could be used to treat the different syndromes under various diseases, and the research can be tuned fine by referring to the efficacious or pro-prietary Chinese formulae. A recent study (Wang *et al*., 2004), was based on such a formula, Zuo Jin Wan, which has a long-standing use for the treatment of gastrointestinal disorders especially inflammations. Berberine and evodiamine, another natural antitumour alkaloid, composing of the two major active ingredients of the formula, were combined to treat human hepatocellular carcinoma SMMC-7721 cells. The cell apoptosis, cell-cycle distribution, and the level of TNF- were remarkably elevated.

It is worthwhile to note that berberine induced apoptosis in U937 cells without affecting the cell-cycle or induction of necrosis in B16 or HeLa cells (Letasˇiová *et al*., 2006). Berberine could also antagonize the hypoxia-induced angiogenesis in gastric adenocarcinoma via HIF-1 repression. Therefore, the antineoplastic effects of berberine against various cancer cells may involve different mechanisms and is dependent on cell types and concentrations of the agents used.

Based on the above studies and our ongoing research results on berberine, we can conclude that berberine has multiple anti-neoplastic effects mainly through cytotoxicity, anti-proliferation, anti-invasion and anti-inflammation mechanisms. The main mechanism underlying induction of cytotoxicity, growth inhibition of berberine on cancer cells, could be mediated through DNA topoisomerase I inhibition and cell-cycle arrest which eventually induces apoptosis via the caspase-3 or Fas/FasL signal pathways. Berberine-induced anticancer effects were strongly correlated with its anti-inflammatory properties through cross talk between different signaling pathways, such as NF-B pathway for anti-inflammatory effects and caspase-dependent pathways for apoptosis. The mechanisms underlying anti-invasion of berberine are relatively less understood at present. In addition to down-regulate u-PA, MMP-2 and-9 expressions, the signaling pathway involved in cell motility and invasion, such as the RhoA GTPase/NF-B pathway, may be considered.

Coptidis rhizoma(huanglian)

Huanglian has also been used in many anticancer studies in such cancer types as human breast cancer (Li *et al*., 2000), human colon cancer, human esophageal cancer, human gastric cancer, human leukemia, human liver cancer and human pancreatic cancer (Hara *et al*., 2005). We have also reported that Huanglian had the strongest cytotoxicity among a selection of sixteen anticancer Chinese herbs in rat leukemia L-1210 cells (Luo & Feng, 2004). A much higher inhibitory activity for growth of tumour cells was present in the water extract of Huanglian (or Huanglian extract, the most clinically applied form in CM), compared to extracts using other solvents for extraction. Cells treated for 3h with 5g/ml of the Huanglian extract showed 89% inhibition, while those treated with ethanol extract showed only 28% inhibitory activity.

Fig 1. The structures of berberine and other protoberberine-type alkaloids contained in huangalian

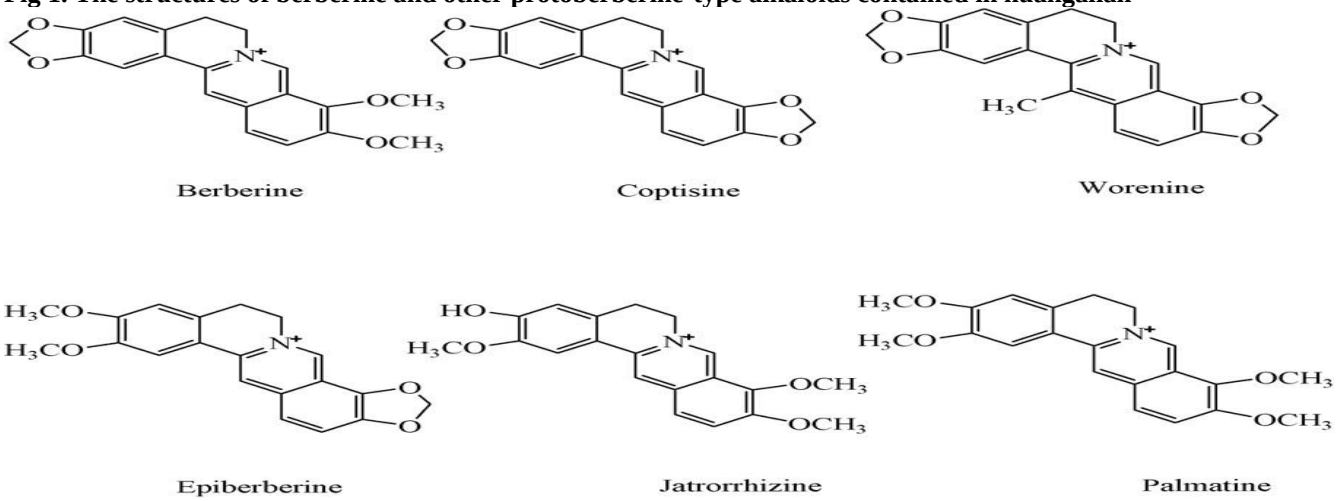


Fig 2. Enzymatic reduction and auto-oxidation in quinones. Q ¼ quinone, SOD ¼ superoxide dismutase

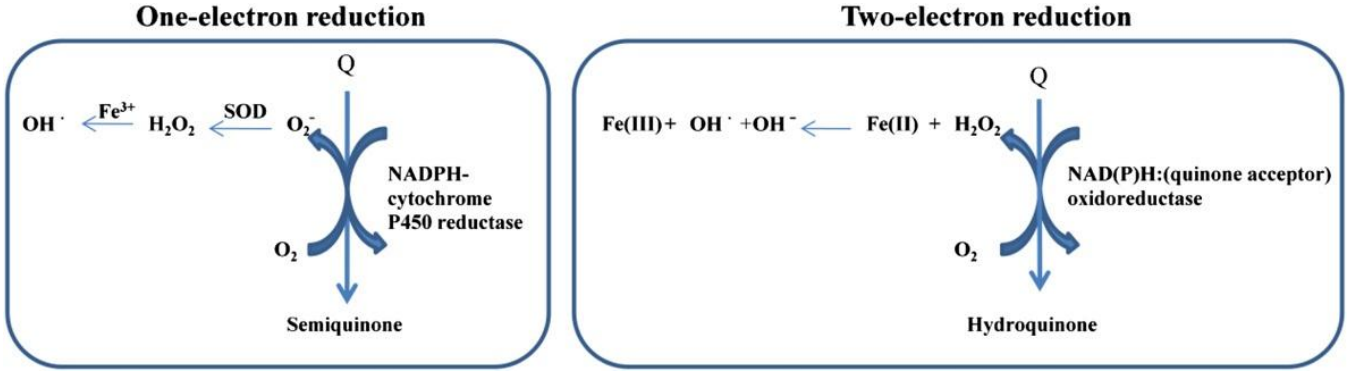
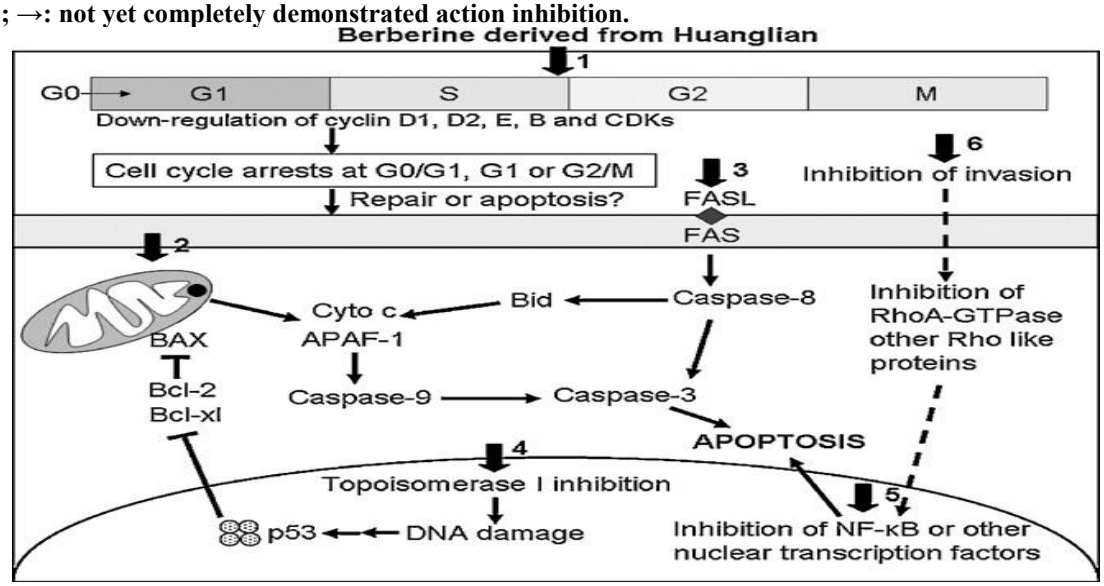


Fig 3. A schematic diagram of molecular machinery for antineoplastic properties of berberine. Berberine has multiple anticancer effects through direct cell toxicity, anti-proliferation and anti-invasion pathways. Bold arrows with number 1, 2, 3, 4, 5 and6 show the different cell signaling pathways targeted by berberine. →: stimulation or positive regulation; →: not yet completely demonstrated action inhibition.



1. Cdki–Cdk-cyclin cascade; 2. Mitochondrial/ caspase - dependent pathway; 3. Fas/ FasL signaling pathway; 4. DNA damage via topoisomerase I inhibition, and followed by p53 pathway in response; 5. NF B pathway; 6. RhoA ATPase/ NF-B pathway.

In some studies, the antitumour effects of Huanglian extract were compared with berberine, in which either IC50 or ID50 values were used for assessment of in vitro cytotoxicity found that only Huanglian had potent antitumour effects on various esophageal cancer cells among all ingredients of the complex prescription 'Oren-to' (Huanglian Jiedu Decoction), which may be due to its major component, berberine. In another study by, purified berberine and coptisine (Fig. 1) were shown to be the active components, which were more potent than Huanglian extract against hepatoma and leukemia cells. However, other studies (Hara *et al*., 2005), on human pancreatic and gastric cancer cell lines demonstrated that Huanglian may not necessarily be less potent than berberine alone after normalization to levels of berberine present in the herb extract. The underlying mechanism may be related to genes or cellular pathways resistant to berberine's action that were overcome or regulated by other components in Huanglian (Fig. 1).

CONCLUSION

Studies performed on diospyrin and its derivatives have been successful in demonstrating apoptotic activity related to these compounds, but not enough information has been generated regarding the mechanisms involved in their anti-cancer activity. Initial studies have implicated ROS as potential key factors in

causing the anti-tumor activity of diospyrin and its derivatives. It has been demonstrated that the effect of ROS on cell viability and proliferation may be influenced by the source and site of production of ROS. Other known sites of ROS generation include autophagosomes and lysosomes. It will be intriguing to investigate the effects of diospyrin and its derivatives on production of ROS at different sites in the cell. Doxorubicin (a known anti-tumor Quinone) cytotoxicity is mediated by autophagy and is also attenuated by transcription factor GATA4 by modulating the expression of Bcl2 and autophagy-related genes. Menadione (2-methyl-1, 4-naphthoquinone) has been shown to affect various aspects of cellular biochemistry such as metabolic activity and mitogen activated protein kinase pathways.

Huanglian is a Chinese medicinal herb and berberine constitutes its major active component. It has been used clinically in certain CM formulae for treating inflammatory diseases and cancers. There are three distinct medicinal agents related to Huanglian which are used in CM, that is, Chinese composite formulae containing Huanglian (e.g., Huanglian Jiedu decoction), the single herb Huanglian, and single compound berberine, however, their physio-chemical nature, pharmacological profiles and clinical indications in CM are somewhat similar to each other. Huanglian is the main active herb of Huanglian Jiedu decoction, both of which contain the main active principal, berberine. Hence, in the standpoint of chemistry, in that berberine is the major active compound against cancer, all three agents are strongly correlated. Actually, their clinical uses in modern CM practice have largely been benefited from basic research achievements on berberine. In return, the clinical successes further promote research activities and the understanding of molecular mechanisms as addressed above. In this review, recent progresses in research of berberine and Huanglian against cancers are highlighted.

In CM, there were no direct claims of Huanglian to cancer therapies in early history. However, there are many indications of anti-tumour effects in Chinese herbs with similar core properties to Huanglian such as clearing damp-heat, fire and toxicity (they can be conceived as characteristics of inflammatory diseases nowadays) and their perspective uses in treatment of diseases with tumour symptoms, which can be derived from both traditional knowledge and modern basic research. The early development of berberine and Huanglian as botanic drugs to treat infections and inflammations may also be helpful to understand more clearly their potential value as anticancer agents. The positive results of carcinostatic research of berberine-containing plants in early 1900s and the purification of berberine have encouraged researchers to explore antineoplastic properties of Huanglian and berberine in conventional approaches. Even though many other pharmacological effects have been reported in berberine and Huanglian including lowering of blood cholesterol and blood pressure and decrease of blood glucose, the potential use of berberine and Huanglian as antineoplastic agents has triggered new excitement and enthusiasm in investigations. The relatively low toxicities at therapeutic level for both Huanglian and berberine also show additional benefit for their further development.

Berberine is an essential anticancer compound in Huanglian. However, it is interesting to note in some studies that Huanglian extract may be more effective than berberine alone. One explanation is that other components in Huanglian may act synergistically with berberine (Fig. 1). the identities of these components and their mode of action are still unknown. This would suggest that berberine alone might not fully represent the therapeutic properties of Huanglian used in CM practice. This point is also best illustrated by the use of Huanglian formulae (with the main herb as Huanglian) in CM, in which various ingredients follow the rule of drug synergism and compatibility. The synergy research in combination with berberine and other typical tumour-killing approaches have drawn a large attention so far; however, it may be greatly promoted with the full understanding of the intrinsic principle implied in CM.

The anticancer effects of berberine and Huanglian mainly involve cytotoxicity, anti-proliferation and anti-invasion. It is clear that berberine is able to inhibit the growth of various types of cancer cells by inhibiting DNA topoisomerase I, inducing cell-cycle arrest and apoptosis mainly through caspase-3 or Fas/FasL signaling pathways. Current studies also demonstrate that berberine-induced anticancer effects are strongly correlated with anti-inflammatory properties through cross talk between different signaling pathways targeted, such as NF-B pathway for anti-inflammatory effects and caspase-dependent pathways for apoptosis. This evidence may further establish a link with the art of CM to treat cancers from its original standpoint by removing heat and toxicity (inflammatory elements in cancerous pathogenesis). In addition to down-regulation of u-PA and matrix metalloproteinase (MMPs) expression, the mechanism underlying anti-angiogenic, anti-invasive and anti-metastatic effects of berberine is less understood at present, which warrant further investigation. We propose that inhibition of the RhoA GTPase/NF-B pathway may be involved anti-invasive effect of berberine according to our current study. *In vivo* studies in animals will also be needed to validate the in vitro studies.

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