The Preventive and Therapeutic Effect of Coptidis Rhizoma and Berberine in Cancer

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1 Introduction

*Coptidis Rhizoma* (Huanglian in Chinese) has been widely prescribed by Chinese Medicine physician for treating damp-heat syndromes for more than two thousand years (J. Tang et al., 2009). Berberine (Figure 1) is an isoquinoline alkaloid in *Coptidis Rhizoma*, which is also a major active compound in *Coptidis Rhizoma* (Enk et al., 2007). Many studies have confirmed that *Coptidis Rhizoma* is a good producer of berberine. High yield of berberine can also be isolated and purified from various medical plants like Berberidaceae Ranunculaceae and Papaveraceae (Galle, Blodt, & Wagner, 1994; Grycová, Dostál, & Marek, 2007) via genetic engineering (Sato et al., 2001) and now can be totally synthesized. It is found that in dried herb of *Coptidis Rhizoma*, the most abundant alkaloid is berberine (5.20 – 7.69%, w/w) (Iizuka et al., 2003).

![Figure 1: Berberine.](image)

In clinic, *Coptidis Rhizoma* and berberine are commonly used for the treatment of gastrointestinal infection including acute gastroenteritis, cholera and bacillary dysentery. Since last century, *Coptidis Rhizoma* and berberine have been widely investigated for their pharmacological and biological activities. The major pharmacological properties of berberine and *Coptidis Rhizoma* include anti-microbial, anti-inflammatory, anti-oxidative and anti-tumor effect (Dharmananda, 2005; Hayashi, Minoda, Nagaoka, Hayashi, & Uesato, 2007). The purification of berberine from *Coptidis Rhizoma* has greatly facilitated investigation studies into the therapeutic effects of *Coptidis Rhizoma*.

The anti-cancer effect of berberine was first highlighted in 1959 (Hano, 1959). In 1990s, Studies focusing on the anti-cancer effects of *Coptidis Rhizoma* and berberine and its underlying mechanisms of anti-cancer activity in various cancer cell types have dramatically increased. Until now, the anti-cancer effects of berberine are still hitting the eyes of many re-

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searchers. Moreover, some studies have obtained promising and interesting results. In this review, the anti-cancer effect and cancer preventative effect of Coptidis Rhizoma and berberine (especially berberine) in recent publications will be discussed in detail.

2 Anti-cancer Effect of Coptidis Rhizoma and Berberine

2.1 Prevent Carcinogenesis

In view of cancer etiology, pathogenic microorganism has a close relationship with carcinogenesis and it’s one of the major factors that causes certain types of cancer. For example, long-term Helicobacter pylori infection is strongly associated with chronic gastritis and peptic ulcer disease, which might gradually develop into gastric cancer (Takahashi, 2002; Uemura et al., 2001). Also, two hepatitis viruses, HBV and HCV, are confirmed to be the major factor that causes HCC (Lavanchy, 2004). Thus, the antimicrobial activity of berberine might contribute to its anticancer effect.

The antimicrobial activity of berberine was first reported in 1960s (Amin, Subbaiah, & Abbasi, 1969). Many studies showed that berberine exhibited a broad spectrum of antimicrobial effect. Berberine could inhibit the growth of various kinds of bacterial and fungi in vitro including Staphylococci (Kowalewski, Kedzia, & Mirska, 1972), Klebsiellapneumoniae, Proteusulgaris, Mycobacterium smegmatis, Candida albicans(Franzblau & Cross, 1986), Helio bacterial pylori (L. Zhang, Yang, & Zheng, 1997), Mycobacterium tuberculosis(Gentry et al., 1998), Candida rugosa (Grippa et al., 1999), and tetrahymenathermophila (T. thermophila) BF(5) (Kong, Li, Xiao, & Zhao, 2010). At a minimum inhibitory concentration of 12.5 mg/ml, berberine could inhibited the growth of Helicobacter pylori in vitro (Mahady, Pendland, Stoia, & Chadwick, 2003). Berberine could reduce viral production as well as inducing toxicity in hepatitis B virus permanently transfected HepG2 2.2.15 cells (H. L. Li et al., 2008). Mechanisms in the antimicrobial effect of berberine included suppressing the biofilm formation in Staphylococcus epidermidis (X. Q. Wang et al., 2009), inhibiting the cell division protein FtsZ (Boberek, Stach, & Good, 2010) and blocking microbial adhesion to the epithelial host cells (D. Sun, Courtney, & Beachey, 1988).

The synergistic activities of berberine with other natural or synthetic anti-microbial agents were conducted in many studies. One study revealed that berberine may be the substrate of MDR enzymes due to multidrug resistance inhibitor (MDR inhibitor) sensitizing the human pathogen Staphylococcus aureus to berberine treatment (Stermitz, Lorenz, Tawara, Zenewicz, & Lewis, 2000). In another study, berberine could sensitize Methicillin-resistant Staphylococcus aureus (MRSA) bacteria to ampicillin and oxacillin treatment (H. H. Yu et al., 2005). Furthermore, synergistic activity with anti-fungi agent fluconazole, strobilurin orfludioxonil was found in berberine (J. H. Kim et al., 2007; Quan et al., 2006). The underling mechanism of berberine’s synergistic activity may be induction of inducing reactive oxygen species (ROS) production (Xu et al., 2009).

2.2 Suppress Cancer Cell Proliferation

Berberine exhibited cytotoxicity effect on different cancer cell lines in a dose- and time-dependent manner and the studied cell lines included HeLa (cervical carcinoma) (Kettmann,
Kostalova, Jantova, Cernakova, & Drimal, 2004), HepG2 (hepatocellular carcinoma) (Hwang, Kuo, Tseng, Liu, & Chu, 2006), HONE 1 (nasopharyngeal carcinoma) and HK1 (nasopharyngeal carcinoma) (Tsang et al., 2009a). At a relatively low IC50, berberine could inhibit the growth of a wide range of cancer cell types including both hematological and non-hematological cancers arising from leucocytes, liver, lung, stomach, colon, skin, oral, esophagus, brain, bone, breast and sexual organs (either hormone-dependent or independent) (Y. Y. Sun, Xun, Wang, & Chen, 2009). In some cases, the IC50 of berberine was lower than 4 µg/mL, which is below the safety limit established by National Cancer Institute (NCI), suggesting the antineoplastic potential of berberine on some human cancers such as cervical cancer, leukemia and colon cancer (Letasiova, Jantova, Cipak, & Muckova, 2006). The effect of berberine on cell cycle redistribution in numerous cancer cells has been reported in many studies and contributes the broad spectrum of antineoplastic activity. In human epidermoid carcinoma cell lines, berberine inhibited cyclin-dependent kinase CDK1 and CDK2, induced G2/M cell cycle arrest, and up-regulated CDK inhibitor of p27 (Roublevskaiia, Polevoda, Ludlow, & Haake, 2000). Berberine showed a similar effect on cell cycle of esophageal cancer (Iizuka et al., 2000), glioblastoma (Gagliano et al., 2007), hepatocellular carcinoma (X. N. Wang et al., 2008), neuroblastoma (Choi et al., 2008), osteosarcoma cells (Liu et al., 2009), breast cancer (J. B. Kim et al., 2010), cervical cancer (Lu et al., 2010), pancreatic cancer (Pinto-Garcia et al., 2010), colon cancer (Hu et al., 2011), bladder cancer (Yan et al., 2011), human cholangiocarcinoma (W. He et al., 2012), ovarian carcinoma (Park et al., 2012b), thyroid cancer (Park et al., 2012a) and colon cancer (L. Wang et al., 2013). Berberine could induce apoptosis via G2 arrest on human nasopharyngeal carcinoma cells (HONE1 cells) by increasing levels of cleaved-PARP, cleaved caspase 3 and cleaved caspase 9 (Tsang et al., 2009a). The induction of cell-cycle arrest was determined by the presence of p53 in some cases, for example, G1 arrest was induced in p53 functional HCO and U2OS cells while G2/M arrest in p53-deficient Saos-2 cells (Z. J. Liu et al., 2009). In p53 expressing SK-N-SH cells, cell-cycle arrest in G0/G1 phase was much higher than those in p53-deficient SK-N-MC cells (Choi et al., 2008). It was the same case when comparing p53 positive LNCaP prostate cells with p53 negative PC-3 cells (Choi et al., 2009). Targeting multiple aspects of cellular metabolism, such as both aerobic glycolysis and mitochondrial oxidative phosphorylation (OXPHOS), has the potential to improve cancer therapeutics. A recent study showed that berberine combined with 2-deoxy-d-glucose synergistically enhanced cancer cell proliferation inhibition via energy depletion and unfolded protein response disruption (Fan et al., 2013). In human multiple myeloma U266 cells, berberine suppressed cell proliferation in dose- and time-dependent manners by suppressing NF-kappa B nuclear translocation via Set9-mediated lysine methylation and reducing miR21 and Bcl-2 level, which induced ROS generation and apoptosis (Hu et al., 2013). Berberine inhibited the proliferation of colon cancer cells by inactivating Wnt/beta-catenin signaling, which indicated the potential of berberine as chemoprevention and chemotherapy agent human colon cancer in human (K. Wu et al., 2012).

2.3 Induce Cancer Cell Apoptosis

The ability of berberine to induce apoptotic cell death has been widely reported in various kinds of human cancer cell lines including leukemia (Jantova, Cipak, Cernakova, & Kost'alova, 2003), melanoma (Burgeiro et al., 2011; Letasiova et al., 2006), Ehrlich ascites carcinoma (Letasiova et al., 2006), prostate carcinoma (Mantena, Sharma, & Katiyar, 2006b), epi-
dermoid carcinoma (Mantena, Sharma, & Katiyar, 2006a), glioblastoma (Gagliano et al., 2007), breast cancer (J. B. Kim et al., 2010; Pazhang, Ahmadian, Javadifar, & Shafiezadeh, 2012), hepatocellular carcinoma (X. N. Wang et al., 2008), neuroblastoma (Choi et al., 2008), osteosarcoma cells (Z. J. Liu et al., 2009), cervical cancer (Lu et al., 2010; Mahata et al., 2011), colon cancer (W. C. Hu, L. L. Yu, & M. H. Wang, 2011; L. Wang et al., 2012), pancreatic cancer (Pinto-Garcia et al., 2010), bladder cancer (K. Q. Yan et al., 2011), human erythro-myeloblastoid leukemia (Pazhang, Ahmadian, Mahmoudian, & Shafiezadeh, 2011a), and myeloma (Hu et al., 2013).

The underline mechanisms of berberine inducing cell apoptosis were also extensively studied in numerous cancer cell lines. In human gastric cancer cells, berberine could release cytochrome C into nuclear by up-regulating Bax expression and down-regulating Bcl-2, which would activate the cleavage of caspase-9/3 and finally induce apoptosis (J. P. Lin, Yang, Lee, Hsieh, & Chung, 2006). In human leukemia cells, berberine reduced mitochondrial membrane potential by inducing production of ROS and calcium influx and then activated the intrinsic apoptotic pathway (C. C. Lin, Kao, Chen, Ho, & Chung, 2006). Study further revealed that berberine could induce the oxidative stress and stimulates the mitochondrial permeability transition by slowing down the mitochondrial respiration (Pereira et al., 2007). Another study showed that mitochondria and caspase activation were involved in the mechanism of berberine inducing apoptosis in melanoma cells, but ROS generation was not essential. The results indicated that inhibition of B-RAF/ERK survival signaling facilitated the cell death response triggered by berberine (Burgeois et al., 2011). Study demonstrated that berberine could increase the expression of FasL and phosphor-c-Jun, and the down-regulation of anti-apoptotic factors c-IAP-1, Bcl-xL, Bid and activation of JNK and p38 MAPK to initiate extrinsic apoptotic pathway (Hsu et al., 2007). In human hepatocellular carcinoma, berberine induced apoptosis and activated the caspase-8/9/3 by increasing the expression of p53 and Fas (G. Y. Wang, Lv, Dong, Xu, & Dong, 2009). In another liver cancer cell line Huh7, berberine induced the apoptosis of Huh7 cells via the mitochondrial pathway (Yip & Ho, 2013). Moreover, berberine selectively inhibited the growth of human hepatocellular cancer cells by inducing AMPK mediated caspase dependent mitochondrial pathway cell apoptosis, and rarely caused cytotoxicity in normal cells (Yang & Huang, 2013). In human oral cancer cells, berberine showed its apoptotic property by down-regulating the expression ofCOX-2 to suppress the expression of Mcl-1 and activation of Akt signaling (Kuo, Chi, & Liu, 2005b). In breast cancer cell line 4T1, berberine enhanced tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis (Refaat et al., 2013). Further study showed that inhuman colorectal cancer cells, p53-inducibleATF-3 and NAG-1 were two key factors involved in berberine-driven apoptosis (Piyanuch, Sukthankanr, Wande, & Baek, 2007). The vital role of p53 in berberine-induced apoptosis was further explained by a study demonstrating that p53-proficient cancer cells were more sensitive to berberine treatment than p53-deficient cells (Katiyar, Meeran, Katiyar, & Akhtar, 2009b). The regulating effect of berberine on p53 was further studied and the result showed that expression of MDM2 (endogenous inhibitor of p53) could be decreased by berberine treatment. The underlying mechanism may be berberine promoting MDM2 binding to death domain-associated protein (DAXX) and subsequent degradation (Li et al., 2013; X. L. Zhang et al., 2010). ROS-induced endoplasmic reticulum stress may contribute to berberine’s pro-apoptotic action by initiating the expression of GADD153 and catalase (J. P. Lin et al., 2007). Further study showed that intracellular ROS and nitric ox-
ide (NO) were up-regulated by berberine treatment, while its anti-cancer effect were diminished by co-treatment of anti-oxidant. This study further demonstrated the role of oxidative stress in berberine-induced apoptosis (Hur, Hyun, Lim, Lee, & Kim, 2009). However, controversial result appeared in another study showing that iNOS production did not contribute to the pro-apoptotic effect of berberine, but berberine-induced survivin suppression played a more vital role (Pazhang et al., 2011b). Similar result appeared in an experiment using human ductal breast epithelial tumor cell line, which indicated that the apoptotic effect of berberine may be mediated by reduction of COX-2 and survivin in T47D cell line, while the iNOS was not involved in the mechanism of apoptosis induced by berberine (Pazhang et al., 2012).

2.4 Anti-angiogenesis effect

The anti-angiogenesis effect of berberine in human tumor has been reported in previous studies (Wartenberg et al., 2003). In the process of tumor angiogenesis, vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF-1) are two key factors. In hypoxic condition, berberine could promote the binding of HIF-1α to E3 ligases and subsequent degradation instead of directly suppressing the transcription activation of HIF-1 in human gastric cancer cells SC-M1. Thus, HIF-1α suppression would inhibit the transcription activation and secretion of VEGF, which is vital in tumor blood vessel formation (S. K. Lin et al., 2004). Berberine could directly suppress the proliferation of VEGF-induced human umbilical vein endothelial cell (HUVEC) and berberine inhibiting VEGF-trigger Erk1/2 activation may be involved in the underline mechanism (Gao, Shi, Lee, Zhang, & Wang, 2009). In human HCC cells, berberine acted as the role of mediation between HCC cells and vascular endothelial cells by inhibiting secretion of VEGF from HCC and down-regulating VEGF mRNA expression (Jie et al., 2011). In human breast cancer cells, a study suggested that berberine may suppress both TPA-induced VEGF fibronectin and VEGF-induced fibronectin through the inhibition of the PI-3K/AKT pathway, which indicated that berberine may be a potential anti-angiogenesis candidate in human breast cancer (S. Kim et al., 2013). The result of in vivo study suggested that the serum VEGF level in mice was significantly suppressed by berberine and the pro-angiogenic factor iNOS and COX-2 decreased when treated with berberine (Hamsa & Kuttan, 2012a).

2.5 Inhibit Cancer cell migration and invasion

Matrix metalloproteinase (MMP) is the major factors in regulating cancer cell migration and invasion. The effect of berberine in suppressing MMPs activation was demonstrated in various cancer cell models. In highlymetastatic A549 lung cancer cell line, berberine could inhibit its motility and invasion ability under noncytotoxic concentrations by suppressing uPA and MMP-2 activity and expression (Peng, Hsieh, Wang, Hsu, & Chou, 2006). In human breast cancer cells, berberine could inhibit TNF-α-induced MMP-9 expression and cell invasion by blocking the DNA-binding activity of AP-1(S. Kim, Choi, et al., 2008). In human tongue cancer cells, berberine down-regulated uPA, MMP-2 and MMP-9 expression via p-JNK, p-ERK, p-p38, IkK, and NF-kB signaling pathways and thus suppress cancer cell migration (Ho, Yang, Li, et al., 2009). Berberine showed metastasis inhibitory effect on mouse melanoma cells by suppressing ERK1/2, NF-αB, ATF-2 and CREB-driven MMPs expression (Hamsa & Kuttan, 2012b). Many studies also showed that berberine significantly inhibited various MMPs pro-

Berberine also exerted inhibitory effect on factors associated with cancer cell migration other than MMPs. Studies showed that berberine may directly suppress human HONE1cell migration by inhibiting RhoGTPase, RhoA, Rac1 and Cdc42 activities (Tsang et al., 2009b). Extended study found that berberine inhibited Rho-driven Ezrin phosphorylation at threonine 567 in human nasopharyngeal carcinoma (NPC) cells, and the mutation at threonine 567 of Ezrin further suppressed the anti-invasive effect (F. Q. Tang et al., 2009). Epithelial to mesenchymal transition (EMT) may also be involved in the underlying mechanism of berberine’s anti-invasion effect. In mouse melanoma cells, berberine could activate AMPK to inhibit the metastasis potential of cancer cells by down-regulating the Erk1/2 signaling and COX-2 expression, which may be involved in the EMT transition and tumor cell migration (H. S. Kim et al., 2012). A recent study showed that berberine is an effective inhibitor of the metastatic potential of lung cancer A549 cells through suppression of TGF-beta1-induced epithelial-to-mesenchymal (Qi, Xin, Xu, Ji, & Fan, 2014). Berberine could inhibit cancer invasion by regulating expression of cell migration related genes. Studies showed that berberine could significantly increase cell migration related genes NM23-H1 and reduce SDF-1 protein level in NPC cells and leukemic cells respectively, which suppressed cancer cell motility and migration (H. Y. Li et al., 2008; S. J. Liu et al., 2008). Furthermore, pro-inflammatory factors play a key role in cancer cell metastasis and berberine exerted inhibitory effect on these factors in many studies. Berberine inhibited melanoma cell migration and invasion by inhibiting pro-inflammatory factors including COX-2, prostaglandin E2 and prostaglandin E2 (PGE) receptors, which may be related to NF-αB alteration in cancer cells with treatment of berberine (Singh, Vaid, Katiyar, Sharma, & Katiyar, 2011). Studies also showed that berberine suppressed colon cancer cells migration by reducing COX-2 expression (Fukuda et al., 1999a). By suppressing AP-1 (a complex of c-fos and c-jun) activation, berberine could inhibit invasion of human hepatoma cells (Fukuda et al., 1999b), oral cancer cells (Kuo, Chi, & Liu, 2005a) and breast cancer cells (S. Kim, Choi, et al., 2008). More new mechanisms have been discovered in recent studies. Berberine inhibited the migration and invasion of T24 bladder cancer cells via reducing the expression of heparanase (L. Yan et al., 2013). Berberine-induced AMPK activation inhibited the metastatic potential of colon cancer cells by decreasing integrin beta1 protein levels and downstream signaling (J. J. Park et al., 2012). At nontoxic concentrations, berberine reduced the migration and invasion of chondrosarcoma cancer cells by modulating the alpha v beta 3 Integrin and the PKC delta, c-Src, and AP-1 Signaling Pathways (C. M. Wu, Li, Tan, Fong, & Tang, 2013). The Possible mechanism of coptis and berberine reducing COX2 expression can be referred to Figure 2.

2.6 In-vivo Study

The in vivo study of anti-cancer effect of berberine began in 1970s. Animal experiments showed that berberine has the protection effect against carcinogenesis. In mice initiated with 7,12-dimethylbenz[a]anthracene, berberine sulfate could suppress the effects of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate and significantly inhibit the promoting effect of teleocidin on skin tumor formation (Nishino, Kitagawa, Fujiki, & Iwashima, 1986). The re
Figure 2: Possible mechanism of coptis and berberine reducing COX2 expression (Berberine inhibited migration and invasion by inhibiting pro-inflammatory factors COX-2, which may be related to NF-κB alteration. Coptis and berberine reduced the binding of AP-1 and resulted in decreased COX-2 expression. Berberine showed its apoptotic property by down-regulating the expression of COX-2 to suppress the expression of Mcl-1 and activation of PI3K/Akt signaling. Berberine could activate AMPK to inhibit the metastasis potential of cancer cells by COX-2 expression, which may be involved in the EMT transition and tumor cell migration. Berberine could also reduce pro-angiogenic factor COX-2 expression, which may be involved in tumor angiogenesis.)

Result of Anis et al. study suggested that carcinogenesis induced by 20-methylcholanthrene or N-nitrosodiethylamine was significantly suppressed after treated with berberine in a dose-dependent manner in both mice and rats. The incidence of tumor in animals after 20-methylcholanthrene injection significantly decreased after berberine administration. It was also observed that animals in berberine treatment group had a longer life span than those in
control group. When treated with a combination of berberine and NDEA, the markers of liver injury (liver weight, aspartate aminotransferase (AST) and alanine transaminas (ALT) level), serum levels of lipid peroxide, bilirubin and glutamate pyruvate transaminase decreased compared with control group. This study revealed the potential chemical carcinogenesis protection effect of berberine (Anis, Rajeshkumar, & Kuttan, 2001).

The effect of berberine on WEHI-3 leukemia cells in vivo was examined in order to understand the berberine action against leukemia. The results showed that Mac-3 and CD11b markers were reduced, which suggested that differentiation inhibition of the macrophages and granulocytes precursors. There was no effect on the CD14 marker but the CD19 marker, which suggested that the promotion of the differentiation of the B-cells precursors. The weights and sizes of spleen from mice treated with berberine were found to be lower when compared to these from untreated animals (F. S. Yu et al., 2007). The treatment with berberine was found to suppress the progression of leukemia induced by FMuLv, which included the effects of elevating the life span of leukemia harboring animals by more than 60 days, decreasing the anemic condition, inhibiting the massive leukemic cell infiltrations to sinusoidal spaces in spleen, decreasing the expression of Bcl-2, Raf-1, Erk-1 IFN-α receptor and erythropoietin and inducing the expression of p53 (Harikumar, Kuttan, & Kuttan, 2008).

Murine xenograft animal models are often used to study the effect of berberine on solid tumor in vivo. The anticancer effect of berberine in vivo was studied by using the transplanted B16 cancer cell line in a dose range from 1 mg/kg to 10 mg/kg. The significant reduction of tumor volume was observed on day 16 at doses of 5 and 10 mg/kg. The dose of 1 mg/kg stimulated the tumor mass, but other tested concentration, 5 and 10 mg/kg, reduced the tumor weight (Letasiova, Jantova, Muckova, & Theiszova, 2005). In SCC-4 tongue cancer bearing xenograft mice model, treatment with 10mg/kg of berberine resulted in a reduction in tumor incidence. Tumor size in xenograft mice treated with 10mg/kg berberine was significantly smaller than that in the control group. The result of the study showed that berberine may be a potential preventive drug for tongue cancer (Ho, Yang, Lu, et al., 2009). The oral administration of berberine inhibited the growth of A549 (p53+/+) and H1299 (p53 −/−) lung tumors inoculated subcutaneously in athymic nude mice, especially more effective in A549 xenograft mice (58% tumor volume reduction vs. 35% in H1299 at a dose of 100 mg/kg) (Katiyar, Meeran, Katiyar, & Akhtar, 2009a). Berberine also strongly suppressed the growth of human prostate cancer xenograft in mice, in which LNCaP xenografts with p53 expression were more easily affected by apoptotic cell death than those inoculated with p53-deficient PC-3 cells (Choi et al., 2009). A recent study investigated the chemopreventive effects of berberine on intestinal tumor development in Apcmin/+ mice. The result showed that berberine inhibits intestinal tumor development, which is correlated with its activity to suppress tumor cell proliferation and increase apoptosis in Apcmin/+ mice. Down-regulation of Wnt and EGFR signaling pathways and COX-2 expression by berberine may be involved in its anti-tumorigenic effects (Cao et al., 2013).

The anti-cancer effect of combination use of berberine and other anti-cancer drugs on tumor-bearing animal models were investigated in many studies. The chemomodulatory activity of Alstoniascholaris extract (ASE) was studied in combination with berberine hydrochloride (BCL) in Ehrlich ascites carcinoma-bearing mice. The combination of 180 mg/kg of ASE with 8 mg/kg of BCL showed the greatest antitumor effect. More tumor-free survivors and longer median survival time as well as average survival time were observed in combined treatment group (Jagetia & Baliga, 2004). It is a clinical challenge to maintain radio-sensitizing
effects in lung cancer treatment. A study showed that the growth of implanted Lewis lung carcinoma in C57BL/6 mice reduced when berberine was given intraperitoneally at 2.0 mg/kg twice weekly combining with irradiation. The underlying mechanism was in consistent with the autophagy-mediated tumor diminishment. The results verified the synergistic cytotoxic effect of berberine and irradiation (Peng, Kuo, Tseng, & Chou, 2008). A study on murine melanoma B16F10 xenograft suggested that combination of berberine and doxorubicin significantly reduced tumor size and tumor weight when compare to control. This drug combination inhibited proliferation and increased apoptosis of cancer cells by reducing PCNA-positive cells and increasing cleaved caspase-3 positive cells (Mittal, Tabasum, & Singh, 2014).

3 Preventive and Curative Effects of Coptidis Rhizoma and Berberine in Hepatocellular Carcinoma: View from Our Studies

Hepatocellular carcinoma (HCC) accounts for most liver cancer. The most common risk factors for HCC include chronic hepatitis B viral (HBV) infection and chronic hepatitis C virus (HCV) infection (Rahbari et al., 2011). Moreover, studies showed that chronic liver injury and live fibrosis are also involved in the progression development of HCC (Baffy, Brunt, & Caldwell, 2012; D. Y. Zhang & Friedman, 2012). However, the prognosis of HCC remains poor since the prevention and treatment for HCC are far more from effective. Our group has conducted several studies to investigate the hepatoprotective effect of Coptidis Rhizoma and its major active component, berberine. The anti-cancer effect of berberine was further extensively studied. From our studies, we conclude that Coptidis Rhizoma and berberine exhibit potential preventive and curative effects on HCC. Coptidis Rhizoma and berberine may prevent HCC by chronic liver injury and liver fibrosis protection, while tumor growth inhibition, anti-angiogenesis, and cancer metastasis suppression may account for their anti-cancer effect.

3.1 Effects of Coptidis Rhizoma and Berberine on Experimental Chronic Liver Injury and Liver Fibrosis

Chronic liver injury may gradually develop into HCC and may be one of the risk factors of HCC. Chronic liver injury leads to insufficiency of liver blood circulation, which is caused by gradually damage of liver blood system, and further induces hypoxia of normal hepatocytes, and then develops into genomic instability (X. Z. Wu, Xie, & Chen, 2007) and consequently leads to tumor genesis. Thus the treatment of liver injury may be an effective way to prevent HCC (Yibin Feng et al., 2009). Many studies have showed that Chinese medicine has a great potential of hepatoprotective effect (Batey, Salmond, & Bensoussan, 2005; Schuppan, Jia, Brinkhaus, & Hahn, 1999) and Coptidis Rhizoma is one of them according to our clinical observation and lab study (YB Feng, Luo, & Zhu, 2008).

Here shows one of our experiment studies reporting on the effect and mechanism of berberine and Coptidis Rhizoma on chronic liver injury. In order to study the effect of Coptidis Rhizoma extract and berberine in diminishing hepatic damage, carbon tetrachloride (CCl4)-induced sustained chronic liver injury rats were used as in vivo animal model in our research. Our results indicated that Coptidis and berberine acted as potential alternative therapeutic agents for chronic hepatic damage. Significant decrease of serum AST and ALT level com-
bined with increased SOD activities were observed in *Coptidis Rhizoma* extract and berberine treatment group, which indicated that anti-oxidative action may be the underlying mechanism of *Coptidis Rhizoma* and berberine during their hepatoprotective action. Moreover, *Coptidis Rhizoma* extract and berberine significantly reduced apoptosis of hepatocyte through Erk1/2signaling inhibition. The result of our study shed light on the utilization of *Coptidis Rhizoma* and berberine for chronic liver diseases as potential complementary medication to protect hepatocyte from damage (Y. Feng et al., 2010; Ye et al., 2009).

Chronic liver injury, alcohol-induced liver damage, HBV infection and HCV infection are the major causes of liver fibrosis. Eliminating primary disease, modulating immune system, suppressing inflammation, inhibiting ECM protein accumulation and reducing complications are the basic principles in the treatment of liver fibrosis (Friedman, 2007). In the view of Chinese Medicine, blood and toxin stagnation and qi deficiency are the major pathological factors inducing liver fibrosis (Yibin Feng et al., 2009). Chinese Medicines widely used in many Asian countries as one of the complementary and alternative treatment for liver fibrosis in clinical practice.

Our previous studies indicated that aqueous extract of *Coptidis Rhizoma* and its major active compound berberine exhibited potential therapeutic effect on CCI4-induced animal liver injury model (Y. Feng et al., 2010; Ye et al., 2009). The promising result provoked us to investigate the possible therapeutic effect of *Coptidis Rhizoma* extract and berberine on liver fibrosis.

In our study, two different animal models were used to imitate the process of liver fibrosis. In bile duct-ligation (BDL)-induced liver fibrosis model, extra hepatic damage was a vital factor inducing liver fibrosis. In the other alcohol-fed-induced liver fibrosis model, intrahepatic damage mainly led to liver fibrosis. The anti-fibrotic effect of *Coptidis Rhizoma* extract and its major compound berberine were determined by serum AST, ALT and total bilirubin (TBil) level. And the underlying mechanism was elaborated by serum superoxide dismutase (SOD) level. 600 mg/kg *Coptidis Rhizoma* extract and 120 mg/kg berberine were used in our study based on our preliminary observation. Animal behavior after *Coptidis Rhizoma* extract and berberine treatment was observed in the study. The results showed that both *Coptidis Rhizoma* extract and berberine diminished liver fibrosis in experimental hepatic fibrogenic model induced by either bile ductligation or alcohol. Both *Coptidis Rhizoma* extract and berberine could recover SOD activity to avoid the per-oxidation damage, protect normal liver cells from cholestatic damage and assist harmful bilious product excreting from liver. Our study suggested that *Coptidis Rhizoma* and berberine were potential complementary and alternative treatment for liver fibrosis. Results from other study also suggested that berberine exerted hepatoprotective effect. The possible mechanism may be activation of AMPK, blocking Nox4 and Akt expression (Li et al., 2014). Another study showed that berberine could be effective in protecting the liver from acute CCI4-induced injury by inhibiting TNF-α, COX-2, and iNOS expression (Domitrovic, Jakovac, & Blagojevic, 2011). More detail mechanism of berberine’s hepatoprotective effect can be referred to Figure 3.

3.2 Effects of Coptidis Rhizoma and Berberine on Proliferation, Angiogenesis and Metastasis in HCC

We’ve conducted comprehensive study to reveal the cancer cell death types induced by berberine and its underlying mechanisms in HCC. Different cell death types were examined in
Figure 3: Possible mechanism of Coptidis and berberine recovering SOD activity (In CCl4-induced liver injury and fibrosis model, Coptis and berberine could inhibit oxidative stress-induced Erk1/2 signaling activation to recover SOD activity. Berberine activated AMPK, decreased the protein expression of Nox4 and inhibited the proliferation of activated hepatic stellate cell (HSC), which resulted in a decrease of serum ALT and AST and an elevation of SOD. TNF-α activated NF-κB, which further stimulates the expression of iNOS and COX-2. The final products of iNOS and COX-2, NO and prostaglandins, respectively, initiated the cascade of inflammatory response.)

human hepatic carcinoma cell lines HepG2 and MHCC97-L. The results of our study showed that berberine can induce both apoptosis and autophagic cell death in human hepatocellular carcinoma cells. Interference with cell death inhibitor 3-methyladenine or essential autophagy gene Atg5 diminished berberine-induced cell death in human hepatic carcinoma cells. Mechanism study showed that berberine may activate mitochondrial apoptosis in HepG2 and MHCC97-L cells by increasing Bax expression the formation of permeable transition pores, cytochrome C release to cytosol, and subsequent activation of the caspases-3 and -9 execution pathways. Berberine may also induce autophagic cell death in HepG2 and MHCC97-L cells through activation of Beclin-1 and inhibition of mTOR-signaling pathway by suppressing the activity of Akt and up-regulating P38 MAPK signaling (N. Wang, Feng, Zhu, et al., 2010; N. Wang et al., 2011). Possible mechanism of berberine-induced autophagy in cancer cells was shown in Figure 4.

Studies has showed that angiogenesis is a key factor in the progressive development from chronic liver diseases to HCC (Coulon et al., 2011). In HCC angiogenesis, HIF-1α/VEGF becomes the most common target. To verified anti-cancer effect of berberine or Coptidis Rhizoma on HCC, we have conducted studies to exam the action of berberine in HCC angiogenesis.

In our experiment, Coptidis Rhizoma aqueous extract (CRAE) was used. To further examine the effect of CRAE on tumor angiogenesis of HCC, we conducted another in-vivo ex
Figure 4: Possible mechanism involved in berberine-regulating autophagy in liver cancer cells. (The schematic signaling patterns display that Bcl-2/Beclin-1 complexes and PI3K/Akt/mTOR signaling pathway are modulated by berberine in regulating autophagy in liver cancer cells. Our study revealed that berberine may activate Beclin-1 from Bcl-2/Beclin-1 complexes via inhibiting the expression of Bcl-2 and on the other hand, inhibition of mTOR by berberine responsible for the inhibition of Akt and activation of p38 MAPK may initiate autophagy. Berberine makes both signaling pathways work together to enhance autophagy in hepatoma cells.)

This study revealed the new molecular mechanism of CRAE regulating VEGF expression at the posttranscriptional level by Coptidis Rhizoma and berberine on HCC. The study also suggested Coptidis Rhizoma may acts as a potential antiangiogenic agent for HCC (Tan, Wang, Tsao, Zhang, & Feng, 2013). Moreover, our preliminary study showed that Id-1 protein in HCC cells was inhibited by berberine. As previous report, Id-1 induces VEGF by enhancing the stability and activity of HIF-1alpha in human breast cancer cells (H. J. Kim et al., 2007). Previous study also found that Id-1 could stabilize the HIF-1α and increase the VEGF expression in hepatocellular carcinoma cells (T. K. W. Lee et al., 2003). The possible regulatory network of berberine on HCC angiogenesis is shown in Figure 5.
We also conducted study to investigate the anti-migration and anti-invasion effect of *Coptidis Rhizoma* in human HCC cell. HCC cell line with highly metastatic property, MHCC97-L cells were used in the study. 7 components were identified as berberine-like alkaloids in CRAE by high-performance liquid chromatography combined with mass spectrometry (HPLC/MS). CRAE was found to significantly inhibit cell migration and cell invasion through extracellular matrix. Mechanism study suggested that CRAE did not affect cell adhesion and attachment factors including E-cadherin/N-cadherin ratio, integrin β4 and had no effect on the expression of migration and invasion related molecules like uPA and MMPs. Interestingly, we discovered that CRAE suppressed MHCC97-L cell motility by acting on F-actin to induce filament reorganization, which may be induced by berberine’s inhibitory action on RhoA/ROCK1 signaling. The inactivation of the Rho/ROCK signaling pathway involved in CRAE’s inhibitory action onMHCC97-L migration indicated CRAE’s role as a Rho small GTPase inhibitor. This study sheds light on CRAE as an alternative therapy for the treatment of metastatic hepatic carcinoma (N. Wang, Feng, Lau, *et al*., 2010).

### 3.3 Effect of Coptidis Rhizoma and Berberine on Epigenetics of Liver Cancer Cells

Micro RNAs (miRNAs), which was first reported in 1993 (R. C. Lee, Feinbaum, & Ambros, 1993), are single-stranded RNA molecules regulating gene expression at either the transcriptional or post-transcriptional level (Ruvkun, 2001). Studies showed that the expression patterns of microRNAs in liver tissue differ between men and women with hepatocellular carcinoma. Patients whose tumors had low miR-26 expression had shorter overall survival but a better response to interferon therapy than did patients whose tumors had high expression of...
the microRNA (Ji et al., 2009). These finding indicates that miRNAs may play a vital role in carcinogenesis as a novel class of oncogenes or tumor-suppressor genes (L. He et al., 2005). Thus, miRNAs have been considered as novel targets for cancer therapy. We have conducted a study to examine the effect of Coptidis Rhizoma on miRNA.

In our study, HCC MHCC-97L cells were treated with CRAE. After treatment, a sensitive miRNA on-chip array was used to examine the variation in the miRNA expression profile. After 48h exposure to 175 µg/ml of CRAE, overexpression of miR-21 and miR-23a in MHCC-97L cells was detected. The modified expression of miRNAs affected several tumor-suppressor genes. The analysis of PicTar and TargetScan databases suggested that CRAE modified several signal transduction pathways by miRNA expression. We concluded from this study that CRAE may be a potential therapeutic agent targeting miRNAs in HCC cells (Zhu et al., 2011).

4 Summary

Both In vitro and in vivo studies proved the anti-cancer properties of Coptidis Rhizoma and berberine in preventing carcinogenesis, inhibiting proliferation, inducing apoptosis, suppressing cancer angiogenesis and inhibiting metastasis and invasion. However, all the evidence comes from bench experiments, clinical potential of Coptidis Rhizoma and berberine still remains unknown. Therefore, further researches are still warranted and more advanced approaches are in need to transfer the anti-cancer properties of Coptidis Rhizoma and berberine from bench side to clinical practice.

Reference


Li, H. L., Han, T., Liu, R. H., Zhang, C., Chen, H. S., & Zhang, W. D. (2008). Alkaloids from Corydalis saxicola and their anti-


Li, H. L., Han, T., Liu, R. H., Zhang, C., Chen, H. S., & Zhang, W. D. (2008). Alkaloids from Corydalis saxicola and their anti-


Singh, T., Vaid, M., Katiyar, N., Sharma, S., & Katiyar, S. K. (2011). Berberine, an isoquinoline alkaloid, inhibits melanoma cancer cell migration by reducing the expressions of cyclooxygenase-2, prostaglandin E(2) and prostaglandin E(2)


Wartenberg, M., Budde, P., de Marees, M., Grunheck, F., Tsang, S. Y., Huang, Y., . . . Sauer, H. (2003). Inhibition of tumor-induced angiogenesis and matrix-metalloproteinase expression in confrontation cultures of embryoid bodies and tumor spheroids by plant ingredients used in traditional Chinese medicine. Laboratory Investigation, 83(1), 87-98. doi: Doi 10.1097/01. Lab. 0000049348.51663.2f


