Chapter 6
An Evidence-based Perspective of *Coptis Chinensis* (Chinese Goldthread) for Cancer Patients

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Abstract *Coptis chinensis* (Chinese goldthread) and herbal complexes containing Chinese goldthread have been proven to possess anticancers effect *in vitro* and *in vivo*. Some herbal complexes containing Chinese goldthread, YiQiJueDu Granule (YQJDG), San-Huang-Xie-Xin Decoction, and ZuoJin Pill possess an inhibitory effect on nasopharyngeal carcinoma (NPC), HepG2, or S180 tumor cells, respectively. YQJDG's inhibitory effects on NPC growth and nasopharyngeal carcinogenesis are associated with reducing-telomerase besides down-regulating cell cycle and inducing apoptosis genes expression. Specially, YQJDG also has a therapeutic effect on the population at high risk for NPC. As a main component of YQJDG, Chinese goldthread has an anticancer effect on NPC, leukemia, melanoma, epidermoid carcinoma, hepatoma, oral carcinoma, glioblastoma, prostate carcinoma, and gastric carcinoma. Berberine and coptisine are two major components of Chinese goldthread. Berberine exerts anticancer effects through modulating Mcl-1, Bcl-xL, COX-2, MDR, TNF-α, IL-6, iNOS, IL-12, intercellular adhesion molecule-1, ELAM-1, MCP-1, CINC-1, cyclin D1, AP-1, HIF-1α, PPAR-γ, topoisomerase II, and inhibiting stress-induced mitogen-activated protein kinase activation. Its mechanisms include inducing cell cycle arrest, apoptosis, antiangiogenic, and anti-metastasis. Berberine also has a therapeutic effect-enhancing and toxicity-reducing effect on other antitumor therapies and preventive effect on carcinogenesis. However, berberine possesses some potential toxicity and adverse effects. Coptisine exerts anticancer effect mainly *via* inhibiting vascular smooth muscle cell proliferation.

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6.1   Introduction

Herbal medications are currently promoted more widely for clinical use in cancer therapy. Many claims of efficacy for these medications are based on anecdotal evidence in traditional Chinese medicine. However, certain Chinese herbs could presumably have potent anti-cancer properties. *Coptis chinensis* (Chinese goldthread or Huanglian) has been widely used in China for several thousand years and is prepared as a herbal tea from its roots. Chinese goldthread has been used for treating inflammatory conditions accompanied by high fever, including pneumonia and infections of the head and face, and is also routinely used in China for the treatment of gastroenteritis. Furthermore, it has been reported to inhibit the growth of *Heliobacter pylori* and the intestinal parasite *Blastocystis hominis in vitro* (Franzblau and Cross 1986; Yang et al. 1996; Zhang et al. 1997).

Chinese goldthread comprises various alkaloids, including berberine, palmaline, jatrorrhizine, epiberberine, magnoflorine, and coptisine (Sun et al. 2006). It exerts diverse activities, including anti-hypertensive (Ko et al. 2000), anti-diabetic (Tang et al. 2006; Jung et al. 2008), anti-inflammatory (Kuo et al. 2004), hypolipidemic (Doggrell 2005), and antioxidant effects (Rackova et al. 2004; Yokozawa et al. 2005; Hsieh et al. 2007; Hung et al. 2007). In particular, Chinese goldthread and its isolated alkaloids have been shown to suppress the growth of various tumor cells, including leukemia (Lin et al. 2006b), melanoma (Letasiova et al. 2006), epidermoid carcinoma (Kettmann et al. 2004), hepatoma (Hwang et al. 2006), oral carcinoma (Kuo et al. 2005), glioblastoma (Sanders et al. 1998), prostate carcinoma (Mantena et al. 2006), and gastric carcinoma (Lin et al. 2006c).

Animal studies have revealed that berberine can suppress chemically inducing carcinogenesis (Anis et al. 2001), tumor promotion (Nishino et al. 1986), and tumor invasion (Peng et al. 2006). Berberine is also a radiosensitizer of tumor cells, but not normal cells (Yount et al. 2004). Berberine modulates Mcl-1 (myeloid leukemia cell differentiation protein) (Kuo et al. 2005), Bcl-xL (Hwang et al. 2006), cyclooxygenase (COX)-2 (Kuo et al. 2005), MDR (multi-drug resistance) (Lin et al. 1999), tumor necrosis factor (TNF)-α and interleukin (IL)-6 (Choi et al. 2006), iNOS (inducible nitric oxide synthase) (Tan et al. 2005), IL-12 (Kang et al. 2002), intercellular adhesion molecule-1 and ELAM-1 (endothelial-leukocyte adhesion molecule-1) (Peng et al. 2006), MCP-1 (monocyte chemotactic protein-1) and CINC-1 (cytokine-induced neutrophil chemoattractant-1) (Cui et al. 2007), cyclin D1 (Mantena et al. 2006), AP-1 (activator protein 1) (Kuo et al. 2004), hypoxia-inducible factor (HIF)-1α (Lin et al. 2004b), peroxisome proliferator-activated receptor (PPAR)-γ (Huang et al. 2006), and topoisomerase II (Kang and Chung 2002). Using yeast mutants, berberine was found to bind and inhibit stress-induced, mitogen-activated protein kinase activation (Kang et al. 2002). Apoptotic, carcinogenic, and inflammatory effects, as well as various gene products (such as TNF-α, IL-6, COX-2, adhesion molecules, cyclin D1, and MDR) modulated by berberine are regulated by the transcription factor NF-κB (nuclear factor-κB). Berberine and coptisine constitute the major components of Chinese goldthread. Herein, evidences supporting

the use of Chinese goldthread are summarized for cancer patients based on the herb itself, compounds containing the herb, and the extracts.

6.2   Anticancer Effects of Herbal Complexes Containing Chinese Goldthread

6.2.1   Effect of YiQiJueDu Granule (YQJDG) on Anti-nasopharyngeal Carcinoma (NPC)

6.2.1.1   Inhibitory Effect of YQJDG on the Proliferation of NPC Cells

YQJDG comprises Chinese goldthread, *Astragalus membranaceus*, *Codonopsis pilosula*, and *Glycyrrhiza uralensis* Fisch (Tang et al. 2001b). Cell dynamics studies have demonstrated that YQJDG inhibits the proliferation of NPC cells time- and dose-dependently (Tang and Tian 1995). YQJDG induces NPC cell apoptosis at low concentrations (<40 μM), possesses a cytotoxic effect at high concentrations (>50 μM) (Tang and Tian 1996), and inhibits NPC cell telomerase activity (Tang et al. 2001a). Proteomic studies have revealed that YQJDG down-regulates expression of aldehyde dehydrogenase, heat shock protein (HSP)27, matrix metalloproteinase (MMP)-2, CD27L receptor, IL-12 alpha chain, DNA-PKcs (DNA-dependent protein kinase catalytic subunit), MPRI (mannose 6-phosphate receptor), and follistatin *in vitro* (Wang et al. 2003). *In vitro*, YQJDG can inhibit NPC cell proliferation and induce apoptosis, and these effects may be associated with telomerase and DNA-dependent protein kinase.

6.2.1.2   Inhibitory Effect of YQJDG on the Growth of Implant Tumors of NPC Cells

The antitumor effect of YQJDG was investigated in nude mice carrying the NPC cancer cell line CNE1. YQJDG can inhibit the implant tumor growth when orally administered. Genomic studies (Tang et al. 2004a) have demonstrated that YQJDG down-regulates expression of genes encoding growth factor receptors [NGF (nerve growth factor), pleiotrophin, interferon-α receptor, and transforming growth factor (TGF)-β receptor 1], protein kinases (STK2, FER, and cGMP-dependent kinase type 1 α), cell cycle regulators (cdc2-related protein kinase CHED, protein kinase CHK1, and CDKN2C), cell signal transduction regulators (TRAF5, MAD3, and p68 kinase), and gene expression regulators (Ets transcription factor, zinc finger protein, and transcriptional activator). Conversely, YQJDG up-regulates expression of genes encoding the apoptosis regulators *TIEG*/*EGRα* and *H731*. Proteomic studies (Tang et al. 2004b) have revealed that YQJDG up-regulates expression of HKR2, phosphoribosyl pyrophosphate synthetase, TNFR superfamily members, BAX, mucin 5B precursor, and GTP:RNA guanylyltransferase, and down-regulates

expression of fibulin-3, zinc finger protein 266, and carboxyl terminus of HSP70-interacting protein *in vivo*, which are associated with cell division, cell cycle, DNA repair, and apoptosis signal transduction. These indicate that YQJDG can inhibit NPC growth *in vivo*, and this inhibition is associated with growth factor, transforming growth factor, cell cycle, and cell division.

6.2.1.3 Inhibitory Effect of YQJDG on Nasopharyngeal Tumorigenesis Induced by N,N′-dinitrosopiperazine

Rat NPC induced by N,N′-dinitrosopiperazine was served as a nasopharyngeal tumorigenesis animal model to investigate the anti-NPC effect of YQJDG. In this model, telomerase was activated and involved in nasopharyngeal tumorigenesis (Tang et al. 2001a). Treatment with YQJDG inhibited nasopharyngeal tumorigenesis and telomerase activation (Tang et al. 2001a). Genomic studies have indicated that *PDGFB*, *M6P/IGFR$_2$*, *ErbB$_3$EGF*, *CCNE*, *CCND$_3$*, *NF*-κB, *JNK*, prothymosin-α, Cu-Zn SOD1, and *MAPK1* are involved in the inhibition of nasopharyngeal tumorigenesis by YQJDG. In addition, proteomic studies have revealed that YQJDG decreased expression of E$_3$ isozyme, mitochondrial processing peptidase, hydroxy-meth-methylglutary 1-coalyase, CD27L receptor, MPIR I, HSP27, MMP2, and metalloproteinase inhibitor in inhibition of nasopharyngeal tumorigenesis. YQJDG may have some preventive effect on nasopharyngeal carcinogenesis by chemicals.

6.2.1.4 Therapeutic Effect of YQJDG on Patients with Nasopharyngeal Pre-cancerous Lesions

237 patients with nasopharyngeal dysplasia (pre-cancerous lesion) were randomized to trial group and control group, and either received YQJDG orally ($n=112$) or did not ($n=125$). In total, 96 g of YQJDG was boiled twice with water and reconstituted into 100 ml of YQJDG decoction each time. Subsequently, 100 ml of YQJDG decoction was administered orally twice daily in the trial group for 3 months as one therapeutic course repeated once each year. These patients were followed up after 2 years, at which time their nasopharyngeal tissues were pathologically detected using a comparison determined before therapy. The results demonstrate that YQJDG could markedly block the development of nasopharyngeal tumorigenesis in the trial group at a total effective rate of 88.4% (99/112) in the short-term, significantly higher than that in the control group ($P<0.05$), which was only 45.5% (57/125) (Ouyang et al. 1999; Tao et al. 2001). YQJDG can block nasopharyngeal carcinogenesis.

6.2.1.5 Therapeutic Effect of YQJDG on Individuals at High Risk for NPC

Epstein-Barr virus (EBV) is associated with nasopharyngeal carcinoma, and individuals harboring the EBV-associated antibodies VCA/IgA and EA/IgA are considered at high risk for nasopharyngeal carcinoma. With YQJDG treatment, it could

reduce EBV potential infection and incidence rate of nasopharyngeal carcinoma (Tian et al. 2000). YQJDG may have some preventive effect on the population with high risk for NPC through reducing EBV infection.

6.2.1.6 YQJDG Enhances Therapeutic Effect and Reduces Toxicity of Radiationtherapy on Patients with NPC

Radiotherapy has been a main therapeutic strategy on patients with NPC for decades, but some patients discontinue therapy due to the adverse effects of radiotherapy. 70 patients with NPC were divided into trial (TG) and control (CG) groups. TG received the routine radiotherapy and YQJDG ($n=35$), 100 ml of YQJDG was administered orally twice daily for 3 months. CG only received the radiotherapy ($n=35$). Rate of tumor reduction was determined using solid tumor WHO objective curative effect standards, and CD3, CD4, and CD8 were detected. Results showed that the rate of tumor reduction, CD3, and CD4 in TG were higher than that in CG, and 3 years survival rate of TG (91.43%) was higher than CG (70%). The adverse-toxicity of radiation in TG was lower than that in CG. These data indicated that YQJDG could effectively reduce the adverse effects of radiotherapy and enhance therapeutic effect (Wang and Tian 2006).

6.2.2 Anti-liver Cancer Effect of San-Huang-Xie-Xin Decoction

San-Huang-Xie-Xin Decoction (SHXXD) consists of *Rheum officinale* rhizomes, *Scutellaria baicalensis* roots, and Chinese goldthread rhizomes. HepG2 cells were treated with extracts of SHXXD and gene expression profiles were analyzed by DNA microarray. Gene set enrichment analysis indicated that SHXXD displayed a unique anti-proliferation pattern *via* p53-signaling, p53-activated, and DNA damage-signaling pathways in HepG2 cells. Network analysis confirmed that most genes were regulated by one molecule, p53. In addition, hierarchical clustering analysis showed that *Rhizoma Coptidis* shares a similar gene expression profile with SHXXD (Cheng et al. 2008).

6.2.3 Anticancer Effect of ZuoJin Pill

ZuoJin Pill (ZJP) is a well-known classic formula in traditional Chinese medicine, comprising Chinese goldthread and *Evodia rutaecarpa* (Juss.) Benth at a ratio of 6:1 (w/w). Anticancer activity experiments were performed by inhibiting the growth of S180 tumors *in vivo*. Tumor growth inhibition rate, spleen index, lymphocyte proliferation activity, apoptosis index, TNF-α level, serum tumor marker activity, increase in life span, histopathology, and gene expression were tested. The results indicated that ZJP could significantly induce apoptosis of cancer cells. The inhibition ratio, increase in life span, and TNF-α level of mice treated with ZJP alone were 50.54%, 64.91%,

and 1.04 ng/ml, respectively, much higher than in mice treated with Chinese gold-thread or *Evodia rutaecarpa* alone. In addition, acid and alkaline phosphatase activities were significantly increased, aldolase and lactate dehydrogenase activities were reduced in serum, and the expression of Bax and wild-type p53 proteins were much higher for mice treated with ZJP alone compared with those treated with Chinese goldthread or *Evodia rutaecarpa* alone. A clear synergistic effect on anticancer activity was observed with ZJP, and the mechanism of antitumor growth may be due to an effect on gene expression and activities of serum tumor markers (Wang et al. 2009).

6.3   Anticancer Effects of the Single Herb Chinese Goldthread

Chinese goldthread exhibited the strongest activity against human liver and leukemia cell lines when used alone. The IC_{50} values for Chinese goldthread in HepG2, Hep3B, and PLC/PRF/5 cell lines were 20, 55, and 35 mg/ml, respectively. The IC_{50} values for Chinese goldthread in K562, U937, and P3H1 cell lines were 29, 29, and 31 mg/ml, respectively (Lin et al. 2004a). Chinese goldthread is a potential anti-carcinogenic agent for treating hepatocellular carcinoma by inducing cell cycle arrest and promoting apoptosis, with *NAG-1* (NSAID-activated gene) as the molecular target. Inhibition of cell proliferation, induction of apoptosis, and cell cycle arrest at the G2/M phase was observed in HepG2 cells treated with Chinese goldthread. The pro-apoptotic effects of Chinese goldthread were associated with a corresponding down-regulation of Bcl-2, activation of procaspase-3 and -9 as well as cleavage of poly (ADP-ribose) polymerase. Further studies demonstrated the involvement of *NAG-1* in the pro-apoptotic events following prior activation of its upstream transcriptional factor *Egr-1* (early growth response gene-1) (Auyeung and Ko 2009). Considering the traditional use, the anticancer effects of Chinese goldthread can be ascribed to its CM trait by removing damp-heat, fire, and toxicity. The Chinese goldthread molecular mechanisms involve cell-cycle arrest, apoptosis induction, and anti-inflammation. Although berberine is an essential anticancer compound in Chinese goldthread, the latter is shown more effective and beneficial than the use of berberine alone in some studies. The clinical application of Chinese goldthread as a novel cancer therapeutic agent requires *in vivo* validation and further investigation of its anticancer mechanisms (Tang et al. 2009b).

6.4   Anticancer Effects of Chinese Goldthread Extracts

Chinese goldthread contains diverse alkaloids, including berberine, palmaline, jatrorrhizine, epiberberine, magnoflorine, and coptisine. Berberine and coptisine are the major components of Chinese goldthread.

6.4.1   Anticancer Effects of Berberine

Berberine could modulate Mcl-1 (Kuo et al. 2005), Bcl-xL (Hwang et al. 2006), COX-2 (Kuo et al. 2005), MDR (Lin et al. 1999), TNF-α and IL-6 (Choi et al. 2006), iNOS (Tan et al. 2005), IL-12 (Kang et al. 2002), intercellular adhesion molecule-1 and ELAM-1 (Peng et al. 2006), MCP-1 and CINC-1 (Cui et al. 2007), cyclin D1 (Mantena et al. 2006), AP-1(Kuo et al. 2004), HIF-1α (Lin et al. 2004b), PPAR-γ (Huang et al. 2006), and topoisomerase II (Kang and Chung 2002). Berberine was also found to bind and inhibit stress-induced mitogen-activated protein kinase kinase activation (Kang et al. 2002).

6.4.1.1   Inhibitory Effect of Berberine on the Proliferating Activity of Tumor Cells

Berberine (Natural Yellow 18, 5,6-dihydro-9,10-dimethoxybenzo-(g)-1,3-benzo-dio-xolo(5,6-a) quinolizinium) is a major component of Chinese goldthread used in traditional Chinese herbal medicine. The chemical structure of berberine chloride, which has a molecular weight of 371.8, is shown in Fig. 6.1. Because of its ability to cause cell cycle arrest and apoptosis in several malignant cell lines, berberine has received much attention as a potential anticancer therapeutic agent. Recently, berberine has been examined for its anticancer activity following evidence of anti-neoplastic properties (Nishino et al. 1986).

Berberine induced G2/M arrest in leukemia cells *via* the inhibition of cyclin B1 and the promotion of Wee1 (Lin et al. 2006b). In addition, treatment with berberine induced cell cycle arrest at G0/G1 in the anoikis-resistant MCF-7 and MDA-MB-231 breast cancer cells (Kim et al. 2010). Furthermore, berberine effectively induced mitotic arrest of HONE1 cells (Tsang et al. 2009), greatly decreased G0/G1 phase-associated cyclin and cyclin-dependent kinase (cyclin D1, cyclin E, Cdk2, and Cdk4) expression, and increased apoptotic gene expression and activation of caspase-3 in SK-N-SH cells (Choi et al. 2008). After treatment with berberine, the proportion of pulmonary giant cell carcinoma (PG) cells at the G0/G1 phase increased, while cells at the S and G2/M phases decreased. Berberine at low doses (12.5–50 μM) was concentrated in mitochondria and promoted G1 arrest. In contrast, higher doses (>50 μM) resulted in cytoplasmic and nuclear berberine accumulation, as well as G2 arrest. DNA synthesis was not markedly affected by low doses
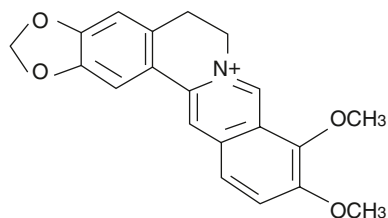

Fig. 6.1 Chemical structure of berberine

of berberine, but 100 μM was strongly inhibitory. Berberine displayed multiphasic effects in these malignant cell lines, which correlated with the concentration and intracellular distribution of this alkaloid (Serafim et al. 2008). Berberine-induced inhibition of proliferation of DU145, PC-3, and LNCaP cells was associated with G1 arrest, which in DU145 cells was associated with inhibition of expression of cyclins (D1, D2, and E) and cyclin-dependent kinases (Cdks 2, 4, and 6), increased expression of Cdk inhibitory proteins (Cip1/p21 and Kip1/p27), and enhanced binding of Cdk inhibitors to Cdk (Mantena et al. 2006). In SNU-5 cells treated with 25–200 µmol/l berberine, G2/M cell cycle arrest was observed, which was associated with a marked increase in the expression of p53, Wee1, and Cdk1 proteins and decreased cyclin B. A concentration-dependent decrease of cells in G0/G1 phase and an increase in G2/M phase were detected (Lin et al. 2006c).

Berberine increased apoptosis through reduction of MMP, release of cytochrome c, and activation of caspase-3 (Lin et al. 2007a). Berberine also suppressed the expression of NF-κB-regulated gene products involved in anti-apoptosis (Bcl-xL, survivin, IAP1, IAP2, and cFLIP). Suppression of anti-apoptotic gene products correlated with enhancement of apoptosis induced by TNF-α and chemotherapeutic agents, as well as with inhibition of TNF-induced cellular invasion (Pandey et al. 2008). Berberine inhibited the levels of anti-apoptotic protein Bcl-2 but increased the levels of pro-apoptotic protein Bax before leading to decreased mitochondrial membrane potential (DeltaPsim), cytochrome c release, and activation of capase-9 and -3 for an apoptotic response. Caspase-8, -9, and -3 were activated by berberine based on the substrate solution (PhiPhiLux-G1D1, CaspaLux 8-L1D2, and CaspaLux 9-M1D2 for caspase-3, -8, and -9, respectively). Each inhibitor of caspase-8, -9, and -3 led to an increase in the percentage of viable C6 cells after exposure to berberine (Lin et al. 2006a; Mantena et al. 2006; Eom et al. 2008; Meeran et al. 2008; Chen et al. 2009; Ho et al. 2009a). Berberine enhanced the apoptosis of CaSki cells through the induction of a higher ratio of p53 and Bax/Bcl-2 proteins, increased levels of reactive oxygen species (ROS) and Ca^{2+}, disruption of the mitochondrial membrane potential, and promotion of caspase-3 activity. In CaSki cells pre-treated with the pan-caspase inhibitor zVAD-fmk, berberine-induced caspase-3 activity and apoptosis were significantly blocked as confirmed by flow cytometric analysis. Expression of GADD153, a transcription factor involved in apoptosis, was induced by berberine. Thus, berberine increased ROS levels, leading to endoplasmic reticulum stress based on the increase in GADD153 and Ca^{2+} release from the endoplasmic reticulum (Lin et al. 2007b). Berberine-induced, dose-dependent induction of apoptosis was accompanied by sustained phosphorylation of JNK and p38 MAPK, as well as generation of ROS. In addition, the induction of apoptosis was alleviated by inhibitors specific for JNK and p38. Furthermore, there was an increase in the cellular levels of phospho-c-Jun, FasL, and t-BID in berberine-induced apoptosis *via* the activation of JNK and p38 signaling pathways. Administration of N-acetylcysteine, a scavenger of ROS, reversed berberine-induced apoptotic effects *via* inhibition of JNK and p38, c-jun activation, FasL t-BID expression (Hsu et al. 2007). Apoptosis, detected as the sub-G0 cell population in cell cycle measurements, was confirmed in 25–200 µmol/l berberine-treated cells by monitoring the apoptotic path-

way. Apoptosis was identified by the sub-G0 cell population, up-regulation of Bax, down-regulation of Bcl-2, release of Ca^{2+}, and decreased mitochondrial membrane potential leading to the release of mitochondrial cytochrome *c* into the cytoplasm, activation of caspase-3, and concomitant apoptosis (Lin et al. 2006c).

Apoptosis mediated by berberine is associated with p53 expression. Treatment of human lung cancer A549 cells, which express wild-type p53, and human lung cancer H1299 cells, which are p53-deficient, with berberine resulted in inhibition of cell proliferation and an increase in apoptotic cell death. However, A549 cells were more sensitive to berberine-induced cytotoxic effects than H1299 cells. In addition, treatment of A549 cells with pifithrin-α, a specific inhibitor of p53, or transfection of A549 cells with a *p53* antisense oligodeoxynucleotide resulted in a reduction in the berberine-induced inhibition of cell proliferation and apoptosis. Berberine-induced apoptosis of both A549 and H1299 human lung cancer cells was associated with disruption of the mitochondrial membrane potential, reduction in the levels of Bcl-2 and Bcl-xL, an increase in Bax and Bak, and activation of caspase-3. Furthermore, berberine administration by oral gavage inhibited the growth of subcutaneous A549 and H1299 lung tumor xenografts in athymic nude mice. However, the growth of tumor xenografts with H1299 cells was faster than with A549 cells in mice and the chemotherapeutic effect of berberine was more pronounced in the p53-positive A549 tumor xenograft than in the p53-deficient H1299 tumor xenograft (Katiyar et al. 2009). Moreover, expression profiling and Ingenuity pathway analysis results showed that the cytotoxicity of berberine was accompanied by activation of BRCA1-mediated DNA damage response, G1/S and G2/M cell cycle checkpoint regulation, and p53 signaling pathways. The activation of these signaling pathways may be caused by berberine intercalating DNA and inducing DNA strand breaks through the inhibition of topoisomerases and induction of DNA lesions (Pinto-Garcia et al. 2010). The p53-expressing SK-N-SH cells were more susceptible to berberine ($IC_{50}=37$ μM) than the p53-deficient SK-N-MC cells ($IC_{50} \geq 100$ μM) without cytotoxic effects on cortical neuronal cells.

6.4.1.2 Anti-angiogenic Effect of Berberine on Tumor Tissue

Tumor-induced angiogenesis is a prerequisite for excessive tumor growth. Blood vessels invade the tumor tissue after degradation of the extracellular matrix scaffold by matrix metalloproteinases (MMPs). Therefore, inhibition of MMPs may be a useful tool to abolish neoangiogenesis of solid tumors. Berberine significantly inhibited angiogenesis in embryoid bodies and decreased intracellular ROS levels (Wartenberg et al. 2003). In addition, berberine could directly inhibit *in vitro* human umbilical vein endothelial cell tube formation and migration. Based on berberine's antiangiogenic property and its clinical potential, it may be an inhibitor of tumor angiogenesis. Furthermore, berberine prevented hypoxic SC-M1 cultures from expressing VEGF (vascular endothelial growth factor) and HIF-1α two key factors in mediating tumor angiogenesis. However, overexpression of HIF-1α in SC-M1 cells dramatically reversed the inhibitory effect of berberine on SC-M1-induced *in vitro*

human umbilical vein endothelial cell migration. HIF-1α repression is a critical step in the inhibitory effect of berberine on tumor-induced angiogenesis. Berberine did not down-regulate *HIF-1α* mRNA but destabilized HIF-1α protein. In addition, berberine -induced HIF-1α degradation was blocked by a 26S proteasome inhibitor and berberine increased lysine-acetylated HIF-1α in hypoxic SC-M1 cultures (Lin et al. 2004b).

6.4.1.3   Inhibitory Effect of Berberine on the Metastatic Potential of Cancer Cells

In cancer cell migration and invasion processes, matrix-degrading proteinases are required. In particular, invasion of cancer cells induced by MMP-9 is a pivotal step in cancer metastasis. Berberine induced down-regulation of MMP-1, -2, and -9, but not MMP-7. Berberine appears to exert its anti-cancer properties by inducing ROS production and preventing cell migration *via* inhibition of the gene expression of MMP-1, -2, and -9 in a human gastric cancer cell line SNU-5 (Kim et al. 2008; Lin et al. 2008a). Berberine inhibited migration and invasion of human tongue squamous carcinoma SCC-4 cells. These processes were mediated by the p-JNK, p-ERK, p-p38, IκK, and NF-κB signaling pathways, resulting in inhibition of MMP-2 and -9 in these cells. Berberine down-regulated the expression of u-PA (urokinase-plasminogen activator), MMP-2, and MMP-9 in SCC-4 cells through the FAK, IKK, and NF-κB mediated pathways and a novel function of berberine was to inhibit the invasive capacity of malignant cells (Ho et al. 2009b). A549 cells treated with berberine demonstrated reduced levels of ECM (extracellular matrix) proteinases, including MMP-2 and u-PA, by gelatin and casein zymography analyses. This inhibitory effect likely occurs at the transcriptional level, since reduction in the transcript levels corresponds to that in the protein levels. Moreover, berberine exerted its action *via* regulation of TIMP-2 (tissue inhibitor of metalloproteinase-2) and u-PAI (urokinase-plasminogen activator inhibitor). The upstream mediators of the effect involved c-jun, c-fos, and NF-κB, as revealed by reduced phosphorylation of the proteins. These findings suggest that berberine possesses an anti-metastatic effect in non-small lung cancer cells and, therefore, may be helpful in clinical treatment (Peng et al. 2006).

Berberine suppressed the presence of phosphorylated ezrin, and this inhibitory effect was dependent on the suppression of Rho kinase activity. Reduction of ezrin phosphorylation at Thr567 by berberine was associated with its inhibitory effect on filopodia formation. In addition, reduction of Rho kinase-mediated ezrin phosphorylation may be involved in the anti-metastatic effect of berberine on NPC (Tang et al. 2009a). Berberine suppressed the activation of Rho GTPases, including RhoA, Cdc42, and Rac1, indicating a novel function of berberine in the suppression of Rho GTPase signaling to mediate its inhibitory action on cell migration and motility. The SDF-1/CXCR4 axis involves in the migration process of leukemic cells and berberine partially inhibited SDF-1-induced AML cells and the migration of leukemia stem cells. Berberine reduced the levels of SDF-1 secreted by bone marrow-

derived stromal cells in the microenvironment but did not affect CXCR4 expression on the HL-60 cell membrane. Therefore, berberine might be a potentially effective agent for the prevention of leukemia (Li et al. 2008).

6.4.1.4 Anticancer Effect of Berberine in Animal Experiments

Retroviral infection with Friend murine leukemia virus (FMuLv) has been used as a model for identifying potential anti-viral medicinal preparations or establishing new treatment strategies. In BALB/c mice, treatment with berberine was found to extend the life span of leukemia-harboring animals by more than 60 days, decrease the anemic condition prevalent in the FMuLv alone-treated group, inhibit the massive leukemic cell infiltrations to sinusoidal spaces in the spleen, and decrease the expression of Bcl-2, Raf-1, Erk-1, IFN-γ receptor, and erythropoietin. Berberine was able to suppress the progression of leukemia induced by FMuLv and further support its chemopreventive potential against virally induced cancers (Harikumar et al. 2008). The effect of berberine on WEHI-3 leukemia cells *in vivo* was studied, revealing the reduction of Mac-3 and CD11b markers and indicating differentiation inhibition of macrophage and granulocyte precursors. No effect was observed concerning the CD14 marker but the CD19 marker demonstrated the promotion of differentiation of B cell precursors. The weights of spleen samples from mice treated with berberine were found to be lower (Yu et al. 2007). In *in vivo* studies, a significant reduction in tumor volume was observed on the 16th day with berberine administration at 5 and 10 mg/kg. The 1 mg/kg-dose stimulated the tumor mass, but the other tested doses, 5 and 10 mg/kg, reduced the tumor weight (Letasiova et al. 2005). In addition, berberine reduced tumor weights and volumes accompanied by apoptotic cell death and increased expression of apoptotic cell death proteins (Choi et al. 2009).

SCC-4 tumor cells were implanted into mice and groups of mice were treated with berberine (10 mg/kg of body weight). Treatment with berberine resulted in a reduction in tumor incidence. Tumor size in xenograft mice treated with 10 mg/kg berberine was significantly smaller than that in the control group (Ho et al. 2009c). Oral administration of berberine for 14 days significantly inhibited spontaneous mediastinal lymph node metastasis produced by orthotopic implantation of Lewis lung carcinoma into the lung parenchyma in a dose-dependent manner, but did not affect tumor growth at the lung implantation site. Combined treatment with berberine and an anti-cancer drug, CPT-11, resulted in marked inhibition of tumor growth at the implantation site and lymphatic metastasis (Mitani et al. 2001). Chinese rhizome supplement significantly attenuated the weight loss of tumor-bearing mice without a change in food or water intake (Iizuka et al. 2000).

6.4.1.5 Effect of Berberine on Prevention of Carcinogenesis

Berberine inhibited neoplastic transformation by the induction of an antioxidant defense system and the ability to induce apoptotic-like changes, thus clarifying its

anticancer role (Thirupurasundari et al. 2009). Oral administration of berberine inhibited hepatocyte proliferation and iNOS expression, as well as inhibited the activities of CYP2E1 and CYP1A2 in diethylnitrosamine (DEN) plus phenobarbital (PB) DEN-plus-PB-treated rats *in vivo*. Moreover, berberine inhibited the activities of CYP2E1 and CYP1A2 in microsomes isolated from DEN-plus-PB-treated rats *in vitro*, suggesting that the anti-hepatocarcinogenic potential of berberine might be due to inhibiting the oxidative metabolic activities of CYP2E1 and CYP1A2 in rats (Zhao et al. 2008). Berberine has been found to significantly inhibit carcinogenesis induced by 20-methylcholanthrene or N-nitrosodiethylamine in a dose-dependent manner in small animals. Administration of berberine could significantly reduce the tumor incidence in animals after injection of 20-methylcholanthrene and increase their life span compared with the control (Anis et al. 2001). COX-2 is abundantly expressed in colon cancer cells and plays a key role in colon tumorigenesis. Compounds inhibiting *COX-2* transcriptional activity could potentially have a chemopreventive property against colon tumor formation. Berberine effectively inhibited *COX-2* transcriptional activity in colon cancer cells in a dose- and time-dependent manner. Thus, these findings may indicate that berberine has antitumor promoting effects (Fukuda et al. 1999). AP-1 (activator protein 1) is a transcription factor that plays a critical role in carcinogenesis. Berberine was shown to inhibit AP-1 activity in a dose- and time-dependent manner. The inhibitory effect on AP-1 activity in cancer cells may further explain the anti-tumor activity of berberine (Fukuda et al. 1999).

6.4.1.6 Berberine Combining with Other Antitumor Therapies Enhances Therapeutic Effect and Reduce Toxicity

The combination of berberine and gamma-radiation enhanced the anticancer effects through the p38 MAPK pathway and ROS generation (Hur et al. 2009). In addition, combined treatment of ER antagonists and berberine conferred synergistic growth inhibitory effects on MCF-7 cells (ER$^+$), but not MDA-MB-231 cells (ER$^-$). Similar results were observed using the combined treatment of berberine and fulvestrant, a specific aromatase antagonist. Analysis of the expression of breast cancer-related genes indicated that *EGFR*, *HER2*, *bcl-2*, and *COX-2* were significantly down-regulated, while *IFN-β* and *p21* were remarkably up-regulated by berberine. This suggests that Chinese goldthread extracts could be promising adjuvants to ER antagonists in ER-positive breast cancer treatment through regulation of expression of multiple genes (Liu et al. 2009). Berberine enhanced As$_2$O$_3$-mediated inhibition of glioma cell growth. The formation of confluent cell layer was inhibited after incubation with As$_2$O$_3$; and this effect was even more pronounced in the presence of berberine. In addition, As$_2$O$_3$-mediated reduction in motility and invasion of glioma cells were enhanced upon co-treatment with berberine. Furthermore, it has been reported that PKC isoforms influence the morphology of the actin cytoskeleton as well as the activation of metalloproteinases MT1-MMP and MMP-2, which were involved in cancer cell migration. Treatment of glioma cells with As$_2$O$_3$ and berberine significantly decreased the activation

of PKC-α and led to actin cytoskeleton rearrangements. The levels of two down-stream transcription factors, myc and jun, as well as MT1-MMP and MMP-2 were also significantly reduced. After co-treatment of glioma cells with As_2O_3 and ber-berine, cancer cell metastasis can be significantly inhibited, most likely due to blocking of the PKC-mediated signaling pathway involved in cancer cell migra-tion. The abovementioned study is potentially interesting for development of novel chemo-therapeutic approaches in the treatment of malignant gliomas, as well as cancer in general (Lin et al. 2008b).

Radiotherapy is the most efficacious strategy for lung cancer. Radiation-enhanc-ing effects and the underlying mechanisms of berberine have been investigated. The cellular ultrastructure revealed the presence of an autophagosome and an in-creased proportion of acridine orange stained-positive cells, demonstrating that berberine enhanced radio-sensitivity *via* autophagy. An animal tumor model veri-fied the synergistic cytotoxic effect of berberine and irradiation that resulted in a substantial shrinkage of tumor volume. Supplementation with berberine enhanced the cytotoxicity of radiation in both *in vivo* and *in vitro* lung cancer models. The mechanisms underlying this synergistic effect involved the induction of autophagy, suggesting that berberine could be used as an adjuvant therapy to treat lung cancer (Peng et al. 2008). In experiments measuring clonogenic survival, treatment with a non-toxic dose of berberine rendered glioblastoma multiforme (GBM) cells more sensitive than vehicle-treated control cells to X-rays. Berberine could be integrated with post-operative radiotherapy to selectively promote residual GBM tumor cell death (Yount et al. 2004).

6.4.1.7   Potential Toxicity and Adverse Effects of Berberine

Electrospray ionization mass spectrometric (ESI-MS) studies indicated that berber-ine shows a binding stoichiometries with d(AAGAATTCTT), d(AAGGATCCTT) and d(AAGCATGCTT). Their relative binding affinities toward these three double-stranded DNA were semi-quantitatively evaluated by measuring the ratios of the complex signals ([ds+alkaloid-5H](4−)+[ds+2alkaloid-6H](4−)) to those of the du-plexes ([ds-4H](4−)) and also by ESI-MS competitive binding experiments (Chen et al. 2005). This binding may block DNA replication in normal cells. Berberine was selectively accumulated by mitochondria, resulting in arrest of cell proliferation, mitochondrial fragmentation and depolarization, oxidative stress, and a decrease in ATP levels. Electron microscopy of berberine-treated cells showed a reduction in mitochondria-like structures, accompanied by a decrease in mitochondrial DNA copy number. Isolated mitochondrial fractions treated with berberine exhibited slower mitochondrial respiration, particularly when complex I substrates were used, and increased complex I-dependent oxidative stress. These results demonstrate that many previously unknown alterations of mitochondrial physiology may be induced by berberine. Pereira et al. suggested that high doses of berberine should not be used without a proper toxicology assessment (Pereira et al. 2007). Berberine up-regulated multidrug- resistance transporter (pgp-170) expression in two oral (KB, OC2), two

gastric (SC-M1, NUGC-3), and two colon (COLO 205, CT 26) cancer cell lines, and decreased the retention of rhodamine 123. Treatment of cells with berberine before paclitaxel treatment resulted in increased viability. In addition, pre-treatment with berberine blocked paclitaxel-induced cell cycle responses and morphological changes. Berberine modulated the expression and function of pgp-170, leading to a reduced response to paclitaxel in digestive tract cancer cells (Lin et al. 1999).

6.4.2   Effect of Coptisine

6.4.2.1   Anticancer Effect of Coptisine

Acceleration of vascular smooth muscle cell (VSMC) proliferation is closely linked to the pathogenesis of vascular diseases. Coptisine (Fig. 6.2), an alkaloid of the Chinese goldthread, was the active ingredient in Chinese goldthread, selectively preventing VSMC proliferation with a GI_{50} of 3.3 µM (1.2 µg/ml). Coptisine selectively prevented VSMC proliferation at lower concentrations (Tanabe et al. 2006). In addition, coptisine was cytotoxic in LoVo and HT29, but less potent in L1210 cells, and it was partially crossresistant in the human colon tumor cell line (Colombo et al. 2001). Furthermore, coptisine exhibited a strong inhibitory effect on the proliferation of hepatoma and leukemia cell lines, with IC_{50} values varying from 1.4 to 15.2 µg/ml and from 0.6 to 14.1 µg/ml, respectively. Thus, coptisine possesses anti-hepatoma and anti-leukemia activities (Lin et al. 2004a).

Coptisine displayed antiproliferative action against VSMCs by blocking the cell cycle at G1 and G2/M phases. The G1 block was shown by inhibition of ^{3}H-thymidine incorporation into VSMCs at coptisine concentrations higher than 15 µM. The mechanism underlying the G1 arrest involved a decrease in cyclin D1 protein, although cyclin E, A, and B were not affected by coptisine treatment. The selective reduction in cyclin D1 protein was mainly attributable to accelerated proteolysis *via* proteasome-dependent pathway, since it was inhibited by a proteasome inhibitor, N-carbobenzoxy-L-leucinyl -L-leucinyl-L-norleucinal (MG132) and further the mRNA level of *cyclin D1*, protein synthesis, and MAPK (mitogen-activated protein kinase) activity remained unaltered. The mechanism underlying the G2/M arrest involved partial inhibition of tubulin polymerization, which was apparent at coptisine concentration of 3 µM. Berberine arrested the cell cycle at G1 phase *via* a mechanism identical with coptisine, but did not cause block at G2/M phase (Tanabe et al. 2005).
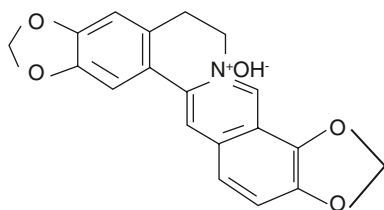

Fig. 6.2 Chemical structure of coptisine

6.4.2.2 Potential Toxicity and Adverse Effects of Coptisine

ESI-MS spectrometric studies indicated that coptisine shows binding stoichiometries with d(AAGAATTCTT), d(AAGGATCCTT), and d(AAGCATGCTT). Their relative binding affinities toward these three double-stranded DNA were semi-quantitatively evaluated by measuring the ratios of the complex signals ([ds+alkaloid-5H](4−)+[ds+2alkaloid-6H](4−)) to those of the duplexes ([ds-4H](4−)) and also by ESI-MS competitive binding experiments (Chen et al. 2005). This effect may block DNA replication in normal cells. In A10 cells (a rat VSMC line), coptisine up-regulated the mRNAs of *Mdr1a* and *Mdr1b*. Coptisine also induced *Mdr1a/1b* protein expression and enhanced the efflux of rhodamine 123 from A10 cells (Suzuki et al. 2010). This implies that coptisine may up-regulate *Mdr* expression in tumors. Coptisine showed an inhibitory effect on MAO-A (type A monoamine oxidase) activity in a concentration-dependent manner using a substrate kynuramine. Coptisine is also a potent reversible inhibitor of MAO-A, and that coptisine functions to regulate the catecholamine content (Ro et al. 2001). Coptisine may possess some potential toxicity and adverse effect.

6.5 Perspectives and Challenges

Chinese goldthread and herbal complexes containing Chinese goldthread have been used in tumor therapy in traditional Chinese medicine. Chinese goldthread has been proved to have an antitumor effect on many cancers, and this antitumor effect distributes to its main components, berberine and coptisine. It possesses both tumor therapeutic and preventive effects. Chinese goldthread or its extract beberine may be a potential cancer therapeutic agent and it may be widely used in clinical therapy, especially at cancer prevention.

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