Natural Medicine

A letter from the editor
> Bringing natural therapy back into medicine

What is Natural Medicine

An evolution is taking place in healthcare, one that emphasizes natural treatments rather than drugs or surgery. Natural medicine encompasses topics such as nutrition, herbs, acupuncture, physical interventions, lifestyles, and mental health. Though fast growing public acceptance of natural therapy has supported the value of natural medicine, more empirical evidence is needed to bring natural medicine into mainstream medicine. Natural medicine will be established based on the existing systems of alternative medicine, such as traditional Chinese medicine, naturopathic medicine, ayurvedic medicine, integrating a wide range of these methods with modern medicine. This column of natural medicine in NAJM welcomes any authors who want to offer their scientific interpretation of natural medicine.

Research Perspective

Rationale and Problems for Use of Coptis and Berberine in Cancer Chemoprevention

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Introduction

Coptis (Huang Lian) is a very popular therapeutic herb in China. The pharmacological name of coptis is *rhizoma coptidis* and the botanical name is *coptis chinesis franch*. Coptis grows in many areas around the globe. There are about 15 major species of coptis in China, including *C. chinesis*, *C. deltoidea*, *C. orneiensis*, *C. teetoides and C. quinquesecta*. *Coptis chinensis*, or duan e huang lian in Chinese, has been widely used in traditional Chinese medicine. *Coptis aspleniifolia* (spleenwort-leaf goldthread) and *coptis occidentalis* (Idaho Goldthread) are popular herbs native to North America.

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The first description of coptis that appeared in China was in an ancient medical textbook called the Divine Husband's Classic of the Materia Medica. The major uses for coptis in traditional Chinese medicine include "clearing heat, toxicity, and dampness from the body system". Traditional use of coptis in Chinese medicine has been widely introduced into modern medical practice for a variety of infectious and inflammatory disorders such as bacterial, viral, and fungal infections. Pharmacological and clinical studies have strongly suggested application of coptis for skin acne, stomach helicobacter pylori bacteria, intestinal parasitic infections, yeast infections and, in particular, conditions that respond poorly to antibiotic treatments. For example, coptis was tested as one of the strongest natural antibiotics for yeast infections. Modern studies also reveal that coptis possesses multiple other pharmacological effects including the modulation of hypertension, hyperglycemia, and hypercholesterolemia.

In the last decade, the roles of coptis and its major constituent berberine on cancer treatments have been extensively investigated. However, most of the results from this research were from laboratory studies. It has been well documented that coptis or berberine possesses anti-proliferative effects on a variety of cancer cell lines from the cancers of the liver,

lung, esophagus, stomach, skin, cervical, and blood (leukemia).[2-7] For example, protoberberine, a compound of berberine in coptis, induced apoptosis of HeLa and L120 cells. The IC_{50} of protoberberine was less than 4 mg/ml, a dose which meets the criteria of the National Cancer Institute for a potential anti-cancer drug.[8] In a previous study, we found that coptis and berberine inhibited cell proliferation and promoted apoptosis of estrogen receptor (ER) positive breast cancer MCF-7 cells but not ER negative MDA-MB-231 cells.[9] A similar result was confirmed by other investigators.[10]

Active Anticancer Constituents of Coptis

It was reported that the most important therapeutic constituent of coptis is isoquinoline alkaloid, occupying 4 to 8% of the herbal body weight. Ninety percent of the alkaloid is berberine, which exists naturally in the form of protoberberine. Protoberberine and berberine are known to generate a wide variety of biochemical and pharmacological effects. There are five types of alkaloids in the berberine family: berberine, coptisone, palmatine, epiberberine and jateorrhizine.[11,12] In fact, berberine exists not only in coptis. More than 10 berberine containing herbs are commonly used in China for a variety of disorders, though mostly for infection and inflammation related conditions. These herbs include *cortex phyllodendri* (Huang Bo), *herba chelidonii* (Bai Qu Cai), *folium mahonae* (Shi Da Gong Lao Ye) and *radix seu cortex berberidis* (San Ke Zhen). The popular berberine enriched herbs in North America are goldenseal, oregona root and barberry. In addition to berberine, other constituents of coptis may also contribute to the anti-cancer effects of coptis.[13] For example, coptisine showed strong anti-proliferative activities.[14] The concentration of coptisine is about 20-30% that of berberine in coptis.[15] The IC_{50} of berberine and coptison for anti-proliferation are 1.4 and 15.2 mg/ml on hepatoma, 0.6 and 14.1 mg/ml on leukemia cell lines, respectively.[12]

The role of coptis has been substituted by berberine in most anti-cancer investigations, because berberine is viewed as the major antitumor constituent of coptis. Iizuka reported that the antitumor effects of berberine are similar to that of coptis rhizoma on six types of esophageal cancer lines. The ID_{50} of berberine positively correlated with ID_{50} of coptis, suggesting that berberine is the major component of the antitumor effect in coptis.[2] In addition, a DNA microarray data from 12,600 genes in eight human pancreatic cancer cell lines were analyzed, of which 27 genes correlated with the antiproliferative effects of coptis. The Hierarchical cluster analysis showed that berberine shared most numbers of the same clusters with coptis, indicating that berberine can account for the majority of the anti-proliferative activities of coptis. Furthermore, the key cancer genes were identified among the 27 genes, of which *PAK1* regulates cell motility and morphology, *PL6* selectively activates the Ras superfamily guanine nucleotide binding protein Rap1A, and *GTF3C1* encodes a component of RNA polymerase III. The result suggests that these genes could be the therapeutic targets of both coptis and berberine.[16] However, the idea that berberine can completely replace coptis in cancer treatment

has not been accepted. For example, it was reported that coptis was more effective in inhibiting cell growth and colony formation of gastric, colon, and breast cancer cell lines.[17]

The Mechanism of the Antitumor Effect of Coptis and Berberine

The mechanism of the antitumor effects of coptis and berberine has been well established in many aspects, including their interferences on DNA, mRNA, mitochondria and a variety of enzymes. Here, we mainly discuss the mechanism involving the cytotoxicity and apoptosis of coptis and berberine.

Effects of coptis and berberine on cytoxicity and apoptosis

Coptis and berberine possess tumor-specific cytotoxicity and apoptosis-inducing activity, suggesting their anti-tumor potential.[18] Cell cycle analysis demonstrated that the cancer cells were accumulated in the G0/G1 phase and decreased in the S phase by treatment of coptis and berberine. Using ^{32}P-labeled human gene DNA fragments, berberine was found to bind with DNA.[19] Berberine interacts in vitro with DNA, poly(A) fragments of mRNA and tRNA by the mechanism of intercalation.[5,20] By treating the cells with berberine, a decrease of the G0/G1 phase and increase in the G2/M phase were detected on human gastric carcinoma SNU-5 cells. This was accompanied by a marked increment in the expression of p53, Wee1 and CDk1 and a decrease of the expression of cyclin B. Concurrently, apoptosis was promoted with the upregulation of Bax and the downregulation of Bcl-2, as well as an activation of caspase-3.[21] The study from Tan found that berberine suppressed the cell cycle and induced DNA damage through the mitogen-activated protein kinase (MARK) phosphoraly signal system.[22] It was also discovered that berberine protected gastric mucosa from the damage by increasing the expression of eNOS mRNA and inhibiting the expression of iNOS mRNA.[23] Cyclin subunit is critical for cell cycle progression. An 8-fold suppression of cyclin B1 protein by berberine treatment was observed, associated with complete inhibition of cdc2 kinase activity and an accumulation of cells in G2. There was no change in the protein expression of cyclins A or E.[17] Activator protein 1 (AP-1) is a transcription factor which plays a critical role in inflammation and carcinogenesis. Fukuda K reported that berberine inhibited AP-1 activity almost completely at a concentration as low as 10 microM after 48 hours of treatment.[24]

Water extract of coptis was found to be able to stabilize the cleavable complex with mammalian DNA topoisomerase I. Two protoberberine alkaloids, epiberberine and groenlandicine, were identified as active components responsible for the topoisomerase I-mediated DNA cleavage. Berberine was discovered as a specific inducer of topoisomerase II-mediated DNA cleavage in vitro.[25] Interestingly, the action of berberine on DNA is similar to that of camptothecin (CTP), a typical chemotherapeutic drug inducing DNA topoisomerase toxicity and hence apoptotic cell death, although the cytotoxic potency of berberine was much lower than that of camptothecin.

Coptis and berberine induce apoptosis on variety of cancer lines. Berberine inhibited the growth of MGC-03 stomach cancer cells by inducing apoptosis and arresting cell in G0-G1. Plus, berberine inhibited tumor metastasis by decreasing the expression of CD44V6 on MCG803. The data from several studies shows that berberine acted on the caspase-3 pathway. It was reported that the effect of apoptosis of human promyelocytic leukemia HL-60 and murine myelomonocytic leukemia WEHI-3 cells, induced by berberine, was through activating caspase-3, accompanied by inducing ROS and Ca^{+2} production and downregulating Bcl-2, as well as upregulating Bax and cytochrome C.[26] The activity of berberine on the mitochondrial / caspase pathway was also observed in human promonocytic U937 cells and the NB4 leukemia cell line. Furthermore, the study showed that berberine induced apoptosis by upregulating the expression of caspase -3 and downregulating the expression of survivin on human hepatoma cells.[27] Besides the caspase pathway, the apoptosis induced by berberine could be also associated with a downregulation of nucleophosmin/B23 and telomerase. Nucleophosmin/B23 plays an important role in the control of the cellular response to apoptotic induction.[4]

Effect of coptis and berberine on cancer related enzymes

Several enzymes involving the anticancer activity of berberine have been identified. For example, berberine inhibits NADH oxidase, reverse transcriptase, diaminooxidase,[20] topoisomerase,[25] activator protein 1 and cyclooxygenase-2.[3,24] It is well known that cyclins promote the growth of cancer. Hence, suppression of cyclin-dependent kinase (cdk) activity is an attractive target for cancer chemotherapy. Coptis inhibited cyclin B1 significantly, which was followed by a decrease of cdc2 kinase activity, resulting in the cancer growth arrest of gastric, colon, and breast cancer cell lines.[17]

Long-term consumption of a high glycaemic index (GI) or glycaemic load (GL) diet may lead to chronic hyperinsulinaemia, which is a potential risk factor for cancer. A positive association for GL and the risk of endometrial cancer is particularly among obese women.[28] Glucose is viewed as high-energy molecules. Adenosine monophosphate-activated protein kinase (AMPK) is coupled to insulin signaling, which is involved in controlling energy metabolism for cell growth and proliferation.[29] The study stated that berberine circumvented insulin signaling pathways and stimulated glucose uptake through the AMP-AMPK - p38 MAPK pathway, and thereby induced anti-hyperglycemic effects,[23] a result which may potentially benefit cancer prevention treatments.

N-acetyltransferases (NATs) plays a key role in arylamine compound metabolism. Polymorphic NAT is coded for rapid or slow acetylators, which is viewed as a factor involved in cancer risk environmental exposure. Berberine displayed a dose-dependent suppression to cytosolic NAT activity of intact mice leukemia cells, a result of the inhibitory effect of berberine on NAT1 mRNA and NAT proteins.[30] The effect of berberine on arylamine N-acetyltransferase activity was also observed on other cell lines, including colon tumor cells

(colo 205), human leukemia cells, human malignant astrocytoma (G9T/VGH), brain glioblastoma multiforms (GBM 8401) cells, Salmonella typhi, and human leukemia HL-60 cells.[31-35]

Matrix-degrading proteinases are required in the cancer cell migration and invasion process. Berberine exerted inhibitory effects on the motility and invasion ability of highly metastatic non-small cell lung cancer A549 cells. The effect was via regulating metalloproteinase-2 (TIMP-2) and urokinase-plasminogen activator inhibitor (PAI). In this process, the upstream modulations involved c-jun, c-fos and NF-kappaB.[36]

Rationale of Coptis and Berberine in Combination with Chemotherapy for Cancer Treatment

The adjuvant agents, such as the inhibitors of cyclooxygenase-2 (COX-2), tyrosine kinase, bcl-2 and angiogenesis, have been intensively studied in order to improve the efficacy and reduce the resistance of chemotherapy. It becomes evident that COX-2 plays a key role in tumorigenesis. Compound inhibiting COX-2 transcriptional activity is therefore potentially a chemopreventive agent.[37] Studies showed that berberine possesses a COX-2 inhibitory effect, which could count for its anticancer effect.[24] At concentrations of higher than 0.3 microM, berberine effectively inhibited COX-2 transcriptional activity in colon cancer cells in a dose-and time-dependent manner.[24] COX-2 inhibitor has been intensively studied for years for its potential in enhancing the efficacy of chemotherapy in a variety of cancer treatments.[38,39] It was revealed that combining treatment of berberine and CPT-11 induced a marked inhibition of tumor growth of lymphatic metastasis of Lewis lung cancer cells compared with either treatment alone.[40] The mechanism is not clear, but the synergistic effect could be due to the common target on DNA that CPT11 and berberine share.[41]

Chemotherapy resistance or multiple drug resistance (MDR) has been the most difficult challenge in clinical cancer treatment. The overactivity of MDR1/P-glycoprotein could lead to the failure of chemoprevention. So far, the most promising resolution is to use the adjuvant agents against the MDR.[42,43] On a model of MDR1 in cervical carcinoma cell line HeLa, coptis was discovered to enhance the palitaxel sensitivity via the inhibition of MDR 1 cellular membrane transport.[44] The effect was through Retinitis pigmentosa 2 (RP2).[14] RP2 encodes a protein involved in beta-tubulin folding. Taxol and palitaxel are tubulin antagonists. A possible synergistic anti-cancer effect could be through such co-targeted activities of herbs and chemotherapy.

Anti-Bcl-2 agents could enhance the efficacy of chemotherapeutic drugs.[45] Bcl-2 is involved in the early stage development of cancer. For example, increased levels of Bcl-2 were followed by a significant promotion of proliferation on stomach mucosa membrane. Berberine downregulated the gene expression of Bcl-2, accompanied by

increased apoptosis on the pre-carcinoma lesion on the mucosa membrane of the stomach. Ma reported that the anti-stomach cancer effect of berberine could be the result of the downregulation of Bcl-2 and mutated p53 gene expression. A similar result was also found from the study using an *in vivo* model.[27] Bcl-2 was viewed as responsible for chemotherapy resistance for variety of cancers.[46] Therefore, we have a reason to hypothesize that berberine may have the potential to reduce chemotherapy resistance through inhibiting Bcl-2. More investigations are needed to clarify the role of coptis or berberine on chemotherapy.

The Paradoxical Effects of Antioxidants on Cancer Chemoprevention

It should be warned that coptis and berberine could possibly counteract chemotherapy because coptis and berberine show antioxidative activity. Antioxidants are viewed to be in potential conflict with chemotherapy.[47] It was shown that coptis protected against ONOO(-)-induced oxidative damage and this effect was mainly attributed to berberine in coptis.[15] Using four different radical scavenging methods, berberine showed its ability to scavenge the free radicals in a concentration dependent manner.[48] The structure-effect analysis illustrated that protoberberine, analogue of berberine, exhibited an inhibitory activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals.[49] Indeed, there were a few reports demonstrating that berberine may enhance multiple drug resistance (MDR).[50] Berberine was claimed to attenuate Paclitaxel-induced apoptosis and G2/M arrest in oral, gastric hepatoma and colon cancer cell lines.[50,51] However, antioxidants may not reduce the cytotoxicity of chemotherapy, as evidenced from randomized controlled clinical trials.[1,52] The discrepancy of coptis and berberine on ROS could explain the contrary effects on the combined berberine and chemotherapy. It is well acknowledged that radiation kills cancer cells by generating harmful concentrations of free radicals. Berberine induced the synergistic, anti-lung cancer effect of radiation in animal models while suppressing ROS and free radicals,[53] implicating that the antioxidant effect of berberine may not necessarily be the only factor which decides its role in the radiation or chemotherapy of cancer. In addition, berberine was reported to significantly inhibit angiogenesis in embryonic bodies as a result of decreased intracellular ROS levels.[1]

On the other hand, berberine was reported to promote ROS. Berberine directly inhibited in vitro human umbilical vein endothelial cell (HUVEC) tube formation and migration. Also, berberine prevented hypoxic SC-M1 cell cultures from expressing vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1alpha, two key factors in mediating angiogenesis through destabilizing hypoxia-inducible factor (HIF)-1alpha. This suggests the antiangiogenic property of berberine and a possible correlation with ROS.[54] Mitochondrial abnormalities that disturb the ROS-HIF-1alpha-Kv1.5 O(2)-sensing pathway contribute to the pathogenesis of cancer, that is, mitochondrial metabolism and redox signaling are reversibly disordered, creating a pseudohypoxic redox state characterized by normoxic decreases in ROS, a shift from oxidative to glycolytic metabolism and HIF-1alpha activation.[55] Therefore, the conversion of decreased ROS and activated HIF-1 alpha of berberine may constitute therapeutic benefits on cancer. The preliminary data from our study suggests that the effects of coptis and berberine on ROS and cancer were mostly cancer cell type-specific and doses of the herbs dependent, the result from observing the responses of five different cancer cell lines to the treatment of coptis and berberine, which may partially explain the paradox of coptis and berberine action on ROS and cancer here.

Interestingly, the crude berberis plant may not show a similar concern on MDR as that of berberine. It was discovered that the berberine-containing plants produce 5'-methoxyhydocarpin, an MDR inhibitor that can counteract the possible MDR stimulating effect of berberine and enhance the anti-cancer effect of berberine.[56] In fact, the data from our study also revealed that the crude coptis extract exerted stronger anti-cancer effects compared with that of berberine in cancer cell lines (data is not published).

Unresolved Problems and Perspective Research

Coptis and berberine demonstrated promising anti-cancer results, evidenced mostly from in vitro assays. Much less data is available from clinical and in vivo animal studies. It was reported that berberine induced 30% to 45 % growth inhibition of S180 tumor by the oral doses of 25uM and 75uM/kg on mouse, compared with 93% in the in vitro study, indicating that the oral absorption of berberine is poor. Berberine can not be absorbed well in the stomach or intestines. The data from the animal tests showed that the blood concentration was 100ug /ml, 30 minutes after an oral dose of 400mg. The $T_{1/2}$ in the stomach was 194 minutes. The result from clinical test demonstrated that the mean maximum plasma concentration (C_{max}) in twenty volunteers was about 0.3 to 0.4 ng /ml after a 300 to 400 mg oral dose of berberine.[57,58] These results indicate that the bioavailability of oral administration of berberine is much lower than that needed for cytotoxicity. Further investigations are needed to improve the bioavailability of berberine or berberine containing compounds. One way to improve the situation is to investigate coptis containing multi-herb formulas, which have been successfully used in clinics for generations, to evaluate if some substances in the formula could enhance the bioavailability and efficacy of berberine or coptis in cancer treatment. Another consideration is to re- structure berberine in order to improve its therapeutic availability or efficacy on cancer. It was reported that extending the alkyl chain at position 8 or 13 of the berberine molecule strongly improved the cytotoxic activity of berberine.[59]

It should be noticed that the cytotoxicity effect of berberine or coptis is not the only value of the herbs in cancer treatment. Our previous study demonstrated that coptis remarkably promoted IFN-β gene expression and protein synthesis of MCF-7 breast cancer cells. The upregulation of this gene is responsible for the anti-proliferative effect of berberine.[9] It will be interesting to know if low blood concentration of berberine (0.3 to 0.4ng/ml) is enough to

positively modulate the IFN-β or other inflammation-immune factors and benefit cancer chemoprevention. An in vitro study showed that coptis and berberine inhibited IL-1-induced IL-6 mRNA expression in a dose-dependent manner in colon 26/clone 20 cells. A similar result of IL-6 suppression induced by coptis and berberine was observed on colon 26/clone 20-transplanted mice.

Improvement of MDR for Coptis and Berberine Treatment

Because of the concern of the paradoxical effects of berberine on MDR, it is reasonable to develop an anti-MDR/P-Glycoprotein agent which could prevent possible berberine resistance.[60] For this purpose, the berberine-enriched coptis or other natural substances should be studied and compared with berberine on their feasibilities of MDR. As we discussed, some constituents in coptis and other natural substances may inhibit MDR and synergistically enhance the anti-cancer effect of berberine.[61-63] For example, one of the constituents of coptis, 8-oxocoptisine, possesses significant activity of P-gp MDR inhibition on MES-SA/DX5 cells and HCT15 cells. Another clue is that hypoxia is one of the factors resulting in P-Glycoprotein overexpression.[64] Therefore, natural substances, which can improve the condition of hypoxia may reduce MDR generation in the berberine treatment. Among these substances, certain antioxidants, such as inflavones, were shown to improve the berberine inducible MDR.[65]

There are hundreds of berberine-containing berberis species on earth, but only a dozen of berberine enriched medical herbs have been widely used in health care. Their therapeutic value on cancer treatment remains to be investigated. The discovery of the potential values of coptis and berberine on cancer treatment has opened a new opportunity to improve the outcome of cancer treatments, but it remains a long way to understand the healing from nature.

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