CJIM 1995;1(4):242-248

FEATURE ARTICLE

Recent Progress of Traditional Chinese Medicine and Herbal Medicine for the Treatment and Prevention of Cancer

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According to the data released by the Ministry of Public Health of China, cancer has been ranked as the number one killer in urban area of China in 1990 and more than one million patients die from cancer every year in mainland China. Although surgery and radiation therapy are the major modality of treatment for cancer, the importance of drug treatment has increased dramatically in the past forty years. For the time being, we are able to cure some advanced cancers by chemotherapy, and the complete remission with increased survival rate induced by chemotherapy or adjuvant chemotherapy has been achieved in some cancers.

The research and development of anticancer drugs from plants has been very fruitful, advances in biochemical and pharmacological studies of plant-derived drugs have exerted enormous impetus on the research and development of new anticancer drugs from plants.

Some new antineoplastic drugs developed from traditional Chinese medicine and herbal medicine are reviewed in this paper.

CYTOTOXIC ANTICANCER DRUGS[1,2]

Indigofera Tinctoria and Indirubin

Guided by the therapeutic principle of "purging the hepatic substantial fire" of traditional Chinese medicine, a group of hematologists from the Institute of Hematology, Chinese Academy of Medical Sciences has demonstrated the efficacy of a prescription called Danggui Longhui Wan (当归龙荟丸) for treatment of chronic myelocytic leukemia, consisting of eleven Chinese medicinal herbs, namely, Angelica sinensis, Aloe, Gentiana scaber, Saussurea lappe, Scutellaria baicalensis, Phllodendron chinensis, Coptis chinensis, Gardenia jasminoides , Rheum palmatum, Indigofera tinctoria and Moschus moschiferus. Pharmacological studies demonstrated that Indigofera tinctoria is active for the inhibition of Walker carcinosarcoma 256 in rats. Phytochemical studies exhibited that indirubin is the active principle of it. Clinical studies showed positive result for the treatment of chronic myelocytic leukemia. Since the content of indirubin in the herb is very low, chemists have succeeded in the total synthesis of indirubin. On the basis of chemical structure-activity relationship studies of these compounds, a derivative called N-methyl isoindigotin has been developed recently. Clinical studies indicated that it is more effective than its parent drug but with less side effect.

Iris Latea and Irisquinone

The seeds of Iris latea has been used in folk medicine in China for treatment of malignancies. The chemists of Tianjin Institute of Materia Medica have isolated an active principle called irisquinone from the seeds of this plant, its chemical structure being 6-methoxy-2-cisheptadecenyl-1, 4-henylquinone. Experimental therapeutic studies have shown that it is effective against transplanted tumors in mice.

Cephalotaxus Hainanensis and Harringtonine and Homoharringtonine[3,4]

Cephalotaxus fortuni was used by the folk

practitioners in Fujian Province for treatment of neoplastic diseases. Powell et al. reported that the active principles of Cephalotaxus harringtonia are active against rodent leukemia. On the basis of these reports, a systemic investigation of Cephalotaxus hainanesis in resource, chemistry, pharmacology and toxicology have been performed. More than 16 alkaloids and two lactones have been isolated from it and characterized by the infrared spectrometry, mass spectrometry, nuclear magnetic resonance spectrometry and X-ray diffraction analysis. All these compounds have been screened on rodent tumor systems. It was found that the major component of these alkaloids called cephalotaxine is inactive, but the esters of it including homoharringtonine, harringtonine, isoharringtonine and deoxyharringtonine are acive against rodent leukemia. To explore the mechanism of action of harringtonine, its effect on the incorporation of radioactive precursors of DNA, RNA and protein were studied. Results showed that the incorporation of ^{3}H-leucine into protein of L-1210 cells was inhibited very quickly and remarkably by harringtonine followed by the inhibition of ^{3}H-thymidine incorporation into DNA. It has no effect on the RNA synthesis. On the basis of preclinical toxicological studies, harringtonine and homoharringtonine were recommended for clinical trial, results indicated that harringtonine and homoharringtonine are active against acute myelocytic leukemia. Through a multiple center studies these drugs are widely used in China for treatment of acute myelocytic leukemia.

In a HOAP Protocol (H: homoharringtonine, O: oncovine, A: cytarbin or AraC, P: prednisone) of combination of chemotherapy developed in China for the acute myelocytic leukemia the complete remission rate is as high as 50%. It is interesting to find that harringtonine and homoharringtonine are good inducers for the programmed death of human acute promyelocytic leukemia cells in vitro at a concentration of $1 \sim 2 \times 10^{-6}$ mol/L in terms of typical apoptosis and DNA "ladder".

Taxus Chinensis and Taxol[6,7]

Originally taxol was isolated from the stem bark of Taxus brevifolia by Wani et al. Horowitz et al. reported that it has a unique mechanism of action in terms of the promotion of tubulin polymerization. On the basis of field survey on the resources of Taxus species in mainland China. Taxus chinensis was found to be the most interesting species. More than 40 chemical compounds including cephalommanine, taxol and baccatin Ⅲ have been isolated and characterized by the Institute of Materia Medica, Chinese Academy of Medical Sciences. Pharmacological studies demonstrated that taxol and cephalommanine are the two major active principles for the inhibition of cancer. Therapeutic experiments indicated that taxol exhibited remarkable inhibition on a variety of human cancer cell lines. Studies in vivo showd that taxol is effective against rodent tumors. Biochemical pharmacological studies demonstrated that taxol could promot microtubule assembly and reduce the lag time for the assembly in vitro. Flow cytometry showed that taxol decreased the cell population in G1 phase at a concentration of 25 nmol/L, while the cell population in G2 + M phases was increased significantly. In addition, a polyploidy cell population with 8C DNA content appeared on the DNA histogram. The action of taxol is time dependent as well as concentration dependent.

Camptotheca Accuminata and Camptothecin[8,9]

Camptotheca accuminata is indigenous to mainland China and has been used in folk medicine in Zhejiang Province for the topical treatment of psoriasis. Wall et al. reported that the active principle of the stem bark from Camptotheca accuminata is comptothecin which is active in inhibition of tumor growth in animals. Chemical and pharmacological studies on it have been performed and reported that camptothecin and 10-hydroxy camptothecin exhibited a wide antitumor spectrum in rodent tumors and the latter is more active and less toxic than the former. Recently a new comptothecin derivatives calles CPT-11 which

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is very prospective against lung cancer was developed in Japan.

INDUCERS OF CELL DIFFERENTIATION OF CANCER

Cancer can be considered as a disorder of cell differentiation which is readily illustrated by the hematologic neoplasms. Oncogenic conversion, defined as the arrest of differentiation without loss of proliferation capacity, can occur at any of the intermediate maturation steps. Consequently, the maturation-arrested cells continue to proliferate and a population of immature "cancer" cells emerges and gives rise to adverse clinical manifestations. It is well known that the traditional target of cancer chemotherapy is to kill the cells of cancer. The historic achievements of cancer chemotherapy is fruitful, but its drawback is the poor selectivity and severe side effects which limited their further development. In addition, the bad news is that the cytotoxic anticancer drugs do very little to the bulk of cancers, such as large bowel cancer, pancreatic cancer, liver cancer, soft tissue sarcomas, stomach cancer, non-small cell lung cancer, head and neck cancer, melanoma and cervix cancer. Therefore it is the general view that further major therapeutic advance is unlikely to come from empirical studies of new candidate agents developed by the traditional cytotoxic approach.

Recently, it was found that some neoplastic cells can be induced to differentiate to normal or normal-like cells in vitro, for example, teratoma, neuroblastoma, leukemia and melanoma. Particularly the finding that human acute promyelocytic leukemia could be induced to a complete remission by all-trans-retinoic acid opened a new frontier —— the so called differentiation therapy of cancer, this approach was regarded as a new modality of cancer treatment.

In order to search for new inducers of cell differentiation of cancer from natural products, researches have been done for more than ten years, so far, more than 1000 samples have been screened by the human acute promyelocytic leukemic cell line (HL-60) and several interesting inducers from TCM have been found.

Pueraria Lobata and Daidzein[10,11]

Pueraria lobata has been used for the treatment of hypertension and angina pectoris for many years in TCM. It was reported that the two major active principles are daidzein and puerarin. There was no information on the activity of these compounds on cell differentiation of cancer before. Our experiments demonstrated daidzein is active for the induction of cell differentiation of HL-60 cells in morphological change, NBT reduction activity and flow cytometry. It was shown that at a concentration of 18 and 22 μg/ml daidzein could induce the cells to differentiate along the myelocyte lineage. Flow cytometry demonstrated that daidzein exhibited a G1 arrest in cytokinetics of HL-60 cells. At a concentration of 14μg/ml of daidzein, 63.6% of the cells were arrested in G1 phase. It is interesting to note that the expression of oncogene c-myc and c-myb was inhibited by daidzein but the expression of oncogene c-fos was enhanced by it, which coincide with the differentiation of HL-60 cells. Interestingly, there is a report that the majority of flavones extracted from Aurantii nogilis Pericarpium and fruit peel of Citrus reticulata exhibited differentiation activity in HL-60 cells.

Boswellia Carterii and Boswellic Acid

Boswellia carterii has been used for many years in TCM for activating blood circulation and relieving pain. Liu et al. reported that one of the active principle of Boswellia carterii (alphaboswellic acid acetate, BC_4) is a potent inhibitor of DNA topoisomerase Ⅰ and Ⅱ. In the searching for new inducers of cell differentiation of HL-60 cells it was found that the BC_4 can induce the cell differentiation of HL-60 cells in NBT reduction, phagocytosis and morphological change. At a concentration of 10 μg/ml of BC_4 80% of the cells become NBT positive and 90% of them showed phagocytosis of polysterene latex pellets.

Panax Ginseng and Ginsenoside Rh_2[12,13].

Panax ginseng is one of the most famous drug used in ancient time. Pharmacologic studies have demonstrated that Panax ginseng is able to strengthen the resistance to harmful stress of the host and stimulate phagocytosis in normal and tumor-bearing mice. Odashima et al. have reported that ginsenoside Rh_2, one of the characteristic components of red ginseng exhibited a significant induction of cell differentiation of melanoma B-16 cells in vitro. It was found that at a concentration of 8μg/ml it inhibited the cell growth of B-16 melanoma completely and the morphology of these cells turned to be differentiated. Flow cytometry showed that most of the melanoma cells were arrested in G_1 phase.

CHEMOPREVENTIVE AGENTS OF CANCER[14~19]

It is generally agreed that the ultimate purpose of cancer research is to lower the mortality and incidence of cancer. In the earliest ancient classic book of traditional Chinese medicine called Huang Di Nei Jing, a famous medical principle is that the role of a good doctor is to treat before sickness. In spite of the great success of early diagnosis and treatment of cencer, the total incidence of cancer is going up. Therefore how to reduce the cancer incidence is a big challenge for the medical community and pharmaceutical community as well. The aim of cancer chemoprevention is to use natural products or chemical compounds to reverse or inhibit the malignant transformation of cells and prevent the metastasis of cancer. In the ideal cases chemoprevention would be the less painful, more economic and more reasonable approach for the control of cancer. Many edible plants and medicinal plants have been described in the classics of Chinese materia medica, they provided a potential resource for the research and development of chemopreventive agents of cancer. In recent years the antimutagenic activity of plants was of considerable interest. It displayed that higher plants contain a variety of preformed secondary metabolites that represents a structurally diverse array of antimutagenic compounds. Japanese scientists have reported that the extract of green tea decreases the mutagenic activity of carcinogen MNNG and tumor promotion induced by TPA. Several groups of Chinese scientists have verified these results.

Since the late nineteen seventies, on the basis of established methodologies of experimental chemoprevention in vitro and in vivo, we have engaged in the screening of chemopreventive agents for cancer in terms of antimutagenesis, antipromotion and anticarcinogenesis. Some herbal medicines of TCM showed very promising results.

Red ginseng[20~22]

A resource of nourishing tonics which was also called Fuzheng drugs in TCM, i.e., the drugs for reinforcing body resistance to pathogens or internal pathological factors. Red ginseng is a processed Panax ginseng which was regarded as typical tonic. Our studies demonstrated that the extract of red ginseng could inhibit the skin papillomas induced by carcinogen DMBA at dosages of 100, 200 and 400 mg/kg orally. Under the action of red ginseng extract, the incidence of papilloma was decreased significantly and the number of papilloma per mouse was also decreased dramatically. These results were coincident with that of the epidemiological observation of Korean scientists in terms of chemopreventive activity of cancer in human.

Curcuma Longa and Curcumin

Curcuma longa is a drug of choice for "eliminating the stagnation and activating the blood circulation" in TCM. It is used to relief abdominal pain and also used as a food additive. It has antibacterial activity in vitro and the major active principle of this plant is curcumin. Experiments have been done to explore the chemopreventive potential of curcumin for cancer, results indicated that curcumin could inhibit the His-revertants of Salmonnela typhimurium induced by methyl methane sulfonate (MMS) in Salmonella/ microsome plate incorporation assay. It also decreased the micronucleus formation induced by Endoxan. As

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an antipromotor of cancer it decreased the ear edema caused by croton oil in mice.

Glycyrrhiza Uralensis and Glycyrrhetinic Acid[23]

Glycyrrhiza uralensis is widely used as an antidote in TCM. It is also used as a food additive. Nishino et al. have reported that one of the predominant components —— glycyrrhetinic acid exhibited antipromotion activity in a two-stage model of chemical carcinogenesis and antimutagenic activity in Salmonella typhimurium TA98 and TA100 system. Our experiments demonstrated that glycyrrhetinic acid inhibited the ear edema induced by croton oil in mice. It also inhibited the activity of epidermal ornithine decarboxylase at a dosage of 50～200 mg/kg for three days. It is interesting that the rapid DNA damage induced by carcinogen benzepyrene was significantly protected by this compound.

Rhus Chinensis and Chinese Gallotannin[24]

Rhus chinensis and its major component, Chinese gallotannin (CG) is widely used as an astringent in TCM as well as in Western medicine. Recently it was found that CG has biological antitumor, antiviral and antifungal activities. Pharmacological studies demonstrated that CG inhibited the malignant transfromation of V_{79} cells induced by benzopyrene in vitro at a concentration of 5～20 μg/ml. It is interesting to note that under the action of CG the benzopyrene induced pulmonary adenoma in A/J mice was inhibited profoundly, the incidence of tumor and the number of tumor per mouse in treated group were significantly lowered. Other experiment indicated that CG also inhibited the DMBA/croton oil induced skin papilloma in mice, the latent period of the tumor was prolonged and the incidence of the tumor was also decreased in a dose dependent manner. In the transplantable tumor system CG exhibited significant antitumor activity in sarcoma 180, Lewis lung carcinoma and colon carcinoma 26 bearing mice. Further studies showed that Chinese gallotannin is a strong free radical scavenger in terms of O_2^- and DPPH. Interestingly, it also inhibited the metabolic activation of benzopyrene by inhibiting the P-450A1 and AHH enzyme activities. CG showed a strong antimutagenic activity in vitro and protected the chromosome damage induced by cyclophosphamide.

Recipe "Antitumor B"[25-28]

Recipe antitumor B (抗癌乙片, RAB) is a complex recipe of TCM consisting of six medicinal herbs, namely, Sophora subprostrata, Patrinia cillosa, Dictamnus dasycarpus, Discorea bulbifera, Prumilla vulgalis and Polygonum historia. It was reported that the RAB exhibited a dramatic inhibition on the chemical carcinogenesis of esophagus induced by a carcinogen nitrosamin in rats. Wei et al. followed Lin et al. reported that RAB had a significant inhibitory effect on DMBA induced buccal carcinoma in hamsters. Fan et al. published that the RAB also inhibited the incidence of bladder cancer induced by N-butyl-(4-hydoxy butyl) nitrosamide (BBN) in rats. On the basis of toxicological studies RAB was recommended for the clinical trial in a high risk area of esophagus cancer in Linxian county, Henan Province. It was reported that under the long term administration of RAB to a high risk population of esophagus cancer for five years, the incidence of esophagus cancer was reduced significantly, and no severe side effect was found. Although it is a preliminary field study which needs further confirmatory study, it is a good start to use TCM for the prevention of cancer.

ANTIMETASTATIC AGENTS OF TUMOR[29]

Tumor metastasis is the major cause of treatment failure for cancer patients. About 30% of patients with newly diagnosed solid tumors (excluding skin cancers other than melanoma) already have clinically detectable metastasis, therefore, the searching for potential inhibitor of cancer metastasis is of critical importance for cancer treatment. In view of this, the invasion of cancer cells plays an important role in cancer metastasis and effort has been made in order to search for potential inhibitors of cancer invasion from medicinal plants. In addition, arresting or retarding

molecular events involved in tumor invasion and angiogenesis have defined a new category of cancer chemoprevention. For patients with newly diagnosed solid tumors, long-term cytostatic therapy could potentially create a state of metastasis dormancy of delay the time to overt relapse following cytotoxic agent-induced remission.

Fagopyrum cymosum (FC) is an herbal medicine used in TCM for the treatment of lung abscess and nasopharyngeal cancer. The extract of FC-92B exhibited significant antimutagenic activities in Ames assay and antipromotion activity in mouse ear edema assay, it is also effective for the inhibiton of chemical carcinogenesis in mouse skin induced by DMBA and croton oil in terms of papilloma formation. In view of the low toxicity and chemopreventive activity for cancer, it is worthwhile to study the effect of FC on cancer invasion and metastasis.

By using the Transwell cell culture chamber assay for invasion of cancer, which was developed by Albini, et al, the effect of FC on the invasion of highly metastatic melanoma B16-BL6 cells in vitro was studied. It was found that under the action of FC, the invasion of tumor cells into reconstituted basement membrane (Matrigel/fibronectin coated filter) was inhibited significantly and this inhibiton is concentration dependent. But FC has no significant effect on cell growth and migration in vitro.

For the study of mechanism of action of cell invasion, the effect of FC on the production and activity of type Ⅳ was investigated. By using gelatin zymograph described by Heussen and Dowdle, the serum-free conditioned media in a type Ⅳ collagen-embedded polyacrylamide gel was analysed by SDS-Page electrophoresis. It was found that FC exhibited a singificant inhibition on the enzyme production by human fibrosarcoma HT-1080 cells and its effect is concentration dependent.

In view of the dramatic inhibiting action of FC on tumor invasion and type Ⅳ collagenase production a spontaneous pulmonary metastasis assay of highly metastatic melanoma B16-BL6 cells in mice was performed. Thirty C57BL/6 mice (male) were used and randomly distributed into three groups. Melanoma B16-BL6 cells (5×10^5) were implanted subcutaneously into the right hind footpad and the footpad bearing tumor was amputated on Day 26, FC was administered orally once a day through Day 39 at a dosage of 200 mg/kg. Two weeks after the amputation, the mice were sacrificed and the metastasized colonies on the surface of the lung were counted after they had been fixed in Bouin's solution. Experiment demonstrated that under the action of FC, the lung metastasis of melanoma B16-BL6 is significantly inhibited in terms of nodule number per mouse.

CONCLUSION

TCM represents the enormous accumulation of daily experiences in medical practice by the Chinese people from ancient time. The description of the therapeutic usage in classic books of Chinese materia medica are informative even today, for example, Angelica sinensis ameliorates menstruation disorder and Artemisia annua is effective against malaria. All these therapeutic effects have been confirmed by contemporary scientific research. Chemical, pharmacological and clinical studies published in recent years have demonstrated that Chinese materia medica and TCM afford a valuable approach for the research and development of new antineoplastic drugs as illustrated by the example of indirubin from Danggui Longhui Wan and irisquinone from Iris latea. It seems that there is a good opportunity to explore chemopreventive agents for cancer as illustrated by red ginseng, Curcuma longa and Recipe "Antitumor B". The research and development of anti-invasion and anti-metastasis agents for cancer is of critical importance and urgent needs. In addition to Fagopyrum cymosum the continuous search for drugs for the prevention and treatment of cancer metastasis will be the important frontier of cancer research. The application of Chemotaxonomic

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principle of related species of plants in reference of the experiences of TCM and folk medicine has furnished an alternative approach for the development of new anticancer drugs. The studies of Cephalotaxus hainanensis and Taxus chinensis provide two typical examples in this field.

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