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Review Article

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NATURAL FLAVONOIDS AND LUNG CANCER

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ABSTRACT

Medicinal plants play an important role in the management of cancer especially in developing countries where resources are meager. This review presents the profiles of flavonoid containing plants with anti lung cancer properties. The profiles presented include information about the scientific name, family, methodology used, the degree of anti cancer activity and the active agents. The article has given the information about some of Indian medicinal plants like Ashwagandha, curcumin, *lithospermum radix* and green tea. The review also includes the scientific research information about the Chinese herb astragalus and Japanese herb Juzen-Taiho-To. These herbs are mainly studied against lung cancer by using various cell lines in recent literatures**.** The natural drugs are showing better results aginst lung cancer in the literature reviews.

Keywords: Lung cancer, flavonoid, cell lines, curcumin, astragalus.

INTRODUCTION

Flavonoids are compounds which are an integral part of the human diet, with an estimated daily intake of 0.023–1 g/day. They exhibit a wide range of biological activities, of which anti oxidation is the most thoroughly explored. The aim of this review is to collate all available data on plants with anti cancer effects reported specially in lung cancer. Many ethnobotanical surveys on medicinal plants used by the local population have been performed in different parts of the world including India, China, Japan, Saudi Arabia, Taiwan, Trinidad and Tobogo.[1] Several plant species have been described as anti cancer agents. These include ashwaganda, pomegranate, curcumin, lithospermum, astragalus etc.

Lung Cancer

Lung cancer is the most commonly diagnosed and leading cause of death by cancer in men in the United States according for 27% and 31% of all cancer death in women and men, respectively. The median age of diagnosis is 70 years. Although many patients achieve disease-free survival, some experience a long-term impairment of their quality of life, and disease recurrence is common. Numerous chemotherapeutic combination regimens are continuously being introduced for the treatment of advanced lung cancer to improve patient outcomes. Although lung cancer death in man have declined substantially [from 1995 to 84 in 100,000 in 2001],death rate in women only recently began to stabilize in 1995 [at approximately 42 in 100.000 between 1995 and 2001] after increasing for two decades between 4% and 6% per year . Lung cancer is a disease of uncontrolled cell growth in tissues of the lung. This growth may lead to metastasis, which is the invasion of adjacent tissue and infiltration beyond the lungs.

The vast majority of primary lung cancers are carcinomas of the lung, derived from epithelial cells. Lung cancer, the most common cause of cancer-related death in men and women, is responsible for 1.3 million deaths worldwide annually, as of 2004. The most common symptoms are shortness of breath, coughing and weight loss.

Types of Lung Cancer

The main types of lung cancer are *small cell lung carcinoma* and *non-small cell lung carcinoma*. This distinction is important, because the treatment varies; non-small cell lung carcinoma (NSCLC) is sometimes treated with surgery, while small cell lung carcinoma (SCLC) usually responds better to chemotherapy and radiation. The most common cause of lung cancer is long-term exposure to tobacco smoke The occurrence of lung cancer in nonsmokers, who account for as many as 15% of cases, is often attributed to a combination of genetic factors radon gas, asbestos and air pollution including secondhand smoke.

Signs and symptoms

- dyspnea (shortness of breath)
- hemoptysis (coughing up blood)
- chronic coughing or change in regular coughing pattern
- wheezing
- chest pain or pain in the abdomen
- cachexia (weight loss), fatigue, and loss of appetite
- dysphonia (hoarse voice)
- clubbing of the fingernails (uncommon)
- dysphagia (difficulty swallowing).

If the cancer grows in the airway, it may obstruct airflow, causing breathing difficulties. This can lead to accumulation of secretions behind the blockage, predisposing the patient to pneumonia. Many lung cancers have a rich blood supply. The surface of the cancer may be

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fragile, leading to bleeding from the cancer into the airway. This blood may subsequently be coughed up.

Causes

The main causes of any cancer include carcinogens (such as those in tobacco smoke), ionizing radiation, and viral infection. This exposure causes cumulative changes to the DNA in the tissue lining the bronchi of the lungs (the bronchial epithelium). As more tissue becomes damaged, eventually a cancer develops.

Smoking: Smoking, particularly of cigarettes, is by far the main contributor to lung cancer. Cigarette smoke contains over 60 known carcinogens, including radioisotopes from the radon decay sequence, nitrosamine, and benzopyrene. Additionally, nicotine appears to depress the immune response to malignant growths in exposed tissue. Across the developed world, almost 90% of lung cancer deaths are caused by smoking. In the United States, smoking is estimated to account for 87% of lung cancer cases (90% in men and 85% in women. Among male smokers, the lifetime risk of developing lung cancer is 17.2%; among female smokers, the risk is 11.6%. This risk is significantly lower in nonsmokers: 1.3% in men and 1.4% in women.

Radon gas: Radon is a colorless and odorless gas generated by the breakdown of radioactive radium which in turn is the decay product of uranium, found in the Earth's crust. The radiation decay products ionize genetic material, causing mutations that sometimes turn cancerous. Radon exposure is the second major cause of lung cancer, after smoking. Radon gas levels vary by locality and the composition of the underlying soil and rocks. For example, in areas such as Cornwall in the UK (which has granite as substrata), radon gas is a major problem, and buildings have to be force-ventilated with fans to lower radon gas concentrations. The United States Environmental Protection Agency (EPA) estimates that one in 15 homes in the U.S. has radon levels above the recommended guideline of 4 picocuries per liter (pCi/L) (148 Bq/m^3).Iowa has the highest average radon concentration in the United States; studies performed there have demonstrated a 50% increased lung cancer risk, with prolonged radon exposure above the EPA's action level of 4 pCi/L.

Asbestos: Asbestos can cause a variety of lung diseases, including lung cancer. There is a synergistic effect between tobacco smoking and asbestos in the formation of lung cancer. In the UK, asbestos accounts for 2–3% of male lung cancer deaths. Asbestos can also cause cancer of the pleura called mesothelioma (which is different from lung cancer).

Viruses: Viruses are known to cause lung cancer in animals, and recent evidence suggests similar potential in humans. Implicated viruses include human papillomavirus, JC virus, simian virus 40 (SV40), BK virus, and cytomegalovirus. These viruses may affect the cell cycle and inhibit apoptosis, allowing uncontrolled cell division.

PATHOGENESIS

Lung cancer is initiated by activation of oncogenes or inactivation of tumor suppressor genes. Oncogenes are genes that are believed to make people more susceptible to cancer. Proto-oncogenes are believed to turn into oncogenes when exposed to particular carcinogens. Mutations in the *K-ras* proto-oncogene are responsible for 10–30% of lung adenocarcinomas. The epidermal growth factor receptor (EGFR) regulates cell proliferation, apoptosis, angiogenesis, and tumor invasion. Mutations and amplification of EGFR are common in non-small cell lung cancer and provide the basis for treatment with EGFR-inhibitors. Her2/neu is affected less frequently. Chromosomal damage can lead to loss of heterozygosity. This can cause inactivation of tumor suppressor genes. Damage to chromosomes 3p, 5q, 13q, and 17p are particularly common in small cell lung carcinoma. The *p53* tumor suppressor gene, located on chromosome 17p, is affected in 60-75% of cases. Other genes that are often mutated or amplified are *c-MET*, *NKX2-1*, *LKB1*, *PIK3CA*, and *BRAF*.

HERBS AS ANTICANCER REMEDY

Withania somnifera

Withania somnifera (Solanaceae) is one of the most highly regarded herbs in Ayurvedic medicine .In Indian system of medicine, large numbers of drugs of either herbal or mineral origin have been advocated for various types of diseases. *Withania somnifera* (L) Dunal is an ayurvedic medicinal plant coming under the family of Solanaceae, which is popular as a home remedy for several diseases. It is mentioned in Vedas as herbal tonic and health food.[2] It has been used for a very long time for all age groups and both sexes[3] and even during pregnancy, without any side effect.[4] The chemical composition and pharmacological and therapeutic efficacy of *W.somnifera* have been established.[5]

Here, an attempt has been made to counteract the toxic side effect as well as to improve the therapeutic efficacy of paclitaxel by combining with the immunomodulatory *W somnifera*. Benzo(a)pyrene activates oxidative stress-induced cell proliferation and carcinogenesis by transcriptional elevation of several genes including c-jun, c-fos, c-myc and inducible nitric oxide synthase(iNOS).[1] These genes are found to be activated by stress signals through the stimulation of tyrosine kinase, which in turn modulates downstream events including the expression of nuclear proto oncogenes.[6] The cellular target for paclitaxel has been identified as the microtubule system that plays a significant role in mitosis, intracellular transport, cell motility and maintenance of cell shape. It promotes the assembly of stable microtubule from α- and ß-tubulin.[7]

The experimental animals were divided into five groups of six animals each. Control animals treated with corn oil (vehicle) orally, cancer induced animals B(a)p treated (50mg/kg body weight dissolved in corn oil, orally) twice weekly for 4 successive weeks, cancer bearing animals treated with paclitaxel (33mg/kg ip) weekly once for 4weeks, cancer bearing animals treated with paclitaxeland *W somnifera* (400mg/kg po) for 4 weeks and the control animals treated with paclitaxel along with *W somnifera*. At the end of the experimental period, the animals were fasted overnight and scarified by cervical decapitation. The blood was collected in two tubes with an anticoagulant. Anticoagulant containing blood was used for counting of the immunocompetent cell[8] and for estimation of immune function test using *Candida albicans*[9] and by the nitrobluete tetrazolium (NBT) reduction test[10] Coagulant blood was used for the determination of IgA and IgM[11,12] and soluble immune complex level.

In this anticancer study, a decrease in cell counts, total leukocyte counts as well as absolute neutrophil and lymphocyte counts were observed in cancer bearing

animals as well as in paclitaxel treated animals. Earlier studies report that the cell counts decrease in cancer bearing animals as well as paclitaxel treated animals.[13] The presently observed decrease may be due to a decline in ATP content in cancer animals, as most of the activities of the immune cells depend on cellular energy supply and also due to poor glycemic condition. *W somnifera* treatment was found to increase the number of total white blood cells, red blood cells and heamoglobin level.[14] The extract of *W somnifera* was found to enhance the total white blood cell count and bone marrow cells in animals treated with non-lethal dose of radiation.[15] In this study, the phagocytic and avidity indices and NBT reduction test were found to be significantly decreased in cancer bearing animals when compared with control animals. The neutrophil function test indicates the bactericidal potency of neutrophils.[16] A significant alteration in the neutrophil functions has been observed in all our study. The killing ability of the neutrophils, as indicated by the NBT reduction and phagocytic ability of the neutrophils, as indicated by the phagocytic index and the avidity index has been significantly decreased in cancer bearing animals which was further decreased upon treatment with paclitaxel.

Serum immune complexes (SIC) serve as an indicator of immune responses either due to presence of excess antigens or antibodies. In our present study, the poly ethylene glycol (PEG) indices were markedly reduced in cancer bearing animals when compared with control animals. This may be due to the decrease in antibody production in cancer. Serum soluble immune complexes serve as an indicator of immune responses either due to the presence of excess antigens or antibodies. This may be due to decreased antibody production during cancer. Immunomodulation through natural or synthetic substances may be considered as an alternative for the prevention and cure of neoplastic diseases.[17]

The rate of immunoglobulin synthesis has been condensed in patients with certain neoplastic conditions with IgG, and IgM levels indicate diminished humoral immunity and reduction in immune system response due to an increased non-enzymatic glycosylation of IgG. The abnormal features of immunoglobin in serum of patients with malignant diseases are well documented.[18,19] IgA content alone was found to be increased in the cancer bearing animals[20] have reported that the elevated serum IgA levels may be due to the failure of clearance mechanism by the damaged liver.

Pomegranate

Pomegranate (*Punica granatum*, Punicaceae), native to semitropical Asia and naturalized in the Mediterranean region, is now cultivated in Afghanistan, India, China, Japan, Russia and in the United States, particularly in Arizona and California. The fruit can be divided into three parts: the seeds (3% of fruit weight), the juice (30% of fruit weight) and the peels that also include the interior network of membranes.[21] Pomegranate is a rich source of many phenolic compounds including flavanoids (anthocyanins, catechins and other complex flavanoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallagic and ellagic acid esters of glucose), which account for 92% of its antioxidant activity.[22] Several studies were conducted in *in-vitro* and *in vivo* models to explore the chemo preventive/therapeutic potential of pomegranate against lung cancer.[23,24]

A relative assessment of the antioxidant capacity of the pomegranate extract in human lung carcinoma A549 cells showed that PFE possessed higher activity than (-)-epigallocatechin-3-gallate, the major polyphenol present in green tea.[23] Interestingly, PFE treatment was found to result in a dose-dependent decrease in the viability of A549 cancer cells with only minimal effects on normal human bronchial cells. Treatment with PFE resulted in marketed induction of WAF1/p21 and KIP/p27 and a concomitant inhibition of cyclins D1, D2, and E and CDKs 2, 4 and 6 protein expressions in A549 cells. In addition, inhibition of MAPK, PI3K/AKT and NF-kB/p65 signaling as well as down-modulation of Ki-67 and PCNA protein levels by the extract suggests that the decrease in growth and viability of lung cancer cells is a result of decreased cellular proliferation. Based on the results of this *in-vitro* data, a study carried out in the mouse model showed that oral administration of a human acceptable dose of PFE to arthymic nude mice implanted with A549 cells resulted in significant inhibition in the progression of tumor growth.[24]

The effect of oral consumption of the extract on tumor growth, progression and signaling pathways involved, was studied further in two other mouse lung tumor protocols.[24] Mice given PFE in drinking water and exposed to benzo (a) pyrene (B(a)P) and N-nitroso-tris-chloroethylurea (NTCU) had statistically significant lower lung tumor multiplicities than mice treated with carcinogens only. Tissue studies showed that PFE treatment caused inhibition of MAPK, PI3K/AKT and NF-kB /p65 signaling pathways, dysregulated in a variety of cancers. Decreased phosphorylation of mTOR protein and downstream targets such as p70S6kinase and 4E-BP1 suggested a suppressive effect of PFE on mTOR signaling, involved in protein translation and cell cycle progression.

In addition, significant inhibitory effect of PFE was observed on B(a)P and NTCU-mediated cell proliferation with an apparent decrease in PCNA and Ki-67 immunostaining in the lung tissues of PFE treated mice. PFE treatment resulted in reduced in related expression of markers of tumor-associated angiogenesis such as inducible nitric oxide syntheses (iNOS), platelet-derived endothelial cell adhesion molecule (CD31) and vascular endothelial growth factor (VEGF) in the murine lungs. c-Met, frequently over expressed, activated and sometimes mutated in cancer cell lines and tumor tissues, was significantly down regulated in mice that received PFE.

This study overall demonstrated that pomegranate inhibited lung tumor genesis by targeting multiple signaling pathways in the mouse model and merits consideration for development as a potential chemo preventive agent against human lung cancer.[23]

Curcuma longa

Curcuma longa (Zingiberaceae) has also been used for culinary purposes. Turmeric has several components with immunomodulatory and antioxidant properties.[24] Curcumin is a hydrophobic polyphenol derived from turmeric: the rhizome of the herb *Curcuma longa*. Curcumin exhibits anticancer effects in various lung cancer cells through a variety of molecular targets. At the cellular level, curcumin derivatives inhibit FPTase in A549 cells.[25] Curcumin inhibits AP-1 transcription and mediastinal lymph node metastasis in Lewis lung carcinoma cells and ornithine decarboxylase activity in rat tracheal epithelial cells.[26,27] Curcumin eradicated the DNA-binding of NF-jB, IjBa kinase activation, IjBa deterioration and phosphorylation, and p65 nuclear translocation and it

down-regulated COX-2.[28-30] Likewise, treatment with curcumin induces apoptosis and inhibits growth in A549 and H1299 cells.[31] In A549 cells, curcumin interferes with cell growth and down regulates NAT activity and STAT1 activation.[32-34] Curcumin regulates the invasive activity of CL1-5 cells and demonstrates antiproliferative properties in NCI-H460 and -H520 cells, suggesting its suitability as an adjunct chemotherapeutic agent.[30,35,36] Orthotopic implantation of a metastatic cell line of Lewis lung carcinoma (LLC-MLN), which was isolated by an in vivo selection method, resulted in greater metastatic growth in mediastinal lymphnodes as compared with that of the original LLCcells. Oral administration of curcumin significantly inhibited the mediastinal lymph node metastasis of orthotopically implanted LLC cells in a dose-dependent manner, but did not affect the tumor growth at the implantation site. Combined treatment with curcumin and cis diamine-dichloroplatinum (CDDP), resulted in a marked inhibition of tumor growth at the implanted site and of lymphatic metastasis, and a significant prolongation of the survival time.[27] Deshpande and Maru[37] showed that curcumin can inhibit BP-derived DNA adducts by interfering with the metabolic enzymes and its physical presence is essential for this effect. In the year 1999 one group studied the activity of curcumin as chemopreventive agent against lung tumor induction in A/J mice by the tobacco smoke carcinogens benzopyrene (BaP) and 4 (methyl-nitrosamino)-1- (3-pyridyl)-1-butanone (NNK). The treatment of curcumin (2000 ppm) 1 week after carcinogen treatment until termination had no effect on lung tumor multiplicity. In another study, oral administration of curcumin (200 nmol/kg body weight) was, however, found to inhibit the lung metastasis of melanoma maximally as seen by the reduction in the number of lung tumor nodules (80%). Consequent to the inhibition of the lung tumor nodules, the life span of animals treated with curcumin was also found to be increased (143.85%).

The results indicate a possible use of these compounds in arresting the metastatic growth of tumor cells. In Wistar rats, however, marker enzymes and plasma lipid levels decreased after treatment with 80 mg/kg of curcumin or a curcumin analog.[30,38]

Green tea

Green tea is a heterogeneous product that contains several antioxidant compounds, known as polyphenols. (-)-Epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallate catechin (EGC), and (-)-epicatechin (EC) are some of the polyphenol compounds found in green tea. EGCG, the most bioactive green tea polyphenol, was demonstrated to be a multipotent chemo preventive and anticancer agent in several animal models, including leukemia, lung, prostate, colon, and breast cancer.[39-44.] It was shown to interact with numerous protein targets and to disrupt biologic/biochemical reactions involved in cancer progression. The EGCG compound can vary its chemical conformation, thus allowing it to behave as a chemical chaperone that is able to interact with an assortment of biologic molecules including DNA, RNA, lipids,and proteins[45] However, it is not entirely clear which of the many functions of EGCG are the most critical with regards to this compound's anticancer activity. Green tea, concentrated GTE, and certain individual components of green tea, such as EGCG, have widely recognized health benefits, which include purported anticarcinogenic and antitumor effects. Besides being used by healthy people as a means to support continued health, this "miracle herb" extract is also consumed by many cancer patients who follow popular trends and self-medicate with complementary and alternative medicine (CAM) in hopes to support their conventional therapy or to lessen the burden of side effects sometimes without the knowledge of their health care provider. In an effort to harness the therapeutic potential of green tea in a more systematic fashion, numerous clinical trials are ongoing to establish the framework for its future therapeutic use. GTE or EGCG may also hold cancer therapeutic potential when combined with established cancer treatments.[41] In our current study presented here, we investigated whether GTE or EGCG would be able to boost the anticancer efficacy of BZM, a proteasome inhibitor that is in clinical use for the treatment of multiple myeloma and is under consideration for the treatment of other cancers as well. To our surprise, we discovered that GTE, as well as some of its individual components, effectively blocked the anticancer efficacy of BZM; that is, in the presence of polyphenolic green tea components, BZM was unable to exert proteasome inhibitory function and, as a consequence, there was no trigger for proapoptotic ER stress and subsequent cell death. The severe antagonistic effect of EGCG appeared to require the presence of the boronic acid moiety in BZM. Among the 6 proteasome inhibitors tested, the boronic acid–containing ones (BZM, MG-262, PS-IX) were similarly incapacitated by EGCG, whereas none of those without this functional group (NFV, MG-132, PS-I) were affected by EGCG. In this context, it is noteworthy that the chemical structures of MG-262 (effective inhibition by EGCG) and MG-132 (no inhibition by EGCG) differ only in the presence/ absence of the boronic acid moiety, indicating that the decisive mechanism of EGCG's antagonism resides with the chemical structure of the target molecule (ie, the boronic acid moiety), not its function (ie, proteasome inhibition). The ability of molecules with a 1,2-diol group to form covalent cyclic boronate moieties with boronic acid in a tight, but reversible manner, is a well-described and long-established chemical process, and is known to be one of the strongest single-pair reversible functional group interactions in an aqueous environment.[46-48] In retrospect, it was therefore not surprising that we were able to verify direct molecular interactions between EGCG and the bortezomib molecule, although similar behavior is expected for other polyphenols that harbor 1,2 benzenediol groups, such as EGC, ECG, and EC.

Lithosperum radix

Protein-tyrosine kinase (PTKs) play important roles in a variety of signal transaction pathways that are involved in cell growth differentiation cell death, and carcinogenesis.[49-51] In this anti cancer study, they started the analysis of genes involved in the β-HIVS-induced apoptosis using a DNA array and found that expression of that expression of a gene for tumor necrosis factor receptor associated protein1 (TRAP1) was significantly suppressed up on treatment of human leukemia HL60 cells with β-HIVS. TRAP1 was initially identified as the type 1 tumor necrosis factor receptor binding protein by yeast two hybrid screening, which is an efficient method for studying interactions among proteins.

An analysis of DNA sequence revealed that human TRAPI is identical to heat shock protein 75 (HSP75), which is a member the HSP family of molecular chaperones that interact with the retinoblastoma protein during mitosis

and after heat shock.[52] TRAP1 is substantially homologous to members of the 90-kDa family of heat-shock proteins (HSP90) and is expressed both is transformed cells and in a variety of normal tissues. Apoptosis can be induced by a variety of extra cellular stress and signals. HSP70 protects cells from a number of apoptotic stimuli, such a heat shock, radiation, and oxidative stress, withdrawal of growth factors, chemotherapeutic agents, ceramide, and tumor necrosis factor.

In this study, while attempting to identify genes that are involved in the mitochondrial function that are associated with β-HIVS-induced apoptosis, we found that β-HIVS suppressed that expression of the gene for TRAP1. The level of expression of TRAP1 fell after the treatment of HL60 and DMS114 cells with β-HIVS and the effects of β-HIVS were both dose and time dependent. VP16 also suppressed the expression of TRAP1 in DMS114 cells. The level of expression of TRAP1 was also higher in untreated DMs114 cells than that in untreated HL 60 cells.

These findings suggest the possibility that high –level expression of TRAP1 might be involved in apoptotic effects, as in the case of Bcl-2. This possibility is supported by suppression of the expression of TRAP1 by siRNA-sensitized DMS114 cells to β-HIVS –induced apoptosis. Results indicated that β-HIVS and VP16 suppress the expression of TRAP1 during apoptosis in human leukemia HL60 cells and human lung cancer DMS114 cells. The β-HIVS – induced suppression of the expression of trap is probably mediated by ROS rather than by inhibition of tyrosine kinase. Such suppression is responsible for the indication of the apoptosis since TRAP1 –specific siRNA increased the apoptosis inducing activity of β-HIVS. Reduction of the level of expression of TRAP1 by siRNA enhanced the release of cytochrome c from the mitochondria during the apoptotic process, suggesting that TRAP1 might involve in apoptosis via regulation of the release of cytochrome from mitochondria.[53,54]

Chinies herbs

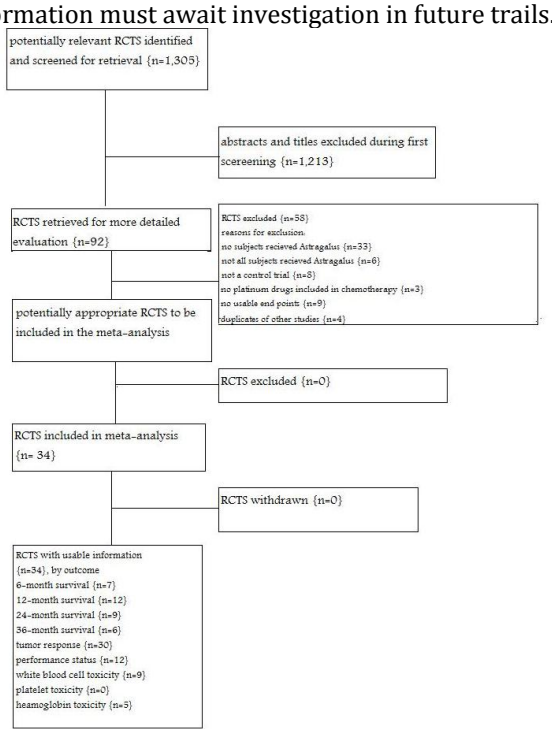
In china medicine frequently is combined with chemotherapy in treatment of lung cancer of particular interest is the herb astragalus membranaceus (fisch), which may potentiate host immune function by stimulating macrophage and natural killer cell activity, and enhance immune recognition of lung cancer cells by inhibiting production of T-helper cell type-2 cytokines (T-helper cell subsets implicated in the development of immunological tolerance to tumor progression).[55] In a recent clinical trial, single agent astragalus herbal treatment in combination with platinum-based chemotherapy, compared with platinum-based chemotherapy alone, has been shown to significantly reduce risk of death at 12 months {risk ratio(RR)=0.62; 95% CI, 0.43 to 0.89} and 24 months (RR=0.75;95%CI, 0.58 to 0.97) in clinical practice and most published trials, however, astragalus rarely is used as single -agent therapy; it usually combined with other herbal medicine.[56]

The meta-analysis was motivated by the large number of published trials of astragalus-based Chinese herbal medicines combined with platinum-based chemotherapy, and the continuing problems with low efficacy and high toxicity in standard chemotherapy treatment of advanced non-small-cell lung cancer. Our prior hypotheses were adding astragalus-based Chinese herbal medicine to platinum-based chemotherapy, compared with treatment with platinum-based chemotherapy alone, could prolong survival, increase tumor response, stabilize are improve performance status and reduce chemotherapy toxicity.

Screened titles and abstracts and retained those that were described as randomized. Recruited patients with advanced non-small-cell lung cancer. Provided the treatment group with Chinese herbal medicines containing the herb astragulus in combination with standard platinum-based chemotherapy provided the control group with platinum-based chemotherapy alone, and reported data on at least one of our outcomes of interest (survival, tumor response, performance status, or toxicity) with sufficient detail to permit calculation of the risk ratio of each out come and 95%CIs.

All inclusion and exclusion criteria and the categorization of outcomes for made before any meta-analysis of the data. Decision to group together for this meta-analysis those studies using platinum-base chemotherapy was based on the fact that this therapy is currently a standard treatment for advanced non-small-cell lung cancer. Following the example set by D'addarioetal and the Cochran Collaboration non-small-cell lung cancer collaborative group, platinum-based chemotherapy was grouped together as a therapeutic class when assessing efficacy of treatment for non-small-cell lung cancer. Each stage of the planning, design, analysis and reporting of this meta-analysis was conducted in accordance with the QUOROM statement guidelines.

These findings were subject to several limitations. These meta analysis results suggest that combining platinum-based chemotherapy with Chinese herbal medicine in treatment of non-small-cell lung cancer may increase survival, tumor response, and performance status, as well as reduce chemotherapy toxicity, when compared with treatment with platinum-based chemotherapy alone; however because the studies be found where of poor quality, this study unable to make firm conclusions and conformation must await investigation in future trails.[54]



Juzen-Taiho-To(Japanes medicine)

Juzen-Taiho-To (JTT) is well known to be one of Kampo (Japanese herbal) medicine consisted of 10 component herbs and used for the supplemental therapy of cancer patients with remarkably success. Digestive cancer patients generally undergo surgical therapy,

chemotherapy, radiotherapy or a combination of these treatments. While the effects of these treatments are significant, it is a fact that most patients suffer from side-effects, such as high fever, general fatigue, loss of appetite, pancytopenia and many kinds of infections. Japan and China, herbal medicine is used as a supplemental therapy for many kinds of chronic diseases such as loss of appetite, anemia and chilliness of the arms and legs with remarkable succes.[57,58] Recent reports clearly showed when herbal medicines are used for cancer treatment, many patients experience fewer or diminished side-effects induced by western medicine, such as chemotherapy and radiotherapy, and the survival period is longer . It is also reported that herbal medicine can prevent the progression of colon carcinoma, gastric and breast cancer as well as the prevention of these cancer metastasis in liver, lung or bone.[59,60]

Moreover, hepatocellular carcinoma has been shown to become smaller without severe side-effects.[61,62] Although these reports strongly suggestthat herbal medicine will be a good alternative for the treatment of several types of cancer, the mechanisms by which herbal medicine could improve clinical status, including cancer metastasis. Preparation of Diet Containing JTT Pure powder of JTT was well mixed with normal powder diet (MF) for maintaining rats and mice (Oriental Kobo Kogyou Co. Ltd., Tokyo, Japan) at concentrations of eithe0.2 or 1.0%. The concentration of JTT in the diet (0.2%) is equivalent to the clinical dose (7.5 g/day/50 kg) (10). Tumor Cells B16 melanoma cells were purchased from Dai-Nippon Pharmaceutical Co. Ltd. (Osaka, Japan) and maintained with RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum (Flow Laboratories, North Ride, Australia).

In the first experiments, B16 cells (2 x 10^5) were injected subcutaneously into the right hind sole in a volume of 0.l ml. After tumor growth reached 1 cm (14 days after cell injection), the tumor was removed under a dissecting microscope. These mice were then maintained for further 21 days and the black dots, showing tumor colony formation, on the lung surface were counted under a dissecting microscope[63,64]. In the second experiments, B16 cells (2 x 10^5) were injected intravenously into recipient mice in a volume of 0.1 ml. After 14 days, mice were killed under ether anesthesia and the number of tumor colonies on the lung surface was counted in a similar manner. In these two experiments, mice were given food containing JTT and tap water ad libitum for 2 or 3 weeks starting 7 days before tumor cell injection. The present results showed that oral administration of JTT inhibited B16 melanoma cell colony formation on the lung surface, when the recipient mice were given tumor cells intravenously. JTT also suppressed spontaneous B16 tumor cell metastasis from hind footpad to the lung surface. The prevention of tumor cell growth and metastasis is well accepted to be through diverse mechanisms, including tumor cell death. Influence of anti-IFN-g monoclonal antibody or amrinone injection was studied on B16 melanoma cell metastasis in mice treated with JTT. C57BL mice were orally administered with 1.0% JTT for 3 weeks, which was started 1 week before 2 x 10^5 melanoma cell injection. Anti- IFN-g monoclonal antibody or amrinone was injected intraperitoneally and number of tumor cell colonies was counted 2 weeks later. +p<0.05. Influence of injection of antibodies against natural killer or natural killer T cells was studied on B16 melanoma cell metastasis in mice treated with JTT. C57BL mice were orally administered with 1.0% JTT for 3 weeks, which was started 1 week before 2 x 10^5 melanoma cell injection. Anti-NK1.1 monoclonal antibody or anti-asialo-GM1 antibody was injected intraperitoneally and number of tumor cell colonies was counted 2 weeks later. +p<0.05, ++p<0.01. These results revealed the absence of cytotoxic effects of JTT on B16 melanoma cells suggesting that immune mediated Mechanisms are responsible for the prevention of tumor cell colony formation on the lung surface. The immune effector responses against tumor cells involve activity by several cellular constituents: (i) T cells carry out immunologic surveillance, then proliferates and destroys tumor cells after recognizing tumor-associated antigens in combination with major histocompatibility complex (MHC) molecules. (ii) Dendritic cells are important antigen presenting cells that can present antigen to both helper and cytotoxic T cells and are able to stimulate a naı¨ve T cell response. (iii) NKT cells are another populations of effector cellswith tumoricidal activity. In contrast to cytotoxic T cells, NK cells can kill tumor cells in a non-MHCdependent fashion. We, therefore, examined the final effector cells that cause tumor cell killing in JTT-treated mice. The results proved that injection of NK-1.1mAb and anti asialo-GM1 antibody eliminate the suppressive activity of JTT on tumor cell metastasis. Anti NK-1.1 mAb is reported to be the effective means of the depleting NK and NKT cells. On the other hand, injection of anti-asialo-GM1 antibody into mice only eliminate NK cells from a variety of mouse strains. Taken together, the results may suggest that NK cells play essential roles in prevention of tumor cells metastasis in the lung. Several cytokines have been shown to affect NK cell proliferation and cytolytic activity. Of these, IFN-g, produced by activated T cells and NK cells, in conjunction with other cytokines such as IL-12, is thought to enhance the cytolytic activity of NK cells to attach and kill tumor cells[65-67], indicating that oral administration of JTT into mice increases the levels of cytokines, including IFN-g and IL-12 in the lung tissues and results in prevention of tumor cell colony formation.

On the other hand, the present results also open the question that whether IFN-g or IL-12 is important for the development of the ability to prevent tumor metastasis observed in JTT-treated mice. These results indicate that administration of neutralizing anti- IFN-g mAb could not abrogate the suppressive activity of JTT on tumor cell metastasis. However, administration of amrinone, which cause specific suppression of IL-12 production[68], caused complete elimination of the ability of JTT to prevent tumor metastasis in the lung. These results strongly suggest that IL-12 is the main mediator in the development of inhibitory action on tumor metastasis observed in mice treated with JTT.

Although the present results indicated that JTT exerts the protective effects on tumor cell metastasis through enhancement of IL-12 production and NK cell activation,the component of JTT which shows immuno-modulatory effects, is not defined. There is evidence that oral administration of extracts from *A radix*, a component of JTT, could enhance the ability of cells to produce several types of cytokines, which increase NK and NKT cell activity[69,70]. It is also observed that *A radix* directly activate NK cells to kill tumor cells *in-vitro*[32] suggesting that *A radix* is the most important component of JTT to prevent tumor cell metastasis. Further experiments are

required to delineate the component, showing the suppressive activity of tumor cell metastasis *in-vivo*. The conclusions re-stated are as follows:

i) JTT could prevent tumor cell metastasis through the enhancement of NK cell activity

ii) This activity of JTT may owe, in part, to its ability to increase in IL-12 production.

CONCLUSION

It can be concluded that natural flavonoids can modulate multiple cellular signaling pathways and interact with numerous molecular targets. Thus, it may have the potential to act against a large number of cancers. More extensive randomized clinical trials are now needed. The safety, low cost, and already proven efficacy of this "ageold" natural medicine makes it a promising agent for the treatment of an "old-age" disease like cancer. Immune dysfunction has been found to be associated with cancer and chemotherapy. Evolving knowledge of the role of naturally occurring compounds in the regulation of cancer cell growth suggests that they could serve as new tools for preventive and possibly therapeutic interventions.

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