PROMOTION OF HEALTH BY SOY ISOFLAVONES: EFFICACY, BENEFIT AND SAFETY CONCERNS

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SUMMARY

Cardiovascular diseases, osteoporosis-related hip fractures, and various cancers of the colon, prostate, uterus, and breast are remarkably less prevalent in Asia than in other industrialized countries. It is believed that the large consumption of soy products in Asian countries is contributory to the reduction of these chronic disorders. Genistein is a major isoflavone found in most soy products and plays an important role in the promotion of human health. Extensive epidemiological, *in vitro*, and animal studies have been performed, and most studies indicate that genistein has beneficial effects on a multitude of human disorders, including cancers, cardiovascular diseases, osteoporosis, and postmenopausal symptoms. To date, there is an abundance of promising studies supporting genistein's potential uses, but further research is still needed to validate its preventative and therapeutic efficacy. In addition, the adverse effects of genistein have drawn public attention. More studies are required to assess the potential detrimental effect of genistein, and a benefit-risk ratio should be considered before future clinical studies are performed.

KEY WORDS

isoflavones, genistein, daidzein, soya, cancer, osteoporosis, cardiovascular disease, phytoestrogens

1. INTRODUCTION

The Asian population has lower incidence rates of many malignancies, including cancers of the prostate, breast, colon and uterus. In addition, osteoporosis-related hip fractures and cardiovascular diseases are less prevalent in Oriental countries. Different influences in the East have been implicated in protecting Asians against many cancers and other chronic diseases. Most notably, it is believed that the Asian diet is rich in protective factors against various health risks. The Eastern diet is largely composed of soy products, vegetables and fiber. In contrast, Americans eat a diet high in animal fat consisting of few vegetables and soy food /1/.

The average Asian consumes 20-50 times more soy products than the average American /2/. Soy products contain a group of compounds

called isoflavones, and the primary dietary isoflavones are genistein and daidzein. Japanese men are found to have high blood isoflavone levels /3/ and one clinical study found that subjects on a plant-based diet had a 3-fold increase in urinary genistein levels /4/. Genistein and daidzein are either found as their glycoside conjugates (genistin and daidzin) or they are metabolized from their plant precursors (biochanin A and formononetin) /5,6/. Isoflavones undergo enterohepatic circulation, and are conjugated in the liver by glucuronic acid and sulfate /7,8/. The intestinal microflora convert daidzin to equol or o-demethylangolesin (O-Dma) and genistein is metabolized to p-ethylphenol. Daidzein, genistein, equol and O-Dma are found in blood and urine samples /7-9/.

Isoflavones are found mainly in plants, including many fruits and vegetables /7-9/. In particular, legumes such as soybeans, chickpeas and lentils have the greatest concentration of isoflavones /5/. Genistein is found in most soy products as a major isoflavone. Although soy products show varying amounts of isoflavones, the intake of many different soy foods containing small amounts of isoflavones are shown to have an additive effect /9/. In recent years, widespread attention has been drawn to isoflavones and their potential impact on human health. In this review article, we focus on the health effects and potential risk of soy isoflavones with an emphasis on genistein.

2. PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS

Phytoestrogens are plant-derived estrogens that may have estrogenic or anti-estrogenic effects /10/. Phytoestrogens are composed of several classes of compounds, including isoflavonoids and lignans. The lignans are enterolactone, eterodiol, and secoisolariciresinol. Phytoestrogens have similar structures to estrogens, such as 17β-estradiol and diethylstilbestrol (DES) /8-10/. Genistein has the highest binding affinity for the estrogen receptor of all known isoflavones /7/. Genistein is a heterocyclic phenol and has approximately 1/10,000 activity of 17β-estradiol /8/.

In vitro studies using MCF-7 hormone-dependent breast cancer cells and genistein show a dual action on estrogen receptors /10/. Genistein stimulated cancer growth at low concentrations and inhibited cellular proliferation at higher concentrations. At high concentrations, genistein was also found to compete with binding of

estrogen receptors in the pituitary gland and hypothalamus. In addition, genistein may act as a weak estrogen to enhance transcription of estrogen-related genes, pS2 and cathepsin D /11/. An increase in pS2 levels was found in nipple aspirate of pre-menopausal women receiving a 14-day soy supplement /12/.

It is generally believed that genistein exerts estrogenic behaviors in the presence of low estrogen concentrations, and genistein exerts anti-estrogenic effects when in an environment containing high concentrations of estrogen /6/. Neonatal and prepubertal rats exposed to genistein are protected against DMBA-induced mammary tumors. However, genistein administration from day 35 until 6 months of age showed no protection from mammary tumor incidence. In one cell culture study, when all estrogens were removed from the media, the breast cancer cells proliferated in the presence of genistein. When 17β-estradiol was added to the cells, no additional growth was observed with genistein /2/.

Genistein's anti-estrogenic effects are thought to contribute to its preventative role in many hormone-dependent cancers. Estrogen-related carcinogenesis is rarely seen in primates, and accordingly, chimpanzees and other primates show a high excretion of isoflavones /7/. Several studies demonstrated that increased binding of estrogen to sex hormone binding globulin (SHBG) decreased the risk of breast cancer development, and that increased urinary and blood phyto-estrogens were correlated with a significant increase in plasma SHBG levels /13/. Dehydroepiandrosterone sulfate (DHS) levels were found to be high in women with breast cancer. In one clinical study, the follicular luteal and DHS levels of 35 Seventh Day Adventist vegetarians were compared with the levels of 40 non-vegetarian adolescent girls. The Adventist girls had a significant increase in all hormone levels. After controlling for confounding factors such as body mass index, age and smoking, it was observed that the greatest difference between the girls' diets was the large portion of soyfoods served to the girls at the Adventist school. Additionally, soy intake had more effects on DHS levels in younger women /13/.

Since genistein inhibits cell growth in estrogen receptor positive and negative cell lines, genistein must have other mechanisms of action besides its estrogenic and anti-estrogenic properties /14/. Genistein has multiple roles in the signal transduction pathway and has been proven to be a potent inhibitor of protein tyrosine kinase

(PTK) and a topoisomerase II. It also inhibits cell growth, and induces G2-M cell cycle arrest and apoptotic cell death /15,16/. PTKs act upon receptors that regulate growth factors, such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), and insulin /17/. Uncontrolled tumor growth is usually associated with high levels of PTK activity. It was originally believed that genistein prevented cell growth via its inhibition of the tyrosine kinase activity of the epidermal growth factor receptor (EGF-R). Subsequent studies have disproved this hypothesis. In human prostate and breast cancer cell lines, genistein has no effect on EGF-R autophosphorylation or the phosphorylation of tyrosine kinases found downstream in the EGF signaling pathway /14/.

Growth of pediatric renal tumors is modulated, in part, by growth factor receptors such as EGF-R. In one study, genistein induced a dose-response inhibition of two renal tumor cell lines at concentrations of 25-50 μM, probably through downregulation of EGF-R phosphorylation /18/. *In vitro* studies have demonstrated that genistein was able to inhibit c-*src* and v-*abl*, two genes encoding for tyrosine kinases. Genistein decreased the production of c-*myc* RNA in two human colon cancer cell lines, whereas the known topoisomerase inhibitors had no effect on gene transcription. Therefore, genistein is believed to be responsible for decreasing gene transcription via its tyrosine kinase inhibitory mechanism. In addition, genistein was also found to inhibit PTK activity in the rat thyroid cell line FRTL-5 /17/.

However, genistein does not inhibit all PTKs equally. Thus, genistein's influence on other aspects of the signal transduction pathway in normal and neoplastic cells has been extensively investigated. Topoisomerase I and II are enzymes involved in transcription, replication and recombination in normal cells /17/. Genistein inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase complex, called the cleavage complex /14/. In tumor cells the cleavage complex leads to double and single strand breaks that result in growth inhibition or cell death in proliferating cells but not non-proliferating cells /14/. Genistein inhibited topoisomerase II activity in IL-2 dependent CTLL-2 cells and human thymocytes to the same degree as known topoisomerase inhibitors /19-20/. Genistein inhibited topoisomerase II activity at IC_{50} levels of approximately 111 μM /14/. However, the IC_{50} levels that show growth inhibition do not exhibit

DNA strand breakage. This suggests that there are other mechanisms contributing to genistein's antiproliferative effects on cancer cells.

Angiogenesis is the process by which existing blood vessels produce new capillaries. This process is virtually absent in mature vessels and is seen in relatively few processes such as wound healing, menstruation and pregnancy /21/. A number of diseased states are associated with increased angiogenesis. Specifically, solid tumors tend to induce pathological angiogenesis. Genistein has been found to inhibit endothelial cell growth and other factors that are essential for angiogenesis. Basic fibroblast growth factor (bFGF) induces production of an angiogenic factor. Genistein reduced the bFGF-stimulated production of the plasminogen activator inhibitor-1. In addition, genistein inhibited the bFGF-induced migration of endothelial cells in wounded confluent monolayers of endothelial cells /21/. Of the isoflavones tested, genistein was the most potent inhibitor of bFGF-stimulated proliferation of brain-derived capillary endothelial cells (BBCE), with IC_{50} levels of 5 μM /17,21/.

Reactive oxygen species (ROS) are thought to play a role in cancer development and tumorigenesis. Genistein inhibited agonist-stimulated ROS production at IC_{50} concentrations from 1.8-29.6 μM /14/. Genistein prevented UV-induced oxidative stress and apoptosis in A431 human epidermal carcinoma cells. Therefore, genistein's anti-apoptotic affect on cancer cells may be due to its anti-oxidant properties /22/. Inflammatory bowel disease is characterized by up-regulation in the gene that encodes nitric oxide (NO) synthase. In guinea-pigs with this disease, genistein was found to relieve the symptoms of the disease and decrease NO synthase expression /2/. A mouse model was used to generate singlet oxygen-induced cerebral strokes. Genistein was administered to the mice at concentrations of 16 mg/kg every six hours, and found to reduce the size of the average cerebral lesion, once again pointing to genistein's anti-oxidant activity /23/.

3. BENEFICIAL EFFECTS ON HUMAN HEALTH

3.1 Cardiovascular disease

Cardiovascular disease is a broad term used to describe many different conditions of the heart and venous and arterial systems. Such

a diverse group of diseases cannot be caused by a single mechanism, but rather by many different pathological conditions. Genistein is thought to be a promising treatment/prevention for cardiovascular diseases because of its protective effect on many different causes of cardiovascular problems. Coronary heart disease is less prevalent in Eastern countries, where there is a higher consumption of soy products than in the West /24/. Some of the mechanisms by which soy and specifically genistein prevent cardiovascular disease are thought to be via antioxidant effects, reduction of platelet aggregation and thrombus formation, relaxation of arteries, reduction in oxidized lipid levels in serum, and suppression of proliferation of smooth muscle cells /9, 24,25/.

Epidemiological studies, as well as animal and cell culture studies have shown a reduction in risk of cardiovascular diseases after hormone replacement therapy in postmenopausal women. This suggests an inverse relationship between estrogen and heart disease /9/. Genistein was hypothesized to have similar effects due to its weak estrogenic activity /25/. Monkeys fed a soy protein- and isoflavone-rich diet had an increase in high density lipoprotein (HDL), a decrease in total serum cholesterol, and a decrease in serum triacylglycerol. In contrast, monkeys treated with the anti-estrogen tamoxifen were found to have an increase in serum triacylglycerol /25/. The mechanism for the hypocholesterolemic effect of genistein is thought to be due to upregulation of the low density lipoprotein (LDL) receptor and an increase in LDL-cholesterol degradation.

Clinical trials that have assessed the effect of isoflavones on serum cholesterol levels have produced inconclusive results. Anderson *et al.* /25/ performed a meta-analysis of 38 controlled clinical trials. Each study looked at the effect of soy intake on serum cholesterol levels. The mean soy intake was 47 g/day. Of the 38 trials, 34 (89%) reported a reduction in serum cholesterol after soy intake. In another study, ten healthy men were assigned to three groups ingesting either soy milk, rice dream (vegetable protein control) or semi-skimmed cow's milk (animal protein control) each day for 4 weeks. The group drinking the soy milk did not have a reduction in serum cholesterol levels or triglyceride levels compared to the control groups, but a decrease in DNA oxidative damage to lymphocytes was noted for those taking the soy supplement /26/. Another study showed that postmenopausal wo-

men receiving 40 g of isolated soy protein source had a reduction in serum LDL and an elevation of serum HDL /9/.

In addition, overectomized rats were fed isolated phytoestrogens at oral doses in amounts from 0.1-3.0 mg/kg body weight each day. Cholesterol levels were significantly lower after five days of genistein supplement and the reduction in serum lipid levels was still maintained after 5 weeks /9/. LDL oxidation is thought to play a key role in the development of atherosclerosis. LDL oxidation causes the progression of endothelial cell damage and atherosclerotic plaques. Genistein is a known anti-oxidant and was therefore postulated to decrease LDL oxidation. In a recent animal study, Sprague-Dawley rats were fed a diet containing different anti-oxidants or genistein supplements for 3 weeks. LDL from the serum of rats fed a genistein-rich soy protein diet showed a decrease in LDL oxidation /27/. In another study, genistein and daidzein were converted into their ester derivatives so they could be incorporated into LDLs. The LDLs containing the isoflavones were shown to be more resistant to oxidation than those LDLs not containing the compounds /28/. However, when genistein's fat-soluble derivative was incorporated into LDL in U937 cells, no anti-proliferative actions were observed /29/.

The effects of genistein, phloretin, biochanin A and zearalanone on rabbit coronary arteries were investigated *in vitro* /30/. Each phytoestrogen was added to the arteries and the developed tension was measured. Significant relaxation was induced by genistein, phloretin and biochanin A in smooth muscle arteries with and without endothelium. It is believed that the mechanism for genistein-induced relaxation involves calcium antagonism /30/. In addition, monkeys fed a soy protein and isoflavone-rich diet demonstrated a dilatation of the coronary arteries similar to that seen after estrogen administration /25/. Genistein has been shown to prevent platelet aggregation, an important step in the formation of atherogenic plaques and endothelial cell damage. However, the mechanism by which genistein exerts this action is not well understood. The phosphorylation of tyrosine kinases on platelet proteins is essential in activating platelet aggregation and downstream signaling of thrombus formation. As a protein kinase inhibitor, genistein has been shown to prevent thrombus formation /9/ with modulation of phosphorylation and activation of human platelet MAP-K /31/. Incubation of human platelets with genistein prevented platelet aggregation *via* the collagen and thromboxane pathway

because genistein prevents collagen and thromboxane A2 from binding to their receptors, not because of tyrosine kinase inhibition /9/.

In a recent animal study, 12 adult female rhesus monkeys were fed an atherogenic diet for 6 months in which the isoflavones were either removed by alcohol or left intact. When collagen was injected into the monkeys' coronary arteries to induce platelet aggregation, there was less reduction in blood flow in the animals on the soy diet. In addition, animals on the soy diet had improved dilatation of the arteries even after the arteries were unresponsive to acetylcholine's dilatatory effects /32/.

3.2 Bone/osteoporosis

The development of osteoporosis is a major concern for post-menopausal women. The decreased availability of estrogen in post-menopausal women is believed to be the cause of accelerated bone loss and the increased susceptibility to developing osteoporosis. There are substantially fewer osteoporosis-related bone fractures in Asia as compared to the United States. In addition, elderly men and women in Hong Kong have one third of the hip fractures that Americans have /9/. These observed differences have led researchers to believe that the Asian diet protects against bone loss and osteoporosis. Specifically, the soyfoods present in Eastern diets are thought to be responsible for the low incidence of osteoporosis.

Estrogen replacement therapy is currently the treatment of choice for postmenopausal women suffering from a reduction in bone mass. Unfortunately, hormone replacement therapy (HRT) may have long-term effects of increasing the risk of breast cancer and thrombo-embolisms /33/. This led researchers to investigate the effects of genistein, a weak estrogen, on preventing bone-related diseases in postmenopausal women. In a clinical trial, the postmenopausal women were given a 40 g supplement of isoflavones and soy protein. The study reported a significant increase in bone mineral density and bone mineral content in the lumbar spine compared to those women on a casein-rich diet /9/. In another study, increased urinary excretion of phytoestrogens was noticed to correlate with decreased bone loss. Kardinaal *et al.* /34/ looked at a previous study that followed 154 postmenopausal women from the Netherlands. They selected 32 women with an annual rate of radial bone loss of $\leq 0.5\%$ over the first 5 years of the study, and 35 women with a rate of $>2.5\%$ per year. The

results of the study found no association between excretion of phytoestrogens and cortical bone mass. Further clinical research is needed in order to assess genistein's ability to prevent bone loss in postmenopausal women.

In two different studies of overectomized (OVX) animals, genistein was shown to prevent the loss of bone mass. In one study, 2 month-old OVX or sham-operated (SHAM) rats were injected daily with 5 μg of genistein per gram body weight for 21 days. The rats treated with genistein had less compact and trabecular bone loss. Genistein blocked the production of tumor necrosis factor-alpha (TNFα) in OVX rats. This is important because cytokine production is thought to play a role in promoting osteoporosis in postmenopausal women. It was also observed that genistein and estrogen prevent bone loss by different mechanisms. Genistein stimulates bone formation, whereas estrogen prevents bone resorption /35/. In a second study of OVX mice, genistein restored trabecular bone volume in the mice. The results from this study revealed that genistein acts like estrogen to regulate β lymphopoeisis and prevent bone loss, but does not have similar effects to estrogen on the uterus /36/.

Over the past two years, Gao and Yamaguchi have conducted multiple *in vitro* studies supporting genistein's role in preventing osteoclast activity and bone growth. However, these studies disagree as to how genistein exerts its suppressive effect. Genistein is shown to inhibit osteoclast activity through protein tyrosine kinase inhibition and cyclic AMP signaling, and induces apoptosis via Ca^{2+} intracellular signaling /37-39/. In a recent study, cortical bone tissue from elderly female rats was cultured with daidzein and genistein. Both isoflavones were found to have an anabolic effect on the bone tissue and were able to rebuild bone /40/.

3.3 Postmenopausal symptoms

Soy supplementation has been shown to reduce the severity of various postmenopausal symptoms. In one clinical trial, 104 postmenopausal women were given a 60 g daily supplement of isolated soy protein or placebo (casein). By the fourth week, the soy supplement was shown to significantly reduce the mean number of hot flushes per 24 hours. At the end of week 12 there was a 15% ($p<0.01$) greater decrease in the number of hot flushes in the soy group compared to the control /33/. In a similar study, postmenopausal

women were randomly assigned to a phytoestrogen-rich diet or control diet. The phytoestrogen-rich diet consisted of approximately 6 mg or more total isoflavones per day. Menopausal symptoms decreased 50% ($p<0.005$) in the treated group. In addition, hot flushes decreased 54% ($p<0.05$) and vaginal dryness decreased 60% ($p<0.005$) in the group on the isoflavone diet /25/.

3.4 Anti-carcinogenic and anti-cancer properties

It is believed that one third of all cancers are influenced by diet /25/. Cancers of the colon, prostate, uterus, and breast are remarkably less prevalent in Asia than in other industrialized countries. This difference in cancer incidence has been attributed to the high intake of soyfoods in Eastern countries. Women in Asia are found to have "10-100 fold" higher serum and urine levels of isoflavones than American women /25/. Western diets are almost completely devoid of soy products. Instead, the Western diet is composed of a high fat, meat based diet that is low in vegetables and fiber content /41/. Many *in vitro* and animal studies have revealed the mechanisms by which isoflavones prevent tumor growth and angiogenesis. However, some of the studies show contradictory results. Clearly, more laboratory and clinical research is needed to properly ascertain genistein's role in cancer prevention.

a) Breast cancer

Isoflavones from soybeans have been hypothesized to have a preventative effect on breast cancer. As previously established /4/, the dietary intake of soy is related to urinary excretion of isoflavones in a dose-dependent manner. In a case-control study conducted in Shanghai, overnight urine samples were collected from 60 breast cancer patients and matched controls. The urine samples were tested for the subject's excretion rates of five major isoflavones (daidzein, genistein, glycitein, equol, and O-desmethylangolensin) and total phenols. Breast cancer patients provided the urine samples before any cancer therapy. There was a significant reduction in urinary excretion of total phenols and all individual isoflavones in breast cancer patients compared to controls. The median excretion of all major isoflavones was 50-65% lower in cancer patients than in controls /42/. The adjusted odds ratio for breast cancer was 0.14 (95% confidence

interval, 0.02-0.88) for women whose urinary excretion of both phenol and total isoflavones was in the upper 50% compared with those in the lower 50% /42/.

In a recent study of immature rats, genistein was found to increase expression of EGF-R in mammary glands /43/. It is hypothesized that early exposure to genistein may decrease the response of the epidermal growth factor signaling pathway. Genistein does not directly inhibit phosphorylation of the tyrosine kinase of the EGF-R. Instead, genistein may regulate the EGF-R by targeting the cell at points distal to the EGF-R, such as phospholipase C-γ, mitogen-activated kinases (MAP kinase), and phosphatidylinositol kinases.

The animal studies involving genistein's effect on breast cancer present conflicting results. Researchers induced N-nitroso-N-methyl-urea (NMU) mammary tumors in rats. One week prior to tumor induction, the rats were fed diets containing different amounts of soy products. The experiment lasted for a total of 18 weeks. Between the five groups, there was no significant difference found in the number of tumors developed, or the tumor size /44/. In a study by Fritz *et al.* /45/, Sprague-Dawley CD rats were fed with 0, 25 and 250 mg genistein/kg AIN-76A diet. On day 50, mammary tumors were induced in the animals using dimethylbenz[a]anthracene (DMBA). Genistein showed a dose-dependent reduction of mammary tumors. In addition, genistein enhanced cell differentiation and reduced proliferation of the terminal end buds in the mammary glands. In another study, rats were treated with 20 μg of phytoestrogen from days 7 to 20, postnatally. Genistein significantly reduced tumor multiplicity, but did not decrease the rate of tumor development. In addition, all of the tumors in the control group were malignant, whereas 60% of the tumors that developed in the genistein group were benign /46/. This study implies that exposure to genistein before puberty may protect against future malignant transformation of breast cancer.

Phytoestrogens have been implicated as inhibitors of human breast cancer cells. Genistein, daidzein, biochanin A, and coumestrol were shown to inhibit the growth of T-47D and MCF-7 breast cancer cells /47/. In addition, genistein may induce apoptotic cell death in MCF-7 breast cancer cells by stabilizing the tumor suppressor gene p53, inactivating bcl-2 and causing a cell cycle delay in the G2-M phase /48/. Genistein has also been shown to inhibit cell growth and induce apoptosis in the MDA-MB-231 breast cancer cell line /49/. It caused

downregulation of Bcl-2 and p53 expression and an increase in Bax and p21WAF1 expression. Furthermore, there was increased apoptosis with longer genistein treatment. The synergistic effects of tamoxifen and genistein on the estrogen receptor-negative human breast carcinoma MDA-MB-435 cells have been investigated /50/. These compounds were used because each one modulates the signal transduction pathways *via* different mechanisms. The study found that the two compounds synergistically caused a reduction in cell growth and an increased cytotoxic effect in the breast cancer cells /50/. This study suggests a possible clinical application in the use of both tamoxifen and genistein for the treatment of breast cancer.

b) Prostate cancer

Prostate cancer is the second most common cancer of men in the US. This disease, as well as benign prostatic hyperplasia (BPH), primarily affects men in the latter half of their life /51/. Therefore, as the average lifespan continues to increase, these health problems will become more prevalent. Epidemiological studies point to the protective effect of isoflavones and soy on prostate cancer development. Black men in the United States have the highest incidence of prostate cancer. In contrast, there is a low occurrence of prostate cancer in the Asian population /51/. This difference has been attributed to the high soy diet of Asian populations /9/. Japanese men eat an average of 20 mg of isoflavones per day, whereas Americans men eat less than 1 mg of isoflavones per day /52/. In addition, when Japanese men migrate to the United States, they have the same incidence rate of prostate cancer as people born in the US within one or two generations. A prospective study showed that drinking soymilk more than once a day was associated with a 70% reduction of the risk of prostate cancer /53/.

Genistein is a dietary estrogen that can accumulate in the prostate and make up a component of prostatic fluid /54/. Prostate cancer is a hormone dependent cancer, as are cancers of the ovary, breast, and endometrium. Androgens have been shown to play a key role in prostate cancer development. The conversion of testosterone to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase plays a significant role in androgen activation and prostate cancer development. Genistein has been shown to inhibit 5α-reductase activity by up to 80%, along with biochanin A and equol, at a concentration of 100 μM /9/. It is believed that genistein's preventative role in prostate

cancer is due to its weak estrogenic activity. Recent *in vitro* studies have shown that genistein can inhibit cell growth in both hormone-dependent and hormone-independent cell lines /54-56/. The proposed mechanism of action for genistein-induced cell inhibition and cell death in prostate cancer cells is *via* a p53 independent pathway. Genistein causes a downregulation of cyclin B1, and upregulation of p21 (WAF1/CIP1) growth inhibitory protein, leading to G2-M cell cycle arrest /57/.

Animal studies also indicated the inhibitory effect of genistein and a high soy diet on prostate cancer. Severe combined immunodeficient mice were injected with a human androgen-sensitive prostate cancer cell line (LNCaP). Mice were randomized into four different groups. Each group was fed a different diet: high-fat + casein, high-fat + soy protein/isoflavone extract, low-fat + casein and low-fat + soy protein /isoflavone extract. The mice fed the diet of low fat + soy protein/ isoflavone extract had a statistically significant reduction of tumor growth rate and final tumor weight /58/. In another study, 125 rats were induced with R3327 PAP prostate tumors. The rats were divided into five groups and fed different types of fiber-containing diets. After 24 weeks it was noted that prostate cancer growth was inhibited only in the group of rats fed a diet containing 33% soy flour /59/. Other studies have corroborated these findings by demonstrating the consumption of soy products reduced tumor size, increased apoptosis and reduced tumor angiogenesis in mouse models /60/.

c) Colon cancer

Colorectal cancer rates are lower in Asia, although the differences between incidence in Eastern and Western countries are not as disproportionate as they are for prostate and breast cancer /61/. *In vitro* studies demonstrated genistein's ability to inhibit cell growth in HT-29, SW620, and SW-1116 colon cancer cell lines. Genistein is believed to exert its anti-proliferative effects by inducing G2-M cell cycle arrest /62/, and causing topoisomerase II-mediated DNA breakage in colon cancer cells /62-63/. In a recent animal study /64/, colon cancers were induced in rats by injection with azoxymethane. The rats were started on diets containing soy fiber, soy flour, genistein, or Ca^{2+}, and the diets were maintained for 12 weeks. Animals on the genistein-containing diet had fewer pre-cancerous colonic lesions than animals on the soy fiber and soy flour diets. This study suggests that

genistein may play a role in preventing the early growth of colon cancer.

d) Bladder cancer

Animal and cell culture studies suggest genistein may play a role in preventing bladder carcinogenesis. Genistein induced significant inhibition of seven human bladder cancer cell lines. G2-M cell cycle arrest was also observed in association with growth suppression /65,66/. Transition of non-invasive bladder cancer to invasive bladder cancer (associated with a poorer prognosis) is characterized by the overexpression of EGFR and the p21ras oncogene. Genistein was shown to inhibit bladder cancer cell growth and motility, preferentially in cells overexpressing EGF-R with wild-type p21ras /67/. In an animal study, mice were fed diets of genistein, soy phytochemical concentrate or soy protein isolate. They were then inoculated with MB49 bladder carcinomas. Each of the experimental diets reduced tumor volume by at least 37% (genistein reduced volume 40%). The soy products also increased apoptosis and reduced angiogenesis, thereby restricting the tumor's blood supply and further proliferation /66/.

e) Pancreatic cancer

An inhibitory effect of genistein on pancreatic tumorigenesis has been reported. The phosphorylation of PTK is thought to play a key role in pancreatic cancer growth and enzymatic activation because genistein, a tyrosine kinase inhibitor, suppressed cell growth and enzyme activation /68/. Douziech *et al.* /69/ used EGF and bombesin to stimulate growth in two pancreatic cancer cell lines, MIA PaCa-2 and PANC-1. Genistein inhibited cell growth and p38 MAP kinase activation in MIA PaCa-2 cells, as well as exerting an anti-proliferative effect on PANC-1 cancer cells by downregulating p42 kinase activation.

f) Lung cancer

Genistein has been shown to inhibit cell growth in non-small-cell lung cancer (NSCLC) cells /70/. Genistein also caused G2-M cell cycle arrest, upregulation of p21Waf-1, and induced a p53-indepen-

dent apoptosis in NSCLC /70,71/. EGF-R is overexpressed in NSCLC cell lines. Genistein prevented the activation of EGF-R in NCI-H596 cells (a type of NSCLC cell line). The combination of chemotherapy agents and genistein was also observed to enhance growth arrest and apoptosis in NCI-H596 cells, but had no cumulative effects on NCI-H358 cancer cells /72/. In addition, genistein may play a role in preventing lung cancer metastasis. The metastasis of small cell lung carcinomas (SCLC) is characterized by the attachment of tumor cells to endothelial cells. TNFα enhances tumor cell attachment by increasing expression of cell adhesion molecules (CAMs). When human umbilical vein endothelial cells were co-incubated with TNFα and an SCLC tumor cell line, there was an increased adherence of tumor cells to the endothelium. Genistein was able to completely block the TNFα-induced tumor cell attachment /73/.

g) Malignant melanoma

Currently in the United States, malignant melanoma is the seventh most common cancer. So far, the etiology of malignant melanoma remains unclear and no effective therapy has been developed against advanced stage and metastatic malignant melanoma /74/.

Genistein has been shown to arrest the growth and induce the differentiation of both mouse and human malignant melanoma *in vitro* /75,76/. Genistein was shown to inhibit the growth of B16 melanoma cells in a dose-dependent fashion. IC_{50} for genistein was 35 μM after 48 hours incubation, and 2.5 μM after a 120-hour period, respectively. Cells treated with genistein showed structural alterations representative of differentiation /75/. Additionally, genistein was shown to effectively inhibit the growth of several human malignant melanoma cells and induce significant differentiation by demonstrating the development of melanin-containing melanosomes and dendritic-like structures /76/.

Soy isoflavones have been also shown to prevent the pulmonary metastasis of melanoma *in vivo* /77,78/. The isoflavone genistein has been shown to be the most effective inhibitor of metastases with a positive correlation between the concentration of genistein administered and its ability to decrease metastasis /77/. Other isoflavones, such as daidzein, its metabolites equol and o-desmethylangolensin, and glycitein have proven virtually ineffective in the inhibition of metastases. The authors attributed the effects of dietary isoflavones on

pulmonary metastasis to inhibition of protein tyrosine kinases, topoisomerase II, and regulation of cell cycle progression and apoptosis.

In another *in vivo* study, mice were inoculated with B16F-10 melanoma cells through the lateral tail vein. At the time of inoculation mice were orally administered isoflavones (genistein and daidzein separately) every other day for twenty days. Lung tumor and nodule formation was measured, and the metastatic markers, sialic acid and lung hydroxyproline, were monitored. The number of pulmonary metastatic tumors and the levels of sialic acid and lung hydroxyproline were significantly reduced in genistein-treated groups compared to the control group. Genistein was found to have a strong inhibitory effect on lung tumor formation as well as pulmonary metastasis, whereas daidzein proved useless in inhibition of lung tumors with a slight effect on lung hydroxyproline and sialic acid levels /78/. These studies indicate that soybean isoflavones, particularly genistein, have a potential role in treatment for malignant melanoma, and in the prevention of metastasis of this aggressive human cancer.

h) Skin cancer

Human skin cancers have significantly increased in the past twenty years, and the etiology of increased incidence of skin cancers is largely associated with ultraviolet (UV) radiation exposure /79/. Although numerous *in vitro* studies have indicated that genistein has potential anticancer properties, evidence is lacking as to whether genistein prevents skin carcinogenesis.

A number of studies have been conducted to investigate the effect of genistein on carcinogen- and UV light-induced skin cancer. Genistein was shown to inhibit tumor promoter phorbol ester (TPA)-mediated H_2O_2 formation and inflammatory responses *in vitro* and in skin tissues /80,81/. Genistein scavenges various ROS and quenches Fenton reaction- and UV-induced DNA oxidation /82/. In addition, genistein suppresses TPA- and UVB-induced protooncogene expression in mouse skin /83,84/, and increases skin antioxidant defense enzymes /85/. Genistein also demonstrated potent antiinitiational and antipromotional activities in two-stage carcinogenesis models in CD-I and SENCAR mice /81,86/.

In recent years, we have conducted extensive experiments to investigate the effect of genistein on UV light-induced photocarcinogenesis and cutaneous damage. Our studies have demonstrated that

genistein is a potent photoprotective agent against UV-induced injuries in animals and humans /87,88/. The effect of genistein on UVB-induced skin carcinogenesis was determined in Skh-1 hairless mice. Mice were topically treated with either vehicle or 10 μmol of genistein, and then exposed to 0.6 kJ/m^2 of UVB twice weekly. The tumor incidence and multiplicity were significantly reduced by more than 90% in the genistein-treated mice. In addition, genistein completely blocked UVB-induced acute skin burns in mice daily exposed to UVB (1.8 kJ/m^2) for 10 days and cutaneous photoaging in mice chronically exposed to UVB (0.3 kJ/m^2 three times per week for 20 weeks).

Genistein also blocks UVB-induced H_2O_2 production, lipid peroxidation and oxidative DNA damage in mouse skin. Oxidative DNA damage, lipid peroxidation and H_2O_2 are known to be involved in different stages of carcinogenesis, such as initiation and promotion. UVB irradiation increases the levels of these intermediate biomarkers in UVB-irradiated mouse skin. Topical application of genistein substantially decreased UVB-induced H_2O_2 production, lipid peroxidation, and 8-hydroxy-deoxyguanisine (8-OHdG) in skins of hairless mice /88/. In an *in vitro* study using purified calf thymus DNA, the effects of genistein and the classic antioxidants, ascorbic acid and glutathione, on UV light-induced 8-OHdG were compared. Genistein was shown to quench UV-induced 8-OHdG more potently than ascorbate and glutathione. Within the comparable concentration range, ascorbate exhibited a moderate effect whereas glutathione had no effect on UV-induced oxidative DNA damage /82/.

The effects of genistein on UVB-induced expression of c-*fos* and c-*jun* mRNA in mouse skin was measured /84/. Mice were topically treated with 20 μmol of genistein 60 min prior to UVB irradiation. Topical genistein substantially inhibited both c-*fos* and c-*jun* expression by a low dose of UVB (5 kJ/cm^2) irradiation. At a high dose of UVB (15 kJ/cm^2), genistein only blocked c-*fos* expression, but had little effect on c-*jun*. Treatment of mouse skin with genistein immediately after UVB exposure also inhibited UV-induced c-*fos* and c-*jun* expression. Jun and Fos proteins are known to be involved in the formation of AP1 transcription factors, leading to subsequent cell proliferation. Overexpression of these protooncogenes is related to the promoting process of carcinogenesis. Thus, substantial suppression of

UVB-induced protooncogene expression suggests that genistein may modulate UVB-mediated tumor promotional activities.

The effect of genistein on filtered UVA- and UVB-induced phosphorylation of EGF-R in human epidermoid carcinoma cells was determined /84,85/. Cells were irradiated with UVA or UVB in the presence and absence of genistein. Total cell lysates were immunoprecipitated by anti-EGF receptor monoclonal antibody, and a Western blot was performed with anti-phosphotyrosine monoclonal antibody. Genistein was shown to significantly downregulate both UVA- and B-activated EGF-R phosphorylation in cultured cells. Phosphorylation of TPK-dependent EGF-R is involved in the initiation of transcription factors, release of inflammatory mediators, such as prostaglandins, and stimulation of cell proliferation, all of which are relevant to the promoting activities. Thus, inhibition of UV-induced EGF-R phosphorylation by genistein may suggest its potential antipromotional effects.

In a pilot human clinical trial involving six human subjects, genistein was topically applied to human dorsal skin 30 min prior to UVB radiation. The results consistently showed that genistein effectively inhibited one MED of UVB-induced erythema in human skin /87,88/. This study indicated that genistein may work as an effective agent in protection of human skin against sunlight-induced skin photodamage. More recently, Kang *et al.* /89/ reported that topical genistein inhibited EGF-R tyrosine kinase, ERK, and JUNK kinase activities in human skin exposed to two MED of UVB by 75%, 60% and 50%, respectively. UVB-induced AP-1 and matrix metalloproteinase genes are thought to be responsible for premature photoaging, and genistein significantly inhibited c-*jun* and metalloproteinase gene expression by 70% and 72%, respectively.

4. POTENTIAL RISK OF USING GENISTEIN

There are numerous studies that report the beneficial effects of genistein, such as its anti-carcinogenic and anti-thrombolytic effects and its role in preventing osteoporosis and postmenopausal symptoms. Unfortunately, genistein's potential harmful effects are rarely mentioned. Genistein may cause certain detrimental impacts, for instance, causing reproductive problems, disrupting endocrine function and causing chromosomal aberrations /90-92/. Reinhart *et al.* /2/ demon-

strated genistein's ability to mimic 17β-estradiol and induce synthesis of leukemia inhibitory factor (LIF) in bovine oviduct cells. LIF is an essential glycoprotein for proper embryo implantation. Thus, genistein may serve as an "endocrine disrupter", displacing 17β-estradiol and potentially leading to tubal infertility. Genistein has also been shown to stimulate androgen synthesis and disrupt cortisol production /93/. Two dietary phytoestrogens, genistein and daidzein, were cultured with fetal, postnatal and adult (H295) adrenal cortical cells. Both compounds reduced cortisol production in all cell types. Genistein, daidzein, and 17β-estradiol increased dehydroepiandosterone (DHEA) levels in H295 cells. In addition, genistein and daidzein inhibited 21-hydroxylase (P450c21) activity.

Although genistein has been shown to be a safe, nontoxic compound /94/, evidence has accumulated that genistein may have a potential toxic effect on the reproductive system. Several studies have investigated the effects of genistein on pregnant animals and their offspring. Between days 15 and 20 of gestation, pregnant rats were injected with 20, 30, or 100 μg of genistein or 20 μg of zearalenone. At 2 months of age, offspring were induced with 12-dimethylbenz-(a)anthracene (DMBA) tumors. Offspring from rats treated with genistein had a dose dependent increase in mammary tumorigenesis. They also had an upregulation of estrogen receptor binding sites and increased protein kinase C activity in the mammary glands /95/. In another study, female offspring of pregnant rats treated with genistein during gestation were found to have lower birth weights, longer estrus cycles, and lower relative uterine-ovarian weight at 35 days of age. The offspring of this group did not have an increased incidence of mammary carcinomas, but did have an increased multiplicity of mammary tumors /96/.

Awoniyi *et al.* /97/ observed that pregnant rats fed a genistein-enriched diet gave birth to female offspring with lower uterine and ovarian weights as well as reduced serum E_2 and progesterone levels. The decline in hormone levels was reversed by day 70 post-partum. The offspring of the genistein group also had more atretic follicles and secondary interstitial glands than the offspring of the control group. In addition, cystic rete ovarii was noted in the offspring of the genistein group. Genistein may also have cytotoxic effects on male reproduction /98/. Genistein, sodium azide (naz) and dexamethasone (dxm) were incubated with testicular cells TM3, TM4, and GC-1 spg. Genistein,

as well as genistein and dxm combined, induced significant apoptotic cell death. The synergistic effect of genistein and naz caused necrotic cell death in the testicular cells.

Although many of the published studies point to genistein's anti-proliferative effect on breast cancer, one study has found genistein to promote cancer growth *in vitro* /99/. This research tested the effects of receptor tyrosine kinase inhibitors on cell proliferation of the hormone-dependent breast cancer cell line, MCF-7. Activation of tyrosine kinases is needed for growth factor dependent cell proliferation. Cells were also supplemented with E_2 to cause a dose-dependent cell proliferation. The epidermal growth factor receptor PTK inhibitors completely blocked the E_2-mediated cell growth, whereas genistein stimulated the E_2- and serum-induced growth of MCF-7 cancer cells. These results reinforce a study in which the ZR-75-1 human breast cancer cell line showed increased EGF-R expression, which was related to a decreased sensitivity to genistein /100/. In these two studies, genistein may be acting as a weak estrogen to stimulate estrogen-induced growth.

Previous studies have associated estrogen with colon cancer development. Postmenopausal women on HRT had a relative risk of 0.54 for colon cancer compared to postmenopausal women who never used HRT. In addition, women who used HRT one year prior to sigmoidoscopy reduced the risk of having colorectal cancers by 50% /61/. Fiorelli *et al.* /101/ observed significant expression of cytochrome P450 aromatase in colorectal adenocarcinomas. Aromatase converts androstenedione to estrone. Genistein substantially increased aromatase activation, whereas tamoxifen (an anti-estrogen) inhibited aromatase activation. In addition, genistein promoted the growth of non-invasive colon adenocarcinomas in animal models /102/. These studies demonstrate genistein's ability to enhance, rather than inhibit, colon cancer proliferation.

Genistein has been shown to stimulate pancreatic cancer cell growth. In one study, two human pancreatic cell lines were used, HPAF-11 from a male and Su 86.86 from a female. Cells were exposed to genistein, biochanin A, equol and coumesterol for 24 hours at concentrations of 1 μM in female cells and 1 and 10 μM in male cells. Genistein was found to stimulate growth in male pancreatic cancer cells, but had negligible effect on female cancer cells. Genistein also increased expression of the multidrug resistant (mdr-1)

gene in male tumor cells /103/. Genistein has also been found to cause genetic toxicity. It is a chromosomal mutagen in human lymphoblastiod cells and may signal apoptosis depending upon the status of p53 in the cells /104/. In addition, after exposure of human peripheral blood lymphocytes to 25 μM genistein for 6 hours, significant chromosomal changes were observed. Thus, soy product was assumed to be a potential etiologic agent for infantile leukemia /105/. Secondary leukemia due to topoisomerase II (topo II) inhibitors and infantile acute leukemia are characterized by breakpoints within scaffold attachment regions of 3′-MLL-bcr near exon 9. Genistein, a known topoisomerase inhibitor, causes similar chromatid aberrations as other topo II inhibitors in the MLL gene. Therefore, genistein may be a causative agent for infantile acute leukemia and secondary leukemia /105/. In addition, genistein has also been shown to inhibit rat myoblast growth *in vitro*. This may be indicative of genistein's negative affect on normal muscle development /106/. Further studies are needed to properly assess genistein's toxicity.

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