Pharmacological activities of Genistein, an isoflavone from soy (Glycine max): Part I—Anti-cancer activity

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Phytoestrogens represent a family of plant compounds such as isoflavones, flavones and lignans. A variety of these plant compounds have been identified in various human body fluids. A wide range of commonly consumed foods contains appreciable amounts of these different phytoestrogens, viz. soy products are particularly good sources of isoflavones and lignans. Accumulating evidences from molecular and cellular biology experiments, animal studies, and to a limited extent, human clinical trials suggests that phytoestrogens may potentially confer health benefits related to various cancers and diseases such as cardiovascular disorder. The evidences reviewed here represent the beneficial effects of most potential and promising isoflavone, Genistein in various types of cancers.

In the past several years, soy and its ingredients have received considerable attention from both researchers and health practitioners. Epidemiological data indicates that people from Asian cultures have lower rates of certain cancers, including cancer of the breast, prostate and colon. Geographical variation in cancer rate has been partially attributed to differences in dietary intake. Phytoestrogen (plant estrogens) is a generic name used to define classes of compounds that are non-steroidal. They are either of plant origin or derived from in vivo metabolism of precursors present in plants consumed by human beings. Phytoestrogen helps to regulate the growth of plants and protect them from stress and the damaging effects of ultraviolet radiation. Phytoestrogens are weaker than natural estrogens (possessing between 1/11,000 and 1/100,000 the activity of natural female hormone estrogens) and differ remarkably from synthetic environmental estrogens in that they are easily broken down, are not stored in tissue and spend very little time in the body. These compounds are able to mimic the effects of estrogens because their structure is remarkably close to those of human estrogens and the body accepts them as such. Evidence continues to accumulate that phytoestrogens may offer protection against a wide range of human conditions, including breast, bowel, colon, prostate, and other cancers.

Genistein and Cancer
Cancer is the second leading cause of death. Cancer has reached epidemic proportions as approximately one out of every four persons die due to cancer in the United States and approximately one out of every three people will develop cancer in his/her lifetime. Pre-clinical and clinical studies
provide substantial evidence that Genistein may function as an effective anti-carcinogenic agent. Genistein works several ways to reduce cancer risk (incidence, latency or tumor cell number).\(^4\)

**Estrogenic/anti-estrogenic activity**

The hormone estrogen is a sex hormone with powerful effects on growth differentiation and function of many cells. They attach to protein called estrogen receptors (ERs). These complexes “dock” at sites (response elements) within selected genes in the cell’s nucleus, switching those genes on or off. This switching makes cells proliferate (like breasts growing during puberty) or causes them to differentiate and make special products (for example, milk following pregnancy). For many years, only a single ER was known ER alpha. In 1996, another ER beta was found which predominates in certain estrogen-responsive tissues such as bone and the bladder.\(^2\) Genistein binds only weakly with ER alpha, but binds with ER beta almost as well as estrogen does. The predominance of ER beta in the bone, cardiovascular system, ovary, lung, bladder, brain, uterus, and testis, probably explains Genistein’s ability to prevent bone loss and be partly responsible for the lower incidence of heart disease and cancer in soy-consuming countries.\(^3\)

A study from the Karolina Institute\(^44\) showed that the distribution of estrogen receptors throughout the human body is wider and more concentrated in some tissues than previously believed, adding credence to the theory that manipulating estrogen binding might have broad effects in cancer prevention. In females, estrogen is necessary for reproduction as well as for the development of the breasts and other secondary sex characteristics. But it can also cause a problem because it can encourage cancerous tumors to develop in breast tissue. Genistein can bind to estrogen receptors but not produce the same negative effect on cell division because it is a weak estrogen. This means that in women normally producing a lot of estrogen, Genistein is acting as anti-estrogen in a similar way to the anti-estrogen drug Tamoxifen, widely used in the treatment and prevention of breast cancer. Tamoxifen is the synthetic counterpart to Genistein and works to block the ability of estrogen to stimulate the kinds of changes in breast tissue that result in the formation of tumors. In order for estrogen to stimulate the growth of breast cancer cells, it needs first to bind the estrogen receptor to its cell surface receptor. Due to its weak estrogenic activity, Genistein acts as anti-estrogen by competing with the human estrogen for binding to estrogen receptors.\(^15\) In summary, when a woman has little natural estrogen production (post-hysterectomy or postmenopausal), Genistein can attach to open estrogen receptor sites on cells and produce a weak estrogen effect. When there is too much estrogen (for example during PMS), Genistein can compete with the natural estrogen for receptor sites and because it is a weak estrogen in comparison, it blunts the estrogen effect.

**Inhibition of tyrosine kinase, topoisomerase II and s6 kinas activity**

Apart from its estrogenic and anti-estrogenic activities, in vitro studies have shown that Genistein inhibits the growth of a wide range of cells including those that are not hormone-dependent. The proposed explanation for these effects is the ability of Genistein to markedly inhibit the activity of enzymes that control cell growth and regulation.\(^6\) Tyrosine kinases and receptor tyrosine kinases are critical components of the biological control networks that govern cellular growth and differentiation.\(^17\) Enhanced activity of tyrosine kinases has been implicated in many cancers. In vitro trials have consistently shown that Genistein is capable of inhibiting the activity of tyrosine kinase\(^18\)–\(^20\). This further implicates the use of Genistein as an anti-carcinogen since tyrosine kinase-specific inhibitors may be potentially employed as anti-cancer agents. Some of the most potent anti-tumor agents currently used in cancer chemotherapy inhibit DNA Topoisomerase I or II. The enzymes involved in the processes of DNA replication, transcription and differentiation.\(^21\) A number of in vitro studies have demonstrated that Genistein is capable of inhibiting Topoisomerase II\(^22\)–\(^26\).

**Inhibition of angiogenesis**

At the developing stage, the tumor develops characteristics that enable it to grow in size and spread its cells to other parts of the body (metastasis). When a cancer cell is growing at full blast, the cells soon run out of oxygen and glucose that are normally supplied in blood. To compensate, they send a chemical SOS, which triggers formation of new vessels to nourish the tumor, a process called angiogenesis. It has been demonstrated that Genistein is the most potent amongst several plant derived inhibitors in preventing angiogenesis.\(^25\)–\(^26\) A recent study have shown that Genistein blocks the action of a transcription factor known as CCAAT binding factor.
This protein normally binds to an important genetic “motif” in DNA and triggers the stress genes. Genistein adds phosphorus to the binding factor, neutralizing it, so the cancer cell starves, withers and dies. Crucially, the researchers found that Genistein has no effect on normal and healthy cells, which are not dividing rapidly like cancer cells. The fact that epidemiological studies have revealed that people consuming the traditional soy-rich Japanese diets are less prone to breast and prostate cancers, supports the hypothesis that Genistein may prevent the development of solid tumor growth by inhibiting neovascularization which would, in turn, interfere with tumor growth.

**Antioxidant effects**

Reactive oxygen or free radicals are produced constantly in the body as the by-products of normal metabolism. Indeed, they are deliberately generated to fight infection caused by bacteria and viruses. However, in excess they can cause damage to key bio-molecules such as membranes, DNA, protein and also the cholesterol-carrying particles called low-density lipoproteins (LDL). Genistein has been shown to be a more potent antioxidant than other isoflavones and is also capable of inhibiting free radical oxygen species generation by xanthine oxidase. Reactive oxygen species are known to play an important role in mutagenesis and carcinogenesis. Genistein may also increase the body’s production of superoxide dismutase (SOD), a powerful antioxidant that quenches superoxide radicals. Genistein also appears to function as a SOD mimic. A recent research study has demonstrated that isoflavones can reduce the long-term risk of cancer by preventing free radical damage to deoxyribonucleic acid (DNA). According to the research, Genistein is the most potent antioxidant among the isoflavones, followed by Daidzein.

**Induction of apoptosis and differentiation of tumor cells**

Apoptosis (programmed cell death) is a significant event in the physiological and pathological situation that controls the development, differentiation and regression of tumor cell. On the basis of in vitro results, it has been proposed that Genistein antagonizes tumor cell growth through cell cycle arrest and induction of apoptosis, or though cell cycle arrest alone.

**Inhibition of multi-drug resistance to anticancer drugs**

Multi-drug resistance (MDR) is the simultaneous resistance to several types of commonly used anti-neoplastic agents and it leads to the failure of cancer chemotherapy due to decreased drug accumulation in resistant cells. These cells contain a protein that moves most conventional cancer drugs out of the nucleus making them less effective. In vitro studies have shown that Genistein is capable of reversing decreased drug accumulation and decreasing the resistance of cells to a number of anti-cancer drugs.

**Suppression of the stress response**

A recent study from the University of Southern California’s School of Medicine found that Genistein suppresses the production of harmful stress proteins in cells. These stress proteins include heat shock proteins (HSPs) and glucose-regulated proteins (GRPs) normally help cancer cells survive destruction by the immune system. In hard times, cells turn on these stress response proteins to protect the body. But in cancer cells, it seems that these proteins may inadvertently worsen disease, helping tumor cells to elude the body’s immune system and resist chemotherapy and other cancer treatments. Moreover, the induction of stress proteins in tumor cells has been shown to protect them against programmed cell death. The study reports “Genistein used either intravenously or orally offers an alternative, pharmacological approach for the suppression of the mammalian stress response. The anti-cancer effects of Genistein may be related to its ability to reduce the expression of stress response-related genes.” Furthermore, Genistein may potentially be useful in the prevention of cancer in addition to its treatment. There is intriguing evidence from animal experiments hinting that early consumption of Genistein can reduce the risk of cancer later in life. In an experiment at the University of Alabama, rats were fed Genistein for three days before they entered puberty and later exposed to a number of cancer-causing chemicals. The rats receiving Genistein developed half the tumors than rats given a placebo. It can be concluded that Genistein may potentially be useful in the treatment of cancer in addition to its prevention.
Genistein and Breast Cancer

The incidence of breast cancer has increased dramatically over the past two decades. Breast cancer is the second most common cause of death in American women, and is the first cause of death in those between the ages thirty-five and forty-four. This survey places breast cancer as the most common cause of cancer deaths in women, behind only lung cancer mortality rates. However, there are striking differences in breast cancer rates among countries. For example, American women are four times as likely to die from breast cancer as Japanese women. These differences seem to be related to environmental factors rather than genetic factors. Estrogens (female sex hormones) are thought to promote breast and endometrial cancer. In order for estrogen to stimulate the growth of breast cancer cells it needs first to bind to its cell surface receptor, the estrogen receptor. That, in turn, could keep out some of the naturally produced estrogen and thereby reduce its ability to cause the production of cancer cells. A research suggests that Genistein inhibit proliferation of cancer cells similar to estrogen receptors in breast cancer cells. Genistein also inhibits breast cancer cells that are estrogen-independent. The results indicate that Genistein antiapoptotic functions in human breast cancer cells may be used as a preventive as well as a therapeutic agent. A study investigated the effect of Genistein on estrogen receptor binding and cell proliferation, which indicates that “Genistein is unique among the flavonoids tested, in that it has potent estrogen agonist and cell growth-inhibitory actions over a physiologically relevant concentration range.” Another theory about mechanism of Genistein for reducing the risk of breast cancer is that it results in less frequent exposure to estrogen by lengthening the menstrual cycle. Studies indicate an increase in the length of the follicular phase where the mitotic rate of breast tissue were fourfold less (low estrogen levels) than in the luteal phase (re: high estrogen levels, the length of this phase was unaltered) was observed, resulting in an overall lengthening of the menstrual cycles. The menstrual cycles of the women increased by two-and-a-half days. Over a lifetime there would be fewer cycles, less exposure to estrogen and thus potentially a lowered risk for breast cancer.

Genistein and Prostate Cancer

Despite the same incidence of latent and small prostatic carcinomas as in the Western countries, the mortality in Japan and some other Asian countries is low. In the United States prostate cancer strikes one in 11 men and causes 38,000 deaths per year. Asian men also fall victim to the disease; but prostate tumors in the Far East are much slower growing, and men there die from other causes long before their tumor become lethal. (They also die later in many cases. The average life expectancy for men in Japan is four to five years longer than for U.S. men).

Estrogen, the female hormone, can slow the growth of prostate tumors by interfering with the working of the male hormone testosterone. Testosterone, like estrogen, has its “good” and “bad” sides. That is, it is needed for the host of male characteristics, from muscles and beards to functional erections. But if a man develops prostate cancer, testosterone can serve to hasten its progression. Estrogen, however, slows testosterone production and thereby retards tumor growth. In fact, some men with prostate cancer elect to undergo estrogen therapy to treat the disease. The problem is that it has undesirable side effects, including a loss of libido, a diminished ability to achieve and maintain an erection, and a certain amount of breast development.

A study assayed the isoflavonoids in plasma of Japanese men and found Genistein occurring in the highest concentration. Other studies confirmed these results in other Asian countries. The high Genistein level may inhibit the growth of prostatic cancer in Japanese men, which may explain the low mortality from prostatic cancer in Japan and may be in other Asian countries. Just as Genistein may be able to help women fight breast cancer, it may also be able to help men by preventing cancer of the prostate, a gland that produces fluid that carries sperm.

Numerous studies indicate that Genistein might primarily influence human prostate cancer development by inhibition of cell proliferation, and delay its metastasis. A study found Genistein and its metabolite, Genistin, to be the most effective phytoestrogen in inhibiting the growth of prostatic cell lines. The inhibition of cell growth by Genistein is accompanied by the suppression of the DNA synthesis and the induction of apoptosis. Some in vitro studies show that Genistein has potential as a therapeutic agent for Benign Prostatic Hypertrophy (BPH) and prostate cancer. Thus, epidemiological studies as well as cell culture and animal experiments...
provide evidence, which suggests that Genistein is protective and lowers the risk of prostate cancer during the promotional phase of the disease.

**Genistein and Colon Cancer**

There was an estimated 47,700 deaths from colon cancer during 1998 (10% of all cancer deaths). To date, there have been eight case-control studies on the relationship of soy and colorectal cancer in China, Japan and ethnic Japanese living in the United States. Some of the studies found soy to be protective with frequent consumption of soybeans or tofu decreases colon cancer risk. Another case control study involving 488 matched pairs, age fifty-four to seventy years, found that higher consumption of tofu or soybeans was inversely associated with adenomatous colorectal polyps, which are precursors to colorectal cancer. Those consuming one or more servings of soybeans per week showed half the risk of polyps compared to those not consuming soy.

Another studies using animal models have shown that Genistein reduces the number of aberrant (atypical) formed crypts (aberrant crypts are precursors of colon cancer). Maurice Bennik investigated the number of colonic tumors appearing in rats when they were challenged with a carcinogen. Different types of soy had differing effects on the number of colonic tumors. Animals on the low-fiber diet without soy had up to five tumors. The animals that were given soy flakes got one tumor. Animals that were given soy concentrates had three tumor. The animals that were given soy flour or Genistein, had no tumors. A study done to determine the effects of Genistein and Daidzein on the growth of human colon cancer cells in vitro found that growth was inhibited significantly.

**Genistein and Ovarian Cancer**

Ovarian cancer is the fourth most common cause of cancer death in women. Furthermore, there has been a steady increase in the age-adjusted cancer death rates in the past 25 years in the United States. Women in China and Japan have historically had lower levels of ovarian cancer, as well as lower rates of endometrial and breast cancer, in part because of the high soy content in their diet. A recent study showed a synergistic action of TR (Tiazofurin - an effective agent that produces differentiation and apoptosis in cancer cells) and Genistein in ovarian cell carcinoma.

A study by the Cancer Research Center of Hawaii found that women who ate the highest amount of phytoestrogen rich foods had a 54 percent reduction in endometrial cancer risk compared with those who consumed lesser amounts. Another study investigated the ability of Genistein to alter response of resistant ovarian cancer cell lines to chemotherapy. Genistein stimulated the drug accumulation in cancer cell lines by modulating the passive permeability of the plasma membrane.

**Genistein and Other Cancers**

More than 100 studies have found that in vitro, Genistein inhibits the growth of a wide range of cancer cells including those that are not hormone-dependent, i.e. melanoma, lung, leukemia and lymphoma, bladder.

**Melanoma (Skin cancer)**

A study conducted for examining the effects of Genistein on the growth of melanoma cells in mice in vivo and in vitro. The results provide additional evidence suggesting that Genistein retards the growth and promotes differentiation of melanoma cells. Another recent study conducted in New York showed that Genistein had anti-initiation and anti-promotional effects on skin carcinogenesis. The traditional forms of chemotherapy have little effect on malignant melanoma. Several tyrosine kinase inhibitors were examined for their effect on the growth and differentiation of malignant human melanoma cells. The tyrosine kinase inhibitors inhibited cell proliferation, but only Genistein and 2,5-dihydroxycinnamate were able to induce specific cell-cycle alteration and morphologic changes in human malignant melanoma cells. The American Academy of Dermatology, 1998, Awards for young investigators in Dermatology is looking for studies on photoprotective action of Genistein and its relevance to clinical dermatology.

**Lung cancer**

A study linking Genistein's inhibition of protein tyrosine kinase to lung cancer treatment and prevention. When Genistein and another inhibitor, tyrphostin-25, were used to block the activation of protein kinase, cultured small-cell lung-cancer cells matured and died a "natural" death of apoptosis. Genistein showed biological exerted effects due to the modulation of cell growth, cell death, and cell cycle regulatory molecules.
Another recent in vitro study investigated the effect of Genistein and Daidzein on inhibition of lung metastasis. The isoflavone, Daidzein had no significant effect on the reduction of lung metastasis while Genistein significantly inhibited lung tumor formation and increased the life span of the tumor-bearing animals. In another study, animals with lung carcinoma undergoing cytotoxic therapy were given Genistein and Sumarin (angiogenic inhibitor). With this treatment combination, 40% of the animals were cured. The authors concluded that, "The results of these studies indicate that anti-angiogenic agents can be very useful additions to treatment regimens for solid tumors." Another study in vitro showed that Genistein inhibits tyrosine kinase, which plays a dominant role in the development of fetal lung cancer in human lung cancer cells.

**Leukemia and lymphoma**

A recent in vitro study, Genistein indicated potent cytotoxicity against human leukemia cells. Another study had also demonstrated that Genistein protects leukemia cells from toxicity of anti-cancer treatment agents. The effect of anti-leukemic-cell efficacy of naturally occurring and synthetic flavonoids and lignans on human leukemic cells were examined and the differences between anti-cell-proliferative activity and cytotoxicity of these compounds were compared with those of clinical anti-cancer agents. The results showed that Genistein was among several compounds, which had the strongest anti-cancer effect, almost equivalent to the effects of current anti-cancer agents.

Tyrosine kinase might play an key role in the control of normal and neoplastic cell growth. A study done showed that both quercetin and Genistein (a well-known tyrosine-kinase inhibitor) are able to down-modulate tyrosine kinase activity with different effects. Quercetin induced apoptosis while Genistein brought about both differentiation and apoptosis.

Continuous exposure of leukemia cells to increasing doses of Genistein resulted in statistically significant suppression of their growth. The authors of the study concluded that the strong inhibition of leukemia cells by Genistein "strongly supports the use of Genistein for marrow purging". Other studies confirmed this conclusion.

**Bladder cancer**

A study examined in vitro effects of soy isoflavones (Genistein and Daidzein) on the progression of bladder cancer. Soy isoflavones reduced angiogenesis, increase apoptosis and reduced proliferation while the normal bladder mucosa was not affected. The authors suggest that "soy isoflavones can inhibit bladder tumor growth though a combination of direct effects on tumor cells and indirect effect on the tumor neovasculature." The effect of Genistein on the growth of human bladder cancer cells was examined and it was found that Genistein had a similar inhibitory effect on bladder cancer cells as tyrophostin, a pure synthetic inhibitor. Another study found Genistein to be the most effective substance (compared to 7-hydroxycoumarin and quercetin) in the inhibition of cell growth in human bladder carcinoma cells.

**Conclusion**

It may be concluded that Genistein is potentially beneficial in preventing chronic diseases such as cancers. In view of the multi-faceted benefits of this soy isoflavone, it may seem prudent to shift towards soy-rich diets. However, soy foods contain the b-glucoside, Genistin and conversion to the active aglycone form is dependent upon the activity of the gut microflora, which in turn results in wide variations in the bioavailability of Genistein from soy products. Consequently, the administration of Genistein in the form of a standardized dietary supplement is a convenient means of ensuring improved absorption with the resulting potential health benefits. Thus, the soybean may be small in size but science is confirming that it packs a huge punch when it comes to protecting the health.

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