Cellular and Physiological Effects of Soy Flavonoids

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Abstract: Recent experimental and epidemiological studies have provided convincing evidence for a variety of health benefits derived from the consumption of soy and soy food products. For example, soy isoflavones are felt to protect against different cancers, cardiovascular disease, and bone loss. Many studies have demonstrated the effect of soy isoflavones on specific target molecules and signaling pathways, including but not limited to, cell proliferation and differentiation, cell cycle regulation, apoptosis, angiogenesis, cell adhesion and migration, metastasis, and activity of different enzymes. Isoflavones also share structural homologies with estrogens and are therefore classified as phytoestrogens with weak estrogenic properties. Since isoflavones bind to estrogen receptors (ERα and ERβ), they are considered to be possible estrogen receptor modulators. However, isoflavones can also exert biological effects independent of their phytoestrogenic activities. Recent studies suggest beneficial health effects of soy and recommend increasing the intake of isoflavone-rich soy protein to the level of intake commonly used in Asian countries.

Keywords: soy, isoflavones, cancer, cardiovascular disease, osteoporosis, and signaling pathways.

INTRODUCTION

Soy and soy products are being increasingly used by the US population [1]. Experimental and epidemiological evidence from Asian countries suggest that soybean-derived products can prevent some diseases such as cancer, cardiovascular disease, and osteoporosis [2 – 4]. Soybeans contain soy protein, isoflavones, fiber, and saponins and are usually consumed as fermented and non-fermented soy foods, such as tofu, miso, doenjang, and tempeh, as well as whole soybeans, soy nuts, soymilk, soy yogurt, and soy cheese [5]. Different soy components are reported to have potential health benefits. For example, soy protein containing isoflavones can prevent cardiovascular disease [6], and soy isoflavones themselves are suggested to prevent the development of cancer and osteoporosis [7, 8]. Since isoflavones are the predominant biologically active components in soy and soy products, the targeted modulation of signaling pathways by isoflavones may help prevent and/or treat different diseases.

ISOFLAVONES

More than 4,000 different polyphenolic compounds have been identified in the plant kingdom. These flavonoids have been described and categorized into flavonol, flavone, flavan 3-ol, flavanolol, flavanone, and isoflavone groups [9]. Isoflavones are predominantly flavonoids identified in soybeans in the form of aglycones (genistein, daidzein, and glycitein); glucosides (genistin, daidzin, and glycitin); acetylglucosides (6''-*O*-acetylgenistin, 6''-*O*-acetyldaidzin, and 6''-*O*-acetylglycitin); and malonylglucosides (6''-*O*-malonylgenistin, 6''-*O*-malonyldaidzin, and 6''-*O*-malonylglycitin) [10]. The structures of commonly found isoflavones in soy are shown in Fig. (**1**). The isoflavone content of raw soybeans is about 1mg/g, and traditional soy food contains approximately 0.2-0.4mg/g isoflavones in the fresh product [11-13]. Therefore, the consumption of soy and soy products in Asian countries (corresponding to 30-40 mg of isoflavones per day) increases serum levels of isoflavones [14, 15]. The traditional fermented soy foods contain higher levels of aglycones, whereas non-fermented foods demonstrate higher levels of glucosides [16, 17]. The soy glucosides are deglycosylated by β-glucosidase in gut microflora to the biologically active aglycones genistein and daidzein, which can be detected in plasma and urine in micromolar quantities [18-21].

BIOLOGICAL ACTIVITY OF ISOFLAVONES

Most prominent among soy isoflavones is genistein (4', 5, 7-trihydroxy-isoflavone), which is felt to be responsible for the major biologic activities of soy. Genistein was originally described and widely used as a specific inhibitor of protein tyrosine kinase (PTK) [22]. Genistein also inhibits topoisomerase II [23] and ribosomal S6 kinase [24]. In addition, genistein inhibits lipid peroxidation and demonstrates antioxidant properties [25, 26].

Since genistein shares structural similarities with estrogens, it was postulated to act as an estrogen antagonist [27]. More recently, the specific binding of genistein to estrogen receptor α (ERα) and ERβ has been demonstrated [28]. Although the relative levels of transactivation were the same for ERα and ERβ, genistein showed higher binding activity for ERβ compared with ERα [28]. An *et al.* demonstrated higher transcriptional activity for ERβ than ERα, indicating that genistein was a potent agonist for ERβ and that its transcriptional activity was dependent on recruited co-regulators by phytoestrogens [29]. Furthermore, estrogen receptor activity also depended on the co-

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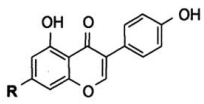
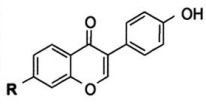
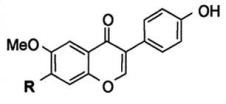
R = OH	Genistein	Daidzein	Glycitein
R = glucose	Genistin	Daidzin	Glycitin
R = 6"-O-Acetylglucose	6"-O-Acetylgenistin	6"-O-Acetyldaidzin	6"-O-Acetylglycitin
R = 6"-O-Malonylglucose	6"-O-Malonylgenistin	6"-O-Malonyldaidzin	6"-O-Malonylglycitin
			

Fig. (1). Soybean Isoflavones

localization and subsequent homo- or heterodimerization of ERα and ERβ. Co-expression of ERα and ERβ enhanced the agonistic effect of genistein [30]. Isoflavones can also mediate their antiestrogenic effects without interacting with estrogen receptors by inhibiting enzymes involved in estrogen metabolism [2]. Thus, isoflavones inhibit aromatase, a cytochrome P_{450} enzyme responsible for the conversion of androgens to estrogenic steroids [31]. Furthermore, isoflavones inhibit 17β-hydroxysteroid oxidoreductases, which convert the weak estrogen, estron, into the more potent estrogen, estradiol, and 3β-hydroxysteroid dehydrogenase, which is responsible for the production of estrone [32, 33].

GENISTEIN AND CANCER

The majority of studies with genistein focus on mechanisms of cancer prevention by isoflavones. Genistein induces differentiation and inhibits growth of leukemia and melanoma cells [34, 35] and inhibits the growth of prostate, breast, non-small-cell lung cancer, and head and neck squamous carcinoma cells [36-41]. Interestingly, although

Table 1. Genistein in the Regulation of Cell Cycle and Apoptosis

Target Molecule		Biological Effect	Cell Type	References
p21	↑	G2/M arrest, apoptosis	breast cancer	38, 40,
Bax	↑			43, 47
cyclin B1	↓			
cdc2	↓			
cdk2	↓			
p53	↓			
Bcl-2	↓			
p21	↑	G2/M arrest, apoptosis	prostate cancer	44, 47
Bax	↑			
cyclin B1	↓			
Bcl-2	↓			
p21	↑	G2/M arrest, apoptosis	non-small-cell lung cancer	39, 47
Bax	↑			
Bcl-2	↓			
p21	↑	G2/M arrest, apoptosis	head and neck cancer	41, 47
Bax	↑			
cyclin B1	↓			
cdk1	↓			
Bcl-2	↓			
p21	↑	G2/M arrest	choroidal melanoma	45
cdk2	↓			
p21	↑	G0/G1 arrest	melanoma	46
cdk2	↓			

Table 2. Inhibitory Effects of Soy Isoflavones on Breast Cancer Cells

	AGLYCONES			GLUCOSIDES		
	Genistein	**Daidzein**	**Glycitein**	**Genistin**	**Daidzin**	**Glycitin**
NF-κB	++	+	-	-	-	-
AP-1	++	+	-	-	-	-
uPA	++	++	-	-	-	-
Adhesion	++	++	-	-	-	-
Migration	++	++	++	++	++	++

For more details see reference 53

genistein inhibits the growth of both ERα- and ERβ-positive (MCF-7) and ERα-negative and ERβ-positive (MDA-MB-231) breast cancer cells at high concentrations (> 5μM), lower concentrations of genistein (< 1μM) stimulate the growth of MCF-7 cells (ERα- and ERβ-positive). This biphasic effect is attributed to genistein acting through estrogen receptors at low (physiologic) concentrations while at higher concentrations genistein acts *via* estrogen receptor-independent mechanisms [2]. Conflicting data exist regarding the interactions between estrogens and genistein, suggesting that more complicated mechanisms than simple receptor interactions are involved [2].

Cancer cells are characterized by deregulated cell growth and cell proliferation. Therefore, agents which are able to induce cell cycle arrest by inhibiting cell signaling proteins may have clinical utility [42]. The growth inhibiting effects of genistein partially result from inhibition of the cell cycle, as demonstrated by genistein-induced G2/M cell cycle arrest in breast, prostate, gastric, non-small-cell lung cancer, and melanoma cells [38-41, 43-45]. Cell cycle arrest in G2/M induced by genistein may result from down-regulation of cyclin B in non-small-cell lung and breast cells and prostate cancer cells [39, 40, 44]. In addition, genistein stimulates expression of cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ in cancers cells compared to control cells [38-40, 43-45]. On the other hand, genistein arrested fibroblast and melanoma cells at G0/G1 phase and induced expression of $p21^{WAF1/CIP1}$, suggesting that effects of genistein on the cell cycle could be cell line dependent [46]. Thus, genistein likely inhibits growth of cancer cells through modulation of the expression of genes that are involved in the regulation of the cell cycle and cell growth [47].

Genistein also induces apoptosis in different cancer cells including breast, prostate, non-small-cell lung cancer, and head and neck squamous carcinoma cells by mechanisms employing up-regulation of the expression of the pro-apoptotic protein Bax and down-regulation of the anti-apoptotic protein Bcl-2 [47]. Therefore, genistein specifically targets different genes involved in cell cycle regulation and apoptosis (Table 1).

Another important characteristic of cancer cells is their invasive and metastatic potential. Tumor invasion and cancer metastases are multifaceted, interrelated processes involving cell adhesion, proteolytic degradation of tissue barriers, and cell migration [48]. Inhibition of one of these important events would be of potential therapeutic interest, because it could inhibit cancer metastasis. We recently demonstrated that inhibition of the constitutively active transcription factors AP-1 and NF-κB suppressed secretion of urokinase plasminogen activator (uPA), which resulted in the inhibition of cell migration of highly invasive and metastatic breast cancer cells [49, 50]. Interestingly, Davis *et al.* [51] demonstrated that genistein abrogated TNF-α-induced and constitutive NF-κB DNA-binding activity in prostate cancer cells, and the inhibition of NF-κB activation by genistein was mediated through the Akt signaling

Table 3. NF-κB Controlled Genes Involved in Cancer Metastasis

Gene	Function	Biological Effect
Bcl-2	protection against apoptosis	survival
Bcl-xl	protection against apoptosis	survival
cyclin-D1	regulation of cell cycle	proliferation
FGF-2	fibroblast growth factor	growth
MMP-9	proteolytic activity	degradation of tissues
uPA/uPAR	cell adhesion, migration, proteolytic activity	invasion and metastasis
TGF-β	transforming growth factor	growth
VEGF	vascular endothelial growth factor	angiogenesis

pathway [52]. Moreover, our data show that genistein inhibits constitutively active NF-κB and AP-1 in reporter gene assays in highly invasive breast cancer cells [53]. This inhibition results in the down-regulation of secretion of uPA and the subsequent inhibition of cell adhesion and cell migration. Furthermore, all the tested soy isoflavone aglycones (genistein, daidzein, glycitein) and glucosides (genistin, daidzin, glycitin) markedly reduce motility of highly invasive breast cancer cells. However, only genistein and daidzein inhibit constitutively active NF-κB and AP-1, suppress secretion of uPA, and inhibit cell adhesion (Table 2). Therefore, our results demonstrate that dietary soy isoflavones can inhibit adhesion and motility of highly invasive breast cancer cells by uPA-dependent and -independent mechanisms.

Because constitutively active NF-κB has been recognized as a characteristic of highly invasive cancers of different origin [54] and because NF-κB controls expression of proteins [55], the activities of which are linked to the invasive and metastatic behavior of cancer cells (Table 3), NF-κB has been suggested as a potential target for anticancer therapies [56]. Thus, proteasome inhibitor PS-341, which inhibits NF-κB, is being evaluated in clinical studies for the treatment of refractory hematologic malignancies and as an adjuvant approach in combination with chemotherapy or radiation for a variety of cancers [57, 58]. As mentioned above, the soy isoflavone genistein inhibits NF-κB and could have potential therapeutic effects.

In addition to epidemiological studies suggesting that soybeans play an important role in reducing the risk for breast, colon, stomach, and uterine cancers [59], *in vivo* studies show direct effects of genistein in animal models of cancer. For example, genistein inhibits tumor growth and stimulates apoptosis in nude mouse xenografts of breast cancer cells [60]. Furthermore, genistein inhibits angiogenesis by decreasing vessel density and decreasing the levels of vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-β1) [60]. The growth of prostate cancer tumors in animals injected with prostate cancer cells is also inhibited by genistein [61, 62]. In chemopreventive studies, genistein suppresses development of chemically induced breast and prostate cancers [63, 64]. Furthermore, topically applied genistein inhibits the initiation and promotion of skin cancers in mice [65]. On the other hand, dietary genistein stimulates the growth of subcutaneously injected estrogen responsive breast cancer cells (ERα- and ERβ-positive) in ovariectomized athymic mice [66]. In addition, isolated soy protein containing genistein increases tumor growth in a dose-dependent manner similar to genistein alone [67]. Thus, in a low estrogen environment, genistein can be estrogenic and have a proliferative effect on breast tissue, whereas in a high estrogen environment, genistein can have an anti-estrogenic and anti-proliferative effect [2].

GENISTEIN AND CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the leading cause of death in the United States and dietary factors are recognized to play crucial roles in the development of CVD*. Interestingly, as in cancer, epidemiological studies suggest that Asian populations with a high consumption of soy product have a lower incidence of CVD [68]. Diet has been shown to modulate risk factors for the development of heart disease such as hypercholesterolemia, hypertriglyceridemia, high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, hypertension, diabetes, and obesity [69]. The effect of soy products on blood cholesterol levels was originally described 60 years ago, and the major component was identified as a soy protein [70]. Recent studies demonstrate that soy protein contains isoflavones, which can significantly decrease the plasma levels of total and LDL cholesterol without affecting the concentrations of triglycerides or HDL cholesterol [71]. In addition, isolated soy protein without isoflavones does not significantly reduce plasma concentrations of total or LDL cholesterol, suggesting that the cholesterol-lowering effect of soy protein is due to the presence of isoflavones [71]. On the other hand, Nestel *et al.* failed to find changes in plasma lipid levels after consumption of soy isoflavones, but demonstrated improved systemic arterial elasticity [72].

Since oxidative damage has been implicated in the development of heart disease, the antioxidant properties of isoflavones may also have a protective effect. For example, genistein inhibits LDL oxidation and inhibits bovine aortic endothelial cell- and human endothelial cell-mediated LDL oxidation and protects vascular cells from damage by oxidized LDL [73]. Genistein also demonstrates an anti-hypertensive effect in spontaneously hypertensive rats [74]. Lowering of blood pressure may have additional protective effects.

Genistein attenuates post-ischemic depressed myocardial function and enhances the myofilament calcium sensitivity in rat myocardium [75]. Furthermore, genistein administered intraperitoneally demonstrates immunosuppressive effects *in vivo* [76]. A high protein soy diet and intravenous genistein significantly prolongs heart survival and delays rejection of rat cardiac allografts [76].

Table 4. Genistein Effects on the Cardiovascular System

Total cholesterol levels	↓
LDL cholesterol levels	↓
LDL oxidation	↓
Hypertension	↓
Arterial elasticity	↑
Calcium sensitivity	↑
Immunosuppression	↑
Rejection of cardiac allografts	↓
Estrogenic activity	

Genistein is a phytoestrogen with estrogenic activity. Estrogen effects on the cardiovascular system can be direct, mediated through estrogen receptors, or indirect (i.e.

* American Heart Association. *1999 Heart and Stroke Statistical Update*; American Heart Association: Dallas, ***1998***.

mediated via changes in serum lipoproteins and antioxidant effects) [77]. Thus, genistein possesses cardioprotective effects, which are mediated by multiple mechanisms (Table 4).

GENISTEIN AND OSTEOPOROSIS

The incidence of osteoporosis among the US population is constantly growing, and presently, 10 million adults are suffering from low bone mineral densities. Although post-menopausal osteoporosis is prevalent among women, osteoporosis is not a sex-exclusive disease [78]. It is well recognized that dietary calcium is a major factor preventing the development of osteoporosis [79]. However, early studies recognized the importance of estrogen on bone density and the improvement of calcium status by estrogen [80, 81]. Although hormone replacement therapy (HRT) prevents bone loss in post-menopausal women, the increased risk for breast and endometrial cancers and cardiovascular disease accompanying HRT resulted in a search for new therapies lacking side effects [82]. Data from numerous studies suggest that diets rich in phytoestrogens can have a protective effect against bone loss [78].

Since genistein is a prominent phytoestrogen, numerous studies have evaluated the effect of genistein on bone cells. Two types of bone cells (osteoblasts and osteoclasts) are involved in bone remodeling. Osteoblasts are responsible for the formation of bone and respond to the activity of osteoclasts, which are responsible for bone resorption. Therefore, the suppression of osteoclast activity prevents bone resorption. Genistein inhibits bone resorption by osteoclasts and suppresses osteoclastic protein synthesis *in vitro* and *in vivo* [83]. Genistein also induces expression of osteoprotegerin (OPG) and suppresses the production of interleukin-6 (IL-6), two regulatory cytokines involved in osteoclastogenesis [84]. The effect of genistein on OPG and IL-6 is probably mediated via a genomic pathway operating through estrogen receptors and gene expression mechanisms [84]. Genistein at low concentrations ($\leq$1μM) acts as an estrogen, stimulating osteogenesis and inhibiting adipogenesis. At high concentrations (>1μM), genistein acts as a ligand of peroxisome proliferator-activated receptor-γ (PPAR-γ), leading to up-regulation of adipogenesis and down-regulation of osteogenesis. Therefore, genistein effects are controlled by two different transcriptional factors, ERs and PPAR. Both have effects on osteogenesis and adipogenesis and are dependent on genistein concentration [85].

In vivo studies demonstrate beneficial effect of genistein upon bone loss. For example, bone mass (femoral weight) of ovariectomized rats treated with genistein is significantly higher than in the control group [82]. Furthermore, genistein reduces both trabecular and compact bone loss after ovariectomy, suggesting that this protective effect differs from the estrogen effect because it depends on stimulation of bone formation rather than on suppression of bone resorption [86]. Furthermore, treatment with low doses of genistein (0.7mg/day) prevents trabecular bone loss in ovariectomized mice without hypertrophic effects on the uterus, whereas higher doses, of genistein (5mg/day) induce uterine hypertrophy [87]. Interestingly, bone mineral density of the femur is markedly decreased in male castrated mice, and this bone loss is prevented by treatment with genistein, suggesting that soybean isoflavones prevent bone loss due to androgen deficiency in males [88]. Therefore, genistein can exert its effects on bone remodeling through the estrogen receptor as well as via non-estrogenic mechanisms.

The experimental studies described above are in agreement with human studies. High dietary intake of soy products is associated with increased bone mass in post-menopausal Asian women [89, 90]. Interestingly, high dietary phytoestrogen intake does not show any effect on the bone mineral density of pre-menopausal women [90]. However, another study demonstrated that soy intake had a significant effect on the maintenance of spinal bone mineral density in women 30- to 40- years of age [91]. Because the intake of soy food during adolescence may reduce the risk of breast cancer in later life [92], it is possible that soy isoflavones can also exert their biological effects in a spatio-temporal manner.

SUMMARY

A variety of health benefits from soy and soy food consumption are suggested by epidemiological as well as experimental studies. Although this short review briefly focused on prevention of cancer, cardiovascular disease, and osteoporosis, soy isoflavones are proposed to have beneficial effects upon other tissues and diseases (Table 5). As emphasized at the Fourth International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, the American public can benefit from an increased intake of isoflavone-rich soy protein. The consumption of even 10g (typical of Asian intake) of isoflavone-rich soy protein per day may be associated with health benefits [1]. Furthermore, replacing some of the animal protein in the diet with soy would help to restore the balance of animal-to-plant protein and would also increase the intake of other non-animal beneficial food components, such as omega-3 fatty acids[†].

Table 5. Beneficial Effects of Genistein and Soy Diets on Human Health

Organ/tissue	Effects on
Bladder	cancer
Bone	osteoporosis
Brain	cognitive function
Breast	cancer
Heart	cardiovascular disease
Lung	cancer
Prostate	cancer
Vagina	post-menopausal dryness

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