Isoflavone Genistein: Photoprotection and Clinical Implications in Dermatology

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ABSTRACT Genistein is a soybean isoflavone with diverse biological activities. It is a potent antioxidant, a specific inhibitor of protein tyrosine kinase, and a phytoestrogen. In recent years, increasing evidence has accumulated that this natural ingredient shows preventative and therapeutic effects for breast and prostate cancers, postmenopausal syndrome, osteoporosis, and cardiovascular diseases in animals and humans. In the past decade we have conducted a series of studies and demonstrated that genistein has significant antiphotocarcinogenic and antiphotoaging effects. Genistein substantially inhibits skin carcinogenesis and cutaneous aging induced by ultraviolet (UV) light in mice, and photodamage in humans. The mechanisms of action involve protection of oxidative and photodamaged DNA, downregulation of UVB-activated signal transduction cascades, and antioxidant activities. In this article, we review the biological activities of genistein, as well as published and unpublished research from our laboratory. In addition, we discuss the potential application of genistein to clinical dermatology.


KEY WORDS: • genistein • photocarcinogenesis • photoaging • dermatology • chemoprevention

Most human diseases have been found to be associated with environmental factors, and the role of dietary modification in reducing disease risk has drawn widespread attention. Differences in cardiovascular disease and cancer mortality in humans exist worldwide and depend on lifestyle and dietary habits (1–3). Soy diets were associated with the reduced incidence of cardiovascular disease, osteoporosis, and certain cancers in humans (4–6). Animal studies also showed that soybean diets block the oxidation of lipoproteins, reduce atherosclerosis of blood vessels, and inhibit radiation- and carcinogen-induced tumors of various tissues in animals (4–6).

Genistein is an isoflavone that was first isolated from soybeans in 1931 (7). Genistein displays a very low level of toxicity in most animal species (6). It was not until the 1950s that the estrogenic and uterotropil activity of genistein was identified (8–10). Genistein and other related isoflavones were found to increase uterus weight in several animal species and to competitively bind to estrogen receptors (9–12). The estrogenic effect on the uterus was not observed in women consuming high amounts of soy (6). However, the estrogenic potency of genistein is much weaker than that of physiological steroids, being only ~1/10,000 to 1/50,000 for that of estril or estradiol (12). Genistein also exhibits antioxidant properties, preventing the hemolysis of red blood cells by dialuric acid or by hydrogen peroxide (13,14) and inhibiting microsomal lipid peroxidation induced by an Fe2+-ADP complex and NADPH (15). In addition, genistein and its related isoflavones inhibit the NADH oxidase and respiratory chain in rat liver mitochondria (16). Genistein and its related isoflavones lack mutagenicity in Salmonella strains at a wide range of concentrations (17,18).

Chea et al. (20) found that genistein is the most potent inhibitor of P450-mediated activation of B[a]P of all tested isoflavones. Genistein has many beneficial effects on human health (4–6). It has been used as an alternative treatment for menopausal syndrome in women on the basis of epidemiologic studies that showed that Asian women consume more soy and have fewer symptoms of menopausal syndrome than non-Asian women (21–22). Genistein is also proposed as a treatment for osteoporosis for postmenopausal women and elderly men.
Hormone replacement therapy has been a mainstay for the prevention and treatment of osteoporosis in postmenopausal women, but estrogen increases the risk of breast cancer, and its use is limited if women have a family or personal history of breast and endometrial cancer. Convincing evidence exists that the consumption of soy isoflavone may decrease the formation of incidence of cardiovascular disease. A reduction of low density lipoproteins and an elevation of high density lipoproteins have been found in women on a soy protein diet containing a high concentration of isoflavones (24,25). Genistein potently prevents the oxidation of low density lipoproteins in vitro and in peripubertal monkeys (25,26). In addition, genistein and other soy isoflavones are thought to improve the arterial elasticity in menopausal women similarly to hormone replacement (27).

Although soybeans contain a number of ingredients with demonstrated anticancer activities, genistein is the most important agent that has been extensively investigated for its chemopreventive and anticancer activity (6). Genistein potently inhibits the activities of tyrosine protein kinase (TPK)³, topoisomerase II, and ribosomal S6 kinase in the cell culture (28–31). Genistein specifically inhibits the growth of ras-oncogene transfected NIH 3T3 cells without affecting the growth of normal cells (32) and diminishes the c-fos and c-jun expression in CH310T1/2 fibroblasts induced by platelet-derived growth factor (33). In addition, genistein inhibits topoisomerase II and ribosomal S6 kinase by stabilizing a cleavable topoisomerase-DNA complex (34) and modulating mRNA translation in vitro (35), which may lead to protein-linked DNA strand breaks, cell growth suppression, and differentiation and induction of several malignant cell lines (35–39). Genistein potently inhibits the production of certain cytokines (40) and eicosanoid biosynthesis (41) through inhibition of TPK, suggesting that genistein can modulate the inflammatory responses that are commonly involved in the promotional stage. Genistein has wide anticancer properties: it suppresses the proliferation of a variety of human gastrointestinal cancer cell lines (42), induces differentiation of leukemia cells (43), and inhibits endothelial cell angiogenesis relevant to tumor metastasis (44). The biotherapy potency of B-cell precursor leukemia was tremendously increased by targeting genistein to CD 19–associated tyrosine kinases (45).

Genistein was shown to arrest the growth and induce the differentiation of malignant melanoma in vitro (46) and effectively inhibit the metastasis of melanoma in animal models (47,48). Genistein more effectively inhibits pulmonary metastasis of malignant melanoma than does daidzein (48). We showed that genistein significantly inhibited 7,12-dimethyl anthracene (DMBA)-initiated, and 12-O-tetradecanoyl phorbol-13-acetate (TPA)-promoted carcinogenesis in a two-stage skin model (49,50). The mechanistic studies demonstrated that genistein inhibited carcinogen-induced production of reactive oxygen species and inflammatory response (51), upregulated antioxidant enzyme activities in tissues (52), and downregulated protooncogene expression in animals (53,54). In addition, genistein blocked carcinogen-induced formation of DNA adducts (49) and Fenton-reaction or tumor-promoted induced oxidative DNA damage (55). In addition, we showed that genistein induced differentiation of malignant cells by modulating TPK and N-myc expression (56).

**Effect of genistein on photocarcinogenesis and photoaging**

The incidence of human skin cancers has significantly increased in the past 20 y and continues to increase at an alarming annual rate of 4% (57). Therefore, the development of safe and effective preventive agents against photocarcinogenesis has become an important subject in dermatological research. Although numerous in vitro studies indicated that genistein has potential anticancer properties, evidence is lacking on whether genistein modifies skin carcinogenesis. We (49–56) demonstrated that genistein significantly inhibits ultraviolet (UV) light-induced oxidative DNA damage in purified DNA and cultured cells, and blocks UVB-induced c-fos and c-jun protooncogene expression in mouse skin. Kang et al. (University of Michigan, Kang, S. and Wan, Y., personal communication, 2001) found that genistein inhibited epidermal growth factor receptor (EGF-R) phosphorylation and metalloproteinase in human skin independent of the sunscreen effect. In the past 10 y, our laboratory has been studying the chemopreventive effects of genistein on cancer in animals and humans. The following is a review of our published and unpublished research.

**Genistein inhibits ultraviolet-B–induced skin carcinogenesis in mice.** We investigated the effect of topical and oral genistein on UVB-induced photocarcinogenesis in hairless mice. Topical genistein was used to test the protective effect...
Genistein inhibits UVB-induced acute and chronic photodamage in mouse skin. We also examined the effect of genistein on UVB-induced acute and chronic photodamage in hairless mice. In an acute sunburn study, mice were irradiated with daily UVB (1.8 kJ/m²) for 10 d. Topical application of 5 μmol of genistein 60 min before each UVB irradiation completely blocked UVB-induced acute skin burns (Fig. 4). In a chronic UVB exposure study, mice were irradiated with UVB twice weekly (0.6 kJ/m²) for 4 wk. The chronic exposure to low dose UVB increased skin roughness and wrinkling in mice (Fig. 5). The application of genistein before and after UVB exposure alleviated photodamage, with pre-UVB application showing stronger effects (Fig. 6). The histological examination showed that genistein substantially inhibited epidermal hyperplasia and reactive acanthoses with nuclear atypia induced by UVB (Fig. 6). The protective effects were histologically confirmed by quantifying the thickness and elastic fibers (data not shown). These experiments demonstrate that genistein substantially blocks the subacute and chronic UVB-induced cutaneous damage and histological alterations related to photoaging.

Genistein protects human skin against UVB-induced photodamage. We investigated the effect of genistein on UVB-induced erythema (sunburn) in the dorsal skin of six men with skin type II to skin type IV. Genistein was topically applied at 250 μg/cm² human skin substantially blocked erythema.
induced by different doses of UVB radiation (lane 3) whereas post-UVB application showed very little protection of cutaneous erythema (lane 4). Further study showed that as low as 0.1 µmol genistein effectively inhibited erythema induced by one minimal erythema dose of UVB (lane 5). Analyses of data from the six subjects showed that pre-UVB application of genistein significantly inhibited both cutaneous erythema and discomfort with a minimal effect on erythema (Fig. 8). This clinical study indicates that genistein effectively protects human skin against UVB-induced skin photodamage.

**Genistein inhibits UVB-induced DNA damage and restores proliferating cell nuclear antigen (PCNA) in vivo.** We previously showed that pre- and post-UVB application of genistein effectively suppressed UVB-induced expression of c-fos and c-jun mRNA in mouse skin (54). We further investigated the effect of genistein on UVB-induced DNA damage and expression of PCNA in mouse skin. Two types of DNA damage can be induced by UVB irradiation: oxidative DNA damage represented by 8-hydroxy-deoxyguanosine (8-OHdG) and photodynamically damaged DNA exemplified as pyrimidine dimmer (PD). The former is a hallmark of carcinogenesis and aging and the latter is a precursor of signature mutation of p53 genes. PCNA is profoundly expressed in proliferating and metastatic tumors and serves as a marker of DNA repair. In the study, hairless mice were topically treated with 1 and 5 µmol of genistein 60 min before UVB irradiation. Mice were killed 24 h after the UVB irradiation and skins were harvested for the above-mentioned biomarkers. UVB irradiation substantially induced formation of PD and 8-OHdG in mouse skin (Figs. 9B, 10B) compared to the sham irradiation (Figs. 9A, 10A). Genistein substantially inhibited both PD and 8-OHdG formation in a dose-dependent manner (Figs. 9C, D; 10C, D). In addition, PCNA expression in mouse skin was...
almost completely diminished 24 h after UVB exposure (Fig. 11B), and topical application of genistein fully restored the PCNA expression (Fig. 11C, D). Expression of PD, 8-OHdG, and PCNA is known to be associated with tumor initiation and promotion. Overproduction of PD and 8-OHdG is considered to be related to p53 and ras gene mutation. Thus, substantial suppression of UVB-induced DNA damage and PCNA expression restoration suggest that genistein may modulate UVB-mediated initiation and promotional activities.

**Genistein blocks UVB-induced phosphorylation of EGF-R and mitogen-activate protein kinase (MAPK) activation in vitro.** We measured the effect of genistein on filtered UVB-induced phosphorylation of EGF-R and MAPK in human keratinocytes. Cells were incubated with genistein for 2 h, washed, and then irradiated with UVB. Cell lysates were used

**FIGURE 6** Effect of genistein on histological alterations in mice exposed to UVB. Skh-1 hairless mice were treated topically with 5 μmol genistein 60 min before UVB irradiation at a dose of 0.3 kJ/cm² twice weekly for 4 wk. Mice were killed 24 h after the last UVB irradiation and skin specimens were taken for histology. (A) Negative control (sham irradiation); (B) vehicle plus UVB; and (C) 5 μmol genistein plus UVB.

**FIGURE 7** Effect of genistein on UVB-induced erythema in human skin. The study was performed in the phototherapy unit in the Department of Dermatology, Mount Sinai Hospital. UVB fluences used a range from 0 to 100 mJ/cm² as described in the text. Genistein was applied to dorsal skin either 60 min before or 5 min after UVB exposure. Photographs were taken 24 h after UVB irradiation. A minimal erythema dose (MED) for this individual was 40 mJ/cm². Lane 1: Vehicle plus UVB; lane 2: blank plus UVB; lane 3: pre-UVB application, 1 μmol genistein/cm² of skin plus UVB; lane 4: post-UVB application, UVB plus 1 μmol genistein/cm² of skin; and lane 5: dose response of topical genistein at a dose ranging 0.05—5 μmol. UVB dose was 1 MED (40 mJ/cm²).

**FIGURE 8** Effect of genistein on UVB-induced erythema and discomfort in human skin. Six healthy male subjects were recruited to the study, and the UVB irradiation was performed in the phototherapy unit in the Department of Dermatology, Mount Sinai Hospital. UVB doses used were two MED for the tested subjects. Genistein was applied to dorsal skin either 60 min before or 5 min after UVB exposure. Photographs were taken 24 h after UVB irradiation. Erythema was quantitated using a Minolta chromometer and discomfort scores were evaluated by two dermatologists blinded to the samples. (A) Erythema index; (B) discomfort score. Legend: (open bar) sham radiation, (solid bar) UVB radiation, and ( striped bar) genistein plus UVB radiation.
for assaying phosphorylation of EGF-R and activation of MAPKs using the Western blot technique. UVB irradiation substantially induced phosphorylation of EGF-R (Fig. 12A, lane 3) and MAPK (Fig. 12B, lane 2), and incubation with genistein dose-dependently inhibited the phosphorylation of EGF-R and MAPK. Phosphorylation of TPK-dependent EGF-R and activation of MAPK are thought to be involved in the initiation of transcription factors; release of inflammatory mediators, such as prostaglandins; and stimulation of cell proliferation. All of these events are relevant to the promoting activities. Thus, the inhibition of UVB-induced EGF-R phosphorylation and MAPK activation by genistein suggests its potential antipromotional effects.

Genistein protects mouse skin against photodamage induced by psoralen plus UVA (PUVA). PUVA therapy is widely used in dermatology in the treatment of psoriasis, vitiligo,
cutaneous T-cell lymphoma, and other inflammatory dermatoses. However, recent clinical studies showed that long-term PUVA treatment significantly increases the risk of squamous cell carcinoma and malignant melanoma in humans (58). Therefore, development of chemopreventive agents that retain the therapeutic efficacy but reduce the toxicity of PUVA becomes an important task in dermatology. We conducted a series of experiments to test whether genistein protects against PUVA-induced photodamage. Hairless mice were orally dosed with psoralen and exposed to UVA in a similar setting to those for human PUVA treatment (59). PUVA caused severe skin damage evidenced by cutaneous ulceration in the gross view (Fig. 13B) and hydropic and acanthotic changes histologically (Fig. 13F). Topical application of genistein significantly protected PUVA-induced cutaneous damage morphologically and histologically (Fig. 13C, D, G, H). In addition, PUVA activated apoptosis by inducing caspase 3 and poly(ADP-ribose) polymerase (PARP), as well as diminution of PCNA in mouse skin (Fig. 14B, E, H). Topical application of genistein reversed PUVA-induced alteration of above-mentioned biomarkers (Fig. 14C, F, I). These studies provide preliminary evidence that genistein may serve as a chemopreventive agent affecting the toxicity and cancer risk caused by PUVA therapy in humans.

Summary of the photoprotective effects of genistein

Our studies indicate that genistein potently inhibits the UVB-induced skin carcinogenesis and photodamage in animals. The possible mechanisms of the anticarcinogenic action include scavenging of reactive oxygen species, blocking of oxidative and photodynamic damage to DNA, inhibition of tyrosine protein kinase, downregulation of EGF-receptor phosphorylation and MAPK activation, and suppression of oncoprotein expression in UVB-irradiated cells and mouse skin. Most importantly, we demonstrated that genistein effectively blocked UVB-induced skin burns in humans as well as PUVA-induced photodamage and molecular alterations in hairless mouse skin. The antipromotional activities are primarily associated with the antiinflammatory pathways, downregulation of TPK activities, and expression of protooncogenes associated with cell proliferation. Considering all these factors, we conclude that the soybean isoflavone genistein has potent antiinflammatory and antiinvasive properties, making it a potential therapeutic agent for dermatological indications.
antiphotocarcinogenic and antiphotoaging effects and will have promising applications in the field of dermatology.

LITERATURE CITED


FIGURE 13 Effect of genistein on photodamage induced by psoralen plus UVA (PUVA) in hairless mice. Skh-1 hairless mice were orally dosed with psoralen and 2 h later were exposed to UVA. Mice were topically treated with 5 and 20 μmol genistein 60 min before UVA irradiation. Photographs were taken at 24 h after UVA irradiation. Mice were killed and skin specimens harvested for histological examination. Animal bioassay: (A) Negative control (oral psoralen plus sham irradiation); (B) PUVA; (C) 5 μmol genistein plus PUVA; (D) 20 μmol genistein plus PUVA. Histological examination: (E) Negative control (oral psoralen plus sham irradiation); (F) PUVA; (G) 5 μmol genistein plus PUVA; (H) 20 μmol genistein plus PUVA.

FIGURE 14 Effect of genistein on PUVA-induced caspase 3 and PARP and PCNA in hairless mice. Hairless mice were treated with PUVA and genistein as described in Fig. 13. Mice were sacrificed 24 h post UVA irradiation and skin specimens harvested for immunohistochemical staining using different antibodies. (A, D, G) Negative control (oral psoralen plus sham irradiation) for caspase 3, poly(ADP-ribos)e polymerase (PARP), and PCNA; (B, E, H) biomarkers in mouse skin treated with PUVA only; (C, F, I) 20 μmol genistein plus PUVA.

Caspase 3

PARP

PCNA

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