Review Article

Genistein Could Have A Therapeutic Potential for Gastrointestinal Diseases

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Genistein is a common precursor in the biosynthesis of antimicrobial phytoalexins and phytoanticipins in legumes, and an important molecule found in soybean seeds. It is one of the naturally occurring isoflavones[1]. belongs to the flavonoids family with three or more phenol hydroxyl residues and it is therefore called a soybean polyphenol[2]. Genistein is a major subject of discussion in the context of nutraceuticals and functional foods, and may soon provide a case study for evaluating the delivery of health-promoting compounds through genetically modified plants.

Soy-food sources and genistein composition

Soybeans and soy products are the major foods containing nutritionally relevant amounts of genistein (Table 1)[3]. The content of genistein varies with the crop variety, geographic location, soil type, crop year and environmental factors[4].

Pharmacokinetic studies and safety

Isoflavones exist primarily in plants in the inactive form as glycosides. Once ingested, isoflavone glycosides (genistin and daidzin) are hydrolyzed in the intestines by bacterial β-glucosidases are converted to corresponding bioactive aglycones (genistein and daidzein). Further fermentation proceeds in the distal intestine with the formation of specific metabolites. The aglycones are then absorbed from the intestinal tract and conjugated mainly in the liver to glucuronides, which are either re-excreted through the bile and reabsorbed by enterohepatic recycling or excreted unchanged in the urine[5].

In animal, the previous study demonstrated that in vivo toxicity profile of epidermal growth factor-genistein (EGF-Gen). No toxicities were observed in mice treated of EGF-Gen at dose levels as high as 40 mg/kg administered i.p. as a single dose or 140 mg/kg administered i.p. over 28 consecutive days[6]. At this time, there is considerable controversy as to whether genistein are net estrogen agonists or whether they act via anti-estrogenic effects. Marjorie G Busby *et al*[7]. investigated the in thirty healthy men ingested a single dose of 1 of 2 genistein preparations purified from soy at the delivered doses of genistein (1, 2, 4, 8, or 16 mg/kg body wt). They found normal dietary intake many

Table 1. Genistein contents of soy products[3].

Soy products	Total Isoflavones (μg/g)	Genistein	Daidzein
Roasted soybeans	2661	1426	941
Soy-protein isolate	987	640	191
Tempeh	865	422	405
Tofu	532	245	238
Soy drink	28	21	7

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fold resulted in minimal clinical toxicity and genistein (free and total) were rapidly cleared from plasma and excreted in urine. In addition, a phase I double blinded clinical trial was conducted to evaluate the effects of a high oral dose of soy isoflavones administered daily for 84 days to healthy, postmenopausal women. Elena A. Pop *et al*[8]. treated in postmenopausal women there was no indication that high doses of soy isoflavones caused DNA strand breakage, increased AP sites, or increased apoptosis in peripheral lymphocytes. They conclusion that unconjugated soy isoflavones appear to be safe and well tolerated in healthy postmenopausal women at doses of 900 mg per day.

The medical tests for genistein exposure

Blood tests

Genistein persist in plasma for about 24 hours and the plasma half-life of it assumed an average half-life of 6-8 hours, as has been reported by various investigators[9], peak blood concentrations occur approximately 4-8 hours after ingestion of single dose[10]. Plasma concentrations of 50-800 ng/mL are achieved for daidzein, genistein and equol in adults consuming modest quantities of soy-foods containing in the region of 50 mg/day of total isoflavones. These values are similar to those of Japanese consuming their traditional diet[11].

Urine tests

Genistein have short-half lives (approximately 8 hours), and nearly all ingested genistein are excreted within 24 hours in both urine and feces. There is considerable interindividual variation in gut bacterial metabolism of genistein which leads to markedly different urinary concentrations of it and its metabolites in different individuals. The mean urine concentration for genistein in the total population age 6 and older was 33.0 μg/L. The range from the 50th percentile to the 95th percentile was 28.9-613.0 μg/L[12].

Activities of genistein

Extensive epidemiological, animal studies and in vitro experiments with genistein have indicated beneficial effects in a multitude of disorders. It has been suggested to be a chemopreventive substance[13]. The cardioprotective[14] and anti-osteoporosis activity[15] of genistein has also been shown. Some of the observed pharmacological effects of genistein can be associated with its estrogenic activity[16] and treatment for acute menopausal symptoms[17]. Interestingly, in 1999, the U.S. Food and Drug Administration approved a health claim for the cholesterol-lowering effects of soy protein, largely based on a meta-analysis of 38 clinical trials that reported significant decreases in total and LDL cholesterol and triglycerides with soy protein intake compared with animal protein consumption[18]. In addition, Tetsu Akiyama *et al.*[19] demonstrated that genistein inhibits the activities of tyrosine-specific protein kinases, on the other hand, the compound proposed mechanisms of interaction with topoisomerase II[20]. A high dietary consumption of genistein has been linked to a number of potential health benefits, as summarized in Figure 1[1].

Genistein as a phytoestrogen

The basic structural feature of isoflavones compounds is the flavone nucleus, which is composed of 2 benzene rings linked through a heterocyclic pyrane ring as well as genistein was originally identified as having a close similarity in structure to 17β-estradiol (female endogenous estrogen), particularly the phenolic ring and the distance between its 40- and 7- hydroxyl groups such, was labeled as a phytoestrogen (Figure 2). Because of its structural similarity to 17β-estradiol, genistein has been shown to compete with 17β-estradiol in estrogen receptor binding assays[21], it is able to bind to estrogen receptors (ER) and elicit either a weak estrogenic (agonistic) or anti-estrogenic (antagonistic) effect, depending on the levels of endogenous estrogens present and the tissue and the estrogen receptor (ER) subtype[22].

Genistein is capable of binding to the ER, with a preference for ERβ, the predominantly expressed ER subtype in the gastrointestinal tract[23,24]. The estrogen

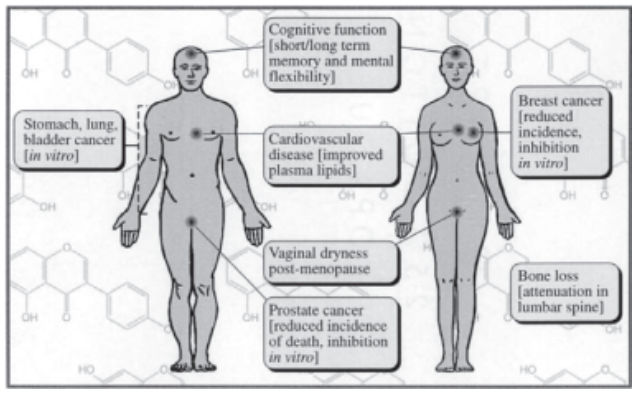


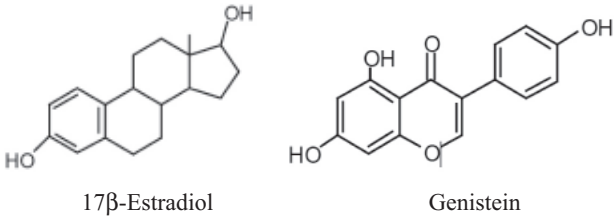
Figure 1. Proposed targets for beneficial effects of dietary genistein or a high soy diet on human health[1].

receptor activity of genistein may play a major role in their effects against cancers of tissues that express estrogen receptors[25].

Antioxidant property and anti-inflammatory

Genistein inhibited xanthine oxidase-one of the enzymes responsible for the production of superoxide radical anion as well as hydrogen peroxide that the concentration of 83.0 μM required to give 50% inhibition (IC_{50}) was evaluated[26]. It is now understood that the proliferative signal is generated by members of a family of protein tyrosine kinases (PTKs) which catalyze the phosphorylation of cellular substrates which in turn is accompanied by T cell proliferation. The T cell PTK, $p56^{ICk}$ may be involved in this process. Genistein, a selective PTK inhibitor blocked the activity of $p56^{ICk}$ in a concentration-dependent manner (IC_{50}= 40 PM). Inhibition of enzyme activity correlated with reduced interleukin-2 (IL-2) secretion and IL-ZR expression but not with TCR-mediated PI hydrolysis[28,29]. Chunyeon Choi *et al.*[30] investigate that genistein may ameliorate oxidation in the lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophage cell line. They found that results suggest that genistein has mild antioxidant activity to suppress intracellular oxidative stress and NF-κB activation. In addition, Mari H am al ainen *et al.*[31] demonstrated the inhibitory effect of genistein in activation of both of the important transcription factors for iNOS, that is, STAT-1 and NF-κB Furthermore, genistein undergoes extensive metabolism (e.g. conjugation with sulfuric acid) in the gut and liver, which may affect its antioxidant properties.

Gerald Rimbach *et al.*[32] investigated the antioxidant activity and free radical-scavenging properties of genistein, genistein-4′-sulfate and genistein-4′-7-disulfate as well as their effect on platelet aggregation and monocyte and endothelial function. They found that sulfation of genistein, with the associated loss of hydroxyl groups, decreases its antioxidant activity and its effect on platelet aggregation, inflammation, cell adhesion and chemotaxis. Interestingly, the reactivity of genistein toward reactive radical species has been investigated by means of pulse radiolysis. It has been concluded on the basis of these calculations that monoanionic form of genistein existing at physiological pH is more powerful radical scavenger than the neutral molecule[32]. Irena Kruk *et al.*[33] reported the results of a complex study of antioxidant activity of genistein, using electron spin resonance (ESR), chemiluminescence, fluorescence and spectrophotometric techniques for confirm good scavenging activity towards O_2-, HO' and ROO' and the antioxidant effect of genistein.



17β-Estradiol Genistein

Figure 2. Structures of genistein (4', 5, 7-trihydroxyisoflavone) in relation to 17β-estradiol[27].

Antibacterial activity

The therapy of bacterial infections often involves antibiotics. However, due to the increasing prevalence of antibiotic-resistant bacteria, the search for new antibacterial compounds has attained a high priority. The prokaryotic type II topoisomerases (DNA gyrase and topoisomerase IV) are targets for broad-spectrum antibiotics[34]. Type II topoisomerases are ubiquitous enzymes that play an essential role in the control of replicative DNA synthesis share structural and functional homology among different prokaryotic and eukaryotic organisms[35].

Genistein is a topoisomerase II inhibitor, and has been shown to stimulate topoisomerase IV-mediated DNA cleavage in *E. coli.*[36] Margareta Verdrengh *et al.*[37], found that exposure to genistein exhibited an inhibitory effect on all staphylococcal strains tested, including methicillin-resistant strains. Furthermore, the growth of *Streptococcus pasteurianus, Bacillus cereus,* and *Helicobacter pylori* was clearly inhibited by genistein as well as the recent study, genistein has been reported to inhibit activity of *Helicobacter pylori,* a bacterium that plays a pathophysiologic role in gastritis and gastric ulcer formation[38]. In addition, Paul Erasto *et al.*[39], investigated three new flavonoids-5,7,40-trihydroxy-6-[1-hydroxy-2-methylbuten-2-yl]isoflavone (isogancaonin C), 7,20-dihydroxy-40-methoxyisoflav-3-ene (bolusanthin III), 6,60-dihydroxy-40-methoxy-2-arylbenzofuran (bolusanthin IV), in addition to eight known flavonoids; derrone, medicarpan, genistein, wighteone, lupiwighteone, gancaonin C, 7-hydroxy-40-methoxyisoflavone and

7,30-dihydroxy-40- methoxyisoflavone were isolated from the root wood of *Bolusanthus speciosus.* The compounds showed strong antimicrobial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Candida mycoderma.*

Effect in the gastrointestinal tract

Effect in stomach

L. Rainova *et al.*[40] demonstrated the ulceroprotective action of several flavonoid compounds isolated from *Genista rumelica* Vel. (total flavonoid mixture, luteoline-7-glycoside, genistine) was established on five models of gastric ulcer in rats, genistine being the most active. Comparison was made against several standard antiulcer durgs (Succus Liquiritiae, Caved S, Almagel). A combined drug Flavogastrol, containing total flavonoid mixture, Semmen Lini gel and antacid compounds, was prepared and demonstrated ulceroprotective activity and low toxicity. Sachio Takekawa *et al.*[41] found that genistein administration resulted in significant suppression of WIR stress-induced gastric mucosal injury and MPO activity. Further, genistein significantly elevated SOD activity and significantly suppressed the TBARS level production of TNF-α and CINC-1, and secretion of gastrin, histamine, and somatostatin. These data suggest that genistein protected against gastric mucosal injury, likely via its ability to inhibit oxidation, inflammation, and secretion of gastrin and histamine. The previously reported that CGRP plays a critical role in the reduction of stress-induced gastric mucosal injury by increasing gastric prostacyclin (PGI2) levels in rats and estrogen has been shown to increase the production of CGRP in sensory neurons. Genistein has estrogen-like effects and is referred to as a phytoestrogen[42].

Furthermore, Nobuhiko Shimozawa *et al.*[43] observations suggest that estrogen and isoflavone might inhibit OVX induced decreases in CGRP levels in DRGs by promoting transcription, thereby contributing to the attenuation of stress-induced gastric mucosal injury in OVX rats. In addition, Yu Li *et al.*[44] found that genistein postconditioning has a protective effect on hypoxia/reoxygenation-induced injury in human gastric epithelial cells. The mechanism by which genistein exerts this protection may be via activation of capsaicin receptor, resulting in the generation of an endogenous protection substance, CGRP.

Interestingly, Dong-Shu Du *et al.*[45] examined whether capsaicin receptor-mediated genistein postconditioning protects against gastric ischemia-reperfusion injury via the PI3K/Akt signal pathway. They found that CGRP secreted by activated capsaicin-sensitive neurons played an important role in the protective effects of genistein and PI3K/Akt pathway was also involved in the protective effects of genistein. Smain Amira *et al.*[46] demonstrate that genistein can affect the relaxation of mouse isolated stomach. Kwang-Pil Ko *et al.*[47] examined the association between soybean products and gastric cancer risk by measured phytoestrogen biological markers in a nested case-control study. These findings suggest a beneficial effect of high soybean product intake for gastric cancer risk.

Effect in intestine

Biguang Tuo *et al.*[48] demonstrated that genistein stimulates duodenal HCO_3^- secretion mainly through estrogen receptor and PI3K-dependent pathway. These findings contribute to the understanding of the molecular mechanism of genistein-induced anion secretion and further pharmacotherapeutic development and use of genistein or related substances in the treatment of diseases of epithelial tissues. In addition, a study in daily subcutaneous injections with genistein, 600 mg/kg genistein/day (600G) significantly increased intestinal chloride (Cl^-) secretion in C57BL/6J female and male murine jejunum after 1-2-weeks treatment[49]. Furthermore, Yukun Zhang and Hong Chen[50] investigated the effects of genistein on WNT signaling, which is involved in colon epithelial cell growth and apop-tosis. They found that genistein inhibits b-catenin-mediated WNT signaling through increasing FRP2 gene expression by demethylating its silenced promoter in colon cancer cell line DLD-1.

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