REVIEW

Isoflavones: Chemistry, Analysis, Functions and Effects on Health and Cancer

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Abstract

Isoflavones are phytoestrogens and natural plant compounds which are similar to 17-β-estradiol in chemical structure. It is known that they can act as estrogen agonists or antagonists, depending on endocrine estrogenic levels, but actions of isoflavones are rather complex due to large number of variables such as chemical structures and mechanisms. Some hypotheses on biological mechanisms have not satisfactorily been confirmed to date and human epidemiological and experimental studies have been relatively limited. Nevertheless, isoflavones and isoflavone rich foods have become a focus onf interest due to positive health benefits on many diseases, especially prevention of hormone-related cancers, cardiovascular disease, osteoporosis, and adverse postmenopausal symptoms, and improvement of physiological condition such as maintaining cognitive function. This review provides an overview of chemistry, analytical techniques (focused on human biospecimens), functions including biological mechanisms, and effects of isoflavones, on the basis of the available meta-analysis and review articles and some original articles, on health and cancer.

Keywords: Isoflavone - chemistry - quantification - health effect - cancer-prevention

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Introduction

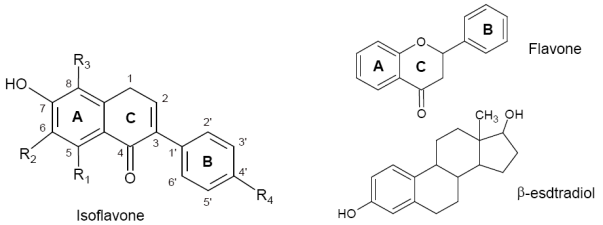
Traditionally, breast cancer, prostate cancer, and diabetes were less common diseases in Asian population than in Western populations and it has been suggested that soy foods may contribute to the prevention of these hormone-related diseases (Kim, 2008; Kang et al., 2012). Soybean products contain organic compounds related to the isoflavone which act as phytoestrogen. Phytoestrogens are called as diphenolic non-steroidal estrogens because those are structurally similar to 17-β-estradiol and thus physiologically functioned as weak estrogens (Figure 1).

Endogenous estrogens are circulated and bound with biologically active unconjugated form, while dietary isoflavones are nearly conjugated in circulation. There are a few unconjugated isoflavones in circulation (1-5% of the total isoflavones) (Barnes, 2010). Moreover, compared to endogeneous estrogens, isoflavones have approximately 100 times weaker affinities of estrogen receptor (Kuiper et al., 1997). So isoflavones have estrogenic or anti-estrogenic effects according to estrogen hormone level in body.

Although recently many researchers have attention to beneficial effects of soybean products on health, the health benefits of soy for many diseases are limited and inconsistent in epidemiologic studies. This paper introduces chemistry including major foods and natural plants, metabolism and mechanisms of isoflavone and reviews *in vitro*, *in vivo*, and epidemiological studies, specifically in terms of their plausible biological effects in human.

Isoflavones in Foods and Natural Plants

Isoflavones are naturally-occurring plant compounds. More than 300 kinds of plants, in particular roots and seeds, include isoflavones (Klejdus et al., 2005). The sources are the Fabaceae/Leguminosae family, including various legumes such as kudzu, lupine, broccoli, cauliflower, barley, fava beans, and soy, are the major



Compound	R1	R2	R3	R4	Substitution
Daidzein	H	H	H	OH	7,4'-$(OH)_2$
Genistein	OH	H	H	OH	5,7,4'-$(OH)_3$
Glycitein	H	OCH_3	H	OH	7,4'-$(OH)_2$, 6-OCH_3
Formononetin	H	H	H	OCH_3	7-OH, 4'-OCH_3
Biochanin A	OH	H	H	OCH_3	5,7-$(OH)_2$, 4'-OCH_3

Figure 1. Chemical Structures of Isoflavones (Mortensen et al., 2009)

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source of isoflavones (Prasad et al., 2010). Also red wine extracts contain small amounts of isoflavones and natural plants such as red clover, germs of alfalfa, and linseed contain isoflavones (Pilsakova et al., 2010). Of these sources, soybeans and their products are one of the richest sources of isoflavones in human.

As other source in food is soy oil and soy protein isolates. Textured soy protein is a kind of soy protein isolates. Vegetarians like to eat food made textured soy protein instead of meat and cheese. Textured soy protein usually containing 50-70% soy protein is commonly used as a meat substitute in hotdogs, hamburgers, sausages and other meat products. Another soy protein isolate containing 90% soy protein is used to enrich energy bars, sports drinks, infant formula, cereals, imitation dairy products, ice cream, cheese and even doughnuts (Rakosky, 1975; Thomas and Lutz, 2001). Soy protein isolate is popularly added in many canned food and bakery products to improve the appearance of the food or to control dietary intake because it is a cholesterol-free, vegetable protein rich in complex carbohydrates and unsaturated fats, high fiber, and free of lactose (Patisaul and Jefferson, 2010). For those reasons, soy is used to make low fat soy mild and tofu, and fermented food in the school breakfast and lunch programs (Thomas and Lutz, 2001).

Dry soybeans contain 1.2-4.2 mg/g isoflavones. Their exact concentration depends on many environmental factors and cooking methods, including the soil in which they are grown, climate, and stage of their maturity or processing (Pilsakova et al., 2010). High processed foods contain lower isoflavone contents than the amount found in unprocessed soybeans. For example, the second generation of soybean such as tofu contains only 6-20% (5.1-64 mg/100g) of the isoflavone amount in unprocessed soybeans (Duncan et al., 2003). Miso, doenjang, and natto contain a large amount of isoflavones in foods (20-126 mg/100g) and tofu and tempeh contain a moderate amount of isoflavones (5-64 mg/100g) (Liggins et al., 2000). New generation soy foods such as soy milk, yogurts and cheeses have a small amount (1-33 mg/100g) (Wiseman et al., 2002). Canned foods including tuna or meatless chili, meat products like sausages and bakery products like bread and muffins have a very small amount (usually 2 mg/100g) (Pillow et al., 1999; Horn-Ross et al., 2000). However, nutritional supplements such as infant formulas contain high concentrations of isoflavones (up to 31 mg/100g instant product). In natural plants, red clover contains moderate levels of isoflavones (nearly 21 mg/100g), however other plants such as green tea, flaxseeds, and raw broccoli have a very small amount (0.02-0.07 mg/100g) (Mortensen et al., 2009). The isoflavone content of an array of foods is now published in numerous on-line databases the most accessible of which for consumers is maintained by the US Departments of Agriculture (USDA, 2008).

There is a big difference of isoflavone intake according to race. Intakes of isoflavones are lower in Western populations than in Asian populations since soybean is rarely consumed food in Western diets. In western countries, soymilk is frequent soybean containing products. In contrast, in Asia, tofu, natto, soymilk and fermented foods such as miso, doenjang (fermented soybean paste in Korea), soy sauce, tempeh are frequent sources of isoflavone intake in food.

Usually mean daily isoflavone intake in Asians is about 8-50 mg (expressed as aglycone equivalents). Blood genistein levels are generally in the range of 25 ng/mL for Asian women, slightly less for vegetarian women, and under 2 ng/ml for US women (Verkasalo et al., 2001). Generally, isoflavone contents in food are measured as sum of daidzein, genistein, and glycitein in aglycone equivalents (Mortensen et al., 2009).

Chemistry

Biosynthesis

Isoflavones are the secondary metabolite formed by symbiotic relationship with the rhizobial bacteria and the defense responses of leguminous plant (Yu et al., 2000). Isoflavons are synthesized as part of the phenylpropanoid pathway, the same biosynthetic pathway of flavonoid biosynthesis (Figure 2) (Barnes, 2010). Phenylalanine converts 4-hydroxycinnamoyl CoA by reaction with malonyl CoA. Chalcone synthase catalyzes the reaction of this intermediate to convert to

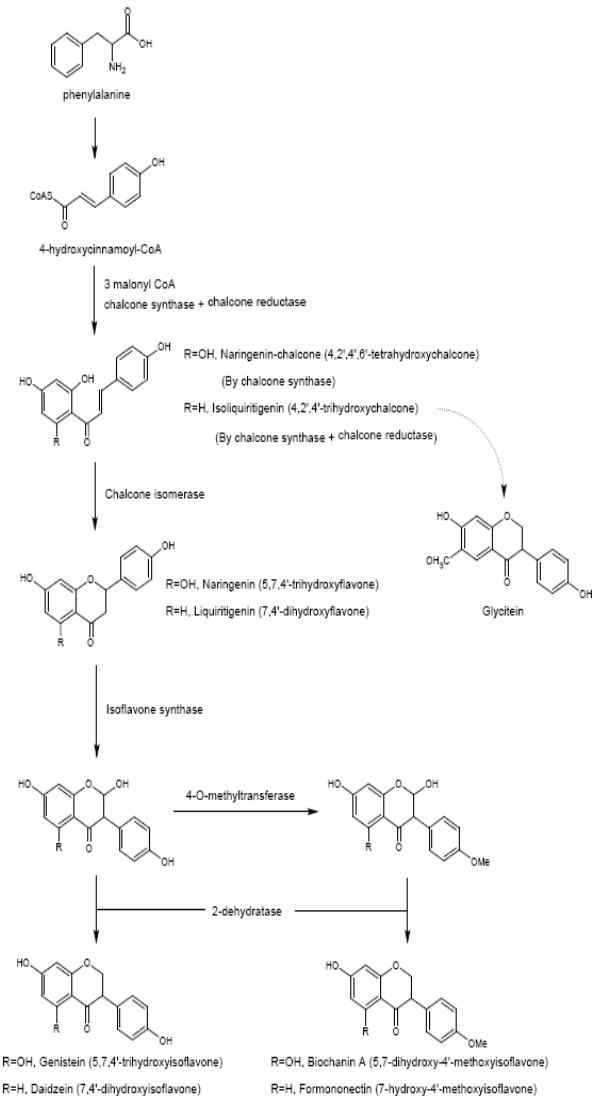

Figure 2. Biosynthesis Pathway of Isoflavones (Barnes, 2010)

4,2',4',6'-tetrahydrozychalcon (naringenin chalcone) and the combined enzyme reaction of chalcon synthase and chalcone reductase convert this intermediate to 4,2',4'-trihydrozychalcone (isoliquiritigenin). And then, chalcone isomerase catalyzes the ring closure of the heterocyclic ring to form 7,4'-dihydrozyflavone (liquirintigenin) and 5,7,4'-trihydroxyflavone (naringenin). The B-ring is moved from the 2-position to 3-position by isoflavone synthase. Isoflavone dehydratase removed water to generate the 2,3 double bond in the heterocyclinc ring. The products generated by this reaction were daidzein (7,4'-dihydroxyisoflavone) and genistein (5,7,4'-trihydroxyisoflavone).

Chemical change by processing

Natural form of isoflavones is biologically inactive glycoside conjugates but some isoflavones such as aglycone of unconjugated form are biologically active. Usually, isoflavones are changed to 7-O-β-glucosides by a glucosyltransferase and then to their 6''-Omalonates by a malonyl transferase (Barnes, 2010). The 6''-Omalonates is the major form in harvested soybeans. The proportion of conjugated glycosides to unconjugated aglycones varies among kinds of foods and depends on processing condition of soybeans. Soybean processing to make foods changes the chemical structure of isoflavone and contributes to the variability of soy isoflavone content.

During fermentation, the glucosidic group from isoflavone structure was removed and the long fermentation process over several months to make miso, doenjang or soy sauce induces additional oxidative process to form hydroxylated derivatives (Esaki et al., 1999). Fermented soy foods contain comparatively high levels of free aglycones than other soy-based foods due to the action of β-glucosidases from the fermentation organisms (Nakajima et al., 2005). By the larger bioactive contents of aglycones, of fermented foods, fermented foods are rapidly absorbed in small intestine to transport to blood (Patisaul and Jefferson, 2010). Under hot water extraction to make soymilk, they are degraded to glycosides and aglycones. Upon dry heat treatment to make a dry compounds (soy protein concentrate, toasting of soybeans, soy flour or the hypocotyls), malonyl glucosides are decarboxylated to acetyl glucosides. Acetyl glucosides can themselves be degraded to glycosides and aglycones (Mathias et al., 2006).

Absorption, metabolism and excretion

After isoflavones ingestion, isoflavones are hydrolyzed in intestinal wall by intestinal enzymes or intestinal micro-organism with glucosidases, and the conjugated isoflavones are changed to bioactive aglycones such as daidzein, genistein, and glycitein by the hydrolysis (Cederroth and Nef, 2009).

Genistein and daidzein are the major isoflavones in soybean. Genistein and daidzein can be produced from their glucosides or from the precursors biochanin A and formononetin by intestinal glucosidases. Genistein and daidzein are extensively metabolized in the intestine and liver. Daidzein undergoes conjugation with glucose either to form the 7-O-glucosede daidzin (in soybean) or

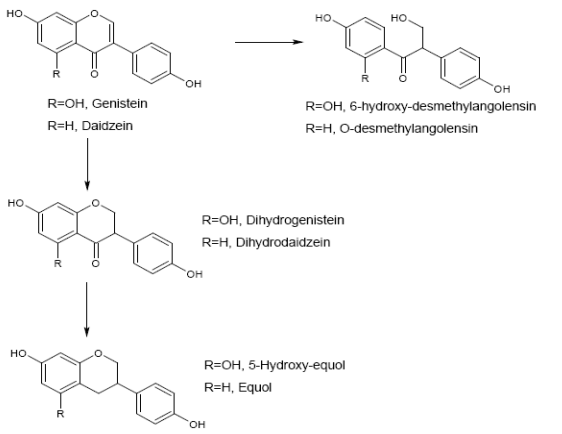

Figure 3. Microbial Metabolisms of Isoflavones Genistein and Daidzein (Mortensen et al., 2009; Barnes, 2010)

8-C-glucoside puerarin (Kulling et al., 2002). Daidzein is metabolized to dihydrodaidzein, which is further metabolized to desmethylangolensin and equol. Genistein is metabolized by gut bacteria to dihydrogenistein, which is further metabolized to p-ethyl-phenol, respectively (Figure 3). Glycitin undergoes little metabolisms by gut bacteria prior to excretion due to being resistant to enzymatic hydrolysis (Cederroth and Nef, 2009; Barnes, 2010).

Bacterial metabolism of isoflavones is main pathway, and intestinal metabolism has been extensively studied. The conjugated isoflavones undergos enterohepatic circulation, are deconjugated in intestinal bacteria and reabsorbs again (Sfakianos et al., 1997; Barnes, 2010). In addition to bacterial metabolism, isoflavone metabolized by phase-I and II isoenzymes in liver. Genistein and daidzein undergo hydroxylation catalyzed by Phase-I enzymes (cyptochrome P450) and glycetin is metabolized to mono- or dihydrozylated glycetin metabolites (Kulling et al., 2000). And conjugated metabolites by phase-II enzymes such as Uridine 5'-diphospho-glucuronosyl transferase and sulfotransferase were synthesized (Kulling et al., 2001).

Isoflavone aglycones are passively absorbed in the upper small intestine from blood (King et al., 1996). There is little evidence that isoflavone are actively absorbed in intestine up to date. Isoflaveones are mainly excreted into urine by glucuronidation and sulfation (5-35%). A few isoflavones are excreted via feces (nearly 1-4%) (Xu et al., 1995).

Analysis

Recently a lot of assay techniques for isoflavone quantification are introduced and development against the cons of each assay method and changes to very fast and accurate techniques. In particular, over last decade many new and interesting HPLC (High-performance liquid chromatography) techniques have been developed using different mass spectrometry (MS) analyzers and internal standards. However, in this section, I'd like to focus on the describing the fundamentals of assays of commonly-using, long-lasting methods and analytic methods for isoflavone

Table 1. Comparison of Analytic Methods for Isoflavone Quantification in Human Biospecimens (Wang et al., 2000; Wahala et al., 2002; Wilkinson et al., 2002; Wu et al., 2004)

Methods	Sensitivity	Specificity	Advantages	Disadvantages
Immunoassays (ELISA, RIA, FIA)	1–100 fmol	Good	Very sensitive Easy sample preparation High throughput assays Well suited technique for epidemiological and intervention studies	Long time to generate key reagent (anti-analyte antibodies) Full characterization of anti-analyte antibody specificity Single analyte determination
HPLC (UV, DAD, etc)	2 pmol	Low	Simpler sample preparation than GC–MS Multi-analyte detection per assay Good for soy food and conjugates Most laboratories have HPLC	Low sensitivity, Low specificity, Slow sample throughput limits analysis of large sample numbers
HPLC-Fluorescence	200 fmol	Moderate	Sensitive than HPLC-UV	Limited to fluorescent analysis
HPLC-ED	20-200 pmol	Moderate	More faster than HPLC-UV In particular, proper in detection of genistein, daidzein, and coumestrol	Required an operation electrochemical potential, but the stability is problematic Considerable expertise required for assays
GC-MS	50 fmol	High	Sensitive (very low detection limits) and specific Multi-analyte detection per assay May be used for new metabolite discovery and identification	Expensive instrumentation Mass spectrometry not a technique available in all laboratories Considerable expertise required for instrument operation Complex, labour intensive sample preparation Unsuitable for analysis of large sample numbers
LC-MS	1–500 fmol	High	Sensitive (very low detection limits) and specific Multi-analyte detection per assay Glycoside and aglycone analysis possible Easy sample preparation Higher sample throughput than other HPLC methods possible	Limited chromatographic resolution

quantification using human biospecimens. Most assay methods can detect isoflavones and their metabolites using human serum, plasma and urine in recent years through continual improvement and development in techniques, however the kind of isoflavone compounds detected in the specific method differ according to each assay technique.

A lot of analytics methods are applied in the analysis of isoflavones in human biospecimens (Table 1). Although the range of detection limits contain a wide variety between laboratories, generally reported sensitivity of analytics methods is as follows: immunoassay >HPLC-MS=HPLC-multichannel electrochemical detection (coularray)>GC-single ion monitoring-mass spectrometry>HPLC-UV diode array>HPLC-single channel electrochemical detection (Wilkinson et al., 2002).

Immunoassays are an assay method using specific antigen and antibody reaction. In radioimmunoassay (RIA) using a radiolabeled tracer and fluoroimmunoassay (FIA) using europium-labeled or other appropriate tracer, radioactivity or fluorescence of the fractions is measured and quantitative results are obtained by comparing the counts against a standard curve (Wahala et al., 2002). Immunoassays are very sensitive with very low detection limits such as 1-500 fmol and highly specific, however their specificity is a little lower than GC-MS due to their cross-reactivity (Wang et al., 2002). Immunoassays are reasonable in epidemiological studies and mass screening with larger population since they are fast and expedient due to some advantages such as multi-analyte detection per assay, easy sample preparation, and higher sample throughput. Moreover only a very low level of biospecimens is needed to be analyzed (Wang et al., 2000; Wahala et al., 2002).

The HPLC-UV method is an assay using the characteristic of isoflavones with heterocyclic ring. Isoflavones with intact heterocyclic ring such as genistein and daidzein more strongly absorb UV light with a maximum wavelength in range from 250 to 270 nm than those without the ring or with opened ring such as equol, dibydrodaidzein, and O-demethylagolensin (Franke and Custer, 1994). Diode array detection (DAD) can differently measures the degree of wavelengths for maximum UV absorption according to the kind of isoflavones (Wang et al., 2002). The HPLC-UV (or DAD) method is proper when high levels of isoflavones are presented in biospcimens (Wahala et al., 2002). The detection limit of this method is too high for the measurement of isoflavonoids in non-supplemented samples or in food products not containing large amounts of isoflavonoids (Wilkinson et al., 2002). HPLC-UV (or DAD) methods are less sensitive with high detection limits such as 2 pmol and their specificity is low. The analysis time is slow. However, their sample preparation is simpler than GC-MS and a lot of laboratories have HPLC

maneuvers. In particular, they are good in quantifying isoflavones from soy and their products (Wang et al., 2002).

HPLC-fluorecence method is more sensitive than UV absorption methods. Fluorescence is measured against a nearly zero background, whereas UV absorption is determined from decreases in the incident light source. It should be noted that the number of phytoestrogens that are naturally fluorescent is quite limited. HPLC-ED (electrochemical detection) uses electroactive characteristics of isoflavones. HPLC-ED has better sensitivity than HPLC-UV method since isoflavones contains phenolic groups, which are electroactive (Jones et al., 1998). The assay method is much sensitive for coumestrol, genistein, and daidzein, but baseline stability for detection is problematic although a required an operating electric potential needed to be proper electrical voltage and electrooxidation of impurities present in the mobile phase (Wahala et al., 2002).

Two chromatography methods using mass spectrometry (GC-MS, LC-MS) are more sensitive methods to detect isoflavones. These methods uses stable isotope analogues as internal standards and an accurately weighed internal standard uses in sample preparation. The analytes and internal standard are extracted, purified, derivatized, separated by GC or LC, and finally analyzed by MS. LC-MS has some benefits of higher precision, less manipulation, and applicability to non-volatile analytes with direct injection of the liquid samples. These methods provide a fast moving field for compound detection. LC-MS analysis, which is both sensitive and requires relatively simple sample pretreatment, is particularly suitable for determining the phytoestrogens and metabolites in biological matrices (Wu et al., 2004). The advantages and disadvantages are shown in Table 1 (Wahala et al., 2002; Wang et al., 2002; Wu et al., 2004).

Function

Hormone-like properties

Estrogenic and anti-estrogenic function: isoflavones have an estrogenic effect and an anti-estrogenic effect because of similarity of structure with 17β-estradiol. Based on the similarity with 17-β-estradiol, isoflavones can bind to estrogen receptor (ER) such as ER-α and ER-β. ER-binding properties of isoflavones indicate that they have the potential to affect intracellular signaling mechanisms which is important for regulating cellular growth and protection. Isoflavones and estradiol are competitively binding on ERs. Genistein has comparable affinity of 17- β-estradiol, while isoflavones have approximately 100-500 times weaker affinities than 17- β-estradiol due to high concentration of conjugated form (inactive form) (Breinholt and Larsen, 1998). Therefore, isoflavone activity is usually lower than that of 17-β-estradiol. At sufficiently high levels (over about 100 nmol/l for genistein), the effect of isoflavones may approach the effect of endogenous 17-β-estradiol at its physiological level (Kuiper et al., 1997).

The function of isoflavones depends on the kind of ER. The affinity of genistein in ER-β is much higher than that in ER-α (nearly 20-fold) (Kuiper et al., 1998). ER-α and ER-β are expressed in varying concentrations in different organs and different cell types. The expression of ER-α is documented in most target tissues of estrogen such as breast, ovaries, uterus, and testis, hypothalamus/pituitary glands, liver. The expression of ER-β is mainly found in prostate, kidney, bladder, lung, bones, circulatory system including heart, endothelial cells and blood cells, thymus and intestines (Kuiper et al., 1996; van Pelt et al., 1999). ER-β is closely relates to inflammation (Yakimchuk et al., 2013). The biological function of isoflavones also depends on endogenous estradiol levels. When the natural levels of estrogens are high, isoflavones bind with the ER-α and inhibit the activity of natural estrogens. In contrast, when the natural levels are low in women (postmenopause, oophorectomy), isoflavones demonstrate estrogenic activity by biding on ER-β. In men, estrogen levels may be not altered regardless of isoflavones concentration and soy diet (Kuiper et al., 1998; Pilsakova et al., 2010).

Interaction with steroid hormone metabolisms and transport: isoflavones inhibit the activity of 5α-reductase, which catalyzes the conversion of testosterone to 5α-dihydrotestosterone, and CYP19 (aromatase), which mediates the conversion of testosterone to estradiol in low isoflavones concentration. In contrast, when isoflavones concentration is high, aromatase activity is rather increased to increase estradiol conversion (Adlercreutz et al., 1993). Isoflavones can bind to the sex hormone binding globulin (SHBG), which stimulate SHBG synthesis and alter the concentration of circulating steroid hormones (Adlercreutz et al., 1987). With high concentration of isoflavones and soy-rich diet, total testosterone levels in men can be increased but free testosterone levels is not increased due to increased SHBG uptake and serum testosterone levels is not altered in women (Berrino et al., 2001; Celec et al., 2005). Moreover, isoflavones-rich soy diets can decrease follicle stimulating hormone (FSH) and luteinizing hormone (LH) in premenopausal women, which may secondarily increase estradiol in postmenopausal women (Hooper et al., 2009).

Affecting thyroid hormone: high risk soy-diet may affect thyroid hormone levels through stimulating thyrotropin, however this effect do not cause too much changes in T3 (triiodothyronine) and T4 (thyroxine) in human (Dillingham et al., 2007). Isoflavone can competitively bind to thyroid peroxidase, which converts T3 to T4 (Doerge and Chang, 2002). Isoflavones inhibit this conversion by the binding and they themselves changed to tri-idoisoflavone. However, the effect may be also generally small.

Antioxidant function

Genistein has antioxidant properties as scavenger of radicals and chelaters of metals. This function is occurred by affecting gene expression of enzymes that react with antioxidants such as catalase and superoxide dismutase, and inhibiting with secondary oxidant production such as hydrogen peroxide or hypochlorous acid (Mortensen et al., 2009). Genistein is more active as antioxidant than daidzein due to having its third hydroxyl group in the C-5 position and equol is another better antioxidant actions

than its precursor compounds due to the absence of the 2,3-double bond in conjunction with a loss of the 4-oxo group which is enhancing antioxidant properties (Arora et al., 1998).

Isoflavone metabolism pathway in intestine and liver affect the antioxidant properties of isoflavones and the metabolites. Although genistein significantly reacts with superoxide dismutase, catalase, and glutathione peroxidase, isoflavone metabolites such as equol, 8-OH-daidzein, O-desmethylangolensin (O-DMA), and 1,3,5-trihydroxybenzene have also played a role as potent scavengers. 8-hydroxy-daidzein is the most potent scavenger for hydroxyl and superoxide anion radicals. These isoflavone metabolites are highly chelating with ferrous compound relative to genistein and daidzein. However sulfated isoflavones can decrease antioxidant activity. Isoflavones can reduce low-density lipoprotein oxidation and lipid peroxidation by reacting with lipid radicals (Tikkanen et al., 1998).

Anti-tumor/disease function

Genistein is known to be a protein tyrosine kinase (PTK) inhibitor. PTKs catalyze the addition process of a phosphate (PO_4^{3-}) of tyrosine residues to proteins and other molecules, which activates or deactivates broad-spectrum proteins and enzymes, especially in carcinogenesis. The PTK inhibition suppresses or slows down a consequent signal transduction pathway inducing carcinogenesis and neuronal degeneration. PTKs are known to be highly expressed in breast, prostate and stomach cancers and several brain regions, such as hippocampus. At high doses of isoflavones, isoflavones suppress PTK actions in these tissues, which is related to reduce the carcinogenesis and neuronal degeneration (Yu et al., 2012). Moreover, inhibition of PTK activity may also play a role in improving cardiovascular function (Furspan and Freedman, 1998). Genistein and daidzein isoflavones directly inhibit growth and proliferation of gastric cancer cells through apoptosis process using down-regulation of apoptosis-regulated gene Bcl2 and up-regulation of apoptosis-regulated gene Bax and cell cycle arrest at G/early M phases (Piontek et al., 1993).

Furthermore, in *in vitro* study, isoflavones genistein can inhibit DNA replication enzymes such as DNA topoisomerases I/II and matrix metalloprotein-9 (MMP-9) (Kurzer and Xu, 1997). Genistein and other isoflavones interact with peroxisome proliferator activated receptors as transcription factors, and thus isoflavones can act as a regulating factor for gene expression in carcinogenesis, insulin sensitivity, metabolisms of carbohydarate, lipid and protein.

In cardiovascular system, animal or *in vitro* studies concentrated on the effects on endothelial and vascular smooth muscle cells, isolated arteries, and live animals such as monkeys. These studies shows that Isoflavones have a role in increasing prostacyclin, activating endothelial nitric oxide synthase, inhibiting cell proliferation and DNA synthesis, relaxing vessel and reducing plaque to control circulatory system (Cano et al., 2010).

Anti-inflammation function

Isoflavones genistein and daidzein, in particular genistein, inhibit deregulated activation of NF-κB (nuclear factor kappa-B), which can suppress inflammation. Suppression in NF-κB activation may occur through inhibiting IκBα kinase, leading to inhibition of phosphorylation and degradation of IκBα and consequent NF-κB DNA binding by p65 nuclear translocation. Isoflavones daidzein can also inhibit STAT-1 (signal transducer and activator of transcription 1, a transcription factor for iNOS (inducible nitric oxide synthase)), which also suppress inflammation pathway. Genistein directly affect to inhibit interleukin-8. Some isoflavone extracts from plant sources affect to suppress NF-κB and NO activation and may down-regulate several inflammation related genes such as COX2 (cytochrome oxidase-2), MMP-9, ICAM1 (inter-cellular adhesion molecule 1), iNOS to reduce inflammation (Prasad et al., 2010).

Immunity function

Isoflavones affect immune functions by estrogen-like properties and the properties as PTK inhibitor of isoflavones themselves. Most *in vivo* studies are those for genistein and the function of genistein is summarized as follows (Sakai and Kogiso, 2008). Isoflavone genistein is known to control general immune function not to excessively function, however increase tumor-specific immune function. A high concentration of genistein inhibits lymphocyte proliferation and thymocyte differentiation, and consequently decreases the number of peripheral lymphocytes and thymocytes and the size of thymus. Genistein also decreases the numbers of peripheral CD-4 and CD-8 T cells as the consequence by thymic atrophy. It affects cellular and humoral immune response: genistein suppress delayed type of hypersensitivity reaction, antigen-induced antibody production and antigen-specific T-cell and cytokine production. However, against tumor, genisteins increase cytotoxic T cell and NK cell activities. Genistein has a suppression function in antigen-induced asthma and arthritis and autoimmune diseases due to estrogen like properties of isoflavones. The estrogen-like action of genistein modulates immune functions mediated by ERs. Isoflavone daidzein can up-regulate interleukin-4 production in activated T cells and increase phagocytic response of peritoneal macrophages. Daidzein may control hyperallergic response through enhancing interleukin-4 production in T cells (Sakai and Kogiso, 2008).

Effects

Positive benefit effect in menopausal women

Isoflavones reinforce their estrogenic effect after endocrine estrogen levels going down through binding to ER-α and thus isoflavones protect ER-α against endocrine estrogens and help reduce estrogen-relating cancer risk. Through binding to ER-β, isoflavones induce estrogen-like effect and thus show positive health effects after menopause. In postmenopausal syndrome with hot flushes and night sweat, isoflavones prescription can decrease the symptoms of menopause (Cassidy et al., 1994; Setchell, 1998; Setchell and Cassidy, 1999). Dietary soy products have slightly and modestly reduced climacteric vasomotor

symptoms affecting hot flushes in menopausal women relative to placebo (Bolanos et al., 2010). However, treatment effect by only isoflavones themselves is not large because severe symptom may be not improved.

Isoflavones suggest some benefit effect for cognition in postmenopausal women however, the evidence is not compelling.

Bone health

Isoflavones estrogen-like effect on bone by binding to ER-β and thus are expected to have positive benefit effects for bone health including osteoporosis. *In vitro* and animal studies involving isoflavones and bone health suggest a positive relationship between isoflavones and positive action on both osteoblasts and osteoclasts. Through stimulating bone formation and inhibiting bone reabsorption, isoflavones can maintain bone health. Human studies including observational and clinical trial studies also support favorable effects of isoflavones showing the results such as increasing bone mineral density and bone mechanical strength, and inhibiting bone turnover in postmenopausal women (Atmaca et al., 2008). Ingestion of isoflavones (more than 90 mg/day of isoflavones) at least 6 months have a significant effect for increasing spine bone mineral density in meta-analyses for randomized controlled trials however the effects on hip and leg bones are controversial (Taku et al., 2010). Long term safety and efficacy for isoflavones ingestion is needed to be confirmed.

Reduction in cancer risk

In vitro and animal studies presented that isoflavones can be related to reduce cancer risk through antioxidant and anti-tumorogenic effect, such as blocking ER-α protein and stopping carcinogenesis pathway through inhibiting PTK, tumor cell growth by suppressing DNA replication and various growth factors, and controlling enzyme activities on signal transduction pathway of carcinogenesis.

Epidemiological studies also show that the soy isoflavones may be associated with a reduction in cancer risk. Soy intakes yield a reduced risk for prostate cancer and breast cancer in recent meta-analyses among Asian population although dose-response relationship is not clear (Qin et al., 2006; Yan and Spitznagel, 2009; Dong and Qin, 2011). However the protective effect of soy isoflavones is not significantly found among non-Asian populations who soybeans and soy-related products intake is not frequent (Yan and Spitznagel, 2009; Dong and Qin, 2011). After stratification for fermentation, non-fermented soy is only significant preventive effect for breast cancer (Dong and Qin, 2011). Soy food intake was associated with not only prevention of breast cancer but also longer survival and low recurrence among breast cancer patients (Zhu et al., 2011; Kang et al., 2012; Zhang et al., 2012).

The effect of isoflavones for gastric cancer is more complicated and it depends on food processing. Both Korean and Japanese populations have high incidence rates of gastric cancer and frequently eaten a wide variable soy foods. In recent meta-analyses among Korean and Japanese population, non-fermented soy foods shows protective effect for gastric cancer, whereas fermented soy foods presents no effect in reducing risk for gastric cancer (Kim et al., 2011). Fermented soy foods including fermented soy sauce and soybean pastes such as Doenjang and miso which Korean and Japanese are used to add to make foods contains salt of high amounts, and therefore fermented soy foods can be associated with higher risk for gastric cancer due to effect and high salt and N-nitroso compounds.

There are few human studies using blood concentration of isoflavones. In Korean study, isoflavones including genistein, daidzein and equol have an effect for reducing gastric cancer risk, and the higher concentration of three isoflavones are, the much lower gastric cancer risk are (Ko et al., 2010). This protective effect for gastric cancer can be explained as an anti-inflammatory, anti-tumorogenic, and anti-oxidative effect of isoflavones.

Although the effect of isoflavones for other cancer prevention has little compelling evidences up to date, Japanese recent study using nested case-control reports an inverse effect for lung cancer (Shimazu et al., 2011) and possible preventive effects of isoflavones for other cancers are expected to be released in future studies.

Cardiovascular health

By binding to ER-β at low endocrine estrogen level, isoflavones have an agonist for estrogen in cardiovascular system. Also isoflavones can directly relax vessels by possibly enhancing the promotion of prostacyclin release and anti-inflammatory action and indirectly reduce plaques in vessels by inhibiting collagen-induced aggregation and platelet activation, although the clear mechanisms are not known (Cano et al., 2010). Physiologically isoflavones are related to reduce lipid profiles such as low-density lipoprotein against global oxidation on endothelial cells. However, the role in total cholesterol and triglycerides is controversial (Cano et al., 2010). In the view of nutrition, many soy products contain high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat. Based on these mechanisms and nutritional point of view, isoflavones is beneficial to cardiovascular health by reducing cardiovascular burden in human. Recent meta-analyses using clinical trials support the cardiovascular healthy role of isoflavones, and isoflavones significantly increase flow-mediated dilatation in vessels and decrease arterial stiffness relative to placebos although heterogeneity across the studies was presented (Li et al., 2010; Pase et al., 2011).

Anti-diabetes effect

After menopause, women's glucose tolerance capacities deteriorate and accumulate central fat. As a consequence of these menopausal changes, many menopausal women have an experience in insulin resistance. Although some epidemiological and clinical trial studies have reported that isoflavones has a beneficial effect on insulin sensitivity and glucose metabolism, it is consistent in that isoflavones has slight decreasing insulin resistance observed after menopause. Recent meta-analysis of randomized clinical trials supports the weak effect of isoflavones for diabetes and shows no

sufficient evidence in improvement of glycemia (Ricci et al., 2010). Despite of limited of evidences, fermented soy products may have better effect for preventing the progression of type 2 diabetes relative to nonfermented soy products (Kwon et al., 2010). In normoglycemic women, isoflavones is expected to have an association with reduced insulin resistance. In summary, the effect of isoflavones as a treatment medicine for diabetes has no compelling evidences, however they seems to have a mild effect in preventing diabetes through reducing insulin resistance.

Isoflavone and Epidemiologic Studies

Although good evidences from animal or *in vitro* studies for isoflavone's beneficial effects has been obtained, the results from epidemiologic studies have been inconsistent, and we need to further consider these inconsistencies. First, measurement error could result in inconsistent results. Previous studies mainly used food frequency questionnaire to measure soybean or isoflavone intake. Because food frequency questionnaire is dependent on the subject's memory, it is generally sensitive to measurement error. Second, there may be difference between dietary intake and its acting level of blood circulation due to variability in food processing and storage, cooking method, individual variability in absorption, metabolism and excretion, and intestinal bacteria variability. Third, the inconsistent results may result from geographic variation in soybean consumption. Total daily and energy-adjusted isoflavone intakes are known to differ by race and ethnicity. Isoflavone serum concentrations in Asian populations have been found to be more than 10 times higher than those in Western populations. In Western populations, consumption of isoflavone may be too small to gain health effect from soybean, and health effect of isoflavone may be underestimated or masked. Forth, the discrepancy among epidemiologic studies on soy isoflavones could be attributed to a failure to distinguish between equol producers and non-producers in the metabolism of isoflavones. Equol is bio-transformed from daidzein by bacterial metabolism in intestine, some individuals cannot convert daidzein to equol. After consuming soy products, up to 80% of Asians produce equol in contrast to only 30-40% of Western populations (Lampe et al., 1998; Morton et al., 2002). There is increasing evidence that the clinical efficacy of isoflavone may be affected by equol-producing status (Shor et al., 2012; Minatoya et al., 2013; Sugiyama et al., 2013). A large intervention study is necessary to verify the effect of isoflavone and equol.

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