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Chemopreventing Effects of Soy Isoflavones in Breast Cancer

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Abstract: *Soy and red-clover isoflavones are mostly consumed in diet as it is present within it, or as a dietary supplement due to a huge range of presumed health benefits. These isoflavones are thought to protect against heart diseases and cancerous diseases like breast and other types of cancer. Isoflavones are structurally similar to estrogens that's why may act as estrogen agonists or antagonists by binding to estrogen receptors. In the present time increased use of isoflavones in processed foods and dietary supplements as well as the greater consumption of soy products, dietary intakes of isoflavones are increasing among children and adolescents. In numerous hormone related cancers including breast cancer estrogens are a known component causing cancer. Keeping these facts in mind we reviewed the existing epidemiological and experimental animal studies for a resolution to a proposed correlation between increased isoflavone consumption and breast cancer. The soybean isoflavones having antioxidant activities, may contribute to their potential role in reducing the formation of certain types of cancers, that's why daidzein, a soybean isoflavone might be a more appropriate cancer preventive neutraceutical because of its lower cytotoxicity. Isoflavones are weak estrogens and their effect is controlled by several factors like dose, time of exposure and species involved. It would, therefore, not be safe to indisputably accept soy or red-clover as a source of isoflavone resource to prevent breast cancer.*

Keywords: *Isoflavones, Red-Clover, Breast Cancer, Phytoestrogen, Cytotoxicity, Risk Assessment.*

INTRODUCTION

Soy foods have received considerable limelight attention for their potential and promising role in reducing the formation as well as progression of certain types of cancers specially breast cancer and some chronic diseases such as cardiovascular disease, Alzheimer's disease, and osteoporosis. Because Oxidative stress is always associated with several cell toxic processes such as oxidative damage to protein and DNA, gene mutation that may lead formation of metabolites which are carcinogenic, so lowering oxidative stress might be an effective to antagonize the formation of cancers and other chronic diseases. A lot of anti-carcinogens, including isoflavones, have been identified in soybeans, but soybean isoflavones might be the most promising one because of they possess antioxidant activity through its structure based on phenol ring, which work in maintaining the redox balance of normal cells. Interest in phytoestrogens, specifically isoflavones such as genistein and daidzein, found abundantly in soy and red clover were first came from suggestion given in one report that the relatively low-cancer incidence were observed among Asian populations which was known to consume high phytoestrogen-containing soy products in Asian diets (Buc-Calderson, Latour and Roberfroid,

1991). Numerous epidemiological and experimental studies have since been established in an attempt to know an association between increased phytoestrogen intake and due to that reduced rates of breast cancer (Cappelletti et al., 2000), prostate cancer (Constantinou Krygier and Mehta, 1998) and cardiovascular disease (Mossman, 1983; Adlercreutz et al., 1995). Soy products are increasingly being touted as 'health' foods despite conflicting evidence. The force to consume soy as a beneficial component to the diet is proponents of the hypothesis that phytoestrogens effectively possess chemoprotective and anticarcinogenic charcteristics. The hypothesis furthers elaborates that all these properties are good to impart protection against various adverse health effects- particularly cancers which are hormone dependent in particular (Lamartiniere, 2000; Holzbeierlein, McIntosh and Thrasher, 2005). Beneficial health effects have continued to emerge. In 1999, the U.S. Food and Drug Administration (US FDA) approved the use of labels for food that includes, if soy protein 6.25 g is given per serving, it will as protective against heart diseases especially coronary blockage (Adlercreutz, Heinonen and Penalvo-Garcia, 2004). Due to the outstanding and side effect free health benefits, a large population of post-menopausal women are choosing soy and red clover supplements as a 'safer' replacement to hormone therapy used traditionally in allopath medication (Key et al., 2002). Despite the suggested and declared benefits, the study of phytoestrogens, their role in the development as well as protection of cancer never escaped controversy, causing the trend of increased consumption of soy become both a cause for concern and sustained study. As our knowledge of the actions of endocrine disrupting compounds (EDCs) have expanded, interest in the potentially harmful effects of genistein and other isoflavones acting as selective estrogen receptor modulators (SERMs) has also grown. In recent epidemiological studies, researchers have found that initially beneficial qualities claim of increased soy intake, including the protection against coronary heart disease, may be unsubstantiated (Coglino et al., 2005).

Soy Isoflavones: A Phytoestrogens

The term phytoestrogen is related to a large group of non-steroidal, phenolic chemicals in plants which have close structural similarity to estrogenic compounds (Zhang et al., 2007). Phytoestrogens consist of two main families of plant-derived compounds:

i) Flavonoids and
ii) Lignans.

Flavonoids can be further divided into two subgroups: first coumestans and second Isoflavones. Compounds belonging to each family of phytoestrogens are present in a variety of food and dietary articles. But still soy and red clover is the main sources of isoflavones, chemically they found in four different forms: glucosides, acetylglycosides, aglycones and malonylglycosides. Coumestans, for example coumestrol, and 4_ methoxycoumestrol, are majorly present in legumes in high concentration, but in clovers and soybean sprouts there is retained the highest amount of coumestans. Lignans, meanwhile, are more widely distributed in all the variety of food like fruits, vegetables, berries, whole grains, and tea and most abundantly in flaxseed (Rice, Whitehead, 2006). The following are the some examples of isoflavones (aglycones) and their molecular weight will be considered in this review:

Table 1: Soy Isoflavones

Sr.No.	Isoflavones	Molecular weight
1.	Daidzein	254.24
2.	Genistein	270.24
3.	Glycitein	284.30
4.	Biochanin A	284.27
5.	Formononetin	268.27
6.	O-Desmethylangolensin	258.27
7.	Equol	242.27

Among phytoestrogens, even those belonging to the same family of compounds, the variation in biological activity and metabolism occur with frequency. The glucoside moiety of Isoflavones from soy and red clover get hydrolyzed of the by intestinal bacteria to get converted into free aglycone, which is actually estrogenically active.

Extraction of Soybean Isoflavones:

Soybeans were ground to flour. The flour was defatted using hexane. The mixture of 100 mL of hexane and 50 g of soy flour was shaken at room temperature for 1 hr. The supernatant was removed after the mixture was centrifuged at 1000g for 20 min. The defatted soy flour was dried under a hood overnight. Methanol was used as solvent to extract isoflavones from the defatted soy flour. The defatted soy flour was mixed with 80 mL of methanol. The extraction conditions and procedure were the same as that in defatting. The supernatant was placed under nitrogen flow to evaporate the solvent to a final volume of approximately 10 ml.

Pharmacokinetics of Isoflavone

In plants, isoflavones occur as glycosides, and as per the general assumption as such they are not absorbed from human gastrointestinal tract because they possess high molecular weight and high lipophilicity, so first they get metabolized by intestinal bacteria to the give free aglycone form which having less molecular weight and somewhat hydrophilic. However, as conclusion of a recent study, the bioavailability of the free aglycone form is higher, as compared with glycoside which are poorly absorbed (Key, Sharp and Appleby, 1999) and metabolism by intestinal bacterial is not necessary for the absorption of the isoflavones. After oral administartion, their deconjugation (hydrolysis) occurs in the gastrointestinal tract by gut microflora (Greenstein, Kushi and Zheng, 1996) or mucosal ß-glucosidases to produce the free aglycone. After reaching into blood circulation, they are again metabolized or goes into synthetic reactions in liver with glucuronic acid (re-conjugation) and or sulfate by phase II enzymes (UDP-glucuronyl transferases and sulfotransferases). This re-conjugation increases their molecular size prone them to enter into enterohepatic circulation. Only 2.7 percent of daidzein and 1.6 percent of genistein are in free form after ingestion of aglycone or glycosides. Among the total absorbed dose almost 30 percent is excreted in urine and only 1 to 4 percent in feces. Ninety percent of the absorbed dose is excreted within the first 24 hours.

The poor recovery rate may be the result of extensive metabolism to as-yet unknown compounds (Horn-Ross et al., 2002; Yamamoto et al., 2003). The metabolism of glycoside genistin, daidzin and glycitin follow similar metabolic pathways. Their hydrolysis by ß-glucosidases results from their respective aglycones, i.e., genistein, daidzein and glycytein. It has been observed that aglycone genistein is metabolized to dihydrogenistein and 6-hydroxy-O-desmethylangolensin (6-OH-DMA) by utilizing human fecal bacteria. Also, once absorbed, the aglycone genistein is reconjugated in the liver to genistein glucuronide and genistein sulphate. Daidzein is metabolized to dihydrodaizein, O-demethylangolensin (ODMA) and cis-4-OH equol. Equol occurs in two isoforms; S- and R-equol.

Humans exclusively produce S-equol, which has a higher binding affinity with ERß as compared to ERa (Dai et al., 2001). Now there is a great deal of inter individual variation in equol and O-DMA production. Out of hundred, thirty to fifty percent of the population is tested to produce equol metabolites (Dai et al., 2003), and rest 80 to 90 percent of the population produce O-DMA. Since CYP 1A2 plays a role in the metabolism of daidzein, conclusion is that it may be that the equol production is related to the CYP polymorphism. However, it has also been suggested that if consumption of carbohydrates is more as a source of energy or higher intake of dietary fiber they produce higher amount of equol as compared to non- or lower-producers of equol or (Wu et al., 1996). The other reason of higher equol production is phenotypes in the Asian population may be due to their genetics and higher fiber intake. Since equol has a higher affinity for ERß, it may also provide some extra and added benefit to the Asian population in terms of reduced cancers like breast cancer risk (Wu et al., 2002). Glycitein is a minor isoflavone of soy and red clover and it is metabolized to 5-methoxy-O-desmethylangolensin and dihydro-6,7,4-trihydroxy-isoflavone via dihydroglycitein (Russo, Balogh and Russo,

2007). The red clover isoflavones are mainly ingested as supplements.

About 90% of the isoflavones in red clover extracts are formononetin and biochanin A, and the remaining are the corresponding 4-O-demethylated daidzein and genistein (Lee et al., 1992). Once ingested, they are readily demethylated to form daidzein (formononetin) and genistein (biochanin A). Liver and intestinal CYP1B1 catalyzed O-demethylation of biochanin A and formononetin produces genistein and daidzein. It has been reported that when equal amounts of genistein and daidzein are given orally to women, plasma genistein concentrations are higher than daidzein and this was related to a higher distribution of daidzein (236L) than genistein (161L). This would suggest a higher amount of daidzein in tissues as compared to genistein. A serum t1/2 of daidzein and genistein should be 8 and 10 hours, respectively. Because of the t1/2, the steady state levels are more likely to be achieved by frequent intakes of soy foods throughout the day. The isoflavone aglycone of soy milk was absorbed faster and in a greater amount than their glycosides in healthy adults and the metabolism of the isoflavone may be affected by the type of soy milk consumed (Hirose et al., 1995).

Mechanism of Action: Estrogens Signalling

Isoflavones are similar in structure to 17ß-estradiol (E2) and function by binding to either of two intracellular receptors, ERa and the more recently discovered ERß similar to E2. Both ER subtypes belong to the nuclear steroid/thyroid receptor superfamily of ligand-activated transcription factors, and are active in regulating tissue-specific physiology via several cellular and molecular pathways:

- **Ligand-dependent,**
- **Ligand-independent,**
- **Estrogen DNA response elements (ERE)-independent, and**
- **Nongenomic**

Ligand-dependent pathways

In the classical ligand-dependent pathway, E2 functions by binding and activating the ER. Ligand binding initiates an ER conformational change in the ER, facilitating subsequent ER homodimerization and binding to an estrogen responsive element (ERE) in the DNA. These EREs are frequently located in promoter regions of target genes, thus at promoter sites that allow the homodimerized E2-ER unit to acts as a regulator of transcriptional activity, resulting in either up-regulation or down-regulation of downstream target genes.

Ligand-independent pathways

The ligand-independent pathway of estrogen signalling involves peptide growth factor cross-talk, whereby compounds such as epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) together with ER mimic E2-ER homodimer actions. On the other hand, various tyrosine kinase growth factor receptors such as EGF, IGF-1, MAPK and PI3K–Akt kinase cascades can activate estrogen receptors by phosphorylation in the absence of ligand. The phosphorylated ER will form either homodimer or hetrodimer and continue the following downstream regulation.

Estrogen DNA response elements (ERE)-independent:

ER also regulates the expression of genes that do not contain ERE in the promoter region via an independent ERE mechanism. This is done by binding to the other classes of transcription factors through protein- protein interaction in the nucleus. For example, the interaction of ER with transcription factors that contain the activation protein (AP-1 protein complex) is through such mechanism.

Nongenomic

Through the nongenomic mechanisms of estrogen activity are still debated, many believe nongenomic effects are thought to be mediated through plasma membrane-bound and mitochondria ERs and involve rapid activation of many signalling molecules such activation of intracellular signal transduction. This is closely linked with protein-kinase cascades and calcium channels activation. Like peptide growth factors, estrogens also cause activation of various protein kinases such as mitogen-activated protein kinases. They also increase

levels of second messengers via calcium channel activation, such as cyclic AMP (cAMP) within minutes (Hirose et al., 2013). These nongenomic, non-transcriptional effects involve a membrane-bound form of ERa and ERß. Both facilitate cross-talk between the membrane estrogen-receptor-signalling process and other signal transduction pathways.

Acting through such mechanisms as described above, estrogen is responsible for regulating numerous target genes, many of which have been identified as key in cell cycle control, particularly in estrogen-responsive breast cells corresponding to the development of breast cancer. As such, estrogen signalling has been implicated in stimulating cell proliferation and promoting the progression of ER-positive human breast cancer as well as cancers at other sites among other forms of cancer-including uterine cancer, for which estrogen is also a highly correlated element. According to theories of endocrine disruption, phytoestrogen mimicry of endogenous E2, especially at high levels, could potentially trigger cellular pathways normally regulated by the hypothalamic pituitary-gonadal endocrine axis and result in the unrestricted cell proliferation characteristic of cancer in estrogen responsive organs. These organs include the breast and uterus. Breast and uterine cancers have been demonstrated as high estrogen-responsive forms of the disease (Xu et al., 1998).

Non-ER Mediated Effects of Isoflavone

Non-ER Mediated Effects of Isoflavone A variety of biological effects have been ascribed to isoflavones without interacting with ER, including the effects on estrogen biosynthesis and metabolism, the inhibition of protein tyrosine kinases, antioxidant activities and the inhibition of DNA topoisomerase, etc. Many of these effects have been observed in vitro studies at levels far higher (10 to 100 μM) than can possibly be achieved with the consumption of a normal diet. While many of these effects would be very desirable as an anticancer agent if prescribed to a patient on a risk/benefit basis, they have no role in an everyday diet utilized by all age groups.

Conclusion

Isoflavones are weak estrogenic compounds when compared to 17 ß-estradioland diethylstilbestrol. Following ingestion, isoflavones are absorbed and distributed to various organs throughout the body, reaching higher levels in endocrine-related tissues. They are able to cross the placenta and thus accumulate in amniotic fluid along with other estrogenic compounds. Many epidemiology studies show an inverse association of breast cancer risk with an increased consumption of soy products (isoflavones, miso soup, soy protein, tofu, or soy bean curd) especially when consumed during adolescence. However it is to be noted that studies supporting an inverse association with breast cancer were conducted in the Asian population where soy is a staple food.

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