The Phyto-Oestrogens: Its Anticarcinogenic and Antioxidant Activity - A Review

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ABSTRACT

The isoflavonoids comprise a group of phyto-oestrogens that have useful biological activities including oestrogenic, antioxidant and anticancer. As dietary components for humans, they are bioavailable from leguminous vegetables (such as genistein from soybean), and have been well-documented to have numerous health benefits. A wide range of epidemiological studies in humans and limited studies in animals have identified isoflavonoids as a potential chemopreventive agents against hormone-dependent cancers. Therefore, this review focuses on the mechanisms of isoflavonoid phyto-oestrogens in inhibiting cancer in vitro and in vivo in the models of human cancers.

Key word: phyto-oestrogens, oestrogenic properties, antioxidant activity, anticancer activity

INTRODUCTION

Phyto-oestrogens that occur in many plants of dietary significance to humans have been found to have diverse biological effects. They can be divided into two major categories: the isoflavonoids and the lignans (Kurzer and Xu, 1997) which have similar molecular weights and metabolism as steroids, but clearly different biological effects on the cells (Adlercreutz, 1995). The isoflavonoid phyto-oestrogens are heterocyclic phenols with a close similarity in the structure to oestrogen and a diphenolic character similar to that of lignans. The precursors of the biologically active compounds originate in the soybean products (mainly isoflavonoid), whole-grain cereal products, seeds, clovers, and probably berries and nuts (mainly lignans) (Adlercreutz, 1997). The structure of the most important dietary isoflavonoids and lignans described in human biological samples can be seen in Figure 1.

BIOLGICAL EFFECTS OF PHYTO-OESTROGENS

Previous studies have shown that isoflavonoid phyto-oestrogens have significant oestrogenic effects in animals and humans (Price and Fenwick, 1985; Gavaler et al., 1991; Van Thiel et al., 1991). The most well-known oestrogenic effect in animals is clover disease in Australian sheep (Price and Fenwick, 1985). When consumed, the plant isoflavonoids and lignans undergo metabolic conversion in the gut, which results in the formation of further hormone-like compounds with oestrogen activity and the ability to bind weakly to oestrogen receptors (Setchell and Adlercreutz, 1988; Smith and Yang, 1994; Tham et al., 1998). Approximately 10 to 100 fold less than 17-β-oestradiol (Davis et al., 1998). However, isoflavonoids may have antioestrogenic potency, when the circulating
Isoflavoids might counteract endogenous oestrogens, through competitive binding to oestrogen receptors, although Shutt and Cox (1972) reported that the relative binding affinity of isoflavonoids for oestrogen receptor is only 0.05-1% of the binding affinity of 17-B-oestradiol. However, Santiti et al. (1998) reported that dietary isoflavonoids competed with endogenous oestrogens for active sites of oestrogen biosynthesis and metabolism enzymes and thus altered the concentration of biologically active endogenous oestrogens at the target cell level. Hence, on the basis of both weak oestrogen receptor binding capacity and competition for oestrogen metabolising pathway, isoflavonoids might exert significant modulation of oestrogen-dependent mechanisms.

The isoflavonoids and the lignans have now been shown to influence not only sex hormone metabolism and biological activity (Smith and Yang, 1994; Tham et al., 1998), but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation and angiogenesis (Adlercreutz, 1995; Adlercreutz and Mazur, 1997; Kapistis et al., 1997). It has been reported that some isoflavones (e.g., genisteen) have the ability to inhibit tyrrosine-specific protein kinase in a redox-sensitive manner (Akayama et al., 1987; Adlercreutz, 1995), also topoisomerase II (Adlercreutz, 1990), and protein histidine kinase (Huang et al., 1992). Phyto-oestrogens are also antioxidants, as discussed in antioxidant effects of phyto-oestrogens. The potential of isoflavones to modify cell proliferation and cancer is discussed in antineoplastic effects of phyto-oestrogens. Hence, the ability of isoflavonoid phyto-oestrogens to influence sex hormone metabolism, biological activity, intracellular enzyme, protein synthesis, growth factor action, malignant cell proliferation, differentiation and angiogenesis in a way to make them strong candidates for a role as natural cancer protective.

Figure 1. Structure of the most important isoflavonoids and lignans identified in human biological samples (Adlercreutz, 1995; Kelly et al., 1993).

Concentration of the isoflavone aglycones becomes 100-fold greater than oestradiol (Davis et al., 1998). Hence the oestrogenic or anti-oestrogenic potency of isoflavones may depend on the level of natural oestrogens. A recent report also indicates that the metabolite (equol) of an orally consumed isoflavonoid (e.g., daidzein) has greater oestrogenic activity than the parent compound (Wiseman, 1999).
METABOLISM OF PHYTO-OESTROGENS

The isoflavonoids genistein and daidzein are derived from the precursors biochanin-A and formononetin present in the plant. When ingested, daidzein can be metabolised by bacteria in the large intestine to form equol (oestrogenic) or O-demethylangolensin (non-oestrogenic) (Witman, 1999), whereas genistein is metabolised to the non-oestrogenic p-ethyl phenol. However, not all subjects who consume soy products can metabolise daidzein to equol, or they exceed this isoflavone in a very low amount, thus there is an interindividual metabolic variation between subjects (Morton et al., 1994; Kelley et al., 1995).

The following isoflavonoid phyto-oestrogens have been identified in human urine: genistein, daidzein, O-demethylangolensin and equol (Axelson et al., 1992; Adlecrcrutz et al., 1991, Adlecrcrutz, 1995). Moreover, studies on absorption and metabolism have found these compounds in plasma (Adlecrcrutz et al., 1993), breast milk (Franke et al., 1998) and faeces (Adlecrcrutz et al., 1995). Bannwart et al. (1984, 1985) also reported that a small amount of plant lignans (matairesinol, lariciresinol, isoferulicresinol, and secoisolariciresinol) have also been identified in human urine.

ANTICARCINOGENIC EFFECTS OF PHYTO-OESTROGENS

Studies of cancer prevention have assessed isoflavonoid phyto-oestrogens for their efficacy in inhibiting cancer in vitro and in vivo particularly in models of the hormone-dependent human cancers. Interestingly, protection against carcinogenesis was provided by very low concentrations of isoflavones. The potential mechanisms for inhibition of cancer by isoflavonoid phyto-oestrogens, whether via their oestrogenic properties or their antioxidant activity, will be described below.

A. Oestrogenic and anti-oestrogenic activity

Breast cancer

The soybean isoflavones, genistein and daidzein have been studied for anti-breast cancer activity, because of their oestrogen receptor antagonist and agonist activities. Constantinou et al. (1996) reported that injection of genistein and daidzein (0.8 mg daily for six months) had the ability to protect against N-methyl-N-nitrosourea-induced mammary tumours in rats. In this study, both genistein and daidzein moderately reduced the number of tumours, but only marginally reduced the tumour incidence. Furthermore, Constantinou et al. (1998) demonstrated that the growth of both the oestrogen-receptor positive human breast cancer cell line (MCF-7), or the oestrogen-receptor negative human breast cancer cell line (MDA-MB-468), was inhibited by genistein. In addition, treatment of these cells with genistein prior to implantation into nude mice decreased their growth in the animal (Constantinou et al., 1998). These investigators suggested that inhibition of human cancer cell growth by genistein, was unrelated to the oestrogenic activity of this compound.

Considering that neonatal oestrogen was known to inhibit both spontaneous and chemically-induced breast cancer, and that dietary isoflavones in early life have been suspected of playing a role in human breast cancer, it may be asked whether isoflavonoids can inhibit the formation of breast cancer. Lamartine et al. (1995) have reported that the development of mammary tumours induced by DMBA, could be delayed by giving 5 mg genistein to neonatal rats on days 2, 4 and 6 postpartum. Furthermore, administration of genistein (0.25 and 250 mg/kg diet) from conception to 21 days postpartum prior to treatment with DMBA at 50 days postpartum, resulted in a dose responsive inhibition of mammary tumours by altering the opening of mammary gland development in rats (Lamartine et al., 1998). Thus there were fewer terminal end buds and few undifferentiated terminal ductal stuctures of mammary glands in the rats at 21 and 50 days of age (Fritz et al., 1998), before carcinogen treatment.
In contrast with oestrogen or genistein in early life protecting against breast cancer, further studies determined that genistein (750 ppm in the diet), like oestrogen, when administered during tumour development, enhanced the growth of oestrogen-responsive tumours (Hsieh et al., 1998; Allerd et al., 2001). Genistein (10 nM to 10 μM) was found to enhance the proliferation of MCF-7 human breast cancer cells, both in vitro and in ovariectomised athymic mice. Genistein (1 μM) acted as an oestrogen agonist that induced expression of the oestrogen-responsive gene pS2 (Hsieh et al., 1998). These findings suggest that caution should be used in considering cancer prevention by soybean isoflavones in humans. This is of particular concern because high-potency isoflavone preparations are now available as dietary supplements.

Endometrial cancer

Studies on endometrial carcinogenesis in mice have shown that administration of genistein or daidzein (1mg/30g body weight) significantly decreased the level of oestriadiol-induced expression of mRNAs for c-jun, c-fos, IL-1α and TNF-α in the uterus of ovariectomised mice (Lian et al., 2001). These investigators further reported that the incidences of endometrial adenocarcinoma and atypical endometrial hyperplasia, were significantly lower in the group of mice given oestriadiol plus genistein or plus daidzein, than the group with oestriadiol alone. These findings indicate that both genistein and daidzein have an inhibitory effect on oestrogen-related endometrial carcinogenesis in mice, possibly by suppressing expression of the oestrogen-induced genes c-fos and c-jun.

Prostate cancer

Mitchell et al., (2000) have shown shown that genistein, daidzein, coumestrol and equol inhibited cell growth and DNA damage in the human prostate tumour cell lines, androgen-receptor positive LNCaP and androgen-receptor negative PC-3. Genistein induced DNA damage and inhibited cell growth in both cell lines at <10 μM. Daidzein inhibited cell growth at 10-100 μM and yet had no effect on DNA damage up to 500 μM. Hence, despite their structural similarity, these phyto-oestrogens inhibit prostate tumour cell growth by different mechanisms.

In humans, the effect of isoflavones has been studied on prostate cancer patients (Stephens, 1997). Peroral administration of 160 mg of a phytooestrogen preparation from red clover (Trifolium pratense) daily for 7 days prior to operation, was shown to reduce the prostate specific antigen level, PSA, and to induce apoptosis in the prostatectomy specimen. These results, especially the apoptosis, indicate androgen deprivation and resemble the typical response to oestrogen therapy (Hellstrom et al., 1993; Ford et al., 1994).

B. Anti-proliferation

Cancer prevention is generally associated with inhibition, reversion, or retardation of cellular hyperproliferation. Isoflavonoids in general appear non-toxic to humans and animals, and have been demonstrated to inhibit proliferation in many kinds of cultured human cancer cell lines. Le-Bail et al., (1998) have reported that genistein in high concentrations (50 μM) has anti-proliferative activity in breast-cancer MCF-7 cells through an oestrogen-independent mechanism. Moreover, Kuo (1996) demonstrated that the two most potent isoflavonoids, quercetin and genistein (1-100 μM) have dose responsive anti-proliferative potency in human colon carcinoma HT29 and Caco-2 cell lines via an apoptosis induction mechanism. Genistein and synthetic analogues, at 0.1-25 μg/ml, were found to have anti-proliferative potency in transformed human (SW620, HT29) intestinal epithelial cell lines and to have induced apoptosis (Booth et al., 1999). Genistein and biochanin A have also been shown to inhibit epidermal growth factor in the human prostate cancer cell lines LNCaP and DU-145 (Peterson and Barns, 1993), with IC50 values from 8.0 to 2.4 μg/ml and 4.3 to 15 μg/ml respectively.
Although the anti-proliferative effects of isoflavonoids in cultured cells appear well established, relatively little data have been published regarding anti-proliferative activity in vivo. Studies by Wei et al. (1998) focused on the ability of the isoflavone genistein to inhibit skin tumorigenesis in mice. These studies demonstrated that topically applied genistein (10 μmol), prior to the carcinogen DMBA, reduced tumour multiplicity and tumour incidence. This was associated with genistein blockage of DMBA-induced bulky DNA-seduct formation (Wei et al., 1998).

C. Cell cycle arrest and apoptosis

The investigators of the anti-proliferative effects of isoflavonoids noted that these compounds may inhibit the cell cycle or induce apoptosis. It has been demonstrated that genistein, at 5-20 μg/ml, produced cell cycle arrest at both the G1/S and G2/M phases in human myelogenous leukaemia HL-60 cell lines and the lymphoecytic leukaemia MOLT-4 cell lines (Traganos et al., 1992). Moreover, human gastric cancer cells were arrested at G2/M by genistein, up to 60 μM (Matsukawa et al., 1993). Studies in a non-small-cell lung cancer cell line, demonstrated that genistein, at 30 μM, induced G2/M arrest and apoptosis induction (Lian et al., 1998). Zhou et al., (1998) reported that the isoflavones (genistein, daidzein, and biochanin A), at 0 to 50 μM, inhibited growth of murine and human bladder cancer cell lines, by inducing cell cycle arrest and apoptosis. Cell cycle arrest and induction of apoptosis, could be functionally related to the activation of p53 (Plaumann et al., 1996) and the inhibition of cell cycle kinase activity (Kyle et al., 1997).

D. Regulation of host immune function

The role of host immune function, has become important in understanding the mechanisms that are involved in cancer prevention. Middleton (1998) has reported that a number of immune cell systems, do not appear to be affected significantly by flavonoids, while they are resting. However, once a cell becomes activated by a physiological stimulus, a flavonoid-sensitive substance is generated and interaction of flavonoids with that substance alters the outcome of the activation process (Middleton and Kandaswami, 1992; Middleton, 1998). Zhang et al., (1999) skin demonstrated that daidzein, genistein and genistein glucuronide; in nutritionally relevant concentrations (0.1 to 10 μM), enhanced the activation of NK cells in vitro.

In vivo, the isoflavone daidzein administered orally at 20 to 40 mg/kg body weight, stimulated murine nonspecific immunity, activated humoral immunity and enhanced cell-mediated immunity (Zhang et al., 1997). Moreover, daidzein at the physiologically relevant concentrations (0.01 to 10 μM) potentiates lymphocyte activation in murine spleen, suggesting that the immunostimulatory effects of daidzein may be involved in cancer chemoprevention (Wang et al., 1997). Our study in mouse skin showed that topically applied equal at 1 to 20 μM markedly reduced UV-induced inflammation and abrogated the UV-induced immunosuppression (Widyasmithi et al., 2001). Furthermore, we found equal protected similarly from immunosuppression, induced by the putative epidermal mediator, cis-UCA, indicating a potential mechanism of action involving inactivation of this UV-photoprotein. Recent study in mouse skin model demonstrated that topically applied equal at 10 μM reduced UV-induced skin cancer (Widyasmithi et al., 2005), indicating that immunostimulatory and antiinflammatory effect of equal involved in cancer chemoprevention.

ANTIOXIDANT EFFECTS OF PHYTO-OESTROGENS

The antioxidant properties of genistein and other isoflavones have been demonstrated in several experimental models (Arora et al., 1991; Mitchell et al., 1998), such as protection from 12-O-tetradecanoyl phorbol-13 acetate (TPA)-induced ·O₂⁻ or H₂O₂ formation, and particularly from UV-induced oxidative damage to DNA in vitro (Wei et al., 1993; Wei et al., 1995; Wei et al., 1996; Cai et al., 1997). In mice, dietary genistein has been
shown to stimulate the endogenous antioxidants, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R) and glutathione-s-transferase (GSH-S-T) (Cai and Wei, 1996), with the effects found mainly in the small intestine and the skin. Furthermore, genistein has also suppressed prostaglandin synthesis, both basally and UV-stimulated, in cultured human cells and human skin (Isoeharen et al., 1999), in a manner similar to the antioxidant N-acetylcysteine.

The antioxidant activity of isoflavonoids was suggested to be related to the number and position of hydroxyl groups. The active compounds all have 4'-hydroxyl and 5'-hydroxyl groups, which is consistent with other observations indicating that these groups are crucial for antioxidants activity due to their ability to scavenge free radicals (Wei et al., 1999; Araora et al., 1998). In contrast, both biochanin A (4'-O-methyl group) and genistein (4'-hydroxyl group) were found to inhibit UV induction of oxidative lesions in DNA in vitro, although the reactive oxygen scavenging properties of the two compounds were shown to be very different (Wei et al., 1996; Ruiz-Larrea et al., 1997). Araora et al. (1998) have also reported that equal 4'-hydroxyl group and genistein (4'-hydroxyl group) were effective scavengers of metal ion radicals such as iron Fe (II) and Fe (III) in vitro. However, whereas the chemical structures of phytoestrogens may play an important role in providing protection against oxidants by scavenging free radicals, the precise structural requirements remain unclear.

It has been suggested that the oestrogen receptor may activate an antioxidant response, and it is possible that isoflavonoid phyto-oestrogens may possess antioxidant activity dependent on their receptor-binding characteristics. Natural oestrogens have significant radical-scavenging antioxidant activity (Ruiz-Larrea et al., 2000) post-menopausal women had significantly elevated plasma antioxidant thiol levels after 6 months of hormone replacement therapy (Konukoglu et al., 2000) and rabbits receiving transdermal oestrogen for 4 months doubled their total reactive antioxidant potential (Blumel et al., 2000). There is also evidence that intraperitoneal injection of 17-α-1-estradiol increased heme oxygenase (HO)-1 activity in the brain of rats (Lai et al., 2002). The lipid protecting antioxidant activity of a diet of increased soy protein content; a source of phyto-oestrogens, was not accompanied by altered urinary sex hormone activity, however (Dentzins et al., 2000). Therefore, it remains to be clarified, whether the protection against oxidative damage by phyto-oestrogens might be regulated via its oestrogenic action.

CONCLUSION

To conclude, in vitro and in vivo studies in the models of human cancers shown that isoflavonoid phyto-oestrogens are natural cancer-protective compounds. Very low concentrations of isoflavonoid phyto-oestrogens (in μM), have the potential mechanisms in inhibiting cancer progression via their oestrogenic properties, antiproliferation activities, and their ability to regulate host-immune function. Moreover, antioxidants properties of isoflavonoid phyto-estrogens generally agreed to be involved in the cancer chemoprevention. Epidemiologic investigations strongly support these above studies because the highest level of phyto-oestrogens in the diet are found in the countries or regions with low cancer incidence (Adlercreutz, 1995).

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