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Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment*

Dietary phyto-oestrogens and cancer

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INTRODUCTION

The significance of the structural similarity of the lignans and isoflavones to mammalian oestrogens and possible effects on cancer prevention were first promulgated in the early 1980s in publications of Setchell and of Adlercreutz (ref. 1). Since that time the literature on the possible health benefits of the isoflavones found predominantly in soy beans, has expanded exponentially, mainly in response to funding initiatives by the US government and soy bean industries, and more lately by European and UK Ministries of Food. Despite their more widespread occurrence in foods, and greater consumption in western populations, the lignans have received comparatively little attention.

In vitro studies have established that plant oestrogens are oestrogenic, since they have the ability to bind to mammalian oestrogen receptors. Their affinity to receptors (from rabbit, sheep and rat uterine receptors, and a human cancer cell line) has been compared with oestradiol. Coumestrol has the greatest affinity, only 10–20 times lower than oestradiol; and genistein about 100 times less. Daidzein and equol bind about 1000 times less. Similar findings are evident when their ability to increase uterine weight of mice is studied (ref. 2).

However, although these weak oestrogens bind to the oestrogen receptor complex, they fail to stimulate a full oestrogenic response of replenishment of oestrogen receptors and protein synthesis. They have thus been characterised as antagonising oestradiol and acting as both oestrogens and anti-oestrogens, at least in rats. Newer studies have shown that they bind especially to β oestrogen receptors (refs 1–3). Since they are eaten in comparatively high amounts, they are therefore able to interfere with the feedback system of the release of gonadotrophins and thus account for fertility problems in animals (ref. 4). Enterolactone has also been shown to have anti-oestrogenic effects, suppressing rat uterine RNA synthesis induced by oestradiol (ref. 5).

The cancers most closely linked to plant oestrogens are the hormone related sites of breast and prostate, and bowel cancer, which are less common in soy consuming populations. Cross sectional studies demonstrated higher levels in urine and plasma samples from individuals in these populations and also in vegetarians (refs 6–9). However, as yet there have been few detailed epidemiological studies relating intakes of plant oestrogens to individual risk of these diseases. For the time being evidence that increased intakes would be beneficial is derived from experimental and physiological findings.

BREAST CANCER

Breast cancer is the commonest cancer of women living in western populations, and incidence is rising. As with most cancers, rates increase with age so that premenopausal cancer is less common than post menopausal. However, the rate of increase begins to slow after the menopause. Development is highly dependent on the hormones associated with ovarian function, and events that occur premenopausally, and perhaps during adolescence, set the scene for later postmenopausal breast cancer. Established hormone

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related risk factors include early menarche, late age at menopause, delayed age at first pregnancy, and, in post menopausal women, elevated plasma free oestradiol concentrations. Family history is also important, although established genetic factors account for only about 4% of breast cancers in most western populations (refs 10–12). Dietary factors currently linked to breast cancer include high meat, fat and low vegetable and fibre intakes, together with high alcohol consumption (ref. 13). Adipose tissue is able to synthesise oestrogens from the precursor hormone androstenedione. After the menopause adipose tissue is the major source, and serum levels of oestrogens can also be shown to fall in women who lose weight. Overweight women are therefore at about a two fold increased risk of postmenopausal breast cancer.

The anti-oestrogen Tamoxifen is known to substantially reduce the risk of mortality from breast cancer by about 20% over 2 years and to reduce the incidence of cancer in the contralateral breast (ref. 14). Some of the hormonal effects of plant oestrogens are similar to those of Tamoxifen, and it is therefore suggested that the plant oestrogens would have a Tamoxifen like effect in reducing the risk of breast cancer, suppressing gonadotrophins and increasing sex hormone binding globulin (ref. 15). In sheep, coumestrol and genistein bind to receptors isolated from the pituitary and hypothalamus, although direct inhibition of gonadotrophins has not been shown (ref. 16). In humans, gonadotrophins are generally suppressed, increasing the length of the menstrual cycle, with no consistent effects on serum oestrogens with either lignans or isoflavones (refs 15,17–22). It has been suggested that plant oestrogens would increase sex hormone binding globulin (SHBG) levels, an effect similar to that of Tamoxifen (ref. 23). However, SHBG is affected by for example changes in body weight, making cross sectional comparisons difficult. In most controlled studies, plant oestrogens do not affect sex hormone binding globulin, but a in a recent study of women given sufficient soybean foods and flax to increase serum levels 100 fold, there was a marked increase in SHBG levels. (refs 15,17–24).

The gonadotrophic effects of plant oestrogens entail longer menstrual cycles. This effect would induce a lower lifetime exposure to oestradiol levels in the luteal phase which has been related to lower risk of both pre and post menopausal breast cancer (ref. 25). These effects of soy in women are coupled with the fact that breast cancer rates used to be low and menstrual cycle lengths longer in Far east populations where the traditional diet is rich in soy (ref. 26). However, the evidence is conflicting since irregular rather than long menstrual cycles are generally related to lower breast cancer risk (refs 25,27,28).

Despite the hypothesised beneficial anti-oestrogenic effects of plant oestrogens in human cancer, two reports suggest that caution is necessary at this stage because these may be oestrogenic effects on the breast. In one study 29 women took 60 g soy (containing 45 mg isoflavones) for 14 days and breast cell proliferation was assessed by 3H thymidine labelling and the antigen Ki67 in biopsies before and after supplementation. Preliminary reports showed a significant increase in the proliferation rate of breast lobular epithelium (ref. 29). In another report, 24 women were studied for 6 months whilst taking a soy drink containing 38 mg of genistein. Fluid was aspirated from the nipple of the breast for analysis of cytology of epithelial cells, and to determine whether the volume and colour would be reduced, towards levels previously found in low risk Japanese and Chinese women. Contrary to expectations however, the risk factors increased, with an increased frequency of hyperplastic cells after soya supplementation (ref. 20).

Two early prospective investigations reported significant protective effects of soy on breast cancer risk in Japanese women (refs 30,31). In a later case control study in Singapore, a significant protective effect was shown with premenopausal breast cancer risk (ref. 32). No relation was shown in a case control study in China, although post and premenopausal risk was not studied separately (ref. 33). Messina (ref. 34) summarised existing epidemiological studies; in general there was a trend towards a protective effect of soy in breast cancer but these studies had generally used rather crude methods and did not distinguish between pre and postmenopausal cancer. Newer epidemiological studies are beginning to report results derived from more specific methods, for example excretion in urine has been used to indicate consumption of plant oestrogens. One recent case control study showed that cases excreted significantly less equol and enterolactone in 72 hour urine collections compared with matched controls, although genistein was not measured (ref. 35).

In animal models, soya products have generally reduced tumours induced by chemical carcinogens. Seven out of nine studies have shown lower numbers of tumours in rats whose diet was supplemented with soya compared with those fed a standard diet, and no study showed an increase in the numbers of tumours. These effects were not seen when soy products which had been treated to remove the isoflavones were fed (ref. 36). The chemopreventive effects are generally attributed to the anti-oestrogenic characteristics of isoflavones. In a careful study where a purified laboratory diet free of isoflavones was given, administration of genistein to new born rats also reduced the numbers of tumours later induced by administration of carcinogen injections to fully grown animals, perhaps because the early administration of genistein resulted in a more mature gland with less susceptible structures to later initiation by the chemical carcinogen (ref. 37).

ENDOMETRIAL CANCER

Cancer of the endometrium is more common in developed countries, with a similar pattern of hormonal risk factors to breast cancer. However, there is clearer evidence for a role for unopposed oestrogens in increasing risk from studies of different types of the contraceptive pill, and the increased risk from hormonal replacement therapy (ref. 38). After the menopause, the other main source of oestrogens is from endogenous formation in adipose tissue, and obesity is a marked risk factor (ref. 39).

Endometrial cancer risk is increased in women treated with the anti-oestrogen Tamoxifen, raising the possibility of a similar effect with plant oestrogens. However, there are no reports of increased risk associated with plant oestrogens. Incidence rates in Japan are somewhat lower than in the UK (ref. 40) and one report in Asian migrants to Hawaii found that soy consumption is associated with a reduced risk (ref. 41).

PROSTATE CANCER

Prostate cancer is the most common hormone related cancer in men, and incidence has been rising rapidly by about 3 to 4% a year in the UK. High fat and meat, low non - starch polysaccharide (fibre) diets are currently linked to increased risk of the disease, and like breast cancer age adjusted rates are comparatively rare in Far east populations consuming soy. Isoflavone levels in prostatic fluid are much higher in Hong Kong and Chinese men consuming soy than in Portuguese and British men (ref. 42). Of the human epidemiological studies, conducted in Japan and Japanese migrants to Hawaii, there was a tendency for increased soy consumption to be associated with lower risk, but only three studies have been conducted. In animal models, all three studies investigating the effects of soy showed reduced tumorigenesis. (refs 34,36).

There have been few studies of the effects of plant oestrogens on hormone levels. In small trials, both soya and linseed supplements did not affect testosterone levels (refs 19,22,43). In one trial the gonadotrophin FSH was suppressed, but no effects were shown with linseeds in another (refs 22,44). Clinical trials of soya in men with abnormal prostate specific antigen are reported to be in progress (ref. 44).

There has been little investigation of the effect of the lignans, although they too have been proposed to be protective in prostate cancer (ref. 23). Like isoflavones, they also inhibit 5α-reductase (ref. 45). Of the population groups studied, Portuguese men have markedly higher levels of lignans in prostatic fluid compared with British, Hong Kong and Chinese men (ref. 42). The incidence of prostate cancer in Portugal is higher than in Hong Kong and China, but about half that of Britain (ref. 40).

OTHER ANTI-CANCER EFFECTS OF PLANT OESTROGENS

There are several other anticancer effects of isoflavones which are not related to their anti-oestrogenic properties. Genistein is known to inhibit tyrosine kinases, which are responsible for phosphorylating proteins required for the regulation of cell functions, including cell division. Hence, it has been shown to inhibit growth in many cell lines. These lines include those which do not have oestrogen receptors, which

suggests that these effects may be independent of any anti-oestrogen effects. Genistein has also been shown to inhibit the DNA repair enzyme topoisomerase, and to act as an antioxidant thus potentially preventing oxidative DNA damage (ref. 46). In one cell line, genistein has been shown to cause changes characteristic of apoptosis, or programmed cell death, a protective mechanism induced in cells that have been damaged in order to prevent the proliferation of harmful mutations and possibly cancer (ref. 47). It has also been shown to inhibit ras gene expression in a rat pheochromocytoma cell line (ref. 48). In addition, genistein has been shown to inhibit angiogenesis, the formation of new blood vessels, an abnormal event which occurs as part of the growth and expansion of malignant tumours (ref. 49). It has been pointed out that many of these effects have been shown with very high concentrations, and not in cells treated with the levels likely to be achieved in plasma of humans eating foods containing plant oestrogens (ref. 36). 100 umols were needed for a significant suppression of angiogenesis for example, although proliferation was inhibited at 5 µmol levels and below (ref. 49). This compares with plasma levels of about 0.4 µmol/L in Japanese men and women fed dietary supplements of isoflavones (refs 8,50) and peak levels of about 4 µmol/L in women fed 126 mg isoflavones of soy milk, rising to 12 µmol/L when fed 480 mg isoflavones (refs 51,52).

BOWEL CANCER

Bowel cancer is the second most common cancer in the UK, after breast in women and lung in men. Up to the 1960's it was rare in Japan and Far East countries, but incidence has increased rapidly, so that age specific large bowel cancer rates in males are now greater in Japan than in the UK (ref. 53). These changes are attributed to westernisation of the Japanese diet over the same period of time, although there has been no formal examination of soy consumption in relation to trends in bowel cancer rates.

There have been 8 case control studies examining a role for soy in protection against colorectal cancer, in China, Japan, and in Japanese migrants to the US. There is not a clear relationship, because studies have yielded either non significant results, or protective or causative associations in about equal numbers (ref. 35). The initial hypotheses suggested the lignans to be the active chemopreventive agent , but there has been little investigation of lignan intake or excretion in relation to bowel cancer (refs 54,55).

Colon cancer does not have a strong association with hormone status, but there are a number of other possible mechanisms whereby lignans or isoflavones could be involved in the aetiology of bowel cancer, see above. For example, via the suppression of inducible NO synthase by genistein, there may be a role for isoflavones to inhibit endogenous N-Nitrosation that occurs when meat is consumed (ref. 56). In animals, aberrant crypts are accepted early markers of colon cancer which can be induced by standard chemical carcinogens. One study has shown that linseed is able to reduced the numbers of aberrant crypts formed in such an animal model, and another that genistein has the same effect (refs 57,58).

OTHER CANCERS

Messina *et al.* (ref. 59) have collated results from case control investigations of soy in relation to cancers at other sites, mainly conducted in Far east populations. Results tend to show that increased soy consumption is also protective in lung and stomach cancer. In addition, in a variety of different animal models of liver cancer, various soy products have protective effects and, as discussed above, there are a number of mechanisms to account for a general reduction of tumours induced by such means (refs 36,46). High levels of raw soy flour are known to cause pancreatic carcinomas in rats, probably due to the presence of a trypsin inhibitor (ref. 60). Levels are reduced on cooking or processing, however, and there is no epidemiological evidence that human populations consuming soy are at greater risk of pancreatic cancer (ref. 61).

CONCLUSIONS

Potentially the plant oestrogens would seem to have wide ranging anti cancer effects. Similar to the 'anti-oestrogen' Tamoxifen, the indications are that both oestrogenic and anti-oestrogenic effects, characteristic of weak oestrogens, may occur with supplementation but a consensus awaits the findings of further trials in humans. Results from cell lines have shown that genistein seems to have fundamental effects in controlling cell signalling, growth and gene expression, which is probably also particularly important in cancer prevention.

The lignans have hardly been investigated both epidemiologically and in animal models, despite their known anti-oestrogenic effects. This is partly a result of lack of industrial funding, but also problems with analytical techniques, especially lack of internal standards, so that little information on food level is available in order to investigate them epidemiologically. Prospective studies in which biological specimens have been collected are being conducted in order to assess hormone and gene nutrient interactions, and biomarkers of plant oestrogen intake in blood and urine.

There is insufficient evidence at present for these compounds in most existing epidemiological studies, which have used crude methods, and have mainly been conducted in soy consuming populations, with no distinction between pre and post menopausal state. No account of interaction with other dietary and constitutional factors has been taken. For example hormonal status, *Helicobacter* infection, and genetic polymorphisms make a substantial contribution to most common cancers when combined with dietary factors. However, new evidence relating these compounds to cancer risk is emerging.

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