

Phytoestrogens after breast cancer

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Abstract

The current extension of the indications for adjuvant chemotherapy, which predisposes to early menopause, and the media coverage of the benefits of hormone replacement therapy (HRT) have led patients with a history of breast cancer to seek treatments for estrogen deprivation. In breast cancer survivors, most physicians avoid HRT because of concern regarding the potential promotion of growth of occult malignant cells by estrogens, due to the estrogen dependence of breast cancer. Soy phytoestrogens are being promoted as the 'natural alternative' to HRT and have been available without restrictions for several years as nutritional supplements. In this paper, data on the complex mammary effects of phytoestrogens in epidemiological studies, in *in vitro* studies, as well as in *in vivo* studies on animal carcinogenesis are reviewed. The potential benefits and risks of phytoestrogens are analyzed, and the prescription of phytoestrogens to postmenopausal women after breast cancer and the coprescription with the anti-estrogen tamoxifen are discussed. The absence of controlled trials and technical checking of extraction and titration in these preparations on 'free sale' raise a new problem in terms of public health and justify close reasoning and a cautious attitude of physicians, as well as straight information given to women, especially after breast cancer.

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Introduction

The current extension of the indications for adjuvant chemotherapy predisposing to early menopause, the media coverage of the benefits of menopausal hormone replacement therapy (HRT), and the well-founded desire of an optimal quality of life have led more and more patients with a history of breast cancer to seek treatments for estrogen deprivation.

The prescription of HRT after breast cancer raises a number of problems due to the estrogen dependence of breast cancer, while the possible increased risk of breast cancer in healthy women taking HRT has to be considered (Schairer *et al.* 2000). Some authors have proposed the prescription of HRT after breast cancer (Vassilopoulos-Sellin *et al.* 1999), but these prescriptions remain limited to a few experimental studies; most physicians continue to avoid HRT in breast cancer survivors because of concern regarding the promotion of growth and dissemination of occult malignant cells while on HRT. Besides these treatments, which are prescribed under medical control, soy phytoestrogens derived from plants are being promoted as the 'natural alternative' to HRT, and have been available without restriction as nutritional supplements for several years. After breast cancer, some

women decide, sometimes 'by themselves', to take these phytoestrogens to alleviate menopausal symptoms. Recently, Ginsburg (2000) underlined the marked contrast between the rigid testing procedures required for synthetic estrogens and the absence of controlled trials or standardization of dose in those preparations on 'free sale' to the public. But patients who present contra-indications to traditional HRT are the potential users of these products. Can they be used safely after breast cancer? What will be the real benefits of these preparations? And what about the association of phytoestrogens and tamoxifen (Tam), the anti-estrogen widely prescribed as a long term adjuvant treatment of breast cancer?

Interests of phytoestrogens for the treatment of estrogen deprivation

Estrogen deprivation induces three orders of symptoms: in the short term, women will present climacteric symptoms, hot flushes, vaginal dryness, and sometimes, psychological and sexual disorders. Some years later, they will present osteoporosis and cardiovascular events.

What are phytoestrogens?

Phytoestrogens have a similar chemical structure to estrogens (for reviews see Murkies *et al.* 1998, Setchell 1998, Tham *et al.* 1998). They are classified into two main categories: isoflavones and lignanes. The two main isoflavones are genistein and daidzein which are essentially found in leguminous vegetables and soy beans. Lignanes are found in high concentrations in linseed. The two main lignanes are enterolactone and enterodiol.

Many factors influence the availability of isoflavones and lignanes after ingestion, especially the presence of fibers, the individual characteristics of metabolism, the ingestion of antibiotics, etc.

Phytoestrogens and menopausal disorders

The rarity of hot flushes in Japanese women has led some authors to test the efficacy of a soy-rich diet on this symptom. Albertazzi *et al.* (1998) carried out a double-blind parallel multi-center randomized placebo-controlled trial of 104 post-menopausal women: patients were taking 60 g of isolated soy protein daily (containing 76 mg isoflavones) versus 60 g of placebo. Soy was significantly superior to placebo ($P > 0.01$) in reducing the mean number of hot flushes per 24 h after 4, 8, and 12 weeks of treatment. This study did not find an effect of phytoestrogen on the other symptoms of menopause (Kupperman index).

On the other hand, Quella *et al.* (2000) recently conducted a double-blind placebo-controlled trial of the effect of 150 mg isoflavones/day on hot flushes in patients treated for breast cancer: no significant difference was observed compared with placebo, but the duration of treatment was brief (4 weeks) and 68% of patients in each group also took tamoxifen, which competes with genistein for binding to estrogen receptors (ER).

We can conclude from these studies that phytoestrogens have a moderate effect on hot flushes, but do not appear markedly to improve other parameters of the Kupperman index that reflect the other symptoms of menopause.

Phytoestrogens and osteoporosis

Osteoporotic fracture rates are lower in Asian women. Once again, it has been hypothesized that a soy-rich diet has a protective effect on bone (Tham *et al.* 1998), but there are many confounding factors (lifestyle, socio-cultural and morphological factors) distinguishing Asian women from Western women.

Animal studies have demonstrated a favorable effect of isoflavones on bone: a diet rich in isoflavones partially prevents bone loss induced by ovariectomy in female rats (Arjmandi *et al.* 1996).

Studies on ipriflavone, a synthetic isoflavone, have also shown that this derivative slightly prevents bone loss in

women treated with a gonadotropin-releasing hormone agonist (Gambacciani *et al.* 1994). However, this is a synthetic derivative used at pharmacological doses and its effects therefore cannot be extrapolated to those of natural phytoestrogens.

Only a few clinical trials have been reported. Potter *et al.* (1998) showed in postmenopausal women that a diet rich in isoflavones taken for 6 months resulted in slightly increased lumbar bone mineral density (2%), but this was a short-term study conducted on a small number of women. Although isoflavones appear to prevent bone loss under certain experimental conditions, their effect must be confirmed by longer term studies on sufficient sample sizes, and by focusing, in the intermediate term, on intermediate factors such as bone mineral density, and in the long-term, on the fracture risk.

Phytoestrogens and cardiovascular protection

The protective role of a soy-rich diet on cardiovascular diseases has been suggested by several authors, based on the decreased incidence of cardiovascular disease in Asian countries. Once again, this is only an hypothesis, as Asian populations have a completely different lifestyle and their diet also differs from Western diets by its much higher fiber content and lower saturated fat content (Tham *et al.* 1998). However, studies concerning the effects of soy on lipid parameters appear to show a reduction of cholesterol in response to soy-rich diets. These studies are delicate (difficulties of retrospective evaluation of soy content in the diet) and are often based on small sample sizes. The meta-analysis of 38 studies by Anderson *et al.* (1995), taking into account the fat content of these diets, showed that a diet containing an average of 47 g soy protein decreased total cholesterol by about 9.3%, low density lipoprotein (LDL)-cholesterol by 12.9%, and triglycerides by 10.5%. The change in serum cholesterol and LDL-cholesterol concentrations were directly related to the initial serum cholesterol concentrations. In contrast, high density lipoprotein (HDL)-cholesterol was not significantly modified. Although these results are encouraging, it should be noted that they were obtained with soy-rich diets, and not isoflavone supplements. Once again, further studies are required to prove that dietary phytoestrogen supplementation has a real long-term cardiovascular protective effect.

Phytoestrogens and the breast

Soy intake and breast cancer: epidemiological data

There is a geographical variation of breast cancer risk. It is higher in the United States and in Western Europe, and lower

in Chinese, Japanese, and Asian women in general, when they live in their country of origin (where the risk is sixfold lower). In contrast, the risk of breast cancer in Asian women increases after several generations when they emigrate to the United States. Therefore this variation of incidence is probably not related to a genetic factor, but to an environmental factor. The diet of Asian women differs considerably from that of Western women, and contains less fat and more fiber. Several investigators have proposed the hypothesis that the lower incidence of breast cancer in Asia is related to the consumption of soy rich in phytoestrogens.

Case-control studies evaluating the breast cancer risk according to the soy content of the woman's diet have been conducted to validate this hypothesis (for review see Wu *et al.* 1998). Some of these studies demonstrated a reduction of the relative risk (RR) of breast cancer (RR of about 0.4) in women consuming large quantities of soy, but exclusively in premenopausal women, while other studies indicated a moderate and non-significant reduction of this risk.

These studies also present a number of methodological problems: it is difficult precisely to define the exact quantities of phytoestrogens ingested by means of sometimes nonspecific nutritional questionnaires.

This problem was overcome by Ingram *et al.* (1997) who, in a case-control study of 144 women with breast cancer and their matched controls, showed that the risk of breast cancer was significantly decreased in women with a high urinary excretion of two phytoestrogens, equol and enterolactone.

Although these data are interesting, it must be remembered that the protective role of soy phytoestrogens for breast cancer is only an hypothesis: the protection provided for Asian women could also be due to other characteristics of their diet, other ingredients of soy beans, or other socio-cultural or medical factors (for example contraceptive habits).

Phytoestrogens and breast tissue: experimental data

Pharmacology

The chemical structure of phytoestrogens and their structural relationship with estrogens allows them to bind to estrogen receptors (ER). Phytoestrogens can be considered as weak estrogens, presenting an activity 100 to 1000 times lower than that of 17 β -estradiol (E2), depending on the system studied (Martin *et al.* 1978, Markiewicz *et al.* 1993, Zava & Duwe 1997). However, in individuals with a moderate soy intake, plasma concentrations of phytoestrogens are 1000 times higher than endogenous estrogen concentrations in women of reproductive age. Plasma genistein concentrations are of the order of 0.1 to 3 μ M/l (Zava & Duwe 1997). The affinities of genistein and daidzein for ER are also lower than

that of estradiol. The order of magnitude varies according to the system studied; for example, the affinity of genistein for ER is 20 to 100 times lower than that of E2 (Martin *et al.* 1978, Kuiper *et al.* 1997). Finally, a second type of ER has recently been demonstrated and cloned, ER β , which has a specific anatomical distribution (bone, brain, vascular endothelium), and for which phytoestrogens appear to have a higher affinity than for ER α (Kuiper *et al.* 1997).

In vitro studies on breast cancer cells

Over recent years, many *in vitro* studies on mammary cells have identified the effects of the main phytoestrogens, especially genistein and daidzein (Martin *et al.* 1978, Sathyamoorthy & Wang 1997, Wang & Kurzer 1997, Zava & Duwe 1997, Hsieh *et al.* 1998). Studies of the effects of increasing doses of genistein on induction of an estrogen-dependent protein (pS2) and on the quantity of DNA, reflecting proliferation, in cultures of MCF7 cancer cells (expressing the ER α estrogen receptor), demonstrated a biphasic effect of genistein on mammary cells, depending on the concentrations in the culture medium: (i) at 'physiological' doses (i.e. at doses corresponding to plasma concentrations achieved with a high soy intake (100 nM/l to 1 μ M/l), genistein stimulates cellular proliferation and this effect is dependent on ER; (ii) at physiological doses, in the presence of physiological doses of estradiol, genistein behaves like a competitive inhibitor for the binding site of E2 to ER and slightly inhibits cellular proliferation, since it has a lower activity than E2; (iii) at pharmacological doses (>10 μ M/l), it markedly inhibits cellular proliferation. This effect is not ER-dependent and is probably related to inhibition of the tyrosine kinase activity of growth factor receptors.

On clearly defined systems, the key determinants of the activity of genistein therefore appear to be the genistein and estradiol concentrations in the culture medium.

How can these results be extrapolated to man?

Theoretical inhibitory concentrations of genistein are much higher than the concentrations obtained as a result of moderate soy intake, providing between 20 mg and 80 mg genistein (soy intake in the USA is 1 to 3 mg/day). There is also a marked individual variability of phytoestrogen metabolism: for example, 30% of individuals metabolize daidzein into equol, which has a higher affinity for ER (Sathyamoorthy & Wang 1997).

Therefore, this raises the problem of the steroid concentrations actually available in breast tissue: without a precise knowledge of intramammary estradiol and genistein concentrations, it is impossible to predict whether the final effect will be markedly inhibitory (due to inhibition of tyrosine kinase), slightly inhibitory (due to competitive inhibition of the binding of E2 to ER), or stimulatory (when local E2 concentrations are very low).

Animal carcinogenesis studies

When newborn female rats are treated with genistein and then exposed to a chemical carcinogen (DMBA), genistein induces an increased latency and a decreased incidence and number of induced mammary tumors compared with animals treated with vehicle alone (Lamartinière *et al.* 1995). Barnes (1997) demonstrated the fundamental role of the time of exposure to isoflavones. This protective effect is much more marked when animals are treated with genistein during the neonatal or prepubertal period; the number of tumors is less markedly decreased when genistein is administered later. These *in vivo* studies suggest an antitumor activity of genistein.

Recently, Hsieh *et al.* (1998) found that dietary genistein may act as an estrogen agonist *in vivo*. MCF7 cells were implanted in ovariectomized athymic nude mice, and the growth of the estrogen-dependent tumors was measured weekly under various conditions: a control diet, a diet with genistein, and a diet supplemented with estradiol. Tumors were larger in the genistein diet group than in the control group, and after 12 weeks they were similar in size to tumors after two weeks of estradiol treatment, demonstrating that dietary genistein was able to enhance the growth of the MCF7 cells tumors *in vivo*. This study underlines the potential for dietary genistein to stimulate the growth of estrogen-dependent tumors in humans with low circulating estrogen levels, such as those found in postmenopausal women.

Whether genistein acts as a chemopreventive agent or as an agent to stimulate tumor growth will probably depend on the age at which females receive genistein, on the timing of genistein administration (i.e. before or after the induction of the tumor), and on the dose of genistein.

Some authors believe that exposure at a young age to high concentrations of isoflavones may induce the differentiation of mammary cells and a subsequent decreased sensitivity to estrogenic stimulation. The studies by Setchell *et al.* (1997) showed that new-born infants fed with soy milk have considerable plasma isoflavone concentrations (6 to 11 times higher than the concentrations per weight which induced a hormonal effect in adults). Rather than daily consumption during adulthood, the protective effect may be due to exposure to high doses of isoflavones very early in life.

In vivo studies in women

In vivo studies in women are rare and methodologically difficult, as they require either sampling of breast tissue (selecting patients with pre-existing disease), or the study of markers which are more difficult to interpret (nipple fluid secretions). The time in the menstrual cycle at which the tests are performed must also be taken into account.

McMichael-Philips *et al.* (1998) studied the effect of

supplementation with 45 mg isoflavones for a fortnight in women requiring surgery for a benign or malignant breast tumor, in whom breast tissue was taken from a healthy zone for the study of proliferation markers (thymidine incorporation index and Ki67). Cell proliferation rates were increased in women treated with isoflavones, taking into account the woman's age and the phase of the menstrual cycle.

Hargreaves *et al.* (1999) studied the effect of 14-day supplementation with 45 mg isoflavones in 84 non-postmenopausal women. The study of nipple aspiration fluid showed increased levels of the estrogen-dependent protein, pS2, in women treated with isoflavones.

In conclusion, the effect of phytoestrogens on breast tissue is complex: intramammary genistein and estradiol concentrations, and the timing of exposure appear to play a major role in determining agonistic or antagonistic effects.

Indications for phytoestrogens

In postmenopausal women with no contraindications to HRT

Current descriptive epidemiology data in Asian women, and the absence of any obvious harmful effects of soy-rich diets constitute arguments which encourage Western women to adopt a high-fiber diet, low in saturated fats and comprising a soy supplement.

However, the supplementation of a diet with extracts containing isoflavones needs to be considered more carefully. Various preparations extracted from soy and containing isoflavones are now freely available on the market. The media coverage of treatments of the menopause, the fear of the adverse effects of 'classical' treatments and the 'ecological' trend towards consumption of plant extracts, often considered by patients to be 'inoffensive', all incite women to use phytoestrogens. In view of the theoretical risk of self-prescribed medication, physicians must define precisely the place of phytoestrogens in the treatment of disorders of the menopause and inform their patients accordingly.

The general indications for phytoestrogens in postmenopausal women are based on the current data in the literature: there is no sufficient scientific evidence, at the present time, to recommend phytoestrogen supplements for either prevention of osteoporosis (either prevention of 'physiological' postmenopausal bone loss or prevention of the fracture risk in women with a low bone mass), or prevention of cardiovascular disease.

Therefore, the current indications for phytoestrogens appear to be limited to the treatment of hot flushes in postmenopausal women who either refuse or do not tolerate conventional estrogen-based HRT because of metrorrhagia or weight problems.

In postmenopausal women presenting a contraindication to HRT

A distinction must be made for patients presenting a contraindication to HRT, especially after a hormone-dependent cancer such as a breast cancer: it would be very tempting to propose, in patients in whom the prescription of estrogens is theoretically contraindicated, a 'substitute' for HRT which, in addition, would also possess possible antitumor properties.

We have seen that animal carcinogenesis data demonstrate an antitumor effect of phytoestrogens, but only at high doses, and only when they are administered during the neonatal or peripubertal periods. We have also seen that *in vitro* data have clearly established the biphasic and largely dose-dependent effect of phytoestrogens especially genistein, which must be considered to be true weak estrogens rather than selective estrogen receptor modulators (SERM) (molecules binding to ER and exerting either agonist or antagonist effects, depending on the tissue). The final effect on breast tissue depends on the intramammary genistein and estradiol concentrations which, in turn, depend on the individual tissue metabolism. Some groups have recently underlined the importance of the intratissue biosynthesis of steroids (intracrine concept) (Blankenstein *et al.* 1999, Chetrite *et al.* 2000). Current scientific data are unable to predict the final effect of phytoestrogens in the breast (Zava & Duwe 1997). On the other hand, the efficacy of anti-estrogen adjuvant therapy after breast cancer (Early Breast Cancer Trialists' Collaborative Group 1998) suggests the possibility that the use of a weak estrogen could be harmful in patients after a hormone-dependent cancer at the stage of micrometastases, and justifies a cautious attitude (Hargreaves *et al.* 1999, Ginsburg 2000).

A combination of phytoestrogens and Tam for the control of hot flushes after breast cancer (Quella *et al.* 2000) also raises the problem of their possible competition. In the case of genistein, this corresponds to administering two molecules, an antagonist and a weak agonist, at equivalent plasma concentrations, with approximately the same affinity for ER α (Kuiper *et al.* 1997)! According to the cell culture studies by Zava and Duwe (1997), genistein probably partially displaces Tam from ER, thus decreasing its inhibitory effect. In contrast, Tam and genistein at very high doses effectively exert a synergistic anti-proliferative action, but at these doses, the effects are no longer dependent on estrogen receptors (Zava & Duwe 1997, Shen *et al.* 1999). As precise scientific data on the intramammary concentrations of these two molecules are not yet available, it does not seem logical to coprescribe a product with Tam that is likely to decrease its efficacy.

In conclusion, prescription of phytoestrogens after breast cancer can only be considered in patients (i) fully informed about the real benefits and risks of these preparations, (ii) in

order to treat postmenopausal hot flushes, (iii) after seeking the oncologist's advice (good prognosis breast cancer, a long time after the diagnosis), (iv) in the absence of coprescription of Tam, and (v) with careful breast surveillance. This treatment should only be continued when it has a marked efficacy on the symptoms.

Future prospects

The unrestricted sale of plant products constitutes a new situation for physicians with little training in phytotherapy. The qualitative and quantitative diversity of the commercially available preparations, their variable phytoestrogen contents (essentially depending on extraction techniques), the absence of precise prescribing guidelines, and the risk of self-prescribed medication justify the introduction of 'phyto-vigilance' procedures and practical proposals.

A list of the available preparations should be established, patients should be clearly informed about the properties and uncertainties of phytoestrogens, and randomized placebo-controlled clinical trials should be conducted, in parallel with ongoing research, to determine the efficacy of phytoestrogens on the improvement of quality of life, and their safety (especially endometrial). A register of patients taking phytoestrogens after breast cancer could be proposed but, in order to be rigorous, it would ideally have to take into account the precise qualitative and quantitative content of the products consumed, including those in the diet, and the individual metabolism of phytoestrogens.

Finally, combined administration of Tam and phytoestrogens should only be considered in the context of randomized trials designed to test the *in vivo* impact of this combination, not only on menopausal symptoms, but also on the efficacy of adjuvant treatments for breast cancer.

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