Phytoestrogens and hormone dependent cancers

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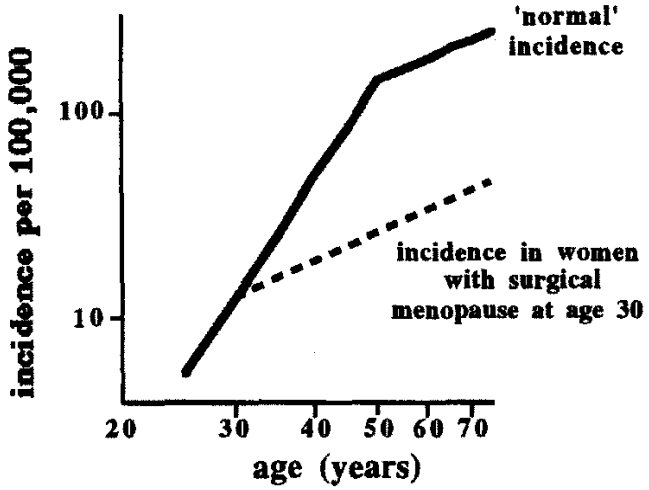
Summary

The potential for specific compounds found in fruit, vegetables and cereals to influence the synthesis, metabolism and effects of steroid hormones has raised the exciting possibility that specific foods, especially those containing soy and flaxseed, may significantly influence the risk of cancers whose genesis and progression are known to be hormone dependent. To date there is weak ecologic evidence; an increasing number of studies in animals and cell lines; but very little analytical epidemiology on which to base claims that these phytoestrogens are protective, either against breast cancer in women, or against prostate cancer in men. Nevertheless, on the basis of possible health benefits of a more short-term nature (eg relief of perimenopausal morbidity, and osteoporosis), penetration of soy and flaxseed-containing foods into the Australian food supply is set to increase.

Introduction

Excluding non-melanocytic skin cancers, the breast is the most common site of cancer in Australian women (1); and the prostate gland is the most common site in men (2). The sensitivity of both the breast and prostate to steroid hormones is well established. Epidemiologic evidence that normal menstrual cycling determines a woman's risk of breast cancer has been elegantly synthesised by Pike and coworkers (3), whose age-risk profiles show that women who undergo a surgically induced 'menopause' early in life have dramatically lower risks than women in whom cycling continues until menopause occurs naturally (see Figure). That a cyclical exposure to oestrogen is the mediating step in this very strong association is highly probable—but the exact mechanisms remain obscure. Early age at



Age dependence of the risk of breast cancer - adapted from (47).

menarche, late age at first birth, late onset of menopause and short cycle-lengths are all consistently associated with higher risks for breast cancer and they all impact significantly on steroid hormone exposure (4)—but attempts to relate breast cancer risk to plasma concentrations of oestrogen at specific times in the menstrual cycle have yielded disappointingly inconsistent findings. Despite this, however, there remains a strong conviction that oestrogen, or a metabolite, is closely involved in the aetiology of breast cancer—sand

deprivation of oestrogen has been recognised for a very long time as an effective, if temporary, control in the treatment of many breast cancers.

Just as oestrogen deprivation has a major effect on breast cancer, androgen blockade is the cornerstone of most treatments of prostate enlargement and cancer (5); and just as surgically induced menopause significantly reduces subsequent risk of breast cancer, men castrated sufficiently young have very low risks of prostate cancer (6). However, in a further parallel with the role of oestrogens in breast cancer biology, attempts to relate plasma testosterone concentration to prostate cancer risk have yielded inconsistent findings.

What is a phytoestrogen?

There is still no agreement on how to define a phytoestrogen. Since much of our understanding of these compounds stems from work conducted on 'clover disease' in farm animals over fifty years ago (7), it might seem reasonable to adopt the old agricultural definition of a phytoestrogen, viz, 'a compound that acts on the central nervous system and induces 'oestrus' (mating) in female mammals' (8). Given that sexual activity in women is not closely linked to oestrogen secretion, such a definition is now manifestly unsuitable. In the intervening fifty years we have greatly increased our understanding of how oestrogens exert some of their effects. One major signal transduction pathway involves oestrogen binding to a receptor within an inhibitory protein complex which includes, inter alia, heat-shock proteins, dissociation of the receptor from its complex followed by dimerisation, and the subsequent binding of this dimer to 'oestrogen-receptor-elements' or EREs in the genes. Binding of the receptor to the ERE then induces changes in transcriptional activity at sites distant to the ERE (9). This knowledge has led to a recent definition of phytoestrogens as 'plant derived compounds that can regulate gene expression that is mediated by an ERE, in a manner either comparable or apparently antagonistic to 17β-oestradiol, as a result of binding to the oestrogen receptor' (10). But such a definition would seem to be unnecessarily restrictive and preclude interactions which, while not as complex as the signal transduction pathway just described, are nevertheless quite specific, and are becoming increasingly associated in the literature with the term 'phytoestrogenic'.

Agonists or antagonists?

A confusing aspect of the usage of the term 'phytoestrogenic' is the fact that compounds to which this adjective is commonly applied may either mimic the effects of natural endogenous oestrogen, or they may block them—and the reason that there has never really been any serious suggestion that it would be useful to coin a term like 'phytoantiestrogen' is that the same compound may be an agonist in one situation and an antagonist in others. A substance which is sufficiently similar to endogenous oestrogens to occupy an oestrogen binding site, but not sufficiently similar to reliably induce the event which normally ensues when natural oestrogen binds, would clearly be acting as an antagonist. However, when oestrogen is either absent, or present only in very low concentrations (postmenopausally, for example), then the limited ability of the phytoestrogen to induce the secondary event may still be significant, and warrant its reclassification as an agonist. Given the demonstration in the Figure of an increasing risk of breast cancer with increasing cumulative oestrogen exposure it is perhaps not surprising that our interest in the relevance of phytoestrogens to breast cancer stems from their ability to act as antagonists of oestradiol.

Sources of phytoestrogens in the diet

Major classes of phytoestrogenic substances include bioflavonoid compounds (flavones, isoflavones, flavanones), lignans, coumestrol from legume sprouts, and zearalenone, a mycotoxin derived from fungal moulds. While bioflavonoid compounds are widespread in foods of plant origin, the most significant compounds with oestrogenic activity in this class are

genistein and daidzein, found in largest amounts in the soybean (11), and formononetin from clovers. Lignans, which are characterised chemically by a 2,3-dibenzylbutane structure are also widespread in plant foods, although the flaxseed contains concentrations which are two orders of magnitude higher than any other known source (12). Oestrogenic activity is critically dependent on the metabolism of these compounds by the microflora in the large bowel (or the rumen of farm animals), where daidzein may be either generated from formononetin or metabolised to equol (13,14), and the 'mammalian lignans' enterolactone and enterodiol are generated from less oestrogenic precursors such as matairesinol and secoisolariciresinol (15).

Effect of phytoestrogens on breast cancer cell lines

Growth responses of breast cancer cell lines when exposed to phytoestrogens in the growth medium have proved to be difficult to interpret. The emerging consensus appears to be that phytoestrogens do not act as direct antioestrogens in their effects on cell growth (16)—and in some situations they appear to actually promote growth. The report by Welshons et al of the lignan enterolactone and the isoflavonoid metabolite equol stimulating growth of breast cancer cells in vitro is one example (17). However in competition studies with mixtures of phytoestrogens and oestradiol, antioestrogenic effects are often observed, and Adlercreutz et al found that oestradiol and the lignan enterolactone stimulated the growth of MCF-7 cells on their own, (ie the lignan alone was apparently oestrogenic) but when they were mixed together in the growth medium enterolactone completely inhibited the response to oestradiol (18). While a number of studies have found genistein to be an effective growth inhibitor of cell lines—the observation that it is equally effective in both MCF-7 cells which are oestrogen dependent, and MDA-468 cells which are not, is further support for the notion that these effects are not necessarily mediated via direct competition with oestradiol for an oestrogen growth receptor. In cell lines derived from non-hormone dependent cancers at other sites, genistein has also been found to be an effective inducer of differentiation and the expression of the mature phenotype (see (19) for references). While breast cancer cell lines have been useful in increasing our understanding of the effects of phytoestrogens, it must be emphasised that no-one has ever claimed that the inhibitory effects observed with, say, genistein would be sufficient to have a significant effect on established breast cancer. The benefits which might accrue from regular consumption of phytoestrogen rich foods are more likely to be chemopreventive than chemotherapeutic.

Effects of phytoestrogens on breast cancer - in vivo animal studies

Messina et al (19) identified eight studies involving soy products and experimental mammary cancer. One study used x-ray irradiation; the others all used dimethylbenz[a]anthracene or N-methyl-N'-nitrosourea as the carcinogenic agent. Five of these studies reported a protective effect, which is not overwhelming evidence for soyfoods being anticarcinogens—although the same review article also summarised additional studies which observed protective effects at several other sites. Very little work has been done with lignans, although Thompson et al reported favourable effects of a linseed enriched diet on cell proliferation and nuclear aberrations in rats, and on mammary tumour initiation and promotion in weanling rats given dimethylbenz[a] anthracene (20).

Effect of phytoestrogens on prostate cancer cell lines

Both genistein and daidzein appear to be able to inhibit proliferation of prostate cancer cells in vitro (21-24) although this ability was not always observed when the cell lines were implanted subcutaneously in rats fed genistein (24). The lignan enterolactone and its plant precursor matairesinol have also been found to inhibit prostate cancer cell growth in vitro (22).

Phytoestrogens and angiogenesis

Angiogenesis, the process by which new capillaries develop from pre-existing vessels, and on which 'solid' cancers are critically dependent for growth, has been shown to be sensitive to phytoestrogens—especially genistein (25). Oestrogen stimulates the production of Vascular Endothelial Growth Factor during the extensive vascular remodelling which accompanies the normal cycle-specific changes in the human female reproductive tract (26)- and although the ability of genistein to inhibit angiogenesis is often discussed separately from its more obviously oestrogenic properties, it is interesting to speculate, that this ability to inhibit angiogenesis may also be an antiestrogenic effect.

Effects of phytoestrogens on sex hormone binding globulins

Sex hormone binding globulins (SHBG) are circulating proteins which are synthesised in the liver, and which exhibit a high affinity for both oestradiol and testosterone (27). Since the biological activity of steroid hormones bound to SHBG is very low, their bioavailability is determined to a significant extent by the circulating levels of SHBG. Indeed SHBG concentrations increase in response to rising levels of either sex hormone and hence appear to be acting as regulators. The notion that phytoestrogens might stimulate synthesis of SHBG and thereby significantly reduce the bioactivity of endogenous oestrogens has been championed by Adlercreutz and coworkers (28, 29)—but their human work was based on very heterogeneous groups of participants, and other studies, including unpublished work of our own, have failed to observe any dietary dependence of SHBG (30). The important finding by Mousavi and Adlercreutz that genistein effectively stimulates SHBG synthesis in human liver cancer cells (Hep-G2), and suppresses their growth, is a much stronger argument for an SHBG-mediated effect of phytoestrogens (31).

Effects of phytoestrogens on markers of carcinogenesis

The observation that some phytoestrogens can inhibit growth in tumours with and without oestrogen receptors underscores the potential importance of these mechanisms in the prevention of malignancy. Genistein, for example, appears to be able to inhibit the tyrosine-protein kinase intimately involved in determining the activity of proteins which regulate cell proliferation (32); to inhibit topoisomerase II (33); and to arrest the cell division cycle around the G2 to M phases (34).

Some effects of phytoestrogens on steroid hormone synthesis and metabolism

Within the context of breast cancer, an important property of phytoestrogens may be their ability to inhibit the cytochrome P450 aromatase, which catalyses the final step in the synthesis of oestrogen and oestrone from testosterone and androstenedione respectively (35,36).

For prostate cancer, the ability of phytoestrogens to inhibit the reductase which converts testosterone to its bioactive form in the prostate, dihydrotestosterone may be an important chemopreventive mechanism. Certainly this reductase has been the target of chemotherapeutic drugs such as finasteride that are currently undergoing Phase III clinical trials in the US. Enterolactone was found to be a much stronger inhibitor of the 5α-reductase than genistein in homogenates prepared from benign prostatic hyperplasia tissue (37).

Human epidemiologic studies of breast and prostate cancer

Descriptive studies examining urinary excretion or plasma levels of phytoestrogens in groups with different experiences of hormone dependent cancers have recently been summarised by Adlercreutz and Mazur (38). While phytoestrogen intakes are highest in the populations with

the lowest cancer risk, this evidence remains circumstantial—and experience with the correlational studies of per capita fat consumption and breast cancer should have taught us to regard this kind of evidence as encouraging, but open to many alternative interpretations.

To date only a few human studies have directly investigated the dietary intake of phytoestrogen-rich foods in relation to breast cancer risk. An extremely large cohort study in Japan reported a 'dose-dependent' decrease with increasing consumption of soybean paste (miso) soup (39). While this early study failed to collect data on ancillary risk factors, the sheer weight of numbers (142,857 women) would require some extreme biases of a systematic nature (eg large age-related differences in consumption of miso) for this trend to be nullified. Another cohort study, which used diets of 6860 Japanese men in Hawaii as surrogate measures of the dietary habits of their wives, found that reduced risks of breast cancer in the wives were associated with high intakes of miso and tofu by their husbands during the years 1971-75, but not during the earlier years of 1965-68 (40). A case-control study in Singapore reported significantly reduced risks of breast cancer with increasing dietary intakes of 'soy protein'; the ratio of soy to total protein; and total soy products (41). Another case-control study in Japan found no association, but the emphasis of this study was more on the consumption of fat (oil) from soy rather than soy itself (42). An especially exciting finding, from a case-control study conducted in Western Australia by Ingram et al, of substantially reduced risks of breast cancer associated with high urinary excretion of phytoestrogens is currently in press (43).

Even fewer data are available for phytoestrogens and prostate cancer. Hirayama's cohort study found a non significant reduction in risk with increasing consumption of miso among the 122,261 Japanese men studied (44)—and in another cohort of 7999 Japanese men in Hawaii there was an inverse (protective) association with intake of tofu but not miso (45). The only available case-control data were also collected in Japan, but found no consistent relationship between prostate hyperplasia and miso consumption (46).

Concluding remarks

In many ways the phytoestrogen and cancer story is a case-study in the contemporary status of the nutritional epidemiology of cancer. Weak ecologic data shows that countries whose populations consume the largest amounts of phytoestrogen-rich foods also have the lowest incidence of hormone dependent cancers. Laboratory studies in animal models of breast and prostate cancer together with observations of the effects of phytoestrogens on cell lines either in vitro or implanted, have been encouraging, and have assisted in the identification of a considerable number of mechanisms, although the relative importance of these mechanisms individually is largely undetermined—and the ability of many phytoestrogens to act as weak agonists of endogenous oestrogens is confusing. Some of these mechanisms directly involve oestrogen signal transduction pathways, but others clearly do not. The evidence from analytical human epidemiology is both sparse and of variable quality—but generally supportive of the hypothesis that phytoestrogens may be chemopreventive agents. In 1997 the information is still not sufficiently convincing, either with respect to their anti-carcinogenic properties or the 'doses' needed to achieve them, in order to make dietary recommendations of a public health nature—but with other potential health benefits of phytoestrogens becoming more widely known, the consumption of phytoestrogen rich foods, especially those containing soy and/or linseed has already begun to rise.

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