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The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids

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ABSTRACT The female flowers of the hop plant have long been used as a preservative and a flavoring agent in beer, but they are now being included in some herbal preparations for women for "breast enhancement". This study investigated the relative estrogenic, androgenic and progestogenic activities of the known phytoestrogen, 8-prenylnaringenin, and structurally related hop flavonoids. 6-Prenylnaringenin, 6,8-diprenylnaringenin and 8-geranylnaringenin exhibited some estrogenicity, but their potency was less than 1% of that of 8-prenylnaringenin. 8-Prenylnaringenin alone competed strongly with 17β-estradiol for binding to both the α- and β-estrogen receptors. None of the compounds (xanthohumol, isoxanthohumol, 8-prenylnaringenin, 6-prenylnaringenin, 3'-geranylchalconaringenin, 6-geranylnaringenin, 8-geranylnaringenin, 4'-*O*-methyl-3'-prenylchalconaringenin and 6,8-diprenylnaringenin) nor polyphenolic hop extracts showed progestogenic or androgenic bioactivity. These results indicate that the endocrine properties of hops and hop products are due to the very high estrogenic activity of 8-prenylnaringenin and concern must be expressed about the unrestricted use of hops in herbal preparations for women.

The female flowers ("cones") of hops *(Humulus lupulus* L.*)* are used primarily as a preservative and as a flavoring agent in beer. A recurring suggestion over the years has been that hops have a powerful estrogenic activity. When hops were picked by hand, menstrual disturbances amongst female pickers were common (1). In Germany, hop baths were used for the treatment of gynaecological disorders and hop extracts have been reported to reduce hot flushes in menopausal women (2). Currently hops are being incorporated into a number of commercial preparations for women with claims of "breast enhancement".

We recently identified 8-prenylnaringenin as a very potent phytoestrogen in hops, with an activity equal to or greater than other established plant estrogens (3). The lupulin glands of the hop flowers contain 8-prenylnaringenin along with other prenylflavonoids and the hop acids essential in brewing. The prenylated chalcone, xanthohumol (Fig.1) is the major flavonoid component of the lupulin secretion, reaching a content of about 1% of the dry weight of the hop cone (1). Other prenylflavonoids including isoxanthohumol, 6-prenylnaringenin and 8-prenylnaringenin as well as geranylated flavonoids are minor constituents and some of these may be derived from non-enzymatic mechanisms (4). The relative proportions of the individual flavonoids can change considerably due to conversions during drying, storage and processing of hops and, furthermore, during brewing, hence various amounts of these compounds are found in hop products and beers (5, 6).

In view of the potent estrogenic activity of 8-prenylnaringenin in hop cones and the variable amounts of the individual prenylflavonoids in hop products, the present study was undertaken to characterize the endocrine activities of the various hop flavonoids to which humans may be exposed. The estrogenic, androgenic and progestogenic actions of the compounds were investigated using sensitive *in-vitro* bioassays (7, 8, 9). In addition, following the reported high affinity of some phytoestrogens for the β-estrogen receptor (ERβ) (10), competitive binding studies between 8-prenylnaringenin and 17β-estradiol were performed with purified α- and β- estrogen receptors.

Materials and methods

Polyphenolic extracts of hops. Polyphenolic hop extracts and prenylated/geranylated compounds (xanthohumol, isoxanthohumol, 8-prenylnaringenin, 6-prenylnaringenin, 3'-geranylchalconaringenin, 6-geranylnaringenin, 8-geranylnaringenin, 4'-*O*-methyl-3'-prenylchalconaringenin and 6,8-diprenylnaringenin) were isolated and identified from hops, as previously described (3, 4). Hop extracts and pure compounds were diluted in ethanol to prepare 1 mM stock solutions. For use in the yeast screens, 20 µl aliquots were added to individual wells in a 96-well plate and the ethanol was evaporated before adding the hormone-inducible yeasts.

Determination of estrogenic, androgenic progestogenic activity. Hormonal activity was investigated using three separate recombinant yeast screens. Estrogenic activity was determined using an estrogen-inducible yeast screen *(Saccharomyces cerevisiae)* expressing the human estrogen receptor and containing expression plasmids carrying estrogen-responsive sequences controlling the reporter gene lac-Z (encoding the enzyme β-galactosidase) (7). Estrogenic activity was determined from the metabolism of chlorophenol red β-D-galactopyranoside by monitoring the absorbance at 540 nm. The principle of the androgen yeast screen was similar to that of the estrogen screen, with the yeast strain PGKhAR expressing the human androgen receptor (8). The androgen screen responded to testosterone and 5α-dihydrotestosterone at levels of 10^{-9} M. Both the estrogenic and androgenic yeast screen were originally developed in the Genetics Department of Glaxo Wellcome plc (Stevenage, Herts, UK) and were a gift from Professor J. Sumpter, Brunel University, UK. The progestogenic yeast screen was based on a yeast strain (DY150) containing the DNA sequence of the human progesterone receptor and expression plasmids carrying progesterone-responsive sequences expressing the enzyme β-galactosidase (9). In this assay, the stimulation of β-galactosidase-activity was detected after lysing the cells with 5% CHAPS (11). The progestogen screen responded to progesterone at levels of $>10^{-9}$ M.

Receptor binding. Human recombinant estrogen receptors α and β (ERα and ERβ) were obtained from PanVera Corporation (Madison, USA). Dilutions of the compounds were incubated in 100 µl buffer (10 mM Trizma preset crystals (pH 7.5), 10% glycerol, 2 mM DDT, 1 mg/ml bovine serum albumin) with 15 nM [2,4,6,7-^{3}H] 17β-estradiol (84.0 Ci.mmol^{-1}; Amersham Life Science, Amersham UK) and ER (1.5 nM). The mixture was incubated overnight at 4°C and the free and bound hormone were separated using 100 µl 50% hydroxylapatite slurry (in 50 mM Tris-CL, pH 7.4, 1 mM EDTA). After three washes in buffer (ERα: 40 mM Tris pH 7.5, 100 mM KCl, 1 mM EDTA, 1 mM EGTA; ERβ: 40 mM Tris, pH 7.5), the slurry was extracted with 2 washes of 200 µl ethanol.

Chemicals. All chemicals were obtained from Sigma Chemical, Dorset, UK.

Results

Estrogenic activity was detected in four hop flavonoids (Fig 2), with their activity in the order: 8-prenylnaringenin >> 6-prenylnaringenin, 8-geranylnaringenin and 6,8-diprenylnaringenin. No estrogenicity was found in xanthohumol, isoxanthohumol, 3'-geranylchalconaringenin or 4'-*O*-methyl-3'-prenylchalconaringenin. Also, 6-geranylnaringenin was inactive. None of the compounds, nor polyphenolic extracts of hops, showed any activity in either the androgenic or the progestogenic yeast screens (data not shown).

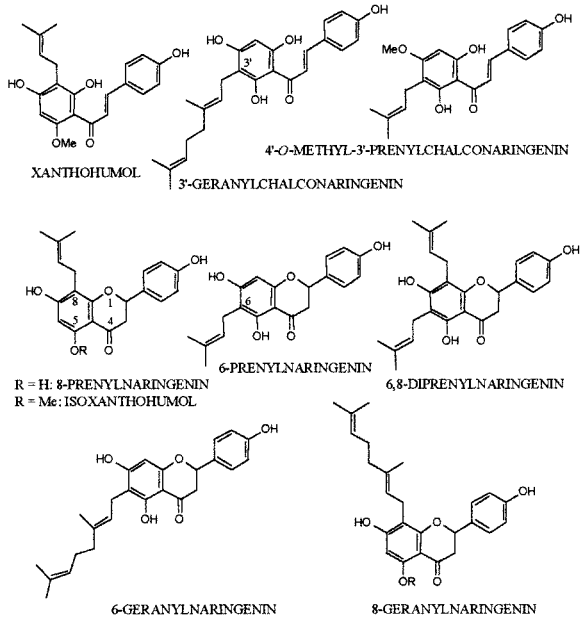

Fig. 1: Structures of hop flavonoids

The estrogenic activity of 8-prenylnaringenin was confirmed in competitive binding assays with purified ERα and ERβ. 8-Prenylnaringenin competed strongly with 17β-estradiol for binding to both receptors with a relative binding affinity of about 0.1 (17β-estradiol = 1). 8-Geranylnaringenin showed some competition (Relative Binding Affinity > 0.001). None of the other compounds was able to displace ^{3}H-17β-estradiol from either receptor (Relative Binding Affinity > 0.0001).

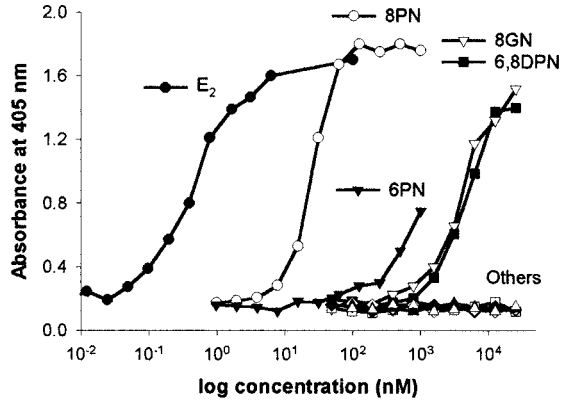

Fig. 2: Assessment of the estrogenic activity of hop-derived prenylflavonoids determined by a yeast screen containing the human estrogen receptor. All points are means ± s.e.m. of at least 4 determinations. E_2 = 17β-estradiol; 8PN = 8-prenylnaringenin; 8GN = 8-geranylnaringenin; 6,8DPN = 6,8-diprenylnaringenin; 6GN = 6-geranylnaringenin; Others = xanthohumol, isoxanthohumol, 3'-geranylchalconaringenin and 4'-*O*-methyl-3'-prenylchalconaringenin.

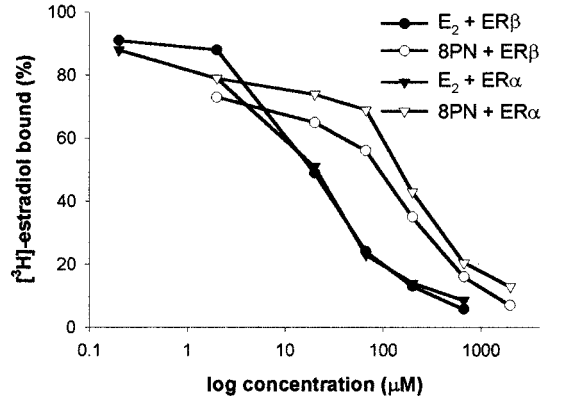

Fig 3: Competitive displacement of [2,4,6,7-^{3}H] 17β-estradiol from isolated ERα and ERβ by 17β-estradiol and 8-prenylnaringenin. All points are means ± s.e.m. of 3 determinations. E_2 = 17β-estradiol; ER = estrogen receptor; 8PN = 8-prenylnaringenin.

Discussion

The results indicate that the endocrine activity of hops and hop products is mainly due to the high estrogenic activity of 8-prenylnaringenin. Some estrogenicity is present in a few other hop-related prenylflavonoids, but the activity is very weak and these compounds are only minor constituents of hops (5). There was no evidence of either androgenic or progestogenic activity in either polyphenolic hop extracts or pure prenylflavonoids. Like some other phytoestrogens, 8-prenylnaringenin also shows strong affinity for ERβ as well as for ERα (10), suggesting that 8-prenylnaringenin may have many potential sites of action within the body.

In addition to the estrogenic activity of 8-prenylnaringenin, a number of other biological activities have been ascribed to individual prenylflavonoids, including antiproliferative effects on breast and colon cancer cell lines, inhibition of cytochrome P450-mediated activation of procarcinogens, inhibition of bone resorption and inhibition of diacylglycerol acyltransferase activity (4, 12-17). This range of potential bioactivities raises the question of whether the exposure to the prenylflavonoids in hops has any physiological significance to humans. The main use of hops is in the flavoring of beer and the content of prenylflavonoids in beers can be up to 4 mg/L. Isoxanthohumol, xanthohumol and 6-prenylnaringenin constitute more than 90% of the total amount of prenylflavonoids in beer, with 8-prenylnaringenin and 6-geranylnaringenin being minor components (≤ 0.24 and 0.07mg/L, respectively) (5, 6). Whether any of the reported health-beneficial effects of moderate beer consumption (18, 19) can be attributed to prenylflavonoids remains to be determined.

The current-day incorporation of hops into a number of herbal preparations for women, including those claiming "breast enlargement", is of more immediate concern because it may result in much higher levels of human exposure to prenylflavonoids and other hop compounds. Controlled clinical trials of hop constituents have not been done so far. It is conceivable that the claimed efficacy of 8-prenylnaringenin reflects the action as an estrogen either centrally (hot flushes) or peripherally (breast tissue). If this were the case, the potential for adverse effects (e.g. in relation to fertility and hormone-related cancer) must also be considered. The biological effects of prenylated flavonoids within the body are poorly understood and scientific evaluation of the safety of high exposure levels is essential.

Acknowledgements

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