Binding of Phytoestrogens to Rat Uterine Estrogen Receptors and Human Sex Hormone-binding Globulins

Pablo Ibieta Hillerns[a], Yuangang Zu[b], Yu-Jie Fu[b], and Michael Wink[a,*]

[a] Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany. Fax: +049-6221-544884. E-mail: wink@uni-hd.de

[b] Northeast Forestry University, Key Laboratory of Forest Plant Ecology, Harbin 150040, China

* Author for correspondence and reprint requests

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The interaction of phytoestrogens with the most important binding sites of steroid hormones, *i.e.* sex hormone-binding globulin and estrogen receptors, was investigated. Relative binding affinities and association constants for 21 compounds among them isoflavones, flavones, flavonols, flavanones, chalcones and lignans were determined. The lignan nordihydroguaiaretic acid weakly displaced 17β-[^{3}H]-estradiol from estrogen receptor and Scatchard analysis suggests non-conformational changes. Compounds from *Glycyrrhiza glabra*, liquiritigenin and isoliquiritigenin, showed estrogenic affinities to both receptors. 18β-Glycyrrhetinic acid displaced 17β-[^{3}H]-estradiol from sex hormone-binding globulin but not from the estrogen receptor. Phytoestrogens compete with 17β-estradiol much stronger than with 5α-dihydrotestosterone for binding to sex hormone-binding globulin.

Key words: Phytoestrogens, Estrogen Receptor, Sex Hormone-binding Globulin

Introduction

Some naturally occurring plant-compounds (phytoestrogens) such as flavonoids and lignans are known to exhibit hormone-like activities, such as estrogenic activities. These compounds are produced by plants or in the human body after metabolisation by intestinal microorganisms (Day *et al.*, 2000; Setchell *et al.*, 2002). The interaction of phytoestrogens with molecular targets could strongly influence the normal cellular control in different organs through agonistic and/or antagonistic activity. This activity depends on tissue, hormonal conditions and interaction with other cellular pathways (Birt *et al.*, 2001; Cos *et al.*, 2003). Dietary factors such as phytoestrogens may contribute to a lower incidence of certain cancers in Asian populations (*e.g.*, prostrate and breast cancer), lower the risk of cardiovascular disease, or improve bone stability in menopause women (Birt *et al.*, 2001).

Phytoestrogens can interact with two of the most important receptors of steroid hormones, the sex hormone-binding globulin (SHBG) (Jury *et al.*, 2000; Martin *et al.*, 1996) and the cytosolic estrogen receptor (ERα and ERβ) (Kuiper *et al.*, 1998; Branham *et al.*, 2002). SHBG is the most significant blood sex hormone steroid transport and blood sex hormone steroid reservoir. Furthermore, SHBG interacts with membrane receptors in sex hormone target tissues (Rosner *et al.*, 1999). Human SHBG specifically binds 5α-dihydrotestosterone (DHT) with high affinity and 17β-estradiol (E2) with lower affinity (Petra, 1991). There are two known ER isoforms, α and β, which are expressed in estrogen sensitive tissues; ERα but not ERβ is highly expressed in the uterus (Kuiper *et al.*, 1997).

Although the interaction of some of the most common phytoestrogens with ER or SHBG has already been studied (Martin *et al.*, 1996; Kuiper *et al.*, 1998; Branham *et al.*, 2002; Morito *et al.*, 2001, 2002), various other secondary metabolites have not been fully evaluated.

In this communication, we present and compare the binding affinities of various common phytoestrogens, of liquorice constituents and the lignan nordihydroguaiaretic acid (NDGA) to rat uterine ER and to human SHBG. Compounds isolated from liquorice root (*Glycyrrhiza glabra*, Fabaceae) exhibit an estrogenic activity in cell cultures (Tamir *et al.*, 2000; Maggiolini *et al.*, 2002). We demonstrate the binding affinities of common liquorice constituents such as liquiritigenin, isoliquiritigenin, 18β-glycyrrhetinic acid and glycyrrhizinic acid to the estrogen receptors and to

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SHBG. Association constants (K_a) of phytoestrogens such as isoflavones, flavanones, flavones, flavonols and lignans were determined for these receptors.

Materials and Methods

Steroids and phytoestrogens

[2,4,6,7-^{3}H]-Estradiol (3.3^{12} Bq/mmol) ([^{3}H]-E2) and 5α-dihydro [1,2,4,5,6,7-^{3}H]-testosterone (3.3^{12} Bq/mmol) ([^{3}H]-DHT) were obtained from Amersham Bioscience Europe (Freiburg, Germany). E2 was obtained from Riedel-de Haën (Seelze, Germany); DHT, isoflavones, lignans and flavonoids were purchased from Sigma Chemical Co. (St. Louis, MO) or Fluka (Buchs, Switzerland), and chrysin, pinocembrin, datiscetin, galangin, and 4′,7-OCH_3-quercetin were generous gifts from Prof. Dr. Kurt Egger (Heidelberg University).

Preparation of rat uterine estrogen receptors

Uteri were excised from freshly killed rats (Sprague-Dawley). Tissues were immediately homogenised in ice-cold homogenisation buffer [1 mM EDTA, 10% glycerol and 0.5% protease inhibitor cocktail (P 2714 Sigma), 10 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.4]. The homogenate was centrifuged for 10 min at 1,500 × *g* at 4 °C. The pellet was discarded and the supernatant was centrifuged for 60 min at 100,000 × *g* at 4 °C. The second supernatant containing estrogen receptors was used for binding studies. Protein concentration was measured by the BCA method (dilution without glycerol) (Pierce, Rockford, IL).

Binding to estrogen receptors (ER)

100 μl of uterus extract (protein concentration 4 mg/ml) was mixed with [^{3}H]-E2 (final concentration 2.7 nM), buffer or E2 (concentration 4 pM to 20 μM), or phytoestrogens (concentration 0.05 nM to 0.5 mM). Determinations were carried out in triplicate. After 4 h incubation at 4 °C, the incubation was terminated by addition of dextran coated charcoal (DCC; dextran T70 0.63%, active charcoal 1.3%) under constant stirring for 10 min at 0 °C. Then unbound [^{3}H]-E2 was removed with DCC by centrifugation for 10 min at 1,500 × *g*. The supernatant was mixed with scintillation cocktail (100 μl/4 ml UltraGold) and radioactivity was measured in a liquid scintillation counter (LKB Wallac 1209; Rackbeta).

Sex hormone-binding globulins (SHBG)

Human plasma was obtained from the blood bank of Heidelberg University; it was treated with DCC (dextran T70 0.05%, active charcoal 0.5%) to remove endogenous steroids. Then plasma glycoproteins were bound to solid phase Concanavaline A-Sepharose 4B (Con A Seph): 200 μl of diluted plasma and 200 μl of Con A Seph 50% (v/v) were mixed in 50 mM Tris, pH 7.4, for 30 min at 4 °C, followed by 5 min centrifugation at 2,200 × *g*. The pellet was washed three times with 400 μl Tris. Bound SHBG was incubated with 400 μl [^{3}H]-DHT (6 nM) or [^{3}H]-E2 (7 nM) and/or various concentrations of E2 (cold ligand), DHT (cold ligand), or phytoestrogens at 37 °C for 60 min; samples were mixed every 10 min. Then the tubes were incubated for additional 30 min without further mixing. Phytoestrogens were added in concentrations between 10 nM to 1 mM, determinations were made in triplicate. The pellet was centrifuged at 2,200 × *g* and washed three times with 300 μl Tris (pH 7.4). The pellet was mixed with scintillation cocktail as described above.

Data analysis

Scatchard plot was used to determine the maximal binding (Bmax) and dissociation constant (K_d). The relative binding affinity (RBA) of each phytoestrogen was calculated as ratio of the effective concentration 50% (EC_{50}) of the steroid hormone to the EC_{50} of the phytoestrogen. The association constants (K_a) were calculated as described by Schöttner *et al.* (1998). The dissociation constant (K_d) and maximal binding concentration (Bmax) of [^{3}H]-E2 at rat ER were obtained from saturation experiments and Scatchard analysis under equilibrium conditions. Scatchard analysis for ER gave a K_d of 0.04 nM and a Bmax of 0.19 nM. These results are in agreement with previous studies (Kuiper *et al.*, 1998; Branham *et al.*, 2002). For SHBG K_d and Bmax values for E2 alone were 7.09 nM and 2.34 nM, respectively, and 0.93 nM and 1.79 nM for DHT.

Results

We focused our experiments on phytoestrogens such as isoflavones, flavonols, flavanones, flavones and natural compounds of liquorice root (for

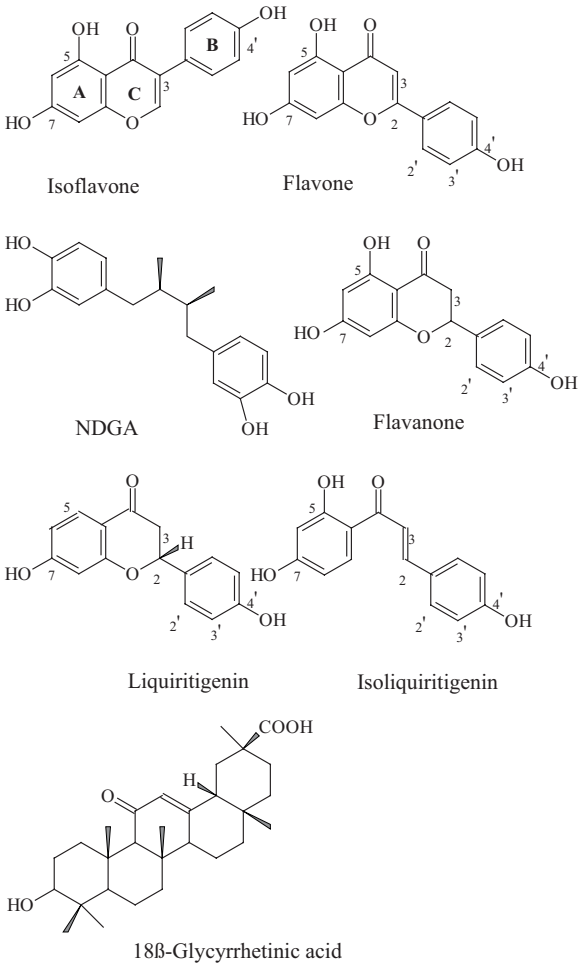

Fig. 1. Structures of isoflavones, flavones, flavanones, nordihydroguaiaretic acid (NDGA) and liquorice naturally compounds liquiritigenin, isoliquiritigenin and 18β-glycyrrhetinic acid.

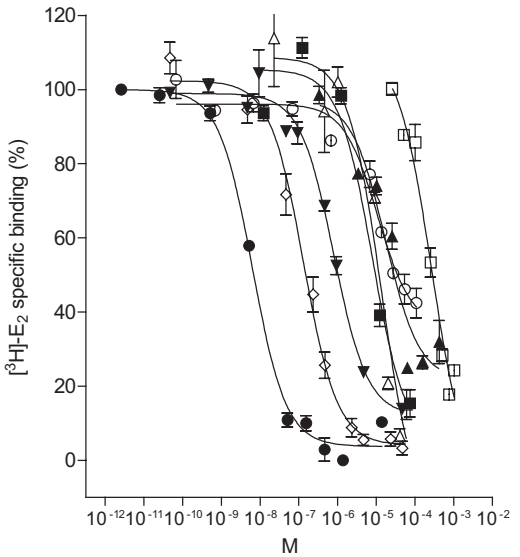

Fig. 2. Competitive displacement of [^{3}H]-E2 from rat uterine ER by unlabeled 17β-estradiol (E2) and phytoestrogens. The results are plotted as percent of bound [^{3}H]-E2 to ER versus concentration [M] of ligand. Uterus extract (0.19 nM ER) was incubated with 2.7 nM [^{3}H]-E2 for 4 h at 4 °C in the presence of E2 (●), coumestrol (◇), genistein (▼), daidzein (■), isoliquiritigenin (○), NDGA (△), apigenin (▲), liquiritigenin (□). Estrogen receptor binding assay was carried out by charcoal absorption technique (see Materials and Methods).

structures see Fig. 1) and determined their binding to rat uterine estrogen receptor (ER) and human sex hormone-binding protein (SHBG). The effective concentrations (EC_{50}), relative binding affinities (RBA), and association constant (K_a) of different phytoestrogens were evaluated in relation to 17β-estradiol (E2) and DHT. Fig. 2 shows displacement curves for different phytoestrogens; in addition, the data were used to determine EC_{50}, RBA and K_a for rat ER (Table I). The tested compounds displaced [^{3}H]-E2 from the rat ER with a decreasing affinity in the following order: coumestrol > genistein > phloretin > daidzein > isoliquiritigenin > NDGA > prunetin > apigenin = formononetin > biochanin A >> liquiritigenin. Despite the fact that we tested enterolactone and enterodiol up to a concentration of 500 μM, a displacement of [^{3}H]-E2 from the rat ER was not detectable. Also the liquorice root compounds 18β-glycyrrhetinic acid and glycyrrhizinic acid were tested at high concentrations (500 μM) but they did not displace [^{3}H]-E2 from ER. In addition, we assayed datiscetin, galangin, fisetin, 4′,7-OCH_3-quercetin, chrysin, pinocembrin and liquiritigenin. Only liquiritigenin, which carries two hydroxyl groups at the 7 and 4′ positions, showed a very weak binding to ER.

It was already known that NDGA can change the conformation of proteins as SHBG and α-fetoprotein (which suggest an external conformational change due to the interaction in the primary or in a secondary binding site) (Garreau *et al.*, 1991; Martin *et al.*, 1996). To determine the ability of NDGA to displace [^{3}H]-E2 from the rat ER, we tested NDGA in concentrations between 0.020 to 500 μM (Fig. 2). Between 0.020 and 60 μM NDGA shows a typical competitive displacement of

Compound	EC_{50} [μM]	$K_a \times 10^{-10}$ (± SEM) [M]		RBA
17β-Estradiol (E2)	0.006	51.1578	± 4.5544	100.000
Coumestrol	0.133	13.8468	± 0.9372	4.199
Genistein	0.803	1.8049	± 0.1443	0.696
Phloretin	3.085	0.5193	± 0.0326	0.206
Daidzein	7.943	0.2002	± 0.0059	0.080
Isoliquiritigenin	10.807	0.1470	± 0.0062	0.059
NDGA	13.780	0.1152	± 0.0026	0.046
Prunetin	14.677	0.0949	± 0.0041	0.038
Apigenin	17.200	0.0922	± 0.0028	0.037
Formononetin	17.709	0.0896	± 0.0025	0.036
Biochanin A	21.100	0.0660	± 0.0051	0.026
Liquiritigenin	267.900	0.0059	± 0.0002	0.002

Table I. Effective concentration 50% (EC_{50}), association constant (K_a) and relative binding affinity (RBA) values of estradiol (E2) and phytoestrogens for rat uterine estrogen receptors (ER).

[^{3}H]-E2 from the binding site (Fig. 2). To evaluate whether NDGA produces changes of Bmax or K_d, saturation was determined in the range from 0.39 to 19.54 μM. Fig. 3 shows corresponding Scatchard (A) and Lineweaver-Burk plots (B). Whereas maximal binding (B_{max}) of high to low NDGA concentrations remained unchanged (0.18 ± 0.02 nM; $p > 0.05$) a dose-dependent K_d increase was observed in the range between 0.09 and 2.75 nM. This suggests that the displacement of estradiol from the binding site by NDGA is competitive without further conformational changes.

In another set of experiments we determined the ability of phytoestrogens to displace DHT and E2 from SHBG. Fig. 4 illustrates the competitive displacement of E2 and DHT by phytoestrogens from SHBG; most of the tested compounds displaced E2 two to 30 times more effective than DHT (Table II). Some phytoestrogens were specific, either for E2 or DHT: prunetin, daidzein, formononetin, liquiritigenin, datiscetin, fisetin, and isoliquiritigenin displaced E2 only with weak RBA between 0.02 and 0.14. 18β-Glycyrrhetinic acid and chrysin showed a selective affinity for DHT (albeit 200-times weaker than that of estradiol) (Table II). The lignans enterolactone but not enterodiol displaced both steroid hormones from SHBG. The isoflavones showed an affinity pattern of E2 displacement at SHBG that was almost similar to that of E2 displacement at ER: coumestrols > genistein > prunetin > daidzein > formononetin > biochanin A (Tables I, II). In addition, among flavones, flavonols and flavanones only apigenin, galangin and pinocembrin were able to displace DHT from SHBG.

Discussion

In this contribution the ability of some phytoestrogens to compete with steroid hormones and displace them from two of the most significant receptors, *i.e.* SHBG and ER (here mainly α-recep-

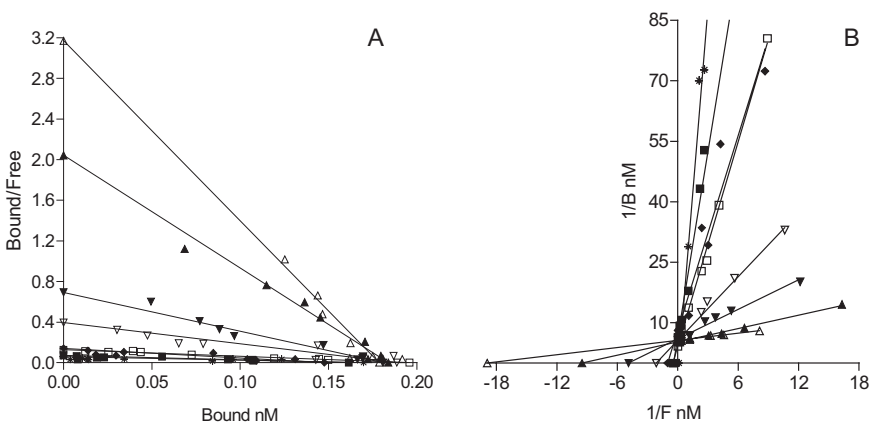

Fig. 3. Scatchard plots of [^{3}H]-E2 binding to rat uterine ER in the presence of NDGA (A) and Lineweaver-Burk analysis (B). Uterus extract (0.19 nM ER) was incubated for 4 h at 4 °C, with [^{3}H]-E2 (5.5 nM to 0.1 nM) in the absence of NDGA (△) or in the presence of NDGA, 0.39 μM (▲), 1.09 μM (▼), 2.44 μM (○), 6.11 μM (□), 9.77 μM (◆), 12.22 μM (■), 19.54 μM (✷).

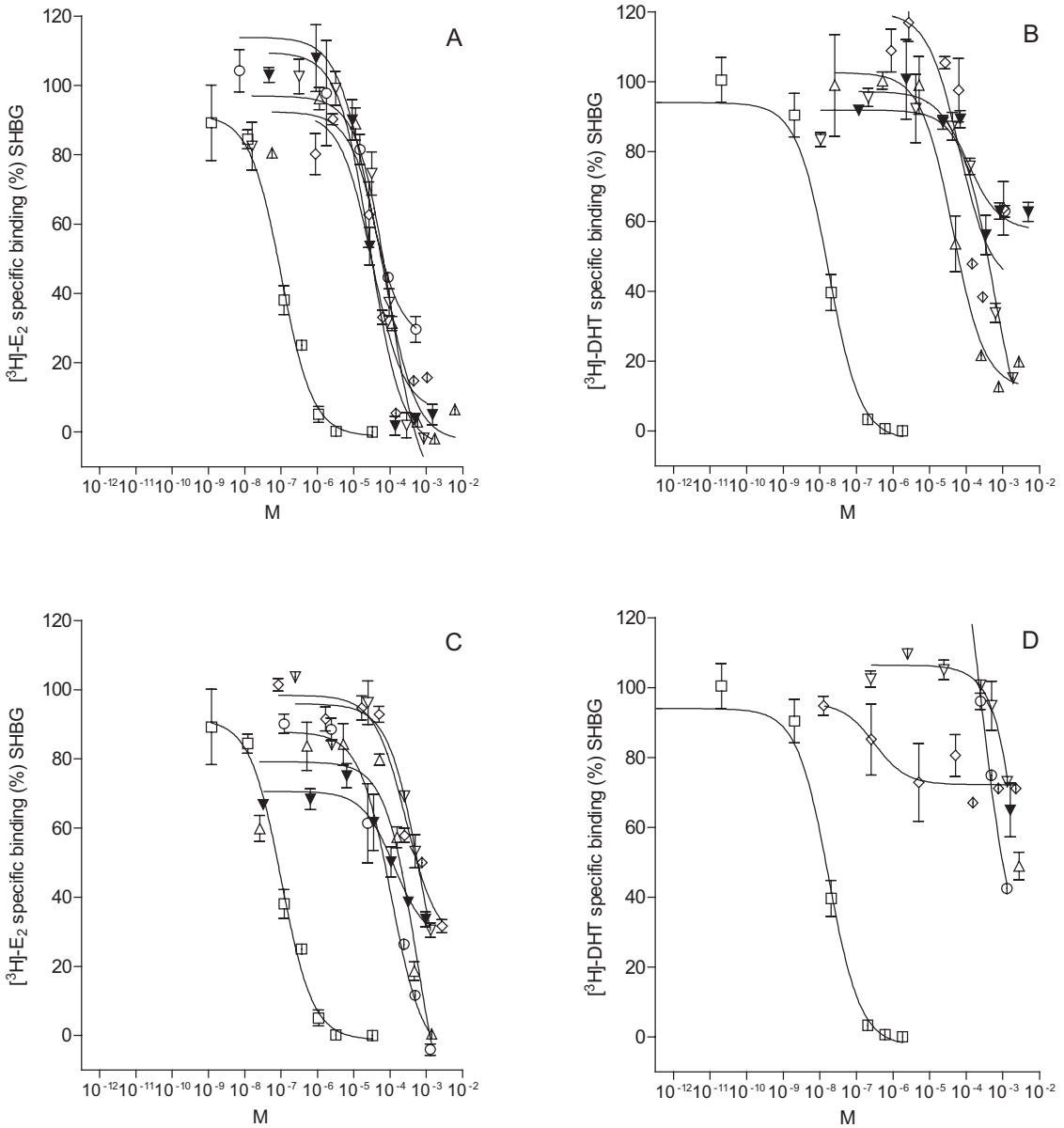

Fig. 4. Displacement of [^{3}H]-E2 or [^{3}H]-DHT from SHBG by unlabeled E2 or DHT and phytoestrogens. The results are plotted as percent of bound [^{3}H]-steroid to SHBG versus concentration [M] of ligand; (A, B) in the presence of E2 (A) or DHT (B) (□), glycyrrhetinic acid (○), chrysin (▼), galangin (◇), NDGA (△), pinocembrin (▽); (C, D) in the presence of E2 (C) or DHT (D) (□), phloretin (○), liquiritigenin (▼), datiscetin (◇), isoliquiritigenin (△), fisetin (▽). For SHBG binding assay the Concanavaline A-Sepharose method was employed (see Materials and Methods).

tor), was studied. Isoflavones displaced [^{3}H]-E2 from ER with differing affinities that are strongly correlated to the position of hydroxyl and methoxy groups of the phenolic ring A and phenolic ring B (positions 7, 5 and 4′) (Fig. 1). The relative binding affinities (RBA) of these compounds are in general agreement with previous results of Branham *et al.* (2002). Surprisingly, Branham *et al.* (2002), who also used rat uterine ER, could not determine a RBA for formononetin and prunetin

Table II. Effective concentration 50% (EC_{50}), association constant (K_a) and relative binding affinity (RBA) values for estradiol (E2), 5α-dihydrotestosterone (DHT) and phytoestrogens for binding to SHBG.

Compound	E2 (SHBG)[a]				5α-DHT (SHBG)[b]			
	EC_{50} [μM]	$K_a \times 10^{-8}$ (± SEM)		RBA	EC_{50} [μM]	$K_a \times 10^{-8}$ (± SEM)		RBA
17β-Estradiol (E2)	0.101	9.794	± 2.264	100.000	–	–		
DHT	–	–		–	0.017	41.933	± 11.540	100.000
Coumestrol	13.010	1.057	± 0.358	0.776	21.660	0.652	± 0.232	0.078
Glycyrrhetinic acid	23.130	0.566	± 0.252	0.437	nd	–	–	–
Chrysin	28.460	0.454	± 0.108	0.355	nd	–	–	–
Galangin	32.650	0.394	± 0.138	0.309	64.770	0.216	± 0.135	0.026
Genistein	67.450	0.186	± 0.063	0.150	162.200	0.086	± 0.042	0.010
NDGA	71.880	0.175	± 0.045	0.141	43.730	0.320	± 0.116	0.039
Prunetin	72.780	0.172	± 0.106	0.139	nd	–	–	
Daidzein	78.230	0.160	± 0.087	0.129	nd	–	–	
Apigenin	80.190	0.156	± 0.038	0.126	260.400	0.053	± 0.031	0.006
Pinocembrin	95.560	0.131	± 0.037	0.106	540.600	0.026	± 0.009	0.003
Phloretin	106.900	0.117	± 0.041	0.094	249.400	0.056	± 0.015	0.007
Liquiritigenin	125.700	0.099	± 0.052	0.080	nd	–	–	
Formononetin	133.900	0.093	± 0.049	0.075	nd	–	–	
Enterolactone	201.900	0.061	± 0.015	0.050	175.000	0.080	± 0.036	0.010
Datiscetin	274.600	0.045	± 0.012	0.037	nd	–	–	
Biochanin A	444.100	0.028	± 0.016	0.023	132.100	0.105	± 0.051	0.013
Isoliquiritigenin	552.800	0.022	± 0.011	0.018	nd	–	–	–
Fisetin	669.300	0.018	± 0.009	0.015	nd	–	–	–
Enterodiol	nd	–	–	nd	–	–	–	–
Glycyrrhizinic acid	nd	–	–	nd	–	–	–	–
4′,7-OCH_3-Quercetin	nd	–	–	nd	–	–	–	–

[a] Diluted plasma 1/10.
[b] Diluted plasma 1/20.
nd, not detectable.

and obtained a low RBA for coumestrol (0.9). However, these authors employed a solid-phase method (hydroxyapatite slurry) to determine binding affinities (Branham *et al.*, 2002). It has been reported that RBA for ERα can differ substantially between solid-phase or soluble receptor competition experiments (Kuiper *et al.*, 1998).

The affinity of various flavonoids to ER had already been demonstrated whereas the affinities for NDGA, liquiritigenin and isoliquiritigenin have not been reported before (Miksicek, 1995; Kuiper *et al.*, 1998; Branham *et al.*, 2002; Morito *et al.*, 2001, 2002). The chalcone isoliquiritigenin showed a binding affinity that was about 1800-fold lower than that of E2. In addition, liquiritigenin, a 4′-hydroxyl flavanone, exhibited a very weak binding to ER. Neither glycyrrhizinic acid nor its aglycone sufficiently displaced E2 from rat ER to allow the determination of RBA. Polar groups such as carboxylic and glucuronic acids seem to reduce a binding to ER.

The ring B of apigenin is in the 2 position instead of the 3 position as in isoflavones (such as genistein); this difference decreases the RBA about 20-fold (Kuiper *et al.*, 1998; Branham *et al.*, 2002). The additional lack of a double bound in liquiritigenin decreases the RBA about 40-fold as compared to the isoflavone daidzein.

Among lignans, only NDGA displaced E2 from ER; this may be due to the polarity of the additional 3,4-dihydroxyl substituents between the aliphatic core. We evaluated the effect of NDGA on ER: NDGA exhibited a competitive displacement of E2 by keeping the number of binding sites for E2 unchanged while increasing K_d 30-fold in a dose-dependent fashion. A high concentration of lignan NDGA decreased up to 30% binding sites for E1 and changes K_d in human and rat α-fetoprotein (AFP) (Garreau *et al.*, 1991). Furthermore, NDGA decreases the binding sites without changing the K_d for testosterone (T) and for E2 of SHBG and reduces the immunorecognition of SHBG by anti-SHBG (Martin *et al.*, 1996). A binding study showed that aromatic rings, their substitution and aliphatic core determine the affinity to SHBG (Schöttner *et al.*, 1997). Hence a

markedly difference between affinities of diol-lignan (as enterodiol) and ester-lignan (as enterolactone) was found. Mammalian lignans appear to have certain estrogenic or anti-estrogenic effects that might be due to non-classical ER pathways and interference with SHBG (Rosner *et al.*, 1999; Schöttner *et al.*, 1998).

The present binding studies showed that isoflavones displace E2 from SHBG in a similar profile as from ER. A 4′-methoxy group significantly decreases the affinity whereas a 7-methoxy group does not alter it. In contrast, a 4′-methoxy group does not decrease the affinity to DHT binding, but a 7-methoxy and/or lack of 5-hydroxyl groups reduces it. These results support the known structure-activity relationships (Miksicek; 1995; Schöttner *et al.*, 1997; Blair *et al.*, 2000; Branham *et al.*, 2002).

The liquorice constituents liquiritigenin, isoliquiritigenin, and 18β-glycyrrhetinic acid displaced only E2 from SHBG in a wide range of affinities. Glycyrrhizinic acid and 18β-glycyrrhetinic acid have shown endocrine interference by the inhibition of 5α- and β-steroid reductase (Latif *et al.*, 1990). We suggest that some of the endocrine effects of liquorice compounds are due to interference with [^{3}H]-E2 binding to SHBG.

Surprisingly, compounds such as chrysin, galangin, fisetin, pinocembrin, and datiscetin exhibited a displacement of [^{3}H]-E2 from SHBG but not from the ER. Flavonols do not seem to alter the binding affinity of E2 to SHBG when their ring B is not hydroxylated (as chrysin and galangin). But, the addition of either a single 4′- or 2′-hydroxyl or both hydroxyl groups reduces the RBA 10 to 20-fold. Furthermore the lack of a double bound (ring C) decreases the affinity over 3-fold (as in chrysin and pinocembrin). Only 4′,7-OCH_3-quercetin had such a weak affinity for E2 binding to SHBG that a RBA calculation was not possible.

Our results show various affinities to ER and to SHBG, suggesting that phytoestrogens interact in a specific and differential manner with these proteins. The tested compounds displaced E2 much stronger than DHT from SHBG. In addition, structure-binding relationships between phytoestrogens and estradiol at ER do not automatically correspond to those at SHBG nor to DHT at SHBG. These findings also provide more evidence that plant compounds can bind to more than one molecular target with a wide range of affinities (Wink, 1999a, b; Birt *et al.*, 2001; Ososki and Kennelly, 2003).

Prior to considering the benefits and risks to human health by consuming active endocrine substance (*e.g.* phytoestrogens), we should determine which proteins or receptors are affected by them. In addition, the potential change of gene expression and of protein profiles after exposure to phytoestrogens should be analysed in several organs (uterus, mammary glands, liver). Since phytoestrogens are active metabolites their utilisation as functional food or nutraceuticals should be under similar safety considerations that are imposed on phytopharmaceuticals.

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Birt D. F., Hendrich S., and Wang W. (2001), Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol. Ther. **90**, 157–177.

Blair R. M., Fang H., Branham W. S., Hass B. S., Dial S. L., Moland C. L., Tong W., Shi L., Perkins R., and Sheehan D. M. (2000), The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. Toxicol. Sci. **54**, 138–153.

Branham W. S., Dial S. L., Moland C. L., Hass B. S., Blair R. M., Fang H., Shi L., Tong W., Perkins R. G., and Sheehan D. M. (2002), Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. J. Nutr. **132**, 658–664.

Cos P., De Bruyne T., Apers S., Van den Berghe D., Pieters L., and Vlietinck A. J. (2003), Phytoestrogens: recent developments. Planta Med. **69**, 589–999.

Day A. J., Canada F. J., Diaz J. C., Kroon P. A., McLauchlan R., Faulds C. B., Plumb G. W., Morgan M. R., and Williamson G. (2000), Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett. **468**, 166–170.

Garreau B., Vallette G., Adlercreutz H., Wähälä K., Mäkelä T., Benassayag C., and Nunez E. A. (1991), Phytoestrogens: new ligands for rat and human α-fetoprotein. Biochim. Biophys. Acta **1094**, 339–345.

Jury H. H., Zacharewski T. R., and Hammond G. L. (2000), Interactions between human plasma sex hormone-binding globulin and xenobiotic ligands. J. Steroid Biochem. Mol. Biol. **75**, 167–176.

Kuiper G., Carlsson B., Grandien K., Enmark E., Häggblad J., Nilsson S., and Gustafsson J.-Å. (1997), Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology **138**, 863–870.

Kuiper G., Lemmen J. G., Carlsson B., Corton J. C., Safe S. H., Van Der Saag P. T., van der Burg B., and Gustafsson J.-Å. (1998), Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology **139**, 4252–4263.

Latif S. A., Conca T. J., and Morris D. J. (1990), The effects of the licorice derivative, glycyrrhetinic acid, on hepatic 3α- and 3β-hydroxysteroid dehydrogenases and 5α- and 5β-reductase pathways of metabolism of aldosterone in male rats. Steroids **55**, 52–58.

Maggiolini M., Statti G., Vivacqua A., Gabriele S., Rago V., Loizzo, Menichini F., and Amdo S. (2002), Estrogenic and antiproliferative activities of isoliquiritigenin in MCF-7 breast cancer cells. J. Steroid Biochem. Mol. Biol. **82**, 315–322.

Martin M. E., Haourigui M., Pelissero C., Benassayag C., and Nunez E. A. (1996), Interactions between phytoestrogens and human sex steroid binding protein. Life Sci. **58**, 429–436.

Miksicek R. J. (1995), Estrogenic flavonoids: structural requirements for biological activity. Proc. Soc. Exp. Biol. Med. **208**, 44–50.

Morito K., Hirose T., Kinjo J., Hirakawa T., Okawa M., Nohara T., Ogawa S., Inoue S., Muramatsu M., and Masamune Y. (2001), Interaction of phytoestrogens with estrogen receptors α and β. Biol. Pharm. Bull. **24**, 351–356.

Morito K., Aomori T., Hirose T., Kinjo J., Hasegawa J., Ogawa S., Inoue S., Muramatsu M., and Masamune Y. (2002), Interaction of phytoestrogens with estrogen receptors α and β (II). Biol. Pharm. Bull. **25**, 48–52.

Ososki A. L. and Kennelly E. J. (2003), Phytoestrogens: a review of the present state of research. Phytotherapy Res. **17**, 845–869.

Petra P. H. (1991), The plasma sex steroid binding protein (SBP or SHBG). A critical review of recent developments on the structure, molecular biology and function. J. Steroid Biochem. Mol. Biol. **40**, 735–753.

Rosner W., Hryb D. J., Khan M. S., Nakhla A. M., and Romas N. A. (1999), Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane. J. Steroid Biochem. Mol. Biol. **69**, 481–485.

Schöttner M., Ganßer D., and Spiteller G. (1997), Interaction of lignans with human sex hormone binding globulin (SHBG). Z. Naturforsch. **52c**, 834–843.

Schöttner M., Spiteller G., and Ganßer D. (1998), Lignans interfering with 5α-dihydrotestosterone binding to human sex hormone-binding globulin. J. Nat. Prod. **61**, 119–121.

Setchell K. D. R., Brown N. M., Zimmer-Nechemias L., Brashear W. T., Wolfe B. E., Kirschner A. S., and Heubi J. E. (2002), Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am. J. Clin. Nutr. **76**, 447–453.

Tamir S., Eizenberg M., Somjen D., Stern N., Shelach R., Kaye A., and Vaya J. (2000), Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cells. Cancer Res. **60**, 5704–5709.

Wink M. (1999a), Biochemistry of Plant Secondary Metabolism. Annual Plant Reviews 2. Sheffield Academic Press, Sheffield.

Wink M. (1999b), Function of Plant Secondary Metabolites and their Exploitation in Biotechnology. Annual Plant Reviews 3. Sheffield Academic Press, Sheffield.