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Review

Overview of in vitro tools to assess the estrogenic and antiestrogenic activity of phytoestrogens

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Abstract

There is an intense discussion in the scientific and even more so in the public community as well as regulatory agencies about the potential benefits or detrimental effects of plant-derived compounds that may affect the endocrine system, especially estrogen signaling pathways. These so-called phytoestrogens are found in the normal western diet and predominantly in an eastern or soy-based diet and the potency of the isolated compounds to interact with the known receptors for estrogen varies tremendously. The estrogen receptors, ERα and ERβ, mediate the effects of endogenous estrogens, i.e. regulation of reproductive function, tissue development, cell proliferation and differentiation. In this review, in vitro test systems available to date for the screening of estrogenic and antiestrogenic activity including mechanism-based assays are described. The potency of phytoestrogens determined using these in vitro assays are compared with the potency of endogenous estrogens and results obtained in vitro are compared with effects in vivo. Finally, the impact of in vitro assays to determine estrogenicity on human hazard assessment is discussed as well as other non ER-mediated mechanisms that may contribute to potential beneficial or adverse effects of phytoestrogens in man.

Keywords: Reviews; Phytoestrogens, activity

Contents

1. Introduction

Phytoestrogens, a term coined to describe plant-derived chemicals that exert estrogenic activity, include a vast variety of structurally diverse compounds (Fig. 1). These include isoflavones found in soy, lignans often found in grains, stilbenes found in the skin of grapes and fungal metabolites, e.g. macrolides (Fig. 1) [1–3]. Other less investigated compounds include the prenylflavonoids, flavones, flavans, isoflavanes and phytosterol esters (Fig. 1) [4–7].

The estrogenic or antiestrogenic activity of any

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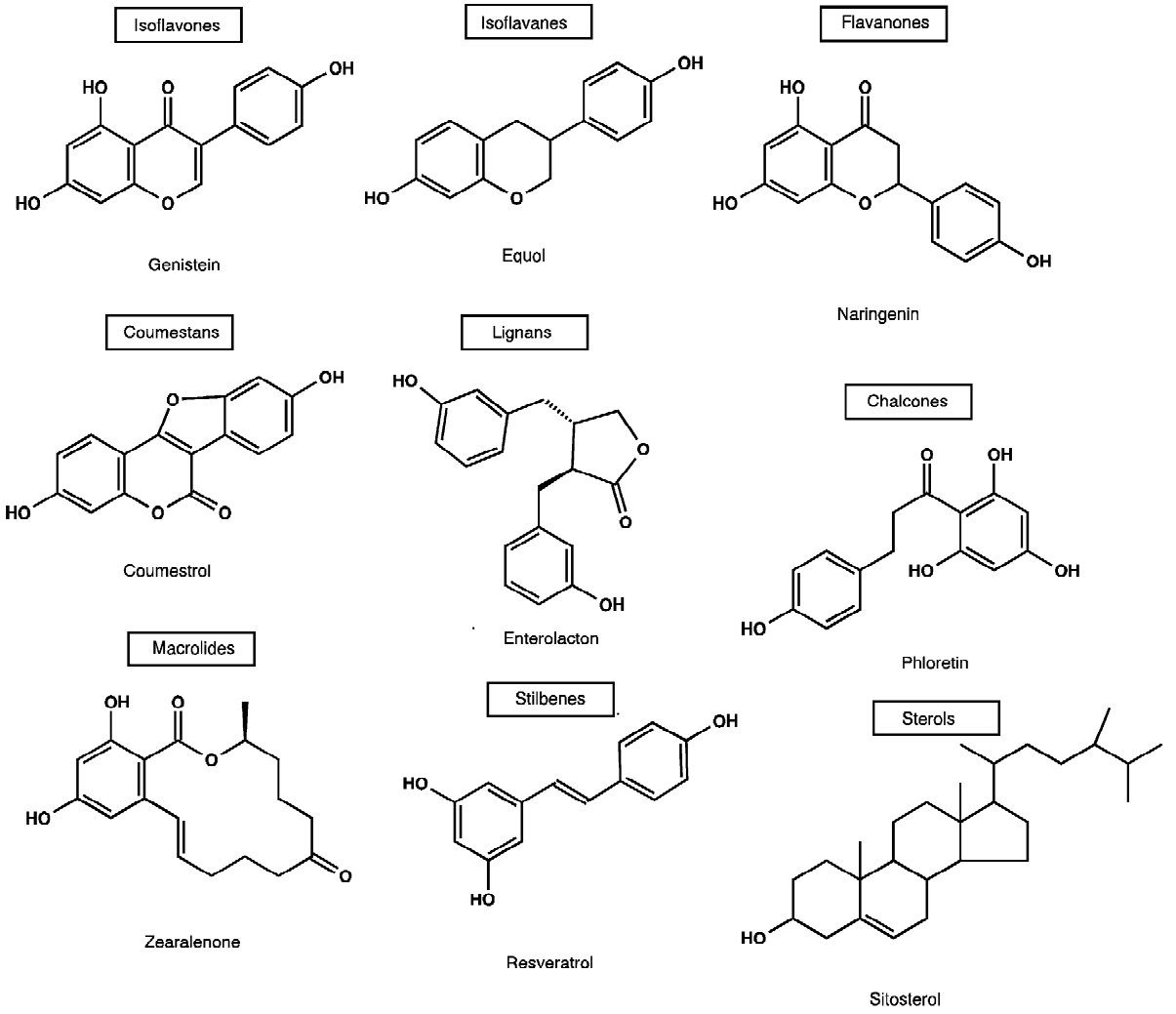

Fig. 1. Chemical structures of major phytoestrogen classes.

chemical depends on the ability of the compound to interact with the estrogen receptor (ER). Two subtypes of the ER are known to date, the ERα [8,9] and ERβ [10,11] and both receptors have a distinct tissue distribution and play a distinct role in physiology [12–14]. The ER is a ligand inducible transcription factor which binds to specific regulatory sequences in target genes to regulate processes in development and neoplasia [15,16].

Due to the important role of the ER in cell proliferation and differentiation, disruption of the ER signaling pathways may contribute to infertility, developmental abnomalities or endocrine cancer seen in both wildlife and humans. This endocrine disruption could be linked to the exposure to chemicals with estrogenic or antiestrogenic activities [17]. Plant-derived chemicals that showed estrogenic activity in vivo and in vitro are therefore a potential risk to the health of humans and wildlife [18–20]. Regulatory agencies and the scientific community have put a lot of effort in the identification of the estrogenic potential of synthetic and natural compounds [21]. For that reason several in vivo and in vitro assays for testing the estrogenicity of these

compounds have been described in the literature [22–25].

The scope of this review is to describe validated in vitro screening tests for estrogenicity and antiestrogenicity and to bring mechanism based assays for ER function that could contribute significantly to the assessment of estrogenic potency and their relevance to human health to the reader's attention. Taking into account the available data for phytoestrogens in vitro and in vivo including man, the value of in vitro assays for human risk assessment is discussed.

2. In vitro assays for estrogenicity and antiestrogenicity

The molecular mechanism of ER action is depicted in Fig. 2. The ER dimerizes upon binding of a ligand and forms a complex with an estrogen responsive element (ERE) which is located within the promoter regions of the target gene. By interaction with transcription factors and cell-type specific co-activators of the ER, chromatin remodeling takes place and the ER-co-activator complex facilitates transcription of its target gene [16,26,27]. The transcribed mRNA codes for a specific protein which elicits the estrogenic effect in the target tissue dependent on the developmental stage and the current cellular environment including crosstalk with other growth factor and kinase pathways [28]. This molecular signaling cascade induced by ligands like phytoestrogens, can be monitored at various stages (see Fig. 2) and allows the characterization of the estrogenic or antiestrogenic potency of the compound of interest.

In this section I will discuss the in vitro assays available to date for the measurement of chemical-induced estrogenicity and antiestrogenicity (Table 1). A hallmark physiologic response to estrogenic stimuli is the proliferation of cells in vivo which may promote tumor growth. This cellular proliferation can also be mimicked in vitro. The 'classic' estrogenicity assay, termed E-screen, uses established cell lines that are known to respond to estrogens and measures cell proliferation in response to increasing doses of the test compound [29,30]. The prerequisite

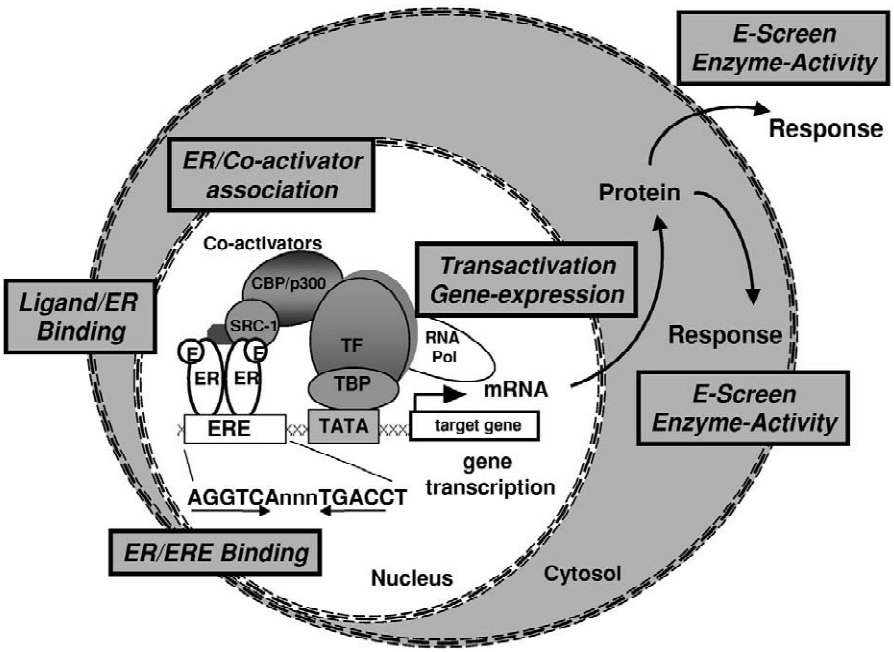

Fig. 2. Molecular mechanism of estrogen receptor mediated action (see text for details). Assays for estrogenicity covering the steps of ER activation are given in boxes in italic letters. CBP/p300, co-activator protein; E, ER-ligand; ERE, estrogen responsive element; RNA pol, RNA polymerase; SRC-1, co-activator protein; TATA, TATA box; TBP, TATA binding protein; TF, general transcription factor.

Table 1
Compilation of in vitro assays for the measurement of estrogenic and antiestrogenic compounds

In vitro assay	Measured endpoint	Advantages	Limitations	Reference
E-Screen	Proliferation of ERα-positive cells	Measures physiological endpoint of estrogen action, measures estrogens and antiestrogens	No defined ER expression No mechanistic data	[e.g. 30]
Ligand-binding	Binding affinity to ERα or ERβ	Simple, high-throughput,	Does not measure ER activation	[e.g. 14,37,38]
ER-binding to ERE	Binding affinity of ERα or ERβ to various EREs	Simple, high-throughput, various EREs can be used	Does not measure ER activation, low sensitivity	[46,47]
GST pull-down /FRET /two-hybrid assay	Ligand-dependent association of ERα or ERβ with co-activators	Analysis of molecular interaction, versatile: defined ER subtype or ER domain as well as co-activators can be used, measures estrogens and antiestrogens	Artificial system, does not measure ER activation, low throughput	[e.g. 51,54,55,56]
Transactivation assay in yeast or mammalian cells	ERα or ERβ mediated activation of reporter	Simple, high-throughput, measures estrogens and antiestrogens	Artificial system	[e.g. 58,59]
Analysis of gene and protein expression	Expression of ER-regulated genes and proteins	Analysis of physiological response, versatile, measures estrogens and antiestrogens	Low-throughput	[e.g. 74,75,76,77]
Analysis of enzyme activity and steroid biosynthesis	Activity of stereoidogenic enzymes, ER regulated enzymes and analysis of estrogen biosynthesis	Analysis of physiological response, measures estrogens and antiestrogens, ER and ER-independent pathways	Only cell lines with active steroidogenesis and marker enzymes suitable	[81,82]

for this assay is a cell line that expresses functional endogenous ER. Exogenous ERα expressed in cell lines lacking endogenous ERα yields an unexpected effect. ER agonists like estradiol and antagonists like tamoxifen inhibit cell growth and kill the cells [31–33]. Therefore, cell proliferations assays can only be performed with cells expressing endogenous ER and the constitutive and stable expression of the endogenous ER should be confirmed before performing the E-screen [34]. Currently, no proliferation assay with a cell line expressing functional ERβ has been used successfully as an E-screen. The description of an immortalized cell line with expression of functional endogenous ERβ derived from mouse testis may provide a tool to analyze estrogen induced proliferation transduced by ERβ [35]. In conclusion, the E-screen assay is widely used and acknowledged as a reliable and valid test for a physiological response of estrogen action and can also discriminate agonists from antagonists [36].

Fig. 2 is a schematic of the molecular mechanism of ER action. The first step in the ER transduction cascade is binding of a ligand to the ER, which induces dimerization and binding of the ER dimer to specific sequences (EREs) within the promoter region of its target gene (Fig. 2). The measurement of ligand binding is therefore an important assay to characterize the potential of test compounds to interact with the ER. The ‘classic’ ligand binding assay uses radioactively labeled estradiol, which competes with the test compound for ER binding sites. This competitive binding assay provides relative binding affinities of test compounds to ERα or ERβ compared to estrogen which is usually unlabeled estradiol or diethylstilbestrol (DES) [37]. The availability of fluorescent probes and labeling

made it possible to use fluorescence polarization to measure binding affinities [38]. This assay, which is commercially available, uses a fluorescein labeled estradiol instead of radioactive label. One drawback is clearly the sterical big fluorescein label of estradiol which results in quantitative distinct binding affinities compared to the radioactive binding assay. However, data to date indicate that relative binding affinity rankings of various compounds are identical in both assays [Mueller et al., unpublished observation, 38]. The advantage of the radioactive binding assay is that, crude protein extracts from any tissue or cell line can be used and, in addition, ligand binding can be measured in living cells that express ER [39]. Thus both, quantification of ER binding sites of the tissue or cell sample can be obtained as well as binding affinities of compounds are determined. In contrast, fluorescence polarization requires the uses of purified ER due to the high background fluorescence in crude extracts. However, the advantage of fluorescence polarization is that it can be done in a high-throughput format within a day or less and therefore it is better suited than the radioactive assay to screen many compounds for ER binding affinity (Table 1).

A novel assay to analyze the conformational change of the ER upon ligand binding rather than measuring binding affinities was described by Paige et al. [40]. This assay is extremely powerful and defines conformational changes that can be linked to specific agonist and antagonists of ERα and ERβ and serves as another assay for the analysis of mechanisms of estrogens and antiestrogens [41]. Since this assay is suitable mainly in defining the mecanistic differences between known estrogen/antiestrogens rather than to screen for unknown potential phytoestrogens, the interested reader is referred to the published literature [40–42].

The second step in the ER signaling pathway is binding of the liganded ER dimer to the promoter region of its target gene (Fig. 2). The specific sequence or ERE to which the ER dimer binds is a palindromic 13-base-pair inverted DNA sequence. The sequence shown in Fig. 2 is the so-called consensus ERE (cERE) derived form the vitellogenin A2 (Vit) gene. However, nonconsensus EREs (nERE), which differ from the cERE in 1–3 nucleotides, predominate in endogenous estrogen-responsive genes [43,44] and ERα and ERβ exhibit differential binding affinity to various EREs [45,46]. These reports indicate therefore, that the binding to the target gene promoter is dependent on the ligand and the ERE sequence. As with the ligand binding assay, radioactively labeled or fluorescein labeled ERE can be used to analyze binding of ERα and ERβ to the ERE [46,47]. The biggest constraint for the use of these assays to measure ligand-dependent binding is the high constitutive binding of the ER to the ERE [e.g. 48]. Accordingly most reports so far have failed to detect any significant change of the affinity of ER to the ERE with ligand [47,48]. However, Nikov et al. were able to detect a ligand-dependent change in the affinity of ERα and ERβ to a consensus and nonconsensus ERE [46]. Regardless of the potential of a ligand-dependent binding of ER to an ERE, the described constitutive ligand-independent binding makes this assay less suitable for the screening of phytoestrogens (Table 1).

In contrast to the ligand-independent binding of the ER to its ERE, the association of the ER dimer bound to an ERE with co-activators within the transcription assembly that leads to gene transcription is a cooperatively regulated event that is dependent on the ligand-induced conformational change in the ER [27,49,50] (see also Fig. 2). Assays have therefore been developed that measure the interaction of ERα or ERβ with co-activators. The most widely used test to measure protein–protein interaction is the glutathione S-transferase (GST) pull-down assay [e.g. 51]. In this assay a fusion protein of GST and the protein of interest, i.e. the ER are incubated in vitro with ^{35}S-labeled co-activator, e.g. SRC-1 [52] in the presence of ER ligand [51,53,54]. The protein complex is purified by glutathione affinity chromatography, subjected to gel electrophoresis and the interaction of labeled co-activator with the GST-ER fusion protein is monitored by autoradiography. The association of co-activator with the ER is dependent on the nature and dose of ligand, thus enabling the molecular analysis and quantification of the potency of a compound to induce an association of ERα and ERβ with co-activators [54]. Similar to the GST pull-down is the use of fluoresecence resonance energy transfer (FRET) to measure ligand-dependent receptor–co-activator interactions [55,56]. This assay makes use of either fluorescently labeled co-ac-

tivators and receptor [55] or inherently fluorescent ligands for the ER [56,57]. In both types of FRET the energy transfer of receptor or ligand to co-activator can be quantified and correlates with the extent of receptor–co-activator association. In the first approach, various ligands can be investigated and their activity to induce or inhibit ER/co-activator association can be analyzed in a dose-dependent manner, thereby allowing an assessment of their estrogenic or antiestrogenic potency [55]. The two-hybrid assay, that can be performed in yeast or mammalian cells, is an excellent system to analyze protein/protein interaction in vivo (i.e. in living cells) and can also be applied to estrogenic compounds [51]. All three described systems, GST pull-down, FRET and two-hybrid are powerful tools to analyze the mechanisms of ligand-dependent or independent receptor–co-activator interaction. The advantages of these systems are clearly the opportunity to dissect functional domains of the ER and to define the interaction with ubiquitous as well as cell and tissue-specific co-activators. This versatility may help to identify a potential tissue-specific effect of alleged phytoestrogens. However, these mechanism-based assays are tedious experiments and not applicable to high-throughput screen and should therefore be applied selectively for potent estrogens and antiestrogens (Table 1).

Transient transactivation assays or recombinant cell assays, in which cells are co-transfected with the cDNA for ERα or ERβ and a reporter gene containing an ERE, are widely used to measure ligand-induced ER-mediated gene activation [e.g. 58,59,60]. In this assay yeast or a mammalian cell line lacking endogenous ER is transfected with an expression plasmid carrying the cDNA of ERα or ERβ or any desired receptor variant together with an ER responsive promoter or ERE linked to a chloramphenicol acetyltransferase (CAT) or luciferase reporter cDNA (see also Fig. 2). Addition of ER ligands induces dose dependent transcription of the reporter protein CAT or luciferase and can easily be monitored and quantified (Table 1). The obvious versatility of this assay is that yeast or various mammalian tissue-specific cell lines and any ER subtype or functional ER domains can be used. Also, the reporter can contain the entire promoter region of any known or unknown ER target gene as well as synthetic consensus and nonconsensus EREs [61,62]. Due to the high sensitivity of available luciferase reporter vectors very weak to highly potent estrogens can be analyzed. Furthermore, single compounds and chemical mixtures can be analyzed dependent on ERE, ER-subtype and cellular context for their estrogenicity and antiestrogenicity. This versatility is especially important in light of the tissue specific estrogenic/antiestrogenic activity of so-called selective ER modulators (SERMs), such as tamoxifen and raloxifene [28,63]. This tissue selectivity of SERMs may apply also for certain phytoestrogens (discussed in more detail later). The transient transactivation assays are versatile, allow for a defined receptor expression level and a rapid throughput of many compounds. However, since exogenous receptor is forced into a cell accustomed to the lack of ER some effects measured may not reflect the physiological response of the analyzed cell type. To account for this limitations, cell lines with endogenous ER expression, similarly to the E-screen, can be used for transactivation assay. For that purpose, permanent cell lines like MCF-7 or BG-1, immortalized cell lines [35] and primary cell lines [Mueller et al., manuscript in preparation, 64] can be employed to measure transactivation.

Cell lines with endogenous ER expression can also be used to measure the expression of endogenous ER target genes. As schematically described in Fig. 2, transcription of ER target genes upon ER ligand binding into mRNAs and subsequently proteins is dependent on ligand, ER expression and cellular context, e.g. available co-activators. The genes regulated by estrogens are dependent on the tissue or cell type and their induction transduces the estrogenic response, e.g. the induction of proto-oncogenes like c-fos and c-jun is likely to mediate the mitogenic effects of estrogens in the breast and the uterus [65] and the protective effects of estrogens in the cardiovascular system and bone are most likely linked to expression of tissue-specific genes [66,67]. The measurement of endogenous ER target gene expression represents therefore a valuable physiological assay for tissue-specific estrogenicity or antiestrogenicity. Two general approaches can be persued, one being the measurement of mRNA the other the analysis of protein expression. Both endpoints can be monitored on a gene by gene or protein by protein

approach using real-time RT-PCR, Northern-blot or RNase protection assay and western blot, respectively. Using these methods several known estrogen regulated genes and proteins, respectively, like progesterone receptor (estrogen regulated in the breast and the uterus) [68], lactoferrin (uterus responsive protein) [69], pS2 (estrogen regulated in breast cancer and MCF-7 cells) [70,71], or cathepsinD (estrogen regulated in the breast but not the uterus) [72,73], can be monitored in cell lines or tissues upon estrogen exposure [74,75]. Importantly, dependent on the chosen tissue or cell line the right marker gene has to be monitored and a time-course should be performed since gene expression levels are time-dependent. The advent of high-density cDNA arrays and proteomics techniques enables now the analysis of thousands of known as well as unknown estrogen target genes and proteins simultaneously [76,77]. The latter approaches are certainly not high-throughput assays, but offer the opportunity to look at several signal transduction pathways in one snapshot. Both types of assays are very valuable for analysis of mechanisms of actions and to determine tissue-specific effects of phytoestrogens (Table 1).

Next to the analysis of estrogen regulated gene and protein expression, the activity of the translated proteins or enzymes (Fig. 2) and the analysis of the steroidogenesis of endogenous estrogens offers other endpoints to measure the physiological response to endocrine disrupters (Table 1). The activity of alkaline phosphatase can be measured in human endometrial Ishikawa cells and osteoblastic cell lines and is induced by estrogens and inhibited by antiestrogens [78,79]. This biochemical assay offers therefore a simple tool for assessing the physiological activity of estrogens and antiestrogens in vitro. Other assays that measure the interference with estradiol biosynthesis rather than direct interaction with the ER include the measurement of the activity of steroidogenic enzymes like aromatase inhibition [80] and quantification of the pattern of steroid biosynthesis [81–83].

3. Estrogenicity and/or antiestrogenicity of phytoestrogens in vitro

The in vitro tools available to date for the analysis of chemicals that interfere with the ER signaling cascade were described in Section 2. Several phytoestrogens were characterized in some but not all of these assays and the results of these assays are summarized in Table 2 for the most potent phytoestrogens and are compared to 17β-estradiol (E2) and diethylstilbestrol (DES). Available data regarding the potency to interact with both ERα and ERβ were predominantly obtained with ligand-binding and transactivation assays. The E-screen assay is widely used for many xenoestrogens but lacks the possibility to compare ERα with ERβ. Therefore, only a qualitative potency comparison based on ligand-binding and transactivation was compiled in Table 2.

Strikingly, only a few of the vast number of plant-derived compounds investigated have proven to exert a distinct effect on ERα and/or ERβ. Genis-

Table 2
Transactivation and binding affinity in vitro of selected phytoestrogens for ERα and ERβ compared to 17β-estradiol and diethylstilbestrol[a]

ERα		Estrogen/ phytoestrogen	ERβ	
Ligand-binding	Transactivation		Ligand-binding	Transactivation
+++	+++	17β-Estradiol	++	+++
+++	+++	Diethylstilbestrol	+++	+++
+	+	Genistein	+(+)	++
++	++	Coumestrol	++(+)	++
+(+)	++/(−)	Zearalenone	+(+)	++/−
(+)	+	Equol	+(+)	+
(+)	(+)/−	Resveratrol	(+)	(+)/−−

[a] Transactivation and binding affinity given as qualitative measures. (+), Weak agonistic activity; +, agonistic activity; ++ and +++, strong and very strong agonistic activity, resp.; (−), weak antagonistic activity; −, antagonistic activity; −−, strong antagonistic activity. Data compiled from [Mueller et al., mansucript in preparation, 14,84–86,93].

tein, a metabolite of biochanin A, the fungal metabolite zearalenone and coumestrol are the most potent agonists for ERα and ERβ [Mueller et al., manuscript in preparation, 14,84,85,86]. Accordingly, as observed with estradiol, genistein, coumestrol and zearalenone induced growth in ERα positive cell lines and expression of ER-regulated genes [7,87]. Equol, a metabolite of the inactive daidzein is also an ER agonist [Mueller et al., manuscript in preparation, 88]. However, DES or E2 exerted 10- to 100-fold higher activity on ERα and ERβ than the most potent phytoestrogens described here.

Only two tested phytoestrogens showed antiestrogenicity in vitro. Zearalenone showed weak antagonistic activity on ERα and higher potency to inhibit ERβ activity [Mueller et al., manuscript in preparation, 84]. Results obtained with the stilbene derivative resveratrol were puzzling. One report showed growth promoting effects in the ERα positive cell line MCF-7 [89], but two others showed growth inhibitory effects in ERα positive mammary cell lines concomitant with downregulation of ER-regulated genes in a similar dose range of resveratrol [90,91] indicating antiestrogenic activity on ERα. In osteoblasts, resveratrol induced DNA synthesis and also activity of alkaline phosphatase [92], which points to an estrogenic activity in bone. Another report showed weak agonistic activity and no antagonistic activity on ERα, but weak antagonism on ERβ in transient transactivation assays [93]. Our own results [Mueller et al., manuscript in preparation] using human endometrial cell lines stably expressing ERα and ERβ showed only weak ERα and ERβ agonism, but stronger antagonistic activity on ERβ than on ERα on two different EREs. Taken together, these results indicate a potential of resveratrol to inhibit ERα and to a higher extent ERβ mediated gene expression as well as regulating cell proliferation in a cell-type specific manner. Thus, the observed differences in agonistic and antagonistic activation of ERα and ERβ by resveratrol may be due to a tissue or cell type selectivity as it is seen with SERMs [63]. Certainly, more studies have to be done in several tissue specific cell lines, with various EREs and target gene promoters to pin down resveratrol as a potential SERM.

Other ‘usual suspects’ include phytosterols, like β-sitosterol and the flavanone naringine (Fig. 1) but both lacked any significant estrogenicity [5,85] as well as the chalcone phloretin (Fig. 1), that had weak ERα binding affinity and was an agonist of ERα and to a lesser extent of ERβ [84,85]. Glabridine, an isoflavan isolated from licorice, showed ERα agonistic activity in vitro and in vivo and exerted also a growth-inhibitory potency on breast cancer cells at high doses [4].

The biggest shortcoming of an in vitro assessment of phytoestrogens to date is the lack of studies done using multiple endpoints in various cell lines. Especially in light of the potential tissue-selective estrogenic or antiestrogenic action of some phytoestrogens, like resveratrol and glabridine more comprehensive screening and mechanistic assays have to be performed to draw any valid conclusions. As outlined in the preceding section, test systems for the analysis of mechanisms of phytoestrogens are available. Tissue-specific gene expression profiles and the analysis of ligand-dependent association of the ER with tissue-specific co-activators, like AIB1 (SRC-3), that is predominatly expressed in mammary tissues, ovary and brain in mice [94] could help to identify tissue-selective phytoestrogens that may be of use for the treatment of endocrine related diseases. Furthermore, the analysis of co-activator interaction with ERα and ERβ may help to elucidate a potential ER-subtype specific agonism or antagonism of phytoestrogens [53,54].

4. Do in vitro assays have an impact on human risk assessment?

Clearly, in vitro assays have sped up the process in identifying potential estrogenic and antiestrogenic compounds that could be of benefit or risk for humans and wildlife. But how has human hazard or risk assessment been supported by results obtained in these assays and how do effects observed in vitro compare with the results obtained in vivo?

Limitations of in vitro assays are that metabolic activation or deactivation and the bioavailability of the test compound cannot be determined. In consequence, the active metabolite of the tested phytoestrogen must be known to determine the maximum estrogenicity in vitro. Daidzein for example is not estrogenic in vitro but it is in vivo, because it is

metabolized to equol [2], a weak ERα and ERβ agonist in vitro [Mueller et al., manuscript in preparation, 86]. Biochanin A, itself a weak ER agonist, is metabolized to genistein, one of the most potent phytoestrogens in vitro [reviewed in 2,84,85]. If the metabolism of phytoestrogens is taken into account, in vitro assays provide a good system to estimate estrogenic and antiestrogenic potencies. However, to draw any conclusions with regard to potential target tissues and effects in vivo, data regarding the mechanisms of action have to support the phenotypical characterization in vitro and in vivo (see also Section 3).

Animal studies have shown that potent phytoestrogens identified in vitro exert estrogenic and/or antiestrogenic effects in vivo. Genistein and daidzein showed uterotropic action in the rat, whereas resveratrol showed no uterotropic activity [95–97] consistent with their in vitro estrogenic activity. In contrast, coumestrol, an ERα and ERβ agonist in vitro, showed similar estrogenic effects to DES in neonatal rats [98,99] but repressed ERβ mRNA expression in rat brain [100]. These results make it clear that results obtained in only one cell line cannot be extrapolated to effects of the tested phytoestrogen in several tissues in vivo. In addition, plant-derived compounds with estrogenic potency likely have effects also on other nuclear receptors [101], they exert antioxidative activity [89], are potent kinase inhibitors [102], show antiandrogen action including inhibition of prostate carcinomas [103–106] and induce apoptosis [107]. Due to the pleiotropic effects of phytoestrogens in vivo a broad panel of in vitro assays covering not only estrogenic action but also other growth regulating processes have to be used to assess the potential of plant-derived compounds to beneficially or adversely affect human health.

Due to the abundance of phytoestrogens in the diet, especially in a vegetable rich diet, potent phytoestrogens can reach doses that would be sufficient to activate ER in vivo [3,108]. Epidemiological data indicates that a diet rich in isoflavones and lignans may contribute to beneficial effects on the cardiovascular system, bone maintenance and also on breast and prostate cancer [3,109–112]. Whereas some effects can be linked directly to compound-induced effects, e.g. anti-androgenic action of isoflavones inhibiting prostate cancer [103] and estrogenic activity of genistein in bone maintenance [113] others are most likely due to the plethora of effects induced by phytoestrogens.

The question of the potency of phytoestrogens to act as estrogens or antiestrogens can easily be addressed by in vitro screening methods. Together with mechanistic data in vitro and in vivo the potential of phytoestrogens to act as SERMs can also be determined. Nevertheless, the risk assessment for the intake of potential endocrine disrupters with pleiotropic effects in a complex matrix like a vegetable diet cannot be done with a one-compound-one-mechanism approach but one has to consider all other possible beneficial or detrimental effects of the diet.

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