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Comparative study of oestrogenic properties of eight phytoestrogens in MCF7 human breast cancer cells

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Abstract

Previous studies have compared the oestrogenic properties of phytoestrogens in a wide variety of disparate assays. Since not all phytoestrogens have been tested in each assay, this makes inter-study comparisons and ranking oestrogenic potency difficult. In this report, we have compared the oestrogen agonist and antagonist activity of eight phytoestrogens (genistein, daidzein, equol, miroestrol, deoxymiroestrol, 8-prenylnaringenin, coumestrol and resveratrol) in a range of assays all based within the same receptor and cellular context of the MCF7 human breast cancer cell line. The relative binding of each phytoestrogen to oestrogen receptor (ER) of MCF7 cytosol was calculated from the molar excess needed for 50% inhibition of [^{3}H]oestradiol binding (IC_{50}), and was in the order coumestrol (35×)/8-prenylnaringenin (45×)/deoxymiroestrol (50×) > miroestrol (260×) > genistein (1000×) > equol (4000×) > daidzein (not achieved: 40% inhibition at 10^4-fold molar excess) > resveratrol (not achieved: 10% inhibition at 10^5-fold molar excess). For cell-based assays, the rank order of potency (estimated in terms of the concentration needed to achieve a response equivalent to 50% of that found with 17β-oestradiol (IC_{50})) remained very similar for all the assays whether measuring ligand ability to induce a stably transfected oestrogen-responsive ERE-CAT reporter gene, cell growth in terms of proliferation rate after 7 days or cell growth in terms of saturation density after 14 days. The IC_{50} values for these three assays in order were for 17β-oestradiol (1×10^{-11} M, 1×10^{-11} M, 2×10^{-11} M), and in rank order of potency for the phytoestrogens, deoxymiroestrol (1×10^{-10} M, 3×10^{-11} M, 2×10^{-11} M) > miroestrol (3×10^{-10} M, 2×10^{-10} M, 8×10^{-11} M) > 8-prenylnaringenin (1×10^{-9} M, 3×10^{-10} M, 3×10^{-10} M) > coumestrol (3×10^{-8} M, 2×10^{-8} M, 3×10^{-8} M) > genistein (4×10^{-8} M, 2×10^{-8} M, 1×10^{-8} M)/equol (1×10^{-7} M, 3×10^{-8} M, 2×10^{-8} M) > daidzein (3×10^{-7} M, 2×10^{-7} M, 4×10^{-8} M) > resveratrol (4×10^{-6} M, not achieved, not achieved). Despite using the same receptor context of the MCF7 cells, this rank order differed from that determined from receptor binding. The most marked difference was for coumestrol and 8-prenylnaringenin which both displayed a relatively potent ability to displace [^{3}H]oestradiol from cytosolic ER compared with their much lower activity in the cell-based assays. Albeit at varying concentrations, seven of the eight phytoestrogens (all except resveratrol) gave similar maximal responses to that given by 17β-oestradiol in cell-based assays which makes them full oestrogen agonists. We found no evidence for any oestrogen antagonist action of any of these phytoestrogens at concentrations of up to 10^{-6} M on either reporter gene induction or on stimulation of cell growth.

Keywords: Phytoestrogens; Resveratrol; Daidzein; Genistein; Equol; Coumestrol; 8-Prenylnaringenin; Miroestrol; Deoxymiroestrol; Oestrogen agonist; Oestrogen antagonist; Antioestrogen; Breast cancer cells; MCF7 cells

1. Introduction

Phytoestrogens are compounds produced naturally in plants and which have the ability to interfere with oestrogen action either by interacting directly with oestrogen receptors or indirectly by modulation of endogenous oestrogen concentrations [1,2]. Early studies noted adverse effects on fertility in animals that had been grazing on plants rich in phytoestrogens [3]. Today, there is a wide interest in phytoestrogens for their potential health benefits in countering menopausal symptoms and in lowering incidence of hormone-dependent diseases including breast cancer [4].

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Such diverse actions of phytoestrogens may involve non-oestrogen mediated mechanisms, such as inhibition of protein tyrosine kinases [2,5,6], inhibition of cell cycle progression [2,6–8], inhibition of DNA topoisomerase [2,6,9,10], inhibition of angiogenesis [2,6,11,12] or as antioxidants [2,6]. Other actions include alteration of levels of steroid hormone binding globulin (SHBG) [2,13], disruption of oestrogen metabolism [2,14] or alteration to cellular levels of oestrogen receptors [15,16]. However, a major mechanism of their action is thought to result from their ability to interact directly with oestrogen receptors [1,2]. Phytoestrogens could therefore interfere with endogenous oestrogen action either by acting as agonists in their own right at times of low endogenous oestrogen or by acting as antagonists at times of higher endogenous oestrogen levels. The molecular basis of oestrogen action involves the binding to intracellular receptors (ERα, ERβ), which function as ligand-activated transcription factors [17]. Therefore, phytoestrogen effects could result either from their competition for binding to ERα and/or ERβ, or from inducing patterns of gene expression different from those induced by 17β-oestradiol. In this respect, it is interesting that some phytoestrogens have been reported to bind more strongly to ERβ than to ERα [18] and that the two receptors have different patterns of tissue distribution [19]. More recent studies have begun to identify specific phytoestrogen-regulated genes [20].

In order to investigate these mechanisms further, studies have been carried out in a wide variety of in vitro assays. However, not all phytoestrogens have been tested in each assay, which makes both inter-study comparisons and ranking oestrogenic potency difficult [2]. Assays have varied from binding to rodent uterine receptors or to recombinant receptors (either full length or ligand binding domain), to gene expression assays in yeast, to reporter gene assays in human cell lines, to assay of endogenous oestrogen-regulated genes in human cell lines and then finally comparing to effects on growth of the oestrogen-sensitive MCF7 human breast cancer cell line [2,21–24]. Variations in the reported potency of phytoestrogens may relate as much to differing cellular receptor content and differing cellular context as to differences in the phytoestrogen actions. Insight into phytoestrogen actions in different cell types is not gained usefully by comparing action of one phytoestrogen in one cell type with action of another phytoestrogen in a different assay in another cell type. Many of such comparisons may turn out to be correct, but validation can only be achieved through performing for each phytoestrogen, all assays (ER binding, gene expression and cell proliferation) within each cell line. In this report, we have made a direct comparison between the oestrogenic actions of phytoestrogens in a range of assays all based in a single cell line, the MCF7 human breast cancer cell line.

Genistein and daidzein are isoflavones found in human diets in leguminous plants, especially soybeans [1,2]. Equol is a related phytoestrogen derived from metabolism of daidzein [1,2]. Equol was actually the major form of phytoestrogen present in sheep suffering fertility problems following the grazing on subterranean clover [25], but ability for metabolic conversion of daidzein can vary in the human population [2,26]. In MCF7 cells, genistein has been shown to have a biphasic effect on cell growth [27]. Low concentrations stimulate cell growth and enhance pS2 gene expression [28], whilst high concentrations (above 10 μM) inhibit cell growth by blocking the cell cycle at the G2-M phase [7,29]. The proliferative action of genistein at low concentrations can be inhibited by antioestrogen [16] indicating that it is an oestrogen receptor-mediated mechanism. However, the inhibition of cell growth at high concentrations is not prevented by antioestrogen or oestrogen [16], indicating it is not ER-mediated, but may be due to other mechanisms including inhibition of tyrosine phosphorylation [7,30].

Deoxymiroestrol and its derivative miroestrol have been reported as phytoestrogens with high oestrogenic potency [31], but their action in MCF7 cells has only ever been reported once and then only on their ability to antagonize antioestrogen action [32]. 8-Prenylnaringenin is a phytoestrogen found in hops and found to be a potent stimulator of Ishikawa cell growth [33] and of E-cadherin-dependent aggregation in MCF7 cells [34].

Coumestrol is a phytoestrogen found in alfalfa and animal foodstuffs and is thought to have potent oestrogenic activity. However, reported relative binding affinities have varied from <0.01% in sheep uterus to 94% for some human ER [1], oestrogen-responsive reporter gene expression has been reported in yeast, HeLa, LeC9 and prostate cells [1], and no studies have been reported on MCF7 cell growth [22].

Resveratrol, a polyphenolic compound found in grapes and wine, has a variety of biological effects, among which its oestrogenic activity is thought to contribute to the cardiopreventive effects associated with red wine consumption [35]. Like genistein, resveratrol has been reported to give a biphasic response on cell growth, but the differences in concentrations between stimulatory (3–22 μM) and inhibitory (above 25–44 μM) responses are rather smaller [36–38]. One report attests to an ability of resveratrol to function as a superagonist to reporter gene expression in MCF7 cells, producing a greater maximal response than oestradiol [36], but this may be promoter and receptor dependent [39] and relate to the presence of steroid response elements in the luciferase reporter gene [40]. Despite these reported superagonist effects in vitro [36], resveratrol is too weak in its oestrogenic activity to allow any measurable oestrogenic response in an in vivo uterotrophic assay [41].

In this study, we have made a comparison between the oestrogenic activities of eight phytoestrogens (genistein, daidzein, equol, miroestrol, deoxymiroestrol, 8-prenylnaringenin, coumestrol and resveratrol) in a variety of assays all based in the same MCF7 cell system, so that direct comparisons can be made within the same receptor and cellular context. We have compared their relative ability to bind to oestrogen receptors of MCF7 cell cytosol, their relative ability to induce a stably transfected oestrogen-responsive