Steroids, Steroid Receptors and Disease

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New advances in the understanding of the role of steroids and steroid receptors in disease

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Abstract

Steroids and related compounds are important in disease development and prevention, via steroid receptor-mediated and receptor-independent mechanisms. Interaction of endogenous oestrogens with the oestrogen receptor has unfortunate mitogenic effects in breast cancer. However, dietary consumption of non-steroidal weak oestrogens, such as the soy isoflavone phytoestrogens genistein and diadzein, is associated with a decreased breast cancer risk. This may arise in part from the suboptimal configuration induced in the transactivation helix of oestrogen receptor-β.

Introduction

The role of steroids and steroid receptors in the occurrence of disease and as targets for disease prevention is widely recognized and is currently an active area of research. For example, endogenous mitogenic oestrogens are known to play an important role in the development of breast cancer, and mutations in the oestrogen receptor are implicated in the development of tumour resistance to therapy with drugs such as tamoxifen.

Key words: oestrogen, phytoestrogen, oestrogen receptor, genistein, breast cancer, antioxidant.

Abbreviations used: PPAR, peroxisome-proliferator-activated receptor; ERα, classical oestrogen receptor; ERβ, ER sub-type β; TGF-β, transforming growth factor-β; LDL, low-density lipoprotein; HHT, haemorrhagic telangiectasia.

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Peroxisome-proliferator-activated receptor (PPAR), thyroid hormone receptor, androgen receptor and their ligands

The oestrogen receptors (α and β), androgen receptor, thyroid hormone receptor, retinoic-acid receptors, retinoid-X receptors and PPAR all belong to the same family of nuclear receptors. Although many of these receptors have well-characterized physiological ligands e.g. steroids, thyroxine and retinoids, 'orphan' receptors make up more than half of this gene family. Frequently, these receptors are likely to be important in development and gene regulation, but are termed 'orphan' receptors because they have not yet been associated with a physiologically relevant ligand [1]. The PPAR is one such 'orphan' receptor. PPARs may be involved in a number of chronic diseases, including diabetes, obesity, atherosclerosis and cancer [2]. There are three known human PPAR subtypes, α, γ and δ, and these are associated with selective ligands and show distinct tissue distributions. Some ligands are common to all three PPAR isoforms, and include polyunsaturated fatty acids, and probably oxidized fatty acids [2]. PPARγ regulates adipogenesis and mediates the action of thiazolidinediones, which are used pharmaceutically for the treatment of Type II diabetes, because they sensitize tissues to insulin [1–3]. PPARγ mutants have been found in subjects with severe insulin resistance. The PPARγ mutants are transcriptionally impaired as a result of altered ligand binding and co-activator recruitment, and analogous to resistance to thyroid hormone, they inhibit the function of wild-type PPARγ when co-expressed [3]. Resistance to
Oestrogens, phytoestrogens and oestrogen receptors

Oestrogens play a vital role in the growth, development and homoeostasis of oestrogen responsive tissues. The oestrogen receptor mediates the biological activity of oestrogens and is a ligand-inducible nuclear transcription factor: oestrogen binds to the ligand-binding domain of the oestrogen receptor, resulting in either the activation or repression of target genes [6]. Phytoestrogens, such as the soya isoflavones genistein and daidzein, are plant-derived non-steroidal oestrogen mimics that are being extensively investigated to determine their therapeutic potential, particularly in disease prevention [7]. Breast and prostate cancer is much less prevalent in Far-Eastern countries, where there is an abundance of soya phytoestrogens in the diet, compared with Western ones. Emigration of people from Pacific Rim countries to the U.S.A. has been shown to increase their risk of breast and prostate cancer [8]. These changes in breast and prostate cancer risk have been mostly attributed to change to a low-soya Western diet. In countries such as Japan, Korea, China and Taiwan, the mean daily intake of soya products has been estimated to be in the range of 10–50 g compared with only 1–3 g in the U.S.A. [9].

The phytoestrogen family includes the lignans and members of the flavonoid family, such as the isoflavonoids; also some of the flavones, flavanones, chalcones, coumestans and stilbenes [10]. Isoflavonoids include the isoflavones genistein and daidzein, which occur mainly as the glycosides genistin and daidzin respectively, in soya beans and, consequently, in a wide range of soya-derived foods and, to a lesser extent, in other legumes [11]. Some alcoholic beverages such as beer contain significant amounts of isoflavonoids [12].

Daidzin and genistin are hydrolysed in the large intestine (by the action of bacteria) to release genistein and daidzein. Daidzein can be metabolized in the large intestine by bacteria to form the isoflavan equol (oestrogenic) or O-desmethylangolensin (non-oestrogenic), whereas genistein is metabolized to the non-oestrogenic p-ethyl phenol. Inter-individual variation in ability to metabolize daidzein to equol could thus influence the potential health protective effects of soya isoflavones. Plasma isoflavonoid levels in Japanese and Finnish men have been measured, and the means of the total daidzein, genistein, O-desmethylangolensin and equol levels of these men were approximately 17-fold, 44-fold, 33-fold and 55-fold higher for the Japanese subjects compared with the Finnish ones [13]. In post-menopausal Australian women following consumption of soya flour, mean plasma levels of daidzein and equol of 68 ng/ml and 31 ng/ml respectively were observed [14]. Interestingly, only 33% of subjects were able to metabolize daidzein to equol [14]. The bioavailabilities of genistein and daidzein are similar (despite differences in urinary excretion, as shown by the approximately equal areas under their plasma concentration-time curves) [15]. A variable metabolic response to isoflavones has been shown for subjects following consumption of soya flour; urinary excretion concentrations of genistein, daidzein, equol and O-desmethylangelensin were increased 8-fold, 4-fold, 45-fold and 66-fold respectively, compared with baseline levels [16]. Great interindividual variation in metabolic response was reported, with the peak levels of equol showing the most variation [16]. In
healthy male and female subjects, where diets high or low in isoflavones (textured soya protein product containing 56 mg/day or 2 mg/day respectively) were each consumed for 17 days separated by a 23-day washout period, considerable inter-individual variation in metabolic response was found [17]. In addition, the good equol excretors (36% of subjects) consumed significantly less fat and more carbohydrate (also greater amounts of non-starch polysaccharide: NSP) compared with the poor equol excretors [17].

The bioavailability of soya isoflavones has been shown to depend on the gut microflora [18]. Metabolism by the gut microflora is an important factor influencing the disposition of chemicals in the gut, and can result in activation of substances to more biologically active products. The presence of different populations of microflora in the human gut may influence the bioavailability of soya isoflavone phytoestrogens, and studies concerning this are currently in progress. In particular, the metabolism of daidzein to equol by certain types of gut bacteria has important implications.

The selective oestrogen receptor antagonist raloxifene (structurally related to the anticancer drug tamoxifen [19]) can inhibit the mitogenic effects of oestrogen in reproductive tissues, while maintaining the beneficial effects of oestrogen in other tissues. The crystal structures of the ligand-binding domain of the oestrogen receptor complexed with either 17β-oestradiol or with raloxifene have been reported [20], thus providing structural evidence for the mechanisms of oestrogen receptor agonism and antagonism. A combination of specific polar and non-polar interactions enables the oestrogen receptor to selectively recognize and bind 17β-oestradiol with great affinity. The oestrogen receptor is the only steroid receptor able to interact additionally with a large number of non-steroidal compounds, including phytoestrogens and environmental and drug xenoestrogens (and their metabolites), which frequently show a structural similarity to the steroid nucleus of oestrogen. In particular, a phenolic ring analogous to ring A in oestradiol is required and these structural features enable them to bind to oestrogen receptors to elicit responses ranging from agonism to antagonism of the endogenous hormone-ligand [21].

Originally, it was accepted that only one oestrogen receptor existed (the classical oestrogen receptor, ERα). This is in contrast with other members of the nuclear-receptor superfamily where multiple forms have been found. A separate sub-type, termed oestrogen receptor β (ERβ) has subsequently been identified in cDNA libraries from rat prostate and ovary tissues [22]. ERβ shows a different tissue distribution from ERα. ERβ was first reported to be strongly expressed in ovary, uterus, brain, bladder, testis, prostate and lung [23]. Expression of ERβ appears to occur at different sites in the brain from ERα [23]. Evidence has now been found for the presence of ERβ in normal human breast tissue [24]. ERβ has also been found to be expressed in both bone [25] and the cardiovascular system [26]. Furthermore, in the rat carotid injury model, following endothelial denudation of rat carotid artery, ERα is expressed at a low level, whereas the expression of ERβ increases by more than that 40-fold and treatment of ovariectomized female rats with genistein provides a similar dose-dependent vasculo-protective effect in this model to that observed with 17β-oestradiol [26].

Although genistein is a much better ligand for ERβ than for the ERα (20-fold higher binding affinity) [23], it can also act as an oestrogen agonist via both ERα and ERβ in some test systems [27]. However, genistein also behaves as a partial oestrogen agonist in human kidney cells transiently expressing ERβ, suggesting that it may be a partial oestrogen antagonist in some cells expressing ERβ [28]. Furthermore, although genistein binds to the ligand-binding domain of ERβ in a manner similar to that observed for 17β-oestradiol, in the ERβ-genistein complex, the AF-2 helix (H12) does not adopt the normal agonist-type position, but instead takes up a similar orientation to that induced by ER antagonists such as raloxifene [29]. This sub-optimal alignment of the transactivation helix is in keeping with the reported partial agonist activity of genistein in ERβ [29].

Many other potentially beneficial mechanisms of action for phytoestrogens and genistein in particular have been suggested in addition to those mediated through the oestrogen receptor. These include: (i) inhibition of DNA topoisomerases [30]; (ii) inhibition of cell cycle progression [31]; (iii) inhibition of angiogenesis [32] (iv) tumour invasiveness [33]; (v) inhibition of enzymes involved in oestrogen biosynthesis [34]; (vi) effects on the expression of the DNA transcription factors c-Fos and c-Jun [35], and reactive oxygen species [36]; (vii) oxidative membrane damage [37]; and (viii) oxidative damage in vivo [38] (ix) by the transforming growth factor-β (TGFβ) action [31,39].
Antioxidant properties have been reported for isoflavones both in vitro and in vivo. Equol, in model membrane systems, was a more effective antioxidant than genistein or the parent compound daidzein [37], and shows structural similarity to the tocopherols [7]. Furthermore, soya isoflavone consumption (56 mg/day for 17 days) has been reported to decrease plasma isoprostane (biomarker for lipid peroxidation) concentrations [38]. Antioxidant action could contribute to anticancer ability because reactive oxygen species could initiate signal transduction through the mitogen-activated protein (‘MAP’) kinases [7]. The antioxidant action of phytoestrogens may contribute also to their cardioprotective properties. Isoflavones can protect low-density lipoprotein (LDL) against oxidative modification in vitro [37]. Furthermore, in a recent study, resistance of LDL to oxidation showed a significant increase following the high isoflavone dietary treatment [38]. These observations suggest that soya isoflavones may protect against the oxidative damage implicated in atherosclerosis and cardiovascular disease generally [38].

Genistein may enhance the action of TGFβ [31,39]. This action may be a link between the effects of genistein in a variety of chronic diseases, including atherosclerosis and hereditary haemorrhagic telangiectasia (HHT; the Osler–Weber–Rendu syndrome), in which defects in TGFB signalling have been characterized [40,41]. Indeed, pilot studies have shown that genistein consumption significantly alleviated symptoms in patients with HHT [41].

However, many flavonoids, such as the flavonol quercetin and also the isoflavone genistein, have been shown to inhibit the action of enzymes such as sulphotransferases important for inactivating the endogenous oestrogens implicated in breast cancer [42], suggesting that further human studies to gain a complete picture of all the biological actions of isoflavones in vivo are clearly required.

**Oxytocin and the oxytocin receptor**

In contrast with the steroid hormones and their receptors already considered, oxytocin is a nonapeptide hormone that induces uterine contractions and may contribute to the onset of labour and parturition. The steroid hormone progesterone is vital for establishing and maintaining pregnancy in mammals. Uterine quiescence is maintained by progesterone by decreasing uterine sensitivity to oxytocin. Classically, it is considered that steroid hormones, such as progesterone, act at a genomic level by binding to nuclear receptors and modulating the expression of target genes. It is of considerable relevance therefore that the function of the oxytocin receptor (a member of the G-protein-coupled receptor family) has been reported to be inhibited by direct binding of progesterone [43]. This work appears to provide the first evidence for a direct interaction between a steroid hormone and a G-protein-coupled receptor, and delineated a new level of ‘cross-talk’ between the peptide-hormone and steroid-hormone signalling pathways [43]. Subsequently, however, other research groups have been unable to repeat this finding [44]. Furthermore, although there is experimental evidence to suggest that, in humans, rats and ruminants, sex steroids are involved in regulating the genes for both oxytocin and the oxytocin receptor, oestrogen action appears to be indirect for both of these genes [44].

**Conclusions**

In conclusion, further studies are clearly needed to elucidate the precise molecular basis for the action of related compounds and their receptors in disease and, ultimately, in disease prevention. It is predicted that a combination of human intervention studies and the techniques of proteomics and functional genomics [45] will be particularly helpful in achieving this aim.

**References**

Do dietary phytoestrogens influence susceptibility to hormone-dependent cancer by disrupting the metabolism of endogenous oestrogens?

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Abstract

Phytoestrogens are natural constituents of our diets that have been suggested to protect against hormone-dependent breast cancer. Some of the diverse effects of these compounds may be attributed to ligand-dependent differences in their interaction with oestrogen receptor sub-classes. However, phytoestrogens can also inhibit enzymes that are involved in the generation and removal of endogenous steroid hormones. Among the most potent effects of dietary phytoestrogens is their ability to inhibit the sulphotransferases that sulphate both oestrogenic steroids and a variety of environmental chemicals, including dietary procarcinogens. Circulating steroid sulphates are thought to be the major source of oestriadiol in post-menopausal breast tumours and sulphation is a key step in the activation of some dietary pro-