Modulation of the oestrogen receptor: a process with distinct susceptible steps

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The selective oestrogen receptor modulators (SERMs) constitute a group of substances which are capable of regulating the agonistic/antagonistic profile of the oestrogen receptor in distinct tissues. Their potential utility is considerable since, among the pleiotropic range of effects that oestrogens exert on their target tissues, they may provide a selective profile that better suits each clinical necessity. This review summarizes the principal steps of oestrogen action where modifications have resulted in changes of the effect profile. Three different steps of oestrogen action have been highlighted as being susceptible to modulation: type of ligand, particular species of oestrogen receptor, and particulars at the target tissue. Two main families of SERMs, the triphenylethylene derivatives, with tamoxifen as the main actor, and the benzothiophene derivatives, mainly represented by raloxifene, provide much of the basic and clinical knowledge on the influence of the type of ligand. Two types of oestrogen receptor, α and β, add the second variable susceptible to modulating the response to receptor activation. Finally, the ligand–receptor complex may define particular events in its interaction with DNA, such as binding to promoters other than the oestrogen response element, recruitment of concrete sets of local transcription factors, or other options.

Key words: mechanism of action/oestrogen receptor modulators/oestrogen receptor species/tissue specificity

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Introduction

In 1958, Lerner et al. published their outstanding paper on the pharmacological properties of ethamoxytriphetol or MER-25, the first non-steroidal anti-oestrogen (Lerner et al., 1958). Only 2 years later, Jensen and Jacobson identified a specific target for that compound, the oestrogen receptor (ER) (Jensen and Jacobson, 1960). Those two discoveries paved the way for the development of a new area in reproductive endocrinology and for the discovery of tamoxifen, a drug that increases survival in patients with breast cancer, no matter what the status of positivity or negativity of the axillary lymph nodes may be (Early Breast Cancer Collaborative Group, 1998).

The experimental and clinical use of tamoxifen has revealed that its benefits include other areas of great importance for the health of women, e.g. protection against osteoporosis, and perhaps also against cardiovascular disease. Those two are functions characteristically ascribed to oestrogens and therefore, their integration into the traditional concept of anti-oestrogen was made only with difficulty. The experience gained with tamoxifen, therefore, opened a new scenario where functional versatility was linked to a range of variables, such as the type of drug, animal species and target tissue (MacGregor and Jordan, 1998; Spencer et al., 1999).

That versatility was particularly attractive since, in theory, it would allow the design of drugs with either agonist or antagonist properties, according to the circumstantial necessity. The perception of that controlled functional versatility associated with the type of compound has opened the concept of selective oestrogen receptor modulators (SERMs), a series of substances that may operate on the ER as either agonists or antagonists, according to a set of variables. In fact, as detailed in another paper of this symposium (Goldstein et al., 2000), the pharmacological research is actually being very active in producing a wide group of substances with SERM properties.

Determinants of the agonist/antagonist activity of SERMs

Steroid receptors integrate a family of proteins which act as true transcription factors (Beato and Klug, 2000). Once they receive an agonist, the receptors become ‘activated’ and orchestrate a
transcriptional oestrogen response element; RRE = raloxifene response element.

**Table I.** The three main levels of modulation of the oestrogen receptor (ER)

<table>
<thead>
<tr>
<th>Type of ligand</th>
<th>Species of ER</th>
<th>Type of effector site</th>
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<tbody>
<tr>
<td>Triphenylethylenes</td>
<td>ERα</td>
<td>Distinct promoters:</td>
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<td></td>
<td></td>
<td>Simple</td>
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<tr>
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<td></td>
<td>Composite</td>
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<tr>
<td>Benzothiophenes</td>
<td>ERβ</td>
<td>Distinct sites at the promoter:</td>
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<tr>
<td></td>
<td></td>
<td>ERE</td>
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<td></td>
<td></td>
<td>RRE</td>
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<td>Pure anti-oestrogens</td>
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</table>

ERE = oestrogen response element; RRE = raloxifene response element.

**Type of ligand**

Among the six domains recognized in the ER, the three most relevant functional domains include the region A/B, the DBD (region C), and the LBD (region E). In the non-stimulated receptor, the heat shock protein, hsp90, is positioned in such a way that prevents dimerization and strong binding to the oestrogen responsive element (ERE) in DNA (Figure 1). The addition of oestrogen is followed by the separation of hsp90 (Figure 1), which is accompanied by dimerization, with strong interaction between the LBDs, and weaker interactions between the DBDs. The resulting dimer binds to the ERE and activates transcription through the intervention of the ligand-inducible transcription-activating function 2 (TAF-2) and the constitutive transcription-activating function 1 (TAF-1), located at the LBD and the region A/B respectively.

The SERMs generate modifications at distinct levels of that scheme. As described by Goldstein et al. (2000), SERMs may be divided into two major families, the triphenylethylene derivatives, where tamoxifen has accumulated most of the experimental and clinical studies, and the benzothiophenes, led by raloxifene. In addition, there is another category, the pure anti-oestrogens (Table I), whose mechanism of action differs in that no agonist activity can be detected in the majority of the models assayed at present (Hermenegildo and Cano, 2000). Although SERMs bind to the same LBD as oestradiol, their receptor-binding affinity, their binding mechanism, and the conformational change that they induce on the ER differ from those of oestradiol (Figure 1).

Much of the knowledge gained in this context has been obtained through the use of mutant receptors and modern crystallographic studies of the ER. Mutations in the mouse ER at residues 525 and 527, for example, can abolish the ability of the ER to bind oestrogen; however, the mutant receptors retain their partial agonist response to tamoxifen similar to that of the wild-type ER in the presence of tamoxifen (Danielian et al., 1993). Structural differences among the various ligands that bind the ER induce distinct conformational changes in the receptor and, therefore, different patterns of oestrogen-induced responses. The consequences of that change on the transcription activation functions, TAF-1 and TAF-2, have contributed to clarify the agonist/antagonist activities of raloxifene and tamoxifen. The TAF-2 activity depends on the presence of a $\alpha$-helix made up of residues 538 to 552. Mutational analyses have identified several additional residues that influence the function of TAF-2, suggesting that although centred on helix 12, probably that activity also encompasses parts of the surrounding helices H3, H5/6 and H11.

The enlightening of the crystal structure of the LBD of the ER with oestradiol or raloxifene (Brzozowski et al., 1997) confirms that the hydroxyls of oestradiol bind to the amino acids 353 and 394 in the LBD and this causes the large helix 12 to fold over and trap the steroid (Figure 2). Helix 12 contains amino acids, such as 540, 543, and 547, which are required within the TAF-2 region for binding co-activators. In the raloxifene–ER complex, however, helix 12 does not overlie the cavity, and this may prevent co-activator recruitment to the LBD of the ER.
The promoter context also can affect the transcriptional activity of both the TAF-1 and the TAF-2 of the ER. Again, the use of receptor mutants has shown that those TAFs function in a cell-specific manner, and depending on the cell and the promoter context, it was determined that the requirement for these activation functions varied (Tzuckerman et al., 1994). Using the ERs mutated at amino acids 538, 542, or 545, it has been shown that the antagonist activity of tamoxifen results from its inability to activate the TAF-2 function. However, in contexts where TAF-1 is required, tamoxifen functions as a partial agonist manifesting 30–40% of the agonist activity of oestradiol. Given the inescapable limits of the in-vitro experiments, these observations cannot be used, as yet, to explain what occurs in vivo. Hence, it is remarkable that together with tamoxifen, droloxifene, toremifene, clomiphene and nafoxidine, compounds that behave as agonists in the uterus and antagonists in the breast, all function as selective TAF-1 agonists in vitro (Tzukerman et al., 1994; McDonnell et al., 1995a). To further illustrate how the differential activation of the TAF-1 and TAF-2 domains may also explain tissue-selective actions within the benzothiophene family, deletion of the TAF-1 domain is followed by suppression of the oestradiol-induced, but not raloxifene-induced, transforming growth factor (TGF)-β promoter activation. Vice versa, deletion of the TAF-2 domain blocks the activation of the TGF-β promoter by raloxifene but not by oestradiol (Yang et al., 1996).

**Type of oestrogen receptor**

The identification of a new species of ER, named ERβ (Kuiper et al., 1996), has introduced a new level of complexity. It is not only that ERα and ERβ vary in their tissue distributions (Mosselman et al., 1996; Kuiper et al., 1997), but also that the agonist/antagonist profile of a compound may also be changed as a function of both the receptor type to which it is bound and the promoter context. At activating protein-1 (AP-1) containing promoters, e.g. ERα and ERβ were shown to signal in opposite ways when complexed with oestradiol: with ERα, oestradiol activated transcription, whereas with ERβ, oestradiol inhibited transcription. Moreover, tamoxifen, raloxifene, and a pure anti-oestrogen, ICI 164384, were potent transcriptional activators with ERβ (Paech et al., 1997).

**The effector site**

Implicit in the differences linked to the preservation or inhibition of the TAF-2 site, or the interaction with promoters containing AP-1 sites that have already been analysed, is the concept that underlines the role of local transcription factors able to modify the cellular response to a given ER ligand. These factors integrate an array of co-activator and co-repressor proteins which inhibit or enhance transcription (Horwitz et al. 1996). Co-activators and co-repressors interact with nuclear receptors through various mechanisms, including mutual competition (Horwitz et al., 1996;
Jackson et al., 1997; Smith et al., 1997). Consequently, the direction of transcription by antagonist-occupied steroid receptors can be controlled by the ratio of co-activators to co-repressors recruited to the transcription complex by promoter-bound receptors. Among the co-activators, the steroid receptor co-activator-1 (SRC-1) has been shown to enhance the transactivation of all the steroid receptors tested (Onate et al., 1995). Another co-activator, L7/SPA, a 27 kDa protein which increases the partial agonist activity of the antagonists by 3–10-fold, has no effect on agonist-mediated transcription (Jackson et al., 1997). The list of co-repressors includes distinct factors, where SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) and hN-CoR, the human homologue of the 270 kDa thyroid/retinoic acid receptor co-repressor, have demonstrated the ability to reduce the 4-hydroxyltamoxifen (SMRT and hN-CoR) and RU 486 (hN-CoR) partial agonist activity (Jackson et al., 1997; Smith et al., 1997).

Three functional variations have been described (Williams, 1997): (i) activation of a ligand–receptor complex in a specific cell; (ii) changes in the rate of transcription of a specific gene by the ligand–receptor complex; and (iii) changing a ligand–receptor complex from an activator to a repressor.

As a result of the presence or absence of transcription factors, two types of promoters have been proposed: simple (only requiring the receptor–ligand complex to produce a change in gene expression) and composite (requiring not only the receptor–ligand complex but also transcription factors) (Williams, 1997). It has been suggested that, with composite promoters but not with simple promoters, the TAF-1 and TAF-2 sites are critically important in inducing the appropriate conformational changes capable of activating gene transcription (Funder, 1993; Kraus et al., 1995; McDonnell et al., 1995b). As previously mentioned, tamoxifen seems capable of activating the TAF-2 site, acting therefore as a TAF-1 agonist only. Accordingly, only if there are other transcription factors which can serve as a substitute or bridge for the TAF-2 function, tamoxifen will be a full agonist in a given tissue. Also in this regard, the activating effect that some ligands display on promoters containing AP-1 sites may be explained by the modifying role that the proteins fos and jun exert in this system. These two proteins are, therefore, transcription factors required for transcription at this specific AP-1 site (Umayahara et al., 1994).

Finally, some other mechanisms have been described to explain the changing activities of SERMs in the different tissues. The specific molecular structure of a particular SERM may, once bound to the LBD in the ER, induce a singular conformation change of the ligand–receptor complex. The possibility exists that such a change may lead to a special conformation of the DBD which enables it to bind to a site distinct from the traditional ERE. This is the case of raloxifene, which acts as an agonist for a so-called raloxifene response element, which regulates the TGF-β gene in bone (Yang et al., 1996). Also, it is possible that the binding of different SERMs to the ER may affect the kinetics of ER interaction with specific DNA elements, which then affects gene transcription (Cheskis et al., 1997). In addition the distinct SERMs may interfere with the formation of ER-associated proteins with the ER, or display non-genomic effects, as confirmed for tamoxifen and raloxifene (Weiss and Gurpide, 1988; Coletta et al., 1994; Halachmi et al., 1994).

Conclusions

The agonist/antagonist profile of the activated ER seems to offer three possible main steps for regulation. The first step is determined by the type of compound which is bound to the LBD of the receptor. The peculiar structure of each ligand may induce specific conformational changes in the ligand–receptor complex which, therefore, may affect the DBD. The second step is determined by the particular species of ER which is available, since the ERα and the ERβ may condition the agonist/antagonist effect of a certain compound. Finally, there is a complex group of ER profile determinants which are linked to the effector site. At this level, the simple or composite type of promoter is an obligatory first consideration. In the composite promoters, the direction of transcription can be controlled by the ratio of co-activators to co-repressors recruited to the transcription complex by promoter-bound receptors. The type of simple promoter which is available may also condition the transcriptional activity, since sites other than the traditional ERE, with capacity of activation by ligand–ER complexes, have been described.

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