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From empirical to mechanism-based discovery of clinically useful Selective Estrogen Receptor Modulators (SERMs) ☆,☆☆

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ABSTRACT

Our understanding of the molecular mechanisms underlying the pharmacological actions of estrogen receptor (ER) ligands has evolved considerably in recent years. Much of this knowledge has come from a detailed dissection of the mechanism(s) of action of the Selective Estrogen Receptor Modulators (SERMs) tamoxifen and raloxifene, drugs whose estrogen receptor (ER) agonist/antagonist properties are influenced by the cell context in which they operate. These studies have revealed that notwithstanding differences in drug pharmacokinetics, the activity of an ER ligand is determined primarily by (a) the impact that a given ligand has on the receptor conformation and (b) the ability of structurally distinct ER-ligand complexes to interact with functionally distinct coregulators. Exploitation of the established relationships between ER structure and activity has led to the development of improved SERMs with more favorable therapeutic properties and of tissue-selective estrogen complexes, drugs in which a SERM and an ER agonist are combined to yield a blended activity that results in distinct clinical profiles. Remarkably, endogenous ligands that exhibit SERM activity have also been identified. One of these ligands, 27-hydroxycholesterol (27HC), has been shown to manifest ER-dependent pathological activities in the cardiovascular system, bone and mammary gland. Whereas the physiological activity of 27HC remains to be determined, its discovery highlights how cells have adopted mechanisms to allow the same receptor ligand complex to manifest different activities in different cells, and also how these processes can be exploited for new drug development.

1. Introduction

The estrogen receptor (ER) is a well-validated therapeutic target that has been exploited in the development of drugs that are currently used as (a) treatments for the climacteric symptoms associated with menopause, (b) oral contraceptives, (c) fertility agents and (d) breast cancer therapeutics. Until relatively recently it was considered that the pharmacology of ER ligands was relatively simple in that classical agonists (steroidal or non-steroidal) phenocopied the actions of the potent agonist 17β-estradiol, while antagonists exerted their activity primarily through competitively inhibiting the binding of estrogens to their cognate receptors. Not surprisingly, therefore, the pharmaceutical development of most of the ER modulators currently used in the clinic was driven by the simple premise that, when corrected for affinity, all agonists were qualitatively the same and likewise antagonists differed only in their affinity for the receptor. Thus it was long considered that, other than enhancements to delivery and formulation, only minor improvements could be made to the therapeutic activity of ER modulators. This became a particular issue for hormone therapy (HT) in postmenopausal women, where a significantly increased risk of endometrial cancer was observed in women taking unopposed estrogens, an activity that was a property of all estrogens. This liability led to the incorporation of progestins in HT regimens administered to women with an intact uterus in order to prevent estrogen-induced endometrial hypertrophy. Unexpectedly, the inclusion of progestins in these medicines was associated with a whole new series of clinical problems, the significance of which was highlighted by the results of the Women's Health Initiative (WHI) in 2002 in which a slight, but significant increase in the risk of invasive breast cancer was observed in women taking conjugated estrogens (CE) together with medroxyprogesterone acetate (MPA) [1]. Whereas efforts to develop ER ligands that functioned

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in a tissue selective manner preceded the WHI, the results of this trial reinvigorated efforts to exploit the complexities of the ER signal transduction pathway as a means to develop safe and effective medicines for HT. From these efforts emerged the third generation Selective Estrogen Receptor Modulators (SERMs) and more recently the Tissue Selective Estrogen Complexes (TSECs), drugs whose actions on ER are manifest in a cell-selective manner and which do not require the inclusion of a progestin. A discussion of how the development of these new drugs was influenced by an increased understanding of the molecular pharmacology of ER is the subject of this perspective.

2. The discovery of first and second generation SERMs

The SERM concept emerged from a series of preclinical clinical studies which revealed that the "antiestrogen" tamoxifen actually exhibited substantial ER agonist activity in bone and in the uterus [2–6]. Thus, while able to oppose estrogen action in the mammary gland, tamoxifen exhibited agonist activity in other tissues. The potential therapeutic utility of this tissue selective action was first highlighted by clinical studies that reported a significant increase in bone mineral density (BMD) in the lumbar spine of tamoxifen-treated breast cancer patients when compared to controls [7,8]. These were followed by a very informative placebo controlled trial in which the bone sparing activity of tamoxifen in breast cancer patients was confirmed [9]. Together the clinical and preclinical pharmacology of tamoxifen provided strong evidence that it was possible to develop molecules whose ER agonist activity was manifest in a cell-selective manner. Indeed, were it not for the fact that tamoxifen exhibited significant uterotrophic activity in rodents and in humans, it may have been developed to treat and prevent osteoporosis [3]. Interestingly, a second "antiestrogen" keoxifene was also shown to protect against ovariectomy-induced bone loss in rodents [2]. This drug had originally been developed as a treatment for patients with tamoxifen-resistant metastatic breast cancer. Although the initial clinical trials in breast cancer were inconclusive, this drug did distinguish itself from tamoxifen in that it did not exhibit uterotrophic activity [10,11]. The bone protective, uterine sparing, activities of keoxifene were confirmed in the MORE trial, and keoxifene (renamed raloxifene) was subsequently approved for the prevention and treatment of osteoporosis [12]. Interestingly, both tamoxifen and raloxifene were also approved for use as breast cancer chemopreventatives in women at elevated risk for breast cancer [13]. It was also clear from these results that it was inappropriate to classify tamoxifen and raloxifene as "antagonists", and thus a new class label "SERMs" was proposed to reflect their complex pharmacology [14–16]. Several other SERMs, notably idoxifene and droloxifene, were evaluated at around the same time for activity as bone sparing agents in post-menopausal women. However, whereas all of these first/second generation SERMs were found to exhibit similar activities in bone, raloxifene alone functioned as a pure antagonist in the uterus. Raloxifene remains the only SERM mono-therapy registered in the US for the treatment and prevention of osteoporosis.

Unlike classical estrogens, the SERMs were ineffective in the treatment of vasomotor instability and dyspareunia, two of the primary reasons women seek HT. For this reason, it was unlikely, even considering their favorable profiles in breast, bone and uterus, that these early SERMs would find significant use in the pharmacotherapy of the symptomatology associated with menopause. However, it was generally considered that, since the SERM actions of tamoxifen and raloxifene (as well as toremifene, idoxifene and droloxifene among others) were discovered in a serendipitous manner, and further that their activities were optimized for antagonist activity in the breast, with additional exploration drugs with improved action in other tissues could be developed.

3. Molecular mechanisms of SERM action

It was apparent even from the earliest studies that the pharmacology of SERMs was complex and that they were capable of exhibiting agonist, partial agonist or antagonist activities in different tissues [3,17–19]. One of the key experiments that shed light on this complexity was performed by Gottardis and Jordan in the late 1980s in which they showed in xenograft models of breast cancer that over time, tamoxifen "switched" from an antagonist to an agonist [20]. The ability of serially-passaged tumors to recognize tamoxifen as an agonist indicated that resistance was a cell intrinsic process and suggested that dissection of the mechanisms underlying this activity would be informative with respect to ER pharmacology. The first evidence supporting a role for coregulators in NR pharmacology came from genetic studies in which disruption of a transcriptional corepressor switched tamoxifen from an antagonist to an agonist when assessed using a reconstituted ERα responsive transcription system in yeast [21]. Soon thereafter, Onate et al. identified the first mammalian coregulator SRC-1, a protein which interacted directly with ERα (and other nuclear receptors) and increased its transcriptional activity [22]. It was subsequently shown that the relative agonist/antagonist activity of tamoxifen could be manipulated by increasing or decreasing the expression of SRC-1 within target cells [23]. This suggested that although tamoxifen induces a conformational change in ERα that dramatically reduces its ability to interact with coactivators, the impact of this disruptive conformational change can be overcome by increasing the cellular concentration of a specific coactivator. This, coupled with the fact that tamoxifen enables binding of the receptor to DNA and that it also increases ERα levels in cells, explains how this drug can induce significant activation of ER target genes [24]. It was further noted that elevated expression of SRC-1 and/or SRC-3 in breast tumors is associated with tamoxifen resistance and that the locus encoding SRC-3 is amplified in a large number of breast cancers [25–27]. However, even considering the role of coregulators, it remained unclear how the relative agonist/antagonist activities of different SERMs could be dramatically different within the same cell. The answer to this problem was revealed in studies which demonstrated that, contrary to the classical "binary on/off" models of ER action, the overall shape of the receptor was influenced by the nature of the ligand to which is was bound, and that this manifested in the differential presentation of protein–protein interaction surfaces on the receptor. Thus, as a consequence of their impact on ER structure, different ligands can facilitate the interaction of ER with different, functionally distinct, coregulators (Fig. 1). To date over 300 coregulators have been identified although the functions of only a few have been explored in detail. Definition of the specific roles of individual coregulators in ER pharmacology will inform the development of screens for ligands that facilitate the interaction/disengagement of specific coregulators involved in processes of interest.

4. The transition to mechanism-based discovery of clinically useful SERMs

The first clinical experience with raloxifene (keoxifene) was an unsuccessful attempt to identify agents that could be used to treat tamoxifen-resistant breast cancer [28]. The primary rationale at the time for this approach was that resistance to tamoxifen was thought to occur either as a consequence of ERα mutations that disrupted tamoxifen binding or was due to the production of an estrogenic metabolite of the drug within tumors. Initially, the failure of keoxifene as a breast cancer therapeutic was thought to reflect its unfavorable pharmaceutical properties. However, we now know from an abundance of structural studies that the overall