The unexpected science of estrogen receptor-β selective agonists: a new class of anti-inflammatory agents?

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In the nine years since the unexpected discovery of a second form of the estrogen receptor (ER), ERβ has been mentioned in about 2,800 literature citations. Such prolific research is testimony to interest in explaining its role in estrogen physiology as well as investigating its potential as a drug target. Our current understanding is that ERα, not ERβ, is responsible for mediating the effects of estrogens in “classic” model systems such as the reproductive tract and skeleton. The role of ERβ is still being defined, but profiling of ERβ selective agonists in several animal models of human disease indicates these compounds may have utility as novel anti-inflammatory agents. The challenge for the future is to elucidate their mechanism of action and determine the clinical relevance of the impressive preclinical observations.

Introduction

I was not at the 1996 Keystone Symposium on Nuclear Receptors where Jan-Åke Gustafsson unveiled the existence of a second estrogen receptor (ER), ERβ, but I do recall the excitement of the other scientists in our group who attended and heard the news.

There were two primary reasons for the stir around this newly discovered ER. First, it was unexpected and it was intriguing to speculate about which aspects of estrogen physiology were attributable to ERβ. In 1986 two groups cloned the first ER [Green et al., 1986; Greene et al., 1986], now called ERα, and in 1993 a knockout mouse was created that had the severe expected phenotype [Lubahn et al., 1993]. The major actions of estrogens seemed explained, so there was not a large effort to seek other ERs.

The second reason for the excitement was the prospect of developing new and medically useful compounds based on selective interaction with ERβ. At the time of ERβ’s discovery, estrogens were thought to positively influence almost all bodily systems and postmenopausal hormone therapy was prescribed for long-term use. These days, with the results from the Women’s Health Initiative, prescribing recommendations have changed, but it is important to realize the environment that existed at the time of ERβ’s discovery.

This perspective will describe our efforts in evaluating and developing ERβ as a drug target and highlight some lessons learned along the way (see Figure 1). Much of this article is based on a presentation given at the 2005 Keystone Symposium on Tissue Selective Nuclear Receptors.

Designing ERβ selective agonists: a significant medicinal chemistry challenge

The amino acid sequence of the ERβ ligand binding domain is about 60% identical to that of ERα [Kuiper et al., 1996]. Thus, a 40% difference seemed sufficient to allow for the design of selective small molecules. However, among the amino acids that are close to the ligand in the binding pocket, there are only two conservative amino acid differences between ERα and ERβ. While this finding initially discouraged the team, it ultimately helped focus our synthetic chemistry efforts on the regions of the pharmacophore best positioned to make preferential interactions with ERβ [Manas et al., 2004]. A number of ERβ selective agonists have been synthesized (by our group and others; see [Veeneman, 2005] for review), thus highlighting the ability to capitalize even on very subtle sequence differences to gain selectivity.

Once significantly ERβ selective compounds were synthesized, they were used as tools to probe ERβ function. Interpretation of these studies was greatly aided by the use of an ERα selective agonist, PPT [Harris et al., 2002; Stauffer et al., 2000], which was designed by John Katzenellenbogen’s group.

ERβ selective agonists are not classic estrogens (both good and bad news)

From a drug discovery point of view, a key attribute for ERβ was that it was not highly expressed in the uterus. Happily, we found that reasonably selective agonists (>~50-fold) were nonuterotrophic. We were also pleased to find that our lead compounds did not stimulate
mammary end bud development. However, we also discovered that ERβ selective agonists did not inhibit ovulation, prevent ovariectomy-induced vasomotor instability or bone loss [Harris et al., 2003]. PPT, the ERα selective agonist was as efficacious as estradiol on many of these endpoints, suggesting that ERα stimulation is both necessary and sufficient for the “classic” effects of estrogens [Harris et al., 2002]. Thus, although these data predict lower clinical liability for an ERβ selective agonist, they did not point us in the direction of potential indications.

Looking beyond menopause and contraception for an ERβ effect

Based on the data mentioned above, we quickly abandoned looking for ERβ utility in the area of women’s health and instead turned our attention to systems where nonselective estrogens (e.g. 17β-estradiol) had effects. This led us to examine the activity of our ERβ compounds in inflammatory diseases. Several disease models have been examined and four of these are summarized below.

Inflammatory bowel disease and arthritis

The HLA-B27 transgenic rat is a model of chronic inflammation and develops several age-dependent phenotypes. When given orally, both ERB-041 [Harris et al., 2003] and WAY-202196 [Mewshaw et al., 2005] rapidly reversed the chronic diarrhea these rats develop after about 10 weeks of age. Concomitant improvements were seen in intestinal histology with a reduction in lesions as well as inflammatory cell infiltrates, and a return of goblet cells. The minimum fully efficacious dose for these compounds was ≤1mg/kg.

The HLA-B27 transgenic rats also develop arthropathy as they age, and ERβ selective agonists prevented [Mewshaw et al., 2005] and reversed (unpublished observations) this joint swelling. This observation prompted examination of compounds in a second model of arthritis, Lewis rat adjuvant-induced arthritis. In this model, arthritis is induced 8-9 days after an intradermal injection of complete Freund’s adjuvant. Both ERB-041 and WAY-202196 rapidly reduced joint redness and swelling, as well as synovitis and Mankin scores (scores of histological joint damage). Furthermore, ERB-041 substantially normalized the majority of disease-responsive mRNA changes in the liver, popliteal lymph node and spleen, as well as those in the plasma proteome [Follettie et al., 2006].

Endometriosis

Although it is counterintuitive to evaluate an estrogen receptor agonist in an estrogen dependent disease, we tested ERB-041 in an in vivo model of experimentally induced endometriosis because endometriosis is an
inflammatory disease. Endometrial biopsies from normal volunteers can establish endometrosis-like lesions when implanted into nude mice [Grummer et al., 2001]. When administered after lesions have established, ERβ-041 (10mg/kg) caused complete lesion regression in 40-75% of the animals, depending on the study [Harris et al., 2005]. Recovered lesions expressed ERα and no detectable ERβ (regardless of the treatment the animal received), thus ERβ-041 would appear to act on the host, not on the implanted tissue. Although nude mice lack T cells, they have functional macrophages and natural killer cells and thus ERβ-041 may be stimulating the ability of these cells to recognize the implanted tissue as foreign and to clear it.

Sepsis
The logic for testing ERβ selective compounds in models of systemic infection was that, because these compounds were likely immunomodulators, we wanted to assess whether they would be globally immunosuppressive [Cristofaro et al., In Press]. It should be noted that we tested high doses of compound because we were looking for a potential deleterious effect. One model used intact female rats rendered neutropenic and then given an oral bolus of Pseudomonas aeruginosa. To our surprise, rats treated orally with WAY-202196 (50mg/kg) on days 4-11 after Pseudomonas inoculation survived longer than rats treated with vehicle (83% vs 25%). Moreover, intestinal histology was significantly improved.

The second model of sepsis used controlled puncture of the cecum in intact female mice to induce acute bacterial peritonitis. Again, daily oral doses of WAY-202196 (50mg/kg) improved survival over mice treated with vehicle (78% vs 0%) and intestinal histology was improved.

Future directions
We have made great strides in developing tools to evaluate ERβ function in vivo and have uncovered an impressive array of activities in animal models of human disease. Some of this testing was directed, but other discoveries were made purely by serendipity. Currently we are hampered by our lack of a mechanistic understanding of how these compounds work. Defining mechanism is difficult for several reasons. First, there are a wide variety of potential target cells. Many types of immune system cells express ERβ, as do cells of the intestine and joint. Thus it is difficult to decide which cell type to study in depth. Secondly, we have been unable to translate our impressive in vivo activities into in vitro systems. For example, our compounds do not influence T cell proliferation in standard assays nor do they prevent monocyte activation after stimulation with lectins, etc. Thirdly, each in vivo activity may be explained by a different mechanism; after all, ERβ is a transcription factor. Finally, ERβ compounds seem to have minimal, if any, effects in normal healthy animals. This has led to postulation of a “challenge hypothesis”, which says that a target cell or organ needs to be injured/stressed or otherwise compromised in order to respond to ERβ. While this may be convenient therapeutically, it poses a significant scientific challenge to determine mechanism if one is restricted to using complex in vivo models.

It is also important to be aware that, although a number of selective ERβ compounds have been designed, not many have been widely tested in vivo. Moreover, since only DPN [Meyers et al., 2001] is commercially available, confirmation of published data by other groups is problematic. Although our group’s ERβ selective compounds from several different chemical series seem to behave similarly and seem to be full agonists, they may not elicit the full range of ERβ biology in vivo. Many other scientists, both at academic and industrial institutions, are exploring biological activities other than those reported here (e.g. prostate proliferation, vasomotor instability [Opas et al., 2006], anxiety/depression [Lund et al., 2005; Wall and Frye, 2005], and subfertility [Hegele-Hartung et al., 2004]). As a field, we need data from a variety of sources to piece together the full spectrum of ERβ activity.

The discovery of ERβ came at a time when powerful scientific tools were readily available to try and understand its contribution to estrogen physiology. Engineered mice, structure-based drug design, and microarray analysis were just a few of the state-of-the-art techniques that were immediately brought to bear on this problem. And while significant progress has been made, and indeed compounds have been advanced into clinical trials to see if data gathered from preclinical models will translate into human disease, we are really just at the beginning of understanding how this receptor functions.

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