ELSEVIER

Molecular and Cellular Endocrinology 287 (2008) 40–46

MCE
Molecular and Cellular Endocrinology

www.elsevier.com/locate/mce

Activity of three selective estrogen receptor modulators on hormone-dependent responses in the mouse uterus and mammary gland

Judy S. Crabtree [a,*], Bryan J. Peano [a], Xiaochun Zhang [a], Barry S. Komm [b], Richard C. Winneker [a], Heather A. Harris [a]

[a] *Endocrinology & Reproductive Disorders Division, Women's Health and Musculoskeletal Biology, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, United States*
[b] *Osteoporosis Division, Women's Health and Musculoskeletal Biology, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, United States*

Received 30 August 2007; received in revised form 18 January 2008; accepted 22 January 2008

Abstract

Selective estrogen receptor modulators (SERMs) have the unique potential to provide estrogenic effects in the skeletal and cardiovascular system, while minimizing/eliminating side effects on reproductive organs. However, despite the unifying characteristic of mixed estrogen receptor (ER) agonist/antagonist activity, compounds within this class are not interchangeable. In order to define and compare the effects of SERMs on different hormone-responsive tissues, we evaluated effects of bazedoxifene acetate (BZA), lasofoxifene (LAS) and raloxifene (RAL) in the mammary gland and uterus of the ovariectomized mouse. Endpoints measured included those regulated by estradiol alone (uterine wet weight, uterine G protein-coupled receptor 105 (GPR105) mRNA expression and mammary gland indoleamine-pyrrole 2,3 dioxygenase (INDO) mRNA expression) as well as others that required the combination of estradiol and progesterone (uterine serine protease inhibitor Kazal type 3 (Spink3) mRNA expression, mammary gland morphology and mammary gland defensin β1 (Defβ1) mRNA expression). The three SERMs tested had variable agonist and antagonist activity on these endpoints. In the uterus, the SERMs were mixed agonists/antagonists on estradiol-induced wet weight increase, whereas all three SERMs were estrogen receptor antagonists on GPR105 mRNA expression. However, in the presence of progesterone, BZA and RAL were agonists on Spink3 expression, while LAS was primarily an antagonist. In the mammary gland, BZA and RAL were predominantly agonists on the endpoint of mammary morphology and all three SERMs were clear agonists on Defβ1 mRNA expression, an E + P-dependent marker. Finally, LAS and RAL had mixed agonist/antagonist activity on INDO mRNA expression, while BZA had only antagonist activity. These results demonstrate that compounds with small structural differences can elicit distinct biological responses, and that in general, SERMs tended to behave more as antagonists on endpoints requiring estrogen alone and agonists on endpoints requiring the combination of estrogen and progesterone.

Keywords: Selective estrogen receptor modulator; Estradiol; Progesterone; Bazedoxifene; Raloxifene; Lasofoxifene

1. Introduction

Estradiol (E2) is a hormone that plays a key role in reproduction and in the homeostasis of a variety of mammalian systems. When estrogen levels decline at menopause, many women experience vasomotor instability and vaginal atrophy, and are at increased risk for developing osteoporosis and heart disease. Hormone therapy can address many of these consequences of reduced ovarian productivity, but not all women are candidates for these medications.

Classically, estrogens act via binding to intracellular estrogen receptors (ER), two of which have been described (ERα and ERβ). Upon binding hormone, these receptors modulate transcription of target genes. Known ER ligands encompass a large class of steroidal and non-steroidal compounds (Anstead et al., 1997), and within this group is a subset of ligands called SERMs that can mimic the effects of E2 in some situations and block its effects in others. The mechanisms responsible for this activity are poorly understood but known to be cell type and gene promoter dependent (Miller, 2002). The cell-type dependent action of this class of compounds is clinically useful. For example, tamoxifen, a triphenylethylene, was the first SERM that was originally identified for use in breast cancer due to strong antiestrogenic activity in the mammary gland but was later discovered

* Corresponding author. Tel.: +1 484 865 2419; fax: +1 484 865 9389.
E-mail address: crabtrjs@wyeth.com (J.S. Crabtree).

0303-7207/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2008.01.027

to prevent bone mineral density loss (Jordan et al., 1987). Tamoxifen also has significant uterotropic activity, and its clinical use is associated with an increased incidence of uterine cancer (Bernstein et al., 1999; Fisher et al., 1998; van Leeuwen et al., 1994). In rats, lasofoxifene (LAS), a tetrahydronaphthalene, has been shown to prevent bone loss with no effect on the uterine epithelium (Ke et al., 2004, 1998, 2000), and in an *N*-methylurea-induced model of rat mammary carcinoma, LAS demonstrated chemopreventive and therapeutic potential (Cohen et al., 2001). Raloxifene (RAL), a benzothiophene-based SERM, also has antiestrogenic activity in the mammary gland and maintains bone mineral density in the postmenopausal population without introducing the uterotrophic liability seen with tamoxifen (Delmas et al., 1997). Preclinically, RAL caused regression of mammary gland development in intact rats and antagonized estrogen-stimulated mammary gland proliferation in ovariectomized (OVX) rats (Buelke-Sam et al., 1998). A more recently developed SERM is bazedoxifene acetate (BZA). Like other SERMs, BZA preserves bone mineral density and biomechanical parameters in a rodent model of osteopenia, has no effect on uterine epithelium and antagonizes E2-dependent MCF-7 breast cancer cell proliferation *in vitro* (Komm et al., 2005).

In mammals, the development of the mammary gland and uterus is dependent on various hormonal contributions. Estrogens and progestins are well-established modulators of reproductive function in the uterus and play significant roles during pregnancy and parturition. In the mammary gland, these hormones support the development of a branched ductal system and secretory lobuloalveoli during pregnancy. Specifically, estrogens facilitate ductal elongation into the fat pad, whereas progestins enable ductal side branching and morphogenesis of the lobuloalveoli (Silberstein, 2001).

We previously described development of a short-term *in vivo* mouse model to measure uterine and mammary gland responses to estrogens and progestins using both gross tissue responses as well as molecular markers (Crabtree et al., 2006). In this report, we describe characterization of three SERMs (BZA, LAS and RAL) in this model and examine their effect on endpoints dependent on the co-administration of E2 and progesterone (P) in addition to those requiring E2 alone.

2. Materials and methods

2.1. Animal care and treatment regimen

Seven-week old c57BL/6 female mice were ovariectomized, rested for 1 week prior to treatment and dosed as previously described with 6 mice/group (Crabtree et al., 2006). Briefly, E2 and/or SERMs were administered on all 7 days, whereas co-treatment with P occurred only on days 4–7. As relevant, vehicle was administered in control groups and in groups receiving only SERM, SERM + E2 or SERM + P. E2 (Sigma, St. Louis, MA; 1 μg/kg) and P (Sigma; 30 mg/kg) were delivered by subcutaneous injection in a vehicle of 50% DMSO/50% 1× Dulbecco's phosphate buffered saline (DMSO/saline). BZA, LAS and RAL (3 mg/kg) were obtained from the Wyeth compound library and were administered by oral gavage in a vehicle of 2% Tween-80/0.5% methylcellulose. Eight hours after administration of the last dose, animals were euthanized by CO_2 asphyxiation and pneumothorax prior to tissue removal. One inguinal mammary gland was removed and frozen on dry ice for RNA preparation. The other inguinal mammary gland was used for morphological analysis according to published procedures (Rasmussen et al., 2000) except that the fixative was ethanol:acetic acid (6:1). Uteri were removed, weighed, and placed in RNALater (Qiagen; Valencia, CA) for RNA preparation. All animal studies were approved by the Institutional Animal Care and Use Committee of Wyeth Research, Collegeville, PA and were conducted in accordance with Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines.

2.2. RNA preparation/quantitative real-time PCR

Total RNA was prepared and analyzed individually from each organ. For the mammary gland, each sample was homogenized in 2 mL of QIAzol lysis reagent (Qiagen) for 15–25 s using a Polytron homogenizer PT3100 (Brinkmann, Westbury, NY). For the uterus, each sample was disrupted with 1 mL of Trizol (Invitrogen, Carlsbad, CA) using a 5 mM bead in a Tissuelyser (Qiagen) for 3–4 min. After 0.2 mL chloroform extraction of 1 mL homogenate and centrifugation at 4 °C for 15 min, about 0.5 mL aqueous phase was collected. The RNA from the aqueous phase was then purified using Qiagen RNeasy Lipid Tissue Mini prep kits (mammary gland) or Qiagen RNeasy Mini prep kits (uterus) according to the manufacturer's protocol. The genomic DNA in RNA samples was removed by on-column RNase-free DNase treatment during RNA purification.

RNA (100 ng) from each individual organ was used as template in TaqMan one-step RT-PCR reactions using MultiScribe reverse transcriptase (Applied Biosystems) according to manufacturer's instructions. Messenger RNA expression was analyzed using real-time quantitative-PCR using previously identified markers of mammary gland or uterine proliferation including Defensin β1 (Defβ1) and indoleamine-pyrrole 2,3 dioxygenase (INDO) in the mammary gland, and Serine protease inhibitor, Kazal type 3 (Spink3) and G protein-coupled receptor 105 (GPR105) in the uterus (Crabtree et al., 2006). Fragments were detected on an ABI PRISM 7700 Sequence Detection System according to the manufacturer's protocol (Applied Biosystems Inc., Foster City, CA). To compare mRNA expression levels between samples, target mRNA expression was normalized to 18S mRNA expression using primers and labeled probes from an Applied Biosystems TaqMan ribosomal RNA control reagent kit (VIC probe) for 18S mRNA detection.

2.3. Statistics

Results were analyzed using SAS/Excel. A one-way ANOVA using least significant differences was calculated to evaluate statistical significance between groups with a p value of ≤ 0.05 considered significant. Error bars on all graphs are presented as standard error of mean.

3. Results

3.1. Effect of SERMs on uterine wet weight

One of the hallmarks of an ER agonist effect on the uterus is an increase in uterine wet weight. Thus, we evaluated BZA, LAS and RAL on this endpoint in agonist and antagonist modes. As seen in Fig. 1, all SERMs increased uterine wet weight compared to vehicle when dosed alone at 3 mg/kg. The slight increase in uterine weight with SERMs was not unexpected given some previously published reports from studies done in rodents with RAL and LAS (Onoe et al., 2000; Wang et al., 2006). This ER agonist effect was most prominent with LAS treatment, whereas BZA had the least agonist activity on this endpoint (44% increase vs. 217% for LAS and 79% for RAL). When co-dosed with E2, all three SERMs reduced the E2-mediated increase in uterine wet weight to that seen when the compound was dosed alone. Thus, on uterine wet weight, the SERMs have mixed agonist/antagonist activity with the rank