Molecular and Cellular Endocrinology 440 (2017) 34–43

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Effects of raloxifene against letrozole-induced bone loss in chemically-induced model of menopause in mice

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ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form
10 October 2016
Accepted 5 November 2016
Available online 8 November 2016

Keywords:
Letrozole
Raloxifene
VCD
Micro CT
Bone turnover markers
Sclerostin and DKK-1

ABSTRACT

Introduction: The deleterious effects of letrozole, an aromatase inhibitor, used in the adjuvant treatment of breast cancer in postmenopausal women, on bone are well-documented and represent a major drawback to its clinical use. Raloxifene, a selective estrogen receptor modulator and a clinically approved anti-osteoporotic drug, has been recently demonstrated to be efficacious in women with breast cancer. The present study evaluated the effects of preventive and curative treatment with raloxifene on letrozole-induced alterations of bone microarchitecture and turnover markers in a chemically-induced menopause model in mice.

Method: Swiss strain albino female mice were made menopausal by inducing ovotoxicity using vinyl cyclohexene di epoxide (VCD, 160 mg/kg for 15 days followed by 30 days drug-free period) confirmed by ovarian histology and serum estradiol levels. Effects on femoral and lumbar bones were evaluated by micro CT determination of bone volume, trabecular number, separation, thickness, connective density and trabecular pattern factor and bone turnover markers including ALP, TRAP5b, hydroxyproline and RANKL. In addition to these, markers of Wnt signaling (sclerostin and dickkopf-1) were also evaluated. To rule out the involvement of pharmacokinetic interaction, plasma levels of letrozole and raloxifene were measured following drugs alone and in combination.

Results: Though bone loss was observed in VCD treated mice (as indicated by micro CT measurements), it was further enhanced with letrozole administration (1 mg/kg) for one month particularly in epiphysis of femoral bones. Raloxifene (15 mg/kg), whether administered concurrently or post-letrozole was able to revert the structural alterations and changes in turnover markers caused by letrozole to varying degrees ($p < 0.01$ or $p < 0.001$). Further, estrogen deficiency following letrozole treatment in ovotoxic mice was associated with significant increase in sclerostin and dickkopf-1 in both lumbar and femur bones ($p < 0.001$) which was attenuated with preventive and curative treatment with raloxifene ($p < 0.05$). The plasma levels of letrozole remained unaffected by raloxifene administration and vice versa.

Conclusions: Our study indicates the potential of raloxifene in preventing and attenuating letrozole-induced bone loss. Further, these effects were found to be independent of a pharmacokinetic interaction between the two drugs.

1. Background

The third generation aromatase inhibitors have recently emerged as a standard adjuvant endocrine treatment for estrogen receptor positive (ER +ve) breast cancer in postmenopausal women (Lonning et al., 2000). They mainly act by inhibiting the aromatization process (peripheral conversion of estrogen from androgen precursors) and are effective in minimizing the tissue and circulating estrogen levels (Osborne and Schiff, 2005; Winer et al., 2005). Letrozole is a non-steroidal competitive inhibitor of aromatase and has been reported to have improved efficacy and tolerability as compared to tamoxifen in the treatment of early breast cancer (Mccloskey et al., 2007). Despite the advantages, clinical superiority of letrozole over tamoxifen in breast cancer patients is greatly limited by its deleterious effects on bone (Mouridsen et al., 2001, 2003). It has been reported that

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http://dx.doi.org/10.1016/j.mce.2016.11.005
0303-7207/

postmenopausal women with breast cancer receiving aromatase inhibitors experience a rate of bone loss estimated at 2.6% per year (Perez and Weilbaecher, 2006). Since postmenopausal women are at an increased risk of osteoporosis and fracture (Riggs and Melton, 1995), the use of letrozole in the adjuvant treatment of postmenopausal breast cancer patients requires appropriate consideration of fracture risk and management of long-term bone health.

Raloxifene, a selective estrogen receptor modulator (SERM), has been approved for the prevention and treatment of postmenopausal osteoporosis (Ott et al., 2002). It has specific affinity to ERα and ERβ of estrogen receptors and show tissue specific estrogen receptor agonistic or antagonist activity (Martinkovich et al., 2014). While it has estrogen agonistic effects on bone, serum lipids, arterial vasculature, it has antagonistic effects in breast and uterus (Thiebaud and Secrest, 2001). It is interesting to note that raloxifene was found to be equally efficacious as tamoxifen in prevention of breast cancer risk in postmenopausal woman in the STAR (Study of Tamoxifen & Raloxifene) trial (Vogel, 2009). Further, it was also demonstrated to reduce the invasive breast cancer in Japanese women (Ko and Jordan, 2011). However, since tamoxifen is associated with endometrial carcinoma due to its partial agonistic action (an effect that raloxifene doesn't have), it would be worthwhile to evaluate the combination of raloxifene with letrozole for possible prevention of letrozole-induced bony adverse effects. The present study has, therefore, been designed to investigate the effect of raloxifene on letrozole-induced alterations of bone microarchitecture and turnover markers in a chemically-induced menopause model in mice.

2. Materials and methods

2.1. Experimental animals

Swiss strains of adult female albino mice of 30–35 g and 40–45 days old were used. Animals were housed in a room maintained at 25 °C in 12:12 h light/dark cycles. Standard laboratory rodent chow diet and water was provided *ad libitum*. Animals were randomly assigned into various groups (6 animals per group). The study was conducted strictly in accordance with the in-house guidelines of Institutional animal ethics committee of Jamia Hamdard, New Delhi (Protocol No-1004).

2.2. Drugs and doses

The following drugs were used: Vinyl-1-cyclohexene diepoxide (VCD, Sigma-Aldrich, India); Letrozole (Ind-Swift laboratories, Chandigarh India) and Raloxifene (Dr. Reddy's Laboratories, Hyderabad, India). All other chemicals and reagents used in this study were of analytical grade. Dose of letrozole (1 mg/kg) was selected from the previous pre-clinical study performed by Yonden and co-workers (Yonden et al., 2009) while the dose of raloxifene was translated from human dose of 120 mg/kg to 15 mg/kg for mice and previously standardized in our laboratory by Anwar and co-workers (Anwar et al., 2014).

2.3. Induction of ovotoxicity and letrozole treatment

Animals were made ovotoxic by the treatment of VCD 160 mg/kg for 15 days followed by 30 days drug-free period following which letrozole was given for one month. Different researchers have used various doses of VCD like 160 or 80 mg/kg (Hooser et al., 1995; Kao et al., 1999). In our laboratory, 160 mg/kg dose was standardized by Pottoo and co-workers (Pottoo et al., 2014) to induce ovotoxicity in Swiss albino mice.

2.4. Preventive and curative treatment

Preventive treatment with raloxifene was given at the time of letrozole administration for the same period of one month. Control group (0.5% CMC, 2 mg/kg); VCD (160 mg/kg); VCD + L (160 mg/kg+1 mg/kg); VLR {160 mg/kg+ (1 mg/kg+15 mg/kg)}; VR (160 mg/kg+15 mg/kg). Curative (therapeutic treatment) with raloxifene was given for one month after one month treatment with letrozole. Control group (0.5% CMC, 2 mg/kg); VCD (160 mg/kg); VL (160 mg/kg+1 mg/kg); VLR (160 mg/kg+1 mg/kg+15 mg/kg); VR (160 mg/kg+15 mg/kg). At the end of the treatment schedule, blood was collected by retro-orbital route following which all animals were sacrificed; femur and lumbar vertebrae were harvested and analyzed. The age of animals at the end of the experimental schedule were 115–120 days for preventive group and 145–150 days for curative group. A schematic representation of the dosing schedule is presented in Fig. 2.

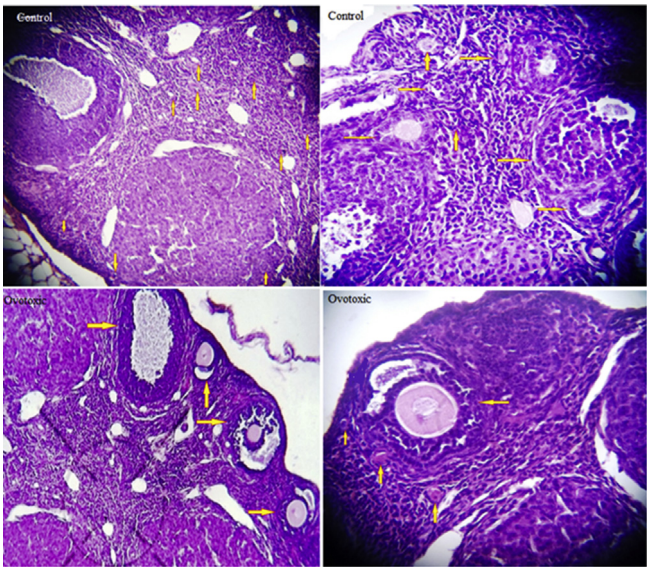

Fig. 1. Ovarian histology of Swiss albino female mice treated with sesame oil as vehicle (Control Group) and 15 days VCD treatment followed by a drug-free period of 30 days (Ovotoxic group).

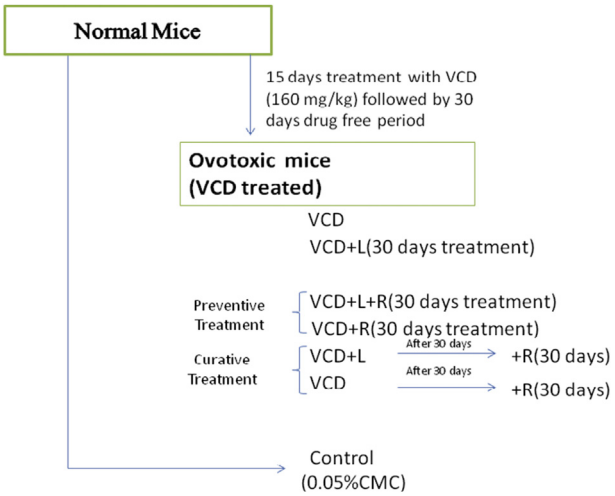

Fig. 2. Schematic depiction of dosing schedule. VCD (ovotoxic group), L-letrozole, R-raloxifene.