Original article

MOLECULAR METABOLISM

Tissue-selective estrogen complexes with bazedoxifene prevent metabolic dysfunction in female mice

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ABSTRACT

Pairing the selective estrogen receptor modulator bazedoxifene (BZA) with estrogen as a tissue-selective estrogen complex (TSEC) is a novel menopausal therapy. We investigated estrogen, BZA and TSEC effects in preventing diabetisity in ovariectomized mice during high-fat feeding. Estrogen, BZA or TSEC prevented fat accumulation in adipose tissue, liver and skeletal muscle, and improved insulin resistance and glucose intolerance without stimulating uterine growth. Estrogen, BZA and TSEC improved energy homeostasis by increasing lipid oxidation and energy expenditure, and promoted insulin action by enhancing insulin-stimulated glucose disposal and suppressing hepatic glucose production. While estrogen improved metabolic homeostasis, at least partially, by increasing hepatic production of FGF21, BZA increased hepatic expression of Sirtuin1, PPARα and AMPK activity. The metabolic benefits of BZA were lost in estrogen receptor-α deficient mice. Thus, BZA alone or in TSEC produces metabolic signals of fasting and caloric restriction and improves energy and glucose homeostasis in female mice.

Keywords Tissue-selective estrogen complexes; Bazedoxifene; Menopause; Metabolic syndrome; Insulin resistance; Type 2 diabetes

1. INTRODUCTION

With the dramatic increase in life expectancy, many women will spend a large part of their lives in a post-menopausal state. Apart from causing degeneration of the cardiovascular, skeletal and central nervous systems [1,2], estrogen deficiency also increases risk for metabolic syndrome and type 2 diabetes [3]. Overall, the role of estrogen deficiency to the pathophysiology of chronic diseases in women is emerging as a novel therapeutic challenge that parallels the increased risk associated with conventional estrogen replacement protocols [4]. It is against this backdrop that novel therapeutic strategies targeting estrogen receptor in non-reproductive tissues may offer therapeutic benefits that reduce metabolic dysfunction associated with both ovarian failure and estrogen deficiency.

Selective estrogen receptor modulators (SERMs) are compounds that exert tissue-selective estrogen receptor (ER) agonist or antagonist activity. For example, bazedoxifene (BZA) is a novel SERM that exhibits estrogen agonist activity in bone but estrogen antagonist activity in breast and uterus [5,6]. Tissue-selective estrogen complexes (TSECs) are drugs in which a SERM and an estrogen are combined to produce mixed estrogen agonist and antagonist activity [7]. The goal of a TSEC regimen containing BZA with conjugated equine estrogens (CE) is to provide the benefits of estrogen such as reducing hot flashes and vulvar–vaginal atrophy [8–10], preventing menopausal osteoporosis [11,12] and promoting favourable effects on cardiovascular risk while simultaneously protecting the endometrium and breast from estrogen stimulation without the need for a progestin [7,13]. Current evidence

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Abbreviations: Akt, protein kinase B; AMPKα, AMP-activated protein kinase α; AUC, area-under the curve; BAT, brown adipose tissue; BZA, bazedoxifene; CE, conjugated equine estrogens; E2, 17β-estradiol; ER, estrogen receptor; FAS, fatty acid synthase; FGF21, fibroblast growth factor 21; GIR, glucose infusion rate; H&E, hematoxylin and eosin; HFD, high-fat diet; HGP, hepatic glucose production; ITT, insulin tolerance test; Lcn2, lipocalin 2; LPL, lipoprotein lipase; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; OVX, ovariectomy; PTT, pyruvate tolerance test; RBP4, retinol binding protein 4; Rd, rate of whole-body glucose disappearance; RER, respiratory exchange ratio; SERM, selective estrogen receptor modulator; TBARS, thiobarbituric acid reactive substances; TG, triacylglycerol; TSEC, tissue-selective estrogen complex; UCPs, uncoupling proteins; VO_2, oxygen consumption; WAT, white adipose tissue.

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indicates that TSEC therapy consisting of the combination of CE and BZA [Duavee (TM)] [14] is an effective alternative to conventional hormone therapy for treatment of postmenopausal symptoms and prevention of osteoporosis [15,16]. However, there is currently no information on the efficacy of TSEC with BZA in preventing postmenopausal metabolic disorders. In a series of experiments that replicated postmenopausal metabolic derangements observed in older women, we investigated the effect of BZA paired with estrogens [CE or 17β-estradiol (E2)] on glucose and energy homeostasis in ovariectomized (OVX) mice fed a high-fat diet (HFD).

2. MATERIALS AND METHODS

2.1. Animals and surgery

Female C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine), 7 weeks of age, were housed with a 12-h light–dark cycle. After a 1-week acclimation period, mice were randomly divided into seven treatment groups as follows: (1) sham vehicle; (2) OVX + vehicle; (3) OVX + CE; (4) OVX + E2; (5) OVX + BZA; (6) OVX + CE + BZA; (7) OVX + E2 + BZA. Mice were subjected to bilateral OVX or sham operation under anesthesia with 1.2% Avertin solution (i.p.). Treatment with CE, BZA, TSEC or vehicle was initiated on the day of surgery. In four separate studies, all compounds were administered orally to mice once daily for 8 weeks with vehicle (saline, 2% Tween 80, 0.5% methylcellulose), CE 2.5 mg/kg, BZA 3 mg/kg or CE 2.5 mg + BZA 3 mg/kg in saline. The dosages or concentrations of CE and BZA administered provided for the optimal maintenance of estrogen action in absence of uterine growth [11,13]. During the ovariectomy procedure, a 90-day release E2 pellet (0.5 mg/pellet, Innovation Research of America, Sarasota, FL) was implanted subcutaneously on the dorsal aspect of the neck of each animal. All mice were provided phytoestrogen-free HFD (TD04059, 52% Kcal from anhydrous milk fat, Harland Teklad, Madison, WI) and water ad libitum during the experimental period. At the end of the study (day 29 or day 57), mice were euthanized by an overdose of Avertin, and blood was collected by cardiac puncture. Mice with a null mutation of the estrogen receptor α (ER$\alpha -/-$) were generated as previously described [17]. All animal work was performed in compliance with the Institutional Animal Care and Use Committee at Northwestern University.

2.2. Biochemical assays

Following euthanasia, serum was separated by centrifugation at 3000*g* for 20 min at 4 °C and used for determination leptin, adiponectin, RBP4, Lcn2 and FGF21 using commercial ELISA kits, RBP4 (Abnova Co., Walnut, CA), Lcn2 (R&D Systems Inc., Minneapolis, MN), leptin, adiponectin and FGF21 (Millipore Co., Billerica, MA) as specified by the manufacturer. TBARS was measured in serum using a commercial kit (ZeptoMetrix Co., Buffalo, NY). For hepatic TG measurement, tissue saponification in ethanolic KOH and neutralization with $MgCl_2$ were performed as previously described [18]. Glycerol content was determined by enzymatic colorimetric methods using a commercially available kit (Sigma-Aldrich, St. Louis, MO). For the measurements of phosphorylated (T172) AMP-activated protein kinase α (AMPKα) in liver and muscle, total protein were extracted from liver and gastrocnemius muscle using tissue extraction reagent I (Invitrogen, Camarillo, CA). AMPKα [pT172] was measured by an ELISA kit (Invitrogen) as specified by the manufacturer. Insulin-stimulated Akt activity was measured in lysates from liver and muscle collected following the clamp study using western blotting of Akt phosphorylation (S473) (Cell Signaling) and expression (Cell Signaling).

2.3. Histological staining

Sections of parametrial adipose tissue and liver were fixed in 10% formalin, embedded in paraffin, sectioned and stained with H&E. Adipocyte area was traced and quantified in 300 cells per mouse using ImageJ software (National Institute of Health, NIH Version v1.32j). The relative adipocyte number was calculated by dividing parametrial fat pad weight by the mean adipocyte size in each mouse ($n = 4$/group) as described [19].

2.4. Studies of energy homeostasis

Food intake, locomotor activity and energy expenditure were analyzed using an indirect-calorimetric system (Labmaster, TSE Systems, Bad Homburg, Germany) in the mouse metabolic phenotyping core at Northwestern University. Mice were subjected to bilateral OVX surgery and received daily drug treatments with vehicle, CE, BZA or CE + BZA as described above for 4 weeks. Mice were then individually placed in air-tight respiratory chambers at a constant temperature (24.0 ± 0.5 °C) and were acclimatized to the metabolic cages for 3 days before measurements. Energy expenditure was measured by indirect calorimetry, while locomotor activity was assessed using an infrared light beam detection system for horizontal and vertical activity. Data were collected every 30 min and averaged over the 3-day period. Energy expenditure was calculated from O_2 consumption and CO_2 production and normalized to fat-free mass. Oral drug treatments were performed at 10:00 am every day during metabolic caging.

2.5. Real-time fluorescent Q-PCR

Perigonadal WAT, BAT, liver and skeletal muscle tissues were homogenized in 1 mL of TRIzol reagent and then total RNA was isolated. Total RNA was reverse transcribed to cDNA using a iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). cDNA was used as a template for the relative quantitation for the selected target genes with predesigned TaqMan gene expression assay kits. Each 20 μL reaction contained 100 ng cDNA, 2 × TaqMan Fast Advanced Mastermix (Applied Biosystems, Carlsbad, CA), forward and reverse primers and TaqMan probe. All reactions were carried out in triplicate with the LightCycler® 480 II Real-Time PCR System (Roche, Mannheim, Germany) using the following conditions: 50 °C for 2 min and 95 °C for 20 s followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. Results were expressed as a relative value after normalization to 18S rRNA.

2.6. FAS enzymatic activity

FAS activity was measured by the incorporation of radiolabeled malonyl-CoA into palmitate as described previously [20]. Briefly, 60–100 mg of liver tissue was homogenized in buffer (20 mM Tris, pH 7.5; 1.0 mM EDTA; 1.0 mM DTT; and phosphatase and protease inhibitors) and centrifuged at 12,000*g* for 30 min at 4 °C. The supernatant was incubated for 20 min at 37 °C with 166.6 μM acetyl-CoA, 100 mM potassium phosphate (pH 6.6), 0.1 μCi [14C] malonyl-CoA, and 25 nM malonyl-CoA in the absence or presence of 500 μM NADPH. The reaction was stopped with 1:1 chloroform/methanol solution, mixed for 30 min at 20 °C, and centrifuged at 12,500*g* for 30 min. The supernatant was vacuum-dried, and the pellet was resuspended in 200 μL water-saturated butanol. After addition of 200 μL ddH_2O, vortexing, and spinning for 1 min, the upper layer was removed for re-extraction. The butanol layer was dried and counted. Protein was quantified by Bio-Rad

