The Effects of a Novel Selective Estrogen Receptor Modulator (pERD) on Bone Health in Intact Female Rats

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Introduction
Selective estrogen receptor modulators (SERMs) are a diverse class of compounds that bind estrogen receptors (ERs) and antagonize or agonize estrogen action in different tissue types.1 Due to a broad spectrum of physiological and pathological processes contributed by ERα, SERMs provide a potential therapeutic benefit for a variety of diseases including cancer, cardiovascular, metabolic and cognitive diseases, and postmenopausal osteoporosis. In a long-term treatment of postmenopausal osteoporosis, SERMs provide a therapeutic potential with less side effects than observed in a traditional hormone replacement therapy.2,3 In preclinical studies, the bone specific actions of SERMs can be studied under physiological estrogen levels in healthy animals and under estrogen deficiency in ovariectomized (OVX) animals.

Aim of the Study
The aim of this study was to characterize the effects of a novel partial estrogen receptor antagonist (pERD) on bone health under physiological estrogen levels in intact female rats. The following reference groups were included in the study: OVX rats and rats treated with a complete antagonistic fulvestrant (FUL) as well as rats treated with SERMs exhibiting agonist activity on bone, namely raloxifene (RAL) and tamoxifen (TAM).

Materials and Methods
Animals
Twenty 10-week-old female Sprague-Dawley rats were obtained from Animal House of University of Turku (Turku, Finland). Rats were kept in a 12-h light/dark cycle with free access to water (26°C) and food pellets (Purina 5001; Purina Mills Inc., St. Louis, MO). After 1 week, rats were randomly assigned to 4 groups (5 rats per group): intact controls, intact rats treated with tamoxifen (TAM), intact rats treated with raloxifene (RAL), and intact rats treated with the pERD (pERD). All animals were housed in individual cages and received standard rat chow (Purina 5001; Purina Mills Inc., St. Louis, MO) and water ad libitum during the experiment. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Turku, Finland.

Study Design
Ten week old female Sprague-Dawley rats were included in the study. They were weighed, and their cortical and trabecular bone structures were characterized using peripheral quantitative computed tomography (pQCT) and microcomputed tomography (μCT) on arrival at the laboratory and after 4 and 6 weeks of treatment with the respective agents. Two weeks after the last treatment, bone mass, bone mineral density, and body weight were determined in all rats using pQCT. The rats were euthanized, and bone biopsies were taken from the left tibia and femur for histological analyses. The left femur was used for biomechanical testing.

Study outcomes included the body weight, cortical and trabecular bone mass, bone mineral density, and bone turnover (bone formation and bone resorption). The bone turnover was assessed by pQCT and μCT microstructural parameters (cortical and trabecular bone parameters), and biochemical markers of bone formation and bone resorption were measured in serum and bone tissue.

Biochemical markers of bone resorption
Biochemical markers of bone formation
Bone tissue and serum samples were collected at the end of the experiment. Serum and bone tissue samples were analyzed for collagen type-I procollagen (CTx), osteocalcin (OC), and bone-specific alkaline phosphatase (BAP) by ELISA. Serum osteoprotegerin (OPG) levels were determined using a commercial ELISA kit. Serum and bone tissue samples were also analyzed for osteoprotegerin ligand (OPGL) by ELISA. The expression of the RANKL to OPG ratio in bone tissue was determined using Western blotting and ELISA analyses.

Data analysis
Statistical analysis was performed using a Statistical Analysis System (SAS, version 9.3; SAS Institute Inc., Cary, NC). Significant differences between treatment groups were established using one-way analysis of variance (ANOVA). Post-hoc analysis was performed using a Tukey test. Relationships between various treatment effects were performed using regression analyses. Data are presented as the mean ± standard deviation. Differences were considered statistically significant when the p value was < 0.05.

Conclusions
The novel pERD decreased trabecular and cortical BMC in tibial metaphysis at 3 mg/kg p.o. (Fig. 3A, B). This reduction in diaphyseal cortical bone was opposite to the effects of OXV and similar with the effects of RAL. pERD did not affect diaphyseal cortical bone at 10 and 30 mg/kg p.o. (Fig. 3C, D). pERD decreased cortical bone area and cortical thickness in tibial diaphysis at 3 mg/kg p.o. (Fig. 4A, B). This reduction in diaphyseal cortical bone was opposite to the effects of OXV and similar with the effects of RAL. pERD did not affect diaphyseal cortical bone at 10 and 30 mg/kg p.o. (Fig. 4C, D). pERD increased serum TRACP 5b activity at 3, 10 and 30 mg/kg p.o. (Fig. 5D) and serum OC levels at 30 mg/kg p.o. (Fig. 5D). The reduction in serum TRACP 5b activity was similar with the effects of OXV and FUL, RAL and TAM treatments, and the reduction in serum OC levels was opposite to the effects of OXV and similar with the effects of RAL and TAM. pERD did not affect serum CTX and PINP levels at 3, 10 and 30 mg/kg p.o. and serum OC levels at 3 and 10 mg/kg p.o.

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References