ELSEVIER

Bone 39 (2006) 523–529

BONE

www.elsevier.com/locate/bone

Effects of stable transfection of human fetal osteoblast cells with estrogen receptor-alpha on regulation of gene expression by tibolone

A. Maran [a,*], K. Shogren [a], M. Zhang [a], M.J. Yaszemski [a], T.E. Hefferan [a], T.C. Spelsberg [b], H.J. Kloosterboer [c], R.T. Turner [d]

[a] *Department of Orthopedics, Mayo Clinic College of Medicine, 3-69 Medical Sciences Building, Rochester, MN 55905, USA*
[b] *Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[c] *Research and Development Laboratories, NV Organon, The Netherlands*
[d] *Department of Nutrition and Exercise Sciences, Oregon State University, Corvallis, OR 97330, USA*

Received 14 October 2005; revised 18 March 2006; accepted 22 March 2006
Available online 16 May 2006

Abstract

Tibolone is a synthetic steroid which undergoes tissue selective metabolism into several metabolites having estrogenic, progestogenic, or androgenic activities. The effects of 3α-hydroxy tibolone (Org 4094), 3β-hydroxy tibolone (Org 30126), and their sulfated metabolites were investigated on human fetal osteoblasts (hFOB). Tibolone had no effect on selected osteoblast marker proteins in estrogen receptor negative hFOB cells. In contrast, 3α-hydroxy and 3β-hydroxy tibolone resulted in dose-dependent increases in alkaline phosphatase activity in estrogen receptor (ER) α-positive hFOB cells. The maximum increase for both metabolites was comparable to the effects of an optimal dose of 17β-estradiol, and occurred at 10 μM. At 20 μM, both metabolites increased mRNA levels for alkaline phosphatase and type 1 collagen and protein levels for osteocalcin. Sulfated metabolites of tibolone also increased alkaline phosphatase activity. The estrogen receptor antagonist ICI 182, 780 inhibited stimulation of alkaline phosphatase activity by sulfated and non-sulfated tibolone metabolites, but was more potent on the former. Taken together, these results suggest that stable transfection of ERα into hFOB cells confers regulation by 3α-hydroxy and 3β-hydroxy tibolone metabolites of osteoblast metabolism.

Keywords: Bone formation; Alkaline phosphatase; Type 1 collagen; Osteoporosis; Antiestrogen

Introduction

Tibolone is a novel synthetic steroid which has tissue-seletive estrogenic activity due to differential metabolism into compounds with estrogenic, progestogenic, and androgenic activities. Tibolone has estrogenic activity on bone, vagina, and brain but not breast and the endometrium [1–4]. Tibolone is rapidly metabolized to the 3α-hydroxy metabolite and the 3β-hydroxy metabolite, which bind to estrogen receptors and have a half-life of ~7 h. These putative estrogenic compounds are further metabolized to sulfated compounds; ~80% of circulating tibolone consists of 3α- and 3β-hydroxy mono- and disulfates. An additional metabolite (Δ^4 isomer) is produced but is rapidly cleared from the circulation [5]. The parent compound and Δ^4 isomer bind to progestrone and androgen receptors while the 3α- and 3β-hydroxy tibolone exclusively bind to estrogen receptors (ER) with a preference for ERα over ERβ [6].

Tibolone is used for the treatment of climacteric symptoms in postmenopausal women and prevention of osteoporosis [3,4,7]. The beneficial effects of tibolone on bone mass were first demonstrated in a clinical study by Lindsay and co-workers [8]. Subsequent studies demonstrated that tibolone prevented bone loss in postmenopausal women [1].

Metabolites of tibolone inhibit bone resorption and as a consequence preserve cortical and cancellous bone in sexually mature rats [1,4,9]. The effects of tibolone on bone in the rat appear to be mediated by metabolites that bind to and activate nuclear translocation and signaling of ER [4,9]. Histomorphometric analysis indicates that tibolone regulates osteoblasts and

* Corresponding author. Fax: +1 507 284 5075.
E-mail address: maran@mayo.edu (A. Maran).

8756-3282/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.bone.2006.03.006

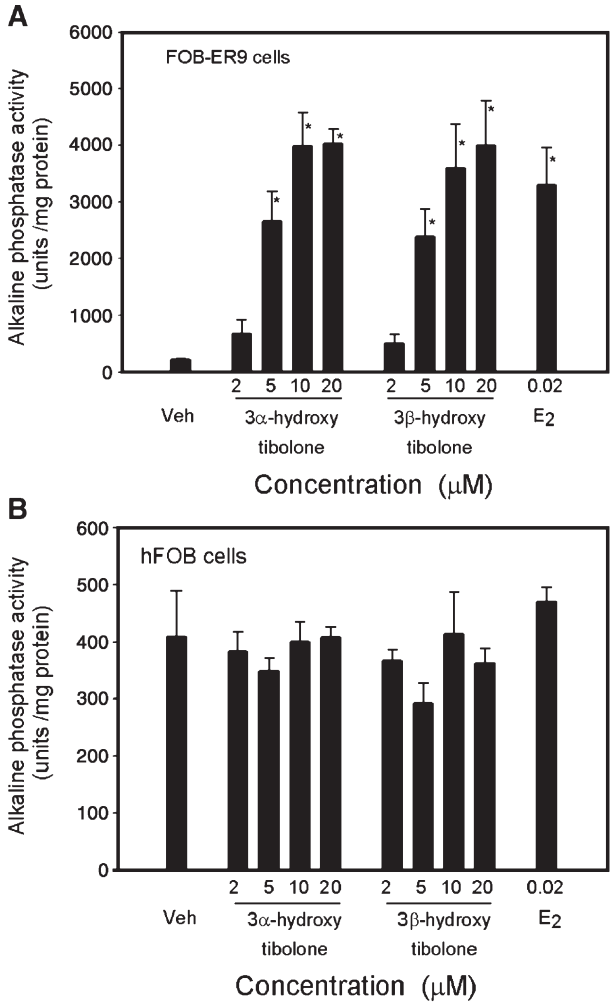

Fig. 1. Effects of tibolone on alkaline phosphatase enzyme activity. (A) FOB-ER9 cells; (B) hFOB cells. The cells were treated for 72 h with indicated concentrations of 17β-estradiol and 3α- and 3β-hydroxy tibolone. The alkaline phosphatase activity in the cell pellet was measured at the end of 72 h. Values are the mean ± SE; $N = 6$. *$P \leq 0.05$ (compared to vehicle control).

osteoclasts and suppresses the accelerated bone turnover induced by ovariectomy and low dietary calcium [10].

The tissue selective actions of tibolone differ from selective estrogen receptor modulators (SERMs) [9]. Although the mechanisms of action of SERMs are incompletely understood, it is clear that they bind to ER in all estrogen target tissues and behave as mixed estrogen agonists/antagonists [11–13]. The relative level of agonism is tissue-specific and depends upon several factors, including the recruitment of tissue specific co-activators and co-repressors [14]. In contrast, the tissue selective action of tibolone depends upon its differential metabolism and enzyme regulation in individual estrogen target tissues [2,3,10].

Tibolone has a very different clinical profile compared to natural estrogens and SERMs. Thus, it is important to determine how tibolone exerts its action on target tissues, including bone. To investigate the direct effects of tibolone on normal human bone cells, we have studied the actions of sulfated and non-sulfated tibolone metabolites on human fetal osteoblast (hFOB) cells.

Materials and methods

Human osteoblast cell lines and culture conditions

Human fetal osteoblast (hFOB) cells express the temperature-sensitive T-antigen expression vector and do not have detectable levels of either estrogen receptor α (ERα) or estrogen receptor β (ERβ) (15). The hFOB/ER9 cell line was transfected with ERα [15]. The cells were grown in DMEM/F12 medium containing 10% charcoal-stripped fetal bovine serum (FBS), penicillin, and streptomycin and maintained at 34°C. To maintain selection, hFOB cells and hFOB/ER9 cells were maintained in media supplemented with geneticin (300 μg/ml) or hygromycin (100 μg/ml) [15,16].

Rat primary marrow stromal cell (RMSC) isolation and culture conditions

Marrow stromal cells were isolated from femora of 5-month-old male Sprague–Dawley rats (Harlan, Indianapolis, IN). The femora were harvested, soft tissue was removed, and the bones placed in media containing DMEM, 10% FBS, penicillin (50 μg/ml), streptomycin (50 μg/ml), neomycin (100 μg/ml), and fungizone (25 μg/ml). The femora were rinsed with sterile PBS, and the epiphyses were removed. The bone marrow was flushed out of the diaphysis by inserting a syringe with an 18-gauge needle into the diaphysis. The media used to flush bone marrow contained DMEM, 10% FBS (v/v), penicillin (50 μg/ml), streptomycin

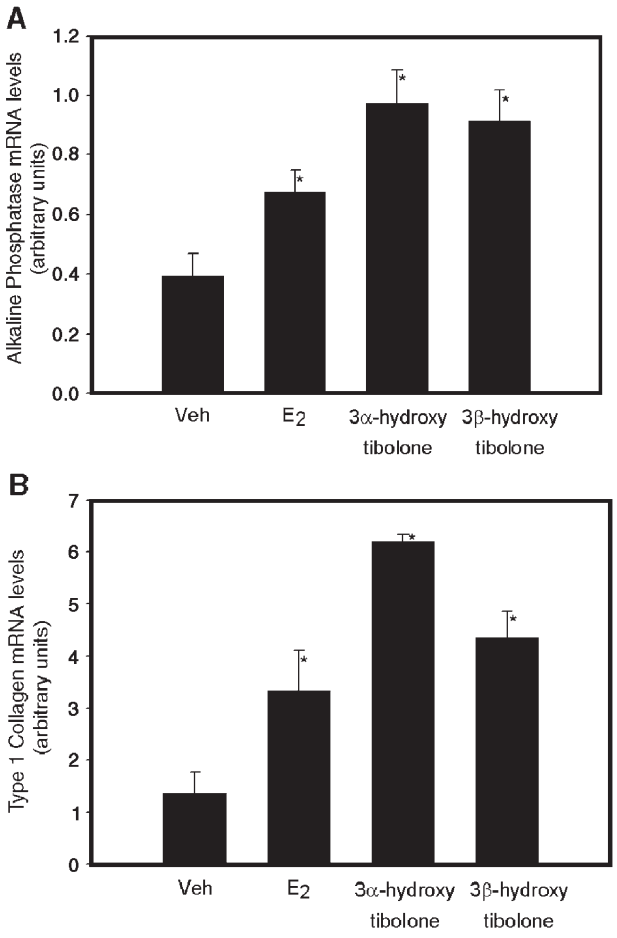

Fig. 2. Effects of tibolone on bone gene expression in hFOB-ER9 cells. (A) alkaline phosphatase mRNA; (B) type 1 collagen mRNA. The cells were treated for 72 h with 3α- and 3β-hydroxy tibolone (20 μM) and 17β-estradiol (E_2) (20 nM). The mRNA levels for alkaline phosphatase and type 1 collagen were determined by Northern hybridization. Values are the mean ± SE; $N = 3$. *$P \leq 0.05$ (compared to vehicle control).