SEXUAL DIMORPHISM IN OSTEOBLAST RESPONSE TO SURFACE MICROSTRUCTURE

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INTRODUCTION. Implant success depends on the integration of the implant with the surrounding bone. This process is affected by the implant surface, bone quality, and local and systemic regulatory factors. For example, unbalanced bone turnover due to estrogen (E_2) deficiency leads to osteoporosis, and potentially impairs bone formation around implants. Previously we showed that human osteoblast differentiation, proliferation and local factor production are dependent on surface roughness and energy [1]. These osteoblasts also exhibit substrate-dependent changes in their responsiveness to osteotropic hormones like $1,25(OH)_2D_3$. Related studies show that there are substrate-dependent changes in the response of female human osteoblasts to E_2 [2], as well. Although E_2 is also necessary for bone strength in males, studies examining the effects of E_2 on other musculoskeletal cells suggest that the mechanism of the E_2 effect is different from that in female osteoblasts. Therefore, it is important to understand how E_2 regulates male and female osteoblasts, particularly with respect to implant surface design.

The aim of the present study was to evaluate if the effect of estradiol on osteoblast phenotype is sex-dependent. To do this, we used osteoblasts isolated from the calvaria of male and female rats. In rat chondrocytes, sex-specific responses to E_2 are mediated by a membrane-associated signaling pathway, although both male and female cells possess nuclear E_2 receptors ERα and ERβ. We also examined whether any differences in male and female osteoblast response to the hormone that resulted from differences in substrate microarchitecture are due to membrane-mediated mechanisms.

MATERIALS AND METHODS. Rat osteoblasts were enzymatically isolated from 8-week old male and female Sprague Dawley rats. The cells from 8 rats of each sex were combined and cultured in 10% FBS-DMEM. Media were changed every 48 hours until cells reached confluence. The osteoblastic phenotype of the cells was characterized by the stimulatory effect of $1\alpha,25(OH)_2D_3$ on alkaline phosphatase activity and osteocalcin. To investigate rat osteoblast responses to surface topography, the cells were cultured on microstructured surfaces. Ti disks (15 mm diameter) had three different surface structures: the smooth pretreatment (PT) surface had an R_a of 0.60 ± 0.02 μm; the coarse grit blasted and acid etched (SLA) surface had an R_a of 3.97 ± 0.04 μm; and the Ti plasma-sprayed (TPS) surface had an R_a of 5.21 ± 0.24 μm. Prior to cell culture, the disks were sterilized by steam autoclaving. The rat osteoblasts were plated on plastic, PT, SLA and TPS surfaces at seeding density of 10,000/cm^2, and cultured as above. When cells reached confluence on plastic surfaces, all cultures were treated for 24 hours with vehicle, 10^{-8}M 17β-estradiol or the same concentration of 17β-estradiol conjugated to bovine serum albumin (E_2-BSA) to prevent the hormone from entering the cell and binding to its nuclear receptors. At harvest the effect of the surface with or without the hormone on cell number alkaline phosphatase activity, and production of osteocalcin, TGFβ1 and PGE_2 was evaluated. For each experiment, there were 6 separate cultures for each variable. Data were analyzed by ANOVA and significant differences between groups determined using Bonferroni's modification of Student's t-test. Experiments were repeated to ensure validity of the results.

RESULTS. Both male and female rat cells exhibited an osteoblast phenotype based on increases in alkaline phosphatase activity and osteocalcin production when treated with 10^{-8}M $1\alpha,25(OH)_2D_3$. Male osteoblasts had higher enzyme activity than female osteoblasts and the response to $1\alpha,25(OH)_2D_3$ was more pronounced.

Male and female cells showed surface roughness dependent changes characterized by decreased cell numbers and increased cellular alkaline phosphatase activity. Osteocalcin levels in the conditioned media were increased on the rougher surfaces (Fig 1, left), as were PGE_2 and TGF-β1 (Fig 1, right). E_2 treatment affected female cells only and the responses were surface-dependent. E_2 decreased cell number on all substrates. It had no effect on cellular alkaline phosphatase activity on plastic and PT, but doubled enzyme activity on rough surfaces. Osteocalcin, PGE_2 and TGF-β1 levels were sensitive to E_2 regardless of surface type. The responses to E_2-BSA were comparable to those of E_2.

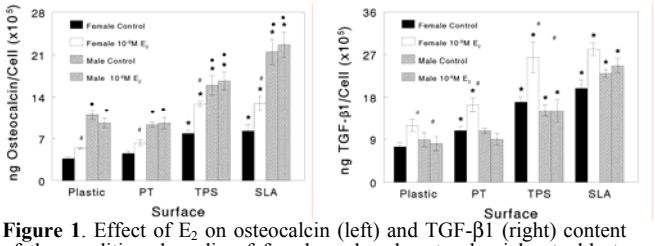

Figure 1. Effect of E_2 on osteocalcin (left) and TGF-β1 (right) content of the conditioned media of female and male rat calvarial osteoblasts grown on tissue culture plastic or Ti. Data are means ± SEM for 6 cultures. $P<0.05$, *Ti v. plastic for each treatment group; #control v. E_2 for each sex; ●female v. male.

DISCUSSION. Primary rat osteoblasts responded to surface microstructure by increased cell differentiation and decreased cell number. These effects were enhanced by E_2. Although the changes due to microarchitecture were not sex-specific, response to E_2 was; only female cells were affected by the hormone. Moreover, the sex-specific response was mediated by membrane-associated mechanisms. E_2-BSA only binds to membrane E_2 receptors because it is too large to penetrate plasma membranes. Both E_2 and E_2-BSA altered osteoblast behavior in a similar manner, suggesting that the components of the membrane signaling pathway are modulated in a surface-dependent manner. Although male osteoblasts also express ERs, neither E_2 nor E_2-BSA affected the male cells, indicating that the membrane pathway was not functional. Other evidence of sexual dimorphism was the observation that male osteoblasts had higher alkaline phosphatase activity and osteocalcin levels than female cells.

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REFERENCES. [1] Zhao et al., Clin Oral Imp Res, 2005; [2] Lohmann et al., J Biomed Mat Res, 2002.