Response of Osteoblasts to 1alpha,25(OH)2D3 and 17beta-Estradiol is Substrate Dependent and Sex-Specific

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Introduction: Successful osseointegration of bone around orthopaedic implants depends on the implant surface, bone quality and local and systemic host environment. Osteoblast differentiation is regulated by substrate properties, such as surface topography, chemistry and energy. To investigate surface effects on osteoblasts, titanium(Ti) is a widely used model substrate because of its good biocompatibility and clinical relevance. Human osteoblasts exhibit increased differentiation and extracellular matrix production on microrough Ti surfaces compared to smooth ones[1]. In addition, the osteoblasts grown on microrough surfaces create an osteogenic microenvironment by producing more osteogenic cytokines and growth factors. These osteoblasts also exhibit substrate-dependent changes in their response to osteotropic hormones like 1alpha,25(OH)2D3 (1,25D) and estrogen (E2). On the rougher surfaces, the effect of the hormones is increased. We previously showed that normal human osteoblasts (NHOst) were more differentiated on microrough Ti surfaces and their response to E2 was enhanced[2]. These experiments used female cells only, based on our related studies showing a clear sexual dimorphism of human articular chondrocytes and rat growth plate chondrocytes to this steroid[3,4]. Only female chondrocytes respond to E2 with a membrane-associated increase in PKC and responses to E2 that are PKC-dependent are also seen only in female cells. In addition, human articular chondrocytes from males were more sensitive to 1,25D. The present study was based on the hypothesis that the osteoblasts also possess sexual dimorphism and this is expressed with respect to their behavior on microstructured Ti surfaces and in their response to E2 and 1,25D. Moreover, the difference in E2 response is due to membrane-associated signaling.

Materials and Methods: Osteoblasts were enzymatically isolated from the calvaria of 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. Expression of an osteoblastic phenotype was determined by response to 1,25D. To investigate rat osteoblast responses to surface topography, the cells were cultured on microstructured surfaces. Ti disks (15 mm diameter) had two different surface structures; the smooth pretreatment(PT) surface had an Ra of 0.60 ± 0.02 μm and the coarse grit blasted and acid etched (SLA) surface had an Ra of 3.97 ± 0.04 μm. Control cultures were grown on tissue culture polystyrene (plastic). The rat osteoblasts were plated at seeding density of 10,000/cm². When cells reached confluence on plastic surfaces, all cultures were treated for 24 hours with vehicle, 10-8M 17beta-estradiol or the same concentration of 17beta-estradiol conjugated to bovine serum albumin (E2-BSA). Alternatively, confluent cultures were treated for 24 hours with vehicle, 10-8M or 10-9M 1,25D. Effects of surface and hormone treatment were assayed as a function of alkaline phosphatase activity of cell lysates and the levels of osteocalcin, PGE2, and latent and active TGF-beta-1 in the conditioned media. 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.

Results: Both male and female rat cells exhibited an osteoblast phenotype based on high levels of alkaline phosphatase specific activity and osteocalcin production that were further stimulated by 10-8M 1alpha25(OH)2D3. Male and female cells exhibited a decrease in cell number and an increase in cellular alkaline phosphatase activity and osteocalcin levels when grown on SLA. PGE2 and TGF-beta-1 (both latent and active form) content of the conditioned media were also increased. E2 treatment affected female cells only and the responses were surface-dependent. E2 decreased cell number on all substrates. It had no effect on alkaline phosphatase on plastic and PT, but doubled enzyme activity on SLA. Osteocalcin (Fig 1a), PGE2 and TGF-beta-1 levels were sensitive to E2 regardless of surface type. The responses to E2-BSA were comparable to those of E2. E2 had no effect on male cells on all the parameters examined (Fig 1b). Male osteoblasts had higher enzyme activity than female osteoblasts and the response to 1alpha,25(OH)2D3 was more pronounced on all the parameters examined with decreased cell number and increased alkaline phosphatase, osteocalcin (Fig 2a,b), PGE2 and TGF-beta-1.

Discussion: Rat osteoblasts responded to surface microstructure as shown previously for human MG63 cells and NHOst cells, indicating that osteoblast responses to surface topography are not species specific. E2 increased the response to surface microstructure noted previously in female NHOst cells. Failure of the male osteoblasts to respond demonstrates that the effects of the hormone are sex-specific. Moreover, the sex-specific response is mediated by membrane-associated mechanisms. E2-BSA only binds to membrane E2 receptors because it is too large to penetrate plasma membranes. Both E2 and E2-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 E2 receptors, they did not respond to E2. Neither E2 nor E2-BSA affected the male cells, indicating that the membrane pathway is not functional. Other evidence of sexual dimorphism was the observation that male osteoblasts had much higher sensitivity to 1,25D in compare to female osteoblast especially in rough surfaces.


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Figure 1: The effect of 10-8M E2 and E2-BSA on osteocalcin. Figure 2: The effect of 10-8M and 10-9M 1alpha,25(OH)2D3 on osteocalcin.