17 β-estradiol (E2) differently affects osteogenic differentiation of mesenchymal stem cells from bone marrow and adipose tissue

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Estrogens confer multiple skeletal protective effects both in vitro and in vivo, facilitating osteogenesis and suppressing osteoclast-induced bone resorption. Estrogen deficiency induces a modulation in osteoblasts and osteoclasts activity resulting in bone resorption increase. Besides, it also act on osteoblast precursors and bone marrow mesenchymal stem cells (BMSCs) impairing their proliferation and osteogenic activity. Mesenchymal stem cells (MSCs) are a heterogeneous population present in several tissues, and, due to their multipotency, low immunogenicity and no tumorigenicity they are an attractive tool in regenerative medicine. Although MSCs isolated from different tissues share common stemness features, recent findings show that they slightly differ in terms of gene and protein expression, suggesting an intrinsic difference among them. Since both BMSCs and ASCs (MSCs isolated from adipose tissue) are the most used in tissue engineering applied to bone regeneration, we evaluated in vitro the effect of 17β estradiol (E2) on their osteogenic differentiation ability. BMSCs and ASCs used in this study derived from two (♂, 55±16 y/o) and six (♂ and ♀, 39±17y/o) patients, respectively. All the MSCs proliferated at constant rate when cultured till passage 10 with no changes in their morphology. In addition, at early passages, high clonogenicity (range 2.4-14.6%) and the typical mesenchymal stem cells immunophenotype were also determined. Additionally, osteogenic and adipogenic differentiation markers, such as ALP activity, collagen deposition and lipid vacuoles formation, became evident when properly induced. At first, MSCs treated with E2 (1, 10, 100, 1000 nM) for 14 days were as viable as control cells. Furthermore, 35 days treatment with E2 (10, 100nM) did not induce any modification in morphology and proliferation rate. In order to identify a pro-osteogenic effect of estradiol on MSCs, cells were pre-treated with 10, 100 nM E2 for 7, 21, 35 days and then both pre-treated and control cells were osteo-differentiated for 7 and 14 days either in the presence or in the absence of the hormone. Estradiol did not reduce their osteogenic differentiation; indeed cells differentiated with or without E2 significantly increased ALP activity and collagen deposition in comparison to control cells. Estradiol increased ALP activity in BMSCs by acting in synergy with osteogenic factors. The effect, already detectable in 7 days, was even more pronounced after 14 days of differentiation, with a significant increase of ALP activity of 49% and 67% in BMSCs incubated with E2 10 and 100nM, respectively, compared to untreated and differentiated cells. Interestingly, a significant dose-related increase of ALP activity was observed in differentiated (for 14 days) BMSCs pre-treated with the hormone for either 7 and 35 days, irrespective of the time of exposure. Surprisingly, E2 treatment never influenced the osteogenic differentiation ability of ASC at the doses and time points we have observed for BMSCs. In conclusion, E2 favoured osteogenic differentiation of just BMSCs and no effect on ASCs was observed, suggesting that either the hormone could activate different pathways or estrogen receptors are differently expressed, in the two mesenchymal stem cell types.