

Proceedings of German Society for Stem Cell Research (PGSSCR)

Short-time zoledronic acid pretreatment stimulates osteogenic differentiation of human mesenchymal stem cells

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Published on 16 May 2007

Pluripotent mesenchymal stem cells (MSC) are the source for the regeneration of bone and cartilage. In osteoporosis anabolic and antiresorptive drugs are applied which respectively stimulate the differentiation of MSC to osteoblasts (e.g. PTH and Teriparatide) or inhibit osteoclasts (e.g. bisphosphonates (BP)). The amino-bisphosphonate zoledronic acid (ZA) inhibits the farnesylation of osteoclasts which leads to inhibition of posttranslational prenylation of proteins like Ras and Rho resulting in osteoclast apoptosis. It was shown that a once-yearly infusion of ZA 5 mg during a 3-year period significantly reduced the risk of osteoporotic fractures. Osteoblasts and precursor cells are also targets of bisphosphonates but little is known about short-time and chronic effects of ZA on MSC cultures and their osteogenic offspring.

To clarify the influence of ZA on MSC, cells were treated with 5, 20 and 50 μ M ZA for 6 and 16 h and the gene expression pattern was determined by RT-PCR. Additionally, MSC were treated with 5 ? 50 μ M ZA for 3 h and the osteogenic differentiation potential of MSC was analyzed after 4 weeks in

osteogenic medium. Cells were harvested and osteogenic marker genes were amplified by RT-PCR. Mineralization of the cell monolayer was analyzed by alizarin red staining. For chronic stimulation with BP MSC were treated with 5, 20 and 50 μ M ZA for 24, 48 and 72 h and apoptosis and proliferation capacity of the cells were determined.

Stimulation of MSC with 50 μ M ZA for 6 to 16 h increased the expression of Dkk1 and runx3 and decreased the expression of L1CAM. Short-time treatment of MSC with 20 and 50 μ M ZA for 3 h stimulated osteogenic differentiation capacity after 4 weeks compared to untreated controls which was shown by alizarin red staining. Osteogenic markers as runx3 and osteopontin were upregulated dose-dependently while osteocalcin was downregulated. In contrast, long-time treatment of MSC with 20 and 50 μ M for 48 and 72 h led to increased apoptosis and decreased proliferation capacity. After 24 h no effect was detected.

We show here that a 3 h exposure to μ M concentrations of ZA is already sufficient to enhance osteogenic differentiation of MSC in

vitro while long-time exposure to ZA impairs MSC proliferation and induces MSC apoptosis. In osteoporosis treatment this might be of clinical relevance to better determine the dosing and the upper threshold of BP accumulation in the bone microenvironment when constantly high local BP concentrations start to impair osteogenic effects in bone.

Supported by Novartis.

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