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EFFECTS OF CERIUM (IV) OXIDE NANOPARTICLES ON RAW 264.7 CELLS ACTIVITY AND RANKL-STIMULATED OSTEOCLASTOGENESIS

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Introduction. Osteoclastogenesis is a complex process that plays a critical role in bone remodeling. Based on detailed knowledge of the molecular mechanisms involved in osteoclastogenesis, new pharmacological agents (including nanoparticles) that selectively influence the differentiation or the activity of bone cells were developed during the last decade. The purpose of our research was to study the molecular mechanisms of influence of 5 μM citrate-stabilized CeO_2 nanoparticles (CNPs) on the RAW 264.7 cells, its proliferative activity and level of multinuclear cells formation during RANKL-stimulated osteoclastogenesis.

Methods. The murine macrophage cell line RAW 264.7 was cultured with CNPs (2-4 nm) in DMEM (4.5 g/l glucose). Cell proliferative activity and apoptosis were assessed and visualized with IncuCyte ZOOMinstrument. Bovine bone slices were stained with *TRAP* and Hoechst 33258 for TRAP-positive multinuclear cells detection. The levels of TNF-α, CCL2, COX2, IL-6, Rel A mRNA expression were examined by RT-PCR analysis.

Results and Discussion. Exposure of RAW 264.7 cells to CNPs (5 μM) during 70 hours decreased cell proliferation and apoptosis by 20 and 12%, respectively compared with control ($P < 0.05$). MTT test has shown a mild cytostatic effect of CNPs on RAW 264.7 cells. On the other hand, a significant 26% increase was revealed in the number of multinuclear cells in bone slices under the effect of CNPs ($P < 0.05$). CNPs led to upregulation of TNF-α and Rel A (4.1- and 1.6-fold respectively) and downregulation of IL-6, CCL2, COX2 and GLUT 1 (1.9-, 1.8-, 1.3- and 1.6-fold, respectively) mRNA expression after 24 hours of RANKL-stimulated osteoclastogenesis compared with control ($P < 0.05$).

Conclusions. Our results demonstrate that CNPs caused a slight cytostatic effect on RAW 264.7 cells and enhanced the fusion of macrophages during RANKL-stimulated osteoclastogenesis. The findings suggest a significant CNPs-induced activation of TNF-α with the lowering effect on the levels of other inflammation factors, as well as GLUT 1 transporters.

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