Introduction
Given that osteoporotic fractures mainly occur in the proximal femur, vertebral bodies and distal radius, their local prevention has recently been considered with interest. This strategy consists in locally reinforcing these specific bone sites by an implantable bioactive drug-combined biomaterial associating a calcium phosphate bone substitute with an inhibitor of osteoclastic activity [1]. Among the potential compounds, gallium (Ga) could be a promising candidate due to its ability to substitute Ca in biological apatite. The chemical characteristics of Ga should presage the possibility of incorporating Ga into CaP biomaterials following an exchange between Ca and Ga. Interestingly, Ga is clinically used for the treatment of hypercalcemia in the case of malignancy and Paget’s disease, thereby suggesting a potent inhibitory effect of Ga on bone resorption. However, the anti-osteoclastic effect of Ga still needs clear deciphering, given that the direct effect of Ga on bone cells has only been partially addressed.

Materials and Methods
By using different osteoclastic models, osteoclasts isolated from long bones of neonatal rabbits (RBC), murine RAW 264.7 cells and human CD14-positive cells, we have performed resorption activity tests, TRAP staining, RT-PCR analysis, viability and apoptotic assays. Moreover, we have evaluated the effect of Ga on osteoblasts in terms of proliferation, viability and activity by using an osteoblastic cell line (MC3T3-E1) and primary osteoblasts.

Results
Ga dose-dependently inhibited the in vitro resorption activity of RBC (Fig 1) and induced a significant decrease in the expression level of transcripts coding for osteoclastic markers in RAW 264.7 cells such as Tartrate resistant acid phosphatase, Cathepsin K, Calcitonin receptor, Receptor activator of nuclear factor kappa B and Osteoclastic stimulatory transmembrane protein. Ga also dramatically reduced the formation of TRAP+ multinucleated cells. Interestingly, Ga down-regulated in a dose-dependent manner the expression of NFATc1. Finally, our results indicate that Ga failed to dramatically affect the primary and MC3T3-E1 osteoblasts.

Discussion and Conclusions
This study demonstrates that Ga exhibits a dose-dependent anti-osteoclastic effect by reducing in vitro osteoclastic resorption, differentiation and formation without negatively affecting osteoblasts. This is the first report indicating that the inhibitory effects of Ga on osteoclastogenesis probably involve a reduction in the expression of NFATc1, a master regulator of RANK-induced osteoclastic differentiation. Considering these data, Ga appears to be a promising candidate to develop a new bone bioactive drug delivery system for the local reinforcement of osteoporotic sites.

Reference

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