CO-CULTURES OF OSTEOBLASTS AND OSTEOCLASTS ARE INFLUENCED BY LOCAL APPLICATION OF ZOLEDRONIC ACID INCORPORATED IN A POLY(D,L-LACTIDE) IMPLANT COATING

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Introduction
The anti-resorptive activity of bisphosphonates such as zoledronic acid (ZOL) has been shown in vitro to be due to their effect on osteoclasts and osteoblasts (Greiner et al. 2007, Teitelbaum 2000, Reinholz et al. 2000). However, whether the effect of ZOL on monocultures might be reproducible on co-cultures and whether cell interactions might influence this effect has not been described. Aim of the study was to investigate the effect of ZOL on co-cultures of osteoblasts and osteoclasts in vitro.

Materials and Methods
In order to evaluate the interplay of primary human osteoblasts (POB) and osteoclast like cells (OLC) two different settings of co-cultivation were selected:
- setting 1 for determination of fusion of mononuclear cells to OLC in presence of POB: POB were seeded in a concentration of 3x10^4 cells per well in 24-well plates and cultivated in 1 ml MEME/HAM’s F-12 for 3 to 4 days. The number of cells was determined with Alamar Blue (BIOZOL, Germany) and 5x10^5 monocytes per well were added. Both cell types were incubated together using MEME/HAM’s F-12.
- setting 2 for determination of bone resorption activity of OLC in presence of POB. Isolated monocytes were seeded in a concentration of 3x10^6 in 24-well plates on dentine chips (obtained from “Bundesamt für Naturschutz”, Germany, diameter: 1.4cm) to allow cell-cell-contact and cultured with ALPHA-MEM (Biochrom-AG, Berlin, Germany) including 10% FCS for 14 days. Nuclear factor-κB ligand (RANKL) (20 ng/ml) (Peprotech Inc, RockyHill, NJ, USA) and macrophage colony stimulating factor (MCSF) (5 ng/ml) (Sigma-Aldrich, Taukirchen, Germany) were added for stimulation of fusion to multinuclear cells. Half of the medium was changed every second day in the first week to allow cell adherence.

ZOL was incorporated in an implant coating based on Poly(D,L-Lactide) (PDLLA) in different concentrations (10-50μM), ZOL-coated implants (ZOL-CI). Cell number was measured and Procollagen I synthesis, osteoprotegerin (OPG) secretion and soluble receptor activator of nuclear factor-κB ligand (sRANKL) were analyzed. Moreover, TRAP positive cells and resorption lacunas on dentin chips (pit formation assay) were counted. All experiments were performed in triplicate and repeated four times (n=12). ANOVA and Bonferroni testing was used for comparison of data using SPSS (release 14.0; SPSS Inc. Chicago, IL).

Results
Results showed that cell viability was significantly decreased of about 20% in total cell number after treatment with 50μM ZOL coated implant (ZOL-CI) after 144h incubation time in comparison to the control (data not shown). Procollagen I and OPG synthesis of POB in coculture was highest when treated with 10μM ZOL-CI (Figure 1a,b), whereas no effect on sRANKL was found (data not shown). TRAP positive OLC formed out of mononuclear cells in setting 1 (Figure 2a). TRAP positive cells (formation of OLC) were decreased when treated with ZOL-CI in a dose dependent manner (Figure 3a). Resorption activity of OLC was not significantly decreased when treated with investigated concentrations of ZOL-CI but showed a trend to a decrease of lacunas with increasing concentrations of ZOL-CI (Figure 2 b,c and Figure 3b).

Discussion
Exposure to specific concentrations of ZOL-CI showed a beneficial effect on osteoblast differentiation and protein synthesis. Formation of osteoclast was decreased, whereas a significant decrease in sRANKL secretion and resorption activity of osteoclasts could not be shown. The investigated effect on co-cultures might be clinically useful to support fracture healing and to reduce orthopedic implant loosening.

Figure 1 a) Collagen I to total protein ratio at 144h after setting in co-cultures exposed to different concentrations of ZOL-CI. (*: p<0.05; control to 10μM). b) OPG to total protein ratio 144h after setting in co-cultures exposed to different concentrations of ZOL-CI. (*: p<0.05; 10μM to control)

Figure 2 a) TRAP positive cell formed out of human peripheral blood monocytes after stimulation in co-cultures (10x magnification) b, c): Analysis of resorption lacunas of control (b) and after exposure to 50μM (c) ZOL-CI. There is a visible reduction of pits with rising concentrations of ZOL-CI (10x magnification).

Figure 3 a) Number of TRAP positive cells in relation to control (100%) after exposure to different concentrations of ZOL-CI (coated implants) 144 hours after setting (*: p<0.05: 10, 30 and 50μM against control). Figure 3 b) Number of pits in relation to control (100%). There is a decrease in osteoclastic activity at 30 and 50μM ZOL-CI, however this decrease was not significant.

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Literature
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