Bone-site-specific responses to bisphosphonates

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General summary

Bone remodeling is a life-long process, and important to maintain a well-functioning skeleton and mineral homeostasis. Multinucleated osteoclasts degrade bone, whereas osteoblasts deposit new bone. Osteocytes experience and respond to mechanical loading and regulate osteoblast and osteoclast activity. When osteoclasts are overactive, for instance in diseases such as osteoporosis and bone cancer, bisphosphonates (BPs) can be used to inhibit osteoclast activity. With the increasing life-expectancy, those bone diseases are expected to become even more common. Therefore, possible side effects of anti-resorptive drugs will also become an emerging problem. Osteonecrosis of the jaw is such a rare, though severe side effect of BPs, and it is not clear how and why specifically the jaw is affected. We hypothesized that osteonecrosis of the jaw is caused by a different response of bone-site-specific osteoclasts and their precursors to BPs. We used several approaches to investigate whether BPs might have a different effect on long bone and jaw osteoclasts and their precursors. With these studies, which are summarized below, we aimed to get more insight into (i) differences between bone-site-specific osteoclasts and precursors, and (ii) the pathogenesis of BP-related osteonecrosis of the jaw.

In **chapter 2**, we investigated the internalization of BPs by long bone and jaw osteoclast precursors and studied the effect of BPs on osteoclastogenesis and apoptosis of long bone and jaw bone marrow cells isolated from healthy mice. Jaw osteoclast precursors internalized more BPs than long bone osteoclast precursors. This was accompanied by an accumulation of more unprenylated Rap1a in jaw osteoclast precursors. Intriguingly, a higher intracellular BP concentration did not differently affect osteoclastogenesis of long bone and jaw bone marrow cells. This could be explained by a higher anti-apoptotic gene expression and lower caspase 3/7 activity in jaw than in long bone osteoclast cultures. Therefore, these data show that jaw osteoclasts or precursors may be less sensitive to bisphosphonates than those from long bone.

In **chapter 3**, the effect of bisphosphonates on long bone and jaw osteoclasts and bone remodeling was assessed *in vivo*. Female C57BL/6J mice were subjected to weekly injections of zoledronic acid for 1, 3, or 6 months. BPs did not significantly affect the number of osteoclasts in both long bone and jaw, thereby confirming the results obtained *in vitro* that BPs did not have a different effect on those different osteoclasts. Also, bone volume fraction and tissue mineral density were similarly increased by BPs in long bone and jaw. Yet, the number of bone marrow cells isolated from the jaw was 4 times lower in the BP-treated animals than in controls. The number of long-bone marrow cells, however, was not affected. These results indicate that BPs may affect osteoclast precursors and/or other bone marrow cells in the jaw specifically. On the other hand, bone formation was inhibited in the long bones on the long term, whereas bone formation in the jaw was not affected by six months of BPs. Furthermore, this study revealed another bone-site-specific effect of BPs;

the drugs were able to induce osteoclast formation at the molar root. These cells also proved to be actively resorbing. Therefore, in **chapter 4**, we used human cells to investigate whether this induction and/or stimulation may be induced by periodontal ligament (PDL) fibroblasts that were treated with BPs. Pre-treatment of PDL fibroblasts with a high concentration of pamidronate was toxic, not only to the fibroblasts, but also to the PBMCs that were co-cultured with the pre-treated fibroblasts. BP concentrations that were not toxic to PDL fibroblasts did not affect osteoclastogenesis in co-culture with PBMCs.

In order to study earlier steps during osteoclast formation, time-lapse microscopy was used to visualize the fusion of long bone osteoclast precursors and multinucleated osteoclasts **(chapter 5)**. Fusion was seen (i) between mononuclear cells, (ii) between multinucleated cells, and (iii) between a multinucleated and a mononuclear cell. Interestingly, cells were also shown to undergo fission, a process that be mediated by small mononuclear cells present in the bone marrow. The cellular compartments that arise as a result of fission may contain apoptotic nuclei. Therefore, we hypothesize that osteoclasts can use the unique process of fission to get rid of apoptotic nuclei.

In **chapter 6**, we used time-lapse microscopy to study early steps of osteoclastogenesis, i.e. proliferation, migration, and fusion, of long bone and jaw bone marrow cells. We also analyzed the expression of genes involved in these processes and the effect of BPs on osteoclast precursor migration. Long bone and jaw osteoclast precursors proliferated, migrated, and fused with similar rates. BPs did not affect the migration of both sets of precursors. Interestingly, jaw osteoclast precursors expressed more CXCL12, CXCR4, and CXCR7, than long bone osteoclast precursors. Those genes are involved in directional migration of osteoclast precursors into the bone marrow and CXCL12 was also shown to inhibit apoptotic pathways in osteoclasts. By showing higher CXCL12 expression in jaw than in long bone osteoclast precursors, we provide more mechanistic insight into the higher anti-apoptotic capacity of jaw than of long bone osteoclasts or precursors.

Collectively, we provide more evidence for the existence of bone-site-specific differences in osteoclasts and their precursors in the bone marrow. The finding that jaw osteoclasts were less sensitive to bisphosphonates may support our hypothesis that those bone-site-specific osteoclasts contribute to the development of osteonecrosis of the jaw. Therefore, we propose a new hypothesis for the pathogenesis of osteonecrosis of the jaw, which includes two hypotheses previously proposed in literature.

Despite the presence of BPs, the more apoptosis-resistant jaw osteoclasts survive longer than their long bone counterparts, and therefore they resorb more bone. Consequently, those jaw osteoclasts are able to release more BPs into the microenvironment, making them available for uptake by surrounding cells. The resulting toxicity to other cells contributes to the onset of osteonecrosis of the jaw. The lethality to

immune cells, and the inability to tackle infections, followed by a decrease in pH, might lead to the release of even more BPs, thereby activating a vicious cycle.

This hypothesis proposes that jaw osteoclasts specifically may play a role in the pathogenesis of osteonecrosis of the jaw, and therefore emphasizes the need for the development of more specific anti-resorptive drugs. Gaining more insight into the differences between bone-site-specific osteoclasts is therefore crucial and requires further research.