Osteoclasts are redundant whereas MMP activity is essential during early endochondral bone repair.

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Introduction

Despite a great deal of experimental insight, the exact role that osteoclastic resorption plays in the process of endochondral ossification remains to be clarified. Until recently osteoclastic resorption has been strongly associated with the removal of cartilaginous soft callus during initial endochondral union. A study examining the effects of the anti-resorptive Bisphosphonates (BP’s) during initial bone healing suggested otherwise, as these potent anti-osteoclastic agents did not interfere with the process of soft callus removal. Further, a study examining RANK:Fc treatment (an inhibitor of osteoclast formation) during bone repair in mice also showed no inhibitory effects on the process of endochondral union. In contrast to the results obtained using anti-osteoclastic agents, when fracture healing was examined in mice lacking matrix metallo-proteinases (MMP’s) endochondral fracture union was considerably delayed. To add to the uncertainty, osteoclasts are known to secrete MMPs as well as more bone specific proteases such as Cathepsin K.

In this study we aimed to assess any delay in fracture healing caused by BP administration using both zoledronic acid (ZA) and clodronate (CLOD) in a well described rat model, and compare this to the specific blockade of osteoclast formation by Osteoprotegerin (OPG). In a final group the effects of broad spectrum MMP inhibitor was assessed. By comparing these interventions in the same model, the cumulative results may allow articulation of the relative roles of the osteoclast and other MMP expressing cells in endochondral fracture repair.

Hypotheses

Endochondral ossification can proceed normally in some circumstances in the absence of osteoclast function

Disruption of MMP function leads to a delay in endochondral ossification in fracture repair

Methods

150 animals were randomized into 5 treatment groups as per Table 1 prior to closed femoral fracture production and samples were harvested at 2, 4 and 6 weeks post fracture. Dosing with the two bisphosphonates ZA and CLOD, human OPG-Fc and the MMP inhibitor MMI270 commenced 2 days prior to fracture and continued up to harvest time points. Serum samples were collected prior to dosing and at harvest time points to examine TRAP levels.

Samples were examined for union rates radiologically before being QCT scanned to determine mineralized callus properties. Histological analysis generated data on union rates and the area of each callus containing avascular cartilage tissue compared to vascularized bone (Figure 1).

Proximal tibiae were assessed for systemic effects of each agent used in the study. QCT scans were performed to assess mineralized tissue properties and histological analysis determined bone volume ratio, growth plate height and osteoclast parameters.

Table I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 wks</th>
<th>4 wks</th>
<th>6 wks</th>
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<tbody>
<tr>
<td>Saline</td>
<td>10</td>
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<tr>
<td>MMI270</td>
<td>10</td>
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<tr>
<td>CLOD 45mg/kg Twice per week</td>
<td>10</td>
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<tr>
<td>ZA 0.1mg/kg Twice per week</td>
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<td>10</td>
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<tr>
<td>OPG 10mg/kg Twice per week</td>
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Results - Fracture Callus

X-rays revealed a delay in union rates with MMI270 treatment only compared to Saline with only 30% completely united at 6 weeks compared to 100% in all other treatment groups (p<0.01, Fishers test).

Figure 1. Saffranin O/light green stained calluses at 4 weeks, avascular cartilage - red, bone - green

Figure 2. Mean percent callus bone tissue content, error bars are 1 SD. *p<0.01 vs Saline.

As outlined in figure 2, only MMI270 treatment affected union grading and the callus area of vascularized bone tissue compared to Saline, with all other treatment groups similar to Saline (p<0.01). At 2 weeks MMI270 showed an average of only 49% bony callus compared to 81%-86% in all other groups. By 6 weeks when all other groups had reached 100% bony callus, MMI270 reached only 90% (p<0.01). This result is clearly seen in figure 1.

QCT scans of fracture samples revealed increases in callus properties with ZA, CLOD and OPG at 4 weeks. By 6 weeks these increases reached 78% in callus BMC and 64% in callus volume with ZA treatment (p<0.01), as well as a 9% increase in callus BMD (p<0.01). OPG treatment at 6 weeks showed a 97% increase in callus BMC and a 66% increase in callus volume compared to Saline at this stage, leading to a 19% increase in callus BMD (p<0.01).

In contrast to the above outcomes MMP inhibition with MMI270 led to a 26% reduction in BMC and 42% in volume compared to Saline at 2 weeks (p<0.01). At 4 weeks a 26% reduction in volume remained (p<0.05), and by 6 weeks no differences were noted. Callus BMD was however increased up to 26% compared to Saline (p<0.01).

Systemic Effects

QCT scans of the proximal tibia revealed expected increases in metaphyseal bone content, volume and density with OPG, ZA and CLOD compared to Saline at all time points (p<0.01). These increases reached a magnitude of 171% with OPG treatment in metaphyseal BMC. MMI270 treatment produced small but significant increases in metaphyseal BMD at 2 and 4 weeks compared to Saline (p<0.05).

Growth plate height was significantly affected with MMI270 treatment with up to an 83% increase at 4 weeks, due to an increased hypertrophic zone (p<0.01). Interestingly OPG also led to a significant increase in growth plate height of 70% at 6 weeks compared to Saline (p<0.01). Osteoclast numbers were significantly reduced up to 99% and accordingly serum TRAP levels decreased with OPG treatment compared to saline (p<0.01).

Conclusion

Inhibition of osteoclast function with BP treatment and inhibition of osteoclast formation with OPG did not interfere with the process of endochondral fracture union in this study, supporting our hypothesis that osteoclasts are redundant in this process. Thus while osteoclasts do secrete MMPs and may normally play a role in endochondral union, other cells rescue the MMP function in the absence of osteoclasts. In contrast, MMP inhibition with MMI270 led to significant delays in cartilage removal, confirming the importance of these proteases, rather than a specific cell type, to the progression of endochondral repair.

Although the anti-osteoclastic agents did not hinder initial union, the larger calluses show signs of delayed remodelling. Additional time points need to be examined to determine reversibility of these effects.

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Poster No. 779 • 55th Annual Meeting of the Orthopaedic Research Society