MRI of Osteoclasts In Mouse Calvarial Bone Organ Cultures Using $\alpha_v\beta_3$ Integrin-Targeted Molecular Contrast Agent

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Introduction: The recent emergence of molecular imaging as an integrated discipline in research and diagnostic imaging may provide a powerful method to monitor the activity of osteoclasts *in vivo*. Studies have shown that $\alpha_v\beta_3$ integrin receptors are highly expressed in both angiogenic vessels and actively resorbing osteoclasts [1]. Antibody targeting of a magnetic resonance imaging (MRI) contrast agent to the $\alpha_v\beta_3$ integrin in angiogenic vessels has been recently reported [2]. In resorbing osteoclasts, $\alpha_v\beta_3$ is distributed throughout the osteoclast cell plasma membrane. Our hypothesis is that Gd particles bound to $\alpha_v\beta_3$ antibody – polylysine peptide – poly metal chelating complexes (APPMCC) can be used to selectively enhance the local MRI intensity on the surface of actively remodeling bone, as shown in a mouse calvarium organ culture model.

Materials and Methods: Calvaria of 1 week old mice were harvested and organ cultured in the presence of parathyroid hormone (PTH-treated group) or its absence (control group). In this bone organ culture system treatment with PTH results in the formation of a large number of resorptive osteoclasts, whereas in the absence of PTH very few or no osteoclasts are observable. These samples were then treated with APPMCC (targeted group) or with Gd-DTPA contrast agent alone (nontargeted group), and washed three times with clean buffer prior to imaging. The four experimental groups (PTH-treated/nontreated, targeted/nontargeted contrast agent) were then scanned. T_1-weighted proton spin-echo MR images of samples immersed in buffer were acquired with a Bruker Avance 4.7 T MRI scanner. Imaging parameters were TR = 500 msec, TE = 10 msec, FOV = 2 × 2 cm, data matrix = 128 × 128, slice thickness = 1 mm, yielding an in-plane resolution of 156 × 156 μm.

Results and Discussion: T_1-weighted imaging of targeted cultured bone specimens demonstrated a substantial signal enhancement effect on the endocranial side (which contains multiple resorbing osteoclasts as visualized by Neutral Red staining) of PTH-treated bone specimens over the 2-hour time course of imaging as compared with the controls (not treated with PTH). There was some localized enhancement in nontargeted specimens observed at one-half hour following treatment with nontargeted agent on either PTH-treated or non-PTH treated samples, which is due to the residual free contrast agent on the specimen surfaces. However, there is no enhancement in specimens imaged one hour or more after treatment of nontargeted agents. Energy dispersive x-ray spectroscopy (EDS) showed a significant Gd peak in the \specimens which were both integrin-targeted and PTH-treated, whereas there was no detectable Gd in EDS spectra of nontargeted PTH-treated specimens.

Conclusion: These studies show that osteoclast activity and bone resorption phenomena can potentially be monitored by means of T_1-weighted MRI with the use of suitable molecular imaging agents.

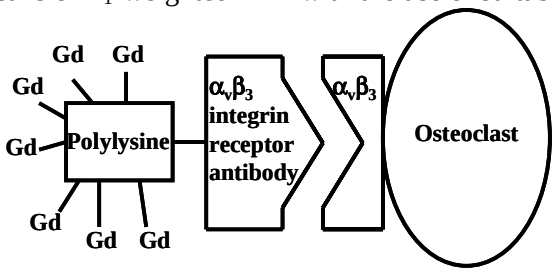

Figure 1. Schematic representation of antibody-linked Gd^{3+} MRI contrast agent (left) binding to an integrin recognition site on an actively resorbing osteoclast (right).

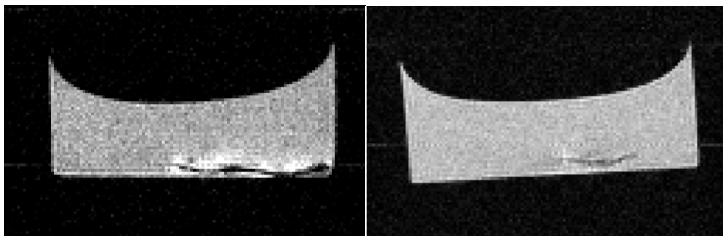

Figure 2. ^1H T_1-weighted spin-echo images of mouse calvarium cultured bone specimens (thin, dark, horizontal feature at bottom of solution). *Left:* treated first with parathyroid hormone, imaged using the integrin-targeted contrast agent. *Right:* treated with parathyroid hormone, imaged using conventional Gd-DTPA contrast agent. Substantial local contrast is observed with the targeted contrast agent.

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