Site-Specific Osteoclast-Mediated Cell Therapy for Vascular Calcification

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Introduction: Vascular calcification occurs during physiological aging and is clinically recognized as a major risk factor for myocardial infarction, systolic hypertension, heart failure and coronary insufficiency caused by loss of aortic recoil[1]. Increasing evidence suggests that vascular calcification share features with skeletal bone formation such as bone matrix deposition and bone resorption[2]. In bone a homeostasis is maintained by its two major cell types; osteoblasts, bone forming cells and osteoclasts, bone resorbing cells, which are derived from the monocyte/macrophage lineage of bone marrow progenitor cells. Bone marrow progenitor cells differentiate into osteoclasts by cytokines released by neighboring cells like activated T-lymphocytes, osteoblasts or bone marrow stromal cells. RANKL and Vitamin D_3 are examples of the cytokines that can stimulate osteoclast activation[3,4]. Osteoclasts have proven effective in demineralizing calcified elastin both in vitro and in vivo[5]. The objective of the study was to evaluate the use of osteoclasts as a cell therapy to treat vascular calcification in an abdominal aortic injury model.

Materials and Methods:

Osteoclast Cell Culture: Rat bone marrow-derived progenitor cells were differentiated into osteoclasts by incubation in Vitamin D_3 / Retinoic acid-supplemented Iscove's media (Sigma) at 37°C and 5% CO_2 for 14 days.

In vivo Study: Adult male Sprague-Dawley rats weighing 250-300g were placed under general anesthesia (2-3% isoflurane) and the infrarenal abdominal aorta treated for 15 minutes with 0.15M $CaCl_2$ by gauze application, which involved placing a rectangle (1.5 x 0.5 cm) of presoaked, 8-ply medical grade sterile cotton gauze on the exposed aorta. The experimental group received an application of collagen gel incorporated with osteoclasts which were fluorescently labeled with the cell-tracker DDAO-SE (Molecular Probes, C34553) following the gauze application. After 7 days, the animals were humanely euthanized using CO_2 asphyxiation; the abdominal aorta excised and segments from each aorta processed for histological analysis and calcium analysis by atomic adsorption spectrophotometry.

Results and Conclusions: Medial calcification was observed in histological analysis and osteoclasts were observed using fluorescent imaging of the arteries. However, osteoclasts were only observed in the adventitial tissue and calcification was seen in the media (Figure 1).

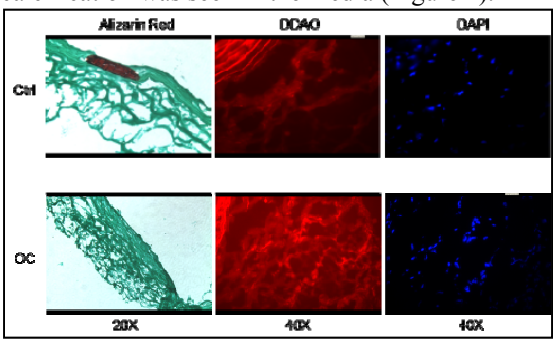

Figure 1. Histological analysis shows medial calcification induced by $CaCl_2$ treatment and the presence of DDAO-labeled OCs in the adventitia.

Calcium analysis did not show a reduction in calcification of the abdominal aorta in the presence of osteoclasts (data not shown). We conclude that osteoclast delivery was successful; but that osteoclasts were unable to migrate to the medial layers where calcification was prevalent. The future challenge will be to deliver osteoclast cells to the medial layer of the artery. Some sort of native macrophage recruitment strategy would be more effective in preventing or regressing arterial calcification. Future work will include incorporating RANKL into the collagen gel to recruit native macrophage /monocyte cells.

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