IN VIVO MECHANISM FOR CALCIUM SULFATE BONE GRAFT SUBSTITUTE

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Introduction

The biological mechanism of calcium sulfate as a bone graft substitute remains unknown. The dissolution of calcium sulfate acidifies the local environment due to a lowering of pH. The reduction in local pH may locally demineralize bone and stimulate bone formation through a mechanism similar to demineralized bone. A bilateral critical size confined cancellous bone defect ovine model was used to examine the hypothesis that the biological response to calcium sulfate is through local demineralization as result of the acidic environment and release of osteoinductive proteins.

Materials and Methods

Forty-two adult crossbred wethers (> 45 kg) were used following approval of the Animal Care and Ethics Committee. Four different groups were examined; Empty defect (Empty); Calcium sulfate (Osteoset®) alone (OS); Autograft alone (Auto); Calcium sulfate (Osteoset®) plus Autograft (OS+Auto). A bilateral medial distal femoral confined cancellous defect (15 deep and 12.6 mm wide) was drilled in stages into both limbs. The dimensions of the defect were reproduced by modification of the instrument used to prepare the patella in total knee arthroplasty. 18 – 20 Osteoset® pellets were randomly placed in the defects. The bone from the drilling remnants was used as the autograft. The periosteum was re-approximated over the defect and the skin closed. An in-vitro study on the pH of a solution of calcium sulfate (Osteoset®) in phosphate buffered saline (pH 7.5) was performed versus time.

All animals were sacrificed at 2, 4, 8 and 12 weeks following surgery. Lateral radiographs and computed tomography (CT) images were taken following harvest. The distal femora were fixed in cold phosphate buffered saline, decalcified in 10 % formic acid – formalin and paraffin embedded. Five-micron thick sections were cut and stained with H&E and Masson Trichrome. Histomorphometric analysis of bone in the defect was quantified using a threshold analysis using Global LabView Image. TGF-ß, BMP 2, 7, 8 and BMP-6 were reproduced by modification of the instrument used to prepare the patella in total knee arthroplasty. 18 – 20 Osteoset® pellets were randomly placed in the defects. The bone from the drilling remnants was used as the autograft. The periosteum was re-approximated over the defect and the skin closed. An in-vitro study on the pH of a solution of calcium sulfate (Osteoset®) in phosphate buffered saline (pH 7.5) was performed versus time.

Results

The in-vitro pH experiment revealed a significant lowering of the pH within 6 hours to pH 5.1 following dissolution of calcium sulfate. All animals recovered from surgery without complication and were load-bearing within 24 hours following surgery. Plain radiographs revealed cortical closure by 12 weeks. CTs confirmed the critical size nature of the cancellous defect in the empty group (E) at 12 weeks. CTs revealed progressive resorption of calcium sulfate with time and new bone formation as early as 2 weeks in the calcium sulfate groups. By 12 weeks the OS group presented a closed cortex with new bone formation demonstrating its bone forming ability. The empty defects (E) did not fill with bone during the 12-week study evaluation period revealing the critical size nature.

The results from the current study support a hypothesis regarding one possible mechanism for calcium sulfate and the bony response due, in part, to local demineralization as the material dissolves in the defect. The in-vitro pH study confirmed the local acidification which establishes an environment which can demineralize bone contained within the defect as well bone along the margins. Other physiochemical mechanism may be involved in the action of calcium sulfate as proposed by Ricci but were not examined in the current study.

The combination of Osteoset® with autograft stimulated the most BMPs and TGFß response. Smad 1, 4, 5 expression supports the molecular response to calcium sulfate mediated through a signal transduction pathway. These results support a relationship between the dissolution of calcium sulfate and the reduction in pH. Calcium sulfates ability to induce bone formation in-vivo may be related, in part, to the local demineralisation of bone and the release of osteoinductive molecules in the bone matrix stimulating the healing process. Calcium sulfate is an osteoconductive bone graft substitute that may also at as a bone graft accelerator through this biological pathway.

Immunohistochemistry: The empty defect did not demonstrate any immunostaining in the defect area. Newly formed bone in the OS group showed the presence of TGFß staining in over 50% of the formed bone at 12 weeks. The OS + Auto group showed higher density of bone islands and a richer cellular response compared to all others. TGFß stained over 60% of bone matrix and most ‘active’ osteoblasts. The Autograft group had less cellular response and rare ‘active’ osteoblasts at 12 weeks. Approximately 30% of the bone matrix present in the defect stained positive to TGFß. Immunostaining for TGFß, BMP-2, 7, 8 was most pronounced in the OS + Auto group in the defect region. The grading for the OS + Auto group at 12 weeks is summarized in table 1.

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<th>BM</th>
<th>AOB</th>
<th>SC</th>
<th>AFB</th>
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<td>BMP-8</td>
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BM: bone matrix, AOB: active osteoblast, SC: stroma cell, AFB: active fibroblast, PC: proliferating chondrocyte, MC: mature chondrocyte

Smads 1, 4 and 5 were not detected in the empty defect. Smad immunostaining was evident in the OS, Auto and Auto + OS group by 2 weeks and continued with time.

Discussion

Calcium sulfate has a long clinical history as a bone graft substitute. The application of calcium sulfate (Osteoset®), alone resulted in new bone formation demonstrating its bone forming ability. The empty defects (E) did not fill with bone during the 12-week study evaluation period revealing the critical size nature.

The results from the current study support a hypothesis regarding one possible mechanism for calcium sulfate and the bony response due, in part, to local demineralization as the material dissolves in the defect. The in-vitro pH study confirmed the local acidification which establishes an environment which can demineralize bone contained within the defect as well bone along the margins. Other physiochemical mechanism may be involved in the action of calcium sulfate as proposed by Ricci but were not examined in the current study.

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