COMPARISON OF AUTO, ISO, AND ALLOGRAFTS AND THE EFFECT OF NSAIDS ON BONE HEALING IN A NOVEL MURINE SEGMENTAL FEMORAL MODEL

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Introduction:

Structural bone allografts are frequently used to reconstruct bone defects in the extremities as well as for spinal fusion surgery. Failed healing at the graft-host junction is one of the most frequent complications of allograft surgery. However, the factors that regulate healing and graft incorporation remain poorly understood. The goal of these studies is to examine the molecular mechanisms of allograft healing and remodeling.

For this purpose, we developed a murine segmental femoral allograft model, and have compared the healing of cortical-cortical junctions in autogenous, isogenic and allogeneic grafts. These studies demonstrate distinct healing patterns of the three grafts. We also examined the effects of non-steroidal anti-inflammatory drugs (NSAIDs), specifically ketorolac and celecoxib on allograft healing in this model. We showed that autografts have superior healing compared with isografts and allografts, based on a robust periosteal reaction. Additionally, both Ketorolac and celecoxib reduced periosteal bone formation and result in increased non-union rate.

Materials and Methods:

10 wk-old C57BL/6 mice were anesthetized, a 7-8mm long incision was made, and the femur was exposed by blunt dissection. A 4 mm mid-diaphyseal segment was removed by osteotomizing the bone using angled wire scissors. Segments of bone graft were obtained from the femoral shaft of the same animal (autograft), inbred C57BL/6 animal (isograft) or from that of a different strain of mice: 129 (allografts). These 4 mm iso and allografts were sterilized with 70% ethanol and then fresh frozen in –70°C. Prior to re-implantation they were thawed at the room temperature and rinsed in saline to remove residual ethanol. Bone graft was secured with a 22-gauge metal pin and placed in the segmented femur through marrow cavity. The incision was closed by silk suture. Graft healing was followed radiographically and mice were sacrificed at was secured with a 22-gauge metal pin and placed in the segmented temperature and rinsed in saline to remove residual ethanol. Bone graft and was monitored until sacrifice by cervical dislocation.

Drugs were administered until sacrifice by intramuscular injection. Celecoxib was administered by gavage to 24 mice note at 4mg/kg /day by intramuscular injection. Drugs were labelled riboprobes in standard hybridization buffer Nonspecifically bound probe was hydrolyzed with RNase A (20 µg/ul), and washed at high stringency at 55°C with 2X SSC/50% formamide. Emulsion-dipped slides were exposed to beta emissions for 14 days.

Results:

The healing of allogenic, isogenic, or autogenous grafts: To compare the healing of the three different grafts, radiographic and histological analyses were conducted at 2, 3, 4 and 5 weeks post surgery. X-rays demonstrated decreased hard callus formation of both allografts and isografts compared with autografts. Histomorphometry was used to quantify new bone formation on the surface of the graft, as well as upon the surface of the host bone. Periosteal bone formation was decreased on both isografts (57%) and allografts (67%) compared to autografts (Figure 1). We also found reduced new bone formation on host bone in isogenic and allogeneic grafts compared with the autogenous grafts, indicating that the autograft is both osteogenic and osteoinductive. In situ hybridization at 2 and 4 weeks confirmed the reduction in bone formation (col2 and colX expression) and bone formation expression in allografts and isografts, compared with autografts.

Bone union occurred in all autografts by 4 weeks and was characterized by abundant new periosteal bone formation that encompassed the entire allograft. In contrast, bone union in allografts and isografts was delayed and occurred with markedly less bone formation, and relied upon creeping callus from the host or by limited intramembranous bone formation at the graft-host junction. Both isografts and allografts had a 30% rate of histological non-union by week 5, whereas all autografts healed by week 4.

Celecoxib or ketorolac treatment reduces bone allograft periosteal bone formation: Histological analyses were performed to examine healing under the influence of celecoxib and ketorolac. We found that the periosteal bone formation over the graft and the total periosteal bone formation was reduced by about 50% in the two drug treated groups after 4 weeks (p<0.05). No significant differences were observed between the celecoxib and ketorolac treated groups in terms of new bone formation. However, the non-union rate in celecoxib group was increased by 30% (p<0.05) above that seen in the allograft controls, while the 12% increase observed in the ketorolac group was not significant.

Discussion:

While autogenous grafts are both osteoconductive and osteoinductive, allografts are primarily osteoconductive. This murine segmental femoral graft model highlights the differences between these grafts and validates use of this animal model to study the molecular and cellular events that govern allograft healing and remodeling. Interestingly, we found no significant advantage in using isografts over allografts. Our finding suggests that the most critical advantage of autograft is the robust periosteal reaction that is likely mediated by surviving periosteal cells.

Recent studies have linked the use of NSAIDs with a reduced fusion rate in patients undergoing spinal fusion surgery. Additionally, we, and others, have demonstrated that COX-2 plays a critical role in fracture healing in animal models. Here we demonstrate that periosteal bone formation in allografts in NSAID treated mice is significantly reduced. This result points toward a role of COX-2 in the bone allograft healing, and we are currently using knockout mice to determine the role this enzyme in bone graft healing and remodeling.

Figure 1

Figure 2