Chronic Heavy Alcohol Consumption Impairs the Ability of Demineralized Bone Matrix to Support Osteoinduction in Rats

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INTRODUCTION: Due to their beneficial osteoconductive and osteoinductive properties, autografts and allografts play an important role in treatment of patients with bone fractures or deformities. However, graft failures are common. Previous studies have focused on intrinsic factors (e.g., age) influencing bone healing. Few studies have investigated the impact of extrinsic factors (e.g., lifestyle) of the graft donor or graft recipient and its impact on the graft's osteoinduction potential. It is well recognized that osteoblast-derived growth factors deposited into bone during growth and remodeling, and activated during resorption, are important for osteoinduction. We have shown that alcohol suppresses IGF-1 gene expression in bone and lowers IGF-1 levels in blood. Also, we have shown that suppression of this important skeletal growth factor by alcohol can be prevented by treatment with parathyroid hormone. The objective of this study was to test the hypothesis that alcohol consumption, a risk factor for fracture repair, can impair fracture healing by two non-mutually exclusive mechanisms: by lowering osteoinductive capacity, potentially by reducing deposition of growth factors into bone matrix, and by suppressing bone formation during fracture healing.

METHODS:

We performed 3 experiments using 2 osteoinduction models: 1) a demineralized allogeneic bone matrix (DBM) model where DBM is harvested from donor rats fed control or ethanol diet and implanted into recipient rats fed control or ethanol diet, and 2) a critical size defect model where a critical size bone defect is created in fibula of recipient rats, and DBM implants harvested from control or ethanol-fed donors are used to close the defect.

Experiment 1a: Donor implants were harvested from male rats fed control or ethanol diet for 12 weeks and demineralized. Bone matrix IGF-1, an important osteoinductive factor, was measured in the DBM using an enzyme-linked immunosorbent assay. DBM implants were then implanted subcutaneously into 3-month-old male recipient rats (n=12). All implants were placed ventrally with each rat receiving 4 implants: 2 thoracic (prior control and ethanol diet) and 2 abdominal (prior control and ethanol diet). The recipient rats were fed control diet for 6 weeks. Bone formed during osteoinduction was measured ex vivo by micro-computed tomography (μCT).

Experiment 1b: Donor implants were prepared and implanted, as described for Experiment 1, into 3-month-old female recipient rats. The recipient rats were fed control or ethanol diet for 4 weeks prior to DABM implantations and for 3 weeks following implantation. Bone formed was measured as in Experiment 1a.

Experiment 2: Bilateral critical size defects were created in fibula of 2-month-old male rats fed control diet. DBM implants from rats fed control or ethanol diet for 12 weeks were used to close the defect in the recipient rats. The recipient rats were treated with PTH (n=12; 5 μg/d) or carrier (n=13) and sacrificed following 6 weeks of treatment. Bone formed was measured as in Experiment 1. Data were analyzed using t-tests, 1-way analysis of variance, or 2-way analysis of variance. (Figure 2.)

RESULTS:

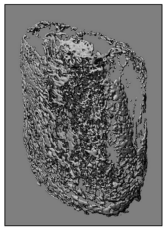
Experiment 1a: IGF-1 was 38% lower in bone matrix harvested from ethanol-fed rats compared to control rats (A). DBM bone volume was 23% lower in DBM recovered 6 weeks following implantation into rats fed control diet, indicating that exposure of the donor rats to ethanol lowered osteoinductive capacity of their bone matrix/

Experiment 1b: Bone volume was greatest in DBM from control donor rats implanted into control host rats (1.57±0.16 mm 3), intermediate in DBM from control donor rats implanted into alcohol consuming host rats (1.12±0.13 mm 3), and lowest in DBM from alcohol fed rats implanted into alcohol consuming rats (0.47±0.12 mm 3) (Figure 1.).

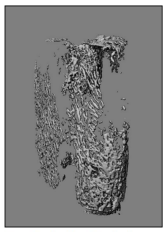
Experiment 2: Ethanol consumption by donor rats resulted in 9% lower DBM bone volume whereas parathyroid hormone treatment resulted in 35% higher DBM bone volume in the critical size defect model. There was no significant interaction between diet consumed by DBM donor rats and parathyroid hormone treatment in recipient rats.

DISCUSSION AND CONCLUSION:

Our findings demonstrate that lifestyle factors may influence fracture healing and graft incorporation by altering the osteoinductive capacity of bone matrix. Specifically, our results suggest that a history of heavy alcohol consumption may impair osteoinduction in allographs and this negative outcome may be worsened by alcohol intake during graft incorporation and fracture healing. Mechanistically, our results implicate reduced bone matrix levels of IGF-1 as contributing to reduced osteoinduction by demineralized bone matrix prepared from alcohol consumers. Additionally, parathyroid hormone, a bone anabolic agent that acts in part by increasing skeletal production of IGF-1, has the potential to increase osteoinduction by demineralized bone matrix generated from control and alcohol-consuming donors. Our findings suggest that a history of chronic heavy alcohol consumption may be an important risk factor for consideration when using bone grafts to augment orthopaedic reconstruction or enhance fracture repair.



Control diet-fed donor DABM implant in recipient fed control diet



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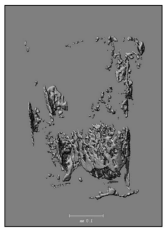

Ethanol diet-fed donor DABM implant in recipient fed ethanol diet