INTRODUCTION

Chronic alcohol consumption can lead to multiple pathologic conditions including osteoporosis. The process of bone remodeling is accomplished by osteoblastic bone formation and osteoclastic bone resorption. It is highly orchestrated, in part, by the molecular proteins receptor activator of NF-κB ligand (RANKL), its receptor RANK, and its decoy receptor and competitive inhibitor, osteoprotegerin (OPG). The RANKL/RANK/OPG triad appears to be dysregulated in chronic alcoholics, resulting in osteoporosis and an increased rate of lumbar spinal fractures. Adipose tissue-derived multipotent stromal cells (ASCs) are being explored in regenerative medicine as a means to promote osteogenesis. It appears osteogenesis can be enhanced with ASCs, but alterations to the microenvironment, as with chronic alcoholism, can limit the efficacy of the cells and, potentially, result in negative outcomes. This study was designed to evaluate the effects of chronic alcohol ingestion on in vivo ASC osteogenesis in a rat spinal fusion model to test the hypothesis that chronic ethanol consumption impairs in vivo osteogenesis of ASCs harvested from alcoholic and non-alcoholic patients via the OPG/RANKL pathway.

MATERIALS AND METHODS

Experimental Design

Inguinal adipose ASCs isolated from two 16-week old male Sprague-Dawley rats and expanded up to four passages. Cells loaded in 1x0.2x0.7cm scaffolds (Vitoss, Orthovita, Malvern, PA) with a perfusion bioreactor (9.33x10^6 cells/cm^3) and cultured overnight in stimulant medium Twenty-four male Sprague-Dawley rats pair fed with an isocaloric liquid diet or 36% ethanol diet from 6 to 16 weeks of age. One member of each feed pair received lumbar fusion with scaffold alone or scaffold-ASCs (n=6/diet/treatment). Spines were harvested six weeks after implantation and sectioned sagittally. One half was prepared for light microscopy and immunohistochemistry (OPG, RANK, RANKL, TRAP) and one half for compositional analysis and mRNOA analysis.

Compositional Analysis

Total protein (Lowery), DNA (picogreen), collagen (hydroxyproline), and sulfated glycosaminoglycan (GAG) (dimethylmethylen blue [DMBB]) were normalized to DNA.

mRNA Quantification

Levels of mRNA corresponding to osteoprotein (OPG), receptor activator of NF-κB (RANK), RANKL, alkaline phosphate (ALP), collagen type 1α1(COL1α1), osteocalcin (OCN), osteopontin (OSP), and 18s rRNA (18S) were quantified with RT-PCR using SYBR Green technology (ABI 7900HT, Applied Biosystems, CA). Values normalized to 18S and fold changes relative to fusion callus from rats on control diets with scaffold alone determined (2-∆∆Ct). Values normalized to 18S and fold changes relative to fusion callus from rats on control diets with scaffold alone determined (2-∆∆Ct).

Statistical Analysis

Data was statistically evaluated with MANOVA models and pair blocking to test group comparisons multivariately (SAS 9.12, Cary, NC). Significance at p < .05 and type I error at P= 0.05.

RESULTS

Spinal fusion callus microstructure varied significantly between cohorts (Fig. 2). Callus microstructure was more mature and organized in the control diet cohorts, and the control diet-scaffold + ASC had the most new bone formation. Spinal fusion was most advanced in the control diet-scaffold + ASC cohort, comparable in both scaffold only cohorts, and least in the alcohol diet-scaffold + ASC cohort. The mRNA levels of bone associated proteins were lowest in the alcohol diet-scaffold + ASC group, while COL1 and RANKL were highest. The alcohol diet-scaffold + ASC cohorts had the highest percentage of positive stained cells for TRAP and RANK, and a higher percentage of osteoclast-like cells positive for OPG and RANKL. The alcohol diet-scaffold + ASC cohorts showed the least OPG staining and the alcohol diet-scaffold with and without showed higher staining for RANK and TRAP (Fig. 4).

CONCLUSION

Based on the results of this study, chronic alcohol ingestions inhibits in vivo osteogenesis, potentially through alterations in the OPG/RANKL cytokine ratio from RANKL up regulation. Down regulation of bone protein mRNA levels in alcohol diet cohorts supports systemic inhibition of bone formation by alcohol ingestion. The effect of alcoholism on bone formation appears to be exacerbated by ASC implantation.

REFERENCES


ACKNOWLEDGMENTS

A special thanks to the members of the Laboratory for Equine and Comparative Orthopedic Research. This study was funded by NIH-NIAAA R15 AA0175.