Mesenchymal Stem Cells (MSCs) Facilitate Fracture Repair in an Alcohol-Induced Impaired Healing Model

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**SUMMARY:**
Repeated pre-injury alcohol exposure impairs fracture healing in the mouse; intravenously administered autologous mesenchymal stem cells are capable of augmenting fracture repair.

**INTRODUCTION:**
Clinical studies have shown alcohol to be a risk factor not only in the incidence of traumatic orthopaedic injuries(1), but also in delayed fracture healing and nonunion(2). Alcohol consumption is a contributing factor in approximately 50% of trauma-related hospital admissions (1), and 25-40% of all orthopaedic trauma patients are intoxicated at the time of hospital admission(3). Data from animal studies suggests that alcohol exposure has an inhibitory effect on fracture repair(4). Our laboratory sought to develop a model of impaired fracture healing based on repeated alcohol exposure. Such a model of impaired healing would add to animal nonunion models employing a critical-sized defect(5). Based on our impaired healing model, we then sought to examine the regenerative effects of an intravenously administered pure population of MSCs. Understanding MSC recruitment patterns and functional contributions to fracture repair will represent one step closer to using MSCs clinically in patients with impaired fracture healing and nonunion.

**METHODS:**
Institutional Animal Care and Use Committee approval was obtained prior to the initiation of experiments. Adult ten-week old C57BL/6 mice were exposed to a two-week alcohol binge via intraperitoneal alcohol injection and subjected to a surgically-created stabilized mid-shaft tibia fracture. Autologous MSCs were isolated by bone marrow immunodepletion from transgenic green fluorescent protein(GFP)-expressing adolescent mice and cultured in highly specific media. Cells were tested for multilineage differentiation potential and MSC surface marker expression. Cultured MSCs were then administered via tail vein to injured animals on post-injury day one. In vivo assessment of MSC localization was performed at daily intervals with a Xenogen fluorescence imaging system. Animals were sacrificed at two weeks following injury, and fractured tibiae were collected and formalin-fixed prior to micro-computed tomography(microCT), biomechanical, and immunohistochemical analysis. Biomechanical analysis was performed via four-point bending with an Instron materials testing machine.

**RESULTS:**
Pre-injury binge alcohol exposure resulted in a significant impairment in biomechanical strength (p<0.001, n=8/group) and callus volume by microCT (p=0.044, n=4/group) of fractured tibiae compared with control animals. Isolated MSCs possessed tri-lineage (osteogenic, chondrogenic, and adipogenic) differentiation potential and expressed MSC-specific surface markers in a high concentration. MSC transplants restored both fracture callus volume (p<0.05, n=4/group, Figure 1) and biomechanical strength (p<0.05, n=8/group) in animals with alcohol-impaired healing. Statistical analyses were performed with one-way ANOVA with Tukey’s post hoc test. In vivo imaging demonstrated a time-dependent MSC migration to the fracture site (Figure 2). Immunohistochemical analysis of GFP-expressing cells showed transplanted MSCs specifically localize to the site of fracture at an endosteal location.

**DISCUSSION:**
We have developed a reproducible model of impaired fracture healing based on repeated pre-injury alcohol exposure in the mouse. Intravenously-administered autologous MSCs are capable of homing to the site of injury and of augmenting fracture repair in animals with alcohol-induced impaired fracture healing. This study provides groundwork for additional studies evaluating the role of MSC therapy in patients with delayed or nonhealing fractures, particularly those patients intoxicated at admission or with a history of alcohol abuse.

**REFERENCES:**

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**FIGURES:**
Figure 1. New bone (callus) volume was impaired in alcohol-treated animals and restored to normal levels in MSC-treated groups (EtOH, alcohol; error bars represent standard error of the mean; n=4/group).

Figure 2. In vivo fluorescence imaging demonstrated time-dependent MSC localization to the fracture site at the right tibia. (PID#, post-injury day).