Local Administration of Non-Diabetic MSCs to Diabetic Femoral Fractures Enhances Callus Remodelling and Deposition of Reparative Bone

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Introduction

It is estimated that 382 million people worldwide have been diagnosed with diabetes, a chronic disorder that compromises the quality of a patient's skeletal tissue and impedes its ability to repair after fracture. Fragility fractures due to low bone strength have become increasingly recognized as a skeletal compli-cation of diabetes mellitus. As a result of this dysfunctional repair process, long bone fractures in diabetic patients often result in complicated cases of fracture non-union or angulated mal-union, thereby reducing an individual's mobility and limb function. The causative aberrant bone mineral density and insufficient bone microstructure of diabetic patients is thought to result from altered osteoblast and osteocyte function, increased bone marrow adiposity, decreased progenitor osteo- and chondral differentiation potential. It is therefore reasonable to hypothesize the root cause of faulty diabetic bone homeostasis and fracture repair is a reduced population of bone marrow progenitor cells (MSCs) and/or their decreased osteochondral capacity. Here we investigated the therapeutic efficacy of locally administered non-diabetic human MSCs to support femoral fracture repair in a murine model of diabetes.

Methods

MSC Isolation, Culture Expansion: Human bone marrow was obtained from the iliac crest of healthy donors aged 18-35 years. All procedures were performed with informed consent and ethically approved by the Clinical Research Ethical Committee at University College Hospital, Galway. The marrow was diluted in phosphate buffered saline and centrifuged. The spun cells were washed and plated at a density of ~40,000 mononuclear cells/cm^2 and 2,800 – 5,600 cells/cm^2 thereafter. Complete MSC expansion medium [alpha MEM with Glutamax, 10% selected fetal bovine serum, 1% penicillin/streptomycin, 1% non-essential amino acids and 1ng/ml fibroblast growth factor] was added to the MSC culture in support of cellular proliferation. Cells were incubated at 37ºC with 2%O_2, 5% CO_2 in a humidified chamber.

Pre-Clinical Model of Diabetes: All pre-clinical experiments were reviewed and approved by the University animal welfare committee as well as the Irish Health Products Regulatory Authority. Diabetes was induced in male C57/B6 mice with intraperitoneal injections of streptozotocin. At surgery, the mouse was anesthetized and placed in a supine position with the knee flexed to 90º. A medial 6mm straight incision was created, a blunt separation was made along the medial patella ligament to expose the joint capsule. The patella was dislocated laterally. A hole was created between the femoral condyles and a 27 gauge needle was inserted into the femoral cavity. The patella was returned to position and the articular capsule was closed with biodegradable 6-0 Vicryl sutures. A transverse fracture was created as described by Marturano et al (2008). Seven days after fracture creation, MSCs resuspended in 50 microliters of saline were directly administered to the fracture site. Fifty six days after fracture creation mice were sacrificed and fracture repair assessed.

Results

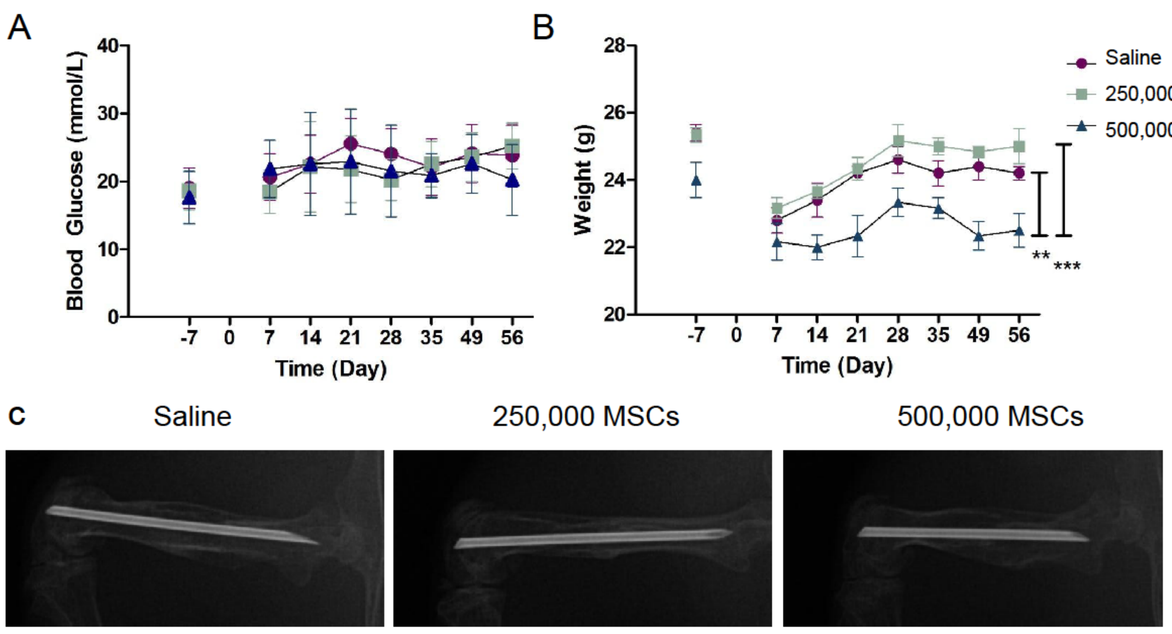

Figure 1: MSC administration does not reverse the diabetic condition. A) All mice enrolled in the study exhibited high glucose content (>13 mmol/L) for 3+ weeks before fracture creation (day 0). Blood glucose levels remained consistent for the remaining 56 days of the study. B) Animal weight was maintained through the duration of the study within each treatment group. Although mice treated with 250,000 MSCs and saline treated animals had group mean body weights significantly increased over animals receiving 500,000 MSCs, this observation was considered incidental. C) Radiographic imaging of fractured femurs demonstrate non-unions in all treatment groups including the absence of bridging, calcified bone and the persistence of a cartilaginous callus. ** p≤0.01, ***p≤0.005

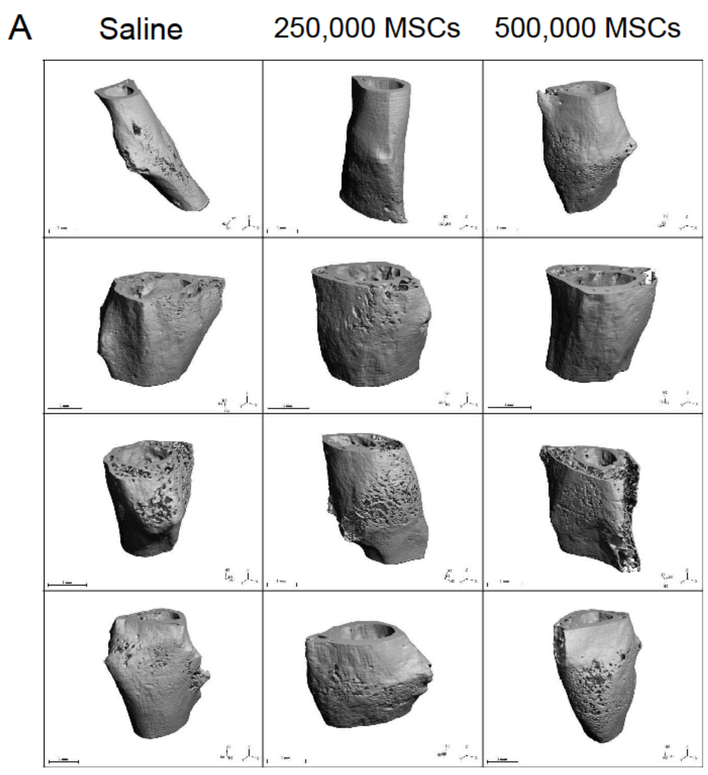

Figure 2: µCT imaging demonstrates callus retention 56 days after fracture creation. A) µCT imaging enables visualization of the fractured cortical bone and the persistence of a mineralized callus 56 days after fracture creation. B) Quantification of the callus circumference indicates a reduction of the callus in fractures treated with 250,000 MSCs.

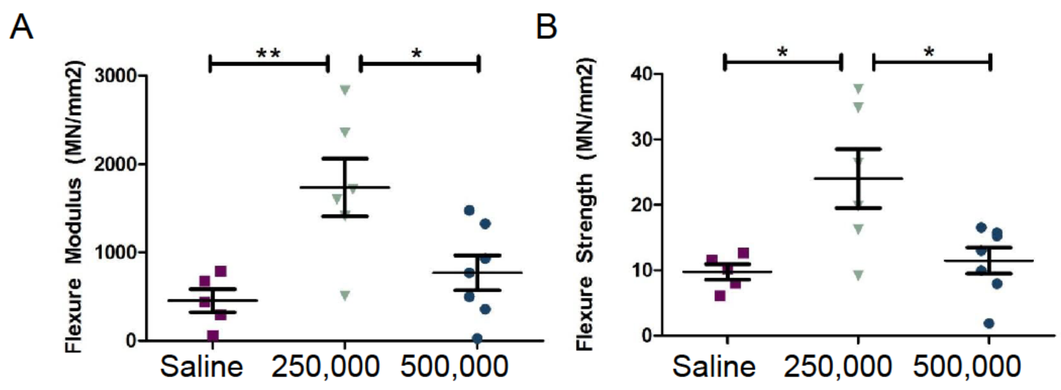

Figure 3: MSC-treatment improves mechanical integrity. Four point bending analysis demonstrated improvement in the (A) flexure modulus and (B) flexure strength withstood by fractures treated with 250,000 cells as compared to control. * p≤0.05, ** p≤0.01

		Heart	Spleen	Muscle	Liver	Stomach	Pancreas	Kidney	Lung	Small Intestine	Large Intestine
Saline	Mean	35.07	34.19	34.55	35.22	34.85	35.14	35.08	35.35	35.34	35.71
	SD	0.60	1.44	0.54	0.93	0.72	0.63	0.35	0.56	0.74	0.47
hMSC	Mean	35.20	34.93	35.26	35.45	35.34	35.28	36.21	35.32	35.93	35.49
	SD	0.58	0.60	0.77	0.97	0.59	0.80	1.02	1.58	0.69	1.17

Figure 4: qPCR analysis for hALU demonstrates the absence of human DNA in distal murine tissues. Whole organ gDNA isolation, followed by qPCR analysis for hALU, enabled the detection of hDNA residing in murine organs. Ct values <32 indicated the presence of hDNA. All samples (n=4) were negative for human DNA, indicating hMSCs are not retained 56 days after administration.

Conclusion

The therapeutic efficacy of locally administered non-diabetic human MSCs to support femoral fracture repair in a murine model of diabetes is here confirmed. Although the diabetic condition is not improved with MSC administration, as indicated by blood glucose levels and persistent fracture mal-union, administration of 250,000 MSCs resulted in reduced callus volume and enhanced mechanical strength in the repairing fracture. There is no evidence of the reparative cells being retained within the host, indicating their mechanism of action is acute, perhaps by supporting the development of an early callus or modulating inflammation. These data warrant the further investigation into the efficacy of treating diabetic fractures with low doses of MSCs and their reparative mechanism.

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