Bone Marrow-Derived Mesenchymal Stem Cells Yield Greater Pain Relief and Tissue Protection than Umbilical Cord Tissue-Derived Cells in a Surgically Induced Instability Model of Osteoarthritis

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INTRODUCTION: Intra-articular mesenchymal stem cell (MSC) injections are rapidly increasing as a viable treatment option to relieve pain and slow disease progression of osteoarthritis (OA). Clinical and preclinical studies have demonstrated efficacious effects, yet variability in treatment protocols, donor source, and patient status have considerably slowed translation to a consistent therapy. Bone marrow (BM) and adipose tissue are the most common MSC sources currently in clinical use (typically as a heterogenous cell mixture such as bone marrow aspirate concentrate (BMAC)), but require point-of-care harvest for autologous use that may have reduced quality in older and/or osteoarthritic individuals (1). Postnatal tissues, including the umbilical cord tissue (UCT), have gained popularity as an alternative, autologous MSC source due to their lack of ethical concerns, compatibility with manufacturing (high proliferation and cell line stability), and high transcriptional consistency (2). Variations in microenvironment, ontogenic age, and cell heterogeneity between sources could impact MSC efficacy for OA. In this project, we directly compared the therapeutic efficacy of human BM- and UCT-MSCs in relieving OA pain and slowing tissue degradation in a preclinical rat model. To investigate the potential role of cellular heterogeneity, we also evaluated BMAC in a pilot experiment. We hypothesized that efficacy would differ between MSC sources, but that UCT-MSCs would provide a less variable source among donors.

METHODS: BMAC and UCT-MSCs were provided by our collaborators from an ongoing multicenter clinical trial (MILES; NCT03818737). BM-MSCs were generated by in vitro expansion of BMAC. We assessed donor variability by including cells from three UCT donors (including one donor with two production lots) and four BM donors. In vivo experiments were completed in four rounds. Male Lewis rats (12 wk, Charles River) were used. Experimental procedures were approved by IACUC. We used the medial meniscal transection (MMT) model to surgically destabilize the left knee and induce post-traumatic (PT) OA (sham was a medial collateral ligament transection). All rats that underwent MMT received i.a. injections at 3 weeks after surgery of 50 µL HBSS with or without $1x10^6$ cells (N=8 per cell donor). Mechanical allodynia and spontaneous gait were measured longitudinally. All gait parameters were corrected against a healthy database for mass and velocity. The study ended at 6 weeks. Fixed tibiae were imaged using contrast enhanced micro-computed tomography. As a pilot experiment, we replicated the study design with BMAC injection from two donors. Differences among groups were first evaluated using ANOVA (or Wilcoxon) with donors pooled prior to assessing differences among donors per cell source.

RESULTS: PTOA was induced with MMT, resulting in tibial cartilage degeneration (**Fig. 1A-D**), gait dysfunction (**Fig. 1E-G**), and mechanical allodynia (**Fig. 1H**). MMT rats displayed a dysfunctional shuffle step gait as detected by a principal component analysis (**Fig. 1E**), including greater hindlimb duty factor (**Fig. 1F**) and shorter hindlimb stride lengths (**Fig. 1G**, p<0.001). BM-MSCs were more therapeutic than the UCT-MSCs in relieving pain, improving function, and slowing tissue degradation. Mechanical allodynia, which was only apparent in untreated rats at 6 weeks, was reduced in the BM-MSCs compared to the saline (p=0.004) and UCT-MSCs (p=0.008) injected rats (**Fig. 1H**). Shuffle step dysfunction was resolved in the BM-MSC (p=0.003) but not the UCT-MSC treated groups (**Fig. 1E**) as UCT-MSC rats walked with a decreased step length (**Fig. 1G**, p=0.002). UCT-MSC rats still displayed pathological thickening of the cartilage (**Fig. 1B**, p<0.001) and increased cartilage attenuation (associated with loss of proteoglycans, **Fig. 1C**, p<0.0001), whereas BM-MSCs returned both parameters to be comparable to sham animals. Once correcting for mass (which differed between studies but did not affect other morphological parameters), both UCT- (p=0.015) and BM-MSCs (p=0.006) decreased cartilage lesions (**Fig. 1D**). We observed variable efficacy among donors of both sources, with differences in correcting gait dysfunction (**Fig. 1E**) and preserving cartilage composition (**Fig. 1C**). We then questioned whether in vitro expansion of the MSC population in bone marrow was required for therapeutic benefit. Our preliminary data suggest that BMAC is effective in reducing allodynic pain (**Fig. 1H**, p<0.001) and can qualitatively preserve tissue quality to similar levels as the isolated BM-MSCs (**Fig. 1I**).

DISCUSSION: In this study, we demonstrated that BM-MSCs decreased allodynia and enhanced tissue preservation compared to UCT-MSCs in a preclinical model. We and others have previously demonstrated that not all MSCs are alike despite exhibiting similar cell surface antigens. This heterogeneity can be classified in three ways: tissue source, donor, and intra-product (single cell). MSCs from various tissues separately cluster in genomic and proteomic profiling based primarily on source. Allogeneic cells, such as UCT-MSCs, are desirable in part due to high manufacturability and cell line stability, potentially reducing inter-donor variability. Contrary to our hypothesis, we observed similar donor variability in both cell sources even though our BM-MSCs were harvested from individuals undergoing management of OA. Our data suggests superiority of BM-MSCs in this controlled, repeatable preclinical model, which could arise for two reasons. First, the microenvironment of the harvested cells may play a more important role in efficacy than ontogenic age. Unlike the cord tissue, bone marrow in OA individuals could be exposed to similar stimuli that cells will encounter intra-articular and thus be primed for response. Second, the BM-MSCs, which were adherent cells cultured from BMAC, are likely to be a more heterogeneous population (3) than UCT-MSCs, which are largely homogenous (4). OA is complex, diverse disease of the whole joint; a broader response, inherent to increased heterogeneity and plasticity in BM-MSCs than UCT-MSCs, could therefore provide superior analgesia and tissue protection. This possibility is supported by our preliminary experiments that indicate that BMAC – a highly heterogeneous cell population – can provide similar benefits as the isolated BM-MSCs.

CLINICAL RELEVANCE: Despite the apparent advantages of allogeneic UCT-MSCs in minimizing systemic donor effects on cell quality and avoiding cell harvest, BM-MSCs provide greater pain relief and disease modification in early PTOA.

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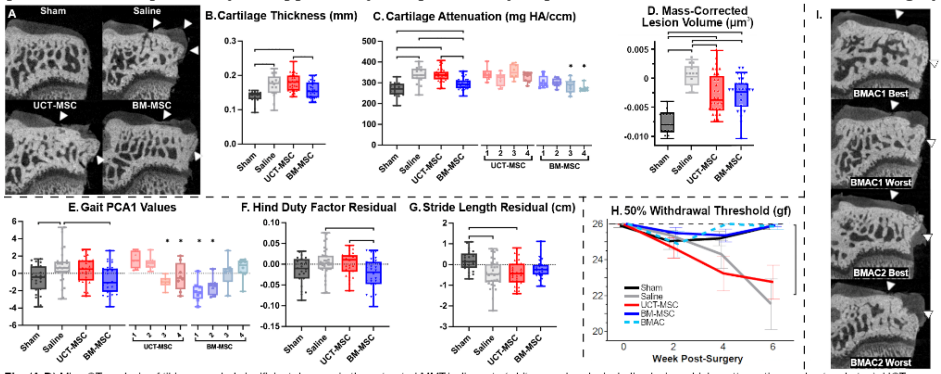

Fig. (A-D) MicroCT analysis of tibiae revealed significant damage in the untreated MMT/saline rats (white arrowheads, including lesions, higher attenuation, and osteophytes). UCT-MSCs did little to prevent tissue damage, but BM-MSCs significantly improved cartilage thickness and attenuation (inversely proportional to proteoglycan content). These effects may be donor dependent however with only 2/4 BM-MSC donors improving attenuation values (indicated by asterisk). After correcting for mass, both cell sources reduced lesion volume. **(E-G)** MMT rats also displayed abnormal gait, as indicated by separation along PCA1. These modifications were associated with a shuffle-step, including increased hind duty factor and decreased stride length. Only BM-MSCs improved gait, although both UCT- and BM-MSC effects were donor specific (asterisks in E). **(H)** MMT rats displayed mechanical allodynia by 6 weeks. BM-MSCs relieved allodynic pain, but UCT-MSCs did not. Our pilot experiment shows that BMAC provides similar analgesia as BM-MSCs. (I) CT images of best and worst tissue preservation per donor in the BMAC pilot shows overall positive protection of cartilage integrity and composition (attenuation) though osteophytes appear similar to saline group.