Evaluation of Safety, Stem Cell Viability, and Osteochondral Response of Repeat Intra-Articular Allogeneic Mesenchymal Stem Cell Injections in MHC Mismatched Horses

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INTRODUCTION: Allogeneic mesenchymal stem cells (MSCs) are a promising source of treatment for osteoarthritis. However, the efficacy and safety of intra-articular major histocompatibility complex (MHC) mis-matched stem cells is controversial. Despite these concerns, studies have demonstrated the safe use of allogeneic bone marrow derived MSCs (BM-MSC). Investigations have been limited to repeat injections from one donor only, and intra-articular synovial fluid viability has only been assessed after a prolonged time (30 days) following stem cell injection [1,2]. There is a lack of reported quantitative information on the effects of intra-articular, allogeneic, MHC mismatched BM-MSCs on bone and cartilage.

METHODS: Two previously tested geldings served as MHC mis-matched BM-MSC donors. Twelve mares (AAEP lameness grade ≤3/5) were divided into control (n=6) and recipient (n=6) groups. Recipient horses had a unilateral limb assigned to MSC metacarpophalangeal joint (MCPJ) injection, with contralateral limbs serving as an internal control. A unilateral limb from control horses served as an external control. Stem cell MCPJs had 20 x 10^6 BM-MSCs resuspended in 1.5 mL sterile saline from Donor Horse A (n=3) or B (n=3) injected on day 0 and from the alternate donor's BM-MSCs on day 21. All procedures were performed as previously described [1]. Control joints (recipient contralateral joints and control horses) had 1.5 mLs of sterile saline injected on days 0 and 21. Synovial fluid (SF) was collected via needle arthrocentesis on days 0, 7, 21, 28, and 42, and cytology was analyzed. Synovial fluid MSCs were cultured (passaged at ≥80% confluency) and DNA haplotyped. Physical exams and quantitative lameness evaluations utilizing a body-mounted inertial sensor system (Equinosis QTM) were performed. Horses received phenylbutazone if an increase in lameness was observed. Markers of cartilage formation (CPII; IBEX Diagnostics), cartilage degradation (C2C; IBEX Diagnostics), bone formation (Osteocalcin; Quidel Corporation), bone resorption (CTX-I; Immunodiagnostic Systems), and inflammation (PGE_2; Assay Design) were performed on samples collected on days 0, 7, 21, and 28. Data was analyzed using mixed model analysis for repeated measures. Significance was set at $p \leq 0.05$.

RESULTS SECTION: Total nucleated cell count (TNCC) was within normal limits for all joints throughout the study. Stem cell limbs (448-1716 cells/uL) had higher TNCC than all control limbs (183-633 cells/uL) at days 21-42 ($p \leq 0.0007$). There was no significant difference in SF color between joints ($p \geq 0.58$). Limb edema and joint effusion were greater in stem cell limbs than control limbs for 1-7 days following injections ($p \leq 0.02$). Horses never demonstrated pain or discomfort observable at a walk. Lameness scores for stem cell limbs were overall greater than control horse limbs 3 and 7 days after initial injection ($p \leq 0.04$), with no difference between groups for the remaining days. Synovial fluid derived stem cells were grown in culture from all collected joint fluid, and were verified to be from recipient horses in stem cell joints. There was an increase in the number of days cells took to grow before first passage in all groups from day 0 to 21 and 42 ($p \leq 0.0095$). Stem cell joint cells took longer to grow than internal control joints from days 7-28 ($p \leq 0.02$), but not day 42. Stem cell joint cells took longer to grow than external controls only at day 7 ($p=0.01$), but not days 21-42. Stem cell limbs were higher in CPII than control joints at day 7 ($p \leq 0.007$), no different from all groups at day 21, and higher than internal control joints at day 28 ($p<0.0002$). Stem cell limbs were higher in C2C than control joints at day 7 ($p \leq 0.0017$), no different from all groups at day 21, and higher than control horse joints at day 28 ($p=0.009$) with no significant difference from internal control joints ($p=0.0575$). There was no difference in Osteocalcin between groups at all time points except day 21, when stem cell joints were higher than internal control joints ($p=0.01$). There was no difference in CTXI between groups at all time points except day 28, when stem cell joints were lower than internal control joints ($p=0.02$). Stem cell limbs were higher in PGE_2 than control joints at days 7-28 ($p \leq 0.03$).

DISCUSSION: While stem cells took slightly longer to grow from joints receiving stem cell injections compared to internal controls, all joints experienced an increase in the length of time it took cells to grow compared to baseline, indicating an effect of arthrocentesis on SF-MSC culture growth rates. Intra-articular allogeneic MSCs affected cartilage biomarkers consistently 7 days following injections, with no appreciable difference at day 21. Cartilage effects seemed to favor formation over degradation. Minor bone biomarker changes were observed, including bone formation higher in stem cell limbs over internal controls at 3 weeks following stem cell injection, and bone degradation lower in stem cell joints 7 days after the second injection. This may indicate favorable responses from bone to MSCs, but poor exposure of bone to MSC with intra-articular injections. It is unknown if increased Type I and II collagen remodeling (degradation and formation) is necessary for healing. MSC injections are used at times to stimulate healing, which would likely be followed by an increase in biomarker levels. However, increased biomarker levels have been associated with osteoarthritis formation. More information is needed in regards to the osteochondral effects of MSC treatments.

SIGNIFICANCE/CLINICAL RELEVANCE: Allogeneic stem cells from two different MHC mis-matched donors were safe for horses, and cartilage effects seemed to favor formation over degradation. Limited change to bone biomarkers with intra-articular injection may indicate that intra-articular MSC injection may not be an effective treatment for bone in osteoarthritis.

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ACKNOWLEDGEMENTS: This study was funded by a Regenerative Medicine Stem Cell Alliance Fund Grant and a Tennessee Equine Veterinary Research Organization Grant. We would like to thank E. Austad, R. Hamilton, I. Heidel, S. Jackson, J. Richards, and J. Wayman for assisting with sample collection.