Age and Sex Influence Bone Marrow Cell Therapy Efficacy in an Aged Rodent Model of Post-Traumatic Osteoarthritis

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INTRODUCTION: There is a critical need to develop treatments that slow, or reverse, post-traumatic osteoarthritis (PTOA) progression in young and middle-aged individuals to prevent chronic pain in later life. Bone marrow aspirate concentrate (BMAC) is a leading point-of-care biologic currently employed to reduce intra-articular inflammation and relieve pain. While clinical data on BMAC efficacy varies due to inconsistent protocols, the consensus supports its safety profile and likelihood of providing at least short-term pain benefits. Many valuable studies have investigated these cellular therapies for PTOA in clinically relevant animal models; yet most have used allogenic cells from young, healthy donors that fail to recapitulate autologous sources (i.e., older and osteoarthritic donors) approved for clinical use. Marrow fat content increases with age while nucleated cell counts decrease, with these changes being particularly pronounced in women[1]. Additionally, mesenchymal stem cells (MSCs) from older donors exhibit increased senescence, decreased proliferation capacity, and limited differentiation potential. In osteoarthritic patients, MSCs further demonstrate reduced proliferation and increased adiposity[2]. These compositional and functional changes likely impact the immune modulatory and pain-relieving properties of BMAC, yet the relationship between patient factors and therapeutic potential remains poorly understood. We hypothesize that age-associated compositional changes in the bone marrow – particularly pronounced in females—will reduce both the pain-relieving and tissue-preserving capabilities of cellular products in a PTOA model using middle-aged rats.

METHODS: All experiments were approved by the IACUC committee. Bone marrow was harvested from healthy 3 mo. and 12 mo. old male and female rats (N=2 per age/sex). We then isolated bone marrow mononuclear cells (BMMC) using Ficoll gradient separation and froze the cells in liquid nitrogen until later use. In order to better reflect the age of patients seeking osteoarthritic pain relief, we modeled PTOA using a surgically induced knee instability mode in 12 mo. old male and female Lewis rats, which is approximately equivalent to 30-year-old human (whereas the 3 mo. old rat typically used in the MMT model is approximately equivalent to a 12-year-old human). These male and female rats underwent a sham surgery (MCL transection only) or a medial meniscal transection (MMT) surgery, which recapitulates a moderate PTOA phenotype by 6 weeks in 3 mo. male rats. At 3 weeks post-surgery, MMT rats received intra-articular injections of saline (control) or $1x10^6$ sex-matched BMMCs in 50 µL saline (N=6/sex/condition). BMMCs were from either 3 mo. or 12 mo. old donors, with individual donors being injected separately. We collected pain behavior (allodynia using the up-down method with von Frey filaments and hyperalgesia using a pressure application monitor) and function (spontaneous gait with the Experimental Dynamic Gait Arena for Rodents) longitudinally until the study end at 6 weeks post-surgery. Tibiae were harvested and imaged for qualitative gross morphology.

RESULTS: MMT resulted in substantial cartilage damage and the development of osteophytes in both male and female rats by 6 weeks post-surgery (Fig. 1A). Damage qualitatively appears to be greater in male rats, with full erosion observed along most of the weight bearing region and larger osteophytes along the periphery. Quantitative characterization of tissue heath via contrast-enhanced µCT and histology are on-going. Pain behaviors in the untreated 12 mo. old MMT rats were sexually dimorphic. Hyperalgesia was observed in the untreated female ($p=0.02$ vs sham) and male ($p<0.01$ vs sham) MMT rats by 6 weeks post-surgery, whereas allodynia was only observed in the female rats (though did not reach statistical significance, $p=0.07$; Fig. 1B-C). Some evidence suggests that male MMT rats walk with a slight limp at 6 weeks (indicated by decreased hind temporal symmetry, though no reaching statistical significance $p=0.15$), with no gait modifications apparent in female rats (Fig. 1D). Injected BMMCs improved tissue health and pain behaviors in a sex- and donor age dependent manner. Large areas of cartilage damage and osteophytes were still observed in BMMC-injected male rats regardless of donor age (Fig. 1A). Cartilage damage may be attenuated in the female rats due to injection of either BMMC, though osteophytes appear larger in those injected with BMMCs from 12 mo. old donors. BMMC injections reduced pain behaviors, with a slight preference towards the younger donors. BMMCs relieved joint hyperalgesia in the female rats by 6 weeks regardless of donor age, though only the 3 mo. cells improved hyperalgesia in the male rats ($p=0.02$ vs untreated MMT). Similarly, female rats injected with 3 mo. old BMMCs had a higher 50% withdrawal threshold than untreated rats ($p=0.01$); the withdrawal thresholds did not differ among females injected with 12 mo. old BMMCs ($p=0.23$). These initial results indicate that 1) MMT results in greater tissue damage in 12 mo. old male rats but greater changes of pain behaviors in female rats and 2) BMMCs from younger donors provided more robust pain relief than cells derived from older donors.

DISCUSSION: Our study addresses a fundamental disconnect in that we often evaluate cellular therapies using young, healthy donor cells in young rodents while employing autologous cells from older, osteoarthritic patients in the clinic. We aimed to bridge this translational gap by using male and female middle-aged rats to evaluate bone marrow mononuclear cells (BMMCs) that recapitulate clinically used BMAC. We found minimal pain behaviors observed in 12-month-old male rats, contrasting sharply with the significant allodynia and shuffle-step gait previously documented in 3-month-old males using the same model. This observation aligns with established age-related changes in pain processing, including elevated spinal cord cortisol levels and increased NF-κB inhibition, which reduce nociceptive responses to inflammatory challenges in rodents aged 12-26 months.[3,4] However, these findings contrast with other reports demonstrating greater short-term responsiveness following injection of TRP activators[5], and more persistent hyperalgesia following MIA[6] in older rats, particularly in females. While our Lewis strain model does not provide truly autologous cells, as required by FDA guidelines for clinical use, the inbred nature minimizes genetic variability and enables focused evaluation of age and sex effects on BMMC therapeutic efficacy. Aging is associated with decreased concentrations and increased senescence of B, T, and mesenchymal stromal cells. While our ongoing cellular characterization and transcriptomic analyses will provide crucial insights into critical quality attributes associated with patient factors and treatment efficacy, our initial evidence suggests that 3 mo. BMMCs may be more effective in reducing pain behavior and cartilage damage.

SIGNIFICANCE: While cellular injections have rapidly increased in the clinic, variations in protocols and product quality have yielded inconsistent results. Our reverse translation approach illustrates how donor sex and age may impact BMAC-based analgesia for PTOA.

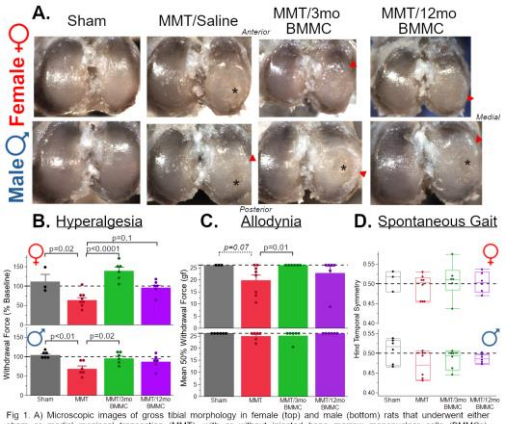

Fig 1. A) Microscopic images of gross tibial morphology in female (top) and male (bottom) rats that underwent either sham or medial meniscal transection (MMT), with or without injected bone marrow mononuclear cells (BMMCs). Substantial cartilage damage (asterisks) and large osteophytes (red triangle) were more apparent in untreated male vs female MMT rats. Damage appeared to be attenuated with BMMC injections in female but not male rats. B) Joint hyperalgesia was observed in both female (top) and male (bottom) MMT rats by 6 weeks, with improvements from injection of BMMCs from 3 mo. (both male and female recepients) and 12 mo. (only female recepients) donors. C) Untreated female MMT rats presented with some signs of allodynic pain at 6 weeks (though did not reach statistical significance), though there was a significant increase in withdrawal threshold due to injection of MBMCs from 3 mo. old donors. D) Gait modifications were subtle, with a possible limb (decreased hind temporal symmetry) observed in only the untreated male MMT rats, though this did not reach significance ($p=0.15$).

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