Fisetin Treated Human Bone Marrow Aspirate Concentrate on Osteoarthritis Rats

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INTRODUCTION: Osteoarthritis (OA) is estimated to affect more than 30 million Americans. Human bone marrow aspirate concentrate (hBMAC) is a source of mesenchymal stem cells (MSCs) and is used as a regenerative medicine treatment in various orthopedic diseases and injuries. One issue is that in older individuals there is an accumulation of pro-inflammatory, anti-regenerative senescent cells that have lost metabolic function and have increased resistance to apoptosis. Fisetin is a flavonoid with senolytic activity to eliminate senescent cells. The purposes of this study were 1) to investigate the therapeutic effect of fisetin on senescence in bone marrow derived MSCs (BM-MSCs) and hBMAC, and 2) to evaluate the efficacy of fisetin treated hBMAC on knee OA model rats. We hypothesize that fisetin will decrease the senescence in both BM-MSCs and hBMAC, which may improve the regenerative quality of the hBMAC orthobiologic product for OA. We further hypothesized that senescence contributes to pain sensitization and, as such, that fisetin treated hBMAC would reduce the pain behavior in knee OA model rats.

METHODS: This study was approved by IRB and IACUC. Purchased BM-MSCs were expanded in normal growth media, then treated with 50μM of etoposide for 24hrs to induce senescence. Fisetin was then added to the cells for 24hrs at 50 μM of concentration. After treatment, senescence was determined using β-galactosidase staining and further verified via flow cytometry with C12FDG staining. In addition, cell viability and gene expression for senescence transcripts p21, and inflammatory transcripts Interleukin (IL)-1β and IL-6 were evaluated. Similarly, hBMAC obtained as discard tissue from our clinic, was tested at four doses (0, 20, 50, 100 μM) for different times (0, 2, 6, 24 hrs). Following ex vivo dosing validation, we transitioned to a rat model of knee OA (destabilized medial meniscus, "DMM") to test efficacy. Nude rats were divided into 5 groups for intraarticular injections 4 week after DMM surgery: Control (PBS), Fisetin only (FIS), BMAC only (BMAC), BMAC co-injection with 50μM fisetin (BMAC+FIS), and BMAC pretreated with 50μM fisetin for 2hr (2h FIS BMAC). Knee bend test and electronical von Frey test were performed to evaluate the pain behaviors before and after injection. Changes in knee diameter (ipsilateral - contralateral) was measured by using a caliper at 4 and 8 weeks after injection. All the data points are presented as the mean ± standard deviation. Statistical significances were calculated based on one-way analysis of variance (ANOVA) and p-value < 0.05 was considered significant.

RESULTS: The results from the *in vitro* experiments on BM-MSCs showed that 50μM was an effective therapeutic dose to reduce senescence. A decrease in senescence was confirmed through a reduction in the amount of senescence-associate β-galactosidase stain, less C12FDG by flow cytometry, and reduced expression of p21. We further show that fisetin improved cell viability and led to a significant decrease in the expression of pro-inflammatory cytokines IL-1β and IL-6. *Ex vivo* time-course and dose-control fisetin treatment experiments on hBMAC suggested that 2hr and 50μM fisetin treatment was optimal to reduce senescence in hBMAC. The results from *in vivo* study showed that the study groups that received hBMAC (Group BMAC, BMAC+FIS and 2h FIS BMAC) significantly reduced the change in the knee diameter at 8 weeks after the injections. DMM surgery caused an increase in the knee bend score and a decrease in nociceptive threshold with von Frey test, and the study groups that received hBMAC (Group BMAC, BMAC+FIS and 2h FIS BMAC) showed a significant improvement in the knee bend score and von Frey test after the injections. However, at 8 weeks after the injections, only BMAC+FIS and 2h FIS BMAC showed significant improvement in knee bend test and only 2h FIS BMAC showed the improvement in von Frey test compared to PBS and FIS groups.

DISCUSSION: This study demonstrated that fisetin treatment was effective in reducing the senescent-cell burden and improving cell viability in both BM-MSCs and hBMAC. In addition, fisetin treatment decreased expression of inflammatory markers in BM-MSCs in vitro, and led to a reduced pain behavior for longer period of time compared to hBMAC without fisetin in rats with knee OA. Ongoing analyses include histology and μCT evaluations of the joint tissue to look for both anatomical and cellular changes of the cartilage.

SIGNIFICANCE: 50μM fisetin treatment for 2 hr was effective to reduce senescence in hBMAC and hBMAC with adjuvant fisetin treatment showed a sustained antinociceptive effects in knee OA model rats.

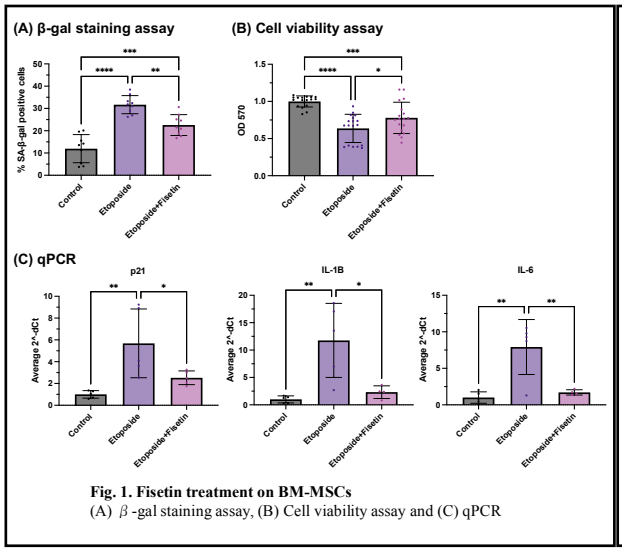

Fig. 1. Fisetin treatment on BM-MSCs
(A) β -gal staining assay, (B) Cell viability assay and (C) qPCR

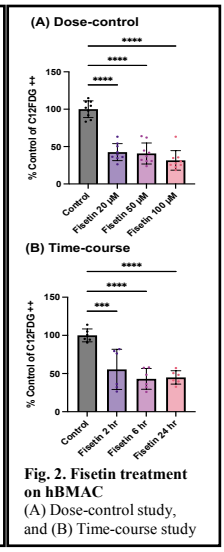

Fig. 2. Fisetin treatment on hBMAC
(A) Dose-control study, and (B) Time-course study

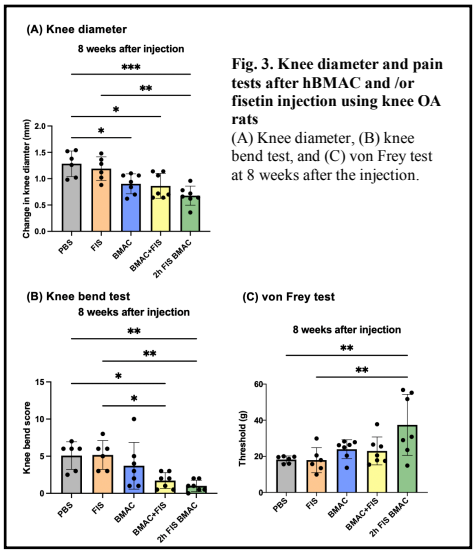

Fig. 3. Knee diameter and pain tests after hBMAC and /or fisetin injection using knee OA rats
(A) Knee diameter, (B) knee bend test, and (C) von Frey test at 8 weeks after the injection.