Low Intensity Vibration Improves Senescence and Differentiation Outcomes during Chronological Aging

Omar Faruq[1], Scott Birks[2], Sean Howard[2], Gunes Uzer[1,2]
[1]Biomedical Engineering Doctoral Program, [2]Mechanical and Biomedical Engineering, Boise State University, Boise, Idaho, 83725.
OmarFaruq@u.boisestate.edu

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INTRODUCTION: Mesenchymal stem cells (MSCs) have significant self-renewal capacity and the ability to differentiate into osteoblasts and adipocytes within the context of bone. Several studies have shown that the aging process can have detrimental effects on differentiation capacity of stem cells. As aging progresses, MSCs lose their renewal properties and become senescent, leading to various age-associated diseases, such as osteoporosis. Therefore, there is a need to combat aging at the cellular level. Clinically, exercise is considered one of the principal sources of mechanical signals needed to maintain a healthy musculoskeletal system during aging, but adherence to strenuous exercise program may not be physically possible for everyone. Low-intensity vibration (LIV), is a mechanical signal delivered by standing on a vertically vibrating plate, and could safely deliver mechanical signals to skeleton. In this study we have used a chronological aging mice model to test the effect of LIV. Daily LIV regimen started at 5 month of age and primary MSCs from non-LIV and LIV treated mice were compared at 24 months.

METHODS: In our preliminary study, we compared the senescence markers and differentiation capacity of MSCs extracted from 5, 12, and 24-month-old C57BL/6J female mice (n=3 mice/grp). To evaluate the LIV effect on primary MSCs, 5-month female C57BL/6J mice were divided into two groups: LIV and non-LIV. In the LIV group (n=10mice/grp), LIV application started at 5 months of age, mice were subjected to 90Hz, 0.7g LIV regimen twice a day for 15 minutes for 19 months. At 24-months, primary stem cells were extracted from the mice bone marrow. Briefly, tibia and femur were dissected, cleared of all soft tissue, and centrifuged at >12,000g to remove the bone marrow. This bone marrow pellet was then resuspended in media and plated in supplemented growth media (alpha-MEM +20% FBS, +100U/mL penicillin, +100µg/mL streptomycin). Cells were washed with PBS after 48 hours to remove non-adherent cells and allowed to proliferate to 50% confluency. At this confluency, cells were lifted, split to produce enough cells for assays, and allowed to proliferate to ~80% confluency. Cells were then lifted and plated for assays. For the immunofluorescence study, cells were stained with Ki67, H2Ax, K9, and P16. For the osteogenic and adipogenesis studies, cells were plated on 6-well plates and differentiation media was changed every 2 days. After 7 days of induced adipogenic differentiation, MSCs were stained with oil red O or lipid droplet staining (Lipid Spot 610, Biotium). After 14 d of osteogenic differentiation, MSCs were stained with alizarin red S or Xyolene orange. RNA samples were collected from MSCs after adipogenic and osteogenic differentiation at days7 and 14, respectively.

RESULTS: In our preliminary study, there is no significant difference of Ki67 expression among the 5,12, and 24-month sample. However, senescence markers P16 and H2Ax had higher expression in both 12-month (45%, $p<0.05$) and 24-month (54.5%, $p<0.05$) samples compared to the MSCs from 5-month samples. On average, 88% of the cells from 12- and 24-month samples showed reduced H3K9me3 loci (95% ($p<0.05$) compared to 5-month. No changes in adipogenesis and osteogenesis were detected. As shown by representative images in Figure 1, following 19 month of LIV treatment the Ki67 expression of MSC extracted from LIV-treated mice increased by 49.94%($p<0.05$) compared to non-LIV. Senescence markers P16 decreased by 64% ($p<0.001$) in the LIV groups. Comparing the adipogenic and osteogenic potential, MSCs from LIV-treated, 24-month old mice exhibited smaller number of fat droplets while alizarin red strained calcium deposits were larger in LIV-treated MSC compared to non-LIV. We are currently quantifying the immunostaining, oil red O, alizarin red and RNA data from 24-month-old samples.

DISCUSSION: Our preliminary study indicated increased levels of senescence markers P16 and H2Ax in primary MSCs extracted from aging mice. Based on our data, application of long-term LIV showed marked increase in of proliferative marker Ki67 and reduced the p16 levels in 24-month-old primary MSCs compared to non-LIV counterparts, suggesting improved proliferative potential. As well, MSCs extracted from LIV-treated mice showed reduced fat accumulation and increased calcium deposition, suggesting that LIV when applied consistently could improve MSC indices often associated with advanced age.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the mechanisms by which LIV affects the *in vivo* aging will be useful in utilizing non-pharmacologic mechanical therapies to combat aging at the cellular level and ultimately improve patient quality of life.

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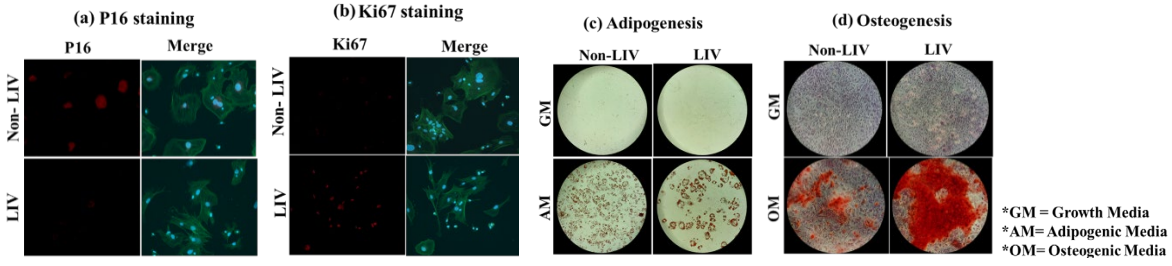

Fig.1 Primary MSCs were extracted at 24-months after 19-month of daily LIV regimen (LIV) and compared to age matched sham controls(non-LIV) (a) Immunostaining against a) senescence marker P16 after LIV treatment showed a decrease while (b) Proliferation marker Ki67 increased after the treatment of LIV sample. Comparing adipogenic and osteogenic potentials, MSCs from LIV-treated, 24-month old mice exhibited (c) smaller number of fat droplets while (d) alizarin red strained calcium deposits were larger in LIV-treated MSC compared to non-LIV.