Therapeutic Effect Of Vitamin E Nanosomes In Adipose-derived Stem Cells Against Oxidative Stress

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INTRODUCTION: Osteoarthritis (OA) is driven by oxidative stress, senescence, and chronic inflammation which disrupt cartilage homeostasis and limit mesenchymal stem cell (MSC) therapy. Adipose-derived stem cells (ADSCs) are a subset of MSCs that have shown promise for OA treatment but are vulnerable to oxidative microenvironments like those found within an OA joint capsule. Senescent cells release catabolic paracrine factors that mimic the effects of oxidative stress, reducing stem cell viability and regenerative potential. Therefore, for cell injections to work we need to find a way to prepare these cells for any potential encounter with oxidative stressors, perhaps in the form of a pretreatment. Molecules like Vitamin E are strong candidates for this treatment because it is readily available and possesses chondroprotective and antioxidant properties[1]. Vitamin E does come with limitations of low solubility and bioavailability, so our study examined to create Vitamin E nanosomes, an antioxidant nanoscale drug delivery system, to try and counteract apoptosis, inflammation, and senescence in ADSCs under oxidative stress[2].

METHODS: Porcine Adipose derived stem cells (pADSCs) isolated from subcutaneous adipose tissue of a 3–4-month-old pig according to the protocol and ethical guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center (UTHSC). pADSCs cultured by digesting fat tissue with 200 µg/mL collagenase II solution (Sigma), followed by incubating with Dulbecco's Modified Eagle Medium-High Glucose (DMEM-HG) (Gibco) supplemented with 10% FBS and antibiotics at 37°C and 5% CO2. pADSC Treatment: pADSCs were seeded at 0.1 x 10^5 before 24-hour incubation period. Experimental groups consisted of control (no treatments), oxidative stress (O.S.: 500 uM H_2O_2), oxidative stress and Vitamin E (O.S. – V: Vitamin E and 500 uM H_2O_2), and oxidative stress and Vitamin E nanosomes (O.S. – V + N: Vitamin E nanosomes and 500 uM H_2O_2). We pretreated O.S. – V and O.S. – V + N groups with Vitamin E and Vitamin E nanosomes, respectively, for 1 hour in serum-free DMEM and antibiotics before treating all O.S. groups with 500 uM H_2O_2 in DMEM containing 10% FBS and antibiotics for 6 h. Media was removed and replaced with fresh media and allowed to incubate for 24 hours. Nanosomes were prepared by incorporating Vitamin E (1 mM) into a lipid mixture, extrusion through a 400 nm membrane and purification via size-exclusion chromatography. Total RNA was extracted for RT-qPCR analysis. For gene expression analysis, qPCR was done using TaqMan™ Gene Expression Assays (Thermo Fisher Scientific). GAPDH was used as an internal control. For additional testing, we duplicated the previous methodology and cells were stained with DAPI, calcein-AM, and EthD-1 for confocal microscopy and cell viability using trypan blue stain. We biochemically examined the medium from treated and control pADSCs to measure various parameters such as cytotoxicity (LDH), cell proliferation (CCK-8), oxidative stress (Total NO), inflammation (PGE_2), and Senescence-associated β-galactosidase (SA-β-Gal).

RESULTS: Vit-E Impact in pADSCs: O.S. – V + N group resulted in significantly improved cell viability compared to O.S. alone ($p < 0.001$). In the live/dead cell staining, the oxidative stress was significantly improved with the administration of vitamin E in nanosomes comparative to the oxidative stress group. The O.S. group composed of mostly orange-reddish tinged cells and with the combination of vitamin E nanosomes, resulted in an image that was similar to the control live/dead cell staining. The CCK-8 assay (cell proliferation) showed a significant increase from O.S. group to the O.S. – V ($p < 0.05$) and O.S. – V + N group ($p < 0.001$). The LDH release demonstrating cytotoxicity showed a significant decrease from the O.S. group to the O.S. – V ($p < 0.05$) and O.S. – V + N groups ($p < 0.001$) where the O.S. – V + N group showed similar LDH release to that of the control group. Oxidative stress (NO) showed a significant decrease between O.S. and O.S. – V + N group ($p < 0.001$). PGE2 assessing inflammation showed significant decrease between O.S. group and O.S. – V + N group ($p < 0.001$). Using RT-PCR, all our pro-apoptotic markers (p53, BAX, CASP3) and inflammatory marker (NFkB) showed a significant decrease in expression between the O.S. group and O.S. – V + N group ($p < 0.001$). Our anti-apoptotic markers (BCL2, MDM2) showed a significant increase in expression between our O.S. group and O.S. – V + N group ($p < 0.001$).

DISCUSSION:

Our findings demonstrate that the controlled delivery of Vitamin E through nanosomes effectively reduces oxidative stress–induced apoptosis, senescence, and inflammation, thereby enhancing stem cell survival. While free Vitamin E provided some protection under oxidative stress, its benefits were less substantial compared to nanosomes containing Vitamin E, likely due to lower stability and limited cellular uptake in the oxidative environment. In contrast, Vitamin E nanosomes significantly alleviated oxidative damage, promoted cell viability, and suppressed pro-apoptotic and inflammatory signaling likely due to faster facilitated cellular uptake of Vitamin E or higher concentrations of Vitamin E absorbed into the cells. These results highlight and support their promise as a potential therapeutic approach for managing osteoarthritis-related oxidative stress and improving survival.

SIGNIFICANCE/CLINICAL RELEVANCE: Healthy MSCs have shown to provide therapeutic benefits in a variety of diseases, but this study presented here clearly shows antioxidant nanosome therapy may enhance MSC-based treatments for OA by mitigating oxidative stress and rejuvenating stem cells, ultimately improving therapeutic outcomes. More studies are needed to determine the effects that nanosomes have on other cell lines, but this study provides a stepping stone for a revolution in how MSC-based therapies are administered.

REFERENCES: 1. R. A. Denu et al., "Effects of oxidative stress on mesenchymal stem cell biology," Oxid. Med. Cell. Longev., Vol. 2016, pp. 2989076, 2016. 2. B. Akbari et al., "Evaluating the effect of liposomes containing vitamin E on bone metabolism," J. Mater. Res., Vol. 39, pp. 1425-1436, 2024.

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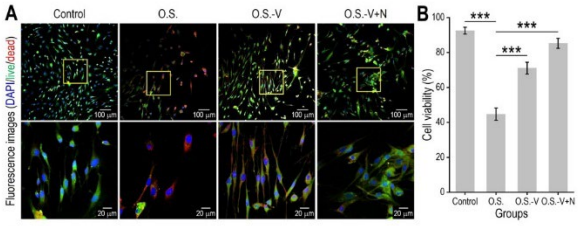

Fig. 1. (A) DAPI (blue)/live (green)/dead (red) images of control, O.S., O.S.-V, and O.S.-V+N groups including the **(B)** measured cell viability result using trypan blue method. (n = 3; ***$p < 0.001$)

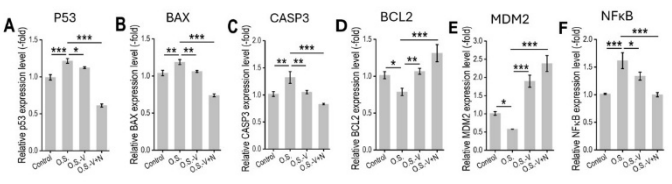

Fig. 2. Pro-apoptotic markers: (A) p53, (B) BAX, and (C) CASP3; anti-apoptotic markers: (D) BCL2 and (E) MDM2; and the inflammatory marker: (F) NFkB after 6 h of cell culture under control (0 uM H2O2 treatment), O.S. (500 uM H2O2 treatment), O.S. - V (O.S. with 1 h vitamin E pretreatment), and O.S. – V+N (O.S. with 1 h vitamin E nanosome treatment). (n = 3; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)