Evaluation of sex-related differences in uptake and chondroprotection of ROS scavenging manganese dioxide nanoparticles

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INTRODUCTION: Nanozymes, engineered antioxidant-mimicking nanomaterials, are an emerging therapeutic strategy for targeting oxidative stress. Nanozymes can be engineered to scavenge reactive oxygen species (ROS) in a manner similar to endogenous enzymes, such as catalase and superoxide dismutase. Oxidative stress is a key contributor to the development and progression of osteoarthritis (OA), but has been an elusive therapeutic target. Conventional therapeutic strategies including small molecule antioxidants and natural enzymes have limited therapeutic impact on OA due to their rapid clearance and poor retention in the joint. In contrast, nanozymes can be engineered with improved stability and bioavailability compared to existing antioxidant therapies while also being easier to produce. Manganese dioxide nanoparticles (MnO_2) are an example of a nanozyme technology that our group has engineered for cartilage localization and retention. We previously demonstrated that MnO_2 are effective in ROS scavenging and chondroprotection in bovine cartilage. The objective of this work was to evaluate the uptake, ROS scavenging, and chondroprotective properties of MnO_2 in human OA chondrocytes from male and female donors. Further, we examined if joint level biodistribution is affected by sex in a rodent model of post traumatic OA.

METHODS: MnO_2 NPs were synthesized with cartilage targeting properties (cationic, <15 nm) following previously reported methods. MnO_2 characteristics were measured via dynamic light scattering and zeta potential. Human OA chondrocytes were isolated from patients undergoing total knee arthroplasty (n = 4/sex). Endocytic mechanisms were measured via flow cytometry and confocal microscopy using temperature (37°C and 4°C), time (3hr and 24hr), and chemical inhibitors (chlorpromazine hydrochloride, amiloride hydrochloride, methyl-B-cyclodextran) (n=3/sex). Chondroprotection was measured via nitric oxide release from cartilage explants (n=4/sex). To evaluate ROS scavenging activity and location, chondrocytes were transfected with plasmids encoding HyPer7, an ultrasensitive genetically encoded hydrogen peroxide (H_2O_2) sensor, treated with MnO_2 (5ug/ml and 20 ug/ml), then washed and exposed to either increasing levels of H_2O_2 or IL-1B (10ng/mL). Oxidation of the probe by H_2O_2 results in a shift in its excitation wavelength - the readout of HyPer7 is presented as ratio of fluorescence emission intensity following excitation at the 2 wavelengths (488 and 405nm). HyPer7 plasmids localized to the mitochondrial matrix, mitochondrial intermembrane space, or cytosol, were used to determine compartment specific H_2O_2 scavenging by MnO_2.. Joint and organ level biodistribution were evaluated in a medial meniscus transection model (Wistar rats, n=3/sex) via IVIS imaging for 7 days. Statistical analysis was done with GraphPad PRISM 10.2, error bars indicate standard deviations and statistical comparison of means were conducted via a Dunnett's test or one-way ANOVA with Tukey's multiple comparisons tests. This work was approved by the UF IACUC and the UF IRB committee.

RESULTS: MnO_2 nanoparticles were engineered to be 11.3±1.2 nm with a zeta potential of 29.4±4.63 mV and were consistent across batches and personnel. Uptake of MnO_2 (20ug/mL) was measurable via confocal microscopy in chondrocyte monolayer within 3 hours of coincubation, with female chondrocytes having increased uptake at early time points (3 hrs.) compared to males (Fig 1). However, there were no significant differences after 24 hours or in the presence of an inflammatory cytokine (IL1B, 10 ng/mL). This result is consistent with flow cytometry analysis. MnO_2 uptake is measurable at 4C and 37C in both sexes, indicating that MnO_2 may utilize active and passive transport mechanisms. Chemical inhibition of clathrin-mediated endocytosis decreased MnO_2 uptake in both sexes, which is consistent with our previous findings in bovine cells. Using Hyper 7 probes, we demonstrated an increase in the Hyper7 ratio in the mitochondrial matrix and mitochondrial intermembrane space in human chondrocytes upon exposure to IL-1B. However, when treated with MnO_2 and IL-1B, chondrocytes demonstrated similar Hyper7 ratios to control chondrocytes (no IL-B), suggesting effective H_2O_2 scavenging in these cellular compartments (Fig 2B). Single-cell imaging confirms MnO_2 co-localization with mitochondria under inflammatory conditions (Fig 2A). MnO_2 had a chondroprotective effect on human OA cartilage, with decreased nitric oxide release in cartilage explants following treatment with MnO_2 with and without IL1B (Fig 3). Joint-level biodistribution was not significantly different between male and female Wistar rats, and there was no measurable accumulation within the organs outside of the joint.

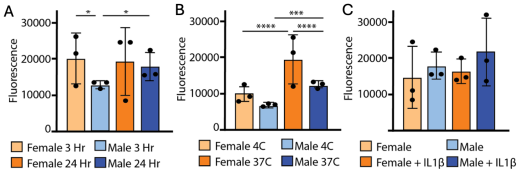

Fig. 1 Temperature and concentration dependent MnO_2 uptake in female and male human OA chondrocytes. Uptake of MnO_2 tagged with Alexa-488 (20 ug/mL) in female and male chondrocytes after (A) 3 or 24-hours of co-incubation at 37°C, after 3 hours of co-incubation at 4°C, and after 3 hours of co-incubation at 37°C with 10 ng/mL IL1B. Fluorescence normalized by cell number comparing MnO_2 uptake between male and female donors based on time (C), temperature (D), and inflammatory conditions. * = p<0.05, **=p<0.01, ***=p<0.005, ****=p<0.001

DISCUSSION: Manganese dioxide nanoparticles are a robust and consistent nanozyme system that can be engineered with specific design criteria - here we have optimized them for chondrocyte localization and retention. Our results showed that human OA chondrocytes under inflammatory conditions with IL1B (10 ng/mL) will increase production of H_2O_2 in the mitochondria, which could be detected by using Hyper7 probes. Under these conditions MnO_2 is readily taken up by chondrocytes, localizes to the mitochondria, and scavenges ROS. This further supports the role of MnO_2 as an ROS scavenging therapy. Currently little is known about sex-related differences in nanoparticle uptake and chondroprotective strategies; this work is the first to consider therapeutic differences in the application of MnO_2 to male and female donor cartilage. Both sexes had robust uptake and localization to the mitochondria and ROS scavenging functions. ROS scavenging in the mitochondria may be a key driver in preventing or slowing the effects of oxidative stress in diseased tissue. Interestingly, female donors showed more rapid MnO_2 uptake and improved chondroprotection, measured by nitric oxide release from the cartilage, compared to male donors. Our *in vivo* model showed that there were no differences in joint retention or biodistribution between male and female rats, when normalized by weight. Our study was not designed to control for other biological or disease-related variables but was aimed at exploring the potential for sex differences in response to the MnO_2. These findings suggest further study may be warranted to determine if or how sex affects nanoparticle uptake and response to ROS scavenging therapeutic strategies.

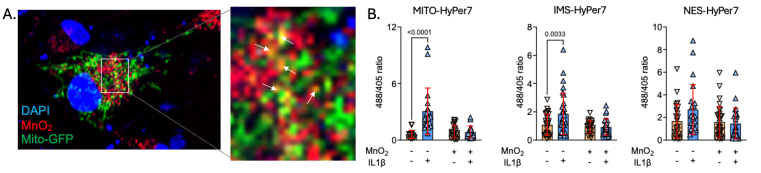

Fig 2. (A) Human OA chondrocytes were treated with AlexaFluor 594 tagged MnO_2 (5 ug/mL) and evaluated for colocalization with mitochondria (labelled with mitoGFP). (B) Average HyPer7 ratio, indicative of probe oxidation, using MITO-HyPer7, IMS-HyPer7, and NES-HyPer7 shows increased H_2O_2 production in the presence of IL1B and a return to baseline levels with MnO_2 (5 ug/mL) treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is the first to investigate sex-related differences in the therapeutic Reffects of a nanoparticle treatment for OA. Specifically, this study compares uptake mechanisms and ROS scavenging functions of MnO_2 in male and female OA chondrocytes which are critical for the future translation and development of this therapeutic strategy. And, this work sheds light on potential tissue and cell-level differences between male and female OA patients.

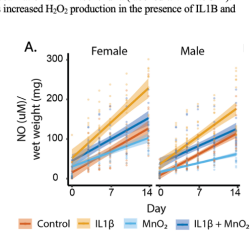

Fig 3. NO release from male and female cartilage explants cultured for 14 days. MnO_2 mitigated NO release in both male and female explants.