Early Detection of Damaged Cartilage in DMM Mouse Using Nanosomes

Hongsik Cho, Ph.D, MBA1,2,3, Byungju Kim, Ph.D4, Karen A. Hasty, Ph.D1,2,3, Byoung-Hyun Min, M.D, Ph.D4.

1University of Tennessee Health Science Center, Memphis, TN, USA, 2Campbell Clinic, Memphis, TN, USA, 3VA Medical Center, Memphis, TN, USA, 4Ajou University, Suwon, Korea, Republic of.

Disclosures:

Introduction: Osteoarthritis (OA) is an extremely common type of arthritis which is the nation’s leading cause of disability. The early lesions are mediated by increased production of matrix metalloproteinases (MMP), a family of enzymes produced by chondrocytes that degrade extracellular matrix (ECM) components [1]. Because cartilage is aneugenetic, these early lesions are not painful and often go undetected until significant damage has occurred. Currently there are no appropriate treatments to cure of cartilage degradation so early detection is critical. An obstacle for OA research with small animals is the lack of good diagnostic tools for efficiently identifying early stages of the disease and monitoring its progression [2]. We have developed nanosomes that are targeted to exposed type II collagen using a monoclonal antibody as a method for early diagnosis in vivo, serial measurement of total disease in individual joints, and for determining the effectiveness of therapeutic intervention in small animals. It was initially shown by Jasin and coworkers that normal articular cartilage poses a barrier to the binding of antibodies [3]. However, when the surface of the cartilage was exposed to proteolytic enzymes, the native type II collagen (CII) was exposed. This CII proved to be accessible to specific antibodies. The nanosomes incorporate a monoclonal antibody (Mab) to CII and a near infrared fluorescent (NIF) dye that can be quantitatively visualized in vivo. Therefore, we hypothesize that CII targeted nanosomes will selectively bind this exposed CII in joint of destabilize the medial meniscus (DMM) mouse and can then be quantified using an external imaging system, IVIS. These results were verified by comparing IVIS data to histological slides.

Methods: Experimental Animal and surgical induction of OA: Male C57BL/6 mice underwent DMM (36) and sham (6) surgery, as described by Glasson et al [4]. Animals were cared for according to institutional animal care and use protocols, and all animal studies were approved by the Animal Care and Use Committee of Ajou University School of Medicine. The experimental groups were arranged according to OA severity using a modified OA scoring system. The groups were then compared to ROI values from IVIS scanning to investigate the correlation between OA score and the fluorescent intensity.

Preparation of Nanosomes: Nanosomes were prepared from lipid films at a ratio of 5.2 μmol 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 4.5 μmol cholesterol, 0.3 μmol DSPE-PEG2000, and 0.015 μmol 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol) 2000] (DSPEPEG2000-maleimide) (all from Avanti Polar Lipids, AL) initially dried under an nitrogen stream, then further dried under vacuum. The lipid film was rehydrated with XenofluorTM680 and multilamellar vesicles extruded through polycarbonate track etched filters with uniform cylindrical pores averaging 200 nm, resulting in unilamellar nanosomes with an average diameter of 200 nm. Free dye was separated by gel filtration chromatography. Antibodies were thiolated and attached to the DSPE-PEG2000-maleimide moiety of the nanosomes and isolated from the free antibody by gel filtration.

Fluorescence imaging analysis and Histopathology: MabCII nanosomes encapsulating the XenoFluor dye (XenoLight CF680 Labeling kit, Caliper Life Science, MA), and targeted with MabCII are injected retro-orbitally (1 μg of MabCII / g body weight; 100ul of targeted nanosomes) into mice for control or DMM mice at 1, 2 and 4 weeks post-operatively. Animals are scanned by IVIS imaging to determine fluorescent binding in knee joint. The euthanized mice’s knee joints are rescanned after dissection to confirm there is no non-specific binding on tibial, femoral, or patellar cartilages. They are then processed for histopathology. Histological scoring was performed on the medial tibial plateaus of safranin-O/fast green staining images [5].

Results: Figures 1 and 2 show the relationship between articular damage, expressed as an OA score, and targeted nanosme binding, quantified as ROI readings. In upper panel of figure 1, groups (a) through (f) correspond to mice in different stages of development of surgically induced arthritis. After scanning, the mice were sacrificed and sections of the knee were stained and evaluated with histopathology using the modified Mankin OA Score. Figure 2 shows that as the Mankin OA score increases (A), binding of fluorescent nanosome also increases (B) during early OA development. In this study, the ROI has the potential to be used as a non-invasive standard for quantifying early arthritic change and therefore, be a helpful tool in determining treatment schedules in patients with early signs of OA.

We do not expect to see any binding of the NIF-MabCII to the sham operated knee or binding of the NIF-MabCon (control mouse IgG) to the DMM knee. In our previous study, significant fluorescence is seen only in the surgically destabilized knee of DMM mice that were injected with MabCII-nanosomes. In these mice, surgical destabilization induced the development of arthritis, resulting in exposure of collagen type II which allowed the targeted nanosome to access the damaged articular surface (data not shown).

Discussion: Our hypothesis, supported by results, is that the MabCII- nanosomes are selectively localized to joints in which the surface of articular cartilage is eroded and CII is exposed. The degree of damage is quantified using IVIS® imaging technology. We
expect that the technique will sensitively identify minimal cartilage damage and that larger and more advanced lesions will bind larger quantities of NIF-MabCII. As our results show, the targeted nanosomes are localized to discrete regions of articular cartilage that have been induced by DMM and did not bind normal articular cartilage. We confirmed by histopathology that MabCII-targeted nanosomes encapsulating a NIF dye bind to the damaged cartilage in the DMM mouse model and quantitatively reflect the degree of cartilage damage during the course of this disease in vivo. This figure show the relationship between articular damage, expressed as an OA score, and targeted nanosome binding, as evidenced by ROI readings. In figure 2, these results show that as the modified Mankin OA score increases, binding of fluorescent nanosome also increases during early OA development. fluorescence intensity appears to be a useful predictor of histopathological damage for mild, early stage OA (OA score <3). ROI has the potential to be in used as a non-invasive standard for quantifying early arthritic change and therefore be a helpful tool in determining treatment schedules in patients with early signs of OA.

Significance: The ability to identify early damage to the articular surface and specifically direct biological agents to OA cartilage offers a new paradigm for treating early osteoarthritis with theranostic (therapeutic and diagnostic) nanosomes. Our nanosome technology has the potential for overcoming the obstacles of current methods for diagnosis and treatment of OA.

Acknowledgments: This work was funded by an NIH R21, VA Merit Award (KAH) and funds from the CTSI at UTHSC.

ORS 2014 Annual Meeting
Poster No: 0350