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Detection of early cartilage damage using targeted nanosomes in a post-traumatic osteoarthritis mouse model

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Abstract

Osteoarthritis (OA) is a major cause of pain and disability in the US. A problem with treatment is that it is very difficult to detect OA before irreversible damage has already occurred. This study was performed in order to characterize a novel method of OA detection in a mouse model of post-traumatic osteoarthritis (PTOA) using nanosomes. In this study, the knee injury was induced in mice by compressive loading. Also, nanosomes encapsulating fluorescent dye and conjugated to collagen type II antibody were prepared to detect cartilage damage. Cartilage damage and OA progression were detected by the use of fluorescence-imaging (IVIS) and histopathology. In results, the histopathology showed that mild osteoarthritic changes had occurred. This corresponded with a higher fluorescence on IVIS imaging due to more nanosome binding. These results suggest that theragnostic nanosomes may be useful for detection of early PTOA as well as for targeted delivery of interventional agents.

Key words: Nanosomes (nano-size of liposome); Osteoarthritis (OA); Type II collagen (CII); Monoclonal antibody (Mab); Theranostic (therapeutic and diagnostic); Extracelluar matrix (ECM); Near-infrared fluorescent (NIF); Post-traumatic osteoarthritis (PTOA)

Introduction

Osteoarthritis (OA) is one of the most prevalent causes of pain and disability in older individuals.[1] Progression of OA is complex and develops over decades. Although there have been substantial advancements in treatment of rheumatoid arthritis, treatments for osteoarthritis (OA) or traumatic injury resulting in post-traumatic osteoarthritis (PTOA) are primarily palliative until joints become dysfunctional and prosthetic replacement is needed.[2,3] Post-traumatic osteoarthritis is a prevalent form of OA, developing as a common sequela to joint injury and resulting in OA in younger individuals.[4–6] Approximately 50% of ACL ruptures develop PTOA within 10-20 years.[7,8] One complication with developing treatments for PTOA is that it is difficult and expensive to detect early cartilage damage before irreversible changes have occurred. Treatment of later stages of PTOA that manifest significant loss of chondrocytes and cartilage matrix becomes a more difficult therapeutic target[9] than pharmacological intervention in the early stages when chondrocyte metabolism might be modulated.

Mechanical loading of the mouse knee has been used as a model of PTOA.[10,11] Histopathology shows an initial lesion after a two week loading interval that progresses in OA severity in mice. This model provides a valuable resource to researchers for investigating arthritic progression induced by repetitive, over-loading of a joint. Traditional evaluation requires the sacrifice of the mouse and histopathological evaluation. A reliable in vivo method of identifying and quantifying articular cartilage damage

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in an individual animal would reduce the number of animals needed for qualitative analyses.

Early OA begins at the superficial surface,[12] eventually exposing subchondral bone and causing debilitating joint pain.[13] In experimental surgical models of OA, articular cartilage initially shows reversible aggrecanase-mediated aggrecan degradation[14] followed by production of proteinases that degrade the structural proteins. The loss of aggrecan induced by FN fragments is reversible.[15] Proteolysis at the articular surface has been shown to allow access and binding of type II collagen (Col-II) antibodies.[16–18] This observation forms the basis for our antibody-targeted nanosomes binding to the sites of early lesions on the articular cartilage.[19]

Drug targeting by antibody-conjugated liposomes represents a technology that has been applied for specific delivery sites of drug action, such as brain, lung, cancer cells or cells of the immune system.[20–22] Nanosomes are small liposomes of approximately 100-200 nm in diameter. In this study, we have developed nanosomes targeted with monoclonal antibody to type II collagen (MabCII) and encapsulating near infrared fluorescent dye (NIF) that can be readily detected and quantitated in the knees of mice using IVIS imaging. By correlating IVIS measurements of fluorescence intensity to histological damage in mechanically loaded mouse knees, we show a non-invasive method for diagnosis of early stages of cartilage injury and PTOA that could conceivably be used as a targeted delivery system.

Materials and methods

Experimental animals

Thirty-six C57BL/6 male mice (age 10 weeks at time of mechanical loading; body weight of ~20 g with <10% variance) were obtained from Jackson Laboratory (Maine, USA). Mice will be randomly divided into several test groups: Normal which has no exposure to any mechanical load and an experimental group to which a mechanical load has been applied to the left knee. Each group has been divided into two different groups according to injection materials: control antibody (mouse IgG) conjugated nanosome or Type II collagen targeted nanosome using monoclonal type II collagen antibody. In a separate study, not presented here, we investigated the injection of nanosome without any antibody on its surface. These un-targeted nanosomes did not accumulate in the joint space. Using fluorescence imaging, it was determined that 90% of these un-targeted nanosomes were cleared from the body within 48 h. Animals were kept in a housing facility for a 1-2 week acclimation period before experimentation was begun. All procedures, in this study, were performed according to approved protocols and experimental procedures of IACUC at the University of Tennessee Center for the Health Sciences.

Mechanical loading to induce PTOA

Mice were placed in an anesthetic induction-chamber to induce sedation and continuously anesthetized throughout the procedure using 2% isoflurane. The left leg of each mouse was positioned within the ElectroForce® 3200 (Bose Corp., Minnesota, USA) biomaterials test instrument with a custom made mechanical loading apparatus with the proximal tibia resting in the upper cup and the dorsiflexed ankle inserted into the bottom cup (Graphical Abstract). The left knee joint of each mouse then received 40 cycles of compressive loading at 9 N, three times weekly over a period of two weeks. The load was administered with a static offset load of 2 N to maintain contact between the specimen and the load cell. These methods were adapted from Poulet's protocol.[10,11] Using the same placement procedure, the right knee was positioned under the loader without loading to act as a non-loaded control. After loading, the mouse was allowed normal cage activity and any abnormal behavior, weight loss or a diminished food intake was monitored. An ex vivo calibration of the mechanical loading apparatus was done before each loading session. Loading data were collected continuously for each mouse using WinTest software (Bose Corp., Minnesota, USA).

Analysis of gene expression

Gene expression in the joint was analyzed at the end of the two week loading regimen after a four day interval without loading. Articular, meniscal and patellar cartilages from the mechanically loaded joint and the contralateral control were isolated by dissection and pooled RNA was extracted by Trizol. Quantitative gene expression (qPCR) was analyzed by polymerase chain reaction using primers specific for type II collagen (Col-II), aggrecan, matrix metalloproteinase-13 (MMP-13), tumor necrosis factor alpha (TNF-α) expression and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).[23] Gene expression was calculated as $2^{-\Delta\Delta CT}$ after normalization to GAPDH expression.[24,25] Gene expression in the loaded knees is shown relative to that of the contralateral non-loaded knee set as 1 +/− standard error of the mean (SEM).

Preparation of targeted nanosomes

This method of nanosome synthesis and antibody conjugation was adapted from Huwyler et al.[26] All lipids were purchased from Avanti Polar Lipids (Alabaster, AL) as pure powders, and dissolved in 2:1 (chloroform:methanol). A lipid film was prepared by mixing 5.2 μmol 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 4.5 μmol cholesterol, 0.3 μmol 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000), and 0.015 μmol 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[maleimide (polyethylene glycol) 2000] (DSPE-PEG2000-maleimide). The lipid film was rehydrated with PBS containing near infrared fluorescent dye (NIF: XenoFluor-™680, Caliper). The rehydrated lipids were repeatedly extruded through a 200 nm porous membrane to generate 200 nm nanosomes with 1-3 lamellar membranes.[19,27] The extruded liposomes (Mini-extrusion kit, Avanti, AL) were separated from the free molecules using a Sepharose CL-4B (Sigma-Aldrich, MO) size exclusion column before their conjugation to a monoclonal antibody to type II collagen (MabCII) made in the laboratory or to an irrelevant control antibody (MabCon, Mouse IgG2A Isotope Control from R&D Systems, Inc.). To thiolate antibody for coupling to the $DSPE\text{-}PEG_{2000}$-maleimide, 200 mg of purified monoclonal antibody was suspended in 200 ml of 150 mM sodium borate, 0.1 mM EDTA. Traut's reagent (2-iminothiolane HCl) was added at a 1:40