In Vitro Effects of Oxidized Low Density Lipoprotein on Canine Joint Tissues

Kennedy CR, Kuroki K, Stoker AM, Cook JL

Comparative Orthopaedic Laboratory, University of Missouri, Columbia, Missouri

www.columc.missouri.edu

Introduction

Patients with primary osteoarthritis (OA) commonly have cardiovascular disease (CVD) and it has been reported that cardiovascular mortality is directly proportional to the extent of OA in affected individuals. Although the high incidence of concurrent OA and CVD may be merely an independent feature of advanced age and/or obesity, major risk factors for both, one can speculate that there is a direct link between the two. Altered lipid metabolism may be the underlying cause and could help link OA and CVD. It has been hypothesized that oxidized low density lipoprotein (oxLDL), a causative material of atherosclerosis, is a key molecule that connects these diseases. The aim of this project is to test the hypothesis that oxLDL would induce histological and biochemical changes compatible with OA in a co-culture model of joint.

Objective

To evaluate the effects of oxLDL on

- Chondrocyte viability and density
- Extracellular matrix (GAG, HP)
- Tissue inflammation (NO, PGE2)
- ECM degradation (ADAMTS4, MMP)
- Cytokine production (IL-6, IL-8, KC, MCP-1)
- Matrix metalloproteinase production (MMP-1,2,3,9,13)

Methods

All procedures were approved by the IACUC and the animals used were euthanatized for reasons unrelated to this study.

Tissue Harvest and Culture Procedures: 6mm normal cartilage explants (n=36) were sterilely harvested from canine humeral heads using a scalpel. 4mm normal synovium explants (n=36) were sterilely collected from the same animals. Cartilage and synovial explants were co-cultured in 2mL of Dulbecco's modified Eagle's medium (DMEM) with oxLDL at concentrations of 0, 10 or 100µg/ml (n=6 of each group) and incubated at 35°C, 95% humidity, and 5% CO_2.

Media Collection: 2mL of media was collected from each well on days 3, 6, 9 and 12 of culture and stored in covered media plates at 0°C. After collection, culture wells were refilled with 2mL of appropriate media. All collection procedures were performed in a laminar flow hood using sterile tools to avoid microbial contamination of culture plates.

Explant Collection: Cartilage and synovium explants were collected on culture day 6 and 12 (n=18 for each group). Synovium explants and half of each cartilage explant were stored in 10% formalin for histological analysis. The other half of each cartilage explant was used for cell viability testing and biochemical analysis.

Chondrocyte Viability and Density: Immediately after explant collection, viability was determined using fluorescent viability dyes ethidium homodimer and calcein AM, and images of live and dead chondrocyes were collected via flourescent microscopy. Live and dead cells were counted and total tissue area was calculated using a computer algorithm. Viable cell density was determined as the ratio of live cells to tissue area.

Biomarker Analysis: Culture media was assessed for ADAMTS4 (aggrecanase) activity, total MMP (matrix metalloproteinase) activity, NO (nitric oxide) concentration (Greiss reagent assay), GAG (glycosaminoglycan) concentration (DMMB assay), cytokine (IL-6, IL-8, KC, MCP-1) concentrations, PGE2 (prostaglandin E2) concentration (EIA assay), and MMP-1,2,3,9,13 concentrations. Cartilage samples were dried and digested then analyzed for GAG and HP (hydroxyproline) concentration.

Statistical Analysis: Group comparisons were performed with SigmaPlot® using t-tests with significance set at $p<0.05$.

Discussion

As we hypothesized, cartilage exposed to oxLDL did result in decreased cell viability causing a decrease in cell density at day 12 of culture (Fig.1). We theorize the decrease in ADAMTS4 and total MMP activity observed in oxLDL treated samples was due to the decrease in the number of viable cells in the tissue during culture. Intriguingly the concentration of nitric oxide, a pro-inflammatory factor, was significantly increased in samples exposed to 100µg/mL oxLDL at all time points (Fig.2). Conversely there was not a significant increase in the production of PGE2 after oxLDL treatment in this model. In previous studies it was found that the synovial explant produces the majority of the PGE2 and the cartilage explant produces the majority of the NO in this model. Therefore it can be speculated that high levels of oxLDL provokes an inflammatory response in cartilage tissue but not synovial tissue in this model. In vivo studies to confirm the NO mediated inflammatory response in articular cartilage resulting from high concentration oxLDL exposure need to be performed but this study provides evidence that a altered lipid metabolism may play a role in the initiation and progression of osteoarthritis.

Significance

- Groups exposed to oxLDL for 12 days showed reduced viable cell density
- High concentration (100µg/mL) oxLDL exposure causes elevated nitric oxide
- High oxLDL levels can induce an inflammatory response in articular cartilage

Results and Figures

* Indicates group is significantly different than 0ug/mL control group

For cytokines IL-6, IL-8, KC, MCP-1, and MMPs-1,2,3,9 there were no significant differences between any of the goups (data not shown). GAG and HP concentrations per gram of dried cartilage were not significantly different between any of the groups (data not shown). PGE2 concentration did not vary significantly between any groups (data not shown).

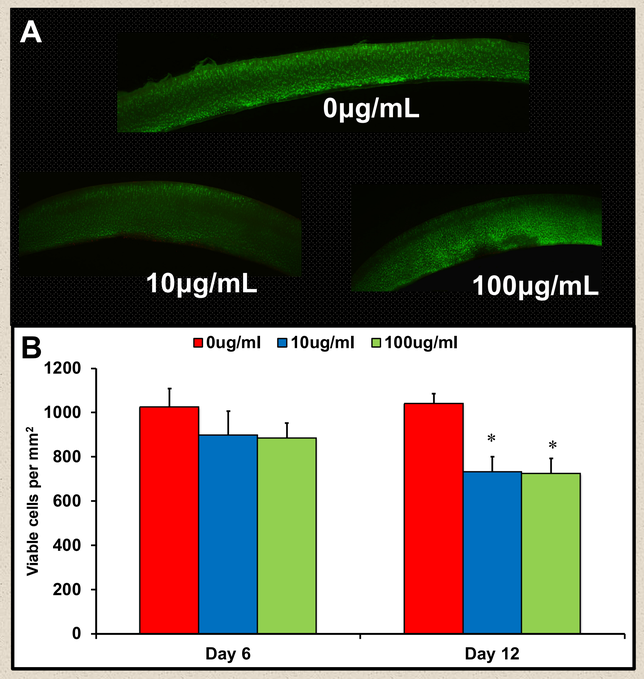


Fig. 1 – A) Representative images of fluorescent live (green) and dead (red) cells from 0, 10, 100 µg/ml oxLDL groups after 12 days of culture. B) Viable cell density. The Day 12 0µg/mL oxLDL group had significantly higher viable cell density compared to both of the Day 12 10µg/mL ($p=0.003$) and 100µg/mL ($p=0.001$) oxLDL groups

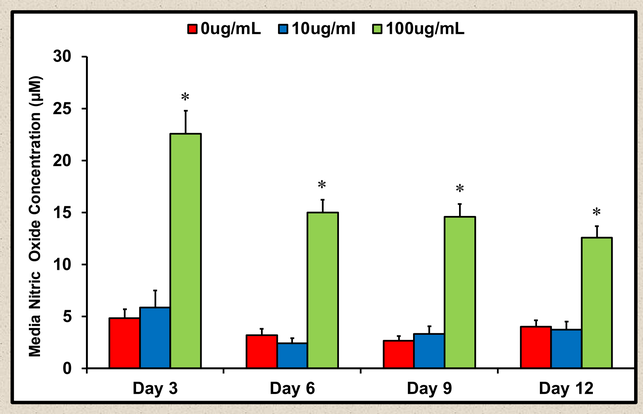


Fig. 2 – Nitric Oxide concentration in culture media. Nitric oxide concentration was significantly higher in all 100µg/mL oxLDL groups compared to their respective 10µg/mL and 0µg/mL oxLDL groups ($p<0.004$ for all).

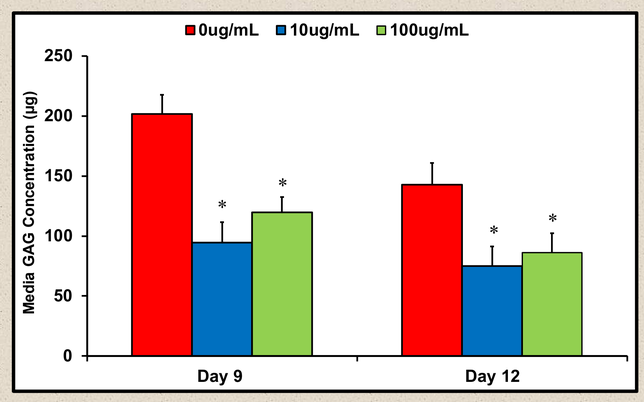
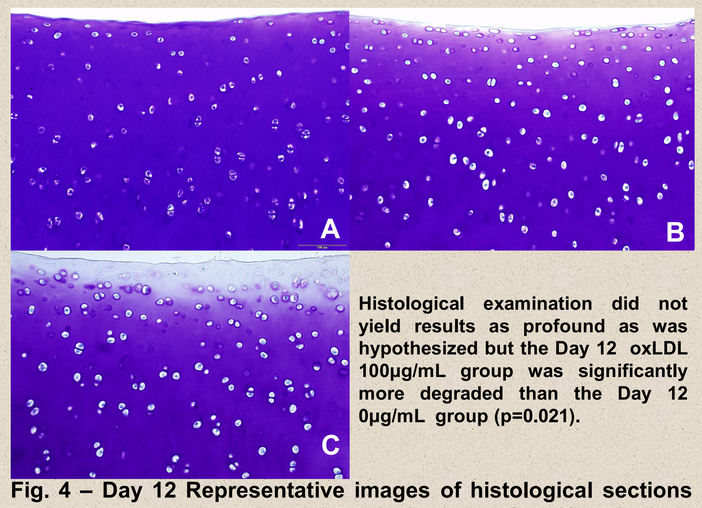


Fig. 3 – GAG concentration in culture media. Day 9 and Day 12 0µg/mL oxLDL groups had significantly higher GAG concentrations in their culture media compared to their respective 10µg/mL and 100µg/mL oxLDL groups ($p<0.026$ for all).



Histological examination did not yield results as profound as was hypothesized but the Day 12 oxLDL 100µg/mL group was significantly more degraded than the Day 12 0µg/mL group ($p=0.021$).

Fig. 4 – Day 12 Representative images of histological sections stained with toluidine blue. A) 0µg/mL B) 10µg/mL C) 100µg/mL

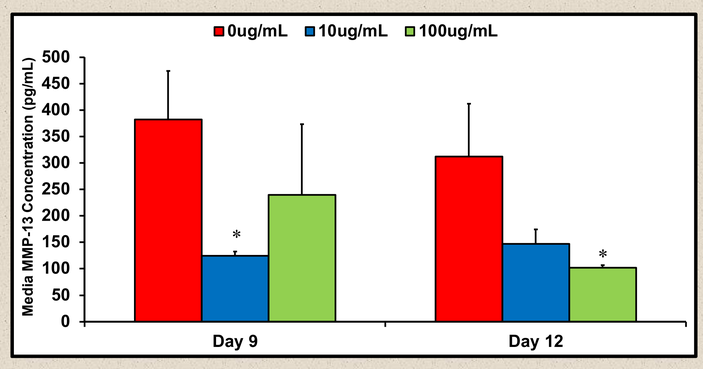


Fig. 5 – MMP-13 concentration in culture media. MMP-13 was significantly higher in the Day 9 0µg/mL oxLDL group than the Day 9 10µg/mL ($p=0.002$) and the Day 12 0µg/mL was significantly higher than the Day 12 100µg/mL ($p=0.002$) .

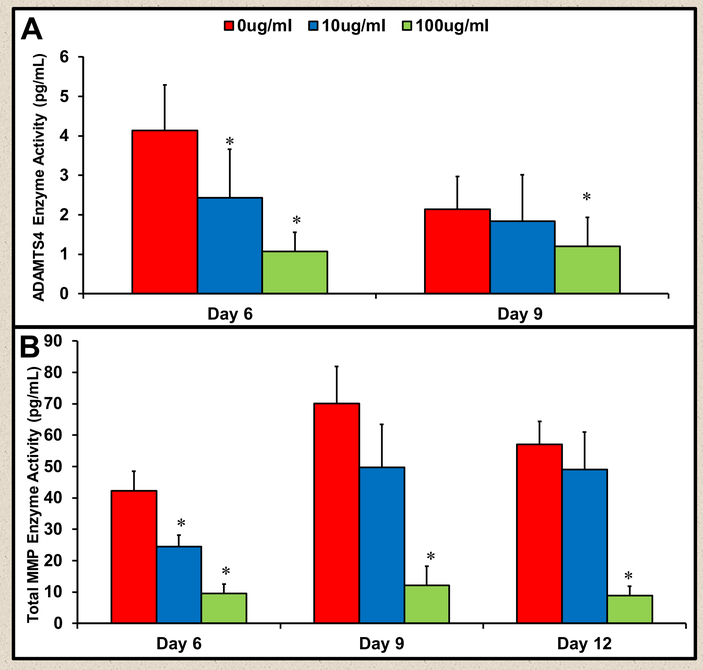


Fig. 6 – A) ADAMTS4 activity in culture media. Day 6 0µg/mL and 10µg/mL oxLDL had significantly higher ADAMTS4 activity compared to the Day 6 100µg/mL oxLDL groups ($p=0.002$, $p=0.012$). Day 9 0µg/mL showed significantly higher ADAMTS4 activity than Day 9 100µg/mL ($p=0.031$). B) Total MMP activity in culture media. Day 6, 9, and 12 0µg/mL oxLDL and 10µg/mL oxLDL groups both had significantly higher MMP activity compared to their respective 100µg/mL oxLDL groups ($p<0.043$ for Day 6, $p<0.004$ for Day 12).

Acknowledgments: *Funded by University of Missouri College of Veterinary Medicine.* The authors thank Mr. Don Connor for assistance with poster preparation.