In Vitro Effects of Oxidized Low Density Lipoprotein on a Canine Osteoarthritic Joint Model

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Results

Methods

Tissue Harvest and Culture Procedures: All procedures were approved by the IACUC. Cartilage (CART) and infrapatellar fat pad (FP) tissue was harvested from 6 dogs euthanized for reasons unrelated to this study. The animals had no orthopaedic disease with grossly normal joints. CART (6mm) and FP (4mm) explants were created, and co-cultured using 24 well plates in 2mL of culture media (DMEM). Co-cultures (n=6/group) were assigned to one of 4 culture groups: 1) Control, 2) IL-1β (2ng/mL) + oxLDL (10μg/mL), 3) IL-1β + oxLDL, 4) IL-1β + oxLDL. Tissues were cultured for 21 days, and media was changed every 3 days and collected for biomarker assessment.

Tissue analysis: On day 21 of culture cartilage tissue was stained for cell viability using calcein AM (live) and ethidium homodimer (dead) fluorescent stains. Viable cell density was determined by dividing the number of live cells by the area of the tissue section counted. Following staining, a portion of the tissue was fixed for histological evaluation, and the other portion was processed to determine the extracellular matrix composition of the tissue.

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

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

Discussion

Contrary to our hypothesis, oxLDL was protective against the decrease in viable cell density seen with IL-1β. OxLDL also mitigated the increase in total MMP activity exhibited by IL-1β. The additive increase in nitric oxide concentration seen in samples exposed to IL-1β over control and oxLDL groups, (+) significantly different from control and oxLDL, (**+) significantly different from oxLDL, (+++) significantly different from control, (+++) significantly different from all other groups, (+) significantly different from all other groups, (+) significantly different than control and IL-1, (+++) significantly different than IL-1β.

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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 molecule of atherosclerosis, is a key molecule that connects these diseases. Previous studies have shown that culturing joint tissues with IL-1β produces histological and biochemical changes compatible to those seen in OA. The aim of this project was to test the hypothesis that oxLDL would exacerbate these changes induced by IL-1β in a co-culture joint model.

Objective

To evaluate the effects of the combination of oxLDL and IL-1β on the metabolism of cartilage and fat pad/synovial tissue co-cultured in vitro

Results

Fig. 1 Day 21 Representative images of T-Blue stained cartilage sections. A) Control B) IL-1β + OxLDL. Histological scoring did not yield significant differences between the groups.

Fig. 2 Day 21 Representative images of H&E stained fat pad sections. A) Control B) IL-1β C) OxLDL D) IL-1β + OxLDL. Histological inflammatory score. IL-1β was significantly higher than oxLDL and IL-1β + oxLDL.

Fig. 4 A) Tissue HP concentration. Groups containing IL-1β were significantly lower than the control. B) Tissue GAG concentration. IL-1β + oxLDL group was significantly different from oxLDL and IL-1β + oxLDL. (**+) significantly different from control and oxLDL.

Fig. 5 PGE2 was elevated in all groups exposed to IL-1β. IL-1β + oxLDL was significantly higher than control, (**+) significantly different from control and oxLDL.

Fig. 6 Nitric Oxide concentration in culture media. Nitric oxide concentration was significantly higher in all IL-1β treated samples compared to all other groups at respective time points (p<0.05 for all).

Significance

• Combination of oxLDL and IL-1β showed additive elevation of nitric oxide levels
• OxLDL was protective against the decrease in viable cell density and increase in total MMP activity seen in IL-1β treated samples

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