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

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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 material 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*

Methods

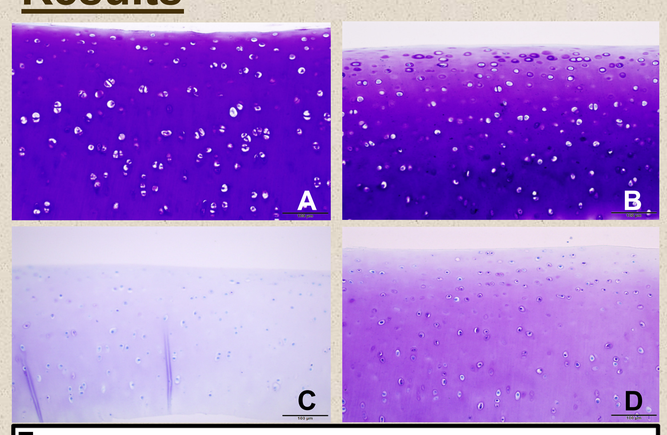
Tissue Harvest and Culture Procedures: All procedures were approved by the IACUC. Cartilage (CART) and infrapatellar fat pad (FP) tissue was harvested from 6 dogs euthanatized for reasons unrelated to this study. The animals had no orthopaedic disease with grossly normal joints. CART (6mm) and FP (4mm) 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), 3) oxLDL (100μg/mL) or 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. After 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 $p<0.05$.

Results



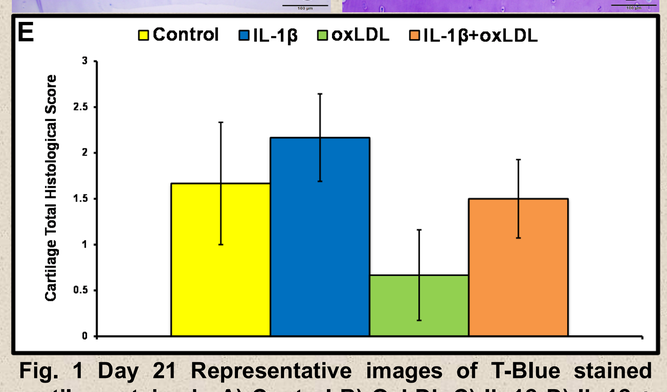
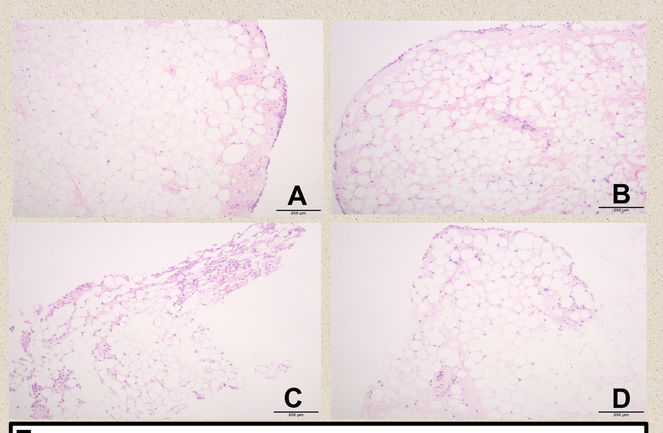


Fig. 1 Day 21 Representative images of T-Blue stained cartilage stained. A) Control B) OxLDL C) IL-1β D) IL-1β + OxLDL. Histological scoring did not yield significant differences between the groups



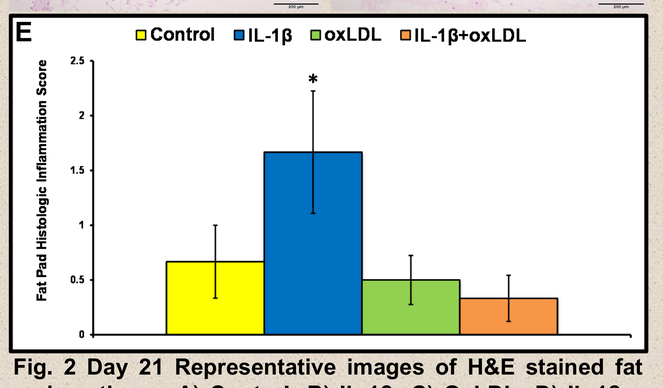


Fig. 2 Day 21 Representative images of H&E stained fat pad sections. A) Control B) IL-1β C) OxLDL D) IL-1β + OxLDL E) Histological inflammatory score. IL-1β was significantly higher than oxLDL and IL-1β +oxLDL.

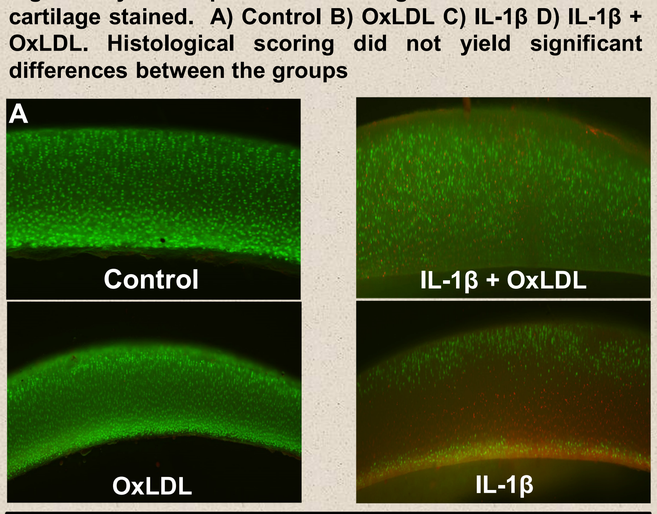


Fig. 3 A) Representative 4x images of fluorescent live (green) and dead (red) cells after 21 days of culture. B) Viable cell density. The IL-1β groups had significantly ($p<0.05$) lower viable cell density than all other groups after 21 days of culture ($p<0.05$).

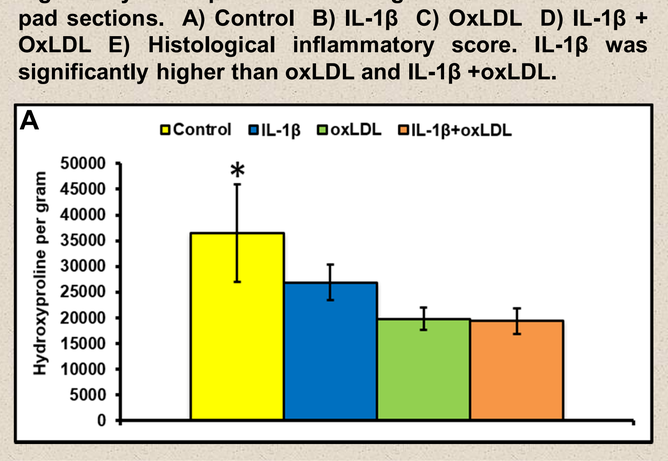


Fig. 4 A) Tissue HP concentration. Groups containing oxLDL were significantly lower than the control. B) Tissue GAG concentration. IL-1β +oxLDL group was significantly lower than control and oxLDL group. (*)=significantly different from oxLDL and IL-1 β+oxLDL, (**)=significantly different from control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

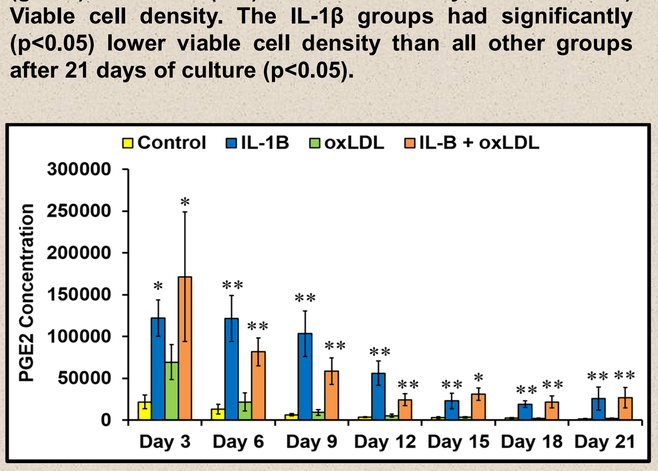


Fig. 5 PGE2 was elevated in all groups exposed to IL-1β. (*)= significantly higher than control, (**)= significantly higher than control and oxLDL

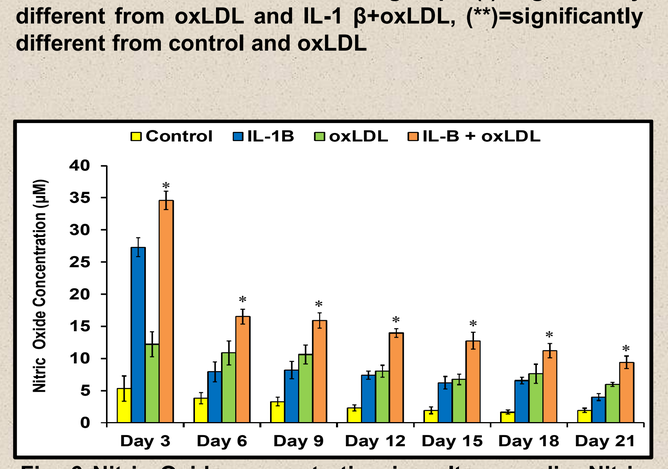


Fig. 6 Nitric Oxide concentration in culture media. Nitric oxide concentration was significantly higher in all IL-1β+ oxLDL groups compared to all other groups at respective time points ($P<0.05$ for all).

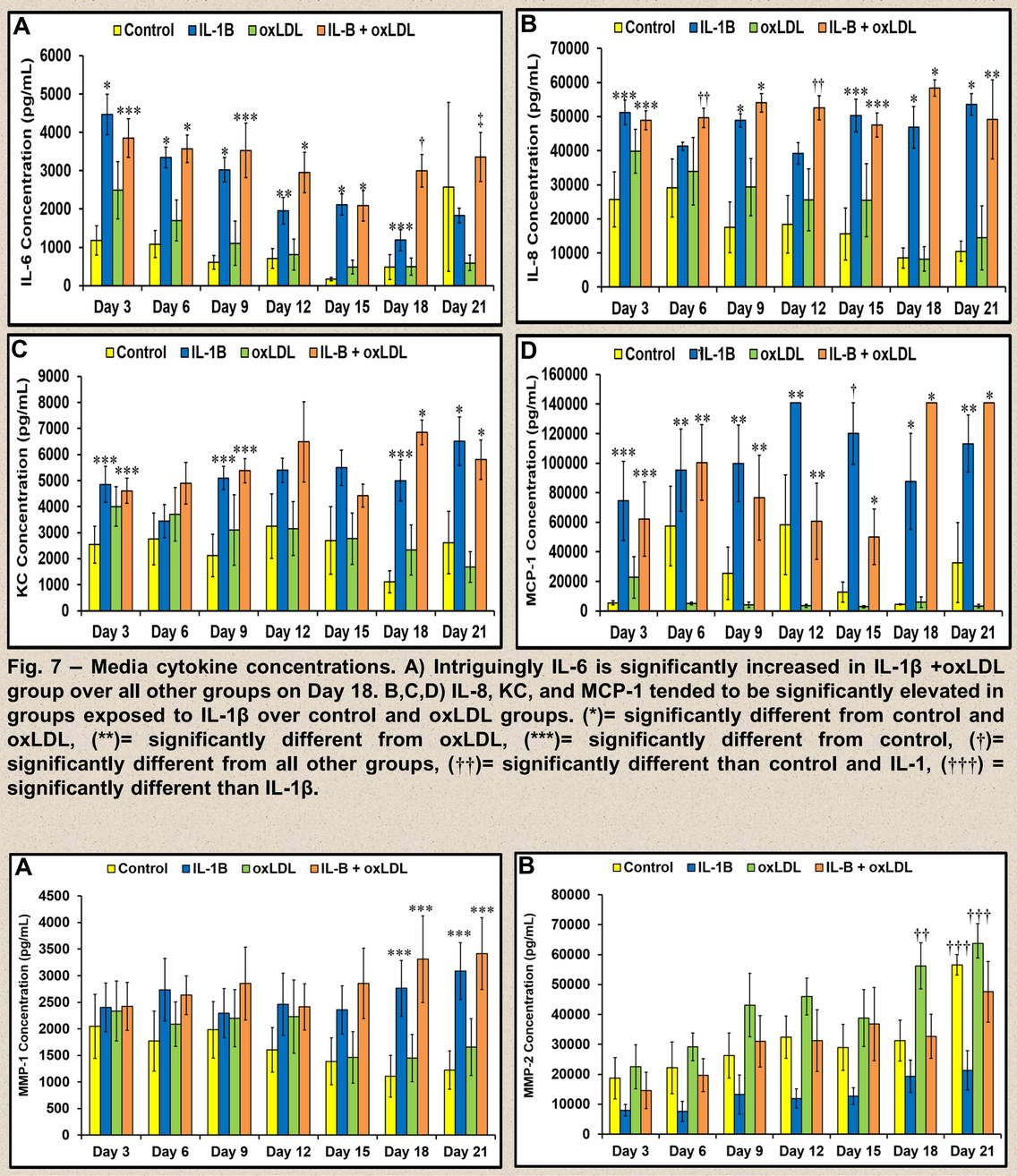


Fig. 7 – Media cytokine concentrations. A) Intriguingly IL-6 is significantly increased in IL-1β +oxLDL group over all other groups on Day 18. B,C,D) IL-8, KC, and MCP-1 tended to be significantly elevated in groups exposed to IL-1β over control and oxLDL groups. (*)= significantly different from control and oxLDL, (**)= significantly different from oxLDL, (***)= significantly different from control, (†)= significantly different from all other groups, (††)= significantly different than control and IL-1, (†††) = significantly different than IL-1β.

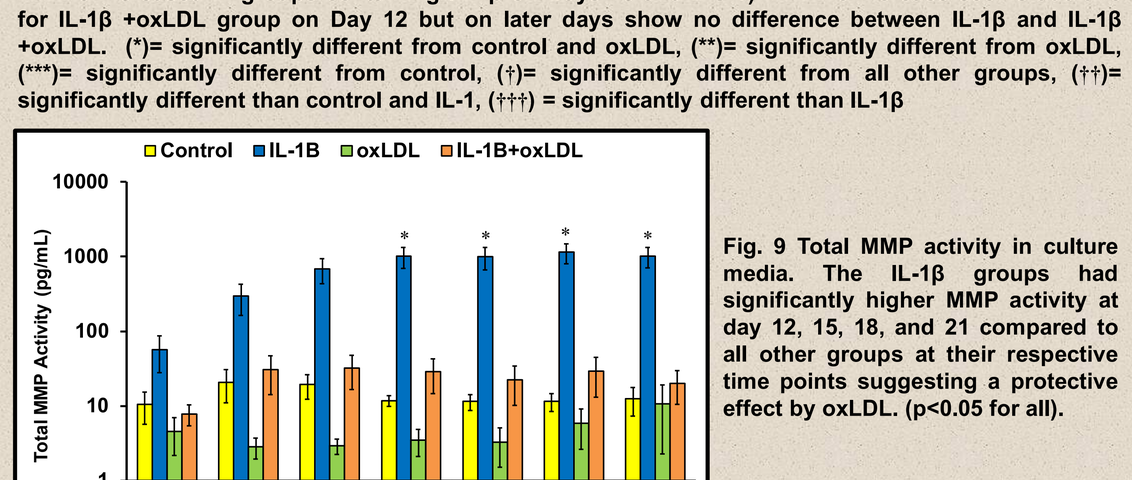


Fig. 8 Media MMP concentrations. A) MMP-1 concentrations are increased in groups containing IL-1 on Day 18 and 21. B) MMP-2 was surprisingly increased in the oxLDL group over the IL-1β group. C) MMP-3 increased in groups containing IL-1β on Days 18 and 21. D) MMP-13 increase seems additive for IL-1β +oxLDL group on Day 12 but on later days show no difference between IL-1β and IL-1β +oxLDL. (*)= significantly different from control and oxLDL, (**)= significantly different from oxLDL, (***)= significantly different from control, (†)= significantly different from all other groups, (††)= significantly different than control and IL-1, (†††) = significantly different than IL-1β

Fig. 9 Total MMP activity in culture media. The IL-1β groups had significantly higher MMP activity at day 12, 15, 18, and 21 compared to all other groups at their respective time points suggesting a protective effect by oxLDL. ($p<0.05$ for all).

Discussion

Contradictory 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 the IL-1β group. The additive increase in nitric oxide concentration seen in samples exposed to the combination of oxLDL and IL-1β however reveals a potentially inflammatory effect. These mixed results suggest the complex and multifactorial nature of metabolic contributions to osteoarthritis. Future studies to further evaluate the effect of oxLDL and other adiposity factors such as adipokines on the joint are needed but this study provides evidence that dyslipidemia may contribute to the initiation and progression of osteoarthritis disease mechanisms.

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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