Effects of Oxidized Low Density Lipoprotein on an in vitro Model of Canine Osteoarthritis

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Introduction: Patients with primary osteoarthritis (OA) often 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 occurrence 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. Our previous study demonstrated that there is an increased synovial fluid concentration of apolipoprotein B100 (ApoB), a protein closely associated with low density lipoprotein (LDL), in OA. It can be speculated that oxidized LDL (oxLDL), one of the important contributors of atherosclerosis, might be a key protein that connects CVD and OA. Further study is needed to elucidate the relationship between oxLDL and joint pathology. As a first step in that process, the study was designed to investigate the effects of oxLDL on joint health. The study hypothesis was that oxLDL would exacerbate adverse joint health effects induced by IL-1β in an in vitro model of canine osteoarthritis.

Methods: All procedures were approved by the institution’s animal care and use committee. 6mm cartilage and 4mm synovium explants were obtained from 6 dogs that were euthanatized for reasons unrelated to the project. Cartilage and synovial explants were co-cultured in media with either 1) media alone (Control), 2) recombinant canine IL-1β (2ng/ml), 3) oxLDL (100 µg/m) or 4) IL-1β + oxLDL for 21 days. Samples of conditioned media (n=6 of each group) were collected and refreshed every 3 days of culture for evaluation of IL-6, IL-8, MCP-1, Groα, MMP-1 and -3, nitric oxide (NO) and proteoglycan E2 (PGE2) concentrations and MMP activity. Explants (n=6 of each group) were collected on day 21 for evaluation of chondrocyte viability, glycosaminoglycan (GAG) content and histopathologic changes. Data from each group were combined and mean ± S.D. were determined. Significance was set at P<0.05.

Results: Viable chondrocyte density was significantly decreased in IL-1β group than all other groups (Figure 1). Nitric oxide concentration in culture media was significantly increased in IL-1β + oxLDL groups compared to all other groups at respective time point throughout the study period (Figure 2). Concentrations of PGE2, IL-6, IL-8, MCP-1, Groα, MMP-1 and MMP-3 in conditioned media were elevated in all groups exposed to IL-1β (IL-1β group and IL-1β + oxLDL group), tissue GAG content was decreased in all groups exposed to IL-1β while total MMP activity in conditioned media was only significantly elevated in IL-1β group compared to all other groups (Figure 3). Histologic examination of the cartilage and synovium explants did not yield significant differences between the groups.

Discussion: Contradictory to our hypothesis, oxLDL did not exacerbate adverse inflammatory effects and was apparently protective against the decrease in viable chondrocyte density seen with IL-1β and mitigated the increase in MMP activity exhibited by IL-1β. Only adverse effect associated with IL-1β that was exacerbated by the presence of oxLDL was nitric oxide concentration. Contrary to the popular concept of proinflammatory cascade mediated by oxLDL, Kannan et al. demonstrated that oxidized LDL was unable to elicit an active pro-inflammatory response from human monocytes and macrophages and that oxidized LDL suppressed LPS-induced proinflammatory cytokines induced in monocytes, similar to
our study results. Although the mechanisms of oxLDL-mediated anti-inflammatory and chondroprotective effects are not fully understood, it has been reported that oxidized LDL inhibits monocyte Toll ligands. Nitric oxide is a well-known catabolic and pro-inflammatory mediator produced by chondrocytes and synoviocytes in OA. NO has also been reported to inhibit cartilage extracellular matrix synthesis and induce chondrocyte apoptosis in OA. It has been reported that oxLDL enhances MMP expression in various cell types. However, similar to our finding, Wilson et al. demonstrated that oxLDL reduced MMP activity in aortic smooth muscle cells. Our in vitro study results reaffirm the complex and multifactorial nature of metabolic contributions to joint pathology. Further study is warranted to elucidate the direct and indirect effects of circulating oxLDL on joint health.

**Significance:** This study implicates a possible and complex role of oxLDL in joint health/pathology. Osteoarthritis is the leading cause of disability while cardiovascular disease is the leading cause of death among adults in the US. It would be important to delineate a possible link of OA and CVD, two of the most problematic and costly disease conditions.

**Figure 1** – Viable cell density. The IL-1β groups had significantly lower viable cell density than all other groups after 21 days of culture. (*P<0.05)
**Figure 2** – Nitric Oxide concentration in culture media. Nitric oxide concentration was significantly higher in IL-1β + oxLDL group compared to all other groups at respective time points. (*P<0.05)*

**Figure 3** – Total MMP activity in culture media. IL-1β group had significantly higher MMP activity at day 12, 15, 18, and 21 compared to all other groups at their respective time points. (*P<0.05)*
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