Oxidized low density lipoprotein (ox-LDL) and its scavenger receptor LOX-1 expression in osteoarthritis

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Introduction: Our study aimed to investigate oxidized low density lipoprotein (ox-LDL) levels in the synovial fluid (SF) of patients suffering from osteoarthritis (OA) and also the mRNA and protein expression of its scavenger receptor (LOX-1) in this common age-related joint disorder. Furthermore, we investigated the effect of ascorbic acid on LOX-1 mRNA expression as well as on chondrocytes viability.

Oxidized LDL is a molecule with many biological functions, widely known for its role on atherogenesis; it causes lipid accumulation and elicits pro-inflammatory changes. Ox-LDL has been detected in the synovium and SF of patients with rheumatoid arthritis (RA) and synergistically with cyclic tensile stretch load affects chondrocytes viability and proteoglycan synthesis.

Lectin-like oxidized LDL receptor 1 (LOX-1) is a type II membrane protein belonging to the C-type lectin family of molecules, which can act as a cell-surface endocytosis receptor for ox-LDL. LOX-1 expression and ox-LDL accumulation have been detected in arthritic joints in a rat zymosan-induced arthritis model. In human cartilage, LOX-1 expression has been detected in patients suffering from RA, whereas regarding its expression in normal and OA cartilage conflicting results come from the same research group.

Although LOX-1 has been shown to be implicated in RA, its presence in OA and normal cartilage remains controversial and its role in OA pathophysiology remains unknown.

Materials and Methods: Articular cartilage samples were obtained from femoral head, femoral condyles and tibial plateau of patients with primary OA undergoing hip and knee replacement surgery at the Orthopaedics Department of the University Hospital of Larissa. A total of 15 patients were included in the study. Normal cartilage was obtained from 5 individuals, undergoing fracture repair surgery, with no history of joint disease.

SF and serum samples were obtained from all patients and ox-LDL was measured using specific enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden) according to the manufacturer’s instructions.

RNA was isolated using TRIzol reagent. LOX-1 mRNA and protein expression was studied with RT-PCR and immunofluorescence respectively.

Primary cultures of human articular chondrocytes, osteoarthritic and normal, were stimulated with ascorbic acid (0-100 μM) (Sigma, St. Louis, USA) and LOX-1 mRNA expression was studied. The effect of ascorbic acid and of ox-LDL (10-40 μg/ml) (Intracel, USA) on chondrocyte viability was studied by the use of an MTT assay.

Results: Ox-LDL was detected in each SF tested with concentrations ranging from 12.3 U/l to 75.85 U/l (mean 34.88 ± 18.45 U/l), being similar to those measured in serum. Gender or age-specific differences of ox-LDL levels were not detected. A significant correlation was observed between BMI and ox-LDL levels in the SF (Pearson rank correlation r=0.881, p<0.01).

Human adult articular chondrocytes derived from osteoarthritic cartilage expressed LOX-1 mRNA, while normal chondrocytes showed no expression. Our LOX-1 protein expression findings were in concordance with the mRNA studies. Immunofluorescence microscopic analysis demonstrated immunolocalization of LOX-1 in osteoarthritic chondrocytes, while no immunoreactive staining was found in normal chondrocytes.

Increasing concentrations of ox-LDL added to human chondrocyte cultures, reduced cell viability dramatically, decreasing the number of cells to half after 24h incubation with 20 μg/ml.

Regarding the effect of ascorbic acid on chondrocyte viability, we observed a 50% increase in cell number 7 days after incubation with ascorbic acid, compared to non-treated cells. Furthermore, we observed that in osteoarthritic chondrocytes LOX-1 mRNA expression decreased as ascorbic acid concentrations were increased.

Discussion: In osteoarthritis, as in other age-related degenerative diseases, such as those of the cardiovascular system, altered lipid metabolism has been implicated as a critical player in disease development. Lipid deposition, seen early in OA process, before histological changes, reflects a lipid involvement in the pathogenesis of OA, while lipid diffusion from SF is depending on the severity of the disease. Age-related changes in lipid composition of cartilage could push the normally contained lipid peroxidation process into a state of uncontrolled oxidative stress, leading to the oxidation of cartilage collagen, making collagen fibrils more brittle and prone to mechanical fatigue failure. It is likely that LDL diffusion from serum into SF could be oxidatively modified to ox-LDL in the joint cavity. Excess weight as a factor of excess mechanical stress may explain our findings that the levels of ox-LDL in the SF correlated significantly with BMI.

LOX-1 mRNA and protein expression was detected in osteoarthritic cartilage, while no mRNA or protein expression was detected in normal chondrocytes. It has been shown that LOX-1 expression is enhanced by oxidative stress, mechanical strain as well as by its ligand, ox-LDL. It is possible that mechanical stress and the diffusion of ox-LDL from the SF may induce LOX-1 expression in chondrocytes.

The binding of ox-LDL to its ligand LOX-1 could oxidatively modify LDL to ox-LDL locally in the surrounding of chondrocytes maintaining a vicious cycle of a redox sensitive regulation.

If oxidative modification of LDL plays a role in osteoarthritis, it is assumed that its inhibition by an antioxidant could slow down disease progression. While ox-LDL reduced chondrocyte viability, revealing a cytotoxic effect on these cells, ascorbic acid increased the number of chondrocytes which, additionally, showed decreased LOX-1 mRNA expression, suggesting that this anti-oxidative nutrient has the ability to stop the cycle of ox-LDL binding to LOX-1.

In conclusion, we detected ox-LDL in the SF of patients with OA, as well as LOX-1 mRNA and protein expression in osteoarthritic chondrocytes, suggesting the implication of ox-LDL and its scavenger receptor LOX-1 in the pathogenesis of osteoarthritis. Furthermore, a potential therapeutic role of ascorbic acid was emerged.