LOX-1-deficient mice are resistant to zymosan-induced arthritis: A mini review

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ABSTRACT

Background: Some reports have shown that metabolic syndrome, including hypertension, hyperlipidemia, and diabetes mellitus, contributes to osteoarthritis (OA) development. Further, lectin-like oxidized low-density lipoprotein (ox-LDL) and ox-LDL receptor-1 (LOX-1), which contributes to atherosclerosis, have also been considered factors contributing to OA development. Several studies have suggested that the LOX-1/ox-LDL system is involved in OA development in vitro. We have suggested the same and conducted in vitro and in vivo studies to validate this concept. However, the role of the LOX-1/ox-LDL system in OA development has not been clarified. This study aimed to identify the mechanism of the LOX-1/ox-LDL system to clarify OA development.

Methods: A zymosan-induced arthritis model was used to identify the mechanism of the LOX-1/ox-LDL system using LOX-1-knockout (KO) mice. Zymosan was administered via the intra-articular route to induce arthritis.

Results: From our experiment, we found that the LOX-1/ox-LDL system contributes to OA development through matrix metalloproteinase-3.

Conclusion: Our findings suggest that the treatment of abnormal lipid metabolism may contribute to the prevention and suppression of arthritis.

Introduction

Osteoarthritis (OA), which is characterized by wear and tear of the articular cartilage, was previously believed to be caused by mechanical stress. However, recent studies have suggested the role of other systemic factors. One of them is metabolic syndrome, including hypertension, hyperlipidemia, and diabetes mellitus, all of which lead to atherosclerosis. Hyperlipidemia is caused by various factors, such as low levels of high-density lipoprotein (HDL), high levels of low-density lipoprotein (LDL), and triglycerides. In recent years, Sawamura et al. have reported that lectin-like, oxidized low-density lipoprotein (ox-LDL) and ox-LDL receptor-1 (LOX-1) contribute to atherosclerosis. Moreover, recent studies revealed the epidemiological relationship between atherosclerosis and OA. However, how atherosclerosis contributes to OA has not been clarified in detail. Interestingly, subsequent studies have shown that the LOX-1/ox-LDL system contributes to the development of rheumatoid arthritis. Previously, we described that ox-LDL binding to LOX-1 induces stress-induced premature senescence of chondrocytes and results in suppression of telomerase activity by inactivating the PI3K/Akt pathway. Recently, we also demonstrated that OA development is downregulated in LOX-1 knockout (KO)
mice. Moreover, development of age-related OA was also found to be reduced in LOX-1 KO mice.

Generally, arthritis such as rheumatoid arthritis differs from OA, as OA is induced by mechanical stress while arthritis is mainly induced by inflammation. Although some studies revealed the relationship between arthritis and LOX-1/ox-LDL, the roles of LOX-1/ox-LDL system in arthritis are still not clarified in detail. Therefore, this study aimed to clarify the role of the LOX-1/ox-LDL system in the development of arthritis. In other words, we tried to identify the role of LOX-1/ox-LDL in inflammatory changes such as synovitis. We also hypothesized that LOX-1 KO would inhibit arthritis-associated changes, which include synovitis and cartilage degeneration.

Summary of the current study

Material and methods

We induced arthritis by administering zymosan intra-articularly. Zymosan is an insoluble fraction of yeast cell walls, which are composed predominantly of polysaccharides. It induces arthritis, which is characterized by cartilage degeneration and synovitis in rodents when injected intraperitoneally or intra-articularly. Zymosan is often used to induce arthritis, especially to induce synovial inflammation. Zymosan was used, as it is the most commonly used method. In addition, in this study, the model was used to evaluate the role of LOX-1/ox-LDL system on arthritis, especially synovitis.

Wild-type (WT) and LOX-1 KO mice (n=10, in each) were used, and we injected with zymosan to induce arthritis (180 μg/6 μL; Sigma-Aldrich, St. Louis, MO, USA). We observed at the time point of 24, 48 and 72 hours after zymosan injection. As a control group, we conducted saline injection to contralateral knee joint with observation at the same time point. Immunohistochemical analysis was performed to evaluate the expression of matrix metalloproteinase-1 and 3 (MMP-1 and 3) and to determine its role in arthritis development.

All data have been presented as mean ± standard deviation. The scores of each group (n=10) were compared using Student’s t-test. P-values less than 0.05 were considered significant. All data were analyzed with Stat Mate (Atms, Tokyo, Japan) software for Windows, version 4.01.15,16. The correlation between the LOX-1 or ox-LDL positive cell rate and cartilage degeneration score was examined using Pearson’s correlation (Excel 2010, Microsoft, Tokyo, Japan).

Results

We compared zymosan-induced arthritis in wild-type (WT) and LOX-1 KO mice and found it to be more severe in the former (Figures 1, 2). Interestingly, both zymosan-induced cartilage degeneration and synovitis were significantly reduced in LOX-1 KO mice compared to WT mice (Figures 1, 2). In the saline-injected control groups, no difference was observed between WT and LOX-1 KO mice (data not shown). Immunohistochemical staining revealed LOX-1 and ox-LDL expression in the chondrocytes and inflammatory synovial cells in WT mice (Figures 3, 4) but not in LOX-1 KO mice (data not shown).
shown). These findings suggest that LOX-1/ox-LDL in the chondrocytes and inflammatory synovial cells are involved in the development of arthritis. In our evaluation of the expression of matrix metalloproteinase-1 and 3 (MMP-1 and 3) by immunohistochemical analysis, MMP-1 was not detected in both WT and LOX-1 KO mice (data not shown); however, MMP-3 expression was significantly higher in the chondrocytes and inflammatory synovial cells of WT mice than in those of LOX-1 KO mice (Figures 5, 6).

**Discussion**

The present study was conducted to clarify the role of the LOX-1/ox-LDL system in the development of arthritis, particularly on inflammatory changes such as synovitis. Our findings indicate that MMP-3 plays a role in the development of arthritis downstream of LOX-1/ox-LDL. These results could provide new findings that LOX-1/ox-LDL system is involved in inflammation such as synovitis and cartilage degeneration induced by synovitis.

This study has limitations. First, in vitro methods were not employed, which need to be included in future studies. Second, we could not evaluate systemic inflammation. Zymosan could induce systemic inflammation as previously described20, 21. In the current study, systemic inflammation as well as the systemic effects of atherosclerosis was not investigated. Second, atherosclerosis was not evaluated. It
would be interesting to investigate the correlation between atherosclerosis and arthritis development. Further research will be necessary.

**Conclusion**

Our findings indicate that treatment of abnormal lipid metabolism may contribute to the prevention and suppression of arthritis. Hence, we believe that the LOX-1/ox-LDL system is involved in the development of arthritis via MMP-3.

**Declarations**

**Availability of data and material**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

None.
Figure 6: Representative tibial chondrocyte immunostaining of matrix metalloproteinase-3 (MMP-3) (a-f). MMP-3 expression in the chondrocyte of wild-type (WT) mice at days 1, 3, and 7 after zymosan injection (a-c) at 400× magnification. MMP-3 expression in the chondrocyte of ox-LDL receptor-1 (LOX-1) knockout (KO) mice at days 1, 3, and 7 after zymosan injection (d-f). MMP-3 positive cells are observed in chondrocytes of both WT (a-c) and LOX-1 KO mice (d-f) in all experiments. The graphs show the positive cell score of MMP-3 expression in the chondrocytes of WT and LOX-1 KO mice after zymosan injection at each experiment (g). Arrows show the MMP-3-positive chondrocytes. Rabbit anti-mouse MMP-3 polyclonal antibodies were used. Scale bars = 100 μm.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgements

We would like to thank Editage (www.editage.jp) for English language editing.

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