A COX-2 inhibitor, Celecoxib, Inhibits IL-1-Stimulated IL-6 Secretion from Ligamentum Flavum-Derived Cells

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INTRODUCTION:
In aged society, there are many elderly people complaining low back pain and lower leg pain due to lumbar spinal stenosis (LSS). As the exact mechanism of pain due to LSS is not yet clear, one of the pain pathomechanisms would be inflammatory response and the involvement of pain-related cytokines such as interleukins (ILs). The importance of pain-related cytokines should be focused because stenosis of spinal canal that is caused by hypertrophy of ligamentum flavum (LF) is not always correlated to pain score of LSS patients. In other words, there is a discrepancy between the degree of stenosis and the degree of pain, that is, some patients with severe stenosis have mild pain, while some with mild stenosis complain severe pain. Therefore, the treatment of LSS patients would be the management of pain-producing substances such as ILs. IL-1 is a strong inflammatory cytokine that is involved in many diseases. Previously, inflammatory cytokines including IL-1 have been reported to be expressed in human LF that were obtained from surgical samples. IL-6 is also an inflammatory cytokine that is involved in pain response. Celecoxib is a cyclooxygenase-2 (COX-2) inhibitor that is used to reduce inflammatory response in many diseases including LSS. However, there is no report that explains the mechanism of medicine in LSS patients. The purpose of this study was to investigate whether celecoxib is truly effective in human LF-derived cells (HFCs).

METHODS:
We conducted this study in compliance with the principles of the Declaration of Helsinki. The study's protocol was reviewed and approved by the Institutional Review Board of Nagoya City University. LF tissues were obtained from patients who underwent surgery due to LSS, who provided written informed consent for this research.

Isolation of cells from human ligamentum flavum: LF from patients was cleaned and digested, and other tissue including ossified parts were removed carefully. HFCs were isolated by sequential enzyme-digestion with collagenase type I. HFCs were then obtained by filtration through a 70 μm membrane.

IL-1 stimulation of HFCs: HFCs were seeded in 6-well plates at a density of 10.0×10^5 cells/well in 10% FBS-DMEM. After sub-confluent, HFCs were stimulated by 10 pg/ml of IL-1, when indicated, 1 μM of celecoxib was added. IL-6 secretion in the conditioned media from HFCs was measured by ELISA. To analyze the expression of COX-2, cells were harvested using RIPA buffer and cell protein was extracted for use in western blotting.

Quantitative data presentation and statistical analyses: Data were analyzed using biological triplicates per condition, and experiments were repeated at least twice to demonstrate reproducibility. A two-tailed Student's t-test was used for statistical analysis and differences were considered significant when the *p* value was less than 0.05.

RESULTS:
Figure 1 shows that IL-1 (10 pg/ml) time-dependently stimulated IL-6 release from HFCs. We confirmed that IL-1 induced COX-2 expression in HFCs (Fig. 2). The expression level of COX-2 was increased from 8 to 16 hours later after IL-1 stimulation. To investigate whether COX2 was involved in IL-1-stimulated IL-6 secretion from HFC, we added celecoxib in HFC culture condition medium. According to the expression level of COX-2, celecoxib was added to condition medium 8 h later of IL-1 stimulation. IL-6 was measured after 48 hours of IL-1 stimulation. The amount of IL-6 secreted from HFCs stimulated by IL-1 was significantly suppressed by celecoxib (Fig. 3).

DISCUSSION:
In the present study, we confirmed that IL-1 stimulates the secretion of IL-6 in LF cells obtained from human surgical samples. IL-1 stimulated inflammatory response such as COX-2 expression. Our findings suggest that IL-1-stimulated IL-6 secretion mediates the inflammatory response in the LF. Therefore, the therapeutic strategy to manage inflammatory response in LF could be a reasonable method to treat the pain of LSS patients. Here, we showed that celecoxib reduced IL-1-stimulated IL-6 secretion, which might be one of the pain-control mechanisms. It is also reported that the inflammatory response is involved in the process of hypertrophy of LF. The results here could be applied to the prevention of hypertrophy of LF using anti-inflammatory drugs such as celecoxib.

SIGNIFICANCE/CLINICAL RELEVANCE:
We showed that celecoxib reduced IL-1 stimulated IL-6 secretion from human ligamentum flavum derived cells. Anti-inflammatory drugs such as celecoxib could be used to reduce pain but also reduce inflammatory response of ligamentum flavum to prevent hypertrophy of ligamentum flavum.

IMAGES AND TABLES:

Fig1: IL-1 stimulated IL-6 secretion from HFCs time-dependently.

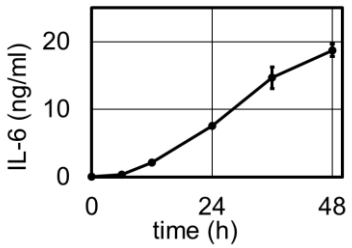

Fig2: IL-1 time-dependently induced COX-2 expression.

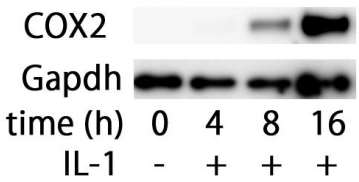

Fig3: Celecoxib suppressed IL-1-stimulated IL-6 secretion from HFCs. # $p<0.05$ to IL-1 alone.