INTRODUCTION:
The lumbar spinal canal stenosis is one of the most common spinal disorders in elderly patients. Canal narrowing (stenosis) in part results from hypertrophy of the ligamentum flavum (LF), which mechanically compresses nerve root or cauda equina. Chemical inflammation follows the mechanical event to aggravate the symptoms. NSAIDs or steroid used to reduce the symptoms have proven effective in certain patients. These medications are believed to decrease the inflammation. However, there is a possibility that these drugs may work by reducing the ligament hypertrophy itself by inhibiting COX-2. Thus, we hypothesized that certain drugs may effectively control LF hypertrophy. To support this hypothesis we undertook a comprehensive biological and histological investigation of the LF obtained in surgery to detect the presence of COX-2.

METHODS:
Histological study: Twenty LF samples were collected at the time of surgery. Two staining techniques were used for evaluation: Trichrome (T) stain for evaluating the degree of fibrosis, and Verhoeff-Van Gieson elastic stain (VVG) to evaluate the condition of elastic fibers. The fibrosis was graded with reference to its severity (range 0 to 4). Grade 0 means normal tissue showing no fibrotic region; grade 1 - fibrosis at 25% or lesser of the entire area; grade 2 - between 25 and 50%; grade 3, between 50 and 75% and grade 4, over 75%. The loss of elastic fibers was also graded using the same scoring system as the fibrosis score. Data were collected from three regions of the LF: dural, middle and dorsal sites. The relationships of these two scores with thickness of ligamentum flavum measured by MRI examination before the surgery were evaluated at each site in the ligament.

Biological study: Standard qualitative and quantitative real time reverse transcriptase-coupled polymerase chain reaction (RT-PCR) was undertaken. Amplification, detection and data analyses for real time PCR were carried out on a BioRad iCycler. The ligament expression of cyclooxygenase-1 and -2 (COX-1 and -2) was evaluated for 18 LF obtained from lumbar surgery. Expression of GAPDH was also evaluated for each sample to serve as an internal control.

Immunohistochemical study: To understand the histological location of COX-2 in the LF tissue, we used immunohistochemical technique. The 18 samples were collected during the surgery and embedded in paraffin. Antibody of COX-2 was purchased from Cayman Chemical (Ann Arbor, MI). A routine 3-stage immunoperoxidase staining technique using avidin-biotin-immunoperoxidase complex was performed.

RESULTS and DISCUSSION:
Histological study:

Overall, the mean fibrosis score of dural, middle, and dorsal side were 1.5, 2.1 and 3.5, respectively. The fibrotic changes were severe at the dorsal side. The relationship between LF thickness and fibrosis score at the dorsal side showed significant positive linear co-relations (p<0.05). The loss of elastic fiber (LEF) score of dural, middle, and dorsal sides were 1.5, 2.1 and 3.5, respectively. The relationships between LF thickness and LEF score at the dorsal side showed significant positive linear co-relations (p<0.05). Figure 1 represents the histology of the hypertrophied ligament. At the dorsal side elastic fibers, which were stained as black color, were scarcely observed in VVG stain, and severe fibrosis was also detected (blue color in T stain). This indicated in the hypertrophied ligaments, there is a thick fibrosis (scarring) formation with loosening elastic fibers, especially along the dorsal aspect of LF. COX-2 expression is reportedly correlated with tissue fibrosis (scarring). We therefore investigated COX-2 mRNA expression of the LF using RT-PCR, and immunohistochemistry, as follows.

Biological study:

COX-1 mRNA expression was not detected in any of the samples. In contrast, all samples showed COX-2 mRNA expression, as determined by standard RT-PCR. The result indicated that COX-2-related inflammation in the hypertrophied LF was likely. As shown in Figure 2, the relative expressions of COX-2 showed a weak (r=0.20) positive correlation with LF thickness. Thus, COX-2 may be correlated with LF hypertrophy due to fibrosis.

Immunohistochemical study: Out of 18 samples, 14 (77.7%) showed positive staining of COX-2. Twelve of them were stained in the endothelial cells of vessels in LF. No fibroblast showed positive staining.

Thus, these biological and immunohistochemical analyses revealed the presence of COX-2, which is released from vascular endothelial cells within the ligamentum flavum, and thus there should also be COX-2-related inflammation in the LF. The COX-2-related inflammation may induce fibrosis in LF, and its accumulation may cause LF hypertrophy.

Drugs that lessen symptoms for patients with canal stenosis such as NSAIDs and steroid can inhibit COX-2; thus, one can assume that these drugs should reduce COX-2 related scar formation in hypertrophied LF. Although there is significant controversy resulting from the adverse effects of COX-2 inhibitors, this study suggests that the hypertrophy in the LF may be controllable in nature.

CONCLUSION:
Hypertrophy of the ligamentum flavum in patients with lumbar spinal canal stenosis may be due to the accumulation of scar tissue, and it can be controlled by drugs that inhibit COX-2 activity.

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Figure 1. Histology of hypertrophied ligament

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Figure 2. Results of real time RT-PCR

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