MMP13 and αSMA are Increased in the Ligamentum Flavum of Rats with Surgically Induced Posterior Lumbar Instability, a Model of Ligamentum Flavum Thickening

Yuta Goto[1], Kenji Kato[1], Kiyoshi Yagi[1], Tomoko Koshimae[1], Hideki Murakami[1], Nobuyuki Suzuki[1]
[1]Nagoya City University, Nagoya, Japan
Email of Presenting Author: 9071ygoto@gmail.com

Disclosures: Yuta Goto (N), Kenji Kato (N), Kiyoshi Yagi (N), Tomoko Koshimae (N), Hideki Murakami (N), Nobuyuki Suzuki (N)

INTRODUCTION:
Lumbar spinal canal stenosis causes various neurological symptoms mainly due to thickening of the ligamentum flavum, and the number of patients is very large. Although there are many patients, the pathophysiological mechanism has not yet been elucidated. Therefore, at present, the main treatment for lumbar spinal canal stenosis is symptomatic therapy. This means that there are no effective drug treatments that directly act on stenotic lesions. Therefore, patients who are poorly controlled with symptomatic therapy may require surgical treatment. However, there are some elderly patients who cannot undergo surgical treatment due to perioperative risks. Therefore, there is an urgent need to develop effective drugs. To that end, we thought that it is necessary to elucidate the pathology of lumbar spinal canal stenosis immunohistologically to uncover pathomechanisms of lumbar spinal canal stenosis. The purpose of this study is to examine immunohistochemical analysis of ligamentum flavum of experimental rats at different timepoints to investigate a mechanism of ligamentum flavum thickening that causes lumbar spinal canal stenosis.

METHODS:
Surgery: This study was approved by an institutional review board conforming to the laws and regulations of the country of origin. Sprague Dawley rats (10-week-old) were used. In this analysis, 9 control rats and 9 experimental rats were used. Experimental rats were anesthetized by ketamine (75 mg/kg) and xylazine (3 mg/kg). With back center incision, the paravertebral muscle was detached from the spinous process and bilateral L4/5 facet joints were removed. The L4/5 interspinous ligament was also resected to increase instability (Figure 1, 2). After confirming instability, the fascia and skin were closed by layer-suture. Control rats were sham-operated. With the same approach, the L4/5 facet was revealed but no resection was done.
Histological analysis: Rats were euthanized at 2 weeks, 4 weeks, and 16 weeks after surgery, and the spine was removed (three experimental rats and three control rats at each time point). Ligamentum flavum of L4/5 level was collected from the resected spine. The isolated ligamentum flavum was fixed in 10% formalin and frozen sectioned. Then, histological analysis was performed by immunostaining with MMP13 and αSMA.

RESULTS:
Both MMP13 and αSMA tended to be stained stronger in the experimental group than in the control group at all time points. With MMP13, the expression level of the experimental group decreased over time (Figure 3).

DISCUSSION:
We have reported this rat model in the 2022 ORS meeting. In that report, we reported the usefulness of this animal model by showing significant differences between experimental and control groups in behavioral analysis and measurement of ligamentum flavum thickness. This time, we decided further histological analysis of the ligamentum flavum using this rat model. MMP-13 is a collagenase that has a high affinity for type 2 collagen and specifically degrades it and has the effect of destroying cartilage and bone matrix. αSMA is a contractile fibrous protein that is expressed in the process of differentiation from fibroblasts to highly contractile myofibroblasts during wound healing. Focusing on tissue destruction and repair, we decided to investigate these proteins immunohistologically. The increase of MMP13, a proteolytic enzyme, suggested that protein degradation occurred due to tissue damage by lumbar instability. αSMA is an index of increased myofibroblasts and tissue fibrosis, so the increase in the surgery group means that fibrosis of the ligamentum flavum was induced in response to increased posterior lumbar instability. This study suggests that the proteolytic enzyme MMP13 is attenuated over time and the fibrosis marker αSMA is prolonged, leading to increased fibrosis and thickening of the ligamentum flavum in the chronic phase. In other words, protein destruction (tissue destruction/damage) occurs in the acute to subacute stages, and tissue thickening occurs due to the prolonged fibrosis reaction in the chronic stage. In this way, to investigate the process of ligamentum flavum thickening at the cellular level in detail may lead to develop drugs that are effective against ligamentum flavum thickening in lumbar spinal canal stenosis.

SIGNIFICANCE:
In our rat model, induced posterior instability of the lumbar spine increased both MMP13 and αSMA in the ligamentum flavum during the acute phase, and only the αSMA expression was prolonged during the chronic phase. We will continue to investigate the mechanism of ligamentum flavum thickening at the cellular level, and these studies may be able to develop an effective disease-specific treatment.

Figures:

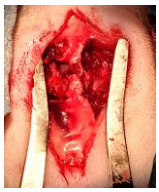

Figure 1. Experimental group after facet joint resection and interspinous ligament resection.

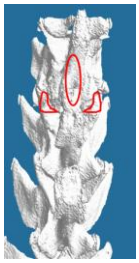

Figure 2. A μCT image. Red-marked areas are the facet joints and interspinous ligament. That were removed.

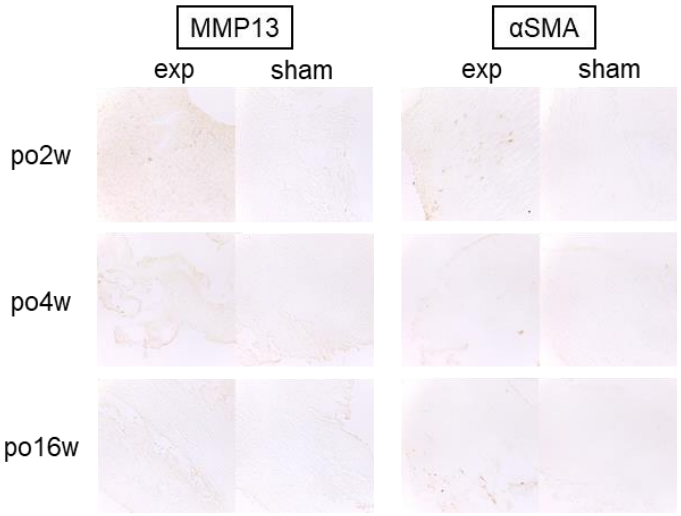

Figure 3. Histological analysis. The collected ligamentum flavum was immunostained with MMP13 and αSMA, respectively. Both MMP13 and αSMA tended to be stained stronger in the experimental group than in the control surgery group at all time points.