Role of Epiligament in Ligamentum Flavum Hypertrophy in Patients with Lumbar Spinal Canal Stenosis: a Pilot Study

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Abstract: Ligamentum flavum (LF) hypertrophy is one of the main factors of lumbar spinal canal stenosis (LCS). The primary object of this study is to clarify the existence of epiligament in the LF and its role in hypertrophy, and to develop an LF hypertrophy animal model. A cadaveric spine from a 30-year-old man was used to investigate the existence of epiligament in LF. Five LF samples from LSCS patients were obtained to evaluate hypertrophied LF. To create a rat model, we destabilized the lumbar spine. Each LF was sagittally cut for histological evaluation. The epiligament was clearly evident in normal LF specimens, which stained pink on Elastica van Gieson and green on Masson Trichrome. One layer was observed on the dural side and another on the dorsal side of the LF. LSCS patients had an enlarged dorsal epiligament, at around 30 times that of the regular thin epiligament on the dural side. The destabilized rat model showed an enlarged dorsal epiligament, with a mean thickness 8-fold that of the control. LF hypertrophy may be due to enlargement of the dorsal epiligament. Mechanical loading of the LF is an important factor for inducing hypertrophy in the rat model.

INTRODUCTION

Lumbar spinal canal stenosis (LSCS) is a common lumbar disorder in the elderly population causing low back pain, radiculopathy, and cauda equina syndrome. Canal narrowing (stenosis) results partly from hypertrophy of the ligamentum flavum (LF), which mechanically compresses the nerve root or cauda equina (1-4). Although numerous investigations have been conducted to clarify the pathomechanism of LF hypertrophy, the exact mechanism has yet to be revealed (1-14). Therefore, surgeons are currently removing the hypertrophied LF since they cannot control its hypertrophy with drugs (15, 16). If the exact pathomechanism could be clarified, it may be possible to control the LF hypertrophy with drugs.

The epiligament is the surface layer of ligaments and consists of woven bundles of collagen fibers (17-19). Bray et al. (17) were the first to report the epiligament of the medial collateral ligament (MCL), which is considered to have several important functions including protecting the MCL against abrasion and being a source of extracellular matrix during ligament growth and healing (18). They created an MCL hypertrophy animal model and showed that epiligament hypertrophy induced MCL hypertrophy in an unstable knee. Thus, the epiligament can be considered to play a major role in ligament hypertrophy. To date, however, no studies have reported on the role of epiligament in LF hypertrophy or even clarified its existence in the LF.

We hypothesized that epiligament surrounds the LF and is the main contributing tissue in LF hypertrophy such as MCL hypertrophy. The purpose of this study was to clarify the existence of epiligament in the LF, to elucidate its role in LF hypertrophy, and then to create an LF hypertrophy animal model.

MATERIALS AND METHODS

Institutional review board approval was obtained for this study, and subjects provided informed consent to participate.

(i) Normal human LF specimens

LF was taken from a fresh cadaveric spine of a 30-year-old man at the Th11-12 level as a human LF control as it did not show severe degeneration or hypertrophy.

(ii) Hypertrophied human LF specimens

LF specimens were collected from 5 patients (1 man, 4 women; mean age, 71.0 years; age range, 66 to 79 years) during surgery for degenerative LSCS.

(iii) Rat model LF specimens

We created posterior destabilization to increase loading on the LF in 6 female Wistar rats (3 for the model and 3 for the control; all 8 weeks old). Under general anesthesia with sodium pentobarbital (32.4 mg/kg body weight), the L5 spinous process, L4-6 supraspinous ligament, and L4-5 and L5-6 interspinous ligaments were removed. We paid special attention not to touch the lamina or LF during surgery. The rats were killed 8 weeks after the operation by pentobarbital overdose for histological study.

Histological processing

Ligaments in the human specimens were sagittally cut, fixed in 10% formalin for 48 h, and embedded into a paraffin block. Intact spinal column, including the vertebral body, disc, and lamina, was taken from the rat specimens at the L4-6 level and fixed in formalin for 48 h. Samples were decalcified with Plank-Rychlo’s solution (Decalcifying Solution A; Wako, Osaka, Japan) and then sagittally cut in the slightly para-sagittal plane from the midline to evaluate...
the LF. Thin-sliced sections (3 µm) of the human and rat specimens were subjected to the following staining: Elastica van Gieson (EVG) to evaluate the condition of the elastic fibers and Masson Trichrome (MT) to evaluate the state of fibrosis.

RESULTS

(i) Normal human LF specimens

The LF consists of elastic fibers rather than collagen fibers. Thus, most parts of the LF stained black on EVG staining and pink on MT staining. In the human control samples, we found two superficial collagenous layers which stained red on EVG staining (Figures 1a-c) and green on MT staining (Figures 1d-f). One layer was present on the dural side and the other on the dorsal side of the LF, indicating that this collagenous layer surrounds the main elastic LF tissue; these layers corresponded to the epiligament. Mean human epiligament thickness at the dorsal and dural aspects measured at five randomly selected sites was 140.9 ± 39.9 µm and 33.8 ± 7.9 µm, respectively. In the human samples, epiligament was thicker at the dorsal aspect than at the dural aspect.

Mean 1162.6 ± 389.4 µm, and that of the enlarged dorsal epiligament in all 5 samples was 1162.6 ± 389.4 µm (Table 1). On the other hand, the mean thickness of the dural epiligament in this sample was 75.4 ± 8.4 µm, and that of the dural epiligament in all 5 samples was 42.6 ± 19.2 µm (Table 1). The dural aspect which had minimal fibrosis and multiple elastic fibers, was mostly of regular size (Figures 2c, f). Thus, the epiligament at the dorsal aspect was about 30-fold thicker than that at the dural aspect in the hypertrophied LF.

(ii) Hypertrophied human LF specimens

In the elderly subjects with LSCS, the dorsal epiligament (thick fibrous area without elastic fibers) was enlarged in all 5 LF samples. Figure 2 shows a representative case of LSCS in a 70-year-old woman. The thick fibrotic area at the dorsal aspect, with loss of elastic fiber, was obvious (Figures 2a, b, d, and e). The mean thickness of the enlarged dorsal epiligament in this sample was 1450.8 ± 412.9 µm, and that of the dural epiligament was 75.4 ± 8.4 µm.

Table 1. Thickness of the epiligament of LF from patients with LSCS

<table>
<thead>
<tr>
<th>Case #</th>
<th>Dorsal aspect (µm)</th>
<th>Dural aspect (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>1450.8 ± 412.9</td>
<td>75.4 ± 8.4</td>
</tr>
<tr>
<td>#2</td>
<td>983.0 ± 344.6</td>
<td>44.0 ± 5.0</td>
</tr>
<tr>
<td>#3</td>
<td>851.1 ± 60.5</td>
<td>29.7 ± 3.7</td>
</tr>
<tr>
<td>#4</td>
<td>832.0 ± 216.7</td>
<td>32.6 ± 5.2</td>
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<tr>
<td>#5</td>
<td>1696.3 ± 317.1</td>
<td>31.5 ± 8.2</td>
</tr>
<tr>
<td>Mean</td>
<td>1162.6 ± 389.4</td>
<td>42.6 ± 19.2</td>
</tr>
</tbody>
</table>

(iii) Rat model LF specimens

The control rat had no fibrosis of the LF, which consisted mainly of elastic fibers. Regular thin epiligaments were seen at both the dorsal and dural aspect (Figures 3a-f). The mean thickness of the dorsal epiligament was 20.9 ± 15.2 µm, and that of
the dural epiligament was 31.2±3.2 µm. On the other hand, a fibrotic area was found at the dorsal aspect in the rat unstable lumbar spine model on MT staining (Figures 4d, e). As this fibrotic area had few elastic fibers on EVG staining (Figures 4a, b), it indicated an enlarged LF epiligament. The mean thickness of the enlarged dorsal epiligament was 165.1±13.8 µm, which was notably thicker than in the control rat. In this model, the dural epiligament was not enlarged, with the mean thickness of 31.7±7.2 µm (Figures 4c, f). Taken together, posterior destabilization could cause enlargement of the dorsal side of the LF while keeping the dural side of the epiligament intact. These features are similar to the histological findings of the samples from LSCS patients.

**DISCUSSION**

This study revealed the following novel findings:

1) The human LF contains an epiligament consisting mainly of collagenous fibers, while the LF itself consists of elastic fibers. Thus, the epiligament has obvious histological differences compared with the main LF.

2) All hypertrophied LF samples from the LSCS patients had an enlarged epiligament in the dorsal aspect consisting of collagenous tissue, not elastic fibers. Epiligament thickness at the dorsal aspect was 30-fold that at the dural aspect.

3) Posterior destabilization in the rat spine probably caused the thickening of the dorsal epiligament, which was 8-fold thicker than that of the control rat. This histological finding was similar to the human samples from the LSCS patients. This is the first animal model of LF hypertrophy to be reported.

**Epiligament of the LF**

The epiligament constitutes the surface layer of ligaments and consists of woven bundles of collagen fibers (17-19). Knee MCL hypertrophy was reproduced in the animal knee instability model, and MCL hypertrophy of the epiligament induced MCL hypertrophy. Thus, the epiligament is considered to play a crucial role in ligament hypertrophy. To date, however, no studies have reported the role of the epiligament in LF hypertrophy. Moreover, the existence of the LF epiligament has been unknown. With this in mind, we first confirmed the existence of the LF epiligament. Normal LF was collected from the fresh cadaveric spine of a 30-year-old man as a human control. This specimen was obtained from the thoracic spine, because even relatively young spines could have degenerative changes in the LF in the lumbar region. The sample clearly showed collagenous membranous tissue on both the dorsal and dural surfaces.

In the MCL investigation, it was difficult to differentiate the epiligament from the main MCL because both consisted mostly of type I collagen (17-19). On the other hand, the epiligament was obvious in the LF, making differentiation on MT and EVG staining easy. As the epiligament consists of collagenous fibers, while the LF consists of elastic fibers, staining is completely different.

**Mechanism of LF Hypertrophy**

LF hypertrophy is one of the major factors of canal narrowing in LSCS. Many studies have investigated the mechanism of LF hypertrophy using anatomical, histological, and biological methods (2, 9, 11-14, 20-22). Our group previously reported that hypertrophy occurs due to the accumulation of fibrosis (scarring) at the dorsal aspect of the LF (22-24). The present study revealed similar histological findings, with apparent enlargement of the thick fibrotic mass at the dorsal aspect of the LF epiligament; the thickness was 30-fold that of the dural epiligament.

This study, as well as previous reports (22-24), indicates that the dorsal fibrous mass, which seems to correspond to enlargement of the dorsal epiligament, is the main pathology causing LF hypertrophy. Chowdhury et al. (18) reported that epiligament is a
source of extracellular matrix, cells, and vasculature during liga-
ment growth and healing, and they further concluded that epliga-
ment is the main source of cells that form ligament scars during ligament healing. Matthews et al. (25) created an MCL hypertro-
phy model using a canine knee joint and induced hypertrophy by transecting the anterior cruciate ligament to destabilize the knee joint. In the hypertrophied MCL, a dense, scar-like tissue mass was found at the medial aspect, and histological findings indicated that the scar-like fibrous mass was connected to the medial epligament. Their histological findings were similar to those of Chowdhury et al. (18). Thus, for the LF hypertrophy mechanism, it is not diffi-
cult to assume that (i) micro injury occurs at the dorsal aspect of the main LF and (ii) healing of the LF causes the dorsal epliga-
ment to create a thick fibrotic mass in the dorsal aspect of the LF.

The present finding are in agreement with previous findings (22, 23) that the dorsal aspect of the LF showed thick scarring and the dural aspect was mostly intact. We previously reported that me-
chanical stress at the dorsal aspect was about 5-fold higher than that at the dural aspect of the LF (22). Thus, during daily activities, higher mechanical stress is likely to be applied to the dorsal aspect, which may induce micro injury on the dorsal side, rather than on the dural side. During the process of micro injury healing, a thick fibrotic mass may be produced, which could cause LF hypertrophy.

Rat LF Hypertrophy Model
In the MCL hypertrophy model of Matthews et al. (25), the dense, scar-like tissue mass found at the medial aspect had similar histological features to the hypertrophied LF from the LSCS pa-
tients. Based on the model of Matthews et al. (25), we surmised that hyper-mechanical stress on the LF could cause hypertrophy in an animal model. Stress in the LF is mainly longitudinal (tensile) stress. Thus, flexion is the most important motion for inducing me-
chanical stress in the LF (22). Based on this concept, we induced posterior destabilization in the rat model to increase flexion. To avoid damaging the LF during surgery, we removed the L5 spinous process, supraspinous ligament, and interpispinous ligaments.

The LF of control rat was surrounded by epligament, as in the normal human LF. Posterior destabilization in the rat spine caused thickening of the dorsal epligament to 8-fold that of the control rat. This histological finding was similar to that of the LSCS patients. The control rat had a regular thin epligament at the dorsal aspect, with the LF consisting of elastic fibers. Thus, these histological features indicate that the LF in the destabilized model is the same as that in human hypertrophied LF. To our knowledge, this is the first report of an animal model of LF hypertrophy.

In conclusion, the LF has two distinctive collagenous layers; one on the dorsal side and the other on the dorsal side. An enlarged dorsal epligament is present in hypertrophied LF.

CONCLUSIONS
This is the first report on the existence of epligament in the LF and its role hypertrophy. LSCS patients had an enlarged dorsal epligament and a regular thin dorsal side epligament. The epliga-
ment at the dorsal aspect was about 30-fold thicker than that at the dural aspect in the hypertrophied LF. To clarify the existence of epligament in the LF and its role hypertrophy, we created a rat model with an unstable lumbar spine. The rat model showed an enlarged dorsal epligament surrounded by thick fibrosis, with a mean thickness 8-fold that of the control. Posterior destabilization could cause enlargement of the dorsal side of the LF while keeping the dural side of the epligament intact.

CONFLICT OF INTEREST
There are no conflicts of interest to declare.

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