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 (LSCS). 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. J. Med. Invest. 65 : 85-89, February, 2018

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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 ligament 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 that at the dural aspect.

(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 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 dorsal 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.

(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 aspect and dural aspect (Figures 3a–f). The mean thickness of the dorsal epiligament was 20.9 ± 15.2 µm, and that of

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

| Case # | Dorsal aspect (µm) | Dural aspect (µm) |
|--------|--------------------|-------------------|
| #1     | 1450.8 ± 412.9      | 75.4 ± 8.4        |
| #2     | 983.0 ± 344.6       | 44.0 ± 5.0        |
| #3     | 851.1 ± 60.5        | 29.7 ± 3.7        |
| #4     | 832.0 ± 216.7       | 32.6 ± 5.2        |
| #5     | 1696.3 ± 317.1      | 31.5 ± 8.2        |
| Mean   | 1162.6 ± 389.4      | 42.6 ± 19.2       |
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 dorsal 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.

In summary, the dorsal epiligament (thick fibrous area) was enlarged in both hypertrophied LF from LSCS patient (Figure 2) and the rat model (Figure 4).

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 histologically 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 8-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 epili-
gament 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 epiliga-
gament. 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 epiliga-
gament to create a thick fibrocartilage 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
fibrocartilage 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 interspinous ligaments.

The LF of control rat was surrounded by epiligaement, as in the
normal human LF. Posterior destabilization in the rat spine caused
thickening of the dorsal epiligaement 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 epiligaement 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 dural side and the other on the dorsal side. An enlarged
dorsal epiligaement is present in hypertrophied LF.

CONCLUSIONS

This is the first report on the existence of epiligaement in the LF
and its role hypertrophy. LSCS patients had an enlarged dorsal
epiligaement and a regular thin dorsal side epiligaement. The epiliga-
gament at the dorsal aspect was about 30-fold thicker than that at the
dural aspect in the hypertrophied LF. To clarify the existence of
epiligaement in the LF and its role hypertrophy, we created a rat
model with an unstable lumbar spine. The rat model showed an
enlarged dorsal epiligaement surrounded by thick fibrocartilage, 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 epiligaement intact.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

REFERENCES

1. Beamer YB, Garner JT, Shelden CH : Hypertrophied ligamen-
tum flavum. Clinical and surgical significance. Archives of sur-
gery (Chicago, Ill: 1960) 106 : 289-292, 1973
2. Park JB, Chang H, Lee JK : Quantitative analysis of transform-
ing growth factor-beta 1 in ligamentum flavum of lumbar
spinal stenosis and disc herniation. Spine 26 : E492-495, 2001
3. Towne EB, Reichert FL : Compression of the Lumbosacral
Roots of the Spinal Cord by Thickened Ligamenta Flava.
Annals of surgery 94 : 327-336, 1931
4. Ulrich CG, Binet EF, Sanecki MG, Kieffer SA : Quantitative
assessment of the lumbar spinal canal by computed tomogra-
phy. Radiology 134 : 137-143, 1980
5. Evans JH, Nachemson AL : Biomechanical study of human
lumbar ligamentum flavum. Journal of anatomy 105 : 188-189,
1969
6. Fukuyama S, Nakamura T, Ieda T, Takagi K : The effect of
mechanical stress on hypertrophy of the lumbar ligamentum
flavum. Journal of spinal disorders 8 : 126-130, 1995
7. Nachemson AL, Evans JH : Some mechanical properties of
the third human lumbar interlaminar ligament (ligamentum
flavum). Journal of biomechanics 1 : 211-220, 1968
8. Nakatani T, Marui T, Hitori T, Doita M, Nishida K, Kurosaka
M : Mechanical stretching force promotes collagen synthesis
by cultured cells from human ligamentum flavum via trans-
forming growth factor-beta1. Journal of orthopaedic research :
official publication of the Orthopaedic Research Society 20 :
1380-1386, 2002
9. Okuda T, Baba I, Fujimoto Y, Tanaka N, Sumida T, Manabe H,
Hayashi Y, Ochi M : The pathology of ligamentum flavum in
degenerative lumbar disease. Spine 29 : 1689-1697, 2004
10. Olszewksi AD, Yaszemetski MJ, White AA, 3rd : The anatomy
of the human lumbar ligamentum flavum. New observations
and their surgical importance. Spine 21 : 2307-2312, 1996
11. Postacchini F, Guminia S, Cinotti G, Perugia D, DeMartino C :
Ligamenta flava in lumbar disc herniation and spinal stenosis.
Light and electron microscopic morphology. Spine 19 : 917-
922, 1994
12. Ramsey RH : The anatomy of the ligamenta flava. Clinical or-
thopaedics and related research 44 : 129-140, 1966
13. Schrader PK, Grob D, Rahn BA, Cordey J, Dvorak J : Histol-
ogy of the ligamentum flavum in patients with degenerative
lumbar spinal stenosis. European spine journal : official publica-
tion of the European Spine Society, the European Spinal De-
formity Society, and the European Section of the Cervical
Spine Research Society 8 : 323-328, 1999
14. Yoshida M, Shima K, Tanigushi Y, Tanaki T, Tanaka T : Hy-
pertrophied ligamentum flavum in lumbar spinal canal steno-
sis. Pathogenesis and morphologic and immunohistochemi-
al observation. Spine 17 : 1533-1560, 1992
15. Ikuha K, Arima J, Tanaka T, Oga M, Nakano S, Sasaki K,
Goshi K, Yo M, Fukagawa S : Short-term results of microen-
doscopic posterior decompression for lumbar spinal stenosis.
Technical note. Journal of neurosurgery Spine 2 : 624-633,
2005
16. Wada K, Sairyo K, Sakai T, Yatsui N : Minimally invasive endo-
coscopic bilateral decompression with a unilateral approach
(endo-BIDUA) for elderly patients with lumbar spinal canal
stenosis. Minimally invasive neurosurgery : MIN 53 : 65-68,
2010
17. Bray RC, Fisher AW, Frank CB: Fine vascular anatomy of adult rabbit knee ligaments. Journal of anatomy 172: 69-79, 1990
18. Chowdhury P, Matyas JR, Frank CB: The “epiligament” of the rabbit medial collateral ligament: a quantitative morphological study. Connective tissue research 27: 33-50, 1991
19. Frank CB: Ligament structure, physiology and function. Journal of musculoskeletal & neuronal interactions 4: 199-201, 2004
20. Mensor MC, Fender FA: The ligamentum flavum: Its relationship to low back pain. Surg Gynecol Obstet 73: 822-827, 1941
21. Naftziger HC, Inman V, Saunders JB: Lesions of the intervertebral disc and ligamentum flavum. Surg Gynecol Obstet 66: 288-299, 1938
22. Sairyo K, Biyani A, Goel V, Leaman D, Booth R, Jr., Thomas J, Gehling D, Vishnubhotla L, Long R, Ebraheim N: Pathomechanism of ligamentum flavum hypertrophy: a multidisciplinary investigation based on clinical, biomechanical, histologic, and biologic assessments. Spine 30: 2649-2656, 2005
23. Kosaka H, Sairyo K, Biyani A, Leaman D, Yeasting R, Higashino K, Sakai T, Katoh S, Sano T, Goel VK, Yasui N: Pathomechanism of loss of elasticity and hypertrophy of lumbar ligamentum flavum in elderly patients with lumbar spinal canal stenosis. Spine 32: 2805-2811, 2007
24. Sairyo K, Biyani A, Goel VK, Leaman DW, Booth R, Jr., Thomas J, Ebraheim NA, Cowgill IA, Mohan SE: Lumbar ligamentum flavum hypertrophy is due to accumulation of inflammation-related scar tissue. Spine 32: E340-347, 2007
25. Matthews JL, Chung M, Matyas JR: Indirect injury stimulates scar formation-adaptation or pathology? Connective tissue research 45: 94-100, 2004