OSSIFICATION OF THE LIGAMENTUM FLAVUM
INDUCED BY BONE MORPHOGENETIC PROTEIN

AN EXPERIMENTAL STUDY IN MICE

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Ossification of the ligamentum flavum and secondary spinal-cord compression were produced experimentally in mice by implanting bone morphogenetic protein (BMP) in the lumbosacral extradural space. The ligamentum flavum became hypertrophied and ossified, and protruded into the spinal canal. The thickness of the ossified ligament increased gradually with time, leading to compression and deformation of the spinal cord which showed various degrees of degeneration. Demyelination occurred in the posterior and lateral white columns and neuronal loss or chromatolysis in the grey matter.

The pathological findings in the experimental animals closely resemble those found in the human disease and suggest that BMP may be a causative factor of ossification of the ligamentum flavum in man. This experimental model may be useful for the study of myelopathy caused by gradual spinal-cord compression.

Ankylosing spinal hyperostosis (ASH) is a well-known condition in which hyperostosis is associated with ossification of the ligaments of the spine (Forestier and Rotes-Querol 1950). Patients with ASH often develop ossification of the posterior longitudinal ligament (Ono et al. 1977; McAfee, Regan and Bohlman 1987) and of the ligamentum flavum (Yonenobu et al. 1987; Okada et al. 1991) with serious neurological complications.

Ossification of the ligamentum flavum (OLF) is a definite clinical entity (Hattori et al. 1976; Miyasaka et al. 1982; Omolola et al. 1982; Spaziante, Divitis and Genovese 1982; Otani et al. 1986; Yoshizawa et al. 1988; Yonenobu et al. 1987; Okada et al. 1991) and is thought to be a form of ectopic ossification but its cause remains unknown. It develops predominantly in the lower thoracic spine in middle age and causes compression myelopathy. Patients with OLF occasionally suffer from severe neurological deficits with gait disturbance, motor weakness in the legs and urinary incontinence. Decompressive laminectomy with resection of the ossified ligaments does not always produce a satisfactory result because of recurrent ossification or progression of the process at other spinal levels (Yonenobu et al. 1987; Okada et al. 1991). No therapeutic agent is known to prevent the development of OLF.

In the pathogenesis of ectopic ossification, growth factors which can cause mitogenesis, cytodifferentiation and matrix synthesis may be important (Urist, DeLange and Finerman 1983) and there has been interest in the response of bone, cartilage, and ligaments to these factors. Among them, bone morphogenetic protein (BMP) induces new bone formation in ectopic sites by a process of endochondral ossification (Urist et al. 1983; Wang et al. 1990). It is a polypeptide, or a group of polypeptides, and is thought to be responsible for the post-fetal differentiation of mesenchymal-type cells into chondrocytes and osteoblasts. BMP is found in normal bone tissue and studies of its physiological and pathological roles are now under way. Because it is a bone-inducing substance, it may be a causative factor in the ossification of spinal ligaments. Our aim was to determine whether BMP could induce ossification of the ligamentum flavum and secondary spinal-cord compression experimentally, in animals.

MATERIALS AND METHODS

We used 48 adult male ICR-strain mice, 24 weeks old, each weighing about 60 g. There were 30 mice in the experimental group and 18 in the control group.

Preparation of BMP. BMP was prepared from a murine osteosarcoma (Dunn) and partially purified as described...
previously (Takaoka et al 1982, 1988). This BMP-active fraction was soluble in saline and diffusible when implanted in vivo (Takaoka et al 1988; Nakahara et al 1989). It was mixed with atelopeptide type-I collagen in 0.01 N HCl solution and then lyophilised. The collagen was used as a delivery system, preventing rapid outward diffusion and allowing the sustained release of BMP (Takaoka et al 1988; Nakahara et al 1989).

**Implantation of BMP.** The mice in the experimental group were anaesthetised with intraperitoneal ketamine. Under aseptic conditions, a longitudinal skin incision was made over the lumbar spine, and the dorsal paravertebral muscles were detached from the spinous processes and laminae. The supraspinous and interspinous ligaments of L2 to L3 or L3 to L4 were resected. A slit was made between the right and left ligamenta flava in the midline through which, using an operating microscope, two sheets of the BMP/collagen composite (0.1 mg) were placed in the posterolateral epidural space. One was in contact with the right ligamentum flavum and the other with the left. The dorsal paravertebral muscles and fasciae were repaired and the skin was closed.

The mice in the control group were operated on in the same way except that collagen alone was inserted into the epidural space.

**Experimental protocol.** Batches of ten mice in the experimental group were killed at four, six and eight weeks after operation (E4, E6 and E8 groups); in the control group batches of six were killed at the same intervals. The whole thoracolumbar spinal column was resected with the paravertebral muscles in every animal. The specimens were examined radiographically with anteroposterior and lateral views and with a soft X-ray apparatus. They were then fixed in neutral buffered formalin and decalcified in formic acid. In each group half were cut sagittally; the other half transversely, and all were embedded in paraffin and sectioned at 5 to 6 μm thickness. Serial sections were stained with haematoxylin and eosin, toluidine blue, elastic Van Gieson and Kluver-Barrera stains for light-microscopic examination.

**RESULTS**

**Neurological findings.** No mouse in either group had any weakness of the hind legs or any gait disturbance during the eight-week observation period.

**Radiological findings.** In all the mice in the experimental group the lateral radiographs showed a pair of beak-like calcified prominences arising from the laminae at the operated segment and protruding into the spinal canal (Fig. 1). The cephalic and caudal prominences did not unite, even when they were large. The degree of narrowing of the spinal canal caused by the bony prominences, determined by dividing the width of the prominences by the anteroposterior diameter of the spinal canal as measured on the lateral radiograph, was 34.7% ± 10.9%

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**Fig. 1a** - Lateral radiograph of a lumbar spine in the E4 group. A pair of calcified prominences (arrow) are seen arising from the laminae and protruding into the spinal canal.

**Fig. 1b** - Lateral radiograph of a lumbar spine in the E8 group. The beak-like bony prominences (arrow) are larger than those in the E4 group.
Photomicrograph of a sagittal section of the ligamentum flavum in the E4 group. The ligament is hypertrophic and bony prominences (arrows) protrude into the spinal canal from the laminae (L) and compress the spinal cord (SC). Fibrous and cartilaginous tissue lies between the cephalic and caudal bony prominences (elastic Van Gieson × 40).

**Table I.** Degree of narrowing of the spinal canal caused by the ossified ligamentum flavum (mean ± SD)

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<tr>
<th>Group</th>
<th>Degree of narrowing (per cent)*</th>
<th>Significance of differences</th>
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<tr>
<td>E4</td>
<td>34.7 ± 10.9 NS</td>
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</tr>
<tr>
<td>E6</td>
<td>42.8 ± 12.3 NS</td>
<td>p &lt; 0.05 (Student's t-test)</td>
</tr>
<tr>
<td>E8</td>
<td>46.4 ± 13.9 NS</td>
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* see text for method of calculation

(mean ± SD) in the E4 group, 42.8% ± 12.3 in E6, and 46.4% ± 13.9 in E8 (Table I). It was significantly different in the E4 and E8 groups (p < 0.05, Student's t-test), indicating that the bony prominences had increased with time.

In the control group, there were no abnormal shadows on the anteroposterior or lateral radiographs.

**Histology**

*Ligamentum flavum.* In the E4 group, the ligamentum flavum was hypertrophied. Two newly-formed bony prominences protruded into the spinal canal from the ventral surfaces of the contiguous laminae (Fig. 2); they were adherent to the dura mater and compressed the spinal cord. Fibrous or cartilaginous tissue was seen between the cephalic and caudal spurs. Ossification was most advanced in the ventral layer of the hypertrophied ligament whereas the dorsal layer had degenerated, its collagen fibres being irregularly hyalinised and its elastic fibres partially disrupted.

In the E6 and E8 groups, endochondral ossification was yet more advanced (Fig. 3). It had extended further along the hypertrophied ligament but the cephalic and caudal parts had not completely united. There was always some intervening fibrous and cartilaginous tissue. A remnant of the original ligament remained in the dorsal layer in which the elastic fibres were decreased in number and scattered within newly-formed fibrocartilaginous tissue.

In the control group, the fibrous structure of the ligament appeared intact and no new bone or cartilage formation was observed (Fig. 4).

*Spinal cord.* In the E4 group, the spinal cord showed deformation from compression by the ossified ligamentum flavum (Fig. 5). Despite this there was little or no degenerative change.

In the E6 and E8 groups, there was more severe
deformation. In moderate cases demyelination was seen in the posterior and lateral white columns but not in the anterior white columns (Fig. 6a). The grey matter appeared intact with no neuronal loss or chromatolysis in the anterior horn (Fig. 6b). In severe deformation, both the white and grey matter had degenerated (Figs 7a, b). There was pronounced demyelination and loss of axonal fibres in the posterior and lateral white columns, but less demyelination in the anterior white column (Fig. 7a). Neuronal loss and chromatolysis in the anterior horn were also seen (Fig. 7b). The degenerative changes in the spinal cord were generally more severe in the E8 group than in the E4 group.

In the control group, no spinal cord was deformed or degenerated (Fig. 8).

DISCUSSION

Ossification of the ligamentum flavum was first reported by Polgar in 1929. It has been recognised as an important cause of thoracic myelopathy as described by Yamaguchi, Tamagake and Fujita (1960). Several reports have discussed the pathology (Hattori et al. 1976; Yoshizawa et al. 1988; Okada et al. 1991), diagnosis (Miyasaka et al. 1982; Okada et al. 1991) and treatment (Yonenobu et al. 1987; Okada et al. 1991) of OLF but its cause is still unknown.

Okada et al. (1991) described the pathology of OLF in detail. The normal fibrous structure of the ligamentum flavum is lost and replaced by hypertrophied fibrous tissue containing numerous fibrocartilaginous cells. OLF results from endochondral ossification which extends along the ventral layer of the hypertrophied ligament.

Our study shows that the pathological findings in the animal model closely resemble those reported in man (Hattori et al. 1976; Yoshizawa et al. 1988; Okada et al. 1991). In mice, BMP induced a lesion in the ligamentum flavum very similar to OLF in human patients suggesting that BMP may be a causative factor of the naturally occurring diseases, ossification of the ligamentum flavum, ankylosing spinal hyperostosis and ossification of the posterior longitudinal ligament. The BMP fraction used in our study was not completely pure, however, and may have contained such contaminants as transforming-growth-factor-β (TGF-β), which is known to be a chondro-osteogenic growth factor (Noda and Camilliere 1989). TGF-β may therefore have played a part in the ossification process.

Although neurological symptoms did not develop in the experimental animals, there was gradual compression...
and deformation of the spinal cord leading to histological changes. This experimental model may therefore be suitable for the study of myelopathy caused by chronic compression. Further studies will be necessary to clarify the pathomechanics of this myelopathy.

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REFERENCES


Fig. 7
Photomicrographs of a spinal cord in the E6 group (transverse section). (A) The spinal cord showed severe deformation with demyelination and loss of axonal fibres, predominantly in the posterior and lateral white columns (Kluver-Barrera stain × 80). (B) Neuronal loss and chromatolysis in the anterior horn were also evident (toluidine blue stain × 400).

Fig. 8
Photomicrograph of a spinal cord in the control group, eight weeks after operation (transverse section). The cord appeared normal (Kluver-Barrera stain × 40).

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