Ultrastructure of early calcification in cervical ossification of the posterior longitudinal ligament

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A case of ossification of the cervical posterior longitudinal ligament was investigated with the electron microscope. The posterior longitudinal ligament was composed of bundles of collagen fibers intermingled with occasional fibroblasts and rare blood vessels. Some ligaments contained matrix vesicles in the vicinity of degenerated cells. Hydroxyapatite crystals were frequently precipitated within the matrix vesicles. These findings are similar to the fine structure of the early stage of calcification in normal and pathological calcifying tissues described previously. In this study, the calcification process of the posterior longitudinal ligament suggests that matrix vesicles originate from degenerated cells, and acquire hydroxyapatite crystal deposits. Some eventually coalesce to form a large calcifying mass. Substantial amounts of collagen fibers comprising the ligament may serve an important role in orienting apatite crystal precipitation.

KEY WORDS: ossification of posterior longitudinal ligament, calcification, matrix vesicles, electron microscope, hydroxyapatite crystals

OSSIFICATION of the posterior longitudinal ligament (OPLL) is a common disease of the spinal canal in Mongolians but an extremely rare one among Caucasians. Since Tsukimoto first described the histological findings in an autopsied case of OPLL in 1960, this clinical entity has gradually become recognized, and the number of reported clinical cases have increased rapidly after 1970. In 1974, the Investigation Committee on OPLL was appointed by the Ministry of Public Health and Welfare of Japan to perform systematic clinical and pathological studies throughout Japan. The pathohistological incidence of OPLL was found to be 20% among those above 60 years of age in Japan.

There have been extensive histological studies of OPLL at the light microscopic level, but only a few ultrastructural investigations. The present communication describes the fine structure of the initial stages of the calcification process in OPLL.

Case Report

This 63-year-old man accidentally fell while riding a bicycle and sustained head and face injuries. He was immediately transferred to the hospital, where he was observed to be well oriented, but could not move his upper extremities. Neurological examination revealed slight motor weakness and dysesthesia of both upper extremities. Roentgenograms of the cervical spine revealed a segmental type of OPLL just behind the C-5 and C-6 vertebral bodies (Fig. 1 left). He was conservatively treated with head traction. On the 3rd day after the injury, his consciousness level began to deteriorate and he became confused. A brain computerized tomography (CT) scan demonstrated a high-density mass consistent with a hematoma in the posterior temporal lobe. An emergency craniotomy was performed to remove the intracerebral hematoma. After the operation, the patient recovered consciousness, but retained bilateral motor and sensory disturbance at the C-5 and C-6 level for 3 months. Excision of the ossified plaque behind the C-5 and C-6 vertebral bodies (Fig. 1 right) was performed through the anterior route, and then anterior fusion was carried out by autograft using the pelvic bone. The neurological deficit of the upper extremities subsided after the operation.

Materials and Methods

A surgical specimen taken from the margin of the ossified plaque with the ligament was investigated, and submitted for electron microscopic study. Representative sections were minced into 1-mm fragments immediately after removal, and fixed in a cacodylate-
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**FIG. 1.** Left: Tomogram of the cervical spine demonstrating segmental type of ossification of the posterior longitudinal ligament just behind the C-5 and C-6 vertebral bodies (arrows). Right: Computerized tomography scan taken after metrizamide myelography showing an ossified plaque behind the C-5 vertebral body. The ossified plaque compresses the spinal cord backward, resulting in narrowing of the subarachnoid space (arrowheads).

**FIG. 2.** Light micrograph of a semi-thin section from Epon-embedded tissue. This section of ligament is composed of both osmiophilic and non-osmiophilic areas associated with scattered cells. Arrows indicate calcified areas. Toluidine blue, × 600.

Buffered 2.5% glutaraldehyde solution for 2 hours. They were postfixed in 1% osmium tetroxide for 1 hour, then dehydrated through graded series of alcohols, and embedded in Epon-Araldite. Thick sections were cut and stained with 2% toluidine blue for light microscopic

**FIG. 3.** Electron micrographs of a ligament. Left: Random arrangement of stout collagen fibers (A) and streamlined bundles of slender collagen fibers with a fibroblastic cell (B) are illustrated. × 5200. Right: Remnants of degenerated cells in a ligament. Membrane-invested matrix vesicles (small arrows) and mitochondria (large arrows) are dispersed. × 23,000.
Ossification of posterior longitudinal ligament

study. Ultra-thin sections were cut and stained with uranyl acetate followed by lead citrate and examined with the electron microscope.

Results

Light Microscopy

Small calcified masses were occasionally present in the fibrous or cartilaginous matrix. In the vicinity of the calcified masses, fibroblast-like cells were frequently observed (Fig. 2). They were located in both osmiophilic and non-osmiophilic areas. Tiny calcified bodies that were strongly osmiophilic were also present (Fig. 2, arrows).

Electron Microscopy

The fine structure of the posterior longitudinal ligament was generally composed of stout bundles of randomly arranged thick collagen fibers and narrow bundles of streamlined fine collagen fibers (Fig. 3 left). Fibroblastic cells were dispersed among the collagen fibers. In some areas, various-sized membrane-invested vesicles, presumably residuals of degenerated cells, were arranged in clusters (Fig. 3 right). Some vesicles were recognizable mitochondria (Fig. 3 right, large arrows), while others were presumed to be morphologically identical to the matrix vesicles described in calcified tissues1 (Fig. 3 right, small arrows). In addition to the remnants of degenerated cells, large numbers of electron-dense small bodies were sometimes observed among the collagen fibers (Fig. 4 left). Some of the electron-dense bodies were identified as heavy deposits of apatite crystals within the matrix vesicles (Fig. 4 right, upper arrow). Matrix vesicles without apatite deposits were also present among the collagen fibers (Fig. 4 right, lower arrow). Furthermore, numerous electron-dense specks were scattered among the thick collagen fibers, comprising stout bundles (Fig. 5 left). These electron-dense particles were composed of accumulations at needle-shaped hydroxyapatite crystals (Fig. 5 right). Among these depositions, apatite crystals within matrix vesicles were also occasionally present (Fig. 5 right, large arrows). Other dense bodies were presumed to be

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Fig. 4. Electron micrographs of multiple electron-dense bodies. Left: A moderately electron-dense extracellular matrix is seen in a ligament consisting of thin collagen fibers. ×8700. Right: Higher magnification of an area similar to that shown left. Matrix vesicles with hydroxyapatite deposits (upper arrow) and without those precipitations (lower arrow) are intermingled with deformed collagen fibers. ×95,000.
FIG. 5. Electron micrographs of electron-dense particles in the ligament.  
Left: Many electron-dense specks are scattered in a bundle of stout collagen fibers. × 8700.  
Right: Higher magnification of electron-dense bodies similar to those shown left. Hydroxyapatite crystals are precipitated in matrix vesicles (large arrows). Aggregates of apatite deposits form small calcified masses (smaller arrows). Cellular debris-like material is also present (double smaller arrows). × 47,000.

large aggregates of apatite crystals within the matrix vesicles (smaller arrows). In addition to the dense bodies, presumed remnants of degenerated cells were frequently observed in the ligament (Fig. 5 right, double smaller arrows).

Discussion

Although various factors, such as trauma, diabetes mellitus, and relation to human leukocyte antigen (HLA), have been proposed for the etiology of OPLL, the pathomechanism of the calcification process is still unknown. Extensive histological investigation of this disease has been carried out in Japan. Some investigators proposed enchondral ossification resembling growth of long bone as an initial manifestation of calcification in the posterior longitudinal ligament. They stressed that cartilaginous cells proliferated in the ligament and were firmly attached to the vertebral body; calcification then occurred within the cartilaginous matrix. Other authors, however, stated that enchondral ossification was rarely observed in the early calcification of OPLL. On the other hand, Yamamura suggested that, in addition to enchondral ossification, connective tissue proliferation associated with numerous blood vessels and hyalinoid, as well as mucoid degeneration, occurred first within the ligament, followed by ossification.

An ultrastructural study of the primary calcification site in OPLL was described by Sako and Morimoto in 1976. They described for the first time the presence of matrix vesicles in the vicinity of chondroblastic cells. We also observed matrix vesicles among the remnants of disintegrated cells in our material, but we failed to identify chondroblastic cells in the ligament. There were precipitated hydroxyapatite crystals within numerous matrix vesicles. We suggest that this is an initial nidus of calcification in OPLL since matrix vesicles are generally accepted as initial foci of calcification at the ultrastructural level. Fine structure similar to calcification of OPLL has been reported in connective tissue calcification such as osteoarthritis, tympanosclerosis, and calcifying tendinitis, in which matrix vesicles were presumably derived from degenerated cells.

Since OPLL usually occurs in patients above the age of 20 years, this disease may be related to aging or degenerating processes. Matrix vesicles observed in our
Ossification of posterior longitudinal ligament material are considered to be remnants of degenerating cells in the ligament and may well be the initial site of calcification. The substantial amount of collagen fibers in the ligament may serve in orienting apatite deposits during the formation of the ossification plaque.

In conclusion, an initial nidus of calcification of OPLL consists of matrix vesicles originating from degenerated cells in the ligament.

References

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