Pathology of lumbar nerve root compression
Part 1: Intraradicular inflammatory changes induced by mechanical compression

Shigeru Kobayashi a,*, Hidezo Yoshizawa b, Shuuichi Yamada b

a University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK
b Department of Orthopaedics, Fujita Health University School of Medicine, 1-98, Dengakugakubo,
Kitukake-cho, Toyoake, Aichi 470-1192, Japan

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Abstract

Study design: This study is to investigate the intraradicular inflammation induced by mechanical compression using in vivo model.

Objectives: The relationship between the intraradicular edema and nerve fiber degeneration induced by mechanical compression was determined in the nerve root.

Summary of background data: Recently some studies reported that mechanical compression increased microvascular permeability of the endoneurial capillaries and resulted in an intraradicular inflammation. These changes may be an important factor of the pathogenesis of radiculopathy. However, the natural courses of the intraradicular inflammation after mechanical compression are still poorly understood.

Methods: In dogs, laminectomy was performed at L7 and the seventh nerve root was exposed to compression at 7.5 gram force (gf) clipping power. The animals were evaluated at 1 and 3 weeks after clipping. After the appropriate period of nerve root compression, Evans blue albumin (EBA) was injected intravenously. The nerve root sections were divided into two groups. The sections were used to investigate the status of the blood-nerve barrier function under the fluorescence microscope. The other sections were used for light and transmission electron microscopic study.

Results: After 1 and 3 weeks, intraradicular edema was observed not only at the site of compression but also in the peripheral zone of a compressed anterior root and in the central zone of a compressed posterior root. The evidence of active Wallerian degeneration was also seen in the area of intraradicular edema. In addition, the nerve roots showing Wallerian degeneration were infiltrated by inflammatory cells, such as macrophages and mast cells.

Conclusions: Inflammatory reaction, such as Wallerian degeneration, breakdown of blood-nerve barrier and appearance of macrophage, may be deeply involved in radiculitis arising from mechanical compression, and these factors seem to be important in the manifestation of radiculopathy.

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Introduction

The lumbosacral nerve roots are often involved in disease processes and injuries, such as disc herniation, spinal stenosis, tumor and vertebral fracture. It is generally considered that the genesis of radiculopathy associated with the pathological conditions of the spine, may result from mechanical compression of the nerve root. This factor may change the intraradicular circulation [20] and produce nerve fiber dysfunction. The mechanical compression may also lead to a series of intraneurial tissue reactions, including edema formation, demyelination, and fibrosis [32,43,53].

A number of papers about the reaction pattern of nerve roots to experimentally applied compression have...
been published. Various morphologic and physiologic changes, in association with acute [6,15,44,51], subacute [4] and chronic [17,52] nerve root compression, have been reported. Some studies reported that mechanical compression increased microvascular permeability of the endoneurial capillaries and resulted in an intraradicular inflammation [44,54]. In recent years, gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) enhanced MR imaging has been of great value in delineating intraradicular edema resulting from increased permeability of blood capillaries in which the blood–nerve barrier is broken down by compression [18]. These changes may be an important factor of the pathogenesis of radicular symptoms. However, the natural course of the intraradicular inflammation after mechanical compression is still poorly understood. The purpose of this study was to investigate the radiculitis induced by lumbar nerve root compression.

Materials and methods

The experiment was carried out under the control of the local animal ethics committee in accordance with the guidelines on animal experiments in Fujita Health University, Japanese government animal protection and management law, and Japanese government notification on feeding and safekeeping of animals. Twelve adult dogs, weighing 7–15 kg, were anesthetized with intramuscular injection of 3 ml of Ketalar (Ketamine 50 mg/ml; Warner-Lambert, Morris Plains, NJ) and ventilated on a respirator under general anesthesia (O2: 3 ml/min, N2O: 3 ml/min, Halothane: 1.5 ml/min). Animals were maintained at constant physiologic levels during experiment. Each animal was placed in the prone position on a flame. The sixth and seventh lumbar laminae were removed, and the seventh lumbar nerve root was exposed widely on one side. The nerve root was clamped with a clip for microvascular suturing (Kouno Co., Chiba, Japan) at the midpoint between the dural sac and dorsal root ganglion. Before using the clip, clipping power was measured by a tension device of Instron type (AGH-2000B, Shimazu Co., Kyoto, Japan). The seventh nerve root was exposed to compression at 7.5 gram force (gf) (approximately 50 mmHg) clipping power [21]. The incision was closed and the animal was allowed to recover. The animals were evaluated at 1 and 3 weeks after clipping. After the appropriate period of nerve root compression, Evans blue albumin (EBA, 10 ml/kg, molecular weight approximately 59,000, Sigma Chemical Co., St. Louis, MO) was injected intravenously and allowed to circulate for 30 min. EBA was prepared by mixing 5% bovine albumin (Wako Chemical Co., Osaka, Japan) with 1% Evans blue (Sigma). The animals were fixed by intraaortal perfusion with 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 at 20 °C.

The nerve root section was divided into two groups. At first, EBA tracer technique was used to investigate the status of the blood–nerve barrier function in the nerve root. After the clamp was released, the tracers were administered intravenously. The EBA was allowed to circulate for 1 h before the nerve roots were removed. After the nerve root sections fixed with 4% paraformaldehyde for 24 h, 20 μm thick sections were mounted with 50% aqueous glycerin to be examined under the fluorescence microscope at 380 mμW. The other sections were rinsed in 0.05 M tris–HCl buffer, postfixed at room temperature for 3 h in 2% OsO4 in 0.1 M sodium cacodylate buffer, impregnated with 2% uranyl acetate, dehydrated in graded ethanol, and embedded in epoxy resin. For light microscopy, 1–3 μm thick toluidine blue stained sections were used. For electron microscopy, ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a JSM2000 electron microscope.

Results

Changes of intraradicular vascular permeability after nerve root compression

On macroscopic examination of the nerve root following intravenous injection of EBA at 1 week after compression, there was blue staining of the dorsal root central to the compressed site (Fig. 1A) and the ventral root peripheral to the site of injury. After 3 weeks compression, blue staining of the dorsal root was extending to the point of spinal cord entry (Fig. 1B).

Fluorescence microscopy showed marked intraradicular edema at the compressed site. Pooling of a serotonin-like neurotransmitter with yellow fluorescence was seen in the axons of dorsal root (Fig. 2A). Intraradicular edema was also evident in the dorsal root central to the site of compression (Fig. 2B). However, the extravasation of EBA did not show in the dorsal root peripheral to the site of compression and the blood–nerve barrier exists in the nerve roots. The nerve fibers emitted a green fluorescence (Fig. 2C). While the blood–nerve barrier was preserved in the ventral root central to the site of compression, extravasation of EBA was evident in the ventral root peripheral to the site of injury.

After 3 weeks compression, fluorescence microscopy showed marked nerve fiber demyelination at the compressed site (Fig. 2D) and revealed ventral root fiber demyelination extending distally from the site of compression, and dorsal root demyelination from the site of compression to the point of spinal cord entry (Fig. 2E).

Fig. 1. Macroscopic findings of the nerve root following intravenous injection of EBA. (A) After 1 week compression. (B) after 3 weeks compression. D: dura mater, DR: dorsal root, LC: lumbar cord, VR: ventral root, Arrow head: compression site.
Pooling of red fluorescent EBA was evident between the degenerating nerve fibers. However, this fluorescence was weaker than that observed in the 1-week group, suggesting a decrease of intraradicular edema. The blood–nerve barrier was preserved in the ventral root central to the site of compression and in the dorsal root peripheral to the site of compression.

Morphological changes of the nerve roots after mechanical compression

One week after nerve root compression, Wallerian degeneration was apparent in the dorsal root central to the site of compression and in the ventral root peripheral to this site. Histological and electron microscopic examination of these regions showed deformation of the myelin sheath (Figs. 3B, C and 4A, B) and the nerve fibers were separated (Fig. 3D). There were a large number of myelin ellipsoids and double membranes caused by folding of the myelin sheath, and the Schwann cells were swollen. In addition, macrophages phagocytizing myelin debris were seen between the separated nerve fibers (Figs. 3D and 4B). On electron microscopy of the intraradicular capillaries, many numerous vacuoles were observed within the endothelial cells (Fig. 4C). After 3 weeks, the intraradicular blood vessels revealed discontinuous capillaries with separation between the endothelial cells, and breakdown of the blood–nerve barrier (Fig. 4F). These findings were also indicative of increased vascular permeability.

Light microscopy of the compressed site 3 weeks after compression showed marked intraradicular nerve fiber degeneration (Fig. 5A). Electron microscopy revealed numerous macrophages in the perivascular space (Fig. 5B), and monocytes capable of passing through the vessel walls were also observed (Fig. 5C). Nerve fiber degeneration affecting the dorsal root central to the site of compression and the ventral root peripheral to it was more advanced than after 1 week (Figs. 3C, D and 4D–F). Destruction of the myelin sheath had progressed to the stage where there was little myelin formation, detachment of the basal lamina, and formation of whorls of various sizes. Accumulation of myelin debris within Schwann cells resulted in the loss of membranous
structures. Furthermore, myelin droplets, fatty aggregates with a strong osmium affinity, had formed and there were large number of nerve fibers with a myelin sheath but no axons (Figs. 3C, D and 4E). In the endoneurial space of some nerve roots, there were edematous regions in which the nerve fibers had virtually disappeared and regions of fibrosis characterized by a marked increase of collagen fibers (Fig. 6). Fibroblasts with long, thin processes extending to form minifascicles on all sides, denervated Schwann cells, macrophages, and mast cells were present in the edematous regions of the endoneurial space. Fibroblast-like cells resembling pericytes were present around the capillaries, and collagen fiber proliferation was observed at the edges of these cells. Fibrosis was particularly marked in the perivascular area and was characterized by the presence of fibroblasts rich in rough endoplasmic reticulum and by marked collagen fiber proliferation. No Wallerian degeneration was evident in the dorsal root peripheral to the site of compression or in the ventral root central to this site even at 3 weeks after compression (Figs. 3E, F, 4A, D and 6A). After 1 and 3 weeks, histological examination of the uncompressed site revealed nerve fiber deformation but no appreciable Wallerian degeneration.

Discussion

Wallerian degeneration of the nerve root after mechanical compression

Wallerian degeneration occurs as follows. When axonal continuity is disrupted by mechanical compression or nerve trunk ischemia, the axons distal to the stump
divide finely and begin to degenerate. At the same time, the myelin sheaths that have lost axons begin to break down. Morphologically, the nerve root is part of the peripheral nerve. The nerve fibers of the anterior root arise from the nerve cell in the spinal cord, while those of the posterior root arise from the nerve cell in the dorsal root ganglion. This is why Wallerian degeneration occurs peripheral to the site of compression in the anterior root and central to this site (i.e., towards the spinal cord) in the posterior root when the cauda equina or nerve root is compressed by a herniated intervertebral disc or spinal canal stenosis. Numerous studies have investigated the degeneration and regeneration of spinal nerve root since the work of Cajal in 1928 [2]. The results show that the nerve roots have a regenerative capacity similar to that of peripheral nerves. Detailed experimental research has been conducted into the degeneration and regeneration process in the nerve fibers of the anterior and posterior roots using cauda equina suturing [3,12–14,27,34,35]. The results of those studies confirm the occurrence of Wallerian degeneration and subsequent regeneration.
have shown that, while regeneration of the anterior root approximates that of peripheral nerves, recovery of sensation presents problems in the posterior root because degeneration extends into the spinal cord. These studies serve as a useful reference when considering the pathophysiology of nerve root compression injury. They also highlight the importance of investigating the degeneration and regeneration of intraradicular nerve fibers following compression injury to roots arising in the unique environment created by the cerebrospinal fluid, as opposed to degeneration and regeneration of peripheral nerves.

**Vascular permeability of nerve roots undergoing Wallerian degeneration**

Some experimental studies on changes in vascular permeability with Wallerian degeneration have focused on the peripheral nerves [28,46,47] and nerve roots [26]. Seitz et al. [46] crushed the sciatic nerves of mice and used various tracers to observe vascular permeability in the endoneurium during Wallerian degeneration. In the degenerating portion of the nerve distal to the site of compression, vascular permeability peaked on day 8. As the nerve regenerated, extravasation of tracers declined and by day 30 the blood–nerve barrier was almost intact. It was suggested that increased vascular permeability was related to the greater demand for energy during neural regeneration. In the present study, the increase of vascular permeability in nerve roots degenerating after compression was more marked at 1 week than at 3 weeks. However, intraneural vasomotion is regulated by neurotransmitters such as vasoactive amines and neuropeptides [16,19], and it is therefore possible that the increased vascular permeability observed in the degenerating regions was the result of loss of neurogenic vascular control.

**Origin of macrophages**

Although there are no lymphatic vessels in the nervous system tissue, macrophages are present as in other tissue and these play a major role in the removal of foreign material. In the spinal cord and nerve roots within the subarachnoid space, the cerebrospinal fluid is believed to have a role similar to that of the lymphatic circulation. This is because macrophages in the cerebrospinal fluid have the capacity to remove foreign material from the subarachnoid space [37], and because its flow can remove metabolites and waste products.
Fig. 6. Light and electron micrographs of the nerve root central to the site of compression after 3 weeks. (A,B) Light micrographs. No Wallerian degeneration was evident in the ventral root (A). In the endoneurial space of dorsal root, there were edematous regions (*) in which the nerve fibers had virtually disappeared and regions of fibrosis (+) characterized by a marked increase of collagen fibers (B). (C–F) Transmission electron micrographs. (C–E) Edematous regions. (F) Fibrous regions. C: capillary, DSC: denervated Schwann cell, En: endoneurial space, F: fibroblast, Fp: fibroblastic process, Ma: mast cell, MΦ: macrophage.

produced in the spinal cord and nerve roots [30,40]. The histological and electron microscopic data obtained in the current study showed that there were virtually a few macrophages in the uninjured nerve roots, and macrophages only increased in regions undergoing Wallerian degeneration after compression. Much remains unknown about the origin of these macrophages [11,40]. They were previously thought to arise from the microglia, but autoradiography and immunohistological investigations have shown that they originate from circulating monocytes [49,50], and can also be found in damaged areas of the brain [22] and in peripheral nerves undergoing Wallerian degeneration [48]. The current study suggested that necrotic substances produced by Wallerian degeneration were primarily removed by macrophages originating from the mononuclear phagocytic system. These cells are believed to have entered the nerve roots as a result of breakdown of the blood–nerve barrier.

Development of chemical radiculitis due to inflammatory cells

Radiculitis and arachnoiditis are often induced by posterior spinal operation and are also associated with lumbar degenerative spine diseases [10]. However, there
are many more unanswered questions than answered questions about pathogenesis of radiculitis. So far the concept of chemical radiculitis is thought to be an inflammatory condition of the nerve root due to the rupture of the annulus fibrosus and dissemination of disc fluid along the nerve root sheath [24,25]. Thus, irritation of nerve roots by substances such as glycoproteins [24,25,36], immunoglobulin G [41], phospholipase A2 [45], and hydrogen ions [8,33] has been proposed.

Olmarker et al. [38] demonstrated that epidural application of autologous nucleus pulposus in pigs, without mechanical compression, induced marked nerve fiber degeneration in the nerve root. They also reported that cytokines such as TNF in the hernia tissue were radiculopathy-causing factors [39]. However, we doubt that intraradicular demyelination is solely caused by chemical factors from the nucleus pulposus because of the following issues. (1) Radiculitis caused by a chemical factor originating in disc tissue has to date been studied only in disc herniation and no adequate explanation has yet been given of the route by which this chemical factor crosses the normally impenetrable diffusion barrier in the arachnoid membrane to cause damage to nerve fibers in the nerve root. (2) Does the mechanism responsible for the radiculopathy seen in lumbar disc herniation differ from the mechanism leading to the radicular pain associated with lumbar canal stenosis and tumors of the cauda equina? (3) Do the underlying pathological features differ for radicular pain caused by herniation of the ring apophysis and annulus fibrosus, and radicular pain associated with herniation of the nucleus pulposus? (4) Cytokines such as TNF are mainly involved in signaling between cells and unlike hormones would not easily be able to travel over a long distance from the hernia to the nerve root. (5) Cervical disc hernias are unlikely to cause myelopathy. (6) The inflammatory reaction around a hernia may be necessary for spontaneous resolution of the lesion. Finally, we have to consider about intraradicular inflammatory reaction induced by mechanical compression. Namely, radiculitis is more likely to be caused by intraradicular edema and nerve fiber degeneration due to mechanical compression of the nerve root and by chemical mediators released from inflammatory cells such as macrophages and mast cells, than it is to be caused by a chemical mediator produced in disc tissue.

Macrophages are the chief effector cells causing inflammatory neuritis together with mast cells. Macrophages can generate a host of inflammatory molecules (e.g., interleukin 1 [7,9,42] and tumor necrosis factor [1,7,9]) and can also exert cytotoxic activity by direct physical contact or through the release of toxic by-products (e.g., nitric oxide [29] and proteases [5,31]). Inflammatory mediators liberated from macrophages

![Diagram](image)

Fig. 7. The nerve root compression was produces increased vascular permeability not only at the site of compression but also in the peripheral zone of a compressed anterior root and in the central zone of a compressed posterior root. After nerve root compression, evidence of active Wallerian degeneration was seen in the area of increased vascular permeability. DR: dorsal root, DRG: dorsal root ganglion, VR: ventral root.
are intimately involved in the inflammatory process by enhancing vascular permeability, providing chemo tactic signals and modulating inflammatory cell activities. Release of histamine induced by mast cell degranulation may play an important role in breaching of blood–nerve barrier [23]. The present study showed that intraradicular edema and the appearance of macrophages and other inflammatory cells not only occurred at the site of compression, but also in other degenerating regions (Fig. 7). This phenomenon may be one cause of chemical radiculitis. In other words, breakdown of the blood–nerve barrier and the appearance of inflammatory cells both at the site of injury and in degenerating regions may play a major role in chemical radiculitis resulting from lumbar canal stenosis, disc herniation, or inadvertent nerve root compression during surgery.

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References


