Ultrastructural changes of compressed lumbar ventral nerve roots following decompression

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The lumbosacral nerve roots are often involved in disease process and injuries, such as disc herniation, spinal stenosis, tumors, and vertebral fractures. 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 and produce nerve fiber dysfunction. The mechanical compression may also lead to a series of intraneural tissue reactions, including edema formation, demyelination, and fibrosis. Various morphological and physiological changes in association with acute, subacute, and chronic nerve root compression have been reported.

Laminectomy has been used to decompress the involved roots. However, chronic low back pain, leg weakness, and lower extremity hyperreflexia complicating post laminectomy surgery have been reported.

Objective: To study whether there will be a permanent lumbar nerve root scarring or degeneration secondary to continuous compression followed by decompression on the nerve roots, which can account for postlaminectomy leg weakness or back pain.

Methods: The study was performed at the Department of Anatomy, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia during 2003-2005. Twenty-six adult male New Zealand rabbits were used in the present study. The ventral roots of the left fourth lumbar nerve were clamped for 2 weeks then decompression was allowed by removal of the clips. The left ventral roots of the fourth lumbar nerve were excised for electron microscopic study.

Results: One week after nerve root decompression, the ventral root peripheral to the site of compression showed signs of Wallerian degeneration together with signs of regeneration. Schwann cells and myelinated nerve fibers showed severe degenerative changes. Two weeks after decompression, the endoneurium of the ventral root showed extensive edema with an increase in the regenerating myelinated and unmyelinated nerve fibers, and fibroblasts proliferation. Three weeks after decompression, the endoneurium showed an increase in the regenerating myelinated and unmyelinated nerve fibers with diminution of the endoneurial edema, and number of macrophages and an increase in collagen fibrils. Five and 6 weeks after decompression, the endoneurium showed marked diminution of the edema, macrophages, mast cells and fibroblasts. The endoneurium was filled of myelinated and unmyelinated nerve fibers and collagen fibrils.

Conclusion: Decompression of the compressed roots of a spinal nerve is followed by regeneration of the nerve fibers and nerve recovery without endoneurial scarring.

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reported and are poorly understood. The pain and weakness have been attributed to spinal facilitation in which there is lowering of pain excitation levels. Epidural and nerve root scarring, and nerve root adherence by scar formation to the underlying disc and adjacent pedicle have been blamed for such persistent pain. Two underlying pain facilitation states are invoked in the clinical condition: 1. an inflammatory state required to achieve wound healing; and 2. a nerve injury state resulting from nerve manipulation and subsequent epidural scarring, spinal nerve scarring, and spinal nerve tethering to the adjacent disc and pedicle. A sequence of events evocative of nociceptive responses has been attributed to the surgical procedure including late spinal nerve scarring and tethering to the disc and pedicle as the proliferative scar process matures, resulting in a neuropathic process analogous to entrapment and scarring of peripheral nerves at other sites.

Surgical correction of anatomical disorders is without side effects and complications. Outcome measures have demonstrated the failure of surgical approaches in a significant number of cases with respect to pain relief. They can also add a considerable risk in some of them. Repeated laminectomy faces additional risks secondary to anatomical changes in cases where proliferative fibrosis has developed within the spinal canal. It is unlikely that corrective surgical structural approaches alone can prevent or correct the chronic postlaminectomy nociceptive process in a considerable percentage of the so-called “failed back” cases.

Researchers have shown that, while regeneration of the anterior root approximates that of peripheral nerves, recovery of sensation presents problems in the posterior root, since degeneration extends into the spinal cord. These studies 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. Consequently, the aim of the present study is to investigate whether there will be permanent nerve root scarring or degeneration secondary to continuous compression followed by decompression on the nerve roots that can account for postlaminectomy leg weakness or back pain.

**Methods.** The study was performed at the Department of Anatomy, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Saudi Arabia during 2003-2005. Twenty-six adult male New Zealand rabbits (weighing 5-7 kg at the beginning of the experiment) were used in the current study. Twenty-four of them were used as the experimental animals and were divided into 6 equal groups according to the length of the post operative period, the remaining 2 animals were used as the control group. The experimental animals were anesthetized with intraperitoneal injection of Ketalar at a dose of 30 mg/gm body weight. Anesthesia was maintained by inhalation of a mixture of nitrous oxide (3 ml/min) and oxygen (3 ml/min). Each animal was placed in the prone position, the back of the animal was shaved and the underlying skin was disinfected with alcohol. Using the dissecting microscope, the skin was incised and the laminae of the fourth and fifth lumbar vertebrae were removed. The ventral roots of the left fourth lumbar nerve were identified, exposed widely and clamped just lateral to the margin of the spinal cord with a clip for microvascular suture then the incision was closed.

Two weeks after surgery, the animals were anesthetized, the clips were removed (decompression) and then the animals were allowed to recover. At set time intervals of 1, 2, 3, 4, 5 and 6 weeks following removal of the clips, the animals of each group were anesthetized, and their tissues were fixed through the left ventricle by 4% paraformaldehyde and 1% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.2 at 20°C. The ventral root of the left fourth lumbar nerve distal to the site of the compression was removed and divided into 1.5-2 mm segments. The segments were labeled, postfixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer, then impregnated with 2% uranyl acetate, dehydrated in ascending grades of ethanol and embedded in epoxy resin. Ultrathin sections of 0.06 micron were obtained and stained with uranyl acetate and lead citrate for electron microscopic study.

**Results.** The normal ventral root of the spinal nerve in the control animals showed compacted and regularly arranged perineurial layers. The endoneurium contained aggregated collagen fibrils and myelinated and unmyelinated nerve fibres (Figure 1a). The endoneurium also contained blood vessels with tight intercellular junctions between their endothelial cells. Spaces surrounding these vessels were filled with collagen fibrils and nerve fibres (Figure 1b).

One week after decompression, the ventral root peripheral to the site of compression showed swollen Schwann cells. These cells contained whorls of degenerated myelin and electronlucent large vacuoles of different sizes and shapes. Their cytoplasm appeared swollen with increased mitochondria and rough endoplasmic reticulum. Some Schwann...
Figure 1 - Electron photomicrographs of a normal lumbar ventral nerve root of an adult rabbit, a) shows compacted layers of the perineurium, (P) and compacted collagen bundles, (C) in the subperineurial space and the endoneurium. It also shows normal myelinated (A) and unmyelinated (U) nerve fibers. × 17,800. b) It shows an endoneurial blood vessel (BV) with tight intercellular junctions (t) between its endothelial cells. The vessel is surrounded by compact collagen bundles (C) and myelinated nerve fibers (A). × 17,800.

Figure 2 - Electron photomicrographs of the lumbar ventral nerve root peripheral to the site of compression, one week after decompression a) Shows swollen Schwann cell (S) containing electron lucent large vacuoles (V) with different sizes and shapes, whorls of degenerated myelin (m) and small unmyelinated nerve axons (A). Their cytoplasm contains increased mitochondria and rough endoplasmic reticulum. Some of Schwann cells wrap many unmyelinated nerve axons (U), some of these axons contain degenerated vacuoles of different sizes and electron densities (long arrows). Other Schwann cells contain regenerating myelinated nerve axons (Ax) with thin myelin sheaths. Few of Schwann cells contain deformed myelin sheaths with the result of formation of 2 or 3 myelin sheaths within the axon (Sc). Macrophages (MG) carry multiple electron lucent fat vacuoles (fg) and degenerated myelin (md). Other regenerated Schwann cells (short arrow) show multiple cytoplasmic processes. Fibroblasts (F) show marked hypertrophy of their cytoplasm with increased rough endoplasmic reticulum (r) and the primary lysosomes (l). Their cytoplasm contains electron lucent fat vacuoles (f). There is also an increased number of flattened fibroblasts (B), most of which have short cytoplasmic processes. × 13,220. b) Shows endoneurial macrophages (MG) carrying myelin debris and fat globules in the vicinity of an endoneurial blood vessel (BV). There is also marked endoneurial edema, albumin extravasation (A) and increased degenerated fat globules (f), some of which are membrane bounded (F) in the perivascular area. The collagen bundles (C) are widely separated due to the endoneurial edema. The endothelial cells of the blood vessel contain electron lucent (fat globules) and electron dense (degenerated myelin) vacuoles (arrows). Endoneurial fibroblasts (Fr) are increased in the perivascular space. They have long cytoplasmic processes and contain cytoplasmic fat globules and increased rough endoplasmic reticulum. Black A: myelinated nerve axon with deformed myelin (m). × 13,220.
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**Figure 3** - Electron photomicrographs of the lumbar ventral nerve root distal to the site of compression, (a) 2 and (b) 3 weeks after decompression. a) The endoneurium shows perivascular edema (O) with an increase of flattened fibroblasts (F), which surround the regenerating nerve fibres and the endoneurial blood vessel (BV). Macrophages (MG) carrying degenerated myelin and fat globules are also increased in the area surrounding the vessel. The endoneurial blood vessel shows increased vacuoles in their endothelial cells (arrow) and luminal membrane-bounded vacuoles (arrow heads). Regenerating thinly myelinated nerve fibres (A) are increased in the endoneurium. × 7,800. b) Shows diminution of the endoneurial edema and extravasated albumin (Ab) with more aggregation of the collagen fibrils (C). The endoneurial fibroblasts (F) contain electron lucent fat vacuoles and have long cytoplasmic processes (pr) that surround the myelinated nerve axons (A). BV: endoneurial blood vessel, m: myelin sheath. × 10,000.

**Figure 4** - Electron photomicrographs of the lumbar ventral nerve root distal to the site of compression, (a) 4 and (b) 6 weeks after decompression. a) Shows a myelinated nerve fiber (Ax) and increased collagen fibrils (C) in the endoneurium. The endoneurial edema and albumin extravasation (A) shows marked diminution. The endoneurial macrophage (MG) carrying myelin and fat globules lies in the vicinity of an endoneurial blood vessel (BV) whose endothelial cells (E) show less hypertrophy and little vacuolation. × 12,400. b) The endoneurium is filled with compacted collagen bundles (C) with marked diminution of the endoneurial edema. There is also an increase in the myelinated (A) and unmyelinated (U) nerve fibers. The number of the fibroblasts (F) is decreased in number with their tendency to wrap the regenerating nerve fibers. M: mast cell. × 17,800.
cells wrapped many unmyelinated nerve axons that contained degenerative vacuoles of different sizes and electron densities. Other Schwann cells contained regenerating thinly myelinated axons, while a few of them circled deformed myelin sheaths. Some Schwann cells showed also multiple cytoplasmic processes. Fibroblasts showed marked cytoplasmic expansion with increased rough endoplasmic reticulum and primary lysosomes. Their cytoplasm contained electronlucent fat vacuoles (Figure 2a).

The endoneurium showed increased number of macrophages containing myelin debris and fat globules. It also showed development of edema, extravasated albumin, fat globules and degenerated myelin, especially in the areas around the endoneurial blood vessels. The blood vessels showed numerous vacuoles within their endothelial cells; some of these cells showed myelinoid debris. The cytoplasm of the endoneurial fibroblasts contained fat globules (Figure 2b).

Two weeks after decompression, the endoneurium showed extensive edema with albumin extravasation that was more pronounced in the vicinity of the endoneurial blood vessels. The endoneurial fibroblasts appeared flattened with multiple cytoplasmic processes and surrounded the regenerating nerve fibres. Macrophages containing degenerated myelin, and fat globules were also increased in the area surrounding the endoneurial blood vessels. The endoneurial blood vessels showed multiple vacuoles in their endothelial cells, in addition to luminal membrane-bounded vacuoles. Regenerating thinly myelinated and unmyelinated nerve fibres were increased in the endoneurium (Figure 3a).

Three weeks after decompression, the endoneurium showed an increase in the regenerating myelinated and unmyelinated nerve fibres. The myelin sheath of the myelinated nerve fibres increased in thickness. The regenerated fibres were wrapped by endoneurial fibroblasts that were increased in number. There was a diminution of the endoneurial edema and the extravasated albumin. The collagen fibrils were also increased and compacted especially between the endoneurial fibroblasts and the regenerating myelinated nerve fibres (Figure 3b).

Four weeks after decompression, the endoneurium showed increased regenerating fibers and collagen fibrils. The endoneurial edema was markedly diminished together with the extravasated albumin. The endoneurial fibroblasts were fewer with short cytoplasmic processes. The endoneurial macrophages were markedly decreased. The endoneurium was filled with regenerating nerve fibres and collagen fibrils. The endothelial cells of the endoneurial blood vessels became less swollen and contained less vacuoles (Figure 4a).

Six weeks after decompression, the endoneurium showed regenerating myelinated nerve fibres with thicker myelin sheaths. The endoneurial edema and extravasated albumin were much decreased and the collagen bundles appeared compacted and the endoneurial fibroblasts were scanty. The endoneurial macrophages and mast cells much decreased in number (Figure 4b).

**Discussion.** The current study showed that, there was an increase in the endoneurial extravasated albumin during the first 2 weeks following decompression. There was also increased vacuolation and hypertrophy in the endothelial cells of the endoneurial blood vessels indicating increased permeability and development of endoneurial edema. After the third week of decompression, such endothelial hypertrophy, vacuolation, and albumin extravasation decreased, leading to reduction of the edema. The possible causes of the increased permeability are numerous, including the secreted histamine by mast cells. It was also reported that intraneural vasomotion of the endoneurial blood vessels is regulated by neurotransmitters such as vasoactive amines and neuropeptides. Consequently, it could be postulated that the increased vascular permeability observed in the degenerating regions was also partially due to loss of neurogenic vascular control secondary to root compression. The increased vascular permeability, and development of the endoneurial edema might affect the vascularity of the endoneurium.

In the present study, there was an increased number of the endoneurial macrophages following the compression injury of the spinal nerve root. Such an increase was more marked in the areas surrounding the endoneurial blood vessels and persisted during the first 2 weeks following decompression. These macrophages were loaded with degenerated myelin and fat globules. Their number decreased during the period from the third to the sixth week following decompression. In the spinal cord and nerve roots within the subarachnoid space, the cerebrospinal fluid is believed to have a similar role to the lymph. This is due to macrophages in the cerebrospinal fluid have the capacity to remove foreign material from the subarachnoid space. The flow of the cerebrospinal fluid can remove metabolites, and waste products produced in the spinal cord and nerve roots. This might explain the increased number of macrophages within the endoneurium in the present study, as to clear the degenerated myelin from the endoneurium.
The present study showed that the Wallerian degeneration, which had started in the nerve segment distal to the compressed area resulted in increased permeability of the endoneurial blood vessels, which led to endoneurial edema and albumin extravasation. Such edema was found to decrease the nerve blood flow, and consequently, adds an ischemic factor that might share in the pathogenesis of radiculitis. As shown in the current study, the Wallerian degeneration also caused increase in the number of the endoneurial macrophages, which might have generated different inflammatory molecules, for example, interleukin 1 and tumor necrosis factor that could exert cytotoxic activity by direct physical contact or through the release of toxic by-products, for example, nitric oxide and proteases. Inflammatory mediators secreted by macrophages can be involved in and are, at least, partially responsible for radiculitis produced by mechanical compression. These mediators increase the permeability of the endoneurial blood vessels resulting in albumin extravasation and accumulation of the endoneurial edema.

In the present study, signs of regeneration were observed in the ventral nerve roots distal to the site of clips one week after decompression. This was in the form of appearance of regenerated unmyelinated axons and thinly myelinated axons in the endoneurium. After the third week, the regenerating nerve fibres were surrounded by the long processes of the proliferated endoneurial fibroblasts, dividing the endoneurium into minifascicles. This indicates that these nerve roots have an expected regenerative capacity similar to peripheral nerve trunks. Six weeks after decompression, the endoneurium appeared nearly normal with increased unmyelinated and thickly myelinated nerve fibres, compacted collagen fibrils with diminution of the number of fibroblasts and macrophages. The endoneurial edema greatly subsided, especially in the vicinity of the endoneurial blood vessels.

The present study also showed that the increased number of the endoneurial fibroblasts in the compressed nerve root was followed by a decrease 4 weeks after decompression. This is in contrast to what was reported by Murphy et al, who stressed on the occurrence of persistent endoneurial fibrosis after nerve root compression. The authors also reported that such endoneurial fibrosis was more obvious in the perivascular areas of the endoneurium. The number of collagen bundles was increased and also, became organized to support the endoneurium, which is considered as a normal feature of regenerating nerves. This is consistent with the improvement of the sensory and motor symptoms after lumbar root decompression. Such improvement indicates that the assumption of the occurrence of endoneurial fibrosis following nerve root compression is remote since fibrosis is generally a permanent and progressive feature.

It could be concluded from the current study that compression of the nerve radicals followed by decompression after a period of time will result in initial accumulation of the endoneurial edema, and increase in the endoneurial macrophages, mast cells, and fibroblasts. All these will produce chemical radiculitis resulting from mechanical compression of the nerve radicals. Nerve regeneration occurred together with the degenerative process. Four weeks after decompression, there was marked diminution of the endoneurial edema, albumin extravasation, and endoneurial fibroblasts, leaving no apparent fibrosis. Therefore, surgical treatment of a herniated disc or decompression of the lumbar nerve roots will obviously correct the neurological deficits that result from compression. Consequently, the recorded cases of postlaminectomy chronic low back pain, leg weakness, and lower extremity hyper-reflexia could not be attributed to endoneurial scarring of the compressed roots as reported by some authors, but due to another cause, such as faults in the surgical techniques, epidural fibrosis or hematomas.

References