Ultrastructural changes in rat peripheral nerve following pneumatic tourniquet compression

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The sciatic nerves of 12 male rats were examined in the electron microscope 14 days after pneumatic tourniquet compression. Tourniquet pressure was maintained at 300 mmHg for varied lengths of time (30 minutes to 3 hours). Nerves compressed for 30 minutes showed very mild fissuring of the myelin without axonal degeneration. Examination of nerves compressed for 1 to 3 hours showed progressively more varied and extensive damage. Changes included splaying of myelin lamellae, axonal shrinkage with periaxonal edema, Schwann cell hypertrophy, and an increase in the number of microtubules and mitochondria per unit area. The myelin sheaths of some fibers, compressed for more than 2 hours, were completely ruptured. These changes resemble nerve lesions which could be induced by a variety of experimental procedures. Ultrastructural changes produced by tourniquet compression are apparently time-related and affect large-diameter nerves more profoundly than smaller-diameter nerves.

The data reported provide an explanation for delayed muscle rehabilitation experienced by patients who have undergone extremity surgery with pneumatic tourniquet application. The evidence presented suggests that the incidence of tourniquet palsy may be far greater than previously recognized.

KEY WORDS • tourniquet • peripheral nerve • nerve compression • nerve injury

Peripheral nerves subjected to compression undergo progressive morphological changes in relation to time, both above and below the site of compression. Seddon and Denny-Brown and Brenner have shown that severe compression may actually crush the nerve fibers and lead to Wallerian degeneration, from which recovery may take several months. Mild compression results in a transient conduction block, but does not lead to any morphological changes. Ultrastructural alterations in peripheral nerves following complete or partial laceration have been well reported. Williams and Hall and Haftek and Thomas have examined anatomical changes in peripheral nerves following crush injury, and Richardson and Thomas have studied the effects of percussive trauma to rat peripheral nerve tissue. These investigators have noted varying degrees of axonal degeneration and demyelination depending on the severity of the precipitating injury. Nerve compression of intermediate severity is routinely induced iatrogenically with application of a pneumatic tourniquet for surgery on the extremities. Clinical complications associated with the use of a tourniquet have been reported by Rudge, Trojaborg, and Dobner and Nitz.

Fine-structure investigation has demonstrated that the nerve lesion under a pneumatic tourniquet is characterized by dislocation of the nodes of Ranvier of the large-diameter myelinated fibers, with slight invagination of one paranode into the adjacent one. These findings were seen most prominently under the proximal edge of the cuff, although varying degrees of nerve damage extended several millimeters below the distal edge of the tourniquet as well. Sequentially, these initial alterations are followed by demyelination, periaxonal edema, and axonal degeneration. Such findings were noted to persist for several months after compression. Ochoa et al. used a tourniquet pressure of 1000 mmHg to create the nerve lesion. This pressure is considerably more than the normal range of tourniquet pressures for extremity surgery, which is 300 to 500 mmHg. In addition, Ochoa et al. were concerned primarily with ultrastructural observations noted in the longitudinal section of single teased nerve fibers. No information is available in regard to tourniquet-induced nerve damage at pressures similar to those used clinically, and sufficient documentation of ultrastructural changes that occur following tourniquet application is lacking. Therefore, the present study was undertaken to describe periph-
neral nerve lesions at the subcellular level caused by pneumatic tourniquet pressure within the clinical range.

Materials and Methods

Observations were made on the sciatic nerves of 15 male Sprague-Dawley rats, weighing 350 to 400 gm each. The right and left mid-thigh of the rat (anesthetized) was compressed with a pneumatic tourniquet, 1.5 cm wide, at a pressure of 300 mmHg for varied lengths of time (30 minutes, and 1, 1 1/2, 2, 2 1/2, and 3 hours). A silk suture was sewn into the skin at the proximal and distal edge of the tourniquet for identification of the compression site at the time of tissue sampling. Four sciatic nerves from two animals were assessed for each compression time. Three animals (six sciatic nerves) served as controls. Fourteen days following tourniquet application, nerve specimens were obtained from all animals.

Prior to sacrifice, animals were anesthetized by intraperitoneal administration of sodium pentobarbital (50 mg/kg). The sciatic nerve was surgically exposed, the epineurium carefully removed, and the nerve irrigated in situ with 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4). Two 1-cm segments of sciatic nerve just distal to the proximal edge of the tourniquet were then carefully excised, cut into 1-mm sections with a sharp razor blade, and immersed in 2% osmium for 2 hours. Following dehydration in ascending concentrations of ethanol, the sections were transferred to propylene oxide and then embedded in Epon 812. Sections 1 μ thick were cut and mounted on glass slides, stained with toluidine blue, and examined for orientation with a light microscope. Ultrathin sections were cut with glass knives on an LKB ultramicrotome,* collected on 300-mesh copper grids, and stained for 2 to 3 minutes with lead citrate. In all, 100 to 300 cross-sectional profiles of nerve fibers per group were examined in a Philips 400 electron microscope.†

Results

Control Group

The ultrastructure of myelinated nerve fibers in the control rats closely resembled the description by Schnapp and Mugnaini19 and Landon and Hall.10 Myelin was well preserved with no lamellar splaying or other disorganization (Fig. 1). The myelin was occasionally mottled in appearance; this was considered to be an artifact. Axons filled the myelin sheaths, and there was no periaxonal edema (Fig. 1). Mitochondria in the Schwann cells and in the axoplasm were normal in number and appearance. Numerous microtubules were observed, oriented predominantly parallel to the main axis of the nerve fibers (Fig. 1).

Compression for 30 Minutes

Many nerve profiles from the group with 30-minute tourniquet application were unaltered and appeared normal. However, some nerves did show minor ultrastructural alterations (Fig. 2). The predominant changes noted were mild separation of concentric myelin lamellar strata and periaxonal edema, suggested by the appearance of a homogeneous fine particulate material which was presumed to be proteinaceous edema fluid (Fig. 2). The axons, however, appeared normal in both myelinated and unmyelinated nerve fibers. The most noticeable morphological changes following compression for 30 minutes were seen in medium- to large-diameter fibers, although some alterations were noted in small-caliber fibers. Subcellular components of Schwann cells and axons appeared normal in this experimental group.

* LKB ultramicrotome manufactured by LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, Maryland.

† Philips 400 electron microscope manufactured by Philips Electronic Instruments, Mount Vernon, New York.
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Fig. 2. Transverse section of sciatic nerve 2 weeks after 30 minutes of tourniquet compression. Mild myelin separation can be seen (arrows) without axonal (Ax) degeneration in myelinated fibers. Nonmyelinated fibers (Nm) appear normal. X 6875.

Fig. 3. Nerve after 1 hour of tourniquet compression. Left: Myelin splaying (Ms) is slightly more pronounced than after 30 minutes of compression. Indentation at the left margin of the axon (arrow) is taken to represent axonal sprouting. Mitochondria (M) and microtubules (Mt) appear normal. X 24,750. Right: Section of nerve fiber showing shrinkage of the axon (arrowheads). An axonal sprout is indicated by an arrow. Bl = basal lamina. X 15,000.

Compression for 1 Hour

Ultrastructural changes following 1 hour of tourniquet compression were characterized by slightly more pronounced myelin fissuring than at 30 minutes (Fig. 3 left). The first evidence of axonal retraction from the myelin sheath was noted at 1 hour (Fig. 3 right). Shrinkage of axons was considered to indicate mild axonal degeneration. The basal lamina of the axon was still adherent to the myelin at one or more sites (Fig. 3). Profiles, axon-like in appearance, indented the basal lamina of the degenerating axon (Figs. 3 and 5 left). These were presumed to represent axon sprouts, indicating axonal regeneration concurrent with degeneration. Normal-appearing mitochondria and microtubules persisted in the axoplasm and Schwann cell cytoplasm (Fig. 3).

Fig. 4. Nerve fiber after 1½ hours of tourniquet compression. Whorled myelin (Wm) is prominent, as is axonal shrinkage (arrowhead). M = mitochondria, Mt = microtubules. X 9000.
Tourniquet compression nerve injury

Compression for 1½ Hours

Nerves with tourniquet compression for 1½ hours displayed more severe concentric myelin lamellar splaying than those of the previous group (compare Figs. 3 left and 4). Some myelin sheaths were torn apart while others were intact but splayed (Fig. 4). Also, axonal degeneration was more pronounced than after 1 hour of compression as evidenced by, in many instances, complete separation of axons from the myelin sheath (Fig. 4). This effect was most likely the result of more severe axonal retraction from the myelin and periaxonal edema within the sheath. Mitochondria, often dilated and swollen, were numerous in axons of the altered nerve trunks when compared to normal nerve fibers. In addition, the mitochondrial crests were altered in such a fashion that they were difficult to observe (Fig. 4). The number of microtubules per area was elevated, giving the axon an almost homogeneous appearance (Fig. 4). On several occasions, axon sprouts were seen in a similar orientation to that observed after 1 hour of compression.

Compression for 2 Hours

After 2 hours of compression, a large percentage of the nerve fibers demonstrated marked disorganization of the myelin lamellae. This finding was especially severe in the large-diameter myelinated fibers (Fig. 5 left). Axonal retraction from the myelin was pronounced, and there was a preponderance of axonal material outside the original axon. Schwann cells were also markedly increased in size. Such hypertrophied cells were most often associated with large-diameter fibers. However, some small-diameter nerve fibers were bounded by large Schwann cells (Fig. 6).

Myelin “ovoid formations” of lamellar structure were first observed at 2 hours of compression. These formations were located external to and distinct from the concentric myelin lamellae surrounding the axon (Fig. 5 right). Similar types of ovoid formations were also noted in nerve fibers of the last two animal groups (with 2½ and 3 hours of compression). As in the two preceding experimental groups, axon sprouts were observed (Fig. 5 left).

Compression for 2½ Hours

The most obvious ultrastructural change observed in nerves of this group was a preponderance of fibers in which actual rupture of the myelin sheath had occurred (Fig. 7). This change was accompanied, in some cases, by extravasation of axoplasm out of the myelin sheath (Fig. 7). Schwann cell hypertrophy continued to be observed. Varying degrees of demyelination were also noted in these nerves.
Fig. 7. Transverse nerve section after 2½ hours of tourniquet application. Myelin rupture is seen (arrowhead) with extravasation of axoplasm (Ap). M = mitochondria. × 12,000.

ulation and remyelination, as well as axonal degeneration, were noted at the injury site. Persistence of abnormally large numbers of mitochondria, as compared to control fibers, was also seen in nerve tissue of this group.

Compression for 3 Hours

Total dissolution of myelin and remarkably disorganized myelin lamellae characterized most nerve fibers in this group (Fig. 8). In many cases, Schwann cells were considerably enlarged (Fig. 8 upper). Some poorly organized large myelin sheaths devoid of axoplasm were observed (Fig. 8 lower). On occasion, medium-sized axons were denuded of myelin. Axon-like profiles adjacent to degenerating fibers, as noted after 1 to 2 hours of compression, were not seen after 3 hours. In general, the nerve tissue at this time was highly disrupted and appeared severely altered, suggesting marked functional impairment.

Discussion

Several ultrastructural changes in peripheral nerve tissue following tourniquet compression have been described in the present report. These changes include separation of concentric myelin lamellae, periaxonal edema, axonal degeneration, increase in number per unit area of mitochondria and microtubules, and Schwann cell hypertrophy. The changes generally became more severe in nature with longer tourniquet compression, although a wide spectrum of nerve-fiber damage was occasionally observed within one major nerve fascicle.

Fissuring of the myelin was attributed to edema, which was suggested by the presence of a fine particulate matter between the myelin lamellae. At higher magnifications, the separations were observed at the intraperiod line (that is, the extracellular space had expanded). This observation is in accord with that reported by Richardson and Thomas followingpercussive injury to peripheral nerve tissue.

Rupture of the myelin sheath, which was observed at tourniquet periods of 2½ and 3 hours, was also reported by Ochoa, et al., after tourniquet compression. Others have observed a similar phenomenon after nerve transection. They suggested that the ruptured sheaths were indicative of the demyelination process. Myelin rupture is considered to be tourniquet-induced and not artifactual in nature for the following reasons: great care was taken in handling the nerve specimens; whole-nerve segments were studied so that teasing of single fibers, with its propensity to damage the nerve, was eliminated; and ruptured myelin sheaths were never observed in the six control nerves.

The first sign of myelin loss following compressive injury was observed by Spencer, et al., on the 3rd day after injury. Trojaborg reported electrophysiological data supporting the contention that demyelination persists for several months after tourniquet compression. Williams and Hall observed traumatized peripheral nerves in vivo by means of oblique incident illumination, and found that nerve fibers of all calibers are involved in the acute reaction to injury. The present observations revealed that ultrastructural abnormalities of myelin resulting from nerve compression persist for at least 2 weeks and involve fibers of all diameters.

In the present study, a progressive increase in axonal retraction from the myelin sheath and periaxonal edema was noted with longer tourniquet compression times. Similar correlation between the duration of compression and the severity of anatomical changes was reported by Ochoa, et al. Dohrmann, et al., suggested that axonal degeneration may result solely from the periaxonal edema itself.

Hypertrophy of Schwann cells, which was noted in tissue compressed for 1½ hours or more, is a well recognized component of Wallerian degeneration, and has been reported by Nathaniel and Pease. Friede and Bischhausen found this cell hyperplasia to be asymmetrical, with twice as much cytoplasm distal to the nucleus as found proximally. The hypertrophy observed with nerve injury is, presumably, in response to a demand for myelin repair.

It has, in general, been believed that degeneration of neural tissue must be complete prior to the onset of regeneration (nerve sprouting), however, we observed these phenomena concurrently in many nerve fibers. This overlap of degeneration and regeneration was also observed by Friede and Bischhausen. This information helps to explain a similar overlap of degeneration and nerve sprouting noted electrophysiologically in patients who have had extremity surgery with pneumatic tourniquet application. Even though axon sprouting in the different groups of animals was not quantified specifically, the impression from viewing a
large number of nerve sectional profiles revealed an interesting corollary. The temporal coincidence of degeneration and regeneration is that, with more profound axonal injury, fewer axon sprouts are noted. This reduction of nerve sprouts was particularly evident in the groups with 2 ½ and 3 hours of compression. Whether the reduced number of nerve sprouts indicates temporal delay or decreased regeneration capacity is not considered in the present study.

The microtubular and mitochondrial increase noted in nerve fibers in this investigation may be the result of the accumulation of organelles secondary to arrested axoplasmic streaming associated with nerve compression, or it may be caused by manufacture of new organelles in the axoplasm, implying a reactive axonal metabolism in response to the lesion. Both of these hypotheses are supported by other studies.\textsuperscript{5,11}

The rather unusual myelin ovoid profiles (ovoids) noted in the nerves of animals with compression for 2 or more hours have been described previously.\textsuperscript{1,11} These formations are thought to originate from aberrant lamellae of the myelin surface.\textsuperscript{11} Residual myelin ovoids are eliminated by macrophage activity in the course of Wallerian degeneration.\textsuperscript{11} The coexistence of severe degenerative changes in the axon has led other authors to consider the ovoid bodies as part of an abnormal regressive manifestation in the myelin sheath.\textsuperscript{11} The present observation suggests that these structures result from prolonged injury to the axons, since the ovoids were only observed in nerves com-

FIG. 8. Nerve section following 3 hours of tourniquet compression. \textit{Upper:} Highly disorganized myelin (My) and a portion of the degenerated axon (Ax) are observed. Sc = Schwann cell \(\times 33,000\). \textit{Lower:} Marked disorganization of myelin is seen. Axons are absent (arrowheads) or highly degenerated (Ax). \(\times 31,250\).
pressed for 2 or more hours. Thus, they most likely represent a deterioration of myelin.

The reported incidence of grossly observed tourniquet palsy for extremity surgery is quite low (that is, one in 5000 cases). However, recent clinical studies, which included electromyographic evaluation following extremity surgery performed with tourniquet application, identified a 70% to 75% incidence of electrical abnormality suggestive of Wallerian degeneration. Previous animal models designed to study tourniquet palsy appear to be inappropriate since they used pressures beyond the normal clinical range (500 and 1000 mm Hg). The present study reveals that peripheral nerve fiber ultrastructure is significantly altered following tourniquet application at a pressure clearly within this range (300 mm Hg). These findings in our model might explain why many patients encounter motor difficulties (delayed muscle rehabilitation) following extremity surgery performed with use of the pneumatic tourniquet. It appears that the incidence of more subtle tourniquet palsy may be far greater than previously recognized. These subtle nerve injuries have clinical importance since patients undergoing surgery involving tourniquet compression exhibit significant motor debilitation, apparently as a direct result of tourniquet application. Therefore, the unquestioned routine use of the pneumatic tourniquet should be reevaluated.

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