Neuromuscular Lesions in Restrained Rabbits

B. MENDLOWSKI

Merck Institute for Therapeutic Research, West Point, Pa.

Abstract. Ten of 16 rabbits restrained 6 h daily for 35 days developed focal to diffuse degeneration of the sciatic nerves. Very small necrotic areas also were found in the skeletal muscles of seven of 16 rabbits, but the muscle lesions did not correlate with the nerve changes.

Rabbits are usually restrained in stocks when they are used in skin irritation studies of topical formulations. They may be kept in these stocks for approximately 6 h daily up to 3 months.

Some animals restrained in this manner develop decreased mobility of the hind legs, which subsides when the rabbits are placed in a regular cage overnight. Some animals also develop lumbar kyphosis.

These observations and whether the restrained rabbits develop anatomical evidence of neuromuscular damage were studied.

Materials and Methods

Random-bred New Zealand rabbits approximately 24 weeks old from a commercial breeder in the southwestern United States were divided into three groups of four males and four females.

One group of animals was restrained in stocks (fig. 1) and the back skin shaved and tightly wrapped with an occlusive dressing (Saran Wrap, Dow Chemical Co., Michigan). A 0.5-ml physiologic saline solution was applied to the skin daily. The hind legs of these animals were extended, and movement of these limbs more restricted than were the front legs. The animals were restrained for 6 h and then were returned to standard rabbit cages.

Another group of saline-treated animals was similarly restrained, but no occlusive dressing was applied.
The third group of control rabbits was confined to standard rabbit cages, and saline without an occlusive dressing was applied daily.

These procedures were repeated daily for 35 days.

The following tissue specimens were taken from each animal at autopsy and fixed in 10% neutral buffered formalin solution: both sciatic nerves and brachial plexuses; spinal cord (cervical, thoracic and lumbar segments); and skeletal muscles on either side of body (biceps and triceps brachii, longissimus dorsi, rectus femoris and semitendinosus). Paraffin sections (5 μm thick) were routinely stained with hematoxylin and eosin (HE) and on several occasions with oil-red-O, Luxol-fast blue in combination with Holmes or periodic acid-Schiff, or MacCallum-Goodpasture.

**Results**

Changes resulting from restraint included degeneration of the peripheral nerves and skeletal muscle necrosis (table I). After removal from stocks, all animals had difficulty moving both hind legs, but this improved overnight in a standard cage. Beginning from the ninth day of restraint a few animals also developed lumbar kyphosis. The lesions and clinical signs were found with similar frequency and degree only in restrained animals, regardless of the use of occlusive dressings.

Moderate degeneration of both sciatic nerves, evidenced by disintegration or loss of axons and myelin sheath, was noted in one restrained rabbit (fig. 2). The endoneurial tubes of a large number of nerve fibers were either collapsed or lined with strands of Schwann cells and also were surrounded by an increased number of fibroblasts and capillaries, in contrast to those of control rabbits (fig. 3). The blood vessels along the epineurium and perineurium were distended and engorged with blood. Numerous internodal vacuoles along the affected nerve fibers were either empty or contained large macrophages with phagocytized or free fragments of axons and myelin debris (fig. 4). Such areas near the perineurium lost the usual birefringence of the myelin sheath and instead revealed numerous aggregations of light polarizing crystals (fig. 5, 6). The centrally located nerve fibers were much less affected.

Degeneration of both sciatic nerves in another restrained rabbit involved a small number of nerve fibers. The fibers were thin because of intraneurial edema or were disrupted and lined with rows of enlarged Schwann cells. Hyperemia and entrapment of axonal and myelin material by a few macrophages were evident in these areas. The blood vessels showed proliferation of the endothelial cells and perivascular mononuclear cellular infiltration.

A very slight disintegration of axons and myelin sheaths, not accompanied by phagocytosis, occurred in both sciatic nerves of four other re-
Fig. 1-2
Table I. Incidence and severity of neuromuscular lesions in restrained rabbits

<table>
<thead>
<tr>
<th>Number of animals affected¹</th>
<th>Peripheral nerve degeneration</th>
<th>Spinal cord vascular lesions</th>
<th>Skeletal muscle necrosis</th>
<th>Lumbar kyphosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restraint with occlusive dressing</td>
<td>5/8 ± ++</td>
<td>1/8 +</td>
<td>3/8 +</td>
<td>3/8</td>
</tr>
<tr>
<td>Restraint without occlusive dressing</td>
<td>5/8 ± +++</td>
<td>0/8</td>
<td>4/8 +</td>
<td>3/8</td>
</tr>
<tr>
<td>No restraint, no dressing (control)</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

¹ Numerator, number of animals affected; denominator, number of animals studied. ± = Trace; + = very slight (very small); ++ = slight (small); +++ = moderate.

strained rabbits. Scattered short chains or groups of internodal vacuoles along the nerve fibers showed enlargement or encroachment of Schwann cells on the internodal spaces. Hyperemia and intraneural blood vessels were prominent in some areas.

Peripheral nerve changes among four other restrained rabbits were limited to a few scattered globular fragmentations and internodal vacuolations of the myelin sheath, without cellular reaction, in one of the sciatic nerves of three rabbits and in the brachial nerve of one rabbit. In general, the nerve changes were more frequent at the periphery than in the center of the nerve bundle, and not all nerve bundles were equally affected. No detectable nerve lesions were seen in the remaining six restrained animals.

Very small foci of mononuclear cells were seen around a few blood vessels in the lumbar spinal cord and the nerve roots of one rabbit with occlusive dressing (fig. 7). A sizable perivascular cuffing and an adjacent granulomatous lesion, the latter devoid of any microorganisms, also were present in the area. This animal also had perivascular lesions in both sciatic nerves. No changes in the spinal cord or the nerve roots were seen in the remaining animals.

Fig. 1. Method of restraint of rabbits. Both hind legs are extended and more restricted than the front legs.

Fig. 2. Moderate degeneration of the sciatic nerve from restrained rabbit without occlusive dressing. Increased number of capillaries (a) and fibroblasts (b). Some internodal vacuoles are empty (arrows). Centrally located nerve fibers (bottom right) appear normal. HE.
Fig. 3. Normal sciatic nerve from unrestrained (control) rabbit. HE.

Fig. 4. Higher magnification of the same sciatic nerve as figure 2. Large macrophages contain axonal (a) and myelin (b) debris. A free fragment of the axon (arrow) is also evident next to a macrophage. Luxol-fast blue and Holmes.
Skeletal Muscles

Very small, necrotic lesions (fig. 8) or nuclear clumps were found in the skeletal muscle fibers of seven of 16 restrained rabbits (three with and four without occlusive dressing). The lesions were widely scattered amidst normal appearing muscle fibers and occurred in one of the biceps or triceps brachii muscles of all rabbits except one. In this animal (without occlusive dressing), unilateral lesions were found in the longissimus dorsi and rectus femoris muscles in association with a moderate degeneration of both sciatic nerves.

Kyphosis in the Lumbar Region

Six restrained rabbits had lumbar kyphosis. Three of these animals had sciatic nerve lesions, and one rabbit had a few scattered necrotic lesions in the dorsal muscles of the back and in the femoral muscles.

Discussion

Prolonged extension of the hind legs during restraint and possibly ischemia may account for the degenerative changes found primarily in the sciatic, but not in the brachial nerves. The changes constituting disintegration of the myelin sheath or Wallerian degeneration were scattered along the nerve fibers, mostly near the perineurium. Those in the center of the nerve bundles were less evident. The changes were associated with hyperemia and edema or, in more advanced lesions, with proliferation of the intraneural capillaries and fibroplasia. Other vascular changes indicating an impairment of the blood supply included mononuclear cell infiltration along intraneural capillaries [8] in one rabbit and dilatation and engorgement with blood of the epineural blood vessels in another rabbit.

Similar nerve lesions attributed to ischemia have been produced by ligation of the nutrient arteries or stripping of the epineurium [7]. The structural changes were confined to the periphery of the nerve, whereas those in the center were sparse because of an intact, intrinsic blood supply. It also was shown in parallel experiments that the nutrient arteries were obliterated by stretching or compression of the nerve. Furthermore, this assumes [7] that a similar situation probably occurs in man exhibiting various neurovascular syndromes after assuming prolonged abnormal postures or positions. The latter assumption is pertinent to this study.

Degeneration of the peripheral nerves also has been produced by stretching [4-6] or compression [1-3]. These structural changes varied in severity
Fig. 5–8
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depending on the magnitude of force, not on the duration of action. The reverse was true in the current study, in which a low-grade pressure exerted by prolonged restraint interfered with the blood supply and produced ischemic nerve changes.

No detrimental effect of restraint was noted in the spinal cord or the nerve roots. In one animal, scattered perivascular cuffs were in the lumbar spinal cord and the nerve roots, but these were considered to represent an extension of similar vascular lesions from the damaged extrapelvic segments of the sciatic nerves; however, a possible coincidental nosema infection that may be represented by granulomatous lesions in the area cannot be ruled out.

Very small, necrotic lesions or nuclear clumps were seen in widely scattered muscle fibers of a few restrained rabbits. The lesions, except in one rabbit, were found in the muscles of the front legs and could not be correlated with the nerve lesions. Only one of seven rabbits affected had unilateral lesions in the dorsal and femoral muscles in association with a moderate degeneration of both sciatic nerves.

The decreased mobility noted among animals that had been restrained could be explained by peripheral nerve lesions or by an expected decrease in conductivity of the nerves [8] in animals without the nerve lesions; however, neither the nerve nor the muscle lesions were found with consistent frequency to account for the deformation in the lumbar region in a few restrained animals.

Acknowledgements


Fig. 5. Formalin-fixed frozen section of the sciatic nerve from the same restrained rabbit as shown in figures 2 and 4. Loss of the usual birefringence and numerous crystals along the nerve fibers at the periphery, but not in the center of the nerve bundle. Oil-red-O, under polarizing light.

Fig. 6. Formalin-fixed frozen section of the sciatic nerve from unrestrained (control) rabbit. Oil-red-O, under polarizing light.

Fig. 7. Longitudinal section of the lumbar spinal cord from restrained rabbit with occlusive dressing. Granulomatous lesion and perivascular cuffs, the latter involving also the nerve root (bottom right). HE.

Fig. 8. Necrotic lesion in the triceps brachii muscle from a restrained rabbit without occlusive dressing. The adjacent muscle fibers appear normal. HE.
References


Dr. B. Mendowski, Merck Institute for Therapeutic Research, West Point, PA 19486 (USA)