Neurotoxicity of Local Anesthetics: Altered Perineurial Permeability, Edema, and Nerve Fiber Injury

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A quantitative, *in situ* experimental method was developed employing the rat sciatic nerve to study the neurotoxicity of local anesthetic solutions applied directly to an intact peripheral nerve bundle. One-milliliter volumes of 2-chloroprocaine, 3%; tetracaine, 1%; lidocaine, 2%; bupivacaine, 0.75%; or sodium chloride, 0.2%; were injected with a 30-gauge needle beneath the mesoneurium but exterior to the epineurium. The wound was closed and the animals were normally maintained until the nerves were reexposed for quantitative biophysical and morphologic testing 24 h to 4 weeks later. The results indicate that topically applied 2-chloroprocaine and tetracaine produce significant endoneurial edema 48 h after treatment. Horseradish peroxidase was used to verify increased permeability of the perineurium. Endoneurial fluid pressure was significantly increased in edematous nerves. Electron microscopy revealed abnormal mast cells and proliferation of endoneurial fibroblasts in addition to Schwann cell injury and axonal dystrophy. This study shows that extravascular administration of clinically used concentrations of local anesthetic solutions can alter perineurial permeability, producing changes in the endoneurial environment that are associated with neurotoxic injury. Perineurial and endoneurial fibrotic changes may be a late consequence of peripheral nerve injury with anesthetic solutions producing altered perineurial permeability with endoneurial edema. (Key words: Anesthetics, local; bupivacaine; chloroprocaine; lidocaine; tetracaine. Nerve injury. Toxicity: local anesthetics.)

Neither the mechanism of action nor the pathologic complications of local anesthetic solution-induced injuries to peripheral nerves are presently understood. In spite of the low incidence of nerve fiber injury associated with local anesthesia, there are numerous clinical reports of injuries. Some of these injuries can be explained through an ischemic mechanism caused when large volumes of drug inadvertently are injected intrafascicularly or by subarachnoid injection when an epidural injection was desired. Controlled laboratory studies suggest a direct toxic effect of some agents, although much of this literature is contradictory, owing in part to different methods, drug dosages, and durations of exposure. It is clear, however, that when local anesthetic solutions are injected intrafascicularly, there are changes in the permeability of the blood–nerve barrier associated with edema and nerve fiber injury. Support for the hypothesis that edema is a pathogenic factor in nerve fiber injury has been gathered during the past few years following introduction of quantitative neuropathologic techniques that have associated increases in nerve fascicular edema with increased endoneurial (interstitial) fluid pressure (EFP) exerted on nerve fibers and the perineurium. Physiologically, it has been noted that osmotic swelling of the nerve bundle is associated with a deficit in nerve conduction. Fink proposed a mechanism of hypoosmotic conduction block based on the development of interstitial edema in isolated peripheral nerves. Since the integrity of the semipermeable and semipermeable perineurium was an essential factor in the block, it was concluded that the mechanism was a variety of compressive nerve block owing to osmotic swelling of the fascicle and the elastic tension of the perineurium. Reduced nerve blood flow (NBF) has been reported in edematous neuropathies with increased endoneurial fluid pressure, and a recent computer model of perineurial biomechanics further supports a direct relationship between edema, increased endoneurial fluid pressure, and reduced nerve blood flow.

Our objective in this study was to develop a reliable model for testing the neurotoxicity of local anesthetic solutions and to use the model initially to identify biophysical and pathologic changes secondary to injection of clinical preparations of 2-chloroprocaine and tetracaine extrafascicularly around intact nerve fiber bundles. Although less severe changes are seen in peripheral nerves bathed in local anesthetic solutions, this approach was necessary in the present study to preclude artifacts that might occur following direct injection into the endoneurium and to test alterations in perineurial permeability associated with external application of the drugs. The focus of this report is on the pathogenesis and morphology of anesthetic solution-induced edema, which were studied using histology, intravenous tracers, electron microscopy, and measurements of endoneurial fluid pressure. The individual effects

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Fig. 1. Experimental preparation for in situ testing of local anesthetic neurotoxicity with drugs applied extraspinally. The sciatic nerve is exposed by a skin incision and reflection of the superficial muscle tissue with care being taken to avoid nerve trauma. A 1-ml volume of test solution is injected through the intact mesoneurum with a 30-gauge needle (arrow) to form a bath adjacent to the nerve (inset). The wound is closed and the animal is maintained normally until removal of the nerve for histology 24 h to 4 weeks later.

of pure local anesthetics and the preservative sodium bisulfite were studied separately and are reported elsewhere.13

Materials and Methods

A double-blind protocol was used in this study for the injection of test solutions and evaluation of histologic results. Young, adult, female Sprague-Dawley rats, weighing approximately 225 g, were anesthetized for nerve exposure with an intraperitoneal injection of a mixture of sodium pentobarbital (50 mg/kg), diazepam (5 mg/kg), and normal saline, 1:1:2. Both sciatic nerves were exposed by lateral incision of the thigh and reflection of superficial fascia and muscle (fig. 1). One milliliter of either 1) 3% 2-chloroprocaine HCl with 0.2% sodium chloride and 0.2% sodium bisulfite (Nesacaine–Clk®); 2) 1% tetracaine HCl with 0.7% sodium chloride and 0.2% sodium bisulfite (Pontocaine®); or 3) the control solution, 0.2% sodium chloride, was injected extraspically with a 30-gauge needle (i.e., directly beneath the clear fascia surrounding the nerve but exterior to the epineurium—fig. 1, inset). Thirty-two extraspicular injections were made with 2-chloroprocaine, eight with tetracaine, and 55 with sodium chloride alone for purposes of control. Injections were performed using an operating microscope to avoid mechanical nerve trauma and to confirm that the test solution formed a local bath adjacent to the nerve. The bath extended 1 cm along the nerve axis. The superficial muscle layer was sutured with 4-0 silk, the wound was closed with metal clips, and the animals were allowed to recover for 48 h before the nerves were excised under pentobarbital anesthesia, immersed in 2.5% phosphate-buffered glutaraldehyde, and processed for light and electron microscopy. Ancillary experiments were conducted on six nerves each with: 1) 2% lidocaine HCl with 0.6% sodium chloride (Xylocaine®) and 2) 0.75% bupivacaine HCl with 0.7% sodium chloride (Marcaine®). The pH of all solutions was between 2.5 and 4.0. Long-term changes in endoneurial hydration were studied following application of 3% 2-chloroprocaine or 1% tetracaine in tissue harvested 2 weeks (n = 13) and 4 weeks (n = 10) postinjection. Permeability studies were conducted in five animals 48 h after treatment by injecting 75 mg of horseradish peroxidase (HRP) suspended in 1 ml of normal saline into the jugular vein 1 h before death. To produce the HRP reaction product, the sciatic nerve was fixed in 1.5% phosphate-buffered glutaraldehyde and stored overnight in phosphate buffer with 5% sucrose. The nerve then was sliced into transverse sections 30–50 μm thick and incubated in a reaction mixture that was prepared by adding 10 mg of Hanks-Yates reagent, 68 mg of imidazole, and 0.1 ml of 1% hydrogen peroxide to 10 ml of 0.05 M TRIS buffer (pH 7.2). The reaction took place in light-shielded vials with continuous mixing for 2–4 h, after which specimens were rinsed in phosphate buffer and postfixed in 1% osmium tetroxide for 45 min. The tissue then was processed normally for microscopy and 1-μm-thick sections were photographed with a Zeiss Axiosmat light microscope for illustration. Endoneurial fluid pressure was measured in the sciatic nerves of three animals 48 h after the nerves were bathed in 3% 2-chloroprocaine or normal saline. The EFP method is based on a bioengineering technique employing a servo-controlled hydraulic system in which a 4-μm diameter glass micropipette is inserted acutely into the subperineurial space.14 Edema was quantified from 1-μm-thick sections of tissue imbedded in araldite and stained with paraphenylenediamine. Approximately 100 sections of tissue were reviewed from each nerve and assigned an edema score ranging from 0 to 4 in 0.5-step increments. A score of 0 indicated no edema, whereas a score of 4 indicated extensive edema in the subperineurial space (1 point), along the endoneurial membrane partitions in the fascicle (1 point), in perivascular spaces (1 point), and in the interstitial spaces separating individual nerve fibers (1 point). Increments of 0.5 points were used to discriminate between differences in edema within any single region of nerve. Edema usually was observed initially in the subperineurial region, where there is an absence of the interstitial collagen matrix and it could be identified as an increase in the "structureless space." More extensive edema widened this structureless space and then progressively could be observed along the endoneurial partitions, in the perivascular region surrounding vessels.
and, finally, in the most severe cases, between nerve fibers in the interstitial space. The results were subjected to statistical analysis. Edema scores at 48 h associated with administration of 3% 2-chloroprocaine and 1% tetracaine and the ancillary drugs 2% lidocaine and 0.75% bupivacaine, were compared separately with the scores from the control group using Student's single-tailed t test (table 1). To test differences between drugs and 0.2% sodium chloride, multiple comparisons between median edema scores were conducted using a Tukey-type multiple comparison test.15,†† EFP values were compared with Student's single-tailed t test.

Results

Direct application of 3% 2-chloroprocaine or 1% tetracaine to the surface of intact peripheral nerves frequently resulted in subperineurial and endoneurial edema within 48 h of treatment, producing edema scores significantly increased above control values (P < 0.001). The anesthetic solutions were applied topically to the epineurium but were not injected endoneurally. Examination of araldite-embedded sections from these nerves revealed subperineurial edema as well as Wallerian degeneration of nerve fibers (fig. 2). A transverse section from a sciatic nerve in the sodium chloride control group is shown in figure 3 for comparison. Three percent 2-chloroprocaine and 1% tetracaine produced edema, which was observed in all regions of the nerve, but especially in the subperineurial region, where there is the least resistance to its accumulation. Application of the other anesthetic solutions produced occasional edema (table 1) in some sections but overall did not produce edema scores that were significantly different from control values (P > 0.05). Penetration of horseradish peroxidase across the blood–nerve barrier also was observed in experimental animals receiving 2-chloroprocaine (fig. 4). No significant changes were noted in animals after 2 or 4 weeks, and there were no pathologic changes seen in control nerves. Electron microscopy revealed mast cell degranulation and proliferation of endoneurial fibroblasts. Schwann cell necrosis characterized by cytoplasmic accumulation of myelin debris and lipid droplets were observed in edematous nerves (fig. 5), as was axonal dystrophy (fig. 6). Endoneurial fluid pressure in nerves bathed in 2-chloroprocaine for 48 h

<table>
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<tr>
<th>Solution</th>
<th>N</th>
<th>Average ± SE</th>
<th>Median</th>
<th>Significance</th>
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<tr>
<td>Control, NaCl</td>
<td>55</td>
<td>0.89 ± 0.11</td>
<td>0.5</td>
<td></td>
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<tr>
<td>Tetracaine, 1%</td>
<td>8</td>
<td>2.62 ± 0.13</td>
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<tr>
<td>2-Chloroprocaine, 3%</td>
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<td>2.05 ± 0.20</td>
<td>2.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Lidocaine, 2%</td>
<td>6</td>
<td>1.58 ± 0.45</td>
<td>1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Bupivacaine, 0.75%</td>
<td>6</td>
<td>0.92 ± 0.30</td>
<td>0.8</td>
<td>NS</td>
</tr>
</tbody>
</table>


Fig. 2. Transverse sections from sciatic nerves 48 h after being bathed in 3% 2-chloroprocaine (left) or 1% tetracaine (right). Note extensive subperineurial edema as well as interstitial edema that has separated individual nerve fibers. Fibrocytes are seen in the subperineurial space. Edema score was 3 (original magnification, ×250).
averaged $4.8 \pm 1.7$ cmH$_2$O, which was significantly increased above the normal values in the contralateral control nerves, $1.3 \pm 0.7$ cmH$_2$O ($P < 0.025$).

**Discussion**

We interpret the results of this study to indicate that local anesthetic solutions applied externally to peripheral nerve bundles can alter the permeability of the perineurium leading to endoneurial edema. Permeability was tested by intravenous injection of horseradish peroxidase (molecular weight = 40,000 daltons), which was allowed to circulate for 1 h before sacrifice. The electron-dense enzyme reaction product stained the perineurium and could be seen in high concentration in the subperineurial space. The reaction product was not observed in perivascular spaces, indicating an intact vasa nervorum. The studies were performed in situ in an experimental preparation that was designed to be clinically relevant. The nerve was exposed without affecting its anastomotic extrinsic circulation, and 1 ml of test solution was injected beneath the clear fascia surrounding the nerve bundle but exterior to the epineurium. The wound was closed with staples, and the animal was monitored until recovery from the associated general anesthesia, at which time it was returned to an approved animal care facility for normal housing. Most of these results were collected 48 h after the injection of test solution to observe early pathologic features of neurotoxic injury. Ancillary experiments were conducted within the time period 24 h to 4 weeks postinjection to review the temporal course of edema.

Our attempt to develop a clinically relevant laboratory model of local anesthetic solution neurotoxicity precluded removal of the nerve for electrophysiologic testing and absolute control of the concentration of test solution in direct contact with the epineurium. These factors, in combination with the inherent biologic variability associated with in vivo testing, contributed to scatter within the data; all local anesthetic solutions tested produced edema in some nerve sections (Table 1). Statistically however, it is clear that 3% 2-chloroprocaine and 1% tetracaine produced endoneurial edema that was greater in magnitude, overall, than did any of the other solutions tested. The study was designed to test a single, large dose of the most common agents used clinically today for a variety of nerve block procedures. The concentrations chosen for study are in direct proportion to their intrinsic anesthetic potencies$^{16}$ and are at or near the upper limits commonly reported to be safe in humans.
Historically, the use of the 2-chloroprocaine solution has provoked a controversy about its suitability for regional anesthesia, since its intrafascicular or intrathecal injection may produce edema and pathologic change in axons and connective tissue. The relative safety of ester-type local anesthetics such as 2-chloroprocaine and tetracaine over the amide-type local anesthetics has been advocated because the ester-type drugs are rapidly hydrolyzed by circulating plasma cholinesterase. Gentili tested the neurotoxicity of local anesthetic solutions (other than 2-chloroprocaine) injected intrafascicularly in large volume and showed that the ester-type local anesthetics were associated with more severe alterations in the permeability of the blood–nerve barrier than were the amide-type local anesthetics. Our data with extrafascicular administration of drug support these conclusions. Other preliminary work in this laboratory has implicated the local anesthetics 2-chloroprocaine and tetracaine rather than their vehicles or antioxidants as the agents responsible for neurotoxic injury; that is, the ester-type local anesthetics 2-chloroprocaine and tetracaine produce more severe edema than the amide-type solutions tested. On the basis of these findings, we would not agree that local anesthetics of the ester type are relatively more safe than the amide type. Dose–response studies are necessary, however, to further test this hypothesis.

Changes in the permeability of the blood–nerve barrier were associated with pathologic damage to peripheral nerve fibers, including Schwann cell injury and axonal dystrophy. Fibroblast proliferation also was observed in affected nerves subjacent to the site of exposure and caused concern that there may be late-occurring changes in perineurial thickness as well as endoneurial fibrosis. A recently developed computer model of perineurial biomechanics suggests that increased perineurial thickness is a pathogenic factor capable of reducing the caliber of vessels from the extrinsic circulation that traverse the perineurium and therefore could contribute to endoneurial ischemia. An even more important factor is the increase in endoneurial fluid pressure associated with nerve edema. Elevation in EFP of the magnitude reported in this study theoretically can reduce the luminal cross-sectional area of transperineurial vessels by approximately 40%. This occurs because of the biomechanical properties of the perineurium, which are unequal in the longitudinal and circumferential axes, and the fact that the extrinsic microvascular circulation of the nerve, which is necessary for normal function, is anchored to the perineurium and must traverse it to Anastomose with the intrinsic nerve vasculature. This may be the mechanism through which nerve blood flow is reduced in edematous neuropathies.
This study has established an in situ model to test the neurotoxicity of local anesthetic solutions and has found that extraneuronal administration of clinically used concentrations of these solutions can alter perineurial permeability, causing endoneurial edema, increased endoneurial fluid pressure, and Wallerian degeneration with Schwann cell injury and axonal dystrophy. Perineurial and endoneurial fibrotic changes may be a late consequence of peripheral nerve injury with these agents.

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References


