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Amitriptyline Neurotoxicity

Dose-related Pathology after Topical Application to Rat Sciatic Nerve

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Background: **Amitriptyline is a tricyclic antidepressant drug used systemically for the management of neuropathic pain. Antidepressants, as a class of drugs with direct neurologic actions, are becoming widely used for the management of chronic pain, although their mechanisms are not entirely understood. Amitriptyline exerts potent effects on reuptake of norepinephrine and serotonin and blocks α_{2A} adrenoreceptors and *N*-methyl-D-aspartate receptors. Because amitriptyline is also a particularly potent blocker of sodium channels and voltage-gated potassium and calcium channels, it has been recommended as a long-acting local anesthetic agent. Unfortunately, amitriptyline has significant toxic side effects in the central nervous system and cardiovascular system that are dose-related to its systemic administration. Therefore, before amitriptyline can be used clinically as a local anesthetic agent, it should be thoroughly explored with respect to its direct neurotoxic effect in the peripheral nervous system.**

Methods: **The left sciatic nerve of Sprague-Dawley rats (12/group) received a single topical amitriptyline dose of 0.625, 1.25, 2.5, or 5 mg; a saline group (n = 2) was used as control. Neuropathologic evaluations were conducted in separate animals (n = 4) 1, 3, and 7 days later.**

Results: **Amitriptyline topically applied *in vivo* to rat sciatic nerve causes a dose-related neurotoxic effect. Drug doses of 0.625–5 mg all caused Wallerian degeneration of peripheral nerve fibers, with the number of affected fibers and the severity of the injury directly related to the dose.**

Conclusion: **Because the effective local anesthetic dose is within this dose range, the authors strongly recommend that amitriptyline not be used as a local anesthetic agent.**

AMITRIPTYLINE is a tricyclic antidepressant drug that has been given orally or intravenously for the management of neuropathic pain.[1–3] Amitriptyline has multiple complex pharmacologic actions that contribute to its analgesic activity: It inhibits norepinephrine and serotonin reuptake[4] and blocks α_2-adrenergic, nicotinic, muscarinic, cholinergic, *N*-methyl-D-aspartate, and histaminergic receptors.[5]

Despite these biologic effects, the clinical efficacy of amitriptyline for pain control remains controversial. It was recently reported in a human controlled trial with an "active" placebo (*i.e.*, a placebo inducing similar adverse effects) that amitriptyline used orally was not effective in reducing chronic pain in patients with spinal cord injury.[6] This might be because of its variable intestinal absorption.[7,8] Amitriptyline also has significant potential adverse effects. In the central nervous system, these effects include sedation, seizures, and coma. Cardiologic toxicity includes QRS complex widening and cardiac arrest.[9]

Nevertheless, amitriptyline is an appealing analgesic and local anesthetic agent because it is a more potent Na^+ channel blocker than bupivacaine when used for sciatic nerve block,[10] and it has been reported to block voltage-gated K^+ and Ca^+ channels.[11,12] Experimentally, amitriptyline seems to have a more efficacious analgesic effect after peripheral administration[13] than after intraperitoneal,[14] spinal,[15] or intrathecal administration.[1,14] Recent studies reported a use-dependent blockade and the prolongation of peripheral nerve blockade by amitriptyline or various derivative amitriptyline solutions (*N*-phenylethyl or *N*-methyl amitriptyline).[16–18] This work has led to the suggestion that amitriptyline with epinephrine could be clinically useful for infiltration and postoperative analgesia.[5] A recent clinical study and editorial in *Regional Anesthesia and Pain Medicine* provides cautious endorsement for this application of the drug.[18,19]

However, these experimental demonstrations and recommendations for the use of amitriptyline as a local anesthetic have apparently occurred without detailed preclinical neuropathologic studies. Because amitriptyline has significant neurotoxic potential, it is imperative that the drug be formally tested for peripheral neurotoxicity using established neuropathologic techniques with greater sensitivity and resolution than the paraffin hematoxylin and eosin preparations that have already been performed.[20] Although useful in assaying gross histologic change, this latter method of tissue processing introduces artifacts in neurologic structures that can obscure early or subtle alterations in the relationships among axons, Schwann cells, and myelin that provide insights into neurotoxicologic mechanisms. Even if the drug is to be applied as a cream on the skin or injected subcutaneously, at some time it will surely come in contact with peripheral nerves in concentrations that were not intended. Because of the rapidly escalating use of amitriptyline as a local anesthetic without these data, we undertook the current study to determine the neuropathologic effect of extraneural administration of am-

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itriptyline on rat sciatic nerve, an established mammalian model for study of peripheral nerve toxicology. Our results clearly demonstrate that amitriptyline is a potent neurotoxic agent that can cause devastating neuropathologic injury when applied topically to peripheral nerve in a dose of approximately 6–8 nmol, a dose that in fact has already been used clinically for local anesthesia.

Materials and Methods

Drug

A commercially available, preservative-free preparation of amitriptyline was used in this study (amitriptyline-hydrochloride, Laroxyl®; Laboratoires Roche, Neuilly-sur-Seine, France). Four doses were evaluated, each dissolved in 0.2 ml saline immediately before application: 5 mg (25 mg/ml, 79.6 mM or 16 nmol), 2.5 mg (12.5 mg/ml, 39.8 mM or 8.0 nmol), 1.25 mg (6.25 mg/ml, 19.9 mM or 4.0 nmol), and 0.625 mg (3.125 mg/ml, 10.0 mM or 2.0 nmol). The pH of the solutions ranged from 4.12 (5-mg preparation) to 5.72 (0.625-mg preparation). We determined in preliminary histology experiments that this pH difference was not significant in the *in vivo* setting where a small volume is neutralized by tissue pH. Each group contained 12 rats. The experimental doses were determined to be compatible with doses used in previous rodent models of transdermal,[21] subcutaneous,[5,22,23] intraperitoneal,[14,24] and intrathecal[13] application or for its use in sciatic nerve block experiments[10,17] for comparison with other local anesthetics (1–5% lidocaine and 0.5% bupivacaine).[5,17,21]

Animals and Surgery

Adult female Sprague-Dawley rats (weight, 200–250 g; Harlan Labs, Indianapolis, IN) were maintained in an animal care facility approved by The Association for Assessment of Laboratory Animal Care and handled in accordance with National Institutes of Health Guidelines for Animal Use. The protocol was approved by the local animal research committee (San Diego, California). Rats were briefly anesthetized with isoflurane (induction box and maintained *via* a facemask; 3–4%). The sciatic nerves were exposed by lateral incision of the thighs and retraction of superficial fascia and muscle. Under magnification, 0.2 ml of the test dose was injected with a 25-gauge needle directly beneath the clear fascia surrounding the nerve but outside the epineurium and perineurium, proximal to the sciatic bifurcation. The perineurium was not breached. The superficial muscle layer was sutured with 4-0 silk, and the wound was closed with metal clips.

Neurobehavioral Evaluation

After recovery from general anesthesia, sciatic nerve function was evaluated by observing the rat's ability to hop and to place weight on its hind limb and by the rat's ability to grasp with its hind paw when suspended by the tail, using a scale that reflected the presence or absence of the result. Resting posture and postural reactions ("hopping" and "tactile" placing) were also recorded. The contralateral side was used as control. Animals were tested every 10 min during the first hour, then every 30 min until 7 h. Animals that had not recovered by that time were evaluated twice per day until the day of sacrifice.

Flinching behaviors (lifting, shaking, or rippling of the haunch) and biting/licking behaviors were recorded. Vocalization and the presence or absence of a withdrawal reflex to pinching of the hind limbs were also recorded. These tests were performed two times each day before sacrifice (days 1–7). Grooming, food intake, responsiveness to environmental stimuli, and exploratory activity were also monitored.

Tissue Processing

For the four dose groups, animals were killed at 1, 3, and 7 days after amitriptyline application (n = 4 at each day). The two sciatic nerves (*i.e.*, treated and contralateral side as control) were excised under the anesthesia protocol used for surgery; then, the rat was euthanized with an intraperitoneal injection of sodium pentobarbital (70 mg/kg). For fixation, the nerves were immersed in 2.5% phosphate-buffered glutaraldehyde for 24 h. They were then rinsed with phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in serial concentrations of alcohol, and embedded in araldite according to the recommended procedure for neurotoxicologic tissue evaluation.[20] One-micrometer-thick semithin sections from the central 2-mm block of each 6-mm-long segment were cut for light microscopy and stained with methylene blue, azure II. Tissue sections were evaluated by an observer (R. R. M.) who was unaware of the experimental groupings.

Semiquantitative evaluation of neuropathologic change was performed in animals receiving saline and 0.625–2.5 mg amitriptyline. The nerves receiving 5 mg amitriptyline were too severely damaged to process for morphologic analysis. This was done to determine if there were statistically significant changes in neuropathology. A value was assigned for each histologic slide based on a scale with one-half unit increments from 0 to 4 in which 0 represented normal nerve and 4 represented a finding of Wallerian degeneration extending throughout the nerve bundle.

Statistical Analysis

The data were analyzed by one-way analysis of variance with Tukey-Kramer multiple comparison tests.

Results

Rat Sciatic Nerve Blockade

After the animals had recovered from general anesthesia, no adverse clinical systemic effects were recorded, *i.e.*, no overt cardiac impairment, seizures, or sedation was observed in any of the rats. Three minutes after the end of surgery and general anesthesia, neurologic evaluation could be performed. All of the rats in the experimental groups receiving 5, 2.5, and 1.25 mg amitriptyline (79.6, 39.8 and 19.9 mM, respectively) had complete motor blockade at this time. In contrast, complete motor blockade was delayed in animals receiving the 0.625-mg (10-mM) dose until 20 ± 5 min after injection, and the duration of complete blockade was 4 ± 0.5 h in this group. All animals in this group recovered complete motor function and had normal behavior evaluations thereafter. However, for the animals receiving doses of 1.25 and 2.5 mg amitriptyline, the durations of block were 7 ± 1.5 and 24 ± 5 h, respectively, and 50% of the these animals (four of eight in each group) did not completely recovery by day 2. Some of the animals in this group showed weakness in motor function (*i.e.*, unilateral hind-limb gait deficit) and increased flinching and biting/licking behaviors until day 7 (two of four in each group). Animals in the 5-mg dose group had no recovery of motor function during the 7 days of evaluation. By day 3, clear hyperalgesic and allodynic behaviors (*i.e.*, avoiding use of the injected leg, biting/licking activity, vocalization when rats were held or when the hind limb was touched) were observed in all rats in this group, which persisted until the animals were killed through day 7.

Neuropathologic Findings

Macroscopic Evaluation. When the surgery was performed for sciatic excision, considerable sciatic edema was observed in animals receiving 5, 2.5, or 1.25 mg amitriptyline, as manifested by gross enlargement of the nerve bundle and a change in gross fascicular color to pale gray from a normal pink. These changes were associated with extensive hypervascularization of the epineurial tissue. The appearance of the muscle also seemed altered because it was pale in the animals receiving 5- or 2.5-mg doses of amitriptyline. In animals receiving the 0.625-mg dose of amitriptyline, the muscle seemed to be normal; however, the sciatic nerve seemed slightly edematous compared to the contralateral control side.

Microscopic Evaluation. All nerves exposed to amitriptyline displayed neuropathologic changes. These changes ranged from relatively mild and reversible in the 0.625-mg dose group to severe and irreversible in the 5-mg dose group, based on the degree of Wallerian degeneration and the likelihood for successful regeneration. It was clearly evident that amitriptyline caused a dose-related injury to the axon and supporting tissue that was manifested as the neuropathologic process called *Wallerian degeneration*. The data are consistent with and parallel in magnitude the motor and behavioral findings described above. That is, low doses of amitriptyline injured relatively few axons and support cells, such that neurologic function was not significantly affected after recovery from the local anesthetic effect of the drug. High doses of the drug caused immediate and lasting injury to the peripheral nervous system that was accompanied by neuropathic pain–like behaviors.

Figure 1 is taken from animals receiving the 1.25-mg (intermediate) dose of amitriptyline and depicts aspects of the neurotoxic injury that can be seen to variable degrees in every animal studied. In these animals, by day 1, there was significant endoneurial edema, particularly evident in the subperineurial region adjacent to the application of the drug, but also evident in the perivascular region and between individual nerve fibers that were tightly packed in contralateral nerves used for control or in vehicle-treated nerves previously studied. There was also dark-staining axoplasm, indicating axonal injury. This finding was more prevalent in the subperineurial region. By day 3, these findings were more severe, with increased edema and axonal pathology throughout the fascicles. Numerous Schwann cells were activated, and myelin lamellae had begun to disintegrate; there was fibroblast proliferation and immune cells adherent to the lumen of intraneural vascular endothelia. By day 7, endoneurial edema was reduced, but there was clear evidence of significant ongoing Wallerian degeneration with nerve fibers exhibiting the processes described above intermixed with degenerated nerve fibers undergoing phagocytosis by invaded macrophages.

Figure 2 shows these same processes in the 0.625-, 1.25-, and 2.5-mg groups, but the magnitude of the changes are dose-related. In the 2.5-mg group and 5-mg group (not illustrated), Wallerian degeneration was extreme, with essentially all nerve fibers affected.

Figure 3 depicts in bar graph form the degree of nerve fiber injury, using a semiquantitative scale for the extent of Wallerian degeneration. All treatment groups displayed were statistically separate from the control group at the level of $P < 0.01$. All treatment groups were also statistically different from each other at the level of $P < 0.01$ except for the 1.25- and 2.5-mg groups, which were significantly different at a level of $P < 0.05$.

Discussion

Amitriptyline is neurotoxic to peripheral nerve fibers, causing direct injury to axons that produce Wallerian degeneration of the nerve fibers. This occurs even though the drug is applied outside the peripheral nerve bundle in relatively low doses. An increased neurotoxic

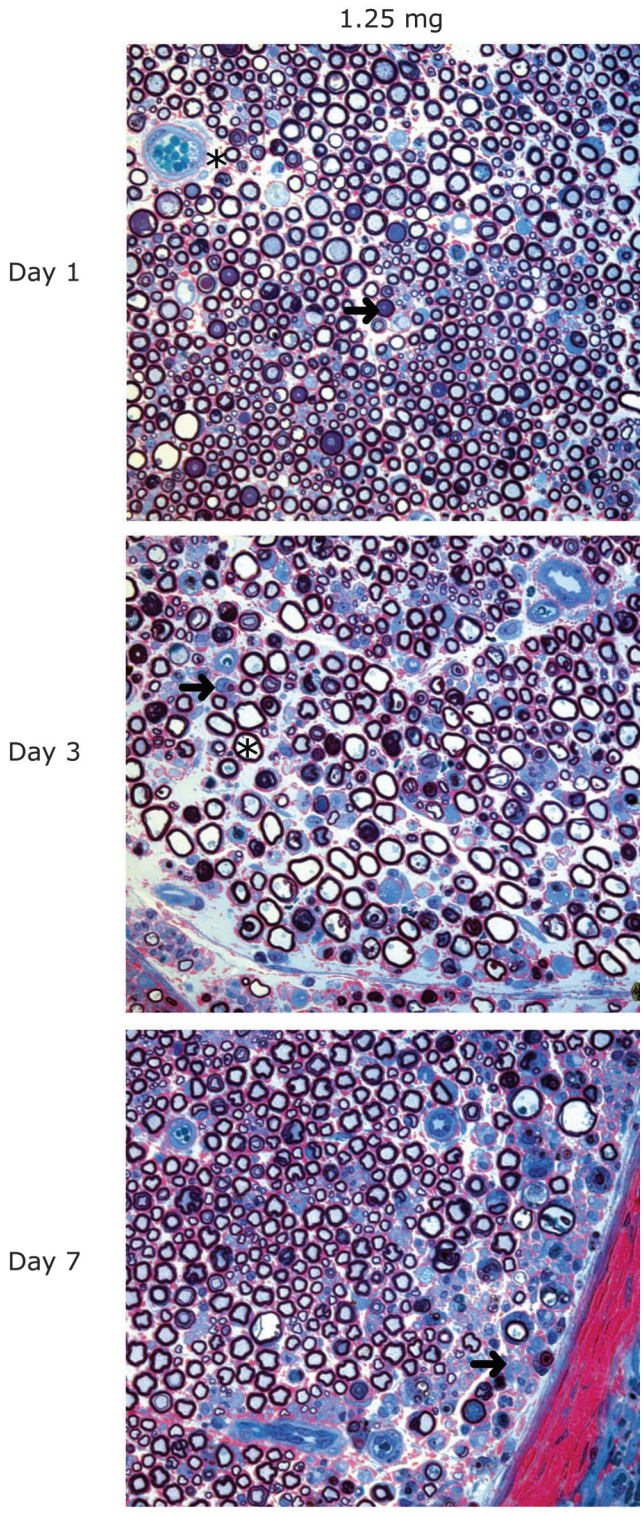

Fig. 1. Light micrographs of rat sciatic nerve after topical application of 1.25 mg amitriptyline in a 0.2-ml volume of saline vehicle immediately adjacent to the nerve. The perineurium was not damaged when the drug was applied, and the vehicle does not cause nerve damage. One day after application of the drug, significant pathologic changes were noted, including extensive edema in the subperineurial and perivascular (*) regions. Axons were darkly stained (*arrow*), indicating the beginning process of Wallerian degeneration. Wallerian degeneration is a progressive pathologic process, so by day 3, there were many swollen axons with pale-staining axoplasm and progressive edema. Schwann cells (*arrow*) were also activated at this time point, and fibroblasts could be seen in the endoneurial space. By day 7, there was evidence of continuing Wallerian degeneration, which peaked at the injury site during this time with disintegration and phagocytosis of myelin sheaths and axon debris. Some axons were devoid of myelin, and these are also phagocytosed by hematogenous macrophages entering the endoneurial space. The stain used in these sections is methylene blue, azure II. Lipophilic structures stain dark blue or black, and collagen stains red. Note the perineurium (a collagen structure) in the lower right of the day 7 micrograph that is thickened by a reaction to the amitriptyline placed in the adjacent epineurial space. Primary magnification: objective ×20, phototube ×16.

effect was observed with increasing doses of amitriptyline applied to the sciatic nerve. This effect of amitriptyline is consistent with the functional and behavioral data we report and with previous studies in which amitriptyline was injected percutaneously on sciatic nerve rats[10] or subcutaneously.[21]

The pathologic process invoked by amitriptyline is *Wallerian degeneration* and was first described by Augustus Waller in 1850 in the context of nerve transection.[25] It is a complex process that begins with injury to the axon and results with degeneration of the axon and its support cells (collectively termed the *nerve fiber*) from the site of axonal injury distally to the terminal tissue. The axon initially becomes electron dense or dark staining, and this is rapidly followed by clumping of organelle and other particles in the axoplasm, swelling of the axon, and disintegration. During this time, axon–Schwann cell communication is altered, and Schwann cells become activated (expressing proinflammatory cytokines) and undergo mitosis. Myelin is disintegrated and phagocytosed by Schwann cells and by invading hematogenous macrophages, which are recruited in large numbers several days after the start of Wallerian degeneration in response to upregulation of tumor necrosis factor α and other proinflammatory chemoattractant proteins. This process occurs in a proximal–distal direction from the site of injury throughout the distal neurologic tissue in a sequential temporal pattern and is often the intended neurolytic process associated with cryolysis or the injection of alcohol. In these cases, it is intended to cause essentially irreversible change in the function of the nervous system rather than the short-term and more reversible changes caused by neurotoxic agents injuring Schwann cells or myelin and sparing the axon.

Although the exact molecular mechanism of amitriptyline neurotoxicity is unclear, it is known that other concentrated local anesthetic agents and severe ischemia can cause Wallerian degeneration, whereas control (vehicle) solutions, such as the saline used in these

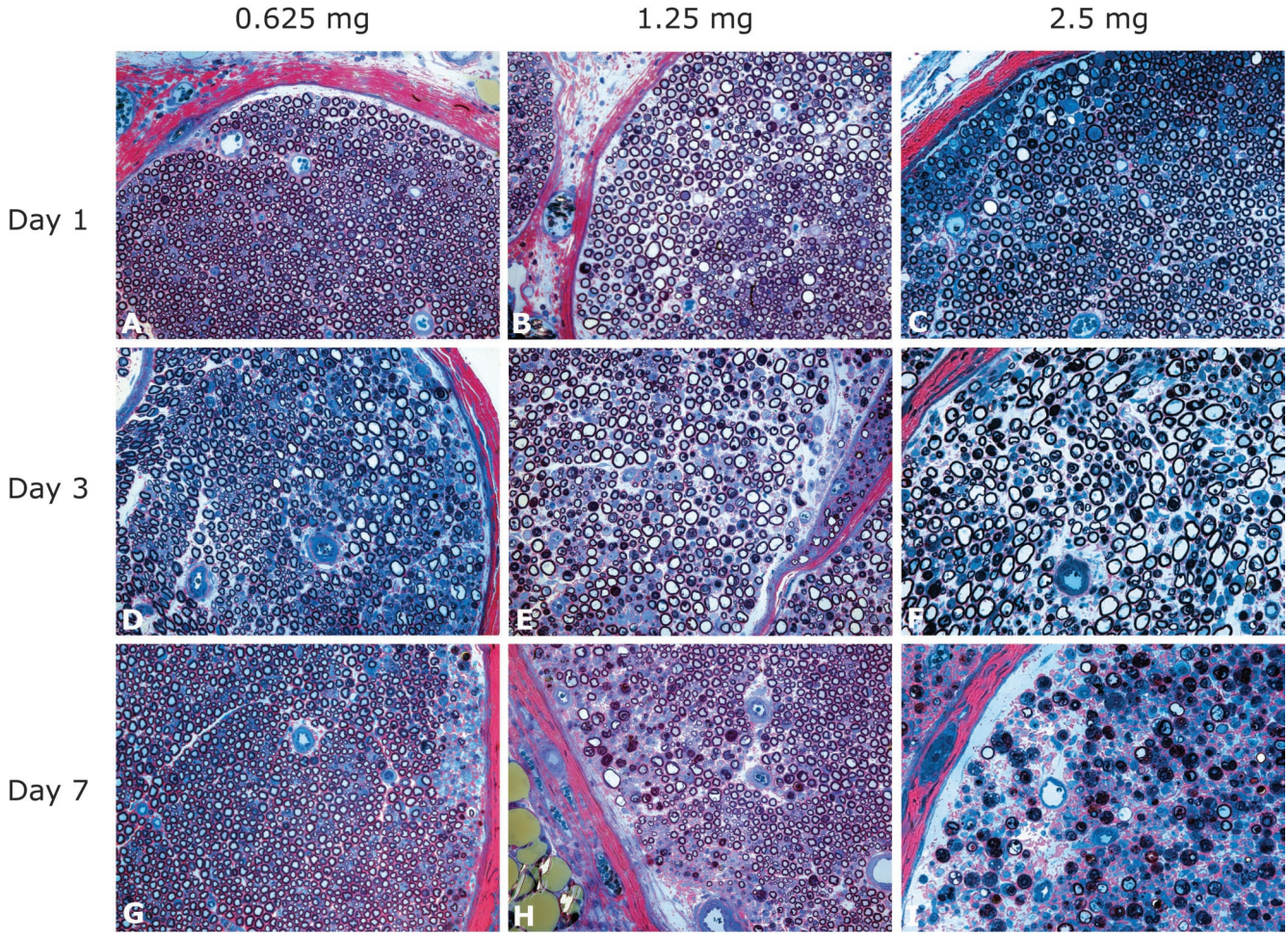

Fig. 2. Light micrographs of rat sciatic nerves exposed to 0.625 (*A*, *D*, *G*), 1.25 (*B*, *E*, *H*), or 2.5 (*C*, *F*, *I*) mg amitriptyline. Using different animals, tissue was studied at 1 (*A*, *B*, *C*), 3 (*D*, *E*, *F*), and 7 (*G*, *H*, *I*) days after topical application of the drug immediately adjacent to the nerve. The pathologic findings are dominated by Wallerian degeneration of nerve fibers, which could be seen even at the lowest dose at the earliest time point. The pathologic effect was dose related and similar in detail to the changes described in figure 1. Methylene blue, azure II stain. Primary magnification: objective ×20, phototube ×12.5.

studies, do not injure nerve.[26,27] It is interesting to note the relation of drug concentration to neurotoxic injury, as indicated by the susceptibility of nerve fibers near the periphery of the nerve bundle in the subperineurial space, which are closest to epineural administered drugs. With even low doses (0.625 mg) of amitriptyline administered in the epineural space (in a 0.2 ml volume), we observed some nerve fibers undergoing Wallerian degeneration in the subperineurial space. With higher doses (delivered in the same volume) the number of degenerating fibers was increased, as was their spatial distribution.

The functional consequences of Wallerian degeneration are severe, as axonal communication with muscles/sense organs is completely interrupted from/to their neurons. Fortunately, peripheral nerve fibers attempt to regenerate in response to neurotrophic factors liberated by denervated and degenerating tissue. There is an important link between nerve degeneration and regeneration in that degeneration must be complete before regeneration can proceed. Both of these processes are driven by proinflammatory cytokines, which we believe are also the principal factors in orchestrating the development of neuropathic pain states.[28] This relation is reinforced by the findings in this study, which link severe Wallerian degeneration with hyperalgesia.

In subcutaneous administration of amitriptyline, a dose of 10 nmol was reported to be inactive in formalin-evoked behaviors.[22] However, at doses of up 100 nmol administered by this route, amitriptyline induced tissue edema (*i.e.*, increase of paw volume),[22] which exhibited a long time course.[23] This edema was not mediated by biogenic amines because it was not blocked by a histamine H1 receptor antagonist (mepyramine).[23] Transcutaneous administration of amitriptyline at a high dose (500 mM) seems to be toxic to the skin.[21] In comparison with the same concentration of bupivacaine administered subcutaneously, amitriptyline at a dose that was

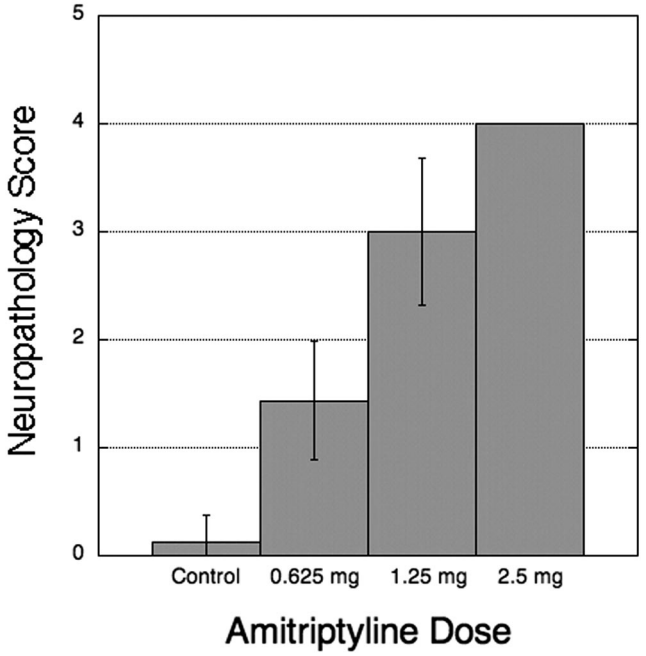

Fig. 3. Comparison of neuropathology scores of nerve sections from experimental groups studied 7 days after application of saline (control) or 0.625, 1.25, or 2.5 mg amitriptyline. A score of 0 indicates normal tissue, and a score of 4 indicates Wallerian degeneration of nerve fibers throughout the tissue section. Each treatment group is statistically different from the control group ($P < 0.01$).

insufficient to produce complete nociceptive blockade was enhanced by the addition of epinephrine or bupivacaine.[5] Intrathecal injection of amitriptyline (5 mg in sheep) was reported to have no significant effect on spinal cord blood flow or hemodynamic variables.[29]

Intravenous administration of 7.5 mg/kg amitriptyline causes severe electrocardiographic changes.[30] Myoclonus occurs at a dose of 30 mg/kg, seizures occur at 50 mg/kg, apnea occurs at 74 mg/kg, and death occurs at 74.5 mg/kg after intravenous amitriptyline administration at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ in anesthetized rats. In our study, no systemic toxicity was observed.

A previous study evaluating the use of amitriptyline for sciatic nerve local anesthesia used a percutaneous approach.[17] This model, although potentially useful for clinical evaluation, is not useful for neurotoxic evaluation because of the variable and uncertain concentration of the drug near the nerve. Our protocol for injection under controlled view outside the perineurium avoids direct needle trauma and guarantees accurate placement of the test dose adjacent to the nerve. However, our motor results with the doses of 10 and 20 mM amitriptyline are in accord with the findings of the pervious percutaneous study (6 and 23 h for 2.5 and 5 mM *N*-phenylethyl amitriptyline).[17] The delay of onset of motor block in both studies suggests that the dose of 10 mM is probably the lowest dose giving a complete motor blockade.

Finally, it has been reported that severe axonal degeneration sometimes occurred with percutaneous administration of 5 mM *N*-phenylethyl amitriptyline, a molecule reported to be even more powerful than amitriptyline hydrochloride.[17] This pathologic finding was correlated clinically with incomplete recovery of the block. Therefore, the demonstration of incomplete recovery or very long differential blocks with experimental local anesthetic agents (5–43 h for complete and full recovery for 2.5 mM, and 22–79 h for 5 mM *N*-phenylethyl amitriptyline[17]; (3–4.5 h for 5 mM, and 4–10 h for 10 mM amitriptyline hydrochloride[10]) should be interpreted cautiously with the suspicion that neurotoxic injury may be part of the mechanism for delayed recovery.

References

1. Onghena P, Van Houdenhouve BJ: Antidepressant-induced analgesia in chronic non-malignant pain: A meta-analysis of 39 placebo-controlled studies. Pain 1992; 49:205–19
2. Mc Quay HJ, Tramer M, Tramer M, Nye BA, Carroll D, Wiffen PJ, Moore RA: A systematic review of antidepressant in neuropathic pain. Pain 1996; 68:217–27
3. Bryson HM, Wilde MI: Amitriptyline: A review of its pharmacological properties and therapeutic use in chronic pain states. Drugs Aging 1996; 8:459–76
4. Sanchez C, Hyttel J: Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptors binding. Cell Mol Neurobiol 1999; 19:467–89
5. Khan M, Gerner P, Kuo WG: Amitriptyline for prolonged cutaneous analgesia in the rat. ANESTHESIOLOGY 2002; 96:109–16
6. Cardenas DD, Warms CA, Turner JA, Marshall H, Brooke MM, Loeser JD: Efficacy of amitriptyline for relief of pain in spinal cord injury: Results of a randomized controlled trial. Pain 2002; 96:365–73
7. Shimoda K, Someya T, Yokono A, Morita S, Hirokane G, Takahashi S, Okawa M: The impact of CYP2C19 and CYP2D6 genotypeson metabolism of amitriptyline in Japanese psychiatric patients. J Clin Psychopharmacol 2002; 22:371–8
8. Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA: A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. Pharmacogenomics J 2002; 2:191–6
9. Harrigan RA, Brady WJ: ECG abnormalities in tricyclic antidepressant ingestion. Am J Emerg Med 1999; 17:387–93
10. Gerner P, Mujtaba M, Sinnott CJ, Wang GK: Amitriptyline *versus* bupivacaine in rat sciatic nerve blockade. ANESTHESIOLOGY 2001; 94:661–7
11. Casis O, Sanchez-Chapula JA: Mechanism of block of cardiac transient outward K+ current (I(to)) by antidepressant drugs. J Cardiovasc Pharmacol 1998; 32:527–34
12. Joshi PG, Singh A, Ravichandra B: High concentrations of tricyclic antidepressants increase intracellular Ca2+ in cultured neural cells. Neurochem Res 1999; 24:381–8
13. Heughan CE, Allen GV, Chase TD, Sawynok J: Peripheral amitriptyline suppresses formalin-induced Fos expression in the rat spinal cord. Anesth Analg 2002; 94:427–31
14. Heughan CE, Sawynok J: The interaction between gabapentin and amitriptyline in the rat formalin test after systemic administration. Anesth Analg 2002; 94:975–80
15. Eisenach JC, Gebhart GF: Intrathecal amitriptyline: Antinociceptive interactions with intravenous morphine and intrathecal clonidine, neostigmine, and carbamylcholine in rats. ANESTHESIOLOGY 1995; 83:1036–45
16. Gerner P, Kao G, Stinivasa V, Narang S, Wang GK: Topical amitriptyline in healthy volunteers. Reg Anesth Pain Med 2003; 4:289–90
17. Gerner P, Mujtaba M, Khan M, Sudoh Y, Vlassakov K, Anthony D, Wang GK: *N*-phenylethyl amitriptyline in rat sciatic nerve blockade. ANESTHESIOLOGY 2002; 96:1435–42
18. Gender P, Haderer AE, Mujtaba M, Sudoh Y, Narang S, Abdi S, Srinivasa V, Pertl C, Wang GK: Assessment of differential blockade by amitriptyline and its *N*-methyl derivative in different species by different routes. ANESTHESIOLOGY 2003; 98:1484–90
19. Strümper D, Durieux ME: Topical antidepressants: The new local anesthetics? (editorial) Reg Anesth Pain Med 2003; 28:268–270
20. Myers RR, Sommer C: Methodology for spinal neurotoxicity studies. Reg Anesth 1993; 18:439–47
21. Haderer A, Gerner P, Kao G, Srinivasa V, Wang GK: Cutaneous analgesia

after transdermal application of amitriptyline versus lidocaine in rats. Anesth Analg 2003; 96:1707–10

22. Sawynok J, Reid A: Peripheral interactions between dextromethorphan, ketamine and amitriptyline on formalin-evoked behaviors and paw edema in rats. Pain 2003; 102:179–86

23. Oatway M, Reid A, Sawynok J: Peripheral antihyperalgesic and analgesic actions of ketamine and amitriptyline in a model of mild thermal injury in the rat. Anesth Analg 203; 97:168–73

24. Esser MJ, Sawynok J: Acute amitriptyline in a rat model of neuropathic pain: Differential symptom and routes effects. Pain 1999; 80:643–53

25. Stoll G, Jander S, Myers RR: Degeneration and regeneration of the peripheral nervous system: From Augustus Waller's observations to neuroinflammation. J Peripher Nerv Syst 2002; 7:13–27

26. Myers RR, Kalichman MW, Reisner LS, Powell HC: Neurotoxicity of local anesthetics: Altered perineurial permeability, edema, and nerve fiber injury. ANESTHESIOLOGY 1986; 64:29–35

27. Kalichman MW, Powell HC, Myers RR: Quantitative histologic analysis of local anesthetic-induced injury to rat sciatic nerve. J Pharm Exp Ther 1989; 250:406–13

28. Myers RR, Shubayev VI, Campana WM: Anatomy and pathology of the peripheral nervous system: Neuropathology of painful neuropathies, Pain due to Peripheral Nerve Diseases. Edited by Sommer C. Basel, Karger, 2001, pp 8–30

29. Cerda SE, Tong C, Deal DD, Eisenach JC: A physiologic assessment of intrathecal amitriptyline in sheep. ANESTHESIOLOGY 1997; 86:1094–103

30. Balcioglu A, Bozkurt A, Kayaalp SO: Comparison of the cardiovascular effects of amineptine with those of amitriptyline and imipramine in anaesthetized rats. Arch Int Pharmacodyn Ther 1991; 309:64–74