Amitriptyline Neurotoxicity

Dose-related Pathology after Topical Application to Rat Sciatic Nerve

Jean-Pierre Estebe, M.D., Ph.D.,* Robert R. Myers, Ph.D.†

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 α2-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 reuptake4 and blocks α2-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 Ca2+ channels.11,12 Experimentally, amitriptyline seems to have a more efficacious analgesic effect after peripheral administration13 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 can 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 amitriptyline.
Bupivacaine.5,17,21 Other local anesthetics (1 determined to be compatible with doses used in previous group contained 12 rats. The experimental doses were (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,5,22-25 intraperitoneal,14,24 and intrathecal13 application or for its use in sciatric nerve block experiments10,17 for comparison with other local anesthetics (1-5% lidocaine and 0.5% bupivacaine).5,17,21

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 ms or 16 nmol), 2.5 mg (12.5 mg/ml, 59.8 ms or 8.0 nmol), 1.25 mg (6.25 mg/ml, 19.9 ms or 4.0 nmol), and 0.625 mg (3.125 mg/ml, 10.0 ms or 2.0 nmol). The data were analyzed by one-way analysis of variance ( Tukey-Kramer multiple comparison tests.

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 ms, respectively) had complete motor blockade at this time. In contrast, complete motor blockade was delayed in animals receiving the 0.625-mg dose of amitriptyline, the muscle seemed altered because it was pale in the animals receiving the 0.625-mg dose of amitriptyline, the muscle also seemed to be normal; however, the sciatic nerve seemed pale gray from a normal pink. These changes were associated with extensive hypervascularization of the epineurial tissue. The appearance of the muscle also changed dramatically. By day 3, clear hyperalgesic and allodynic behaviors (i.e., avoidance 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 sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic sciatic 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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 or subcutaneously. The pathologic process invoked by amitriptyline is Wallerian degeneration and was first described by Augustus Waller in 1850 in the context of nerve transection. 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 cryosurgery 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
studies, do not injure nerve. 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. 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. However, at doses of up 100 nmol administered by this route, amitriptyline induced tissue edema (i.e., increase of paw volume), which exhibited a long time course. This edema was not mediated by biogenic amines because it was not blocked by a histamine H1 receptor antagonist (mepyramine). Transcutaneous administration of amitriptyline at a high dose (500 mg) seems to be toxic to the skin. In comparison with the same concentration of bupivacaine administered subcutaneously, amitriptyline at a dose that was...
Finally, it has been reported that there is a significant increase in the release of norepinephrine and other neurotransmitters with systemic administration of amitriptyline. This finding was correlated 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 $N$-phenethyl amitriptyline), should be interpreted cautiously with the suspicion that neurotoxic injury may be part of the mechanism for delayed recovery.

References

21. Haderer A, Gerner P, Kao G, Sinivas V, Wang GK: Cutaneous analgesia insufficient to produce complete nociceptive blockade was enhanced by the addition of epinephrine or bupivacaine. Intrathecal injection of amitriptyline (5 mg in sheep) was reported to have no significant effect on spinal cord blood flow or hemodynamic variables. Intravenous administration of 7.5 mg/kg amitriptyline causes severe electrocardiographic changes. 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 · kg$^{-1}$ · 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. 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 mg 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). The delay of onset of motor block in both studies suggests that the dose of 10 ms is probably the lowest dose giving a complete motor blockade.