

Lumbar Intrathecal Administration of the Quaternary Lidocaine Derivative, QX-314, Produces Irritation and Death in Mice

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ABSTRACT

Background: We recently found that peripheral administration of the quaternary lidocaine derivative, QX-314, produces long-lasting sensory and motor blockade in animals. The goal of this study was to test whether intrathecal QX-314 has similar properties.

Methods: We conducted a randomized, double-controlled, blinded study with female CD-1 mice. Animals in the treatment group received lumbar intrathecal QX-314 (0.5–10 mM; volume, 2 μ l; each concentration, n = 6). Normal saline and lidocaine (70 mM) served as negative and positive controls (each group, n = 12), respectively. Animals were tested for up to 3 h for lumbosacral neural blockade and observed for adverse effects.

Results: No animal injected with saline and 11 of 12 (92%) animals injected with lidocaine displayed reversible lumbosacral motor blockade ($P < 0.001$). QX-314 (5 mM) produced motor blockade in four of the six (67%) and sensory blockade in five of the six animals (83%; $P < 0.05$ vs. saline). However, six of the six mice (100%) at 5 mM QX-314 and five of the six (83%) at 10 mM exhibited marked irritation; one of the six animals at 5 mM (17%) and two of the six at 10 mM (33%) died. We observed no neural blockade without adverse effects in any animal injected with QX-314. All animals injected with saline and 11 of the 12 (92%) animals injected with lidocaine demonstrated normal behavior.

Conclusion: Lumbar intrathecal QX-314 concentration-dependently produced irritation and death in mice, at lower concentrations than those associated with robust motor blockade. Although QX-314 did produce long-lasting neural blockade, these findings indicate that QX-314 is unlikely to be a suitable candidate for spinal anesthesia in humans.

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What We Already Know about This Topic

- ❖ The lidocaine derivative, QX-314, has been suggested to produce long-lasting local anesthesia and selective blockade of pain after peripheral administration

What This Article Tells Us That Is New

- ❖ In mice, intrathecal injection of QX-314 failed to produce evidence of neural blockade without agitation, motor dysfunction, and, in several cases, death
- ❖ Safety of this agent and its mechanisms for toxicity require further study

QX-314 (*N*-[2,6-dimethylphenyl carbamoylmethyl]-triethylammonium) is a quaternary derivative of the local anesthetic, lidocaine, that features an additional ethyl group attached to the amine function. This modification confers a permanent positive charge and decreased amphotericity to the mother compound; as a result, the traditional view has been that QX-314 is membrane-impermeable and blocks Na^+ -dependent action potentials only when administered intracellularly.^{1–3} Hence, the agent has previously been con-

sidered to be devoid of clinically useful local anesthetic activity. However, we recently found that QX-314, administered peripherally, concentration dependently and reversibly produces robust long-lasting local anesthesia with a slow onset in animal models *in vivo*.⁴ The goal of the current laboratory animal study was to test the hypothesis that QX-314 would have similar properties when administered intrathecally as a spinal anesthetic.

Materials and Methods

The experimental protocol was approved by The University of British Columbia Committee on Animal Care (Vancouver, British Columbia, Canada). All efforts were made to minimize the suffering and number of animals used. All animals used in this study were female, adult CD-1 mice (weight, 20–35 g). The 12 mice were housed in a cage with a 12-h light:dark cycle and free access to food and water. We used a randomized, blinded, and double-controlled experimental design. Animal allocation to treatment groups was randomized by computer with the use of Research Randomizer.[#] The experimenters were blinded to the drug administered in each individual experiment. We conducted the experiments with the use of both negative (placebo: normal saline) and positive (70 mM lidocaine: approximately corresponding to the 2% [*i.e.*, 20 mg/ml] solution familiar to clinicians) controls. All animals were naïve to drug applications, and animals were used only once for an experiment. Each experiment was captured with a video camcorder (Canon, Inc., Tokyo, Japan), and each animal was scored (see Behavioral Observations below) by four senior researchers blinded to group allocation.

Drugs and Chemicals

QX-314 and lidocaine HCl were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). Lidocaine and QX-314 were dissolved in normal saline (NaCl solution of 0.9% weight per volume). Both lidocaine and QX-314 solutions were freshly prepared on each experimental day immediately before the start of the experiments.

Drug Applications

Lumbar intrathecal injections were performed using a modified version of the method described by Hylden and Wilcox.⁵ In brief, under sevoflurane anesthesia, the animals' pelvic girdle (whose superior aspect corresponds to the sixth lumbar vertebral body [L6]⁶) was identified by palpation through the skin. The animals were then firmly held by the pelvic girdle with one hand. The other hand was used to palpate for the spinous processes of the vertebral column. A 30-gauge, 1-inch disposable needle connected to a micro-volume precision syringe (MICROLITER™; Hamilton, Reno, NV) was inserted at an angle of approximately 15° relative to the horizontal plane and gently advanced along the

groove between the spinous and transverse processes until slipping into the intervertebral space between L5 and L6. The L5–L6 position was selected for injection to minimize the risk of spinal cord injury because of its proximity to the terminal end of the spinal cord in mice older than 120 days.⁶ All animals were injected intrathecally with 2 μ l of study solution. While the strength of this technique is that it is well established, reproducible, and highly accurate (*i.e.*, consistently resulting in intradural injections),⁵ we used both positive and negative results for internal validation (*cf.* above [Materials and Methods section, first paragraph]; below [Drug Applications section, last paragraph]; and Results section).

Before the dedicated blinded, randomized, and double-controlled study, we conducted preliminary open-label pilot experiments with QX-314 for lumbar intrathecal dose finding and identification of maximum tolerated doses. Results obtained in this phase showed that animals experienced unacceptable distress or death at a QX-314 concentration of 30 mM: two of the two animals injected with 30 mM QX-314 showed evidences of severe irritation and sustained distress (including flinching, scratching behavior, and vocalization; see Behavioral Observations section below); one died. Hence, in compliance with institutional animal care guidelines and to minimize animal suffering, we limited the range of QX-314 concentrations to be studied to a maximum of 10 mM.

In the blinded, randomized, double-controlled experiments, animals allocated to a control group were injected with normal saline (negative control) or lidocaine (70 mM; positive control; each group, $n = 12$ to maximize robustness of control results). Animals allocated to a treatment group were injected with QX-314 (0.5, 3, 5, or 10 mM; each group, $n = 6$) into the lumbar intrathecal space (L5/L6; volume, 2 μ l).

Behavioral Observations

After emergence from general anesthesia, animals were monitored for up to 3 h and observed for reversible hindlimb paresis and tail flaccidity⁷ as endpoints of lumbosacral subarachnoidal motor blockade at or below the L5/L6 level. To test for the presence or absence of tail flaccidity, we placed animals on the edge of a mesh. A positive response was noted if the tail had no tension and drooped downward over the edge of the mesh surface. As a supplemental assay for motor blockade, we also tested all animals for their ability to hang onto an inverted mesh with their hind limb.⁸ For this assay, we placed the animals in the center of a 20 \times 25 cm inverted mesh. A positive response was defined as an inability of an animal to hang onto the mesh with its hind paws. Animals were tested for lumbosacral neuraxial sensory blockade by their responses in a modified tail clamp assay.^{9–12} For this assay, we placed a vascular clamp in the middle of the animals' tails for a maximum of 5 s and noted as negative responses any resultant flicking of the tail, biting of the tail clamp, or vocalization.

In addition to local anesthetic efficacy, animals were observed for behavioral evidence of any apparent adverse effects, including irritation (scratching behavior, sustained

[#] Available at: www.randomizer.org. Accessed March 19, 2010.

licking, or writhing), agitation (as defined by vocalization on touch or spontaneously, repeated circling, or spontaneous jumping), apparent convulsions, loss of righting reflex, and death.

Observations for all local anesthetic and toxic effects were made at 3, 5, 10, 15, 30, 60, 90, 120, 150, and 180 min postinjection. Animals with sustained signs of toxicity more than 3 h were killed with an overdose of sevoflurane.

Data Analysis

Statistical analysis of categorical data, such as the presence or absence of sensory or motor blockade or evidence of adverse effects, was carried out with the use of Fisher exact test and the chi-square test. We compared time-to-event data with the log-rank test and calculated survival fractions with the use of the product limit (Kaplan–Meier) method. Continuous data were analyzed with the Kruskal–Wallis test, with Dunn’s Multiple Comparison Test for *post hoc* comparisons between individual groups. Differences were considered significant at P less than 0.05. Data are expressed as n = sample size and fraction of animals (%) unless mentioned otherwise. The data were analyzed using Prism version 4 (GraphPad, San Diego, CA) and Microsoft Excel version 2003 software (Microsoft Corporation, Redmond, WA).

Results

None of the 12 animals injected intrathecally with saline and n = 11 of the 12 animals injected with lidocaine reversibly exhibited lumbosacral motor blockade (Fisher exact test, P < 0.0001). The onset to effect was within 3 min in all animals with motor blockade due to lidocaine, with a median duration of tail flaccidity of 10 min and a maximum duration of 15 min (all groups: Kruskal–Wallis test, P < 0.001; Dunn’s posttest, P < 0.01 compared with saline control). In the vascular tail clamp assay, n = 11 of the 12 animals injected with lidocaine reversibly tested positive for sensory blockade, compared with two of the 12 animals injected with normal saline (Fisher exact test, P = 0.0006). The onset of sensory blockade due to lidocaine was within 3 min in all 11 of the 12 animals and the mean duration was 10 min, with a maximum of 30 min.

QX-314 (0.5–10 mM) produced local anesthetic effects in a concentration-dependent fashion (fig. 1). In terms of efficacy, we observed a maximum effect at 5 mM at which QX-314 produced sensory blockade in five of the six animals (83%; P < 0.05 compared with saline control) and motor blockade in four of the six animals (67%; P < 0.05 compared with saline control). The results were similar at 10 mM, at which QX-314 produced sensory blockade in four of the six animals (67%) and motor blockade in three of the six animals (50%; P < 0.05 compared with saline control). The median onset of sensory blockade due to 10 mM QX-314 was 5 min in the four of the six animals (all groups: log-rank test, P < 0.05). Three of the four animals (75%) still tested positive for sensory blockade at the end of the observation time

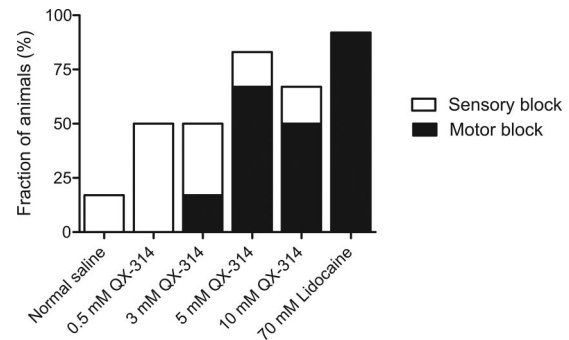


Fig. 1. Concentration-dependent lumbar intrathecal local anesthetic effects of the quaternary lidocaine derivative, QX-314, in mice. Animals were randomly assigned to a treatment group (0.5–10 mM QX-314; for each concentration, n = 6), injected by a blinded experimenter (volume, 2 μ l), and assessed by blinded observers for local anesthetic activity. Normal saline (n = 12) and 70 mM lidocaine (\sim 2%; n = 12) served as negative and positive controls, respectively. Lumbosacral subarachnoid motor blockade was defined by the presence of reversible hindlimb paresis, tail flaccidity, or inability to hang onto an inverted mesh; sensory blockade was defined by a positive vascular tail clamp assay. QX-314 concentration-dependently produced sensory and motor blockade in animals (all groups: sensory block, chi-square test, P < 0.01; motor block, P < 0.0001; details, see text). Sensory blockade occurred at lower QX-314 concentrations than motor blockade; all animals with motor blockade had evidence of sensory blockade.

(*cf.* next paragraph); the minimum duration was 30 min. Similarly, the median onset of motor blockade was 5 min in the three of the six animals (all groups: log-rank test, P < 0.001), and two of the three animals (67%) still tested positive at the end of the observation time. Figure 2 shows the concentration-dependent onset and offset of motor blockade due to QX-314 in the form of Kaplan–Meier survival curves.

However, six of the six mice (100%) at 5 mM QX-314 and five of the six (83%) at 10 mM exhibited behavioral derangements indicative of adverse drug reaction and distress, including severe agitation (sustained circling, jumping, or vocalization), irritation (scratching, flinching, shaking, or writhing), or loss of righting reflex. One of the six animals (17%) at 5 mM QX-314 and two of the six at 10 mM (33%) died. Although the irritable behavior manifested acutely, the deaths occurred at 55 min (5 mM QX-314), 105 min, and 160 min (both, 10 mM QX-314), respectively (*i.e.*, before the end of the observation period of 180 min; *cf.* previous paragraph). We observed no local anesthetic effects without evidence of adverse effects in any animal injected with QX-314 in the blinded, randomized, controlled experiments (0.5–10 mM). None of the animals injected with saline showed signs of any adverse reaction (Fisher exact test, P = 0.0007 compared with 10 mM QX-314; P < 0.0001 compared with 5 mM; and P = 0.02 compared with 3 and 0.5 mM). One single animal injected with lidocaine displayed irritable behavior; all others (11/12 or 92%) exhibited no evidence of behavioral abnormality (Fisher exact test, P = 0.004 compared

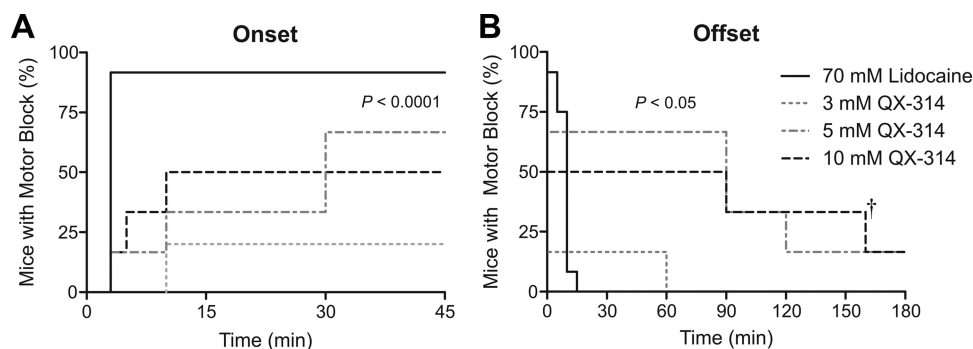


Fig. 2. Time-to-event “survival” curves for onset and offset of lumbar intrathecal motor blockade in mice. After injection, animals were tested at 3, 5, 10, 15, 30, 60, 90, 120, 150, and 180 min. Onset was defined as the first time point at which an animal tested positive for motor blockade, and offset was defined as the first time point at which an animal ceased to exhibit evidence of motor blockade (for detailed experimental procedures, see Materials and Methods). Survival fractions were calculated using the product limit (Kaplan–Meier) method, and survival curves compared with the log-rank test. **A** shows Kaplan–Meier survival curves depicting the concentration-dependent time to onset of motor blockade due to QX-314 (the quaternary lidocaine derivative) (each concentration, $n = 6$), with lidocaine (70 mM [$\sim 2\%$]; $n = 12$) as a positive control. Normal saline as a negative (placebo) control ($n = 12$), and 0.5 mM QX-314 ($n = 6$) did not produce motor blockade in any animal tested (no curves illustrated). **B** shows the concentration-dependent time to offset of motor block due to QX-314 and lidocaine. In the 10 mM QX-314 group, one animal with motor blockade died at 160 min (\dagger the two other animals who died tested negative for motor blockade before death occurred; cf. Results, third paragraph). QX-314 (3–10 mM) concentration-dependently produced lumbar intrathecal motor blockade in animals, with a long duration compared with 70 mM lidocaine.

with 10 mM QX-314; $P = 0.0004$ compared with 5 mM; and $P = 0.08$ compared with 3 and 0.5 mM). Figure 3 illustrates the concentration-dependent adverse effects of QX-314 relative to the positive and negative controls.

Discussion

The two main findings in this randomized, double-controlled, and blinded *in vivo* animal study are that (1) the quaternary lidocaine derivative, QX-314, produced long-

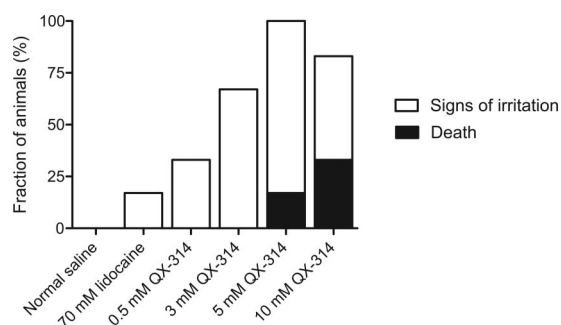


Fig. 3. Adverse effects of lumbar intrathecal QX-314 in mice. Animals were randomly assigned to a treatment group (0.5–10 mM QX-314; for each concentration, $n = 6$), injected by a blinded experimenter (volume, 2 μ l), and assessed by blinded observers for signs of irritation (including scratching behavior, agitation, and persistent loss of righting reflex) and death (details, see Materials and Methods, Behavioral Observations). Normal saline ($n = 12$) and 70 mM lidocaine ($\sim 2\%$; $n = 12$) served as negative and positive controls, respectively. Animals injected with QX-314 concentration-dependently exhibited signs of marked irritation ($P < 0.0001$) and death (details, see Results, third paragraph). In no animal did QX-314 produce subarachnoid neural blockade without evidence of adverse effects.

lasting sensory and motor blockade concentration-dependently when administered intrathecally to mice and (2) lumbar intrathecal QX-314 administration was associated with unacceptable adverse effects, including death. The adverse effects of QX-314 occurred at lower concentrations than those associated with robust motor blockade, indicating a low therapeutic index of intrathecal QX-314 that would preclude its use as a spinal anesthetic. In these investigations, we observed no local anesthetic effects without evidence of adverse effects in any animal injected with intrathecal QX-314.

With regard to local anesthetic efficacy, our results confirm our previous observations in studies on peripheral administration that QX-314 by itself acts as a local anesthetic with a long duration, and that QX-314 produces both sensory and motor blockade.⁴ These findings are somewhat in contrast to a subsequent report of others suggesting that QX-314 produces a nociceptive-selective block and that activation of the transient receptor potential vanilloid receptor-1 (TRPV1) is required to facilitate cell entry and access to the local anesthetic binding site within Na^+ channels.¹³ Although it is possible that such observations of an absence of local anesthetic efficacy relate to either the study of QX-314 in the subeffective range at the foot of the concentration-response curve or observation periods of insufficient duration to pick up the delayed onset of QX-314, our own recent findings have corroborated that TRPV1 agonism can accelerate the onset of QX-314-mediated local anesthesia without affecting its duration.¹⁴ However, in our present and previous experiments, we have shown that coadministration of a TRPV1 agonist such as capsaicin is not required for the local anesthetic efficacy of QX-314. Our findings that QX-314 produces motor blockade after peripheral perineural and intrathecal administration provide further strong evidence for the above notion as

myelinated peripheral axons, including A α fibers do not express TRPV1 receptors to any significant degree.^{15,16}

On the other hand, the observed local anesthetic effects after lumbar intrathecal QX-314 administration in this study were not quite as robust (*i.e.*, close to 100% positive responses in the sensory and motor block assays) as those after peripheral perineural administration.⁴ This is perhaps not surprising because we limited the highest concentrations of QX-314 studied in the present randomized controlled experiments to 10 mM, as the observations of severe distress and death at 30 mM QX-314 in the dose-finding pilot experiments precluded us from systematically studying concentrations equimolar to 70 mM [\sim 2%] lidocaine. Although 10 mM QX-314 was likely insufficient to produce a near maximal local anesthetic effect, the current results in the range of 0.5–10 mM QX-314 are remarkably consistent with our previous observations with peripheral administration. It seems unlikely that experiments with higher intrathecal concentrations would significantly add useful information as our findings indicate that the therapeutic index of spinal QX-314 is unacceptably small.

One single animal in the lidocaine group showed irritable behavior after lumbar intrathecal injection. Although intrathecal lidocaine itself is well-known to be associated with adverse effects, including transient radiculopathy,^{17,18} we cannot entirely exclude the possibility that the observed behavior may have been due to mechanical trauma from the needle. However, none of the animals injected with normal saline exhibited irritable or otherwise abnormal behavior.

Two of the 12 animals injected with the negative control, saline, did not respond to the vascular tail clamp. Although it is conceivable that animals may not respond due to residual analgesic effects of the sevoflurane administered during the injection, these mice did not respond for 30 and 90 min, respectively, rendering this possibility unlikely. Again, we cannot completely exclude that the lack of response may have been due to slight neural injury from the injection; however, because the animals recovered fully and showed normal behavior with no apparent sensory or motor deficit at the end of the observation time, it remains a possibility that the tail clamp assay is less specific than the assays for motor block (our primary outcome variable) in the study of spinal anesthesia in mice.

The striking finding in these studies was that intrathecal QX-314 produced remarkable adverse events, ranging from irritable behavior at low concentrations to disturbingly severe distress and death. On reflection, it even seems possible that our observations that QX-314 in some animals produced sensory and motor blockade that was still present at the end of the observation period of 180 min partly reflected intrathecal neurotoxicity as opposed to long-lasting subarachnoidal blockade.¹⁹ We were surprised by these results as we expected that QX-314, because of its inherently slow transmembrane flux kinetics, could represent a local anesthetic agent with low toxicity in general and the potential to be less toxic than lidocaine in spinal anesthesia specifically.

Although little is known in the peer-reviewed literature about the *in vivo* toxicity profile of spinal QX-314, numerous reports have shown that its tertiary mother compound, lidocaine, can produce adverse spinal effects even at therapeutic doses. These effects are concentration-dependent and in humans range from reversible pain associated with transient neurologic symptoms to irreversible conduction block in cauda equina syndrome.^{20–23} Although the etiology is not entirely clear, the available evidence indicates that lidocaine produces neurotoxicity that arises not from a single action, but from multiple underlying mechanisms. For example, mitochondrial dysfunction, induction of neuronal apoptosis, neuronal membrane disruption, and cell necrosis all have been implicated.^{24,25} *In vitro* studies involving cultured neurons have shown that local anesthetics produce growth changes that may lead to irreversible neural injury; similar to the previous studies, lidocaine was associated with a particularly high risk of toxicity relative to other local anesthetic agents.²⁶ In *in vitro* electrophysiologic studies, lidocaine (\geq 30 mM) irreversibly depolarizes rat dorsal root ganglion neurons and induces cell death.²⁷ In crayfish giant axons, 40 and 80 mM lidocaine irreversibly block action potentials.²⁸ In frog sciatic nerve, lidocaine at \geq 40 mM produces irreversible conduction blockade concentration-dependently.²⁹ Similar results were obtained by others with 5% lidocaine.²¹ However, because lidocaine neurotoxicity primarily manifests as irreversible blockade and not as acute irritable or nocifensive behavior, it seems likely from our present *in vivo* results that the mechanisms of acute irritation due to QX-314 are distinct from those of lidocaine neurotoxicity. In our experiments, we saw no evidence of irreversible motor conduction blockade due to 70 mM (\sim 2%) lumbar intrathecal lidocaine. However, this would not necessarily be unexpected as the effective perineural concentration³⁰ after intrathecal injection and mixing with the cerebrospinal fluid would be predicted to be lower, that is, in the subtoxic range. It is of note that a recent study showed that lidocaine itself can act as an agonist at TRPV1 receptors.³¹ Although the authors did not find QX-314 to produce the same effects in this particular study, these findings provide a possible mechanism for excitation of nociceptive neurons by local anesthetic molecules in general. In the literature, a wide range of targets and molecules have been found to mediate irritation after intrathecal application in rodents. Examples include substance P³²; the tachykinins, neurokinin A, eledoisin, and physalaemin³³; excitatory amino acid receptor agonists³⁴; various nicotinic agonists³⁵; the GABA_A antagonist, bicuculline³⁶; and in our own recent experiments, β -alanine and glycine (albeit not nearly as severely as QX-314).³⁷

Although the precise spinal mechanisms of QX-314 are unknown, it is possible that the quaternary ammonium group of QX-314 is involved in the long-lasting local anesthetic effects and adverse events. For example, although derivatives of the quaternary K⁺ channel blocker, tetraethylammonium, structurally similar to QX-314 without the aromatic group, can produce long-lasting local anesthesia *in*

vitro by blocking both Na⁺ and K⁺ channels,³⁸ these compounds produce neurotoxicity *in vivo*: tetraethylammonium-C12 (triethyldodecylammonium bromide) causes degeneration of myelinated nerve fibers when administered into mouse sciatic nerve as well as disturbance of the blood-nerve barrier.^{39,40} It has been shown that QX-314 can interact with the binding sites of tetraethylammonium derivatives,³⁸ which raises the possibility that QX-314 and tetraethylammonium derivatives produce local anesthesia and neurotoxicity through a shared mechanism. Of note, a variety of other quaternary ammonium compounds have also been shown to have neurotoxic effects. *N*-Phenylethylamitriptyline, *N*-propylamitriptyline, and *N*-propyldoxepin produce axonal degeneration and demyelination when administered to rat sciatic nerve.^{41–43} The long-acting agent, tonaicaine (*N*-phenylethyl lidocaine), causes hematuria, severe axonal degeneration of peripheral nerve roots, and degenerative changes in the spinal cord when administered intrathecally in rats.^{44–46} Although intrathecal tonaicaine produces reversible hyperreflexia to pinching in the area of blockade regression in rats,⁴⁵ no study to our knowledge has demonstrated acute spontaneous irritable behavior or death due to intrathecal application of a quaternary lidocaine derivative.

As far as death is concerned, the precise cause at the relatively low concentrations (5–30 mM) remains unknown in this study. The timing raises the possibility that death occurred due to delayed rostral spread of QX-314; however, no death or apparent cardiorespiratory compromise occurred with 70 mM lidocaine (~2% solution). Notwithstanding, our findings together with those in the literature collectively raise the troubling question if the quest for an ultralong-acting local anesthetic, which with little doubt would be extremely useful for postoperative analgesia and chronic pain, is indeed doomed by concomitant toxicity.⁴⁰ On the other hand, our observations that spinal QX-314 acts as an irritant and produces unacceptable adverse reactions in mice do not necessarily preclude its safe use in the periphery. In our previous studies in mice and guinea pigs, intradermal and peripheral perineural QX-314 infiltration produced reversible, long-lasting local anesthesia with no apparent signs of significant adverse effects.⁴ Further studies clearly are necessary to elucidate the safety and efficacy of QX-314 as a local anesthetic.

In conclusion, the quaternary lidocaine derivative, QX-314, administered in the lumbar intrathecal space, concentration-dependently produced irritation and death in mice, at lower concentrations than those associated with robust local anesthetic effects (*i.e.*, motor blockade). Although lumbar intrathecal QX-314 did produce long-lasting sensory and motor blockade, these findings indicate that QX-314 is unlikely to be a suitable candidate for spinal anesthesia in humans.

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