

The Quaternary Lidocaine Derivative, QX-314, Produces Long-lasting Local Anesthesia in Animal Models In Vivo

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Background: **QX-314 is a quaternary lidocaine derivative considered to be devoid of clinically useful local anesthetic activity. However, several reports document that extracellular QX-314 application affects action potentials. Hence, the authors tested the hypothesis that QX-314 could produce local anesthesia in animal models *in vivo*.**

Methods: **The authors tested QX-314 (10, 30, and 70 mM) in three standard *in vivo* local anesthetic animal models, using a randomized, blinded experimental design with negative (placebo) and positive (70 mM lidocaine) controls. The guinea pig intradermal wheal assay (n = 29) was used to test for peripheral inhibition of the cutaneous trunci muscle reflex, the mouse tail-flick test (n = 30) was used to test for sensory blockade, and the mouse sciatic nerve blockade model (n = 45) was used to test for motor blockade.**

Results: **In all three animal models, QX-314 concentration-dependently and reversibly produced local anesthesia of long duration, at concentrations equivalent to those clinically relevant for lidocaine. In the guinea pig intradermal wheal assay, QX-314 produced peripheral nociceptive blockade up to 6 times longer than lidocaine (650 ± 171 *vs.* 100 ± 24 min [mean ± SD]; n = 6 per group; $P < 0.0001$). In the mouse tail-flick test, QX-314 produced sensory blockade up to 10 times longer than lidocaine (540 ± 134 *vs.* 50 ± 11 min; n = 6 per group; $P < 0.0001$). Finally, in the mouse sciatic nerve model, QX-314 produced motor blockade up to 12 times longer compared with lidocaine (282 ± 113 *vs.* 23 ± 10 min; n = 9 or 10 per group; $P < 0.0001$). The onset of QX-314–mediated blockade was consistently slower compared with lidocaine. Animals injected with saline exhibited no local anesthetic effects in any of the three models.**

Conclusion: **In a randomized, controlled laboratory study, the quaternary lidocaine derivative, QX-314, concentration-dependently and reversibly produced long-lasting local anesthesia with a slow onset in animal models *in vivo*. The authors' results raise the possibility that quaternary ammonium compounds may produce clinically useful local anesthesia of long duration in humans and challenge the conventional notion that these agents are ineffective when applied extracellularly.**

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LOCAL anesthetic research during the past four decades has demonstrated that amino-ester and amino-amide local anesthetics block the generation and propagation of action potentials *via* an intracellular site of action at the voltage-gated Na^+ channel.[1–4] Many of the underlying experiments were performed using QX-314 (lidocaine *N*-ethyl chloride; *N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium chloride; molecular weight, 298.9), a lidocaine derivative whose sole structural difference to the mother compound is in the presence of an additional *N*-ethyl group. This permanently renders the amino group quaternary, *i.e.*, positively charged. As a result, the agent cannot readily pass biologic membranes. Indeed, a series of *in vitro* experiments where QX-314 was applied extracellularly found this agent to be ineffective in blocking action potentials.[5–8] In contrast, intracellular application of QX-314 to peripheral and central neurons produces marked local anesthetic actions, blocking both fast, Na^+-dependent action potentials and voltage-dependent, noninactivating Na^+ conductances.[5–10] However, various studies on a range of other quaternary cationic compounds have shown that these can block electrical conductances in axons when applied outside of the cell. Such studies involved QX-572,[11,12] quaternary tropine esters,[7,13] tonicaine,[14–16] quaternary derivatives of amitriptyline[17–19] and doxepin,[17,20] and methyl and benzyl quaternary derivatives of novocaine, cocaine, dibucaine, and tetracaine.[21] In addition, there are preliminary *in vitro* [5,8,22,23] and *in vivo* [16,23] data to suggest that extracellular QX-314 can, in fact, affect action potentials, although these findings were not further pursued with regard to their potential clinical local anesthetic utility.

On the basis of these observations, we considered the possibility that QX-314 could produce clinically useful local anesthesia in animal models *in vivo*. To test this hypothesis, we compared the actions of QX-314 with those of lidocaine in three established *in vivo* local anesthetic animal models: the guinea pig intradermal wheal assay to test for peripheral nociceptive (infiltration) blockade,[24] the mouse tail-flick test for sensory blockade,[25] and the mouse sciatic nerve blockade model for motor blockade.[26] Concentrations chosen were 10, 30, and 70 mM, approximating equimolar clinically relevant lidocaine concentrations of 0.25, 1, and 2%, respectively.

Materials and Methods

The experimental protocol comprising the three animal models was approved by The University of British

Columbia Committee on Animal Care (Vancouver, British Columbia, Canada). We used a randomized, blinded, and controlled experimental design. The experimenter was blinded to all treatment allocations and drug doses. Animal allocation to treatment groups was randomized by computer with the use of Research Randomizer, version 3.0.‖ Each experiment was conducted with both negative (placebo: normal saline) and positive (70 mM lidocaine) controls.

Guinea Pig Intradermal Assay

A slightly modified version of the method by Bulbrig and Wadja[24] was used. In brief, the backs of guinea pigs (weight, 800–1,200 g; n = 8) were shaved 24 h before experimentation. Each animal received four treatments intradermally on the lower flank using 27-gauge, ½-inch hypodermic needles. Volumes of 0.5 ml were injected, producing wheals with a diameter of 2 cm. Six pinpricks were applied along the perimeter of a 1-cm-diameter circle drawn with the origin at the center of the wheal. This was done to standardize the reversal of local anesthesia, because preliminary observations indicated that the duration of blockade was inversely related to the distance from the center of the wheal. Pinpricks were standardized using a weighted 20-g, 30-gauge needle. The number of pinpricks out of six producing a cutaneous trunci reflex was recorded at 1, 5, 15, 35, 50, 80, 110, 140, and 180 min after injection and every hour afterward. The number of responses out of six was converted to a nociceptive blockade score out of 100%. At 110 min after injection, wheals to which the animal responded six out of six times were no longer monitored.

Mouse Tail-flick Test

A modification of the method of Grant *et al.*[25] was used. In brief, mice (total, n = 30) were placed in restraining tubes, and the 2-cm tips of their tails were submerged into a 50°C water bath. The tail-flick latency was measured, and animals with latencies less than 3 s were selected.

Two 20-μl injections were performed bilaterally 7 cm proximal to the tip of the tail with 29-gauge hypodermic needles. Needles were inserted with the bevel facing medially until their tips contacted the caudal vertebrae, and were then withdrawn by 1 mm before injection. After injection, the tail-flick latency was tested for at 1, 10, 20, 30, 45, 60, 90, and 120 min after injection and every hour afterward until the time to offset was determined. Mice were monitored for a minimum of 60 min. At each sampling time, the maximum exposure to the hot water bath was 4 s, and a tail-flick latency greater than 4 s was defined as sensory blockade. Because of the tendency for the mice to struggle when placed in restraining tubes, the onset of sensory blockade was defined as two consecutive readings with a tail-flick latency greater than 4 s, and, likewise, the offset of sensory blockade was defined as two consecutive readings with a tail-flick latency less than 4 s.

Mouse Sciatic Nerve Blockade Model

The method of Leszczynska and Kau[26] was used. In brief, all mice (total, n = 45) were placed in the middle of a 20 × 25-cm inverted mesh and were taught to climb to the outside and over the edge of the mesh. All mice were able to climb on the inverted mesh with all four limbs before treatments. Mice were placed into restraining tubes for injection, and injections were performed in the area of the popliteal fossa of the left hind limb using 30-gauge, 1-inch hypodermic needles. The bevel tips of the needles faced proximally. After injection, mice were removed from the restraining tubes and positioned onto the inverted mesh. The primary endpoint was the time to loss of ability to hang on to the inverted mesh with the injected hind limb, which was tested for at 1, 5, 10, 20, 30, 45, and 60 min after injection and every hour afterward. The animals were tested on the inverted mesh for a minimum of 60 min. After this time, only mice that were unable to hang on to the inverted mesh with the injected hind limb were monitored.

Drugs and Chemicals

NaCl, *N*-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium chloride (QX-314), and lidocaine HCl were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). Normal saline (NaCl) solution was 0.9% wt/vol. Lidocaine and QX-314 were dissolved in saline, with the NaCl concentration decreased by equivalent moles of lidocaine or QX-314 in solution to maintain equiosmolality. The pH of all solutions was titrated to pH 6.9 ± 0.1 with NaOH and HCl before freezing at −15°C in 1-ml aliquots. Before the start of each experiment, solutions were thawed and placed within syringes up to 24 h before use. While in syringes, solutions were kept refrigerated at 10°C. Before commencement of the individual experiments, drugs were equilibrated at room temperature for 30 min or more.

Data Analysis

Statistical analyses of continuous data were conducted with the use of one-way and repeated-measures analysis of variance for multisample comparisons as appropriate, with the Tukey multiple comparison test for *post hoc* testing. We used the Kolmogorov-Smirnov test to test if values came from a gaussian distribution. The Pearson correlation was used to test for a linear relation between two gaussian variables. The Student *t* test was used for comparisons of two groups of normally distributed data and testing for differences from a theoretical mean. Non-

‖ Available at: www.randomizer.org. Accessed April 13, 2007.

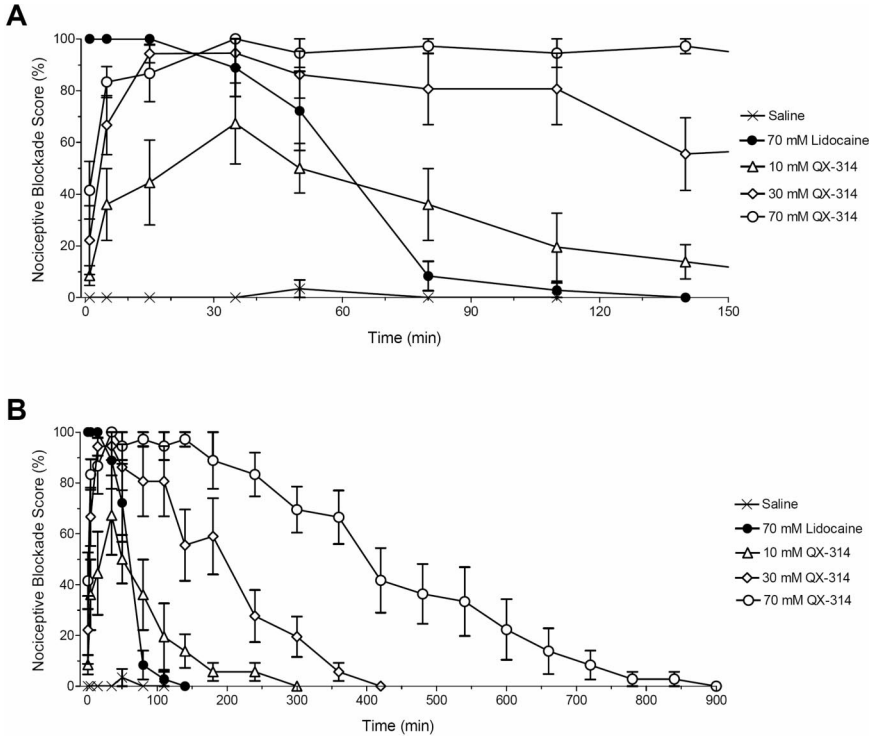

Fig. 1. Inhibition of the cutaneous trunci muscle reflex. In *A*, the time frame from time = 0 min to time = 150 min has been expanded to better illustrate the difference in onset time between the different groups. Note that 70 mM lidocaine produced complete nociceptive blockade within 1 min, whereas QX-314 had a considerably slower onset. In *B*, the time scale is compressed to depict the differences in offset time between the different groups. Both the duration of complete nociceptive blockade and the time to complete recovery of nociception was significantly longer for 70 mM QX-314 than for 70 mM lidocaine ($P < 0.0001$; *cf.* body text). Data are shown as mean ± SEM; control group (saline), n = 5; all other groups, n = 6.

normally distributed data were analyzed with the Kruskal-Wallis test. The Grubbs test was used to test for statistical outliers. Categorical data were analyzed with Fisher exact test. Survival curves of categorical time-to-event data were compared with the log-rank test; survival fractions were calculated using the product limit (Kaplan-Meier) method. Differences were considered significant at $P < 0.05$. Statistical tests were two-tailed, and data are expressed as mean ± SD, n = sample size, 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

In the guinea pig intradermal assay, QX-314 reversibly and dose-dependently inhibited the cutaneous trunci muscle reflex (fig. 1; $F_{4,55} = 5.01$; $P < 0.01$). At 10 mM, QX-314 produced a partial reflex blockade, which peaked at 34 ± 11 min (n = 6). At 30 mM, complete nociceptive blockade (> 90% effect) was observed at 12 ± 5 min (n = 6), and at 70 mM, complete blockade occurred at 18 ± 13 min (n = 6). In contrast, the onset of 70 mM lidocaine was within 1 min in all animals tested (n = 6). Normal saline produced no inhibition of the cutaneous trunci muscle reflex in any animal tested (n = 5). The duration of complete inhibition of the cutaneous trunci muscle reflex was 62 ± 20 min for 70 mM lidocaine. For 70 mM QX-314, this value was 250 ± 132 min, a greater than fourfold increase in duration when compared with the equimolar concentration of lidocaine (Tukey multiple comparison test, $q = 5.58$; $P < 0.01$). The time to complete cutaneous trunci muscle reflex recovery after QX-314 injection also was dose dependent (fig. 2; $F_{3,20} = 26.83$; $P < 0.0001$). At 10 mM QX-314, blockade reversed completely at 175 ± 103 min after injection. At 30 mM, reversal occurred at 323 ± 111 min, and at 70 mM, reversal occurred at 650 ± 171 min. By comparison, in animals injected with 70 mM lidocaine, the cutaneous trunci muscle reflex completely recovered at 100 ± 24 min, representing a sixfold dif-

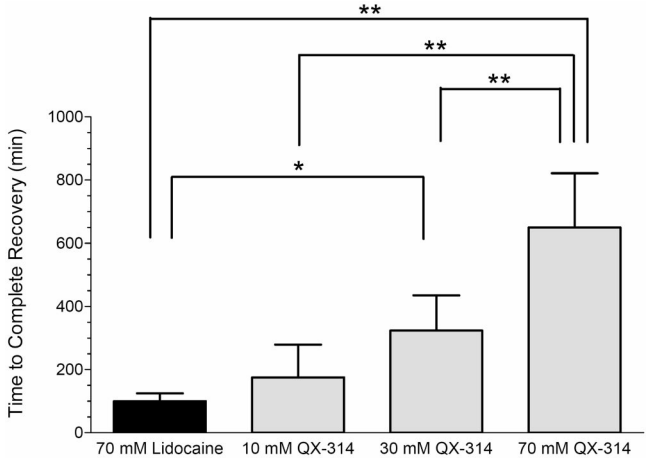

Fig. 2. Time to complete recovery of the cutaneous trunci muscle reflex. The 10 mM QX-314 group did not achieve a complete nociceptive blockade. Cutaneous trunci muscle reflex inhibition was not observed in any control animals injected with placebo (saline wheals). Data are depicted as mean ± SD; each group, n = 6. * $P < 0.05$. ** $P < 0.01$.

Table 1. Tail Nerve Sensory Conduction Block in Mice

Treatment	n	Number of Mice with Sensory Block	Onset Time in Mice with Sensory Block, min	Offset Time in Mice with Sensory Block, min
0.9% Saline	6	0	NA	NA
70 mM Lidocaine	6	6	1 ± 0	50 ± 12
10 mM QX314	6	2§	28 ± 25§	120 ± 0§
30 mM QX314	6	5§	35 ± 17†	228 ± 99*
70 mM QX314	6	6§	20 ± 9§	540 ± 147‡

Each mouse received two 20-μl injections bilaterally on either side of its tail. Injections were made 7 cm proximal from the tip of the tail. The distal 2-cm tip of each tail was placed into a 50°C hot water bath, and the tail-flick latency was determined by stopwatch. Sensory blockade was defined as tail-flick latency greater than 4 s. The onset of sensory blockade was defined as the first time point when tail-flick latency was greater than 4 s for at least two consecutive time points. The offset of sensory blockade was defined as the first time point when tail-flick latency was less than 4 s for at least two consecutive time points. Mice were tested at 1, 10, 20, 30, 45, 60, 90, and 120 min after injection and every hour afterward until offset was reached. Data are expressed as mean ± SD where feasible.

* $P < 0.05$ compared with 70 mM lidocaine. † $P < 0.01$ compared with 70 mM lidocaine. ‡ $P < 0.001$ compared with 70 mM lidocaine. § $P > 0.05$ compared with 70 mM lidocaine.

NA = not applicable.

ference in offset time at an equivalent concentration (Tukey multiple comparison test, $q = 11.69$; $P < 0.001$).

In the mouse tail-flick test, 70 mM lidocaine inhibited the tail-flick reflex response to a thermal stimulus in all animals tested (n = 6). QX-314 blocked the tail-flick response in a concentration-dependent fashion: two of six mice in the 10 mM QX-314 group did not produce a tail flick to a thermal stimulus, five of six mice did not respond in the 30 mM QX-314 group, and six of six mice showed no tail flick with 70 mM QX-314 (all groups, $P > 0.05$ compared with 70 mM lidocaine). The offset of nerve block due to QX-314 also was concentration dependent, with higher concentrations producing longer times to offset (*cf.* table 1; $F_{3,15} = 26.33$; $P < 0.0001$; Pearson correlation coefficient, 0.775). Onset times were fast when 70 mM lidocaine was injected, blocking the response to a thermal stimulus within 1 min in all animals tested, whereas the onset of block in the QX-314 groups was considerably slower (table 1; $F_{3,15} = 7.76$; $P < 0.01$). In this assay, the mean sensory conduction block produced by 70 mM QX-314 was more than 10 times longer than that produced by 70 mM lidocaine (Tukey multiple comparison test, $q = 7.32$; $P < 0.001$; table 1). Figure 3 illustrates the onset and offset of sensory blockade due to lidocaine and QX-314 in the form of survival curves. In all animals, the actions of both lidocaine and QX-314 were reversible. No animal injected with saline exhibited evidence of sensory blockade (n = 6).

In the mouse sciatic nerve blockade model, injection of 50 μl lidocaine, 70 mM, into the popliteal space prevented all mice (n = 9) from using the injected hind limb to hang on to an inverted mesh. On the other hand, only 1 mouse of 10 that received 10 mM QX-314 (Fisher exact test, $P < 0.001$ compared with 70 mM lidocaine), and 5 of 10 mice injected with 30 mM QX-314 ($P < 0.05$ compared with lidocaine) were unable to hang on to the inverted mesh. All 10 mice that received 70 mM QX-314 were unable to hang on to the inverted mesh ($P > 0.05$ compared with lidocaine). Mice that received QX-314 and showed a positive response usually did not respond immediately to the QX-314, whereas each mouse that received lidocaine responded within 1 min. In this assay, 70 mM QX-314 produced nerve blockade for 282 ± 113 min, more than 12 times longer than 70 mM lidocaine (23 ± 10 min; Tukey multiple comparison test, $q = 9.93$; $P < 0.0001$). In each group, there was a single mouse that was unable to hang on to the inverted mesh with the injected hind limb for an extended period of time. The time to recovery of these four animals' ability to hang on to the mesh was 120 min (70 mM lidocaine), 7 days (10 mM QX-314), 15 h (30 mM QX-314), and 6 days (70 mM

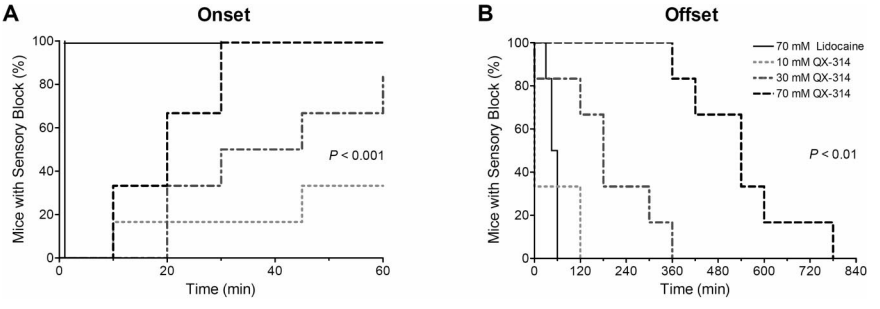

Fig. 3. Time-to-event survival curves for onset and offset of mouse tail nerve sensory conduction block. *A* shows the concentration-dependent time to onset of sensory block due to lidocaine and QX-314 in the in the mouse tail-flick test (for detailed experimental procedures, see Materials and Methods and the legend of table 1). *B* shows the concentration-dependent time to offset of sensory block due to lidocaine and QX-314. The onset of sensory blockade was defined as the first time point when tail-flick latency was greater than 4 s for at least two consecutive time points. The offset of sensory blockade was defined as the first time point when tail-flick latency was less than 4 s for at least two consecutive time points. Mice were tested at 1, 10, 20, 30, 45, 60, 90, and 120 min after injection and every hour afterward until offset was reached. Survival fractions were calculated using the product limit (Kaplan-Meier) method; the survival curves were compared with the log-rank test. Each group, n = 6.

Table 2. Sciatic Nerve Motor Block in Mice

Treatment	n	Number of Mice with Motor Block	Onset Time in Mice with Motor Block, min	Offset Time in Mice with Motor Block, min
0.9% Saline	6	0	NA	NA
70 mM Lidocaine	9	9	1 ± 0	23 ± 10
10 mM QX-314	10	1†	10	120
30 mM QX-314	10	5*	4 ± 2‡	92 ± 70‡
70 mM QX-314	10	10‡	6 ± 6*	282 ± 113†

Each mouse received a 50-μl injection into the popliteal fossa of the left hind limb. Mice were placed on an inverted mesh, and the onset of inability to hang on to the mesh was considered the onset time of nerve blockade. The offset time of nerve blockade was defined as the time point at which the animal regained its ability to use the treated hind limb to hang on to the inverted mesh. After injection, animals were tested at 1, 5, 10, 20, 30, 45, and 60 min and every hour afterward until recovery. Whenever possible, data are expressed as mean ± SD.

* $P < 0.05$ compared with 70 mM lidocaine. † $P < 0.001$ compared with 70 mM lidocaine. ‡ $P > 0.05$ compared with 70 mM lidocaine.

NA = not applicable.

QX-314), respectively. These data met the criteria of statistical outliers using the Grubbs test ($P < 0.01$).[27] Furthermore, each of these four mice was unable to hold on to the mesh with the injected hind limb immediately upon injection (within 1 min), raising the possibility of direct nerve trauma. For these reasons, these animals were excluded from statistical analysis. None of the six control animals injected with placebo (normal saline) exhibited signs of motor blockade. The effects of lidocaine and QX-314 injection into the popliteal space are summarized in table 2. Figure 4 depicts the onset and offset of sciatic nerve motor blockade due to lidocaine and QX-314 in the form of survival curves.

Of the six animals injected with 70 mM lidocaine in the mouse tail-flick test, three exhibited visible erythema which appeared 24 h after injection. This also was observed in two of six animals injected with 10 mM QX-314, one of six injected with 30 mM QX-314, and five of six injected with 70 mM QX-314 (all groups, $P > 0.05$ compared with 70 mM lidocaine). In all animals, the erythema disappeared within 6 days. Saline did not produce any erythema. We observed no signs of local toxicity, such as tissue induration or necrosis, in any animal. Inspection of the tails of mice receiving tail injections 24 h after experimentation also revealed no signs of local tissue toxicity.

Discussion

In this study, we have demonstrated that the quaternary lidocaine derivative, QX-314, concentration-dependently and reversibly produced robust local anesthesia of long duration in three different *in vivo* animal models, at concentrations equivalent to those clinically relevant for lidocaine. Compared with 70 mM lidocaine (*i.e.*, approximately 2%), QX-314 produced local anesthesia up to 6 times longer in the guinea pig intradermal wheal assay, up to 10 times longer in the mouse tail sensory conduction blockade test, and up to 12 times longer in the mouse sciatic nerve motor blockade model. Hence, QX-314 was effective in producing impulse blockade along free nerve endings, C fibers, and motor (Aα) fibers. In each animal model, QX-314 produced local anesthesia with a slower onset compared with lidocaine, but with substantially longer duration. Collectively, these results raise the possibility that quaternary ammonium compounds may be clinically useful as long-acting local anesthetics in humans and challenge the conventional notion that these agents are ineffective when applied extracellularly.[28]

Our findings correspond well to previous observations by others that extracellularly applied QX-314 does affect action potentials. For example, in experiments on squid axons, external QX-314 (10 mM) reduced the action

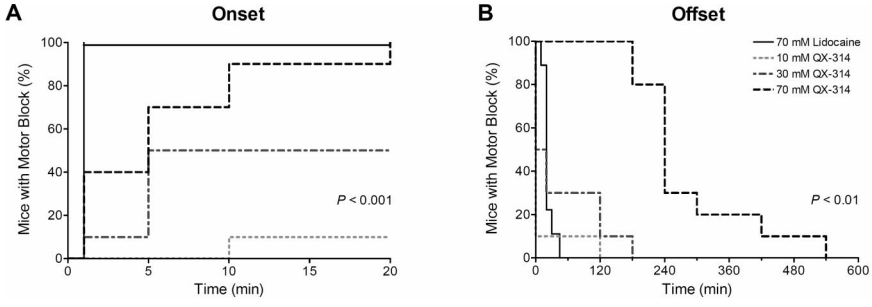

Fig. 4. Time-to-event survival curves for onset and offset of mouse sciatic nerve motor block. *A* shows the concentration-dependent time to onset of motor block due to lidocaine and QX-314 in the mouse sciatic nerve blockade model (for detailed experimental procedures, see Materials and Methods and the legend of table 2). *B* shows the concentration-dependent time to offset of sensory block due to lidocaine and QX-314. The onset of motor block was defined as the time point at which an animal was unable to hang on to an inverted mesh with the injected hind limb. The offset time was defined as the time point at which the animal regained its ability to use the treated hind limb to hang on to the inverted mesh. After injection, animals were tested at 1, 5, 10, 20, 30, 45, and 60 min and every hour afterward until recovery. Survival fractions were calculated using the product limit (Kaplan-Meier) method; the survival curves were compared with the log-rank test. For each QX-314 concentration, n = 10; 70 mM lidocaine control, n = 9.

potential rate of rise by 9%.[8] In single myelinated frog fibers, 5 mM external QX-314 reduced evoked current amplitudes by 7%.[5] In frog sciatic nerve, a 40-min exposure to QX-314 (20 mM) reduced the compound action potential amplitude by 63%.[22] Whereas these results *per se* were not suggestive of clinically useful local anesthesia, it is noteworthy that these experiments were performed at relatively low temperatures (22°, 6°, and 4°C, respectively). Should the activity of extracellular QX-314 be dependent on diffusion through the nerve membrane, the low temperatures would have greatly reduced its ability to do so, possibly explaining the relatively modest activity observed *in vitro*. It is noteworthy, though, that hypothermia potentiated the blocking action of extracellular lidocaine in a study on desheathed rat sciatic nerves.[29]

The precise mechanism of action that underlies the observed local anesthetic effects of QX-314 in the current study remains uncertain. In experiments on frog sciatic nerve, the effect of external QX-314 was noted to last more than 1 h after drug washout, and marked frequency-dependent inhibition of the compound action potential was observed.[22] Similar results showing marked frequency-dependent blockade also were obtained in rat vagus nerve.[23] Because local anesthetic frequency-dependent blockade is mediated by the cationic form of the drug, which becomes "trapped" by the inactivation gate and ion selectivity filter of voltage-gated Na^+ channels,[5,30,31] the observation that use-dependence occurs even when QX-314 is applied extracellularly[22] suggests that the local anesthetic action of QX-314 may be due to intracellular blockade of Na^+ channels through the hydrophilic pathway. It is hence possible that external QX-314 produces local anesthesia by traversing the lipid membrane to bind to its conventional intracellular receptor on the Na^+ channel. The slow onset and long duration of QX-314–mediated local anesthesia provides additional support for the hypothesis that external QX-314 penetrates the nerve membrane (albeit slowly) and then interacts with an intracellular binding site. Because QX-314 is permanently charged, to allow penetration across lipid membranes, an appropriate combination of a large concentration gradient and time would be required. Our observed large differences in local anesthetic duration with increasing QX-314 concentrations further supports the notion that QX-314 penetrates the nerve membrane(s) to act intracellularly, in which case higher QX-314 concentrations would be associated with a higher rate of intracellular penetration and, consequently, a longer duration of blockade.

In the three animal models, 10 and 30 mM QX-314 did not produce local anesthesia with the same consistency as 70 mM. To be effective for conduction blockade, local anesthetics must prevent depolarization at a minimum of three successive nodes of Ranvier so as to prevent axonal saltatory conduction, and the nerve must be exposed to a minimum blocking concentration of local anesthetic.[32] Because QX-314 does not penetrate lipid membranes easily, this agent would be expected to possess a relatively high (extracellular) minimum blocking concentration. Hence, it is likely that the minimal distance from the nerve fiber that a local anesthetic must be applied to achieve effective conduction blockade is lower for QX-314 than for a conventional tertiary agent such as lidocaine. Consequently, the duration and magnitude of blockade due to QX-314 would be expected to vary greatly with concentration, injected volume, and the proximity of the injection to the nerve. Consistent with this hypothesis is our observation in each of the three animal models of a high variability in both local anesthetic efficacy and duration of blockade at the lower QX-314 concentrations.

Collectively, our results suggest that long-acting local anesthetics can be created through quaternization of the molecule. For example, whereas quaternization decreases the potency of lidocaine, it markedly increased the duration of action, an effect that is not exclusive to lidocaine derivatives: In previous experiments, a 2% solution of the quaternary methyl derivative of cocaine produced local anesthesia with an onset within 30 min and a duration of 4–5 h.[21] Similar results were obtained with the quaternary benzyl derivative of diphenyl-piperidinoethyl-acetamide, whereas the tertiary derivatives of these compounds produced local anesthesia within 5 min and possessed a much shorter duration of action, again highly consistent with the results of our study.

In addition to prolonged duration, quaternary local anesthetics have several other potential advantages over conventional tertiary compounds. For example, quaternary agents do not readily penetrate the blood–brain barrier, raising the possibility that these agents are associated with decreased central nervous system toxicity. In fact, one study with QX-314 provides support for this hypothesis.[33] It also is conceivable that quaternary agents produce fewer cardiotoxic effects, because negative inotropism of local anesthetics correlates with lipid solubility.[34] Furthermore, systemic plasma concentrations of quaternary compounds after peripheral nerve blockade are expected to be low, because the permanent charge should impede systemic absorption through capillaries and facilitate rapid renal excretion. Moreover, the use of quaternary local anesthetics may obviate the use of added vasoconstrictors such as epinephrine to prolong the duration of blockade. There are many clinical situations where this would be advantageous, such penile blockade, regional anesthesia in patients with cardiovascular disease, or narrow-angle glaucoma. Finally, one may speculate that quaternary agents also could provide enhanced sensory–motor separation. A major determinant of a local anesthetic's magnitude of sensory–motor separation is its use-dependent blocking activity.[35] The capability for producing use-dependent

blockade inversely correlates with lipid solubility, the latter of which is decreased by quaternization.[36] However, whereas the current study was not designed to investigate differences between tertiary and quaternary agents in terms of sensory–motor separation, we observed no apparent differences in effective blocking doses of QX-314 between the sensory and motor blockade assays.

In summary, we have demonstrated here that the quaternary lidocaine derivative, QX-314, concentration-dependently produces long-lasting, reversible local anesthesia in three standard *in vivo* animal models, at concentrations equivalent to those clinically relevant for lidocaine. Our findings raise the possibility that quaternary local anesthetics may produce clinically useful, long-lasting local anesthesia in humans and challenge the conventional notion that quaternization of tertiary amines obliterates extracellular local anesthetic activity.

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