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Duration and local toxicity of sciatic nerve blockade with co-injected site 1 sodium channel blockers and quaternary lidocaine derivatives

Sahadev A. Shankarappa, MBBS, MPH, PhD^{1,2,*}, Itay Sagie, BS^{1,*}, Jonathan H. Tsui, MEng^{1,2}, Homer H. Chiang, MS^{1,2}, Cristina Stefanescu, BS^{1,2}, David Zurakowski, PhD¹, and Daniel S. Kohane, MD, PhD^{1,2}

¹Department of Anesthesiology, Perioperative and Pain medicine, Children's Hospital Boston, Harvard Medical School, Boston, MA

²Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

Abstract

Background and Objectives—Quaternary lidocaine derivatives (QLDs) have recently received much attention because of their potential application in prolonged or sensory-selective local anesthesia. However, associated tissue toxicity is an impeding factor that makes QLDs unfavorable for clinical use. Based on the proposed intracellular site of action, we hypothesized that nerve blocks obtained from lower concentrations of QLDs would be enhanced by the co-application of extracellularly acting site-1 sodium channel blocker, resulting in prolonged block duration but with minimal tissue toxicity.

Methods—QLDs (QX-314 and QX-222), site 1 sodium channel blockers (tetrodotoxin, 30 μ M or saxitoxin, 12.5 μ M), or both were injected in the vicinity of the sciatic nerve. Thermal nociceptive block was assessed using a modified hot plate test; motor block by a weight-bearing test. Tissue from the site of injection was harvested for histological assessment.

Results—Co-application of 25 mM QX-314 and 100 mM QX-222 with site 1 sodium channel blockers produced an 8- to 10- fold increase in the duration of nerve blocks ($P < 0.05$), compared to QLDs or site 1 blockers alone. QLDs elicited severe myotoxicity; this was not exacerbated by co-injection of the site-1 sodium channel blockers.

Conclusion—Co-administration of site 1 sodium channel blockers and QLDs greatly prolongs the duration of peripheral nerve block without enhancing local tissue injury, but minimal myotoxicity still persists. It is not clear that the risks of QLDs are outweighed by the benefits in providing prolonged nerve blockade.

Introduction

Localized tissue toxicity has become a crucial issue in the field of prolonged duration local anesthesia. Problems with tissue reaction, especially muscle and nerve toxicity, have had a

Corresponding author: Daniel S Kohane, M.D, Ph.D, Daniel.kohane@childrens.harvard.edu, Phone: 617 355 7327, Fax: 617 730 0453.

*Contributed equally

Sahadev A Shankarappa and Itay Sagie are co-first authors on this manuscript

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marked deleterious impact on the clinical implementation of several long-acting local anesthetic agents.^{1,2}

Quaternary lidocaine derivatives (QLDs) such as QX-314 (N-(2,6-dimethylphenylcarbamoylemethyl) triethylammonium bromide) and QX-222 (2-[(2,6-dimethylphenyl)amino]-N,N,N-trimethyl-2-oxoethaniminium chloride) are known to produce prolonged nerve conduction blocks *in-vivo*.^{3,4} QX-314, in particular, has attracted considerable interest because, in addition to producing long peripheral nerve blockade, it provides sensory-selective analgesia when combined with capsaicin,⁵ or chemical permeation enhancers.⁴ However, it has been observed that intrathecally administered QX-314 caused irritation and death in mice,⁶ while systemic administration caused CNS and cardiac toxicity.⁷ In addition, irreversible nerve blockade is seen in animals administered with high concentrations of QX-314.⁴ Since irreversible nerve dysfunction can be associated with severe local tissue toxicity,¹ we sought to determine whether QLD administration caused tissue injury.

Drug synergism, a common feature observed with local anesthetics, could potentially be used to produce prolonged nerve anesthesia while minimizing local toxicity. Synergism has been previously observed between conventional local anesthetics (amino amide and amino ester) and site 1 sodium channel blockers.^{8,9} Amino-amide and amino-ester local anesthetics act by binding to the transmembrane segment S6 of domain IV of the neuronal sodium channels.^{10,11} Such binding blocks action potential-mediated influx of sodium ions, preventing neuronal depolarization. Compared to amino-amide LA's, QLDs have a similar intracellular site of action,¹² but are considered less permeable across the cell membrane.^{7,13} Tetrodotoxin (TTX) and saxitoxin (STX) are naturally occurring neurotoxins that bind to site 1 on the extracellular part of the sodium channel¹⁴ and effectively block peripheral nerve conduction.^{15,16} Synergism has also been previously observed between site 1 blockers and tricyclic antidepressants.¹⁷ Local tissue reaction to site 1 sodium channel blockers is very benign.^{15,18,19}

In this study, we hypothesized that drug synergism between quaternary lidocaine derivatives and site 1 sodium channel blockers could reduce the concentration of QLDs required for prolonged peripheral nerve blockade to the point where there will be minimal tissue toxicity while still achieving very prolonged nerve blockade.

Materials and Methods

This study was conducted using protocols approved by the Massachusetts Institute of Technology Committee on Animal Care, and in accordance with IASP guidelines.²⁰ Adult male Sprague-Dawley rats (Charles River Laboratories International, Inc., Wilmington, Massachusetts) weighing 300 to 350 grams were housed in pairs, allowed standard rat diet and water *ad libitum*, and maintained on a 7 a.m. to 7 p.m. light/dark cycle. Rats were chosen at random for inclusion into specific treatment groups. A total of 64 rats were used in this study, with 4 animals per experimental group.

QX-314 was purchased from Sigma-Aldrich Corp, St. Louis, Missouri. TTX in citrate buffer (pH 4.8) and QX-222 were purchased from Tocris Bioscience Ellisville, Missouri. STX was received as a generous gift from Dr. Sherwood Hall (FDA, College Park, Maryland). All solutions were prepared by dissolving the drug in sterile saline (pH 5.5). The volume of all drug solutions administered *in vivo* was 0.3 milliliters.

Sciatic nerve blockade and neurobehavioral evaluation

Animals were anesthetized using 2% isoflurane with 98% oxygen dispensed through an anesthesia manifold and administered via a facemask. The left hind limb was used for sciatic nerve blocks throughout the study, while the right hind limb served as the untreated control. Nerve block injections were performed using a 23-gauge needle. The needle was introduced postero-medial to the greater trochanter, pointing in an antero-medial direction. Upon contact with bone, the needle was withdrawn approximately 1 mm and 0.3 mL of drug solution injected. Animals were evaluated for sensory and motor blockade, using modified hotplate and weight-bearing tests, as reported previously.⁹ Animals were tested every hour for the first 4 hours post drug administration, and then every 4 hours until block reversal. In brief, towel-restrained animals were carefully held over a 56 °C heated hot plate (IITC Life Science, Inc., Woodland Hills, California), and the hind-paws sequentially placed on the heated plate. Care was taken to ensure that no other part of the body came in contact with the hot plate. The time required by the animal to retract its foot (withdrawal latency) was recorded using a foot-operated stopwatch. Animals that did not withdraw their foot after 12 seconds were removed from the hot plate to prevent heat-mediated injury to the limb. Each test was repeated 3 times with a 10-second pause between each test. A withdrawal response of more than 6 seconds (50% of maximal withdrawal latency) was considered a nerve block. The time required for the thermal latency to return to a value of 6 seconds was assessed as the nerve block duration.

To test motor blockade, the extensor postural thrust of each animal was tested by sequentially placing each hind paw on a digital weighing scale and measuring the maximum amount of weight the animal could bear without its ankle touching the weighing surface. The duration of motor block was defined as the time required for weight bearing returning halfway to normal from maximal block (< 20 g of weight borne).⁹ During all behavioral testing, the investigators were blinded to the drug received by the animal.

Histological analysis

Animals were euthanized by carbon dioxide inhalation 4 days after drug administration and sciatic nerves harvested for histological analysis. The portion of the sciatic nerve and adjacent muscle with connective tissue closest to the nerve block injection site was harvested and fixed in 10% buffered formalin, followed by paraffin embedding and sectioning. Sciatic nerve samples were oriented such that sections were made perpendicular to the longitudinal axis of the nerve, and each tissue sample was sectioned 200 µm apart. Sections were stained with hematoxylin and eosin and imaged using an Olympus FSX-100 optical microscope (Center Valley, Pennsylvania). Two sections were examined per animal. Inflammation and muscle injury were scored on each section by 2 blinded investigators, using a scoring system that has been previously described.¹⁵ Inflammation scoring: 0 = normal muscle tissue morphology; 1 = perimysial inflammation, but inflammatory cells did not invade deeper tissue layers (< 5 cell layers); 2 = deeper infiltration by inflammatory cells (> 5 cell layers); 3 = hemifascicular inflammatory response; 4 = holofascicular inflammatory response. The muscle injury score reflected 2 characteristic findings of local anesthetics-induced myotoxicity: nuclear internalization and regeneration. The muscle injury scoring pattern consisted of a scale from 0 to 6, where 0 = normal morphology, 1 = perifascicular internalization, 2 = deep internalization (> 5 cell layers), 3 = perifascicular regeneration, 4 = deep regeneration, 5 = hemifascicular regeneration, and 6 = holofascicular regeneration.

Transmission Electron Microscopy

Because hematoxylin-eosin staining is relatively insensitive in detecting nerve injury, we used transmission electron microscopy (TEM) to study nerve injury. Sciatic nerve segments were harvested from the site of drug administration and immediately fixed in 0.2 M

cacodylate buffer containing 4% paraformaldehyde, 5% glutaraldehyde, and 0.06% picric acid for at least 48 hours. Nerves were post-fixed in 1% osmium tetroxide/1.5% potassium ferrocyanide for 3 hours, followed by graded alcohol dehydration. Tissue samples were then embedded in blocks containing resin mixture at 60°C for 48 hours. 95 nm sections were cut on a Leica Ultracut microtome, and sections were placed on 100 mesh formvar, and imaged using the Tecnai Spirit Electron Microscope. The TEM nerve sections were then assessed for axon-myelin integrity and perineural edema.

Statistical analysis

Statistical significance for data obtained from neurobehavioral studies was tested using one-way analysis of variance (ANOVA) with post-hoc Tukey pair-wise comparisons to control Type 1 error rate. (Statistical analysis to evaluate components of drug synergism was not conducted in this study.) Histological data for each drug (QX-314, QX-222) were assessed for normality using the Shapiro-Wilk test to determine whether scores should be compared using parametric or nonparametric methods. Consequently, since inflammation scores conformed to a normal distribution, repeated-measures mixed-model ANOVA was applied to account for each tissue section and observer with Fisher *post-hoc* comparisons for determining mean differences between concentrations. Graphical representation of data for muscle inflammation is depicted as mean $\pm$ SD. Since muscle injury scores were significantly skewed, they are reported in terms of medians and scores that range between concentrations compared, using the Kruskal-Wallis H-test followed by pair-wise Mann-Whitney *U*-tests for each drug. Two-tailed values of $p < 0.05$ were considered statistically significant. Data analysis was performed using SAS version 9.2 (SAS Institute, Cary, North Carolina).

Results

Local anesthetic and tissue toxic properties of QX-314 and QX-222

Animals were tested for both sensory and motor nerve conduction blocks using modified hot plate and extensor postural thrust tests, respectively. The time to onset and duration of conduction block was similar (ie, no statistically significant differences) for both motor and sensory functions; consequently only neurobehavioral data for sensory blocks are presented here. Both QX-314 and QX-222 demonstrated a concentration-dependent increase in the duration of nerve block (Table 1), with 70 mM QX-314 causing an almost 8-fold increase in block duration over 25 mM QX-314 ($p < 0.05$, $n=4$). QX-222 did not elicit nerve conduction block (Table 1) until a concentration of 100 mM was reached.

Four days after injection, animals were euthanized for necropsy. Increasing local muscle tissue toxicity was noted with increasing concentrations of QX-314 and QX-222. Hematoxylin and eosin stained sections of muscle tissue harvested from the vicinity of the sciatic nerve of animals administered QX-314 (Figs. 1A–C) showed concentration-dependent worsening of myocyte damage in the form of nuclear internalization, signs of degeneration-regeneration (centralization of nucleus, darker-staining smaller muscle fiber diameter), and increased inflammation with neutrophils, histiocytes, and occasional plasma cells, as reflected in worsening inflammation and muscle injury scores (Figs. 1M,N). Some sections exhibited areas of ischemic necrosis, with large, rounded myocytes without nuclei (Fig. C inset). Tissue injury and inflammation from QX-222 (Figs. 1J–L and O–P) were generally less than for the same concentration of QX-314.

Although the gross appearance of animals was normal at 25 and 45 mM QX-314, at 100 mM the site of injection was very swollen, with ulceration and scabbing of the skin (Fig. 1D and inset). Upon dissection, the subcutaneous tissue appeared injected and/or hemorrhagic (Fig.

1D). The underlying muscle was firm and very pale where not hemorrhagic (Fig. 1E). Animals injected with lower concentration of QX314 (15 mM, data not shown) or vehicle alone did not exhibit any myotoxic features (Fig. 1F).

In addition to myotoxicity, we sought to examine the local effect of QX-314 on nerve tissue. Because hematoxylin-eosin staining is relatively insensitive in detecting nerve injury, we used transmission electron microscopy (TEM) to study nerve injury. In animals injected with 100 mM QX-314, TEM demonstrated empty vacuole-like spaces just beneath the perineurium, indicative of neural edema and degenerating axons, and suggesting nerve fiber injury (Fig 1G) in 2 of 4 rats. Of those 2 rats, 1 had nerve block that had not reversed 4 days after QX-314 injection, while the remaining 3 rats exhibited a mean block duration of 32.8 ± 10.2 hours. The distribution of neurotoxicity features was predominantly found in the periphery of the nerve cross-section. In comparison, nerve tissue exposed to 25 mM (Fig 1E) QX-314 demonstrated tissue morphology indistinguishable from that of untreated nerve (Fig 1F).

Concentrations of 25 mM QX-314 and 100 mM QX-222 were selected for experimentation regarding drug synergism, since these were the minimum concentrations required to produce nerve blocks. Similarly, we used the minimum concentrations of TTX (30 μ M) and STX (12.5 μ M) required to produce nerve block.

Site 1 sodium channel blockers enhance the local anesthetic property of QX-314 and QX-222 without increasing myotoxicity

Co-administration of 12.5 μ M STX with 25 mM QX-314 increased nerve block duration 10-fold compared to 12.5 μ M STX alone ($p < 0.05$, $n=4$) and 5-fold compared to QX-314 alone ($p < 0.05$) (Fig. 2A). 12.5 μ M STX was selected because this is the lowest concentration that provides a detectable nerve block in our animal model. Similarly, co-administration of 30 μ M TTX and 25 mM QX-314 increased nerve block duration by 7- to 8-fold compared to 25 mM QX-314 alone ($p < 0.05$, $n=4$) (Fig. 2A). 30 μ M TTX was selected, as it is at the threshold of eliciting detectable nerve block.

Histological analysis of muscle tissue harvested from the injection site of animals co-administered with 25 mM QX-314 and either 12.5 μ M STX or 30 μ M TTX demonstrated inflammation and muscle injury scores that were similar to those of 25 mM QX-314 alone ($p > 0.05$, $n=4$; Fig. 2B, C, D, E).

Animals co-injected with 100 mM QX-222 and 12.5 μ M STX developed systemic toxicity in the form of respiratory failure within 30 to 60 minutes of administration and died. Upon reducing the STX concentration to 6 μ M, nerve blocks from the combination lasted 3- to 4-fold longer than from QX-222 alone (Fig 3A). Similarly, 100 mM QX-222 co-injected with 30 μ M TTX produced block durations that were 4-fold longer than 100 mM QX-222 alone. Histologically, 100 mM QX-222, in combination with 6 μ M STX and 30 μ M TTX, resulted in muscle inflammation scores similar to those from 100 mM QX-222 alone ($p > 0.05$, $n=4$; Fig. 3D). However, muscle injury scores of tissue harvested from animals co-administered with 6 μ M STX and QX-222 were lower ($p < 0.05$, $n=4$; Fig. 3E) compared to QX-222 alone or QX-222 in combination with 30 μ M TTX.

Discussion

High concentrations of the amino amide quaternary lidocaine derivatives caused prolonged nerve blockade (up to 17 h with QX-314), but with severe myotoxicity. Combination with the site 1 sodium channel blockers TTX and STX could achieve approximately equivalent blocks duration with lower concentrations of the QLDs (Fig. 4). In other words, drug

synergism allowed the achievement of much longer blocks for a given degree of myotoxicity. The site 1 sodium channel blockers did not reduce the considerable myotoxicity of any given concentration of QX-314 and QX-222.

As previously reported by others³ and by us,⁴ QX-314 exhibits a dose-dependent increase in duration of nerve block. Local perineural application of 5, 8, and 15.4 mM QX-314 failed to produce sensory or motor conduction blocks⁴, while even very low concentrations of intracellular QX-314 result in action potential block,^{5,12} suggesting relative cellular impermeability. QLDs have a quaternary ammonium structure that imparts an obligate positive charge at physiological pH²¹ that makes them relatively plasma membrane impermeable.^{13,22} Consequently, a high extracellular concentration of QLD is required for intracellular effect.^{12,23} In this study, we observed nerve blocks starting from extracellular concentration of 25 mM QX-314 and 100 mM QX-222 (Figs. 1A,B).

TTX and STX significantly prolonged nerve block durations with both QX-314 and QX-222. Synergistic activity has been previously observed between site 1 sodium channel blockers (TTX and STX) and vanilloid receptor agonists,²⁴ tricyclic anti-depressant class of drugs,¹⁷ and adrenergic antagonists.²⁵ The interaction of site 1 sodium channel blockers with vanilloid receptor agonists was ascribed to blockade of separate populations of sodium currents; that with tricyclics was believed to be due to the same mechanism as that which pertains to conventional local anesthetics;^{8,26} that with adrenergic antagonists appeared to be due to a non-specific effect at very high concentrations. In retrospect, it is possible that the adrenergic antagonists were, in fact, acting as chemical permeation enhancers (CPEs). CPEs are compounds that help drugs cross biological barriers, and have been shown to markedly enhance block duration from TTX,²⁷ presumably by helping it reach the cell surface. In that context, it bears noting that the amino-ester local anesthetic tetracaine is a CPE.²⁸ Consequently, it is conceivable that QX-314 – and all the amino-ester and amino-amide local anesthetics shown to enhance block with TTX and STX – have some action as CPEs as well, in addition to whatever molecular mechanism they have on the sodium channel itself.

QX-314 and QX-222 induced concentration-dependent myotoxic effects at the site of injection, and injury to connective tissue and skin. At low concentrations, the histological changes noted were similar in type to those seen with conventional anesthetics, such as bupivacaine¹⁵ and tetracaine.²⁹ However, the degree of myotoxicity at all concentrations of the QLDs was much worse than what we have seen with even very high concentrations of bupivacaine,¹ albeit the duration of block was much longer. In fact, the toxicity noted at high concentrations of QX-314 was similar to that seen with the tricyclic antidepressants,¹ particularly with the clear evidence of tissue injury on gross dissection, and the presence of ischemic necrosis on microscopy. This local injury may account, in part, for the severe morbidity encountered by others with epidural administration of QX-314.⁶ This degree of tissue injury should be borne in mind when considering further clinical development of these compounds. TTX and STX, when co-administered with QX-314 and QX-222, did not exacerbate the myotoxicity.

In conclusion, combination of site 1 sodium channel blockers with lower concentrations of quaternary lidocaine derivatives result in prolonged blocks without the toxicity associated with the highest concentrations of the QLDs. Quaternary derivatives can provide very long nerve blocks, but their application may be limited by local tissue toxicity. The potential consequences of local anesthetic-associated local tissue injury^{2,30} suggest caution in the use of QLDs as local anesthetics.

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Figure 1.

Tissue reaction to quaternary lidocaine derivatives 4 days after injection. Hematoxyllin and eosin staining of muscle tissue sections harvested from the vicinity of the sciatic nerve from animals administered 25 (A), 45 (B), and 70 (C) mM QX-314 showed progressive inflammation (M) and tissue injury (N). Some sections exhibited areas of ischemic necrosis (C, inset). 100 mM QX-314 caused swelling and ulceration of the skin overlying the injection site (D and inset); subcutaneous tissue was injected, and hemorrhagic (E) and muscle around the sciatic nerve, N, was very pale (arrow). Animals administered with 45 (J) and 70 (K) mM QX-222 exhibited lesser degrees of inflammation (O) and muscle injury (P), while vehicle-treated animals showed normal muscle morphology (F). TEM images of sciatic nerve sections from rats administered 100 mM QX-314 showed peri-neural swelling with empty vacuoles (white arrows) and degenerating axonal fibers (black arrow) (G), while 25 mM QX-314 (H) resembled untreated controls (I). Data are means $\pm$ SD. N=4 per group. Histological scores for muscle inflammation (data expressed as mean $\pm$ SD) were compared with a mixed-model ANOVA with Fisher post-hoc comparison ($* = p < 0.05$), while scores for muscle injury (data expressed as median and range) were compared non-parametrically, using Kruskal-Wallis H-tests followed by Mann-Whitney U-tests to assess pair-wise differences in median scores between the specific concentrations.

Figure 2.

Effect of co-injection of QX-314 and site 1 sodium channel blockers. (A) Effect on duration of sciatic nerve blockade. Data are means $\pm$ SD, $n=4$ per group. Groups were compared using one-way ANOVA with Tukey post-hoc comparison. (* = $p < 0.05$). (B, C) Representative images of histological appearance of muscle tissues 4 days after injection of 25 mM QX-314 with either 12.5 μ M STX or 30 μ M TTX. (D) Inflammation scores (mean $\pm$ SD, $n=4$) and (E) myotoxicity scores (median and range, $n=4$) of muscle tissues from rats in (A).

Figure 3.

Effect of co-administration of QX-222 and site 1 sodium channel blockers. (A) Effect on duration of sciatic nerve blockade. Data are means $\pm$ SD, $n=4$ per group. Groups were compared using one-way ANOVA with Tukey post-hoc comparison. $*$ = $p < 0.05$. (B, C) Representative images of histological appearance of muscle tissues 4 days after injection of 100 mM QX-222 with either 6 μ M STX or 30 μ M TTX. (D) Inflammation scores (mean $\pm$ SD, $n=4$) and (E) myotoxicity scores (median and range, $n=4$) of muscle tissues from rats in (A). Muscle injury scores were compared non-parametrically, using Kruskal-Wallis H-tests followed by Mann-Whitney U-tests ($*$ = $p < 0.05$).

Figure 4. Synergistic effect of site 1 sodium channel blockers and quaternary derivatives without increasing muscle inflammation. The axial dimensions of each ellipsoid represent the standard deviation of the nerve block duration and muscle inflammation scores. A: QX-314, B: QX-222.

Table 1

Effect of QLDs concentration on duration of sensory block.

Concentration (mM)	Duration of nerve block (h)	
	QX-314	QX-222
25	2.2 ± 2.8	-
45	8.7 ± 2.8	No block
70	17.3 ± 6.2 *	No block
100	32.8 ± 10.2 **	1.05 ± 1.2

Data shown are means ± SD, n=4. Groups were compared using one-way ANOVA with Tukey post-hoc comparison.

* = p < 0.05 compared with 25mM QX-314,

** indicates data from 3 out of 4 animals, where 1 animal did not recover nerve function within the time frame of the experiment.