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Tetrodotoxin, epinephrine, and chemical permeation enhancer combinations in peripheral nerve blockade

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Abstract

Background—Chemical permeation enhancers (CPEs) have the potential to improve nerve blockade by site 1 sodium channels blockers such as tetrodotoxin (TTX). Here we investigated the efficacy and toxicity of CPE-enhanced nerve blockade across a range of TTX concentrations, using two CPEs (sodium octyl sulfate and octyl trimethyl ammonium bromide). We also tested the hypotheses that CPEs could be used to reduce the concentrations of TTX and/or of a second adjuvant drug (in this case epinephrine) needed to achieve prolonged local anesthesia

Methods—Sprague-Dawley rats were injected at the sciatic nerve with combinations of TTX and CPEs, with and without epinephrine. Sensory and motor nerve blockade were assessed using a modified hot plate test and a weight-bearing test, respectively. Systemic and local toxicities of the different combinations were assessed.

Results—Addition of increasing concentrations of TTX to fixed concentrations of CPEs produced a marked concentration-dependent improvement in the rate of successful nerve blocks and in nerve block duration. CPEs did not affect systemic toxicity. At some concentrations, the addition of sodium octyl sulfate increased the duration of block from TTX plus epinephrine, and epinephrine increased that from TTX plus CPEs. The addition of epinephrine did not cause an increase in local toxicity, and markedly reduced systemic toxicity.

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DISCLOSURE

Dr. Daniel S. Kohane has patents relating to prolonged duration local anesthetics, particularly involving site 1 sodium channel blockers. He has a financial interest in a licensed site 1 blocker formulation that does not use drug delivery technology.

Conclusions—CPEs can prolong the duration of nerve blockade across a range of concentrations of TTX. CPEs could also be used to reduce the concentration of epinephrine needed to achieve a given degree of nerve block. CPEs may be useful in enhancing nerve blockade from site 1 sodium channel blockers.

INTRODUCTION

Local anesthetics for peripheral nerve blockade are important tools in the management of postoperative and chronic pain. However, the duration of nerve block from single administrations of commonly used local anesthetics – amino-amide and amino ester drugs – is short in relation to the duration of the perioperative period, for example, 0.5% bupivacaine in a sciatic nerve in a rat model resulted in sensory nerve blockade of 161 minutes.¹ Moreover, those local anesthetics can cause local toxicity to muscle and the peripheral nerves, in direct relationship to their concentration and duration of exposure,^{2,3} and they can cause severe systemic side effects, cardiovascular (e.g. life-threatening arrhythmias) and neurologic (e.g. seizures).^{4–6} Consequently, there is considerable interest in developing formulations that will provide prolonged and safe nerve block from a single injection.^{7–9}

Naturally occurring potent blockers of the extracellular site 1 on voltage-gated sodium channels,^{1,10} such as tetrodotoxin (TTX), are being investigated (and are potentially entering clinical use),⁹ as alternatives to conventional local anesthetics, for their potential to achieve longer durations of peripheral nerve blockade with minimal local toxicity to muscle and peripheral nerves.^{1,11} Tetrodotoxin also lacks significant cardiac and central nervous system toxicity.^{12,13} Nonetheless, the concentrations of TTX needed to achieve significant peripheral nerve blockade can cause hypotension and respiratory failure.¹⁴ Therefore, studies have focused on improving its therapeutic index through the use of adjuvants, such as conventional (amino-amide and amino-ester) local anesthetics, vasoconstrictors, and chemical permeation enhancers (CPEs).^{15–19} Conventional local anesthetics can greatly prolong the duration of nerve block by TTX, in a manner dependent on the concentrations of both compounds. As an example at the high end of dosing, 15.4 mM (0.5% w/v) bupivacaine increases the duration of block of 50 μ M TTX from 154 min to 559 min.¹ However, conventional local anesthetics can cause local tissue injury, cardiotoxicity and neurotoxicity.^{2–6,20} Vasoconstrictors such as epinephrine also prolong nerve block from TTX – and reduce its systemic toxicity^{1,17} – but can cause unwanted hypertension and tachycardia²¹ and local tissue ischemia. The use of chemical permeation enhancers offers an alternative for prolongation of local anesthesia.

Chemical permeation enhancers are molecules that have the ability to increase the permeability of lipid-rich barriers to drug diffusion,²² we previously demonstrated that surfactant CPEs greatly increased the duration of sciatic nerve blockade from 30 μ M TTX.¹⁵ However, the durations of nerve block achieved by the CPEs, using a very low concentration of TTX, were still relatively short. Here, we studied whether increasing the concentration of TTX results in prolongation of CPE-enhanced nerve blockade without incurring local or systemic toxicity. We studied two CPEs, sodium octyl sulfate (SOS) and octyl-trimethyl-ammonium bromide (OTAB), that differ with respect to charge (SOS is cationic, OTAB cationic) in order to enhance confidence that the effects observed are representative of CPEs

in general rather than peculiar to a specific agent. At the concentrations tested here, both CPEs prolonged TTX nerve block duration.¹⁵

We have hypothesized that CPEs enhance nerve blockade from TTX by increasing the fraction of TTX that is able to reach the nerve surface. A similar rationale has been used to explain the effect of vasoconstrictors such as epinephrine, which presumably act by maintaining a high local concentration of drug in the nerve vicinity, allowing drug penetration. Here, we also explored the possibility that the combination of CPEs and epinephrine further increase the prolongation of nerve blockade.

MATERIALS AND METHODS

Animal Care

Adult male Sprague-Dawley rats (350–400 g) were obtained from Charles River Laboratories (Wilmington, MA) and housed in groups of two per cage on a 7 a.m. to 7 p.m. light/dark cycle. Animals were cared for in accordance with protocols approved by the Animal Care and Use Committee at Boston Children's Hospital, as well as the Guide for the Care and Use of Laboratory Animals of the US National Research Council.

Preparation of Nerve Blocking Solutions

Sodium octyl sulfate (SOS) and octyltrimethylammonium bromide (OTAB; both from Sigma, St. Louis, MO) were used as CPEs. Based on previous studies in our laboratory,¹⁵ two different concentrations were selected for each enhancer. These concentrations are the EC₅₀eff, which is the concentration of CPE that caused a half-maximal increase in the duration of nerve block (corresponding to 32 mM SOS and 30 mM OTAB), and the EC_{mbd}, which is the CPE concentration that caused the maximum prolongation of nerve block duration (corresponding to 86 mM SOS and 119 mM OTAB). Each CPE solution was prepared on the same day of scheduled injections in phosphate-buffered saline (PBS) individually and in combination with different concentration of tetrodotoxin (TTX, concentration range: 0 – 50 μ M, Abcam, Cambridge, MA). Tetrodotoxin stock solutions were made by dissolving 1 mg (>98% purity) in 10 ml of 20 mM citrate solution (pH 4.5). For another set of experiments, CPEs and TTX were combined with epinephrine (Novaplast) diluted in PBS (final concentration for injection of 55 μ M, 1.1 μ M or 0.6 μ M).

Sciatic Nerve Blockade

Rats were anesthetized using isoflurane (3.5%) in oxygen. Injections were performed on the left sciatic nerve. A 23-gauge needle was introduced posteromedial to the greater trochanter of the femur pointed in an antero-medial direction. Once the bone was reached the needle was withdrawn 1 mm and the solution was injected, depositing it over the sciatic nerve.¹ The injected volume was 0.3 ml for all test solutions. All injections were performed by the same experimenter, who demonstrated >99% successful sciatic nerve blocks with 0.1 mL of 0.5% bupivacaine before initiating the current experiments, therefore suggesting that differences in block duration reflected actual pharmacological differences rather than operator error.

To generate dose-response curves for the different concentration of TTX, animals were injected with TTX at different concentrations (10–60 μ M, n=6 per group). To evaluate the effect of CPEs on the duration of nerve block produced by TTX, rats were injected with CPEs alone (n=16 for each one of the four CPE concentrations) and in combination with different concentration of TTX (10–50 μ M, n=4–6 per group). Finally, we evaluated the effect of epinephrine on the duration of nerve block produced by TTX alone and in combination with CPEs. Rats were injected with TTX (10–50 μ M) combined with epinephrine (1:100000 or 55 μ M, n=4 per group) and with TTX combined with CPEs (at their EC50eff) and epinephrine (n=4–6 per group). Additional groups of rats were injected with TTX (10–20 μ M) combined with lower doses of epinephrine (1.1 μ M and 0.6 μ M) (n = 4 per group, 4 groups total) and with TTX (10–20 μ M) combined with 32 mM SOS plus epinephrine (1.1 μ M and 0.6 μ M) (n = 4 per group, 4 groups total).

Sciatic Nerve Blockade Assessment

In all experiments, the experimenter assessing sciatic nerve block duration was blinded as to what treatment any given rat had received. Animals that did not survive the injection were not included in the duration of nerve block calculation but were accounted for in the estimation of systemic toxicity. Presence and extent of nerve blockade was investigated as previously described.^{1,11,16,23,24} Testing of nerve blockade was performed at a distal site in dermatomes innervated by the sciatic nerve (i.e. the sole of the left foot), while the right leg (uninjected) served as an untreated control that would demonstrate evidence of contralateral block related to systemic drug effects.

Blockade of thermal nociception was assessed by a modified hotplate test.²⁴ Hind paws were exposed in sequence (left then right) to a 56°C hot plate (Stoelting Co.). This test was repeated three times and the results averaged on each hindpaw for each rat at every time point. The time (latency) until paw withdrawal was measured with a stopwatch. If the animal did not remove its paw from the hot plate within 12 seconds, it was removed to avoid injury to the animal or the development of hyperalgesia. Testing was conducted at the following intervals after injection: 30min, 60min, then hourly four times, then every 2hr until 13hrs postinjection and then every 4hrs until block resolution. Latencies longer than 7s were considered to represent effective blocks, as it is the midpoint between a baseline thermal latency of approximately 2s in adult rats, and a maximal latency of 12s.¹ The duration of thermal nociceptive block was calculated as the time for thermal latency to return to a value of 7.

Motor function was assessed by suspending the animal over a balance and measuring the maximum weight that the animal could bear. Weight borne by the blocked leg of less than 40 g, was considered as present motor block.

Tissue Harvesting and Histology

Animals were euthanized with carbon dioxide, and the sciatic nerves and adjacent tissues were harvested for histologic evaluation. Tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin using standard protocols. Tissues were harvested at 4 days and 14 days after nerve injections. Muscle samples were

scored for inflammation (0–4 points) and myotoxicity (0–6 points).¹¹ The inflammation score was a subjective assessment of severity (0: no inflammation, 1: peripheral inflammation, 2: deep inflammation, 3: muscular hemifascicular inflammation, 4: muscular holofascicular inflammation). The myotoxicity score reflected two characteristic features of local anesthetic myotoxicity: nuclear internalization and regeneration. Nuclear internalization is characterized by myocytes normal in size and chromicity, but with nuclei located away from their usual location at the periphery of the cell.²⁰ Regeneration is characterized by shrunken myocytes with basophilic cytoplasm.²⁵ Scoring was as follows: 0. normal; 1. perifascicular internalization; 2. deep internalization (>5 cell layers), 3. perifascicular regeneration, 4. deep regeneration, 5. hemifascicular regeneration, 6. holofascicular regeneration.

Statistical Analysis

We used the Shapiro-Wilk *W* test in R software (R Foundation for Statistical Computing, Vienna, Austria) to assess normality of the neurobehavioral data for the different experimental conditions. As these tests indicated some departure from normality, we chose to graphically summarize the results using medians and interquartile ranges. The same method was selected for ordinal data (inflammation and myotoxicity scores).

For figures 2a, 2b, 2e and 2f, logistic regression models were fitted in order to model the binary success or failure of nerve blocks. Fitting of models with sufficient numbers of parameters to assess reasonable models revealed complete or quasi-complete separations, meaning that for some subsets of the data, perfect predictions were achieved. Under these circumstances, standard unconditional maximum likelihood estimates do not exist for some parameters. In order to handle these data appropriately, the *logistf* package in Version 3.3.2 of R software was employed to perform Firth's bias-reducing penalized maximum likelihood estimation.^{26–28}

For figures 2c, 2d, 4a, 4b and 4c linear models were fitted using the *lm* package in R. Linear hypothesis tests following the fitting of all models were performed using the utility package *car*'s linear hypothesis function, which performs linear hypothesis tests using model objects from a variety of R model-fitting packages, including *lm* and *logistf*.

In all cases except figure 4c, linear and quadratic trends in TTX dosages were fitted and allowed to interact with CPE levels, with tests comparing nested models used to choose final models for the data in which each figure was based. Once a final model was selected for the data for a given figure, focused comparisons were performed appropriate to that model. For models where TTX dosage was found to interact with the CPE or epinephrine dosage groupings being compared (figures 2c, 2d, 4a, 4b, 4c), group comparisons were performed separately at each non-zero level of TTX. For models where main effects of TTX dosage and CPE dose were chosen (figures 2a, 2b), overall group comparisons among CPE levels were used to compare CPE doses. For models where only TTX dosages were found to be indicated (figures 2e and 2f), it was concluded that no CPE effects were indicated. Local toxicity scores (Table 1 and 2) were compared using the Mann-Whitney U test.

Statistical significance was defined as a *p* value less than 0.05.

RESULTS

Effects of TTX alone on nerve blockade

Injection of 0.3 mL of 10–50 μ M TTX at the sciatic nerve resulted in concentration-dependent increases in the frequency of successful nerve blockade (Figure 1A; see Methods for definition of “successful”) and median duration of nerve block (Figure 1B; durations of block are reported as medians with the interquartile range in parentheses). There was no statistically significant difference between the durations of sensory and motor nerve blockade at any concentration of TTX.

A median duration of sensory nerve blockade of 151 (127–154) min was achieved at 50 μ M TTX. Further escalation of TTX concentration was prevented by systemic toxicity (Figure 1C), as evidenced by sensory deficits in the uninjected (contralateral) leg and increase in the mortality rate.

Effects of chemical permeation enhancers on nerve blockade

SOS and OTAB were injected without TTX at the sciatic nerve at two concentrations which we had previously shown to be effective.¹⁵ The EC₅₀eff [32 mM SOS or 30 mM OTAB] had caused a half-maximal increase in the duration of nerve block from TTX. The EC_{mbd} [86 mM SOS or 119 mM OTAB] had caused the maximum prolongation of nerve block duration. These concentrations will be abbreviated SOS-eff, SOS-mbd, OTAB-eff and OTAB-mbd below. The CPEs alone did not cause sensory block at any of the concentrations tested. However, SOS-mbd caused motor block in 4 of 16 animals with a median duration of 0 (0–0) min and OTAB-mbd caused motor block in 2 of 16 animals with a median duration of 0 (0–0) min.

Effect of chemical permeation enhancers combined with TTX on nerve blockade

CPEs resulted in a much higher rate of successful nerve blockade from TTX (Figure 2A and B). The EC_{mbd} of both CPEs resulted in a 100% rate of successful nerve blockade even with very low concentrations of TTX (e.g. 10 μ M) (SOS-mbd vs. No CPE $p < 0.00002$ and OTAB-mbd vs. No CPE $p < 0.0001$ for all concentrations of TTX).

When using CPEs at their EC₅₀eff, SOS had a greater effect than OTAB on concentrations of TTX $< 30 \mu$ M, but both resulted in a 100% rate of successful nerve blockade when combined with TTX at 30 μ M or higher (SOS-eff vs. no CPE $p < 0.0006$ and OTAB-eff vs. no CPE $p = 0.062$ for all concentrations of TTX).

The duration of nerve block was dependent on the concentrations of both the TTX and the CPE. Both concentrations of SOS demonstrated a concentration-dependent prolongation of nerve blockade ($p < 0.001$) (Figure 2C). The longest durations of sensory nerve block achieved, by 50 μ M TTX with SOS-mbd, was 916 (751–934) min, i.e. > 15 hours compared to 2.5 hours for TTX alone.

OTAB also prolonged the duration of nerve block from TTX (Figure 2D). OTAB-eff prolonged block at TTX concentrations $> 20 \mu$ M ($p < 0.02$), while OTAB-mbd prolonged

block at all TTX concentrations ($p < 0.001$). The longest duration of sensory nerve block achieved, by 50 μM TTX with OTAB-mbd, was 525 (481–570) min, i.e. 8.75 hours.

Addition of CPEs did not affect the incidence of systemic toxicity. (Figure 2E, F).

There was no statistically significant difference between the durations of sensory and motor nerve blockade in animals that received TTX plus SOS or TTX plus OTAB ($P = 0.60$ and 0.73 respectively).

Local toxicity of TTX plus CPE combinations

Animals were euthanized 4 and 14 days after sciatic nerve injection. Tissues were harvested and hematoxylin-eosin stained sections were prepared and scored for myotoxicity and inflammation^{11,29}. TTX alone was associated with virtually no myotoxicity at any concentration (Figure 3A) (see Table 1 for results with the highest concentration of TTX). Administration of SOS-eff and OTAB-eff alone caused mild degrees of myotoxicity, with myocytes showing nuclear centralization (median myotoxicity scores 1 at 4 and 14 days) (Figure 3B, C) (Table 1). Administration of SOS-mbd and OTAB-mbd resulted in higher degrees of myotoxicity with shrunken myocytes with basophilic cytoplasm (median myotoxicity scores 3 at 4 and 14 days) (Figure 3B, C) (Table 1).

TTX plus CPEs combinations had similar myotoxicity to those obtained with the same concentration of CPEs alone, at all concentrations of TTX alone (See Table 1 for results with CPEs plus the highest concentration of TTX).

TTX alone was associated with minimal inflammation (median inflammatory scores 0.5) at 4 and 14 days (see Table 1 for results with the highest concentration of TTX). When using SOS-eff and OTAB-eff, the inflammatory response was mild and limited to the muscle surface (median inflammatory scores 1 at 4 and 14 days) (Table 1). The inflammatory response was stronger when using SOS-mbd and OTAB-mbd (median inflammatory scores 2.5 at 4 and 14 days) (Table 1).

Median inflammation scores decreased from 4 to 14 days after injection, suggesting resolution of the inflammatory response (Table 1).

Addition of epinephrine to TTX plus CPE combinations

Epinephrine is known to prolong the duration of block of TTX in a concentration- dependent manner.¹ Co-injection with 55 μM epinephrine (1:100,000) greatly increased the duration of sciatic nerve blockade from 10–50 μM TTX injected at the sciatic nerve (Figure 4A and B).^{1,18,19}

Addition of SOS-eff to this combination resulted in slightly longer nerve block durations than those from TTX plus epinephrine or TTX plus SOS at TTX concentrations 40 μM ($p < 0.005$ for both comparisons). On the other hand, addition of OTAB-eff to the combination, resulted in substantially similar nerve block durations than those from TTX plus epinephrine or TTX plus OTAB at all TTX concentrations. Maximum median durations of nerve block

obtained with 50 μ M TTX, 55 μ M epinephrine and CPEs were 916 (817–929) min (15.3 h) with SOS-eff and 823 (724–927) min (13.7 h) with OTAB-eff.

SOS was also able to prolong nerve block duration from TTX plus epinephrine, at the lower concentrations of TTX (10 and 20 μ M), using lower concentrations of epinephrine (1.1 and 0.6 μ M) (Figs. 4A, C). For example, the addition of SOS-eff to 20 μ M TTX plus 1.1 μ M epinephrine and 20 μ M TTX plus 0.6 μ M epinephrine prolonged nerve block 1.9-fold and 3.6-fold, respectively. However, this increase was not significant ($p = 0.21$ and 0.91 , respectively). On the other hand, the addition of SOS-eff resulted in a 2.8-fold prolongation of nerve block when combined with 10 μ M TTX plus 1.1 μ M epinephrine ($p < 0.003$), and 7.1-fold prolongation when combined with 10 μ M TTX plus 0.6 μ M epinephrine ($p < 0.001$).

OTAB did not prolong the duration of block from TTX plus epinephrine at any concentration of either compound (Figure 4B).

Epinephrine markedly decreased the systemic toxicity associated with TTX. Animals receiving 40 and 50 μ M TTX, the addition of epinephrine (with or without CPEs) had no signs of systemic toxicity and the mortality rate was 0%.

The addition of epinephrine did not result in statistically different myotoxicity scores than from TTX plus CPE (See Table 2 for results for results with CPEs plus the highest concentration of TTX and the highest concentration of epinephrine).

DISCUSSION

Chemical permeation enhancers were able to increase the duration of nerve block across the range of concentrations of TTX tested without increasing their systemic toxicity. In particular, CPEs enhanced the effectiveness of very low concentrations of TTX, which could be useful in regional anesthetics (such as the transversus abdominis plane [TAP] blocks) that require the administration of large volumes of local anesthetic, by rendering the otherwise ineffective concentrations of TTX effective. Reduction of the TTX dose required for a given anesthetic effect would reduce systemic toxicity.

Sodium octyl sulfate was also able to increase the duration of block from low concentrations of TTX across a broad range of concentrations of epinephrine. These data suggest that CPEs could also be used to reduce the concentration of epinephrine necessary for the prolongation of nerve block, which could be useful in large-volume blocks where the injection of large quantities of epinephrine could lead to undesirable cardiovascular side effects. An analogous situation has been encountered with co-injection of TTX, bupivacaine, and epinephrine.¹⁷ At the relatively low concentrations used of the three components, it was found that a combination of all three was more efficacious than any two alone. Thus, one component could be used to reduce the concentration of the others needed to have a given effect. However, at high concentrations of the components, the use of three did not result in a higher duration of block than did TTX with bupivacaine or TTX with epinephrine.¹

Octyl trimethyl ammonium bromide did not prolong the duration of effect of TTX plus epinephrine at any concentration of the two compounds, while SOS did. SOS and OTAB are similar in many respects. They have the same length of carbon chain (8), and similar molecular weights (232.28 and 252.23 respectively). They differ only in that SOS is anionic due to a sulfate group, and OTAB is cationic due to a quaternary amine. It is not clear how this difference would affect the interaction between CPEs and TTX plus epinephrine.

The addition of epinephrine reduced systemic adverse effects and eliminated mortality at the TTX concentrations tested, while CPEs did not affect associated systemic toxicity. This difference relates to the different mechanisms by which the two types of compounds affect TTX. Epinephrine is believed to improve duration of block by vasoconstriction leading to retention of a high local drug concentration,³⁰ and slows systemic drug distribution by the same mechanism. Chemical permeation enhancers likely prolong block by improving penetration of TTX to the axonal surface, without affecting drug efflux from the site of injection.

The systemic toxicity of TTX and other site 1 sodium channel blockers when they are used as local anesthetics has been recognized for decades,^{1,19} likely rendering them unusable alone. Here with CPEs and in previous work with bupivacaine and epinephrine, we have shown that when other compounds are added that prolong block duration without increasing systemic toxicity, there are effective dosages that provide prolonged local anesthesia safely. The mitigation of toxicity is most striking when epinephrine is in the formulation, as was seen here and previously.^{1,19} We have hypothesized that the toxicity observed with higher concentration of TTX might be greatly reduced when used in larger animals, since the median toxic dose scales in direct proportion to the mass of the recipient, while the duration of nerve block does not.²³ Thus, it is envisioned that the therapeutic index might be better in humans, which would allow the use of higher concentrations of TTX to further extend the duration of the anesthetic effect. This hypothesis seems to have been borne out in the recent clinical trials with the site 1 sodium channel blocker neosaxitoxin in humans, in combination with bupivacaine and/or epinephrine.^{8,9,31} One rationale for the use of CPEs is to reduce the concentrations of adjuvant drugs used, and thus their side-effects. The potential effects of the systemic distribution of CPEs, e.g. in the context of large-volume nerve blocks, is unknown.

In vivo experiments confirmed that TTX alone causes no or minimal myotoxicity or inflammation, as has been previously reported.^{11,32} Tissue toxicity to the CPEs was concentration-dependent, being very minimal at the lower concentration and still relatively modest at the higher. This toxicity was comparable to that seen with clinically relevant concentrations of bupivacaine.^{11,20} For translation, the concentration of CPEs would have to be selected to minimize local toxicity. Alternatively, agents other than the ones studied here may be used in the event of clinical translation. In that regard, there is some literature to suggest that neutral CPEs have milder tissue reaction; the CPEs used here were charged. Addition of epinephrine to TTX plus CPE combinations did not cause an increase in local toxicity.

The inclusion of two CPEs in this study was intended to support the general nature of observed effects on nerve blockade. This study was not designed to provide mechanistic

insights regarding the effects of the CPEs. Although they differed in charge – and therefore potentially in interaction with the cationic TTX – both CPEs increased the duration of block across a range of TTX concentrations (although with differences in the shape of the duration-concentration curves). This suggests that the effect of the CPEs on the barriers to diffusion (rather than their interaction with TTX) was their dominant effect.

Sodium octyl sulfate and OTAB have not been tested in humans, but similar CPEs such as the anionic surfactant sodium lauryl sulphate (SLS) are already FDA approved for topical applications. Therefore, it would be possible for CPEs of the same class to translate into clinical trials after their safety profile for injectable applications has been determined.

In conclusion, CPEs improved the reliability and duration of peripheral nerve block of a range of concentrations of TTX. The addition of SOS also increased the durations of block from low concentrations of TTX with epinephrine. CPEs may be useful in providing prolonged local anesthesia with TTX. They may also help minimize the concentrations of TTX and/or of other agents that are co-delivered. This may be of particular importance when large volumes of local anesthetics are to be used.

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Figure 1.

Effects of TTX on peripheral nerve blockade. (A) Effect of TTX concentration on the frequency of successful blocks. (B) Effect of TTX concentration on the median duration of sensory nerve blockade. (C) Effect of TTX concentration on the frequency of nerve block in the uninjected (contralateral) extremity, and of death (See Results). Data are medians with interquartile ranges. $n = 6$ per experimental group.

Figure 2.

Effects of TTX and TTX with CPE on peripheral nerve blockade. (A) Frequency of nerve block with TTX and TTX with SOS ($p < 0.0006$ for TTX plus SOS-eff vs. TTX alone and $p < 0.00002$ for SOS-mbd plus TTX vs. TTX alone). (B) Frequency of nerve block TTX and TTX with OTAB for the instauration of nerve blockade ($p = 0.062$ for TTX plus OTAB-eff vs. TTX alone and $p < 0.0001$ for TTX plus OTAB-mbd vs. TTX alone). (C) Duration of sensory nerve blockade with TTX and TTX with SOS. Brackets denote significant difference, $p < 0.001$. (D) Duration of sensory nerve blockade with TTX and TTX with

OTAB. Brackets denote significant difference, $p < 0.02$. (E) Contralateral block from administration of TTX with and without CPEs. There was no statistically significant difference in contralateral block between rats receiving TTX alone and those receiving TTX plus either concentration of concentration of either CPE ($P > 0.632$ in all cases). (F) Frequency of mortality from administration of TTX with and without CPEs. There was no statistically significant difference in mortality between rats receiving TTX alone and those receiving TTX plus either concentration of either CPE ($P > 0.966$ in all cases). Data are medians with interquartile ranges. $n = 4 - 6$ per experimental group. SOS-eff = 32 mM SOS, OTAB-eff = 30 mM OTAB, SOS-mbd = 86 mM SOS and OTAB-mbd = 119 mM OTAB. SOS = sodium octyl sulfate. OTAB = Octyl trimethyl ammonium bromide.

Figure 3.

Representative hematoxylin-eosin stained sections of muscles at the site of injection 4 days post-injection. Scale bars = 50 μm . SOS-eff = 32 mM SOS, OTAB-eff = 30 mM OTAB, SOS-mbd = 86 mM SOS and OTAB-mbd = 119 mM OTAB. SOS = sodium octyl sulfate. OTAB = Octyl trimethyl ammonium bromide.

Figure 4.

Effects of the addition of epinephrine to TTX plus CPEs (SOS-eff or OTAB-eff) combinations in peripheral nerve blockade. (A) Addition of 55 μM epinephrine to TTX plus SOS-eff. Brackets denote significant difference, $p < 0.005$. (B) Addition of 55 μM epinephrine to TTX plus OTAB-eff. Brackets denote significant difference, $p < 0.007$. (C) Effect of increasing concentration of epinephrine on duration of nerve blockade from TTX with or without SOS-eff. Data are medians with interquartile ranges. (See Results). Brackets denote significant difference, $p < 0.003$. $n = 4 - 6$ per experimental group. SOS-eff = 32 mM

SOS and OTAB-eff = 30 mM OTAB. SOS = sodium octyl sulfate. OTAB = Octyl trimethyl ammonium bromide.

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Table 1

Data are medians with interquartile ranges.

	Myotoxicity Score		Inflammation Score	
	Day 4	Day 14	Day 4	Day 14
<i>Single compounds</i>				
50 µM TTX	0 (0 – 0)	0.5 (0 – 1)	0.5 (0 – 1.0)	0 (0 – 0)
SOS-eff	1.0 (0 – 1.5)	1.0 (0.5 – 1.5)	1.0 (1.0 – 1.0)	1.0 (0.5 – 1.0)
OTAB-eff	1.0 (0.5 – 1.5)	1.0 (0.5 – 1.5)	1.5 (0.5 – 2.0)	1.0 (0.5 – 1.5)
SOS-mbd	3.0 (2.0 – 4.0)	2.5 (1.5 – 3.0)	2.0 (1.5 – 2.5)	1.0 (0.5 – 1.5)
OTAB-mbd	3.0 (2.0 – 4.0)	2.0 (2.0 – 2.5)	2.5 (2.0 – 3.0)	2.0 (1.0 – 2.0)
<i>CPE plus TTX combinations</i>				
50 µM TTX plus SOS-eff	1.0 (0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (0 – 2.0)	0 (0 – 2.0)
P value vs 50 TTX	0.073	0.683	0.445	0.248
P value vs SOS-eff	0.741	0.498	1.000	0.647
50 µM TTX plus OTAB-eff	1.0 (0 – 1.0)	1.0 (0 – 2.0)	1.0 (1.0 – 1.0)	0 (0 – 1.0)
P value vs 50 µM TTX	0.073	0.445	0.179	0.248
P value vs OTAB-eff	0.498	1.000	0.508	0.186
50 µM TTX plus SOS-mbd	3.0 (2.0 – 3.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 3.0)	1.0 (1.0 – 2.0)
P value vs 50 µM TTX	0.018	0.029	0.029	0.018
P value vs SOS-mbd	0.654	1.000	0.186	0.498
50 µM TTX plus OTAB-mbd	2.0 (2.0 – 3.0)	2.0 (1.0 – 2.0)	3.0 (1.0 – 3.0)	1.0 (0 – 3.0)
P value vs 50 µM TTX	0.018	0.061	0.061	0.077
P value vs OTAB-mbd	0.278	0.117	0.913	0.5

n= 6–8 per experimental groups. SOS-eff = 32 mM SOS, OTAB-eff = 30 mM, SOS-mbd = 86 mM and OTAB-mbd = 119 mM OTAB. SOS = sodium octyl sulfate. OTAB = Octyl trimethyl ammonium bromide.

Table 2

Data are medians with interquartile ranges.

	Score	
	Day 4	Day 14
Myotoxicity Score (0 – 6)		
50 μ M TTX	0 (0 – 0)	0.5 (0 – 1.0)
50 μ M TTX plus 55 μ M epinephrine	0.5 (0 – 1.0)	0.5 (0 – 1.0)
P value ^a	0.157	1.000
50 μ M TTX plus SOS-eff plus 55 μ M epinephrine	1.5 (1.0 – 2.0)	1.0 (1.0 – 1.0)
P value ^a	0.028	0.263
P value ^b	0.196	0.414
50 μ M TTX plus OTAB-eff plus 55 μ M epinephrine	1.0 (0 – 2.0)	0.5 (0 – 1.0)
P value ^a	0.157	1.000
P value ^b	0.760	0.542
Inflammation Score (0 – 4)		
50 μ M TTX	0.5 (0 – 1.0)	0 (0 – 0)
50 μ M TTX plus 55 μ M epinephrine	0.5 (0 – 1.0)	0 (0 – 0)
P value ^a	1.000	0.157
50 μ M TTX plus SOS-eff plus 55 μ M epinephrine	1.5 (1.0 – 2.0)	1.0 (1.0 – 1.0)
P value ^a	0.133	0.025
P value ^b	0.542	0.542
50 μ M TTX plus OTAB-eff plus 55 μ M epinephrine	1.5 (1.0 – 2.0)	1.0 (1.0 – 1.0)
P value ^a	0.133	0.025
P value ^b	0.220	0.182

P values result from the comparison of to ^a 50 μ M TTX or ^b 50 μ M TTX plus CPEs combinations. n = 4–6 per experimental group. SOS-eff = 32 mM SOS, OTAB-eff = 30 mM OTAB, SOS-mbd = 86 mM SOS and OTAB-mbd = 119 mM OTAB. SOS = sodium octyl sulfate. OTAB = Octyl trimethyl ammonium bromide.