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High-frequency, low-intensity ultrasound and microbubbles enhance nerve blockade

Kathleen Cullion[a,b], Claudia M. Santamaria[a], Changyou Zhan[c], David Zurakowski[d], Tao Sun[e], Grant L. Pemberton[a,b], Nathan J. McDannold[e], Daniel S. Kohane[a,d,*]

[a] Laboratory for Biomaterials and Drug Delivery, Division of Critical Care Medicine, Department of Anesthesiology, Boston Children's Hospital, Harvard Medical School, Boston, MA, United States
[b] Department of Medicine, Division of Medicine Critical Care, Boston Children's Hospital, Harvard Medical School, Boston, MA, United States
[c] Department of Pharmacology, School of Basic Medical Sciences, Fudan University & Key Laboratory of Smart Drug Delivery (Fudan University), Ministry of Education, Shanghai, China
[d] Department of Anesthesiology, Boston Children's Hospital, Harvard Medical School, Boston, MA, United States
[e] Focused Ultrasound Laboratory, Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States

1. Introduction

Site 1 sodium channel blockers (S1SCBs such as tetrodotoxin [TTX] and the saxitoxins) are ultrapotent local anesthetics that bind extracellular domains of voltage-dependent sodium channels [1]. S1SCBs do not cross the blood brain barrier readily and have low affinity for the cardiac sodium channel isoform [2,3], and so do not cause seizures [4] or arrhythmias, making TTX an attractive alternative to conventional local anesthetics [5,6]. Local anesthesia with S1SCBs has been described in numerous reports in animal models [7–11], and is now starting to be applied in humans [12,13]. However, due to their very hydrophilic nature S1SCBs have limited penetration to peripheral nerves even with local injection, and require relatively high concentrations to achieve consistent nerve blockade [9]. Those high concentrations can be associated with systemic toxicity. One approach to enhancing the effect of S1SCBs has been to co-inject them with second or third drugs with adjuvantic effects [7,8,14,15]. However, such couplings can entail the side-effects of the second drugs, such as the local tissue toxicity of "conventional" amino-amide and amino-ester local anesthetics or the vasoconstriction and/or tachycardia seen with epinephrine. An alternative approach is to enhance the penetration of S1SCBs to the neuronal surface. Previously we have shown that co-injection of TTX with chemical permeation enhancers could enhance the effect of S1SCBs, albeit with some adverse tissue reaction at high concentrations [16,17].

Ultrasound-mediated drug delivery has the potential to enhance nerve blockade without adverse tissue reaction. Acoustic waves can enhance drug delivery through the sclera [18], the stratum corneum [19,20], across barriers within the gastrointestinal tract [21,22], and the blood brain barrier [23,24]. The effect of high-frequency ultrasound on drug flux across skin (sonophoresis) can be enhanced by exogenous microbubbles [25,26].

In this study, we hypothesized that ultrasound treatment would increase the flux of hydrophilic molecules, such as S1SCBs, across biological barriers to the surface of the peripheral nerve. We used TTX as a model S1SCB. We studied high-frequency, low-intensity ultrasound (HFLIU), parameters similar to those used in clinical imaging. This frequency and intensity range is theoretically safe for a broad range of applications. We evaluated the effects of HFLIU with and without the addition of exogenous microbubbles on TTX-mediated nerve blockade. We also verified whether effects of ultrasound and microbubbles were applicable to local anesthetics in general by testing their effect on the amino-amide local anesthetic bupivacaine.

2. Materials and methods

2.1. Animal care

Young adult male Sprague-Dawley rats (350–420 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. All animals were cared for in compliance 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.

2.2. Microbubble preparation

Microbubble preparation was adapted from previously described work [27]. DSPC, DSPE-PEG2k (90:10, molar ratio) were combined and dissolved in chloroform. Chloroform was then removed by evaporation under a vacuum for 2 h. The lipids were dissolved in a 100 mM Tris buffer (pH 7.4) with glycerol: propylene glycol (80:10:10, volume ratio) to create a lipid concentration of 1 mM. The suspension was mixed well

* Corresponding author at: 300 Longwood Ave, Enders Building, Room 361, Boston Children's Hospital, Boston, MA 02115, United States.
E-mail address: Daniel.Kohane@childrens.harvard.edu (D.S. Kohane).

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and sonicated with a bath sonicator (20 kHz for 3 min), followed by sonication by a probe sonicator (40 kHz, 16 s) to generate microbubbles. Fluorobutane gas was then slowly injected into a glass vial for 20 s, and the glass vial was immediately capped. Microbubbles were evaluated by brightfield imaging. Microbubble size and number-weighted size distribution were determined using a Coulter counter (mean size 6.54 μm +/− 3.35 μm) (SI Fig. 1). Prior to injection, microbubbles were diluted with phosphate-buffered saline to a final concentration of 1×10^7/ml. A more concentrated lipid formulation resulted in increased lipid debris, requiring additional processing (centrifugation).

2.3. Ultrasound

An unfocused transducer (Harisonic i8–0118-P-SU, Olympus, Waltham, MA) with a resonant frequency of 1 MHZ was used for transmitting ultrasound to the targeted peripheral nerve. The transducer was driven by an ultrasonic pulser (Model 5072PR, Olympus, Waltham, MA) with a 5000-Hz pulse repetition frequency (PRF) = 5000 Hz, 26-μJ pulse energy, 50-Ω damping resistance, and 39-dB gain. The acoustic pressure was calibrated using a needle hydrophone (HNC-400; Onda) 0.7 cm from the axial center of transducer. Transverse scan of the targeted plane was also measured to determine the pressure field (SI Fig. 2). Based on the highest acoustic pressure measured in the field (p = 97.87 kPa, Peak negative pressure), the spatial peak temporal peak intensity ($I_{SPTP} = p^2/2\rho c$) was calculated to be 319 mW/cm^2. Where ρ and c refer to the density of water and the speed of sound in water, respectively. With a duty cycle of 0.5%, the spatial peak temporal average intensity I_{SPTA} was calculated to be 1.6 mW/cm^2, based on the following equation.

$$I_{SPTA} = \text{Duty cycle} \times I_{SPTP}$$

After the animal's fur was shaved, and the drug was deposited on the sciatic nerve by injection, the ultrasound transducer was held posteromedial to the greater trochanter of the femur pointing in an anteromedial direction for 5 min, SI Fig. 3. Ultrasound gel was used for coupling. To determine the effect increasing insonation energy has on drug delivery, animals were injected with 30 μM TTX with and without microbubbles. Animals were then treated with 1 MHz ultrasound for 5 min either at an acoustic intensity of 0.1 W/cm^2, continuous pulse, using the Chattanooga Model 2782 (Chattanooga, TN) or using the aforementioned preclinical benchtop setup.

2.4. Sciatic nerve blockade technique

Rats were anesthetized using isoflurane in oxygen and then shaved to provide an adequate coupling surface for ultrasound application. Injections were performed at the left sciatic nerve as described previously [9,14]. A 23-gauge needle was introduced posteromedial to the greater trochanter of the femur pointed in an anteromedial direction, 0.3 mL of the drug was injected upon contacting bone, depositing the drug over the sciatic nerve.

2.5. Preparation of test solutions

Tetrodotoxin stock solutions were made by dissolving 1 mg (> 98% purity, Abcam, Cambridge, MA) in 10 mL of 20 mM citrate solution (pH 4.5). TTX was diluted in 300 μL of phosphate-buffered saline or microbubbles to a concentration of 15–35 μM. Stock solutions of bupivacaine were made in either phosphate-buffered saline or microbubbles to a concentration of 0.05% or 0.5%.

2.6. Assessment of sciatic nerve blockade

In all experiments, the person assessing sciatic nerve block duration was blinded to what treatment the rat had received. Neurobehavioral testing of nerve blockade was performed at a distal site in dermatomes innervated by the sciatic nerve (left foot), while the right leg (uninjected) served as an untreated control.

Assessment of sensory blockade (thermal nociception, perception of pain) was performed by a modified hotplate test, as described previously [9,28]. This is a well-accepted method of testing analgesic responses that we and others have used for over two decades. In brief, hind paws were exposed in sequence (left then right) to a 56 °C hot plate (Model 39D Hot Plate Analgesia Meter, IITC Inc., Woodland Hills, CA). The time until paw withdrawal was measured with a stopwatch. This test was repeated three times and the average time in seconds was calculated. If the animal did not remove its paw from the hot plate within 12 s, it was removed by the experimenter to avoid injury to the animal or the development of hyperalgesia. Testing was conducted every 15 min until the nerve blockade resolved. Latencies longer than 7 s, the midpoint between baseline thermal latency 2 s and maximum latency 12 s, were considered to represent effective blocks. The duration of thermal sensory block was calculated as the time for thermal latency to return to a value of 7 s.

Motor nerve block was assessed by a weight-bearing test to determine the motor strength of the rat's hindpaw, as described [9,28]. In brief, the rat was positioned with one hindpaw on a digital balance and was allowed to bear its weight. The maximum weight that the rat could bear without the ankle touching the balance was recorded. Motor block was considered achieved when the motor strength was less than half-maximal, as described [14,28].

Animals injected with 15 μM TTX, n = 4 animals per treatment group. Animals that were injected with 25, 30, or 35 μM TTX, n = 7–12 animals per treatment group, see SI Table 1. Animals injected with bupivacaine (either 0.05% or 0.5%) n = 3 per group. Any animal that died following injection and treatment was not included in the calculation of the percentage of animals blocked, duration of sensory/motor block, or assessment of contralateral block but was included in the estimation of systemic toxicity and death.

2.7. Tissue harvesting and histology

Animals were euthanized with carbon dioxide, and the sciatic nerves and adjacent tissues were harvested for histology. Tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin using standard techniques. Tissues were all harvested 4 days and 14 days after injections. Muscle samples were scored for inflammation (0–4 points) and myotoxicity (0–6 points) as described [29].

2.8. Statistical analysis

Neurobehavioral and histology data are reported as medians with interquartile ranges (i.e. 25th to 75th percentile) and compared using the nonparametric Mann-Whitney U test. This method was selected because the data are ordinal (inflammation scores, myotoxicity scores), or showed departures from a normal distribution, as judged by the Kolmogorov-Smirov goodness-of-fit statistic (neurobehavioral data). Analysis of the effectiveness of nerve block was performed using logistic regression with a generalized estimating equations (GEE) strategy to account for repeated measures for the same animal with the Wald test used to assess significance and a Bonferroni-adjusted p-value to control the type I error rate. Effect of treatment on systemic effects and death were compared with the two-tailed Fisher's exact test for comparing independent binary proportions. Statistical analyses were performed using Stata 11 SE (StataCorp College Station, Texas) software.

3. Results

Groups of rats were injected at the sciatic nerve with 300 μL of 15, 25, 30, or 35 μM tetrodotoxin (TTX) then underwent neurobehavioral

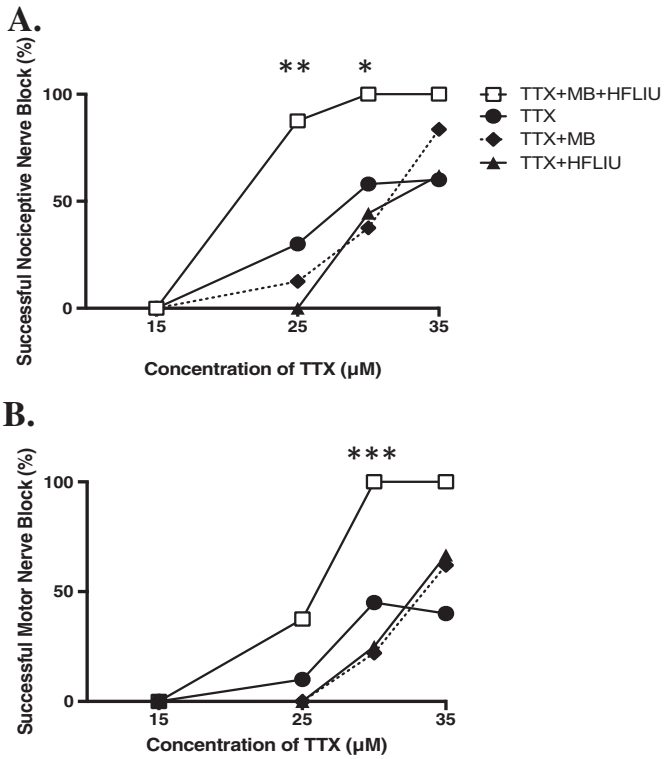

Fig. 1. Effect of treatments on the frequency of successful (A) sensory and (B) motor nerve block with TTX: tetrodotoxin, HFLIU: high-frequency, low-intensity ultrasound, MB: microbubbles. *p < .05, **p < .01, ***p < .001 comparing TTX + MB + HFLIU to TTX, TTX + MB, and TTX + HFLIU. Analysis was performed using logistic regression and generalized estimating equations to account for repeated measures. See SI Table 1 for sample sizes. Differences between the TTX, TTX + MB, and TTX + HFLIU groups were not statistically significant.

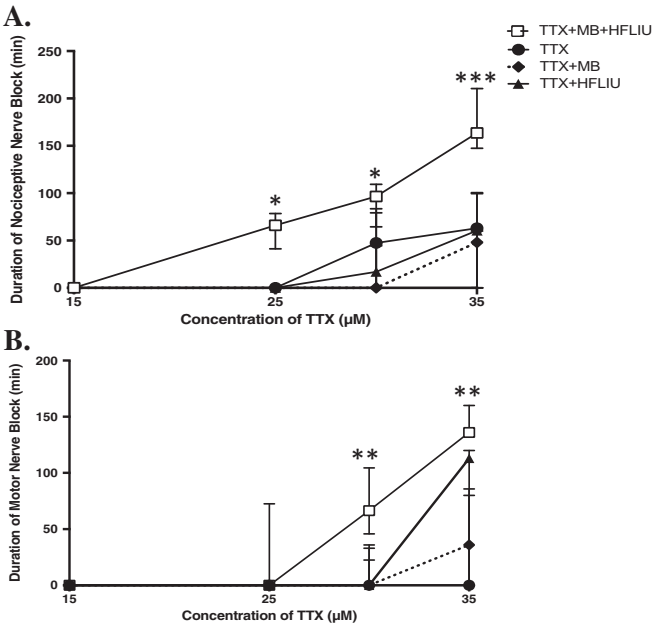

Fig. 2. Effect of treatments on the duration of sensory (A) or motor (B) nerve block with TTX; tetrodotoxin, HFLIU: high-frequency, low-intensity ultrasound, MB: microbubbles. Data are medians with interquartile ranges. *p < .05, **p < .01, ***p < .001 comparing TTX + MB + HFLIU to TTX, TTX + MB, and TTX + HFLIU by Mann-Whitney *U* Test. Differences in duration of sensory or motor nerve block among TTX, TTX + MB, and TTX + HFLIU were not statistically significant.

testing (see Methods) to determine sensory (nociception; perception of pain) and motor (weight bearing) nerve block, from which we determined the frequency of successful nerve blockade (latency > 7; weight-bearing test > 70 g) and calculated the duration of nerve blockade. With injection of 15 μM TTX, n = 4 animals per treatment group; with 25, 30, or 35 μM TTX, n = 7–12 animals per treatment group (SI Table 1).

3.1. Effects of treatments on the frequency of successful nerve blockade

The frequency of successful nerve blocks (see Methods section for definition) increased with increasing concentration of TTX, up to approximately 60% for sensory nerve block (Fig. 1A) or 50% for motor nerve block (Fig. 1B) in the concentration range examined.

Application of ultrasound (defined as high-frequency, low-intensity ultrasound (HFLIU)) after TTX (TTX + HFLIU) did not increase the frequency of sensory or motor nerve block, nor did co-injection of microbubbles with the TTX (TTX + MB) (Fig. 1). In contrast, when TTX was injected with microbubbles followed immediately by application of HFLIU (TTX + MB + HFLIU) there was an increase in the percentage of successful sensory and motor nerve block at concentrations of 25–35 μM of TTX (Fig. 1A, B). For example, at 25 μM TTX, 87.5% of sensory blocks were successful in the TTX + MB + HFLIU group compared to 0% in the TTX + HFLIU group ($p < 0.001$), 12.5% in the TTX + MB group ($p < 0.001$), and 25% in the TTX only group ($p = 0.006$) (Fig. 1A).

Similarly, co-application of HFLIU and microbubbles resulted in a statistically significant increase in the percentage of successful motor block at 30 μM TTX: 100% of the animals in the TTX + MB + HFLIU group developed motor block compared to 25% in TTX + HFLIU group ($p < .001$), 22% in TTX + MB group ($p < 0.001$), and 45% in the TTX group ($p < 0.001$). There was no statistically significant difference in the frequency of sensory or motor nerve block in any control group (TTX, TTX + MB, TTX + HFLIU) at any of the concentrations of TTX tested.

3.2. Effects of treatments on the duration of nerve blockade

In animals treated with TTX alone, the duration of sensory blockade increased with increasing concentrations of TTX (Fig. 2A). Application of HFLIU after TTX (TTX + HFLIU) did not result in an increase in sensory block duration, nor did co-injection of microbubbles (TTX + MB). However, treatment with microbubbles and ultrasound (TTX + MB + HFLIU) resulted in a significant prolongation of sensory nerve block compared to TTX, TTX + HFLIU, and TTX + MB at each concentration of TTX tested (Fig. 2A). For example, at 35 μM TTX, animals in the TTX + MB + HFLIU group had a median duration of sensory blockade of 172 [139–205] min (median [interquartile range]) compared to a median duration of 52 [4–100] min in the TTX group ($p < 0.001$), 37 [4–67] min in the TTX + MB group ($p < 0.001$), and 57 [3–108] min in the TTX + HFLIU group ($p < 0.001$) (Fig. 2A); this was a three-fold prolongation of block due to HFLIU + MB. There was no difference in the durations of sensory nerve block between TTX, TTX + HFLIU, TTX + MB. We observed a similar effect on duration of sensory nerve block when insonation energy was increased to 0.1 W/cm^2 (SI Fig. 4). Animals treated with TTX, microbubbles, and HFLIU underwent neurobehavioral testing 6 and 24 h following treatment. These animals had a mean thermal latency of 3.2 s at 6 h (range 1.8–4.3 s) and 2.5 s at 24 h (range 1.9–3.3 s) post injection, indicating complete resolution of nerve blockade.

As with sensory block, motor block was greatly prolonged in animals treated with TTX + MB + HFLIU at 30 and 35 μM TTX compared to all the other groups tested (Fig. 2B). For example, at 35 μM TTX, the TTX + MB + HFLIU group had a median duration of motor nerve block of 134 [132–157] min compared to 0 [0–82] min and 36 [0–80] min in the TTX ($p < 0.001$) and TTX + MB ($p < 0.001$) groups. In the 35 μM

TTX + HFLIU group, motor nerve blockade had a median duration of 113 min, albeit with a large interquartile range [0 − 120] min ($p < 0.01$) (Fig. 2B). The durations of motor nerve block in the TTX, TTX + MB, and TTX + HFLIU groups were not statistically significantly different.

To assess the possibility that microbubbles or HFLIU alone contributed to nerve block, rats were injected at the sciatic nerve with microbubbles alone then were treated with HFLIU. These animals had a mean thermal latency of 3.19 s (range 3.09–3.69 s) and a maximum weight bearing ability of 138 g (mean 124–155 g), indicating they did not have sensory or motor nerve blockade.

Reliability and reproducibility are important features of peripheral nerve blockade. The ratio of interquartile range to median provides an assessment of variability within treatment groups, analogous to a percent coefficient of variation (the larger the ratio, the more variability). At all concentrations tested, ratios of interquartile range to median of sensory or motor nerve block in the TTX, TTX + MB, TTX + HFLIU groups were > 1, often much higher. In the TTX + MB + HFLIU group, these ratios were ≤ ~1, except in the 25 μM group where variability was high due to the relatively high rate of unsuccessful nerve blocks (SI Fig. 5).

3.3. Systemic effects of treatments

Systemic effects after local injection of TTX suggest systemically distributed TTX. Systemic effects, such as contralateral nerve block, respiratory distress, and/or death, suggest systemically distributed TTX. Animals treated with TTX + MB + HFLIU had a greater percentage of animals with contralateral nerve block (Fig. 3A) and/or respiratory distress (Fig. 3B) than animals treated with single therapy (HFLIU or MB). At 30 μM dosage of TTX, this difference was significant. The percentage of mortality was not statistically significantly different between any groups (Fig. 3C).

3.4. Tissue reaction to treatments

Tissue reaction was assessed by examination of hematoxylin-eosin stained sections of the injection site, adjacent nerve and surrounding muscle, 4 and 14 days after injection. These time points reflect acute and chronic inflammation respectively, and the progression of myotoxicity. Representative hematoxylin and eosin stained tissues at the site of injection 4 days following injection are shown to highlight the mild inflammation and myotoxicity observed (Fig. 4).

Each hematoxylin-eosin section was then scored for myotoxicity and inflammation as described [29]. Scoring was performed on three animals per treatment group, all treatments containing with 35 μM of TTX (Fig. 5). Animals treated with TTX alone had minimal inflammation (score of 1 out of 4) and no myotoxicity (score 0 out of 6) at both 4 days and 14 days, as described previously [29] [30]. Myotoxicity had resolved by 14 days in all groups. Each group had mild residual inflammation at 14 days. There was no statistically significant difference with any other group tested.

3.5. Effects of treatments on nerve blockade with bupivacaine

Animals were injected with microbubbles and the amino-amide local anesthetic bupivacaine (15.4 mM; 0.5%; a clinically relevant concentration) with or without ultrasound application. Treatment with microbubbles and ultrasound did not improve nerve block success or extend sensory or motor nerve blockade mediated by 0.5% bupivacaine (Fig. 6). When a lower concentration of bupivacaine (0.05%) was injected, sensory or motor nerve block was not achieved in any animal tested. Treating with microbubbles, ultrasound, or a combination of ultrasound and microbubbles did not improve nerve block success.

4. Discussion

S1SCBs could offer advantages over conventional anesthetics, as they are less likely to cause local neurotoxicity and myotoxicity [4,29] or seizures and cardiac arrest [2,3]. SISCBs effectiveness is limited by nerve block inconsistency and short duration at low drug concentrations, secondary to limited penetration to peripheral nerves even when injected locally. Nerve blockade reliability and/or duration can be improved by co-injection with adjuvant drugs, which can be associated

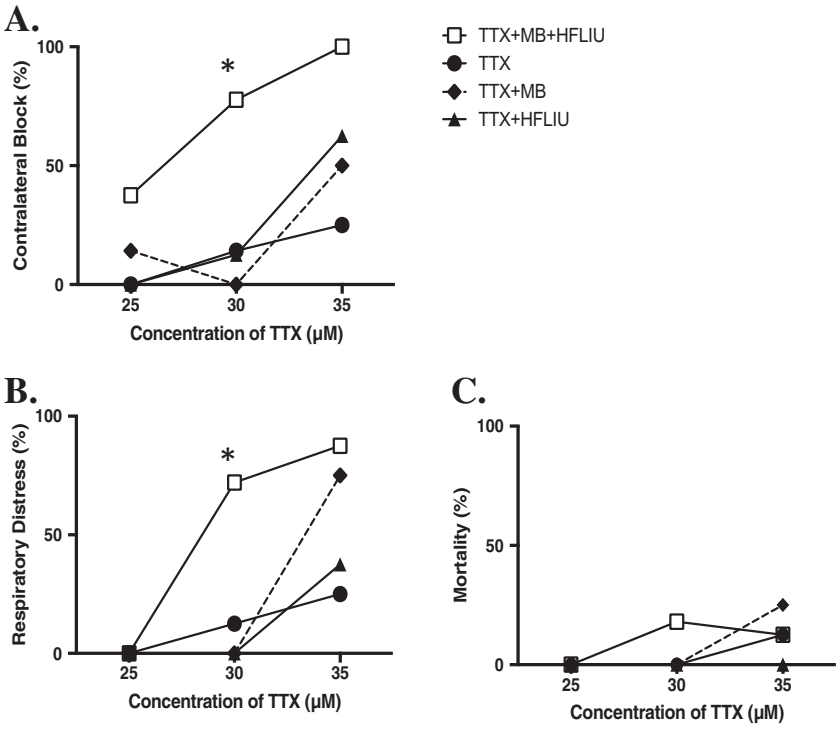

Fig. 3. Effect of treatments on percentage of (A) contralateral nerve block, (B) respiratory distress, (C) mortality. TTX; tetrodotoxin, HFLIU: high-frequency, low-intensity ultrasound, MB: microbubbles. *$p < .05$, comparing TTX + MB + HFLIU to TTX, TTX + HFLIU, and TTX + MB by Fisher's exact test. Differences in percentage of contralateral block or respiratory distress among TTX, TTX + MB, TTX + HFLIU were not statistically significant.

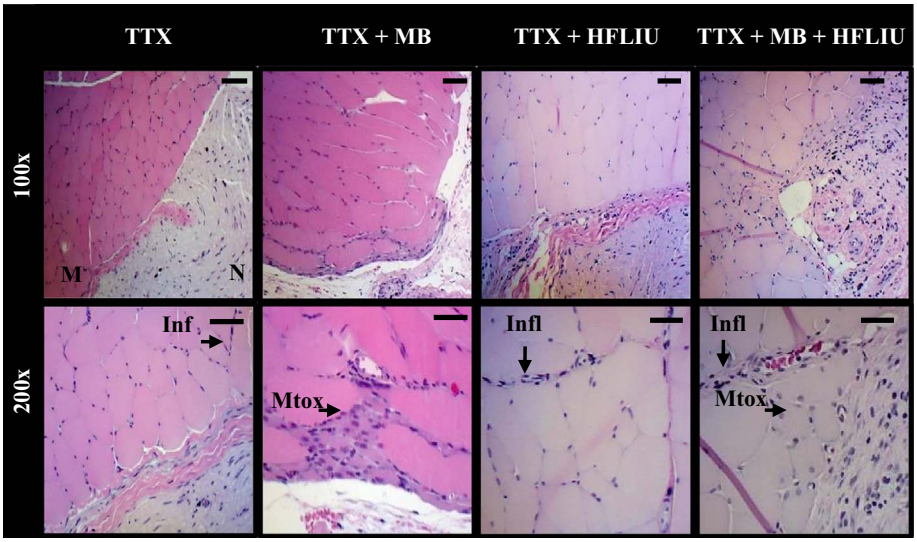

Fig. 4. Representative (of three separate samples) hematoxylin and eosin stained tissues at the site of injection 4 days following injection, shown at 100× and 200× magnification. TTX: 35 μM TTX tetrodotoxin, TTX + US: TTX + high-frequency, low-intensity ultrasound (HFLIU), MB: microbubbles, M: muscle, N: nerve, Infl: inflammation, Mtox: myotoxicity. Scale bar equals 50 μm.

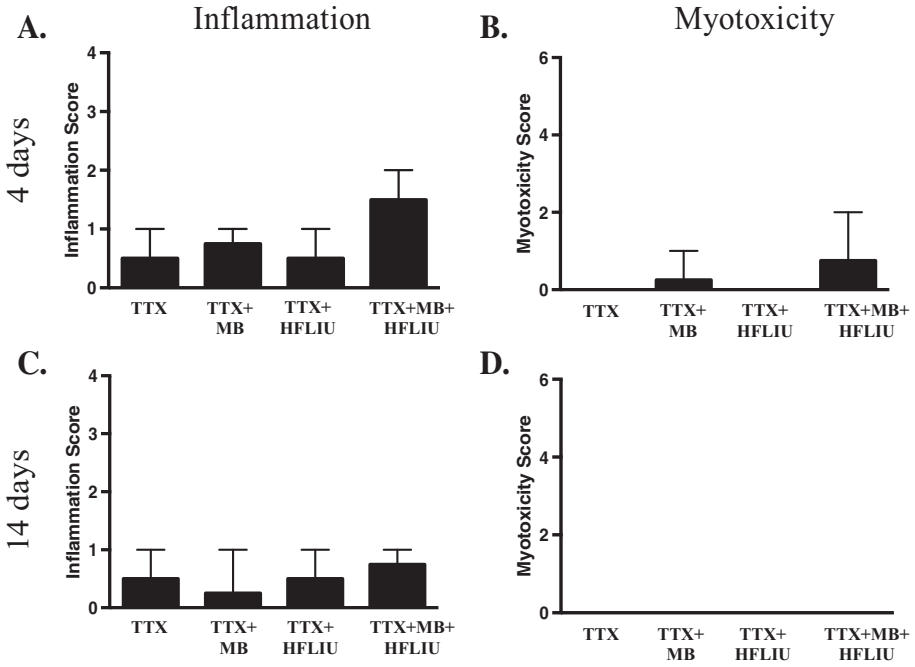

Fig. 5. Inflammation and myotoxicity scores at the injection site at 4 and 14 days. 35 μM TTX (tetrodotoxin), HFLIU: high-frequency, low-intensity ultrasound, MB: microbubbles. Data represent three animals per treatment group. There was no statistically significant difference in inflammation or myotoxicity between treatment groups by Mann-Whitney U Test.

with side-effects [17], by injecting large doses of TTX which are often associated with systemic toxicity [9], or by osmotic shock to peripheral nerve [31]. Here, we have demonstrated that high-frequency, low-intensity ultrasound (HFLIU) in conjunction with microbubbles markedly improved TTX block frequency, and duration of sensory and motor nerve blockade. This enhancement of effect was associated with an increase in systemic distribution. Tissue reaction was unaffected by HFLIU and microbubbles.

At low doses of TTX, animals receiving TTX + MB + HFLIU appeared to have a higher likelihood of developing sensory than motor blockade. However, caution is indicated in describing the effect as sensory-predominant since the effect is modest and the metrics for sensory and motor testing are different and may not be completely comparable. At low doses of TTX (25 μM) combination HFLIU and microbubble treatment resulted in reliable nerve blockade and at 30 μM TTX, nerve blockade was greater than or equal to the duration achieved with 0.5% bupivacaine which is used in a variety of clinical applications including single shot peripheral nerve blocks.

Ultrasound can have intrinsic effects on neuronal function. In animal models of neuronal injury, neuronal suppression secondary to acoustic waves is proportional to acoustic intensity administered, with focused high intensity (35 W/cm^2) resulting in suppressed axonal conduction, intensities such as 390–3000 W/cm^2 generating nerve blockade that last for weeks [32] and with very high intensity (7890 W/cm^2) resulting in eventual complete axon degeneration [33,34]. These intensities are orders of magnitude higher than those used in clinical imaging, or those used here. At lower ultrasound intensities, albeit still significantly higher than those used clinically, ultrasound induces nerve stimulation rather than suppression [35]. The effect of ultrasound parameters similar to those used with clinical imaging ultrasound is not well studied. These data demonstrate that animals treated with TTX, microbubbles, and HFLIU had complete resolution of nerve blockade. In addition, we did not observe clinical evidence of nerve damage (e.g. abnormal ambulation) during a two-week observation period following

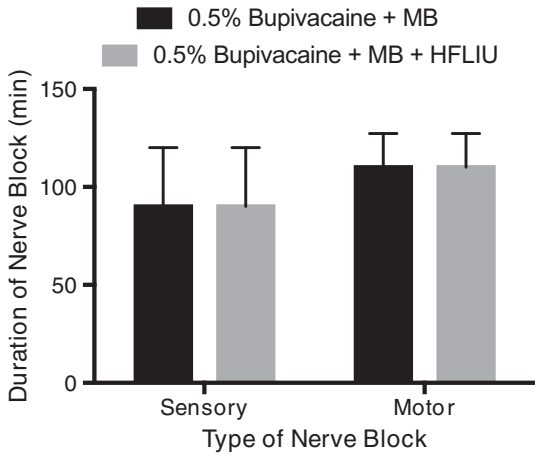

Fig. 6. Effect of treatment on the duration of sensory and motor nerve block with 0.5% bupivacaine and microbubbles with or without ultrasound. HFLIU: high-frequency, low-intensity ultrasound. Data are medians with interquartile ranges. N = 3; see Methods. There was no statistically significant difference between treatment groups by Mann-Whitney U Test.

injection, suggesting permanent nerve damage with these ultrasound parameters and microbubbles is unlikely.

While local tissue injury was not evident, treatment with HFLIU and microbubbles was associated with increased systemic toxicity compared to TTX alone. In contrast, the increase in duration of block caused by chemical permeation enhancers was not accompanied by an increase in systemic toxicity [16,17]. One explanation for this difference might be that while both modalities enhanced the local permeability of the barriers to the penetration of TTX between the site of injection and the axonal surfaces, HFLIU + microbubbles may also have contributed to spread of the injectate, enhancing systemic distribution.

It is likely that the systemic toxicity associated with TTX, with or without HFLIU and microbubbles would be reduced in larger animals, as toxicity of a given dose of anesthetic is in direct proportion to the volume of distribution of the recipient (much larger in a human than a rat) while local anesthetic effect is not [9]. This view is supported by the use of the site 1 sodium channel blocker neosaxitoxin as a local anesthetic in humans, at doses that would be uniformly fatal in small animals [13,14].

The number-weighted size distribution of microbubbles used in this study is larger than the size ranges reported among commercially available products (size range 2.5–4.9 μm) [36]. Microbubbles used clinically as ultrasound contrast agents (e.g. Definity, Sonazoid, Optison, PESDA) are specifically designed for intravascular administration. Of commercially available products, PESDA microbubbles, which have the greatest percentage of bubbles in the 6–10 μm range, demonstrated enhanced *in vitro* and *in vivo* gene delivery at 1 MHz compared to smaller microbubbles [36]. While the size of microbubbles used as ultrasound contrast agents are limited by capillary diameter (7–10 μm), this is not the case for local delivery to the sciatic nerve.

While it is possible to penetrate bone with ultrasound [37], albeit with significant attenuation, in this particular model we did not attempt to propagate ultrasound through bone. In these experiments, the animal was placed in a lateral decubitus position and the ultrasound probe was held posteromedially to the greater trochanter, pointed in an anteromedial direction. With the animal in this position, the sciatic nerve is 0.7 cm below the surface of the skin and only covered by layers of muscle and skin and not by bone (SI Fig. 3). Therefore, the effects of bone might not be important for the anatomic reasons described above.

Treatment with HFLIU and microbubbles did not improve the performance of the conventional amino-amide anesthetic, bupivacaine. This could be because bupivacaine, an amphiphilic and moderately hydrophobic molecule, has the intrinsic capability to pass easily through biological barriers [38], and so enhancement of barrier permeability with HFLIU and microbubbles might not be expected to be beneficial. This view is consistent with the observation that chemical permeation enhancers, which markedly increase the duration of block from the very hydrophilic TTX, have no effect on the duration of block from bupivacaine [16].

Unlike low-frequency ultrasound (< 100 kHz), which can be associated with dose dependent thermal effects [39] the ultrasound used in this study was of high frequency and low intensity, similar to conventional clinical imaging ultrasound [40]. While low-frequency ultrasound has been successful in transdermal drug delivery, it may not be safe [39]or appropriate for all drug delivery uses, such as ocular or perineural uses, making HFLIU a potentially safer alternative. However, we found that high-frequency, low-intensity ultrasound alone does not enhance drug flux to the sciatic nerve. With microbubbles, HFLIU parameters similar to conventional clinical imaging (2–10 MHz) enhanced TTX nerve blockade. Currently, ultrasound-guidance is the gold standard in many centers for identifying peripheral nerves for nerve block. Thus, ultrasound could be used both for needle guidance and to enhance nerve block.

5. Conclusion

Using an *in vivo* model system of local anesthetic delivery, we demonstrate that treatment with high-frequency, low-intensity ultrasound together with microbubbles improved nerve block effectiveness, increased nerve block reliability, and resulted in a prolongation of both sensory and motor nerve blockade mediated by the hydrophilic ultrapotent local anesthetic, tetrodotoxin. The same approach did not work with the more hydrophobic drug bupivacaine.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2018.02.027.

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