A Phase 1, Dose-escalation, Double-blind, Block-randomized, Controlled Trial of Safety and Efficacy of Neosaxitoxin Alone and in Combination with 0.2% Bupivacaine, with and without Epinephrine, for Cutaneous Anesthesia

Kimberly Lobo, M.S., M.P.H., Carolina Donado, M.D., Laura Cornelissen, Ph.D., Joseph Kim, M.A., Rebeca Ortiz, B.A., Roy W. A. Peake, Ph.D., Mark Kellogg, Ph.D., Mark E. Alexander, M.D., David Zurakowski, Ph.D., Katherine E. Kurgansky, M.P.H., James Peyton, M.B.Ch.B., Aykut Bilge, M.D., Ph.D., Karen Boretsky, M.D., Mary Ellen McCann, M.D., M.P.H., Charles B. Berde, M.D., Ph.D., Joseph Cravero, M.D.

CME This article has been selected for the Anesthesiology CME Program. Learning objectives and disclosure and ordering information can be found in the CME section at the front of this issue.

ABSTRACT

Background: Neosaxitoxin (NeoSTX) is a site-1 sodium channel blocker that produces prolonged local anesthesia in animals and humans. Under a Food and Drug Administration–approved phase 1 Investigational New Drug trial, the authors evaluated safety and efficacy of NeoSTX alone and combined with 0.2% bupivacaine (*Bup*) with and without epinephrine.

Methods: The authors conducted a double-blind, randomized, controlled trial involving healthy male volunteers aged 18 to 35 yr receiving two 10-ml subcutaneous injections. Control sites received *Bup*. In part 1, active sites received (1) 5 to 40 µg NeoSTX+Saline (*NeoSTX-Saline*), (2) 5 to 40 µg NeoSTX+Bup (*NeoSTX-Bup*), or (3) placebo (*Saline*). In part 2, active sites received 10 or 30 µg NeoSTX+Bup+Epinephrine (*NeoSTX-Bup-Epi*) or placebo. Primary outcome measures were safety and adverse events associated with NeoSTX. Secondary outcomes included clinical biochemistry, NeoSTX pharmacokinetics, and cutaneous hypoesthesia.

Results: A total of 84 subjects were randomized and completed the two-part trial with no serious adverse events or clinically significant physiologic impairments. Perioral numbness and tingling increased with NeoSTX dose for *NeoSTX-Saline* and *NeoSTX-Bup*. All symptoms resolved without intervention. *NeoSTX-Bup-Epi* dramatically reduced symptoms compared with other NeoSTX combinations (tingling: 0 *vs.* 70%, *P* = 0.004; numbness: 0 *vs.* 60%, *P* = 0.013) at the same dose. Mean peak plasma NeoSTX concentration for *NeoSTX-Bup-Epi* was reduced at least two-fold compared with *NeoSTX-Saline* and *NeoSTX-Bup* (67 ± 14, 134 ± 63, and 164 ± 81 pg/ml, respectively; *P* = 0.016). *NeoSTX-Bup* showed prolonged cutaneous block duration compared with *Bup*, *NeoSTX-Saline*, or *placebo*, at all doses. Median time to near-complete recovery for 10 µg *NeoSTX-Bup-Epi* was almost five-fold longer compared with *Bup* (50 *vs.* 10 h, *P* = 0.007).

Conclusion: NeoSTX combinations have a tolerable side effect profile and appear promising for prolonged local anesthesia. **(Anesthesiology 2015; 123:873-85)**

CURRENTLY available amino ester and amino amide local anesthetics do not reliably provide analgesia beyond approximately 8 to 12 h after subcutaneous infiltration or single-shot peripheral nerve block.[1] Although the duration of analgesia can be extended by catheter infusions, catheters introduce the potential for migration, infection, and the inconvenience of tethering the patient to a pump. In addition, overdoses (or intravascular injection) of amino ester and amino amides can cause systemic and/or local toxicities.[2–6] The risk of local tissue toxicity increases with local anesthetic concentration and duration of exposure. There has been considerable interest in methods of prolonged infiltration analgesia or peripheral nerve blockade using single-injection formulations that are safe, with minimal systemic and local tissue toxicities.

What We Already Know about This Topic

- Clinically available local anesthetics do not reliably provide analgesia beyond 12 h after subcutaneous infiltration or peripheral nerve block
- Neosaxitoxin, a sodium channel blocker which binds to extracellular domains of sodium channels, produces long-lasting anesthesia in animals

What This Article Tells Us That Is New

- In a first-in-human Food and Drug Administration–regulated phase 1 safety study in 84 male volunteers, subcutaneous infiltration of Neosaxitoxin with bupivacaine produced long-lasting anesthesia but no serious adverse events although perioral numbness and tingling were noted at high doses
- Addition of epinephrine reduced circulating Neosaxitoxin concentrations and perioral tingling and numbness and further prolonged sensory block

This article is featured in "This Month in Anesthesiology," page 1A. Corresponding article on page 741. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article.

Neosaxitoxin (NeoSTX) is a site-1 sodium channel blocker that binds to the *outer* pore of the voltage-gated sodium channels, thereby blocking impulse generation and propagation.[7,8] In overdose, site-1 blockers produce reversible skeletal and respiratory muscle weakness.[9,10] Clinical experience with paralytic shellfish poisoning indicates that these toxicities can be treated with respiratory support and that patients make full recovery, without signs of persistent organ toxicities. In a sheep model of repeated intravenous dosing, NeoSTX appeared devoid of cardiotoxicity.[11] Site-1 blockers have also been shown to have benign local effects on nerve and muscle.[12,13]

Several site-1 blockers, including NeoSTX, produce nerve blockade in animals.[11,14] In addition, nerve blockade is markedly potentiated and prolonged when site-1 blockers are administered in combination with bupivacaine or vasoconstrictors (such as epinephrine).[15,16] The effect of vasoconstrictors in reducing systemic toxicity and improving potency and duration appears to be mediated by reducing blood flow in the perineural injection compartment, thereby slowing systemic uptake and maintaining the gradient for drug entry into nerves over the first 30 to 40 min after injection.[17,18] NeoSTX has been shown to produce cutaneous hypoesthesia in volunteers[16,19] and better postoperative analgesia than bupivacaine in a previous human clinical trial.[20]

The primary aim of this study was to assess the systemic safety of NeoSTX, administered by subcutaneous injection, alone and in combination with bupivacaine (with and without epinephrine) in healthy male volunteers. Concerning safety, we hypothesized the following:

1. NeoSTX would have a safe cardiovascular, neuromuscular, respiratory, and laboratory profile at doses 5 to 70 µg.
2. Systemic symptoms (*i.e.*, facial numbness and tingling) would be dependent on NeoSTX dose.

A second aim was to examine the relation between plasma NeoSTX concentration and adverse symptoms or physiologic changes. We hypothesized that addition of epinephrine would reduce peak plasma NeoSTX concentration and reduce systemic symptoms compared with groups receiving NeoSTX without epinephrine.

A third aim was to evaluate the relation between intensity and duration of cutaneous hypoesthesia with NeoSTX when used alone and in combination with bupivacaine with and without epinephrine. We hypothesized that NeoSTX combinations would provide more intense and prolonged cutaneous sensory block compared with bupivacaine. We use the term "efficacy" as a convenience instead of "cutaneous hypoesthesia" in the following discussion, recognizing that clinical efficacy can only be established by subsequent trials in patients, rather than volunteers.

Materials and Methods

Trial Design

After obtaining approval from the U.S. Food and Drug Administration and the institutional review board (Boston Children's Hospital, Boston, Massachusetts), we conducted an investigator-initiated, phase 1, dose-escalation, double-blind, block-randomized, controlled trial of safety and efficacy of NeoSTX alone and in combination with 0.2% bupivacaine, with and without epinephrine, for cutaneous anesthesia (detailed information on drug production and preclinical testing is provided in Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A1). This study was conducted at Boston Children's Hospital (IND 109623), between May 2013 and March 2014. All subjects provided written informed consent. The trial was registered at ClinicalTrials.gov (registration number: NCT01786655; date of registration: February 6, 2013; principal investigator: Joseph Cravero, M.D.) and was overseen by an independent Data Safety Monitoring Board.

Participants. Participants were recruited by local advertisement and were screened before enrollment. Subjects were healthy males between 18 and 35 yr of age. Exclusion criteria included (1) cognitive disabilities; (2) cardiovascular, neurologic, respiratory, or neuromuscular diseases or psychological disorders; (3) known allergy to bupivacaine or suspected allergy to any local anesthetics; (4) prescribed medication known to alter cognition or pain tolerance; (5) skin rashes or disruption involving the skin over the posterior calf; and (6) lack of a stable home environment. Since reproductive toxicology studies have not yet been performed, enrollment was restricted to male subjects.

Interventions. The study was divided in two parts according to the treatment combination allocated to the active treatment site. Each subject received two subcutaneous injections in immediate succession in a 3-cm × 3-cm square over the posterior gastrocnemius (one injection per leg). One injection was administered at the active treatment site and one at the control site. All subjects received 10 ml of 0.2% bupivacaine (*Bup*) at the control site.

Part 1 (Dose Escalation). Sixty-six subjects were randomized to receive one of three treatments at the active treatment site: (1) NeoSTX in saline (*NeoSTX-Saline*); (2) NeoSTX in BUP (*NeoSTX-Bup*); or (3) placebo (*Saline*). The dose escalation was designed to test 5 to 70 µg NeoSTX. Within each dose cohort, one subject was randomized to receive saline, whereas the remaining subjects were randomized to receive *NeoSTX-Saline* or *NeoSTX-Bup* in a 1:1 ratio (fig. 1).

Part 2 (Three-way Drug Combination). Eighteen subjects were randomized to receive one of two treatments: (1) NeoSTX in *Bup* with epinephrine 5 μg/ml (*NeoSTX-Bup-Epi*) or (2) placebo (*Saline*). Subjects were allocated into one of two dose cohorts receiving either 10 or 30 μg NeoSTX, respectively. As in part 1, control sites were injected with *Bup*. Within each cohort, one subject received a placebo and all others received the active drug combination (fig. 1).

Randomization and Masking. The randomization strategy was designed to account for an adaptive design, in which the numbers of subjects in each dose step was planned to vary based on the frequency of symptoms or physiologic changes at that particular dose step, as outlined in figure 1. Therefore, for part 1 of the study, we prepared randomized assignments for 90 potential subjects using computer-generated randomized tables. Each dose cohort included the maximum number of possible randomization slots (15) at that dose to account for the fact that the adaptive design resulted in variable group sizes for many of the doses. At each dose step, of the 15 potential slots assigned to each dose cohort, the first seven were assigned into block 1, the next four into block 2, and the next four into block 3. Within each block, the subjects who

presented sequentially were assigned to the specific treatment and injection side levels. Part 1 of the clinical trial included 66 subjects. Part 2 was an add-on trial at two NeoSTX doses, 10 and 30 μg. A total of 84 subjects were finally randomized and completed the clinical trial. Researchers, clinicians, nurses, and participants were blinded to the identity of the injections throughout the trial.

Study Procedures

Subjects fasted for at least 8 h before drug administration. Safety measurements included an assessment of adverse symptom frequency and severity; neuromuscular, respiratory, and hemodynamic monitoring (noninvasive oscillometric blood pressure, pulse oximetry [SpO_2], respiratory rate, and end-tidal carbon dioxide [$ETCO_2$] monitoring); 12-lead electrocardiograms; and clinical biochemistry (hematologic, renal, and hepatic function) laboratory tests. Efficacy measures consisted of mechanical and thermal quantitative sensory testing (QST) of the active treatment and control sites. Subjects were discharged from the inpatient unit after all the 24-h assessments were completed and returned to the hospital daily for between 2 and 7 follow-up visits. Two weeks
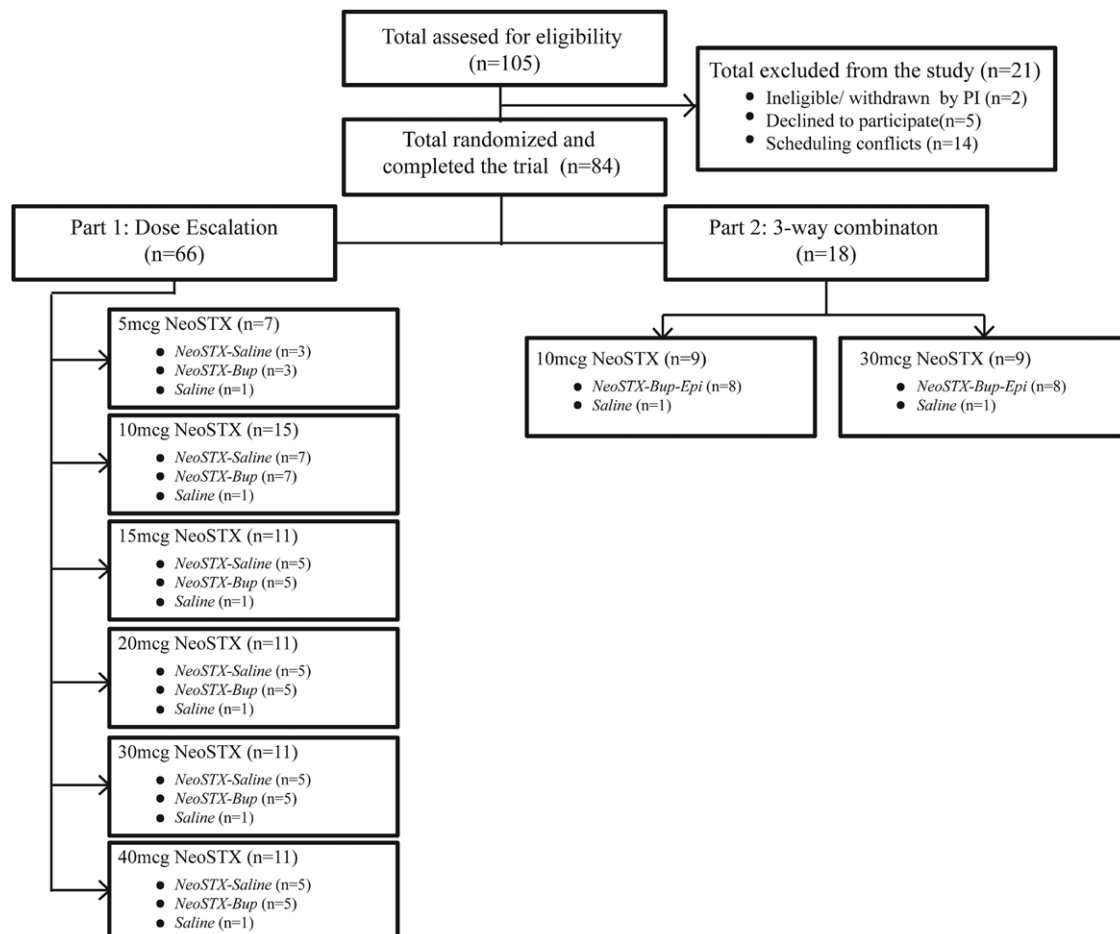

Fig. 1. Flow diagram of study design. NeoSTX = neosaxitoxin; *NeoSTX-Bup* = neosaxitoxin in bupivacaine; *NeoSTX-Bup-Epi* = Neosaxitoxin in bupivacaine with epinephrine; *NeoSTX-Saline* = neosaxitoxin in saline; PI = principal investigator.

after injection, subjects were contacted by phone and asked about any side effects that they thought might be related to the test drug (fig. S1, Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A2 presents the time points at which study tests were performed).

Adverse Symptom Reporting. Subjects were asked to report the presence and intensity of adverse symptoms. Previous studies have reported adverse effects associated with site-1 sodium channel blockers as perioral numbness and tingling of lips, tongue, and fingertips.[9] Data on nausea (no/sometimes/often or most of the time/all of the time), vomiting (no/once or twice/three or more times), dizziness (1 to 5 scales; 1 being none and 5 being severe), and perioral tingling and numbness (0 to 10 scales; 0 being no sensation and 10 being the most unbearable sensation imaginable) were collected from validated scales.[21–23] For purposes of this article, perioral "numbness" is a self-reported symptom, not a measurement of hypoesthesia to stimuli.

Neuromuscular Function (Grip Strength). A dynamometer (Jamar Hydraulic Hand Dynamometer; Sammons Preston, USA) was used to measure hand-grip strength (GS). Subjects were asked to sit in the upright position and to hold the dynamometer using the dominant hand with the elbow flexed at 90°. Subjects were then asked to squeeze their fingers around the grip as tight as they could for 3 s. At each time point, three recordings were documented and averaged for analysis.

Respiratory Strength. Vital Capacity (VC) (CareFusion Corp., USA) and negative inspiratory force (NIF) (NIF Meter; Instrumentation Industries, Inc., USA) were measured by trained research personnel using handheld spirometry per standardized American Thoracic Society guidelines. At each time point, two recordings were documented and averaged for analysis.

Electrocardiograms. Electrocardiograms were acquired digitally with a sampling rate of 250 Hz and analyzed online with manual correction of QT (QTcB), manual heart rate confirmation, and PR/QRS duration. P, QRS, and T wave axes were taken from the automated reading.

Pharmacokinetic Analysis. Blood samples were obtained for all subjects from an indwelling angiocatheter. NeoSTX was measured in plasma using an Acquity Ultraperformance® Liquid Chromatography system coupled to a Xevo TQ-S triple quadropole tandem mass spectrometer (Waters Corporation, USA). The provisional limit of quantification used was 10 pg/ml. Urine samples were taken at baseline and *ad libitum* (further details on pharmacokinetic analytical methods are provided in the Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A3).

Quantitative Sensory Testing (QST). To test the duration and density of sensory blockade, a series of sensory tests evaluating sensitivity to mechanical and thermal stimuli were conducted. The QST procedure started with the evaluation of mechanical detection and pain thresholds, followed by thermal detection and pain thresholds[24] (detailed information

on QST procedures is provided in the Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A4).

Outcome Measures

The primary outcome of interest was severity and frequency of adverse events (AEs). AEs were graded as mild (grade 1), moderate (grade 2), severe (grade 3), life threatening (grade 4), and death (grade 5) according to the Food and Drug Administration guidelines.[25] Subjects were asked to rate their perioral numbness and tingling on a 0 to 10 scale. Clinically significant perioral numbness and tingling were defined as being greater than 3/10 and lasting 30 min or longer. Nausea, vomiting, and dizziness were coded as yes/no. A greater than 30% change from baseline values for neuromuscular (GS) and respiratory (VC and NIF) measures and abnormal vital signs are presented in the Results section.

Secondary measures included changes from baseline for clinical biochemistry; NeoSTX plasma pharmacokinetics for 30 μg *NeoSTX-Saline*, *NeoSTX-Bup*, and *NeoSTX-Bup-Epi* groups; and duration and intensity of sensory blockade as measured by QST. Mechanical detection and pain thresholds (mechanical detection threshold [MDT] and mechanical pain threshold [MPT]) and cool detection threshold (CDT) are presented in the Results section. Time to partial recovery was defined as the time to 50% recovery from the maximum experimental value to the subjects' own baseline. The maximum experimental values for cutaneous hypoesthesia were defined for MDT as 180 g (von Frey hair number 18); for MPT as 300 g (von Frey hair number 20); and for CDT as 5°C. Near-complete recovery was defined as the time required for MDT/MPT to return to subjects' own baseline + 2 hairs and for CDT to return to subjects' own baseline –3°C. Percentage of subjects in each cohort with dense block (greater than the maximum experimental value), moderate block (maximum experimental value to partial recovery), mild block (partial recovery to near-complete recovery), and minimal block (less than near-complete recovery) at 24 and 48 h were also evaluated. Reliability of block at 5 and 30 min is defined as having dense or moderate block for each QST parameter.

Statistical Methods

Sample size for initial dose steps was calculated based on the "rule of 3s" in phase 1 studies in oncology.[26] Our dose-escalation plan specified initial dose escalation with groups of three subjects in each treatment combination or seven subjects per dose step (three subjects receiving *NeoSTX-Saline*, three subjects receiving *NeoSTX-Bup*, and one subject receiving placebo) until two grade 2 AEs occurred. Once we reached that threshold, group sizes were expanded to 11 or 15 subjects per dose step.

Stopping rules based on physiologic endpoints were not met. However, after review with the study Data Safety Monitoring Board, the frequency and severity of AEs (self-reported symptoms) at the 40 μg NeoSTX dose (while not

dangerous) were considered bothersome enough to preclude clinical use at doses higher than 40 μg in awake volunteers. Based on the dose–response of symptoms occurring in part 1 of the trial, a *post hoc* decision was made to include the 10- and 30-μg *NeoSTX-Bup-Epi* groups (part 2 of the trial) with a total of nine subjects in each dose cohort, with eight subjects receiving *NeoSTX-Bup-Epi* and one subject receiving placebo (*Saline*).

Statistical analyses were performed using SAS version 9.3 (SAS Institute, USA) and all hypotheses were two tailed. Mean values with SDs are reported for physiologic measures. Fisher exact test was used to evaluate the effect of treatment combination on the occurrence of perioral tingling and numbness. Neuromuscular, respiratory, hemodynamic, and pharmacokinetic analyses were performed using ANOVA followed by *post hoc* Bonferroni correction when needed.

For efficacy data, all treatment group comparisons were evaluated against the eight subjects receiving 0.2% bupivacaine at the control site and placebo (*Saline*) at the active treatment site. To test for possible systemic analgesic effect of NeoSTX, we performed QST analyses on two sites: (1) the contralateral posterior calf (control) sites (*Bup*; n = 84) and (2) a remote site (thenar eminence, n = 72) (see Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A6).

QST data were tested for normality using the Shapiro–Wilk test. Where deviations from normality were identified, group comparisons were analyzed using nonparametric Kruskal–Wallis test corrected for multiple comparisons using rank-sum tests (Dunn test) or Fisher exact test, where appropriate. Generalized linear models of time to near-complete and partial recovery were used for an exploratory analysis to evaluate the influence and interaction of dose (5 to 40 μg) and treatment combinations (*NeoSTX-Saline* and *NeoSTX-Bup*) (detailed information on this statistical procedure and results are presented in Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A6).

Results

Participants

A total of 84 subjects were enrolled. All subjects completed the trial and were included in the final analysis (fig. 1). The original study plan included evaluation of *NeoSTX-Saline* and *NeoSTX-Bup* at doses from 5 up to 70 μg NeoSTX. Based on the frequency and severity of systemic symptoms (perioral numbness, tingling, and nausea) observed in subjects receiving the 40 μg NeoSTX dose, we ended the dose escalation at this point with a total of 66 subjects, and predefined stopping rules based on physiologic endpoints for this part of the study were not met. The second part of the study included a total of 18 subjects receiving three-way combinations of *NeoSTX-Bup-Epi versus* placebo (*Saline*). No deviation from the inclusion and exclusion criteria occurred. Demographic profiles are shown in table 1.

Adverse Events

No serious AEs occurred (grade 4 or higher) and no subject required any medical intervention. The majority of the AEs were rated as being either mild (grade 1) or moderate (grade 2) in intensity and no subject discontinued the study due to AEs. The most common AEs among all subjects included perioral tingling (48%) and numbness (52%).

One grade 3 AE was observed during the trial. This was characterized by transient intense tingling and numbness in the face and hands. In this subject, symptoms began 3-min postinjection with the maximum intensity of perioral tingling and numbness observed within the first hour; symptoms gradually reduced and were completely resolved by the 6-h time point. There was no impairment of oxygenation or ventilation and no clinically significant changes in cardiac rhythm, rate, or blood pressure. The grading of this AE was based on our investigator-defined criteria of intensity of self-reported symptoms, not by physiologic impairment.

At each NeoSTX dose, at least one subject presented with one or more systemic symptoms though at the lower doses, these were all in the clinically insignificant range. Symptoms of perioral numbness and tingling increased in frequency and severity in a dose-dependent manner for the *NeoSTX-Saline* and *NeoSTX-Bup* groups. Dizziness, nausea, and vomiting were also noted at higher doses (Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A5, table 1). Frequency of clinically significant perioral tingling and numbness was significantly reduced in the 30-μg *NeoSTX-Bup-Epi* group compared with those receiving the same dose of NeoSTX in the other treatment combinations (tingling: 0 *vs.* 70%, $P = 0.004$; numbness: 0 *vs.* 60%; $P = 0.013$; Fisher exact test) (fig. 2).

Neuromuscular and Respiratory Function

Mean GS, NIF, and VC values did not differ between dose cohorts at baseline. Overall, neuromuscular and respiratory function remained stable postinjection. The number of subjects presenting with a greater than 30% decrease from baseline for GS, NIF, and VC values in at least one study time point during the first 2-h postinjection is provided in table 2 (see Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A5, for detailed information on outliers for neuromuscular and respiratory function).

Vital Signs, Electrocardiograms, and Clinical Biochemistry

Overall, vital signs remained stable postinjection. No significant differences in mean heart rate, systolic blood pressure, and diastolic blood pressure were observed between dose cohorts at baseline. Table 2 presents the total number of subjects presenting with vital signs outside of the predefined normal range in at least one study time point up to 2-h postinjection (see Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A5, for detailed information on abnormal vital signs).

Table 1. Dose Escalation and Demographics

Neosaxitoxin Dose (µg)	Total (n)	Age (yr)	Weight (kg)	Height (m)	BMI (kg/m²)
0	8	25 (5)	77.4 (12.0)	1.7 (0.1)	26.9 (3.0)
Part 1: dose escalation					
5	6	25 (4)	71.0 (9.4)	1.7 (0.1)	24.3 (3.3)
10	14	25 (4)	86.9 (9.7)	1.8 (0.1)	26.1 (3.5)
15	10	24 (4)	75.7 (15.4)	1.8 (0.1)	23.6 (4.5)
20	10	26 (6)	74.7 (11.0)	1.8 (0.1)	22.7 (3.4)
30	10	28 (4)	81.6 (16.7)	1.7 (0.1)	27.7 (6.2)
40	10	23 (2)	70.9 (9.3)	1.8 (0.0)	22.6 (3.2)
Part 2: three-way combination					
10	8	26 (3)	72.8 (9.1)	1.8 (0.1)	22.6 (3.1)
30	8	26 (5)	84.6 (12.5)	1.8 (0.1)	26.5 (4.3)

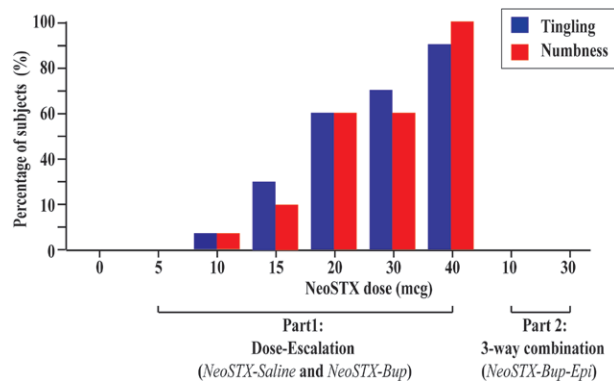Age, weight, height, and BMI presented as mean (SD).
BMI = body mass index.

Fig. 2. Percentage of subjects with clinically significant perioral numbness and tingling after Neosaxitoxin (NeoSTX) injection. Clinically significant perioral tingling and numbness were defined as being more than level 3/10 (with 0 being no sensation and 10 being an unbearable sensation) and lasting 30 min or longer. *NeoSTX-Bup* = Neosaxitoxin in bupivacaine; *NeoSTX-Bup-Epi* = Neosaxitoxin in bupivacaine with epinephrine; *NeoSTX-Saline* = Neosaxitoxin in saline.

Analysis of electrocardiographic data showed no significant arrhythmias and no systematic change in QTcB. When examining the delta-delta QTcB, the mean change was 12.5 ms, with the dominant contributor to that being the 40 µg *NeoSTX-Saline* and *NeoSTX-Bup* dosing groups. There was also a clinically insignificant but statistically significant change in QRS duration, with the maximum change in a decrease of 8 ms in the 40-µg group. No clinically significant changes in electrocardiographic parameters were observed for the two *NeoSTX-Bup-Epi* groups.

Laboratory studies showed no clinically significant changes in any of the hematologic, renal, and hepatic values that were attributed to the study medication.

Pharmacokinetic Data
There was a rapid increase in the mean plasma concentration to a peak value in the first 30 min after injection for the *NeoSTX-Saline* and *NeoSTX-Bup* groups (fig. 3). For the *NeoSTX-Bup-Epi* group, this rapid increase in plasma concentration was markedly suppressed. At the 30-min time point, the NeoSTX plasma concentration for the *NeoSTX-Bup-Epi* group (26 ± 15 pg/ml) was approximately five-fold lower than for the *NeoSTX-Saline* group (134 ± 63 pg/ml, $P = 0.013$; ANOVA) and approximately six-fold lower than for the *NeoSTX-Bup* group (164 ± 81 pg/ml, $P = 0.002$; ANOVA). *NeoSTX-Bup-Epi* peak concentration (67 ± 14 pg/ml) occurred much later around the 12-h postinjection time point and was at least two-fold lower than for the *NeoSTX-Saline* and *NeoSTX-Bup* groups ($P = 0.016$, ANOVA).

Duration and Intensity of Sensory Blockade
Onset of Cutaneous Sensory Block. At the 5- and 30-min postinjection time points, we observed an immediate decrease in cutaneous sensitivity (increase in threshold) to mechanical and thermal stimuli for all treatment groups. Reliability of cutaneous nerve block was greater using NeoSTX combinations than *NeoSTX-Saline* for the 10-µg group particularly for MPT, which could be used as a surrogate for surgical pain (Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A6, table 2).

Time to Partial and Near-complete Recovery. *Bup* had a median time to partial recovery of 7 h for MDT, 8 h for CDT, and 12 h for MPT. For part 1 of the study, exploratory generalized linear models evaluating the effect of dose and treatment combinations on MDT, MPT, and CDT did not show an effect of NeoSTX dose in any of the models; the differences observed were driven primarily by treatment combination. The 10 and 30 µg *NeoSTX-Bup* and *NeoSTX-Bup-Epi* groups showed a significantly longer time to partial recovery than the *Bup* group for MDT (10 µg: 30 h *NeoSTX-Bup*, $P = 0.007$ and 38 h *NeoSTX-Bup-Epi*, $P < 0.001$; 30 µg: 36 h *NeoSTX-Bup* and 40 h *NeoSTX-Bup-Epi*, $P < 0.001$; Kruskal–Wallis) and MPT (10 µg: 21 h *NeoSTX-Bup*, $P = 0.023$ and 38 h *NeoSTX-Bup-Epi*, $P = 0.001$; 30

Table 2. Mean Baseline Values and Postinjection Deviations from Normal Range

Neosaxitoxin Dose (µg)	Total No.	VC (L)		NIF (cm H₂O)		GS (kg)		HR (Beats/min)			SBP (mmHg)			DBP (mmHg)		
		Mean (SD)*	>30% Decrease from Baseline	Mean (SD)†	>30% Decrease from Baseline	Mean (SD)‡	>30% Decrease from Baseline	Mean (SD)§	<45 (n)	>100 (n)	Mean (SD)‖	<90 (n)	>150 (n)	Mean (SD)#	<50 (n)	>100 (n)
0	8	5 (1)	0	−116 (9)	0	37 (6)	0	67 (11)	0	0	121 (8.5)	0	0	72 (9)	0	0
Part 1: dose escalation																
5	6	5 (1)	0	−116 (11)	0	42 (8)	0	68 (13)	0	0	123 (15)	0	0	71 (5)	1	0
10	14	5 (1)	0	−103 (16)	0	42 (8)	0	67 (8)	0	0	127 (11)	0	1	70 (10)	1**	0
15	10	5 (1)	0	−105 (19)	0	42 (8)	0	60 (11)	1	1	123 (12)	0	0	69 (10)	1	0
20	10	5 (1)	0	−112 (12)	0	40 (8)	0	59 (9)	0	2	122 (7)	0	1	71 (7)	0	1
30	10	5 (1)	0	−104 (26)	0	43 (5)	1	63 (11)	0	0	127 (10)	1	0	75 (9)	1	0
40	10	5 (1)	0	−106 (18)	0	34 (11)	1	64 (11)	0	2	126 (7)	0	0	76 (9)	1	1
Part 2: three-way combination																
10	8	5 (1)	0	−108 (14)	0	39 (7)	0	62 (10)	0	0	122 (8)	0	0	74 (9)	0	1
30	8	5 (1)	0	−107 (22)	0	38 (4)	0	63 (12)	0	0	131 (11)	0	1	76 (61)	0	0

Subjects counted only once even if values were out of the predefined range at two or more time points within the same subject. ANOVA test P value models showed no significant differences when compared across Neosaxitoxin doses.

* $P = 0.886$; † $P = 0.701$; ‡ $P = 0.174$; § $P = 0.605$; ‖ $P = 0.458$; # $P = 0.631$. ** This subject had a DBP reading of 47 mmHg at baseline.

DBP = diastolic blood pressure; GS = grip strength; HR = heart rate; NIF = negative inspiratory force; SBP = systolic blood pressure; VC = vital capacity.
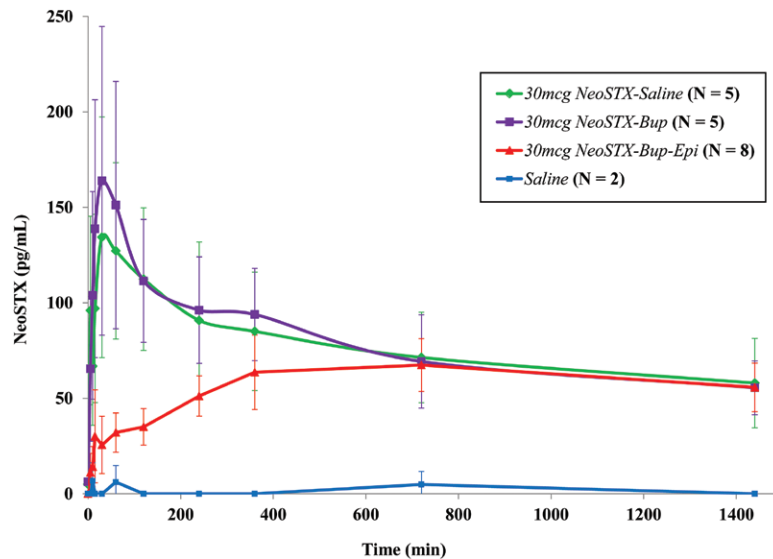
Fig. 3. Neosaxitoxin (NeoSTX) plasma concentrations over time. Data presented as mean and SD for 30-μg Neosaxitoxin treatment groups. *NeoSTX-Bup* = Neosaxitoxin in bupivacaine; *NeoSTX-Bup-Epi* = Neosaxitoxin in bupivacaine with epinephrine; *NeoSTX-Saline* = Neosaxitoxin in saline.

μg: 35 h *NeoSTX-Bup*, P = 0.029 and 47 h *NeoSTX-Bup-Epi*, P < 0.001; Kruskal–Wallis). Similar patterns were observed for CDT (10 μg: 4 h *NeoSTX-Bup*, P = 0.86 and 47 h *NeoSTX-Bup-Epi*, P = 0.012; 30 μg: 1 h *NeoSTX-Bup*, P = 0.999 and 43 h *NeoSTX-Bup-Epi*, P = 0.562; Kruskal–Wallis); however, statistical significance was observed only in the *NeoSTX-Bup-Epi* cohorts. Time to near-complete recovery followed the same distribution as time to partial recovery (fig. 4) (see Supplemental Digital Content 1, http://links.lww.com/ALN/B194, Section A6, table 3, for detailed information on time to near-complete and partial recovery for all dose cohorts and treatment groups).

Density of Block at 24 and 48 h. Median values for each QST parameter at study time points are presented in figure 5. At 24 h, a greater percentage of subjects had dense or moderate block in all QST parameters at 10 and 30 μg for all NeoSTX combinations when compared with the *Bup* group (fig. 6A). At 48 h, for MDT and MPT, the 30-μg *NeoSTX-Bup-Epi* group had a higher proportion of subjects with some degree of block (50.0%), followed by 10 μg *NeoSTX-Bup-Epi* (40.0%), 30 μg *NeoSTX-Bup* (25.0%), and 10 μg *NeoSTX-Bup* (MDT: 14.3%; MPT: 28.6%). At 48 h, no subjects in the *Bup* group had moderate or dense block to mechanical stimuli (both MPT and MDT) (fig. 6B).

Discussion

Researchers have long sought a safe, long-acting, local anesthetic that would be clinically useful for patients during or after surgery or with any painful condition that is amenable to nerve blockade. Such a drug or combination could potentially provide days rather than hours of pain control without the necessity for indwelling catheters and pumps. In this phase 1 clinical trial of NeoSTX subcutaneous injection, we present the first detailed human data on safety and AEs for this drug with symptom scoring and physiologic measurements. We report dose–response for cutaneous sensory block using the combinations in which NeoSTX would most likely be used in clinical practice—NeoSTX in combination with bupivacaine, with and without epinephrine.

Our findings indicate that NeoSTX has few hemodynamic effects. Throughout this study, regardless of dose or drug combination, subjects generally maintained stable vital signs. Of the 84 total subjects enrolled, one subject experienced a brief vasovagal episode associated with mild bradycardia and hypotension, which resolved rapidly without treatment. No treatment was required for any subject. Analysis of electrocardiographic data showed no clinically significant atrial or ventricular rhythm disturbances or changes in QTc intervals in any of the subjects.

Respiratory and neuromuscular function was similarly stable at the doses examined in this trial. We did not observe any clinically significant changes in oxygen saturation or $ETco_2$ in any subject. NIF did not change significantly at any time point for any participant, and VC and GS measurements remained within the normal reference ranges. Small within-subject variations in measurements at times appeared effort related, especially with testing overnight, and were not dose related.

These results are consistent with *previous* animal studies that showed that neuromuscular, respiratory, and cardiovascular effects of NeoSTX were mild and dose dependent using the dose range anticipated for clinical use.[11] In addition, in human studies where subjects received similar NeoSTX doses, no gross signs of weakness or cardiovascular toxicities were observed even with doses significantly higher than those used here.[19,20]
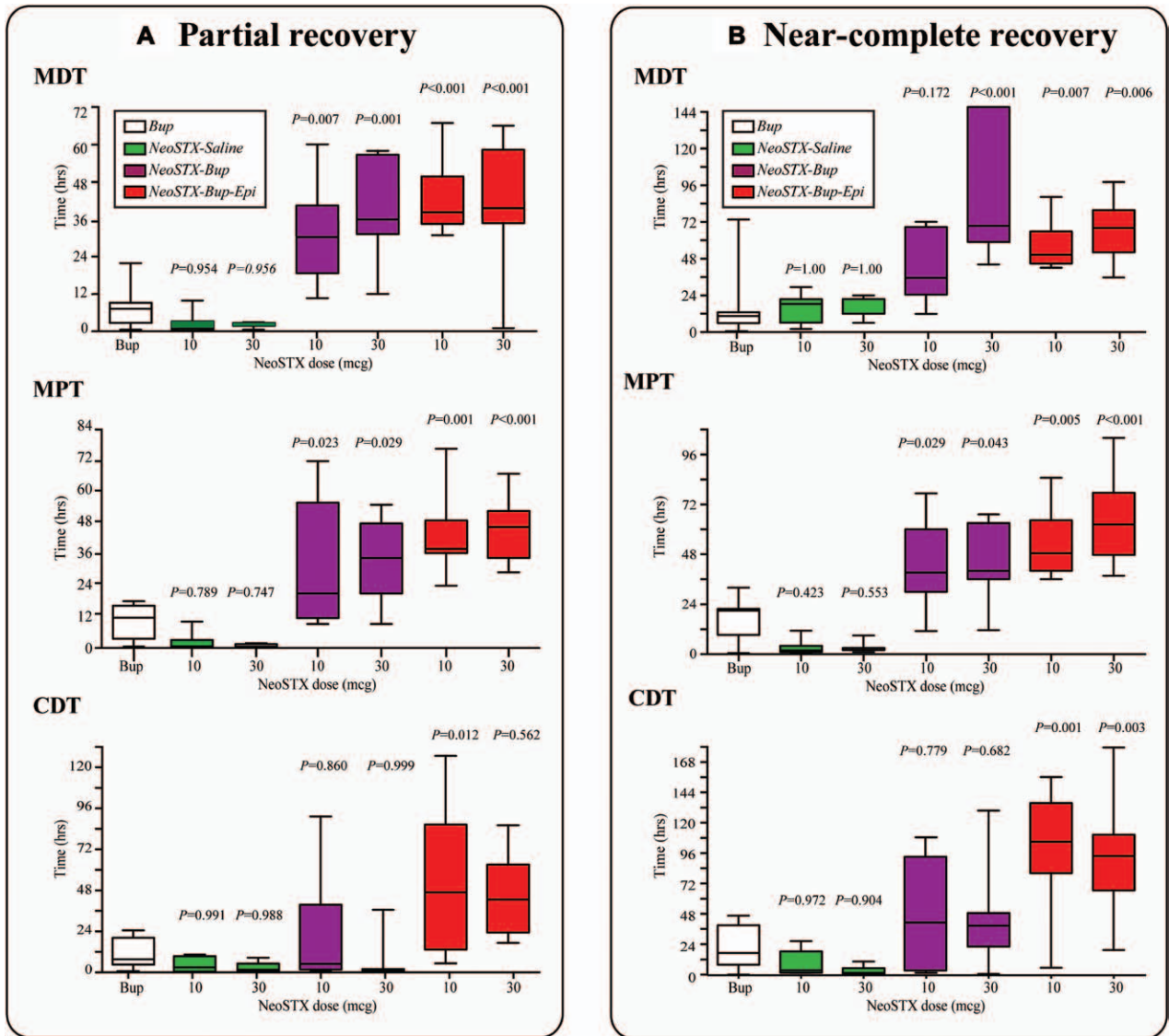
Fig. 4. Time to partial (*A*) and near-complete recovery (*B*) of cutaneous mechanical thresholds in 10 and 30 µg Neosaxitoxin in saline (*NeoSTX-Saline*), Neosaxitoxin in bupivacaine (*NeoSTX-Bup*), and Neosaxitoxin in bupivacaine with epinephrine (*NeoSTX-Bup-Epi*) treatment groups. *Horizontal lines* represent median values, *boxes* represent interquartile ranges, and *whiskers* represent minimum–maximum values. *P* values present the comparison of that group against the bupivacaine (*Bup*) group using Kruskal–Wallis and Dunn test. CDT = cold detection threshold; MDT = mechanical detection threshold; MPT = mechanical pain threshold; NeoSTX = Neosaxitoxin.

Symptoms such as tingling and numbness of the lips and tongue, nausea, and dizziness are common with unintentional ingestion of site-1 blockers as contaminants of shellfish.[9] We noticed a high frequency of these symptoms in our subjects, although (as described) none of the symptoms were accompanied by measurable physiologic impairments or required any hemodynamic and respiratory support. The symptoms usually began approximately 15 to 30 min after injection and dissipated within 90 min after injection. These symptoms increased in frequency and intensity in a dose-dependent manner. At doses *less than* 40 µg in part 1 of the study, subjects reported these symptoms as being mildly bothersome, which did not

affect their regular activity or disrupt further testing. However, at the 40 µg dose in part 1 of the study, the intensity of systemic symptoms observed in subjects increased significantly and were accompanied with nausea, vomiting, and dizziness that decreased in intensity over the next 1 to 2 h. We ended the dose escalation at this concentration (40 µg) because of the frequency and intensity of these symptoms. In part 2 of the study, addition of epinephrine dramatically suppressed the frequency and intensity of these transient symptoms.

Previous human volunteer studies of NeoSTX in doses of 50 µg (NeoSTX alone) or 10 µg (NeoSTX-BUP or NeoSTX with epinephrine) in awake volunteers did not report
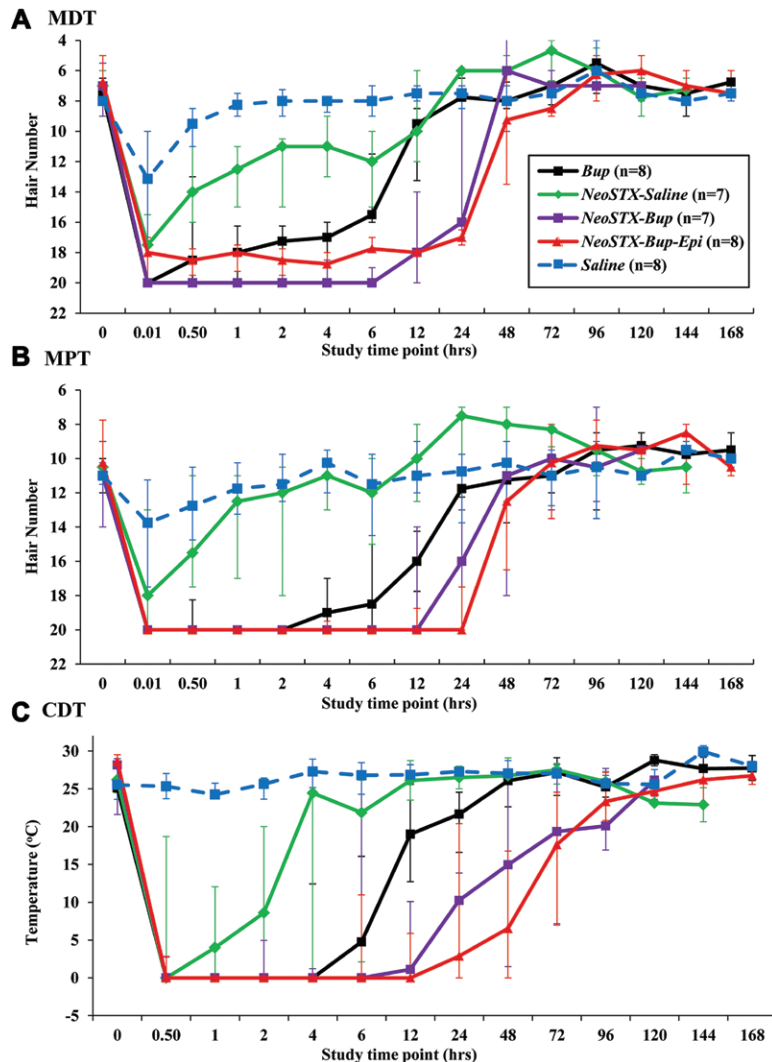
Fig. 5. Median quantitative sensory testing values at study time points for bupivacaine (*Bup*), placebo (*Saline*), and 10 µg Neosaxitoxin in saline (*NeoSTX-Saline*), Neosaxitoxin in bupivacaine (*NeoSTX-Bup*), and Neosaxitoxin in bupivacaine with epinephrine *(NeoSTX-Bup-Epi)* treatment groups. (*A*) Mechanical detection threshold (MDT), (*B*) mechanical pain threshold (MPT); (*C*) cold detection threshold (CDT). *Symbols* represent median values and *whiskers* represent interquartile ranges.

systemic symptoms.[16,19] There are several possible reasons for this discrepancy. Previous studies did not use systematic recording of symptoms using rating scales. In addition, the study using 50 µg involved drug formulations that had a different pH compared with the current study.[19] Another previous study using 100-µg injections was performed in surgical patients under general anesthesia and thus making the subjective symptoms of perioral numbness, tingling, and nausea unreportable.[20] Further studies involving larger sample sizes and a range of patient populations as well as the effect of sex, age, and a full range of medical comorbidities will be required to fully define the recommended maximum safe and tolerable doses for NeoSTX when used in combination with bupivacaine with and without epinephrine.

Previous animal and human studies have shown that the local anesthetic effect of site-1 blockers is potentiated when coinjected with conventional local anesthetics or

vasoconstrictors such as epinephrine.[15,16] Several mechanisms may potentially contribute to these additive or synergistic interactions.[17,18,27,28] Epinephrine is known to slow drug uptake from the injection site, presumably by decreasing local blood flow *via* vasoconstriction,[17,18] thereby reducing the absorption rate of NeoSTX and the occurrence of side effects presented. Our preliminary pharmacokinetic analyses show that the peak concentration for NeoSTX when used either alone or in combination with bupivacaine was reached within the first 30-min postinjection. This correlates with the time of maximum intensity and frequency of systemic symptoms. Addition of epinephrine to the combination of NeoSTX and bupivacaine at NeoSTX doses of 30 µg dramatically reduced the peak NeoSTX concentration. This observation is consistent with the decreased frequency and intensity of systemic side effects in this cohort. More extensive pharmacokinetic analyses of blood and urine
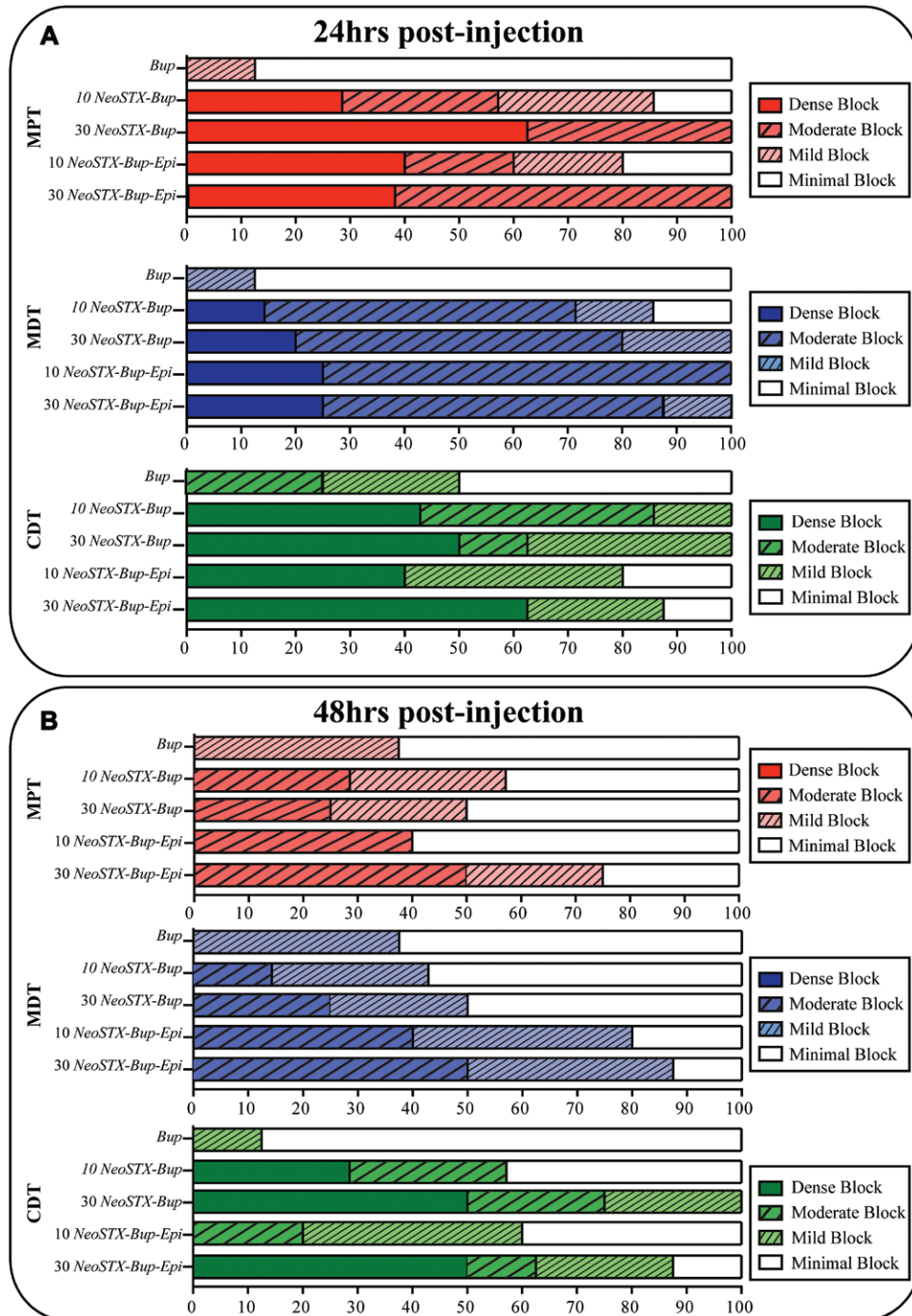
Fig. 6. Percentage of subjects at (*A*) 24 h and (*B*) 48 h postinjection with dense, moderate, mild, and minimal block to mechanical and thermal stimuli in 10 and 30 μg Neosaxitoxin in saline (*NeoSTX-Saline*), Neosaxitoxin in bupivacaine (*NeoSTX-Bup*), and Neosaxitoxin in bupivacaine with epinephrine (*NeoSTX-Bup-Epi*) treatment groups. At 24 h mechanical pain threshold (MPT) *P* < 0.001; mechanical detection threshold (MDT) *P* < 0.001; cold detection threshold (CDT) *P* = 0.074, Fisher exact test. At 48 h MPT *P* = 0.494; MDT *P* = 0.353; CDT *P* = 0.010, Fisher exact test. *Bup* = bupivacaine.

are in progress, along with efforts to characterize metabolites. In future studies, additional cohorts will be required to better define whether further increases in NeoSTX dose in *NeoSTX-Bup-Epi* combinations can be tolerated with minimal symptoms, particularly in patients with a range of medical conditions. Bupivacaine and NeoSTX also may have a

direct synergism by virtue of different site of actions on the sodium channel, and bupivacaine may act to some degree as a chemical permeation enhancer.[29,30]

NeoSTX-Bup and *NeoSTX-Bup-Epi* combinations showed reliable and prolonged cutaneous anesthesia, as assessed by QST. In comparison with *Bup*, *NeoSTX-Bup-Epi* produced

nearly five-fold prolongation of time to near-recovery of MDTs. With *NeoSTX-Bup-Epi* 10 μg, cold detection remained partially impaired (moderate-to-dense block) at 24 and 48 h in 40 and 20% of subjects, respectively.

Quantitative sensory testing is a useful surrogate measure of cutaneous anesthesia, but it is only an approximate predictor of intensity and duration of local anesthesia and analgesia in surgical patients.[31] Nevertheless, the time course of onset and recovery of cutaneous anesthesia from *NeoSTX-Bup* and *NeoSTX-Bup-Epi* combinations in this study appears very promising for further study as a clinically useful prolonged duration local anesthetic. An ideal agent for perioperative use should have (1) very rapid onset of dense blockade, permitting surgery under local or regional anesthesia, (2) persistence of dense and reliable blockade through the first postoperative night, and (3) a prolonged period of partial blockade over the next 2 or 3 days.[32] Based on the time course and intensity of block using QST measures in this phase 1 study, *NeoSTX-Bup* and *NeoSTX-Bup-Epi* appear promising for showing these favorable features when used for surgical patients.

Limitations

Under the constraints of this first U.S. investigator-initiated Investigational New Drug trial that mandated truncation of the trial when symptoms became significantly bothersome, our dose escalation was limited by the occurrence of bothersome numbness and tingling sensations in awake volunteers at a dose of 40 μg. We were therefore unable to escalate NeoSTX dosing to a range that would define a human threshold for clinically important respiratory, neuromuscular, or cardiovascular impairments. Future studies in anesthetized subjects may permit further examination of physiologic effects in doses greater than 40 μg. QST may be an imperfect predictor of adequacy of local anesthetic action for surgical anesthesia and for intensity and duration of postoperative analgesia.

Conclusion

In this first detailed study of safety, symptoms, and physiologic data for NeoSTX (alone and in combination with bupivacaine and epinephrine) in awake humans, our findings reveal that NeoSTX combinations did not produce physiologic impairments when used at the doses studied. The addition of epinephrine reduces the frequency and severity of systemic symptoms. *NeoSTX-Bup* and *NeoSTX-Bup-Epi* combinations appear promising for progressing into phase 2 trials as prolonged duration local anesthetic formulations for surgical anesthesia and postoperative analgesia.

Acknowledgments

Competing Interests

Dr. Berde and his collaborators (Drs. Daniel Kohane, Gary Strichartz, and Robert Langer) hold issued patents on site-1 blockers, including Neosaxitoxin, for prolonged duration local anesthesia. Dr. Berde is Investigational New Drug holder for Neosaxitoxin. Boston Children's Hospital, Boston, Massachusetts, has a collaboration agreement with Proteus S.A., Santiago, Chile, for commercial development of Neosaxitoxin. In the event of future commercial development, Dr. Berde, his coinventors, and Boston Children's Hospital, Boston, Massachusetts, could potentially receive royalties. Dr. Berde has received no research support, equity, consulting fees, or other income from Proteus S.A. or any other commercial partner related to this clinical trial. Proteus S.A. did supply Neosaxitoxin at no charge, but they did not fund the clinical trial. As per Boston Children's Hospital Conflict of Interest Policy, Dr. Berde had no contact with human subjects for this study, however, was involved in study design and editing of the article. Boston Children's Hospital and the Children's Hospital Medical Center Anesthesia Foundation both provided partial funding for this trial. In the event of successful commercial development, both institutions could potentially receive royalties and other payments. The other authors declare no competing interests.

Reproducible Science

Full protocol available at: joseph.cravero@childrens.harvard.edu. Raw data available at: joseph.cravero@childrens.harvard.edu.

Correspondence

Address correspondence to Dr. Cravero: Department of Anesthesiology, Perioperative, and Pain Medicine, Boston Children's Hospital, 300 Longwood Avenue, Boston, Massachusetts 02115. joseph.cravero@childrens.harvard.edu. This article may be accessed for personal use at no charge through the Journal Web site, www.anesthesiology.org.

References

1. Klein SM, Greengrass RA, Steele SM, D'Ercole FJ, Speer KP, Gleason DH, DeLong ER, Warner DS: A comparison of 0.5% bupivacaine, 0.5% ropivacaine, and 0.75% ropivacaine for interscalene brachial plexus block. Anesth Analg 1998; 87:1316–9

2. Butterworth JF IV: Models and mechanisms of local anesthetic cardiac toxicity: A review. Reg Anesth Pain Med 2010; 35:167–76

3. Di Gregorio G, Neal JM, Rosenquist RW, Weinberg GL: Clinical presentation of local anesthetic systemic toxicity: A review of published cases, 1979 to 2009. Reg Anesth Pain Med 2010; 35:181–7

4. Drasner K: Local anesthetic neurotoxicity: Clinical injury and strategies that may minimize risk. Reg Anesth Pain Med 2002; 27:576–80

5. Lambert LA, Lambert DH, Strichartz GR: Irreversible conduction block in isolated nerve by high concentrations of local anesthetics. ANESTHESIOLOGY 1994; 80:1082–93

6. Padera R, Bellas E, Tse JY, Hao D, Kohane DS: Local myotoxicity from sustained release of bupivacaine from microparticles. ANESTHESIOLOGY 2008; 108:921–8

7. Catterall WA: Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. Annu Rev Pharmacol Toxicol 1980; 20:15–43

8. Penzotti JL, Lipkind G, Fozzard HA, Dudley SC Jr: Specific neosaxitoxin interactions with the Na^+ channel outer vestibule determined by mutant cycle analysis. Biophys J 2001; 80:698–706

9. de Carvalho M, Jacinto J, Ramos N, de Oliveira V, Pinho e Melo T, de Sá J: Paralytic shellfish poisoning: Clinical and electrophysiological observations. J Neurol 1998; 245:551–4

10. Isbister GK, Kiernan MC: Neurotoxic marine poisoning. Lancet Neurol 2005; 4:219–28

11. Wylie MC, Johnson VM, Carpino E, Mullen K, Hauser K, Nedder A, Kheir JN, Rodriguez-Navarro AJ, Zurakowski D, Berde CB: Respiratory, neuromuscular, and cardiovascular effects of neosaxitoxin in isoflurane-anesthetized sheep. Reg Anesth Pain Med 2012; 37:152–8

12. Epstein-Barash H, Shichor I, Kwon AH, Hall S, Lawlor MW, Langer R, Kohane DS: Prolonged duration local anesthesia with minimal toxicity. Proc Natl Acad Sci U S A 2009; 106:7125–30

13. Sakura S, Bollen AW, Ciriales R, Drasner K: Local anesthetic neurotoxicity does not result from blockade of voltage-gated sodium channels. Anesth Analg 1995; 81:338–46

14. Kohane DS, Lu NT, Gökgöl-Kline AC, Shubina M, Kuang Y, Hall S, Strichartz GR, Berde CB: The local anesthetic properties and toxicity of saxitonin homologues for rat sciatic nerve block *in vivo*. Reg Anesth Pain Med 2000; 25:52–9

15. Kohane DS, Lu NT, Cairns BE, Berde CB: Effects of adrenergic agonists and antagonists on tetrodotoxin-induced nerve block. Reg Anesth Pain Med 2001; 26:239–45

16. Rodriguez-Navarro AJ, Lagos M, Figueroa C, Garcia C, Recabal P, Silva P, Iglesias V, Lagos N: Potentiation of local anesthetic activity of neosaxitoxin with bupivacaine or epinephrine: Development of a long-acting pain blocker. Neurotox Res 2009; 16:408–15

17. Sinnott CJ, Cogswell III LP, Johnson A, Strichartz GR: On the mechanism by which epinephrine potentiates lidocaine's peripheral nerve block. ANESTHESIOLOGY 2003; 98:181–8

18. Masuda T, Cairns BE, Sadhasivam S, Dunning PS, Berde CB: Epinephrine prevents muscle blood flow increases after perineural injection of tetrodotoxin. ANESTHESIOLOGY 2004; 101:1428–34

19. Rodriguez-Navarro AJ, Lagos N, Lagos M, Braghetto I, Csendes A, Hamilton J, Figueroa C, Truan D, Garcia C, Rojas A, Iglesias V, Brunet L, Alvarez F: Neosaxitoxin as a local anesthetic: Preliminary observations from a first human trial. ANESTHESIOLOGY 2007; 106:339–45

20. Rodríguez-Navarro AJ, Berde CB, Wiedmaier G, Mercado A, Garcia C, Iglesias V, Zurakowski D: Comparison of neosaxitoxin *versus* bupivacaine *via* port infiltration for postoperative analgesia following laparoscopic cholecystectomy: A randomized, double-blind trial. Reg Anesth Pain Med 2011; 36:103–9

21. Wengritzky R, Mettho T, Myles PS, Burke J, Kakos A: Development and validation of a postoperative nausea and vomiting intensity scale. Br J Anaesth 2010; 104:158–66

22. Meier PM, Alexander ME, Sethna NF, De Jong-De Vos Van Steenwijk CC, Zurakowski D, Berde CB: Complex regional pain syndromes in children and adolescents: Regional and systemic signs and symptoms and hemodynamic response to tilt table testing. Clin J Pain 2006; 22:399–406

23. Galer BS, Jensen MP: Development and preliminary validation of a pain measure specific to neuropathic pain: The Neuropathic Pain Scale. Neurology 1997; 48:332–8

24. Gröne E, Crispin A, Fleckenstein J, Irnich D, Treede RD, Lang PM: Test order of quantitative sensory testing facilitates mechanical hyperalgesia in healthy volunteers. J Pain 2012; 13:73–80

25. US Department of Health and Human Services, Food and Drug Administration: Guidances (Drugs). Available at: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. Accessed April 27, 2015

26. Ivy SP, Siu LL, Garrett-Mayer E, Rubinstein L: Approaches to phase 1 clinical trial design focused on safety, efficiency, and selected patient populations: A report from the clinical trial design task force of the national cancer institute investigational drug steering committee. Clin Cancer Res 2010; 16:1726–36

27. Scholz A, Vogel W: Tetrodotoxin-resistant action potentials in dorsal root ganglion neurons are blocked by local anesthetics. Pain 2000; 89:47–52

28. Bernards CM, Kopacz DJ: Effect of epinephrine on lidocaine clearance *in vivo*: A microdialysis study in humans. ANESTHESIOLOGY 1999; 91:962–8

29. Hille B: Local anesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. J Gen Physiol 1977; 69:497–515

30. Khoo X, Simons EJ, Chiang HH, Hickey JM, Sabharwal V, Pelton SI, Rosowski JJ, Langer R, Kohane DS: Formulations for transtympanic antibiotic delivery. Biomaterials 2013; 34:1281–8

31. Krumova EK, Zeller M, Westermann A, Maier C: Lidocaine patch (5%) produces a selective, but incomplete block of Aδ and C fibers. Pain 2012; 153:273–80

32. Berde CB: Developing better local anesthetics. Anest Analg 2015; 120:718–20