Anesthesiology 2007; 106:339–45

Neosaxitoxin as a Local Anesthetic

Preliminary Observations from a First Human Trial

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Background: **Neosaxitoxin is a phycotoxin that reversibly blocks the voltage-gated sodium channels at the neuronal level. Its activity results in blocking the axonal conduction, stopping the propagation of the nerve impulse. The objective of the present work was to evaluate neosaxitoxin as a local anesthetic in a human trial.**

Methods: **The authors conducted a randomized, double-blind, placebo-controlled trial with 10 healthy volunteers. Subcutaneous injections were made in the middle posterior skin of the calf: one leg received 50 μg neosaxitoxin, and the contra-lateral leg received placebo. The anesthetic effect was evaluated using a standardized human sensory and pain model. TSA II Neurosensory Analyzer (Medoc Ltd, Minneapolis, MN) and von Frey technique were used to evaluate five parameters: sensory threshold for warm and cold, pain thresholds for heat and cold, and mechanical touch perception threshold. Measurements were made 0, 1, 3, 6, 9, 12, 16, 24, and 48 h after the injections.**

Results: **For all the patients, effective and complete blocking of the evaluated parameters was obtained. As the blocking began to revert gradually, heat pain was the first to return to normal values after 3 h. Cold pain was the longest sensation abolished, achieving 24 h of blockade. The toxin was undetected in blood and urine samples. No adverse reactions to neosaxitoxin were detected.**

Conclusions: **Neosaxitoxin showed an effective local anesthetic effect when injected in the subcutaneous plane. The efficacy of a 50-μg dose of neosaxitoxin was shown. This is the first report of neosaxitoxin as a local anesthetic in a human trial.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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Received from the Department of Surgery, Universidad de Chile, Santiago, Chile. Submitted for publication July 10, 2006. Accepted for publication October 10, 2006. This study was supported by Programa de Cooperación Cientifica Internacional, Agencia Gubernamental investigación Científica (GRICES), Lisbon, Portugal/Consejo Nacional de Ciencia y Tecnología (CONICYT), Santiago, Chile; Fundación de Estudios Biomédicos Avanzados (FEBA) 264 and 265, Facultad de Medicina, Universidad de Chile, Santiago, Chile; and Organisation for the Prohibition of Chemical Weapons (OPCW), The Hague, The Netherlands. Clinical trials government number NCT00273065.

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VOLTAGE-GATED sodium channels are responsible for the rising phase of the action potential in the membranes of neurons and most electrically excitable cells.[1] At least nine distinct voltage-gated sodium channels have been cloned from mammals, being broadly divided by its affinity to tetrodotoxin. $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.7$ are highly tetrodotoxin-sensitive, whereas $Na_V1.5$, $Na_V1.8$, and $Na_V1.9$ are tetrodotoxin-resistant to varying degrees.[2] Many of these channels have specific tissue distributions, determining distinct excitation properties.[3]

Local anesthetics are compounds that reversibly block the neural conduction by occupying enough sodium channels within an axon to interrupt activity, stopping the depolarization, thus preventing the propagation of action potential and neuronal communication.[4]

Clinically used local anesthetics such as aminoamides (*e.g.*, lidocaine) and amino esters (*e.g.*, procaine) inhibit sodium channel activity by binding in the inner pore, entering from the intracellular side of the cell.[1]

In the last 20 yr, there has been an interest in the local anesthetic activity of a group of highly potent natural occurring toxins that bind the outer opening of sodium channels.[5] These agents, including tetrodotoxin and saxitoxin and its analogs, bind to sodium channels with high affinity and reversibility.[6–13]

Paralytic shellfish toxins are a group of phycotoxins, nonprotein neurotoxins, synthesized by dinoflagellates in marine littorals. These toxins are accumulated by filter feeders bivalves, and, when they are consumed by mammals, paralytic shellfish poisoning may occur.[14–16] More than 20 different natural paralytic shellfish poisoning toxins have been found,[14,16–19] all of them known as analogs of saxitoxin, the first one isolated and the most well studied and described.[20]

Neosaxitoxin, one of the saxitoxin analogs, differs from saxitoxin by the addition of one oxygen atom, wherein the hydrogen (-H) at Nitrogen 1 in saxitoxin is replaced by a hydroxyl group (-OH) in neosaxitoxin. Neosaxitoxin has shown greater potency than saxitoxin and its analogs and is also more potent than tetrodotoxin in *in vitro* and *in vivo* animal studies.[5,12] The relative potencies of these toxic compounds in *in vitro* and *in vivo* experiments are neosaxitoxin > saxitoxin > tetrodotoxin.[5,21]

In studies searching for safer local anesthetics with less local neuronal, cardiac, and central nervous system toxicity, it has been suggested that the risk of neuro- and cardiotoxicity may be reduced by developing anesthet-

ics that selectively bind to the tetrodotoxin receptor site.[22] Nevertheless, clinical applicability of tetrodotoxin and saxitoxin and its analogs has been limited by the possibility of systemic toxicity. However, in recent publications, local infiltration of paralytic toxins has been shown to be safe and effective in clinical use.[23–26]

The aim of the present study was to evaluate the local anesthetic effect of neosaxitoxin in a randomized, double-blind, placebo-controlled trial in humans. To test its anesthetic property, a validated human experimental sensory evaluation model was used.[27,28]

Materials and Methods

Ten healthy male volunteers aged 18–32 yr were enrolled. The study was approved by the University of Chile Clinic Hospital Ethics Committee, and written informed consent was obtained from all the participants. Volunteers were interviewed about their health history and underwent a physical examination by Clinic Hospital physicians. Inclusion criteria were healthy men with the ability to understand and respond to the tests performed. Exclusion criteria were patients who consumed any oral analgesics at least 10 days before the study, patients with drugs abuse history, and patients showing any sign of a psychiatric disorder during the clinical examination. These clinical trials were performed with anesthesiologists in attendance and in a location with immediate access to the operating room, with facilities for respiratory and hemodynamic support.

The study was performed as a double-blind, placebo-controlled, randomized trial. Each volunteer acted as his own control. The volunteers were injected in the posterior middle skin of right and left calves. Subcutaneous injections were made, evenly covering a marked quadrangular area (40 × 40 mm) in a standardized fan-like fashion. The injections were made at the two opposite angles of the square, injecting a total of 10 ml solution. A 50-μg dose of neosaxitoxin in a saline solution of 0.9% NaCl was used as a treatment, and saline solution was used as the placebo. A computer-generated randomized table was used to choose the leg to be injected with either the placebo or the toxin in each volunteer. Neosaxitoxin was purified from toxic shellfish collected in Southern Chilean fjords. Toxin purity (98%) was determined by high-performance liquid chromatography with online fluorescence detection and online mass spectroscopy analysis.[14,29] The toxin was diluted in 0.9% saline solution. No other additives were used. The doses were produced in the Laboratorio Bioquímica de Membrana, Facultad de Medicina, Universidad de Chile with the approval of Instituto Nacional de Salud (National Institutes of Health, Santiago, Chile). The areas were infiltrated with 10 ml of the assigned treatment using a 0.8 × 50-mm (21-gauge) needle. Injections and preparations of syringes were performed by different researchers who were not involved in the sensory testing. Pain on injection of the solution, not needle insertion, was evaluated using a 0-10 visual analog scale. This scale was anchored by the descriptions "no pain" (0) and "worst pain imaginable" (10).

Assessment of pain and sensory thresholds was made before the injections (baseline) and 1, 3, 6, 9, 12, 16, 24, and 48 h after the injections. All sensory testing was performed at the same time of the day in an isolated quiet room with a controlled temperature of 23°C. The subjects were resting in a relaxed position with their eyes closed during all assessments.

The touch detection thresholds were determined by mechanical stimuli with a series of monofilaments of different strength. Ten hairs that covered the range from 0.1 to 100 force grams on a logarithmic scale (Touch Test Sensory Evaluators; Stoelting Co., Wood Dale, IL) were used. The touch detection threshold was defined as the least force of mechanical stimulation that produced a sensation of touch or pressure. Eight stimuli covering the infiltrated area were made with each hair, beginning with the lightest of the 10, until at least one half of the stimulations with one hair caused the specified sensation. The eight stimuli were applied with a rate of approximately 1 Hz.

Thermal thresholds were determined by using a computerized contact thermode (TSA II Neurosensory Analyzer; Medoc Ltd, Minneapolis, MN). All thresholds were evaluated using a 30 × 30-mm thermode. All thermal thresholds were determined as the average of three assessments performed at 10-s intervals, from a baseline temperature of 32°C and with a rate of change of 1°C/s. The upper cutoff limit was 50°C, and the lower cutoff was 0°C. Cold and warm detection thresholds were defined as the smallest change from baseline that the volunteer could perceive, and the volunteer pressed a button as soon as the specific sensation was perceived. The heat and cold pain detection threshold was the temperature perceived as painful, and the volunteers were instructed to react to the first sensation of pain.

Localized reactions at the injection site (erythema, discoloration, hematoma, induration, swelling, and blisters) and neosaxitoxin intoxication symptoms such as nausea, headache, ataxia, perioral, and distal limb paresthesia were recorded. Two weeks after the injections, the volunteers returned to the Clinical Hospital where physicians evaluated the injection sites for persistent and delayed reactions and the volunteers were questioned regarding any abnormality. Two months after the injections, the volunteers were contacted by telephone and asked about any trouble or incidents that they thought might be related to the study applications.

Blood and urine samples were taken 1 h and 4 h after the injection to determine the amounts of neosaxitoxin

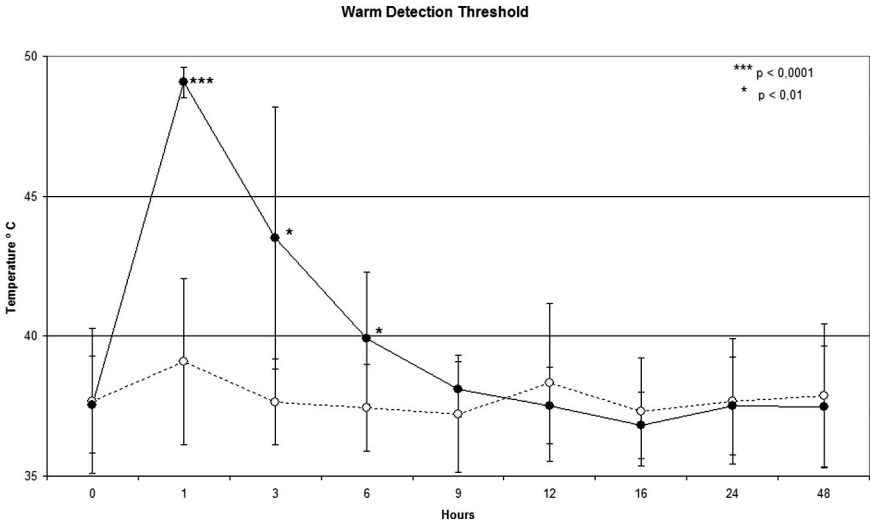

Fig. 1. Warm detection threshold (mean ± SD; $n = 10$). *Open symbols* represent placebo values; *closed symbols* represent neosaxitoxin values.

with the high-performance liquid chromatography technique using online fluorescence.[14,29]

Statistics

Normality of data was evaluated by using the Shapiro-Wilk test. Comparisons were made by using Student *t* test for parametric data and the Mann–Whitney U test for nonparametric data. These analyses were conducted by using Stata Statistical Software (Stata Corp., College Station, TX). *P* values less than 0.05 were considered statistically significant. Neural blockade extent was defined as the period with significantly reduced sensory sensitivity compared with baseline values. Assuming a two-sided type I error protection of 0.05 and a power of 0.90, 10 patients in each of the groups were required to reveal a reduction in mean of perception of 3°C, assuming a SD of 2. Mean ± SD values are reported in figures 1–5.

Results

None of the volunteers presented symptoms of neosaxitoxin intoxication. No toxin could be detected in blood and urine samples, using a cutoff limit of toxin detection by online high-performance liquid chromatography technique of 1×10^{-12} mol. Four volunteers had small hematomas in the infiltration zone (two in the toxin group and two in the placebo group) at the 24-h evaluation; all disappeared after 2 weeks. No other local reactions were presented. None of the volunteers noted any motor disability or discomfort during the follow-up period.

There was no significant difference between the groups in any of the baseline evaluations, giving the values a normal distribution in the five parameters evaluated.

There were no significant differences between the neosaxitoxin and placebo injection pain. The mean pain for the neosaxitoxin injection was 6.5 ± 1.6 and for placebo 5.8 ± 2.1 ($P = 0.41$). Blinding was considered adequate as no differences were observed.

The five parameters presented a complete block at 1 h from the infiltration, showing $P < 0.0001$ in all cases. All parameters reverted to the baseline level in a steady way.

Warm Detection Threshold

All volunteers almost reached the limit of 50°C (mean, 49.1 ± 0.5°C) with no warm sensation (fig. 1). The threshold gradually returned to baseline values 9 h from the infiltration, being significantly elevated for 6 h.

Heat Pain Detection Threshold

All participants reported complete heat pain blockade 1 h post injection, reaching 50°C with no pain sensation (fig. 2). This parameter presented the fastest return to normal values, as the threshold remained elevated for only 3 h after the injection.

Cold Detection Threshold

All participants reported a complete cold sensation blockade the first 3 h post injection, exhibiting the threshold down to 0°C, showing no dispersion (fig. 3). Return to the baseline value was measured 12 h after injection.

Cold Pain Detection Threshold

This parameter took the longest to return to the baseline value (fig. 4). As with the cold sensation, all patients reported a complete block of cold sensation for the first 3 h. The cold pain threshold then slowly returned to its baseline value, achieving a significant 24 h of neural blockade

Touch Detection Thresholds

Data of the applied forces are shown on a logarithmic scale (fig. 5). This parameter presented a significant reduction until 9 h post injection, showing a progressive

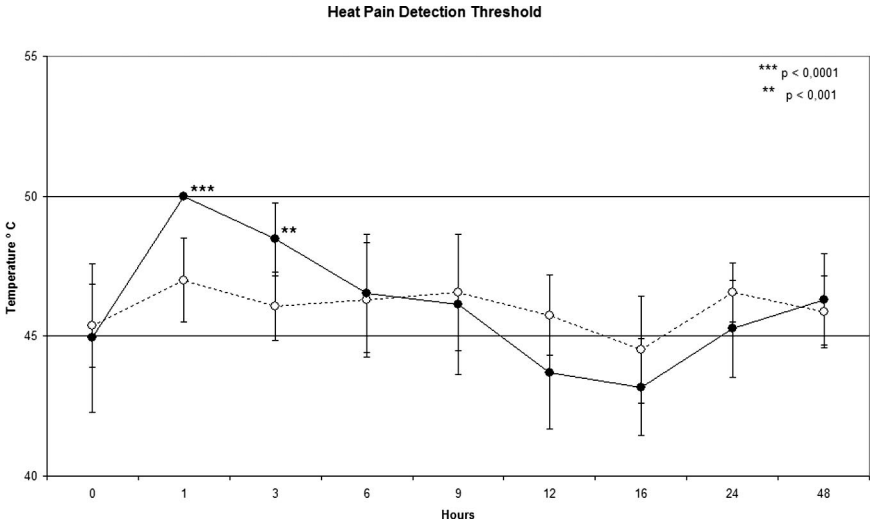

Fig. 2. Heat pain detection threshold (mean ± SD; *n* = 10). *Open symbols* represent placebo values; *closed symbols* represent neosaxitoxin values.

return to normal values, in accordance with the all the other parameters measured.

Discussion

This is the first report of neosaxitoxin testing as a local anesthetic in a human randomized trial. Neosaxitoxin is an extremely potent agent, capable of producing, at a microgram level of dosage, clinically sensitive blockage in a completely reversible manner. Typically, local anesthetics like lidocaine and bupivacaine are used in the 25- to 500-μg range, 1,000 to 10,000 times greater than the neosaxitoxin dose tested in the present study.

In this trial, we found a time-dependant reversal of sensory parameters. The heat pain and warm sensation were the earliest to revert, followed by mechanical perception and cold sensation, and lastly the cold pain sensation. In studies of spinal and epidural anesthesia with lidocaine, a sequential temporal recovery of sensations has been noted, with the pinprick being the first to revert, followed by touch and cold.[30,31] Using transdermal lidocaine applications, it has been reported that pinprick and cold sensations are more strongly affected than the warm sensation measured with the receptor site self-reporting of perceived intensity test.[32] According to nerve fibers involved in perception, C fibers (small unmyelinated) convey afferent sensory information, including that of warm temperatures and a diffuse pain response, whereas A delta fibers (small myelinated) relay the sharp pain response and cold sensation.[33] C fibers are the most resistant to local anesthetics, as shown in the rat sciatic nerve model, and have been shown to be less sensitive to lidocaine than A delta fibers.[34] C fibers are also less sensitive to tetrodotoxin blocking than A delta fibers in the frog sciatic nerve preparation and in the rat dorsal roots.[35,36]

A possible physiologic explanation for this phenomenon could be the fact that C fibers have a higher presence of $Na_V1.8$ and $Na_V1.9$ (tetrodotoxin-resistant sodium channels) than myelinated fibers.[3,37–39] In Ranvier nodes, there is also a predominance of $Na_V1.6$, a tetro-

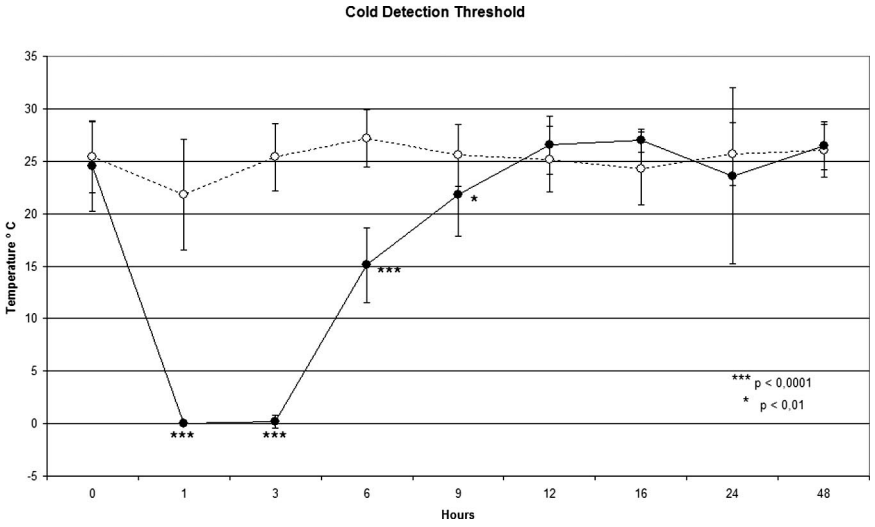

Fig. 3. Cold detection threshold (mean ± SD; *n* = 10). *Open symbols* represent placebo values; *closed symbols* represent neosaxitoxin values. Significance was reached 9 h after infiltration.

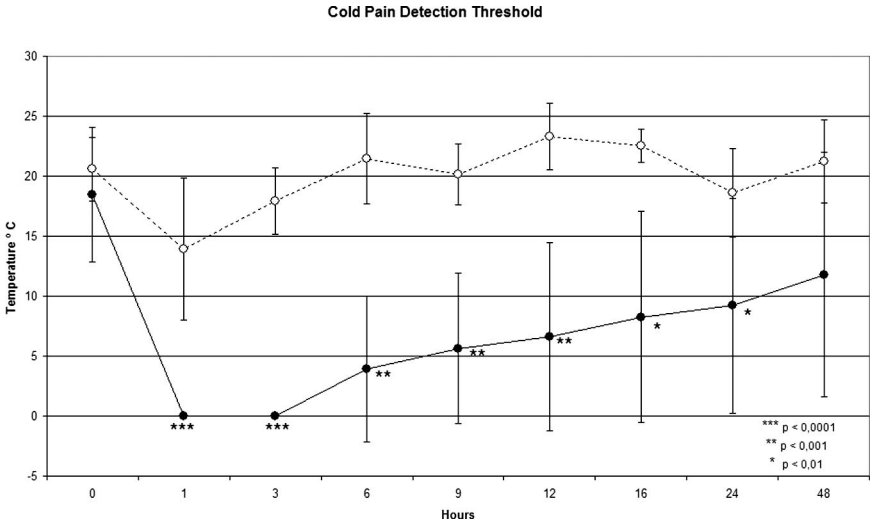

Fig. 4. Cold pain detection threshold (mean ± SD; *n* = 10). *Open symbols* represent placebo values; *closed symbols* represent neosaxitoxin values. There was a complete block of cold sensation for the first 3 h post injection. Significance was reached 24 h after infiltration.

dotoxin-sensitive sodium channel.[40] These data explain the time sequence of sensation reversal shown in this study, in which the warm sensation was the first to revert because of the higher paralytic shellfish poisoning toxin resistance of C fibers.

The anesthetic effect of outer sodium channel-blocking compounds such as tetrodotoxin and saxitoxin has long been known and only been previously tested in animal models[5,13,22,41–43]. Neosaxitoxin's anesthetic effect has been studied only in an animal *in vivo* preparation, and in this experimental model, neosaxitoxin was the most potent anesthetic when compared with saxitoxin, tetrodotoxin, and dc-saxitoxin.[5]

The amide-type local anesthetics like lidocaine, ropivacaine, and bupivacaine show highly toxic effects, mainly cardiovascular (dysrhythmias and cardiac depression), central nervous system toxicity (seizures and coma), and direct nerve cell cytotoxicity.[44,45] These undesirable clinical effects have stimulated the search for safer local anesthetics to reduce the risk of cardiac and central nervous system toxicity produced by accidental intravenous injection.[46–48] Neosaxitoxin should have less cardiotoxic effect than amide anesthetic agents because of its lower affinity for cardiac sodium channel receptors. Relative to axons, cardiac Purkinje fibers have extremely low affinity to tetrodotoxin:[49] the cardiac sodium channels bind tetrodotoxin with 200-fold lower affinity mainly by one amino acid change in domain I, when compared with the binding measured in brain and skeletal channel membrane preparations.[1] In a model of rats with intrathecally placed catheters, tetrodotoxin also achieved an adequate anesthetic effect without causing local neurotoxicity. This result was opposite that of bupivacaine, which produced moderate to severe nerve root injury when evaluated for histologic damage.[22]

The systemic toxicity of the outer pore blockers (tetrodotoxin and saxitoxin) has been described extensively.[13,43] Unlike that with conventional local anesthetics, death from these toxins results primarily from respiratory failure due to diaphragmatic paralysis. The latter occurs by direct action on the diaphragm and phrenic nerves, rather than a central depressing effect.[13] Respi-

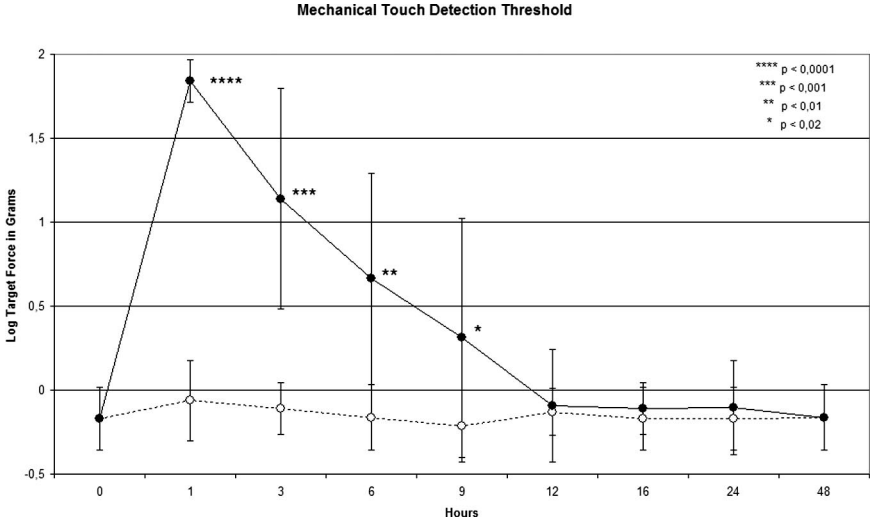

Fig. 5. Touch detection thresholds (mean ± SD; *n* = 10). *Open symbols* represent placebo values; *closed symbols* represent neosaxitoxin values.

ratory support is the mainstay of treatment. On average, 24-72 h are usually required for a complete neurologic recovery.[50] Moreover, severely paralytic shellfish poisoning-intoxicated patients have recovered without neurologic impairment or other clinical damages.[51] If inadvertent intravenous injection were to occur in the presence of a vigilant anesthesiologist, most episodes could be managed successfully without sequelae by proper attention to ventilation.[43] Is important to keep in mind that international official regulations allow consumption of shellfish with a safe limit of 80 μg saxitoxin, equivalent to 100 g shellfish meat.‡‡

In conclusion, this is the first report of neosaxitoxin use as local anesthetic in a human trial. Neosaxitoxin showed effective local anesthetic properties, blocking the five sensitive parameters measured in this trial. Ongoing studies using highest doses of neosaxitoxin will show its limits and maximal effects as a local anesthetic compound. Our data will open a whole new line of research in acute and chronic pain management with this phycotoxin.

The authors acknowledge the support of The Henry Mayer Center, Hospital Clínico Universidad de Chile, and thank David Sisco, B.S., Santiago, Chile, for revision of the manuscript.

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