Effect of Neosaxitoxin on Epidural Anesthesia in Cats: a Promising Alternative to Conventional Anesthetics

Xaviera Varela[1], Miguel del Campo[1], Robin Piron[1], Joaquín Sepulveda[1], Tamara Bustamante[1], Jaime Hinzpeter[2,*], Néstor Lagos[1]

[1]Membrane Biochemistry Laboratory, Department of Physiology and Biophysics, Faculty of Medicine – University of Chile. Independencia 1027, Zip Code 8380000, Santiago, Chile.

[2]Department of Orthopaedic Surgery, Clinical Hospital, University of Chile, Santos Dumontt # 999, Independencia, Zip Code: 8380456, Santiago, Chile.

Abstract

Neosaxitoxin (NeoSTX) is a specific high-affinity inhibitor of voltage-dependent sodium channels, which has shown excellent results as a local anesthetic in various pathologies and post-operative protocols, since it effect is long-lasting and have virtually no side effects.The aim of this study was to analyze the effect of NeoSTX as an epidural anesthetic in female cats, undergoing ovariohysterectomy, compared to Lidocaine in a randomized anddouble-blind study. Two groups of 11 female cats were randomly in the NeoSTX group and the lidocaine group. They were administered, respectively, a single dose of NeoSTX (0.5 μg / kg) or lidocaine (4 mg / kg, 2%) by epidural via. Using the UNESP-Botucatu pain assessment scale, which considers multiple behavioral and physiological factors, the epidural anesthetic effect of NeoSTX and lidocaine was evaluated, up to 240 min after the ovariohysterectomy procedure. NeoSTX no altered the peripheral blood pressure during the cut of uterine cervix, and generated lower values on the pain scale as compared to the lidocaine treatment. None of the cats anesthetized with NeoSTX required an extra dose of pain-relieving drugs (2 mg / kg of tramadol) during the first 150 min after surgery, whereas nine cats from the lidocaine group did need an extra dose of analgesic. NeoSTX is a powerful pain blocker, with a long-lasting anesthetic effect when administered by an epidural procedure. Therefore, NeoSTX emerges as a promising alternative to conventional anesthetics for the treatment of postoperative pain.

Introduction

Epidural anesthesia is a clinical procedure that aims to control pain during surgery and the postoperative period, in which a local anesthetic is administered into the epidural space, outside the meninges of the spinal cord, to desensitize the sensory nerve endings that innervate the abdominal wall and the sacral nerve. The most used technique in veterinary medicine of small animals is to administer a local anesthetic between the vertebrae L7- S1, being lidocaine and bupivacaine the most commonly used drugs. Besides, systemic analgesics such as fentanyl, morphine, tramadol and dissociative anesthetics as ketamine, or combinations of these have been used with different results in terms of their anesthetic effect and duration times [18]. However, for lidocaine and bupivacaine the duration of their anesthetic effects does not exceed 2 hours, and when they are combined with opioids, although the effect was prolonged, multiple side effects have been described, such as pruritus, central nervous system disorders, seizures and cardiovascular depression [18, 10]. Therefore there is a need to find new epidural anesthetics for veterinary use that have a prolonged effect on pain control without side effects.

Biotoxins of microalga origin have been used in biomedicine for various purposes, but it is of special interest to highlight the medical use of the toxins that cause paralytic shellfish poisoning (PSP), because of their long-lasting local anesthetic effect [13]. The PSP toxins correspond to a large group of alkaloids produced by several species of marine dinoflagellates and freshwater cyanobacteria [9]. The basic chemical structure of the PSP toxins corresponds to a tricyclic tetrahydropurine, derived from imidazole guanidine-type, non-peptidic molecules, which allows several chemical substitutions, giving rise to more than thirty compounds that are analogs of Saxitoxin (STX), which is the base structure and reference of the PSP toxins. Neosaxitoxin (NeoSTX) differs from STX by the addition of a hydroxyl group. These molecules are thermostable and hydrophilic [14]. PSP toxins are highly specific blockers of voltage-gated sodium channels. These channels are responsible for the propagation of action potentials in neurons, myocytes and other excitable cells. They are constituted by a subunit α that interacts with the sodium ions on the outer side of the membrane and by one or two subunits that modulate their activity. In mammals, there are 9 types of subunit α, called NAv 1.1 to NAv 1.9, varying in sequence (approximately 50%) and in the tissues in which they are expressed [4]. The major Navs in the central nervous system (Nav 1.1, 1.2, 1.3 and 1.6) are inhibited by NeoSTX at nMolar concentrations [2].

The use of PSP toxins, and specifically NeoSTX, in human and veterinary medicine is extensive and well documented, with more than 20 clinical studies for multiple pathologies and post-operative protocols published, where it has been able to establish its potent effect as muscle relaxant and local anesthetic [20, 13, 15, 16, 19]. Its utilization as an anesthetic is highlighted by the generation of long-lasting effects compared to conventional anesthetics, without side effects [13]. The aim of this work was to evaluate NeoSTX as an epidural anesthetic for the control of postoperative pain after feline ovariohysterectomy, in comparison with the anesthetic effect of lidocaine, in a randomized and investigator-blind study. For this purpose, the multidisciplinary pain assessment scale UNESP-Botucatu was used [5]. It was decided to opt for the UNESP-Botucatu pain scale since it is supported by multiple studies and homologations [6,7], and it has already been used for comparative studies between different anesthetic drugs administered by the epidural route [11]. This evaluation considers multiple behavioral and physiological factors and is divided into three areas of measurement: Psychomotor disorders (posture, comfort, activity, attitude and various behaviors); Protection of the painful area and vocal expression of the pain (reaction to the palpation of the surgical wound, reaction to the palpation of the abdomen and vocalization); and Physiological variables (peripheral blood pressure and appetite). In addition, the need to apply rescue medication (tramadol) to the patients was evaluated in both groups, according to the values obtained in the pain scale.

Materials and Methods

The evaluation of the epidural anesthetic effect of NeoSTX and lidocaine in the postoperative period after an ovariohysterectomy, was performed as follows: 22 feline females, of domestic or common European breed, from 6 months to 2 years of age, were voluntarily

taken by their owners to the Diego Silva Veterinary Clinic, (Conchalí, Santiago de Chile), where the procedure was explained informed consent was given. The procedure was free of charge for the owner. The Bioethical Committee of the Diego Silva Veterinary Clinic; and the Bioethical Committee on Animal Experimentation the Faculty of Medicine, University of Chile (FM 0551), approved the experimental procedures.

The cats were randomly divided into two groups, NeoSTX and lidocaine, using the SPSS software. Both the veterinarian in charge of the ovariohysterectomy and the veterinarian who performed the pain assessment worked blindly, without knowing which anesthetic was being used.

In both groups, the endotracheal intubation was introduced, which was connected to an anesthesia machine, supplying Isoflurane at an initial concentration of 3% using a vaporizer, which was varied according to the patients' requirement (1.5 - 3%). Oxygen was provided at a rate of 20 mL kg^{-1} $minute^{-1}$. Then, the cephalic vein was cannulated with a venous catheter, through which 0.9 % sodium chloride solution at a dose of 10 mL kg^{-1} $hour^{-1}$, was administered as a maintenance fluid. No cat was previously treated with any drug. Anesthesia was induced with a bolus of Propofol at 3 mg / kg intravenously. Once the appropriate anesthetic level was reached, NeoSTX (0.5 µg/kg) or lidocaine (4 mg / kg, 2 %, Virbac®) were placed in epidural via. NeoSTX was purified according to Riquelme et al., 2018.

Then Peripheral blood pressure was measurement with a pediatric cuff and vascular Doppler sphygmomanometer (Sonoline B Doppler 8 mhz) pre-surgical, and an average of 3 continuous measurement was taken. Then, in the pavilion, the peripheral blood pressure was measured throughout the surgical procedure and recorded at the beginning of surgery, post cervix cut and at the end of the surgical procedure.

Afterwards the patients were transferred to the recovery room and 30 min after the endotracheal extubation, the evaluation of postoperative analgesia was started, which was carried out every 30 min until completing a period of 240 min, based on the multidimensional scale of the UNESP-Botucatu [7]. It should be noted that previous to the start of the surgical procedure, patients were evaluated to establish the

baseline value on the pain scale.

The variables were evaluated with response options numbered from 0 to 3, indicating the degree of pain. Thus 0 indicated absence of pain, 1 mild pain, 2 moderate pain and 3 severe pain. The sum of scores of all the variables can give a total of 30 points which defined the degree of pain of the patient: between 1 and 9 points indicated mild pain, between 10 and 19 points indicated moderate pain and between 20 and 30 points severe pain. Patients who scored higher than 10 for more than two consecutive evaluations were given the emergency analgesic tramadol at a dose of 2 mg / kg intramuscularly [7].

The data collected were grouped into criteria based on the multidimensional scale of UNESP - Botucatu, generating averages for each group with a standard deviation equivalent to $P \leq 0.5$ using student's T.

Results

When the peripheral blood pressure of the patients of the NeoSTX group was compared, no difference could be observed between the pressure before the surgery and at the moment of the cervix cut. This is indicative of a total state of anesthesia achieved by NeoSTX, which demonstrates its effectiveness and anesthetic power. However, in the group anesthetized with lidocaine, an increase in pressure was observed when the cervix was cut, as compared to its basal state, and also as compared to the peripheral blood pressure of the NeoSTX group (Figure 1). At the end of the procedure, the peripheral blood pressure in both groups had returned to their basal levels.

The pain assessment scale of UNESP-Botucatu was measured for all patients prior to the surgery to establish the reference level and every 30 min after the procedure, for a total duration of up to 240 min. The higher the score is on this evaluation, the greater the discomfort of the patient is. The final results are shown in Figure 2A. Both groups have the same preoperative score (around 2 points, score described in [7], for control animals), but then, 30 min after the surgery, the score of the lidocaine group shows a sudden and significant increase for up to 150 min, as compared to the NeoSTX group, while the score of the NeoSTX group remains low. These low values reflect a deep anesthetic state that is also observed in the values of the
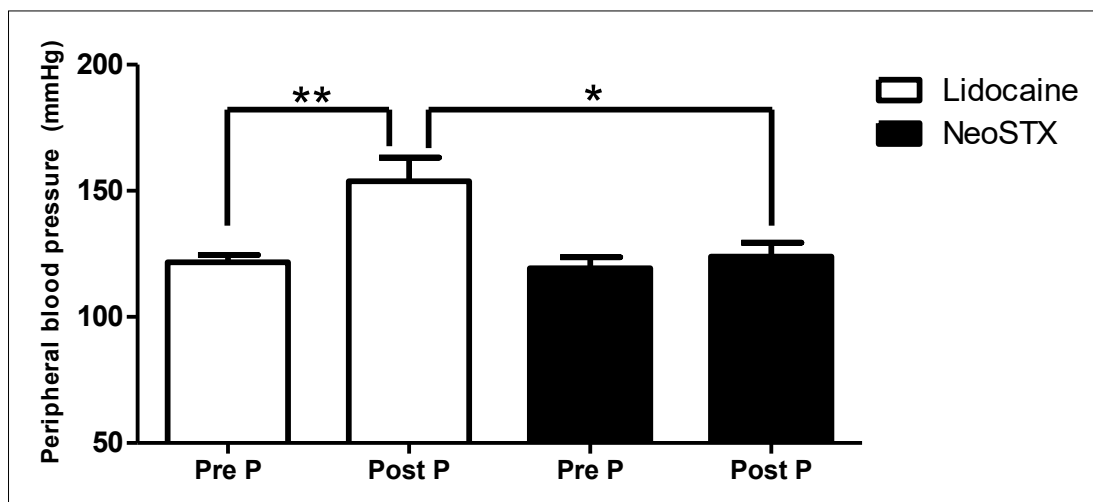
Figure 1. Effect of NeoSTX and lidocaine on peripheral blood pressure, comparison of the pressure before surgery (basal) and the pressure at the moment when the cervix was cut. The NeoSTX group does not show differences, while for the lidocaine group an increase in peripheral blood pressure is observed when both measurements are compared. Significance determined with T test: * $p < 0.05$, ** $p < 0.01$.
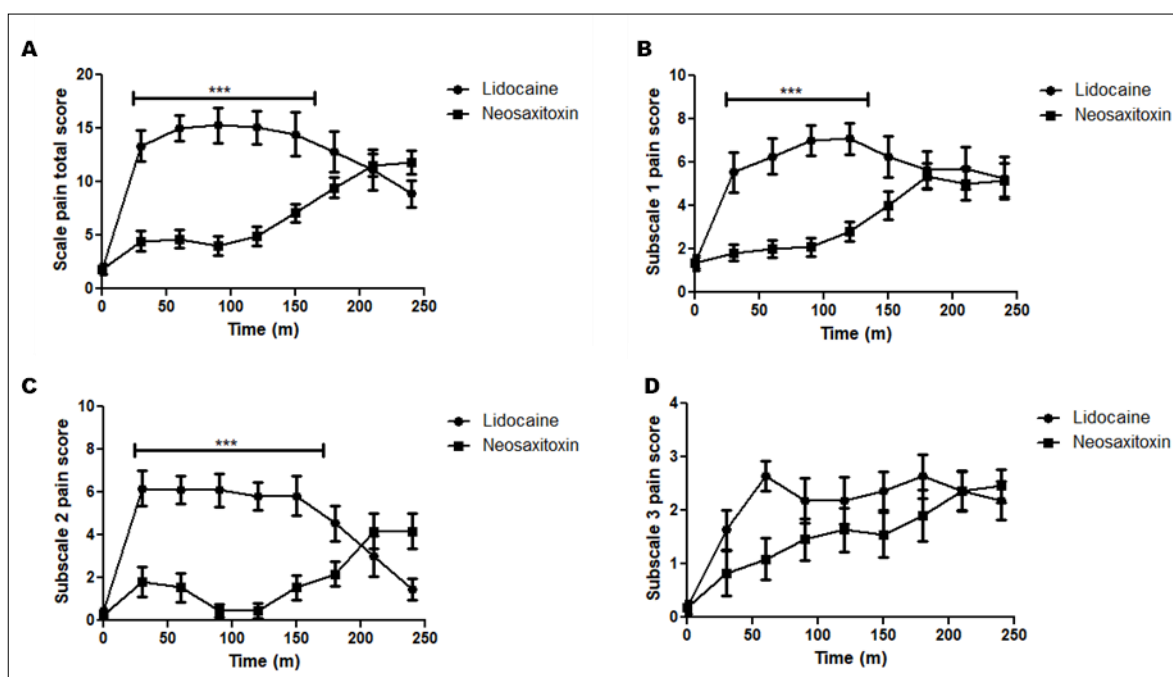

Figure 2. Pain assessment using the UNESP-Botucatu multidimensional scale: A. Total score; B. Subscale 1, Psychomotor disorders; C. Protection of the painful area and the vocal expression of pain and D. Physiological variables. Significance determined with T test: *** $p < 0.001$.

subgroups 1 and 2 of the measurement of the UNESP-Botucatu method, these are: Psychomotor disorders (Figure 2B) and Protection of the painful area and the vocal expression of pain (Figure 2C). For subgroup 3 (Figure 2D), which analyzes the physiological variables, again lower scores were observed for the NeoSTX group as compared to the lidocaine group, albeit not significant.

Already at 30 min after surgery the average pain score of the lidocaine group exceeded 10 (Figure 1A). As a result, 9 out of 11 patients in the lidocaine group had to be given a rescue dose of tramadol at 60 min, the requisite for which were 2 consecutive pain scores of 10 or more for a given patient, while the remaining 2 patients eventually received a dose at 180 min (Table 1). In contrast to the lidocaine group, the average pain score of the NeoSTX group only exceeded 10 after 210 min. Consequently, the first patient had to be rescued at 150 min, and a second one at 180 min, the point in time at which all 11 patients of the lidocaine group had been rescued. Moreover 3 more patients received a dose at 210 min, while the remaining 6 patients (more than half of the cohort) were rescued only after 240 min at the end of the experiment.

Since there were 9 patients in the lidocaine group who received tramadol after 60 min, the values on the pain scale had a tendency to decrease (Figure 1A). The effect of tramadol on the NeoSTX group is not reflected in the overall pain scores that show a tendency to increase towards the end of the experiment, because the majority of the patients in this group received the drug only after 210 or 240 min close to the end of the experiment. It should be noted that even though most of the patients of the lidocaine group were treated with the rescue drug early on (60 min), the group did not show lower values on the pain scale than the NeoSTX group for up to 210 min after surgery.

In summary, the animals that received NeoSTX as epidural anesthesia did not show an increase in their peripheral blood pressure during the surgical procedure or in the post-surgery period in relation to their baseline values. Moreover, they scored significantly lower on the UNESP-Botucatu pain assessment scale as compared to the lidocaine group. Furthermore after 180 min, when the entire lidocaine group had received a rescue dose, only 2 NeoSTX patients needed rescue and half of the NeoSTX cohort was rescued only at the end of the experiment after 240 min. No side effects were observed in any patient anesthetized with NeoSTX.

Discussion

Lidocaine is the most commonly used local anesthetic for epidural administration in cats and dogs during surgical procedures. However, lidocaine alone is not a good analgesic for controlling surgical and postoperative pain in ovariohysterectomy, because it is highly toxic in felines, when compared to canines, as well as generating significant cardiopulmonary depression when administered together with Isoflurane [17]. Although it is a low-risk, low-cost drug that provides rapid-onset anesthesia, its short duration increases postoperative pain scores and increases the requirement for analgesics such as opioids, which generate a series of side effects. In addition, opioids are not effective in the inhibition of A-δ fibers that are responsible for acute pain and are activated by persistent stimuli inflicted by surgical procedures [18].

It has been demonstrated that NeoSTX has a prolonged post-surgery anesthetic effect and is effective at pain control in both human and veterinary

Table 1. Number of patients and time at which tramadol was applied as emergency medication to reduce excessive pain, comparison of lidocaine and NeoSTX treatments for epidural anesthesia

					Time(min)				
	30	60	90	120	150	180	210	240	Total
Lidocaine	0	9	0	0	0	2	0	0	11
Neosaxitoxin	0	0	0	0	1	1	3	6	11

medicine [13, 15, 19]. Lately, it has become a powerful alternative to the use of conventional anesthetics [8]. Specifically in cats, the toxicokinetic and toxicodynamic parameters of GTX2/3, analogous to NeoSTX, have been established, showing the clearance of these toxins at 4.6 mL / min kg, mainly by glomerular filtration [3]. Taking into account that for the related compound Saxitoxin the LD50 in rats is 7 µg / kg, the dose used in this pilot study was 0.5 µg / kg, which was sufficient to showed favorable results compared to lidocaine (4 mg / kg). Although no side effects were seen in patients that received the NeoSXT dose, we believe that it would be of interests in future studies to determine the lower threshold dose of needed to obtain the same effect.

In this study, the epidural anesthetic effect of NeoSTX was compared to lidocaine in an ovariohysterectomy surgical intervention, using the UNESP-Botucatu pain assessment scale, which is widely used in veterinary medicine. This measurement of post-surgery pain includes both the interpretative measurements of the veterinarian in charge and analytical data, particularly the peripheral blood pressure. The visceral pain produced in surgical procedures causes an increase in the sympathetic tone that generates vasoconstriction and an increase in cardiac output, which leads to an increase in peripheral blood pressure [1] The latter is the most reliable physiological parameter as an indicator to measure pain in cats [12]. As seen in Figure 1, patients anesthetized with NeoSTX maintained a peripheral blood pressure level equal to that observed during and after ovariohysterectomy, unlike patients anesthetized with lidocaine, where peripheral blood pressure increased during the surgical procedure, which is indicative of pain.

The results of the postoperative analgesic evaluation with the multidisciplinary scale of UNESP-Botucatu [7] show that the NeoSTX group not exceed 10 points on average on the total pain scale, for up to 180 min, which is indicative of a good level of analgesia, and is the result of a better performance of the NeoSTX patients in the three groups of parameters measured (Psychomotor disorders, Protection of the painful area and vocal expression of pain, and Physiological variables) in comparison to lidocaine (Figure 2). In addition, this score was significantly lower than that obtained by the lidocaine group, which had an average score higher than 13 (moderate pain) after 60 min, which forced veterinarians to administer emergency medications to 9 of the 11 patients.

Even after administration of the rescue dose, the lidocaine group's pain scores remained significantly higher than those of the NeoSTX group for the first 210 min, clearly showing that the NeoSTX treatment was more effective at pain control post-surgery.

It is demonstrated in this work that NeoSTX, through the epidural way, is a powerful post-surgery pain blocker in ovariohysterectomy in cats, being more effective and long lasting than lidocaine, without having side effects.

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