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SHORT COMMUNICATION

Neosaxitoxin, a Long-Lasting Local Anesthetic and its Potential Clinical Applications in Horses

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ABSTRACT. Neosaxitoxin (NeoSTX) is a toxin that binds to the voltage-gated sodium channels therefore, inhibiting the neuronal impulse. The present study was conducted to explore the properties of NeoSTX and to evaluate its effects when injected as a perineural nerve block in horses. A group of five client-owned mature Warmblood horses exhibiting clinical signs of unilateral foot pain were enrolled in the study. For inclusion, lameness should subside after a palmar digital nerve block (PDNB) using 2 mL of 2% lidocaine administered over the medial and lateral palmar digital nerves of the affected limb (Day 0). Lameness was assessed using the AAEP lameness grading scale and skin sensitivity was judged objectively using a pressure algometer. On day 1, 5µg of NeoSTX was injected, then on day 4, 10 µg of NeoSTX was administered. Lameness examination and skin sensitivity were evaluated at 3, 5,10, 15,30, 60, 90 minutes, and every hour until the effects of the nerve block were no longer detectable. When effects of NeoSTX was compared to effects of lidocaine at 2% there were no statistical differences in the onset of the anesthetic effect, measured as the time of start of desensitization of the skin and the time of complete desensitization or lameness resolution, nonetheless there was a significant difference in the return of skin sensation or lameness, showing a clear long-lasting nociceptive blocker effect of NeoSTX. In conclusion, results of this study suggest NeoSTX can potentially be used as an alternative to conventional local anesthetics drugs when a long-lasting effect is desired, for example as a part of a multimodal approach for pain management, as a local anesthetic for surgical procedures or to control chronic pain in some musculoskeletal disorders. However, more studies are needed to evaluate its use as long-lasting anesthetic effects in the aforementioned situations.

Keywords: neosaxitoxin, NaV channels, equine, foot pain, welfare.

INTRODUCTION

Animal welfare is a standard consideration that refers to animal well-being under different situations, such as disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling, and even humane euthanasia. The most frequent painful conditions in horses are the ones related to the locomotor system (Pennel *et al*., 2005; Egenvall *et al*., 2009). Horses can suffer several orthopedic conditions throughout their lives and on many occasions those conditions require surgery or progress to chronic disorders, deteriorating the horse's quality of life (McGovan, 2011; Kelemen *et al*., 2021). For years, the use of conventional local anesthetics (e.g., lidocaine, mepivacaine, or bupivacaine) for diagnostic or local anesthetic to perform surgical procedures have been an excellent tool for equine practitioners. Furthermore, studies have been conducted in horses to evaluate the ability of adjuvants such as epinephrine and sodium bicarbonate to either increase the intensity, hasten the onset, or prolong the duration of local anesthesia in some desired situations (Alvarez *et al*., 2018; Boone *et al*., 2019).

The pharmaceutical industry continues to explore the development of safer and more effective local anesthetics. In the last twenty-five years, researchers have used different toxins obtained from plants and microorganisms in the search for a new drug with a potential property to be used as long-lasting local anesthetic. In recent publications, local injection of Paralytic Shellfish Poison (PSP) toxins such as Gonyautoxins and Neosaxitoxin (NeoSTX) have shown its properties *in vitro* (Montero *et al*., 2020) and *in vivo* in pilot studies and case reports showing to be safe and effective in several clinical applications in humans to control chronic and post-operative pain (Lagos, 2014; Manriquez *et al*., 2015; Hinzpeter *et al*., 2016) and potentially in veterinary medicine in different species (Riquelme *et al*., 2018; Valenzuela *et al*., 2019; Varela *et al*., 2019; Montero *et al*., 2021). NeoSTX biological activity lies in the reversible inhibition of excitable cells impeding neuronal impulse propagation at the neuronal synapses or neuromuscular junction (Catterall, 2000; Lagos & Andrinolo, 2000; Lagos, 2014). The inhibition of the neuronal impulse is due to closure of the voltage-gated sodium channels by high affinity binding of NeoSTX to the site 1 of the alfa unit of the channel, blocking the sodium influx through the channel (Lagos, 2014).

Thus, due to the preliminary information available regarding the NeoSTX, the aim of this study was to evaluate the effect of NeoSTX when injected perineurally in horses to gather more evidence of its safety and capability to be included as a part of a multimodal approach for chronic and surgical pain management or to be used alone as a regional anesthetic for standing surgical procedures in horses.

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MATERIAL AND METHODS

A prospective clinical study was conducted to evaluate NeoSTX when administered as PDNB. Five client-owned mature Warmblood horses (3 mares and 2 geldings), with an age between 8-14 years-old, and a weight ranging from 485-535 kg were included in the study. Horses selected presented clinical signs consistent of unilateral foot pain. The study was approved by the Ethical Committee of the Medicine Faculty, University of Chile (FM 0551) and informed consent was given by horse owners enrolled in the study. Horses were selected from clinical cases presented to the equine hospital (Equestria Equine Medical Center, Quillota, Chile) and as inclusion criteria, the lameness had to disappear after a palmar digital nerve block (PDNB) using 2 mL 2% lidocaine on each nerve. For injection, the distal pastern region was cleaned with an antiseptic solution, and the nerve block was performed inserting subcutaneously a 25-gauge, 5/8-inch needle over the palpable medial and lateral neurovascular bundles of the affected limb. The needle was placed just proximal to the border of the lateral and medial ungular cartilage (Moyer *et al*., 2007). Finally, 2 mL of 2% lidocaine was administered in each point. Lameness assessment was evaluated subjectively using the American Association of Equine Practitioners (AAEP) grading system (Ross, 2011). For lameness evaluation, all horses were walked and trotted in a straight line in a flat hard surface (concrete). Horses were observed from the front, back, and from both sides. Additionally, horses were trotted in circles, on a lunge line. Only one clinician performed the lameness evaluations (CD). The skin sensitivity was judged objectively using a pressure algometer (Baseline® push-pull force gauge, Model: 12-0804. White Plains, NY, USA), this model has been used previously in equine pain/pressure assessment (Riquelme *et al*., 2018). The algometer with a circular push tip supplement was applied over both heel bulbs, pressure was applied until withdrawal response happened and their maximum pressure thresholds were recorded in kg/cm^2 (maximum pressure of 10 kg/cm^2). The pressure measuring procedure consisted in applying pressure on both, the lame and control, limbs in a non-weight-bearing position. As a standardized technique in this study, the algometer was applied 5 consecutive times to the lame limb in each heel bulb before and after the perineural block, then the mean pressure tolerated was documented. The contralateral limb served as the negative control. On day 0, a PDNB using 2 mL of 2% lidocaine was performed. On day 1 (24 hours post lidocaine treatment), the PDNB was repeated by administering 5 μg of highly purified NeoSTX diluted in 1 mL of NaCl 0.9% at pH 6.2 obtained from *cyanobacteria Aphanizomenon sp*. Finally, on day 4 (96 hours post lidocaine treatment and 72 hours post 5 μg NeoSTX treatment) PDNBs for each horse were repeated by administering 10 μg of NeoSTX. Horses were examined at 3, 5, 10, 15, 30, 45, 60, 90 minutes, and every hour until the effect of the injection wore off. Daily clinical examination was performed to all horses over a one-week period to assess for any local inflammation or systemic illness.

Four time points were established (Figure 1). T0 corresponded to time 0 or baseline, T1 to the start of desensitization of the skin measured by the increase of the threshold in the algometer after pressure was applied and/or by partial improvement in the grade of lameness. T2 was the time when complete desensitization of the skin or lameness resolution was achieved and T3 corresponded to the time when the return of skin sensation or lameness was first observed. The duration of the anesthetic effect was calculated from the time points T3 and T1.

Statistical analyses were performed using GraphPad Prism 5 software (USA). Normality assumptions were verified with the Kolmogorov-Smirnov test. Time of start of desensitization (T1), complete desensitization / lameness resolution (T2), and return of skin sensation / lameness (T3) were compared between treatments from the baseline starting point (T0) using a repeated-measures analysis of variance (ANOVA) and the Bonferroni`s multiple comparison test. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

All treatments given were consistently efficient abolishing lameness and skin sensation measured by the subjective gait analysis and objective measurement of skin sensation after administration of the drugs as a PDNB (Table 1). Results analysis showed no statistical differences when T1 and T2 were compared between 2% lidocaine and 10 μg of NeoSTX, nonetheless there was a significant difference in T3 and the duration of the anesthetic effect ($p < 0.05$) showing a clear long-lasting nociceptive blocker effect of NeoSTX. No local or systemic adverse reaction was observed after subcutaneous injection of NeoSTX over a one-week period of daily evaluation.

Even though mepivacaine and lidocaine are the most used local anesthetics, it has been reported that their efficacies vary and that variations could be attributed to the horse, time of interpretation, and deficient technique (Arkell *et al*., 2006; Nagy *et al*., 2009). Furthermore, Silva *et al*. (2015) showed that lidocaine inconsistently alleviated skin sensation, but not lameness and contradictory, Hoerdemann *et al*. (2017) showed that lidocaine inconsistently alleviated lameness, but not skin sensation. Despite the aforementioned, in our study we observed a good correlation between the alleviation of lameness caused by foot pain and skin sensation for both treatments. Other factors to be considered in the efficacy of local anesthetics is the one related to the pharmacology and mechanism of action of each drug. The speed of the onset, potency and duration are directly related to the pKa, lipid solubility, and protein binding site of the drug (Taylor & Mcleod, 2020). Local anesthetics activity lies on interruption of neural conductivity by binding to the

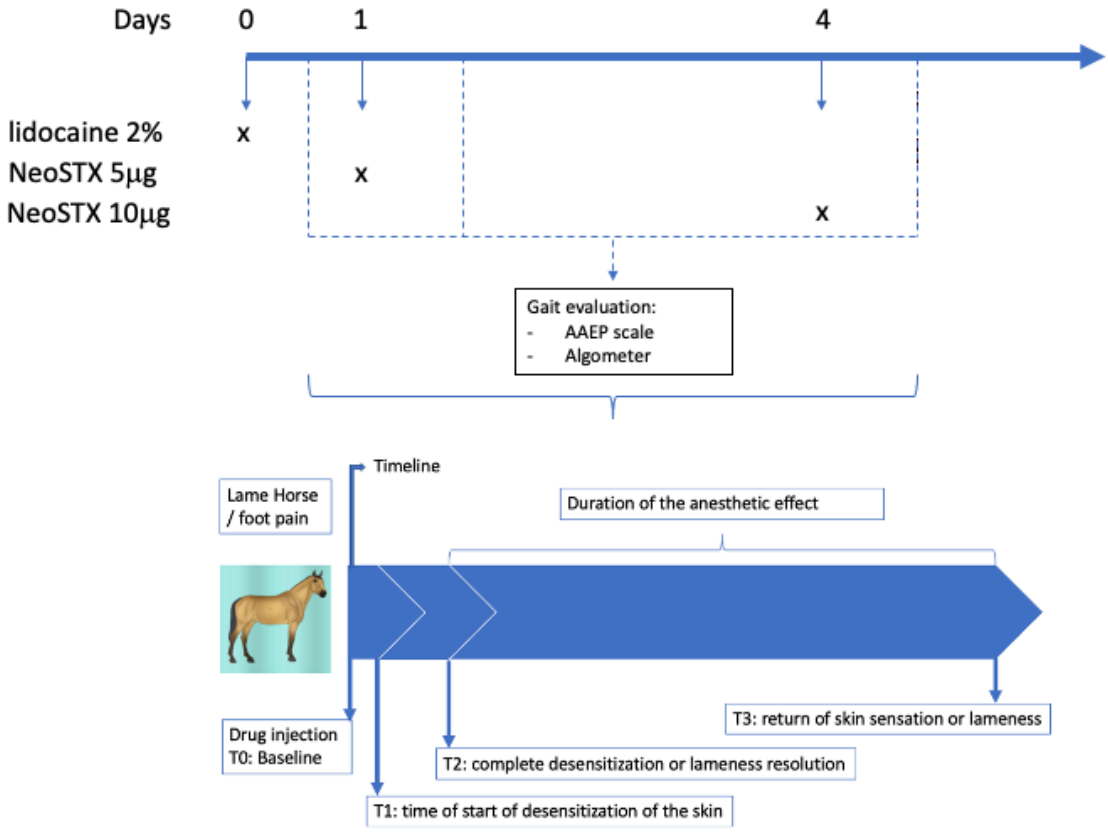

Figure 1. Scheme of timing of palmar digital nerve blocks and evaluation of gait and skin sensation.

Table 1. Mean values recorded of horses with foot pain at different time points.

Local anesthetic	Baseline (T0)			Time of start of desensitization of the skin (T1)			Complete desensitization / Lameness resolution (T2)			Return of complete skin sensation / lameness (T3)		
	Time (hours)	AAEP scale	Algometer (kg/cm^2)	Time (minutes)	AAEP scale	Algometer (kg/cm^2)	Time (minutes)	AAEP scale	Algometer (kg/cm^2)	Time (hours)	AAEP scale	Algometer (kg/cm^2)
lidocaine 20 milligrams	0	3	5.90 ± 0.141	3.42 ± 0.78	0	9.70 ± 0.142	5.58 ± 2.58^{ac}	0	10 ± 0	1.4 ± 0.224^{a}	3	5.90 ± 0.316
NeoSTX 5 micrograms	0	3	5.56 ± 0.205	6 ± 1.98	2	5.66 ± 0.282	16 ± 2.22^{b}	0	10 ± 0	5.8 ± 0.447^{b}	3	5.50 ± 0.228
NeoSTX 10 micrograms	0	3	6.10 ± 0.200	3.42 ± 0.9	1	8.10 ± 0.245	4.98 ± 0.6^{ac}	0	10 ± 0	11.6 ± 0.548^{c}	2	5.96 ± 0.316

Different letters indicate statistically significant differences (P≤0.05).

voltage-gated sodium channels (NaV channel), preventing depolarization, and avoiding further neuronal transmission (Taylor & Mcleod, 2020). Amino amides (e.g., lidocaine and mepivacaine) and amino esters (e.g., procaine), bind to the NaV channel inner pore, in other words the binding occurs from the intracellular side of the cell (Catterall, 2000). More specifically, the binding site for local anesthetics is in domain IV, loop S6 and is only accessible when the NaV channel is open and depolarized (Taylor & Mcleod, 2020). On the other hand, NeoSTX binds with high affinity to site 1 of the alpha unit on the NaV channel, impeding the sodium ion entrance to the nerve cell. Consequently, the foremost effect of NeoSTX is related to their blocking action at the axonal level, inhibiting nerve depolarization and impulse conduction (Lagos, 2014). The mechanism of action of NeoSTX explains the rapid onset of the effect because firstly, NeoSTX does not have to pass through the phospholipid membrane to bind the NaV channel (binding to its site facing in extracellular part of the channel), secondly, is not affected by the intracellular pH, and

finally there is no need for the NaV channel to be opened for it to bind, as it must happen when amino amides and amino esters are used. Accordingly, NeoSTX has shown to be a long-lasting dose-dependent local anesthetic when administered subcutaneously around a painful area without the need of any adjuvant to prolong the duration of the anesthetic effect (Riquelme *et al*., 2018) as also shown in this report where the capability of NeoSTX to abolish the nerve impulse through peripheral nerves when administered perineurally is demonstrated. Therefore, NeoSTX could potentially be used as a local anesthetic to perform surgical procedures in the standing horse due to its dose dependent long-lasting nociceptive blocker properties and in the recumbent anesthetized horse as a part of the regional pain management after a surgical procedure.

This pilot clinical study was not limitations-free, and probably the most important one is the small sample size used which directly affects the representativeness of the statistical results. Additionally, despite we did not specifically evaluated the effect of NeoSTX in the surrounding tissues, according to the previous studies in different animal models no detrimental effects have been reported (Rodriguez-Navarro *et al*., 2007, 2011; Wylie *et al*., 2012; Manriquez *et al*., 2015; Hinzpeter *et al*., 2016; Riquelme *et al*., 2018; Valenzuela *et al*., 2019; Varela *et al*., 2020; Montero *et al*., 2021), however, none of the studies mentioned above did an histopathological evaluation of the injected tissues so it should be considered for future studies.

According to our results, NeoSTX has a rapid anesthetic effect when a dose of 1 mL with 10 μg solution is used to decrease pain arising from the foot. All the above data shows that NeoSTX can be effectively used in a very low volume (1 mL) and can abolish lameness as rapidly as 5 minutes when a PDNB is performed. It is demonstrated that the local anesthetic properties of NeoSTX, when used as a perineural nerve block in horses, are an excellent alternative when a long-lasting anesthetic effect is desired.

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AUTHORS CONTRIBUTIONS

CD participated in the design and implementation of the clinical trial, data analysis and writing of this manuscript. MdC in the analysis of data and writing of this manuscript. NL participated in the clinical design and writing of this manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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