Toxicon 141 (2018) 15–17

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Short communication

Neosaxitoxin, a Paralytic Shellfish Poison toxin, effectively manages bucked shins pain, as a local long-acting pain blocker in an equine model

Gricel Riquelme, Joaquín M. Sepúlveda, Zaki Al Ghumgham, Miguel del Campo, Cecilia Montero, Nestor Lagos*

Membrane Biochemistry Laboratory, Department of Physiology and Biophysics, Faculty of Medicine, University of Chile, Independencia 1027, 8380000, Santiago, Chile

ARTICLE INFO

Article history:
Received 13 July 2017
Received in revised form
9 November 2017
Accepted 11 November 2017
Available online 13 November 2017

Keywords:
PSP toxins
Neosaxitoxin
Bucked shins
Pain management
Long-lasting pain blocker

ABSTRACT

Local anesthesia is an effective method to control pain. Neosaxitoxin is a phycotoxin whose molecular mechanism includes a reversible inhibition of voltage–gated sodium channels at the axonal level, impeding nerve impulse propagation. The present study was designed to evaluate the clinical efficacy of Neosaxitoxin as a local long-acting pain blocker in horse bucked shins, and it was found to effectively control pain. While Neosaxitoxin and Gonyautoxin, another Paralytic Shellfish Poison (PSP) toxin, have been successfully used in humans as long-lasting pain blockers, this finding marks the first time a PSP has been shown to have an established effect in veterinary medicine.

Periostitis of the third metacarpal bone of horses, also known as bucked shins, is the result of repetitive concussive force due to running on firm surfaces in overly rigorous training regimens. Specifically, this medical condition is caused by inflammation of the periosteum, a layer of connective tissue that surrounds bone, and it is marked by tenderness and swelling of the bone and aching pain. Bucked shins is a frequent and serious condition in competitive racing horses and requires immediate attention (Nunamaker, 2002; Burba, 2009; Ross and Dyson, 2011; Hinchcliff et al., 2013).

Microalgae, primary producers that make up the base of both marine and fresh water food webs, also produce secondary metabolites with potent biological activities. One such metabolite is Paralytic Shellfish Poison (PSP), which poses a serious threat to public health due to its high mortality rate in mammals (Lagos, 1998, 2003; Lagos and Andrinolo, 2000; Lagos, 2014). The high toxicity of PSP is due to its reversible binding to a receptor on voltage-gated sodium channels (Na_V channel) in excitable cells (Lagos et al., 2004), thereby blocking neuronal transmission (Andrinolo et al., 2002; Catterall, 2000; Strichartz et al., 1995). Consequently, its main physiological effect is to impede both nerve impulse propagation and neuronal transmission over neuromuscular junction. Therefore, when PSP toxin is applied locally, two clinical activities are manifested simultaneously: (i) control of pain (anesthetic activity) and (ii) control of muscle hyperactivity (relaxant effect) (Lagos, 2014).

A descriptive exploratory study was conducted to test Neosaxitoxin, a PSP toxin, as a local anesthetic in 14 horses diagnosed with bucked shins, from Club Hípico Racetrack in Santiago, Chile. The principles of the Chilean National Ethical Guidelines for Biomedical Research Involving Veterinarian Subjects (*Comité Institucional de Cuidado y Uso de Animales de Experimentación* (CICUAL, CD:812/03)) were strictly followed in the design of this study, which was also approved by the Ethical Committee of the Medicine Faculty, University of Chile (FM 0551).

Neosaxitoxin was purified from shellfish highly contaminated with PSP that were collected from the fjords around the Magellan Strait, close to the city of Punta Arenas in the southernmost part of Chile, and toxin purity (98%) was determined by high performance liquid chromatography with online fluorescence detection and mass spectroscopy analysis (Lagos, 1998; Andrinolo et al., 2002; Lattes et al., 2009, Oshima, 1995). Doses for the horses were

* Corresponding author.
E-mail address: nlagos@med.uchile.cl (N. Lagos).

https://doi.org/10.1016/j.toxicon.2017.11.004
0041-0101/

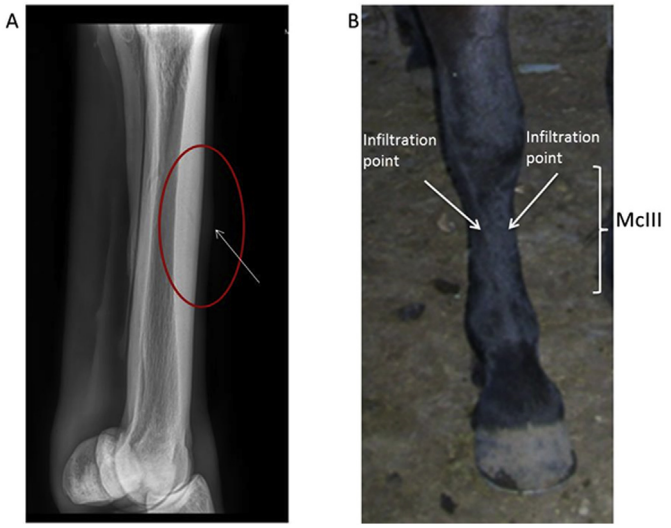

Fig. 1. A. X-ray of the Lateromedial view of the McIII, shows lesion area with increase inperiosteum thickness (circle) and volume increase of adjacent soft tissue (arrow). B. Photograph of the McIII. Subcutaneous infiltration points with Neosaxitoxin are indicated with arrows.

extrapolated from the results of clinical studies performed in humans and were produced in the Membrane Biochemistry Laboratory, of the Faculty of Medicine, University of Chile with the approval of National Institute of Health in Santiago, Chile (Lagos, 2014).

The horses were divided into three groups, according to the dosage of Neosaxitoxin they received (200, 300, or 600 μg), with each administered dose corresponding to pure Neosaxitoxin diluted in 1 mL of NaCl 0.9% at pH 6.2, in a sterile isosmotic solution without additives. For the local infiltration, 5 mL syringes with 25G needles were used. The dose was applied subcutaneously in two infiltration points (2.5 mL each), located on either side of the affected area, as shown in Fig. 1.

Unlike humans, animals cannot explain the level of pain that they are suffering. Horses were chosen as the animal model to test Neosaxitoxin efficacy as a local long-acting pain blocker, since two methods can be utilized to quantify anesthetic activity: the AAEP Lameness test (March Test, Hinchcliff et al., 2013) and pain measurement using a pressure algometer, also called a painmeter (Baseline® push-pull force gauge, Model: 12-0804. White Plains, NY, USA) (Fischer, 1998). The latter procedure involves applying direct pressure with the algometer to the assessed area, to determine the maximum pressure (in kg/cm^2) tolerated by each horse, before the limb was lifted. As a normalized procedure in this study, the algometer was applied 4 consecutive times to the affected limb before and 10 min after infiltration, and the median pressure that was tolerated was recorded. A pressure measurement in the uninjured limb served as the negative control.

The results observed using the March Test were confirmed by the Algometry Test (Table 1). The horses tolerated a median baseline of 2 kg/cm^2 of pressure before Neosaxitoxin infiltration. Meanwhile, after toxin application, the horses tolerated up to a median of 9 kg/cm^2 of pressure. This difference is statistically significant ($p = 0.001$) (Fig. 3) and establishes that Neosaxitoxin infiltration yields a potent analgesic effect, with all horses ceasing claudication post-infiltration, according to the AEEP Lameness Scale. No side effects were observed. Furthermore, the significantly different results according to infiltration dose group (200, 300, and 600 μg) indicates that the Neosaxitoxin long-lasting anesthetic effect is dose dependent (Fig. 2).

To date, nine types of Na_V channels have been described. These are >50% identical, with regard to their amino acid sequences in the trans-membrane segments and extracellular loop regions, though they have different functionality and expression profiles (Catterall et al., 2005). Of these, Na_V 1.7 is predominantly expressed in peripheral damage-sensing neurons and plays a critical role in inflammatory pain response. Furthermore, Na_V 1.7 is highly expressed in afferent dorsal root ganglia and increase its expression when there is chronic inflammation (Ekberg and Adams, 2006; Strickland et al., 2008). Interestingly, aberrant mutations in the gene SCN9A, which encodes the alpha subunit of Na_V 1.7, have been found to determine congenital insensitivity to pain, while multiple polymorphisms of the gene have been associated with the susceptibility to symptomatic regional pain (Cox et al., 2006; Valdes et al., 2011). All Na_V channels are present in high density in the Nodes of Ranvier on peripheral nerves, which are essential to enhance impulse transmission. Thus, it could be postulated that the long-acting pain blockage observed after Neosaxitoxin infiltration could be explained by the reversible binding to the Na_V channel on these Nodes of Ranvier segments (Catterall, 1993; Goldin, 2001).

This study successfully used Neosaxitoxin to manage pain in a horse model for the first time. Our findings are promising for other veterinary applications and open up a new path for the use of this type of phycotoxins in pain control. While classical pain management is still greatly used in clinics, our study highlights the benefits of this innovative method of pain control, with rapid recovery that permits faster horse rehabilitation due to pain relief. Although this exploratory study was successful, there is a limitation since the number of horses used was small, and there was no control group

Table 1
Horse summary list.

Subject	AAEP lameness scale	Total dose (μg)	Lasting time effect (days)	Algometry test before infiltration. (Kg/cm^2)	Algometry test after infiltration (Kg/cm^2)
1	3	600	21	2	13
2	3	600	19	2	4
3	3	600	21	2	N.D.
4	3	600	21	2	8
5	3	600	21	3	8
6	2	300	14	2	11
7	3	300	7	2	8
8	2	300	19	2	8
9	3	300	21	3	9
10	2	200	10	2	9
11	2	200	10	3	11
12	3	200	7	3	10
13	3	200	5	3	8
14	3	200	7	3	10

N.D. No determinate.

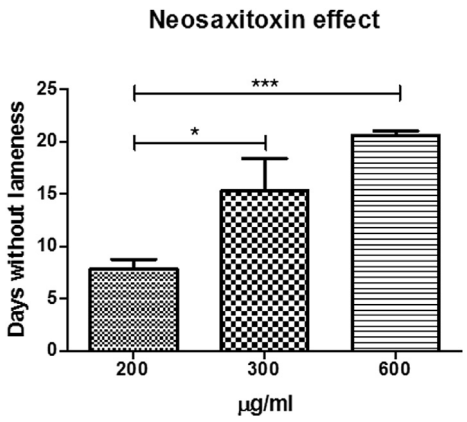

Fig. 2. Effect of neosaxitoxin on cessation of lameness in horses with bucked shins. A significant dose-response effect is observed (* $p < 0.05$ and *** $p < 0.005$).

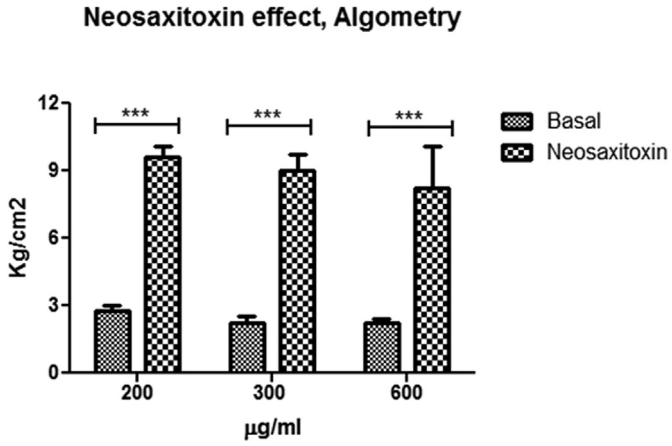

Fig. 3. Effect of neosaxitoxin on the sensation of pain in horses with bucked shins, as measured by algometry. Significant differences were observed between baseline and treated (*** $p < 0.005$).

for comparison. Thus, future studies should compare the effects of Neosaxitoxin on a larger cohort of horses against a control group. Finally, this innovative application could offer a simpler yet more effective pain management option.

Acknowledgements

This study was supported by FONDECYT Grant # 1130037 Chile, directed by Nestor Lagos. The data shown in this manuscript is a part of G Riquelme's M.Sc. thesis.

References

Andrinolo, D., Gomes, P., Fraga, S., Soares-da-Silva, P., Lagos, N., 2002. Transport of the organic cations gonyautoxin 2/3 epimers, a paralytic shellfish poison toxin, through the human and rat intestinal epitheliums. Toxicon 40, 1389–1397.
Burba, D., 2009. The Dilemma of Bucked Shins in the Racehorse. Equine Health Studies Program. LSU School of Veterinary Medicine.
Catterall, W.A., 1993. Structure and function of voltage-gated ion channels. Trends Neurosci. 16, 500–506.
Catterall, W.A., 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26, 13–25.
Catterall, W.A., Goldin, A.L., Waxman, S.G., 2005. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol. Rev. 57, 397–409.
Cox, J.J., Reimann, F., Nicholas, A.K., Thornton, G., Roberts, E., et al., 2006. An SCN9A channelopathy causes congenital inability to experience pain. Nature 444, 894–898.
Ekberg, J., Adams, D.J., 2006. Neuronal voltage-gated sodium channel subtypes: key roles in inflammatory and neuropathic pain. Int. J. Biochem. Cell Biol. 38, 2005–2010.
Fischer, A.A., 1998. Algometry in diagnosis of musculoskeletal pain and evaluation of treatment outcome: an update. J. Musculoskelet. Pain 6, 5–32.
Goldin, A.L., 2001. Resurgence of sodium channel research. Annu. Rev. physiology 63, 871–894.
Hinchcliff, K.W., Kaneps, A.J., Geor, R.J., 2013. Equine Sports Medicine and Surgery: Basic and Clinical Sciences of the Equine Athlete, second ed. Saunders, W. B.
Lagos, N., 1998. Microalgal blooms: a global issue with negative impact in Chile. Biol. Res. 31, 375–386.
Lagos, N., Andrinolo, D., 2000. Paralytic shellfish poisoning (PSP): toxicology and kinetics. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins": Mode of Action, Pharmacology and Physiology. Marcel Dekker, New York, NY, USA, pp. 203–215.
Lagos, N., 2003. Paralytic shellfish poisoning phycotoxins: occurrence in South America. Comments Toxicol. 9, 175–193.
Lagos, N., García, C., Lattes, K., Lagos, M., Garrido, R., et al., 2004. Paralytic Shellfish Poison: toxins that can kill and heal. In: Proceeding Chemical and Biological Medical Treatment Symposium. Spiez Laboratory, Switzerland, pp. 197–202.
Lagos, N., 2014. Clinical applications of paralytic shellfish poisoning toxins. In: Rossini, E.G.P. (Ed.), Toxins and Biologically Active Compound from Microalgae. CRC Press, Taylor & Francis Group, New York, pp. 309–329.
Lattes, K., Venegas, P., Lagos, N., Lagos, M., Pedraza, L., Rodriguez-Navarro, A.J., et al., 2009. Local infiltration of gonyautoxin is safe and effective in treatment of chronic tension-type headache. Neurol. Res. 31, 228–233.
Nunamaker, D., 2002. On bucked shins. In: AAEP Proceeding, vol. 48, pp. 76–89.
Oshima, Y., 1995. Postcolumn derivatization liquid-chromatographic method for paralytic shellfish toxins. J. Aoac Int. 78, 528–532.
Ross, M., Dyson, S., 2011. In: Diagnosis and Management of Lameness in the Horse. Elsevier/Saunders, St. Louis, Mo, pp. 64–80.
Strichartz, G.R., Hall, S., Magnani, B., Hong, C.Y., Kishi, Y., et al., 1995. The potencies of synthetic analogs of saxitoxin and the absolute stereoselectivity of decarbamoyl saxitoxin. Toxicon 33, 723–737.
Strickland, I.T., Martindale, J.C., Woodhams, P.L., Reeve, A.J., Chessell, I.P., et al., 2008. Changes in the expression of Na(V)1.7, Na(V)1.8 and Na(V)1.9 in a distinct population of dorsal root ganglia innervating the rat knee joint in a model of chronic inflammatory joint pain. Eur. J. Pain 12, 564–572.
Valdes, A.M., Arden, N.K., Vaughn, F.L., Doherty, S.A., Leaverton, P.E., et al., 2011. Role of the Na(V)1.7 R1150W amino acid change in susceptibility to symptomatic knee osteoarthritis and multiple regional pain. Arthrit Care Res. 63, 440–444.