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Case report

Gonyautoxins: First evidence in pain management in total knee arthroplasty

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ABSTRACT

Improvements in pain management techniques in the last decade have had a major impact on the practice of total knee arthroplasty (TKA). Gonyautoxin are phycotoxins, whose molecular mechanism of action is a reversible block of the voltage-gated sodium channels at the axonal level, impeding nerve impulse propagation. This study was designed to evaluate the clinical efficacy of Gonyautoxin infiltration, as a long acting pain blocker in TKA. Fifteen patients received a total dose of 40 μg of Gonyautoxin during the TKA operation. Postoperatively, all patients were given a standard painkiller protocol: 100 mg of intravenous ketoprofen and 1000 mg of oral acetaminophen every 8 hours for 3 days. The Visual Analog Scale (VAS) pain score and range of motion were recorded 12, 36, and 60 hours post-surgery.

All patients reported pain of 2 or less on the VAS 12 and 36 hours post-surgery. Moreover, all scored were less than 4 at 60 hours post-surgery. All patients achieved full knee extension at all times. No side effects or adverse reactions to Gonyautoxin were detected in the follow-up period. The median hospital stay was 3 days.

For the first time, this study has shown the effect of blocking the neuronal transmission of pain by locally infiltrating Gonyautoxin during TKA. All patients successfully responded to the pain control. The Gonyautoxin infiltration was safe and effective, and patients experienced pain relief without the use of opioids.

1. Introduction

Adequate pain management has become a priority in health care, as declared by the Joint Commission on Accreditation of Healthcare Organizations (Badner et al., 1996). In surgery, a delicate balance is required between adequate pain relief and early mobilization. Pain after total knee arthroplasty (TKA) is associated with venous thromboembolism, coronary ischemia, pneumonia, insomnia, paralytic ileus, urinary retention, arthrofibrosis, and cognitive disturbance (Carr and Goudas, 1999; Kehlet and Holte, 2001; Ranawat et al., 2003; Singelyn et al., 1998; Maheshwari et al., 2009). In light of this, pain management plays a crucial role in recovery post-TKA (Rankin et al., 2004) and is usually patients' primary concern (Park et al., 2007; Lavernia et al., 2010). Successful pain management can cut the length of hospital stays, decrease opioid use (Wheeler et al., 2002), and reduce costs, as well increase range of motion (Shoji et al., 1990), enhance patient satisfaction, and reduce incidence of chronic pain (Ranawat et al., 2003; Singelyn et al., 1998; Maheshwari et al., 2009; Perkins and Kehlet, 2000; Chang and Cho, 2012). Nevertheless, studies have found that over 50% of patients suffer from undertreated pain (Parvizi et al., 2011).

There are many alternatives for pain management post-TKA, although there is no consensus as to which is the best option (Gibbs et al., 2012). The most frequently used are: epidural analgesia, epidural continuous infusion, intravenous patient controlled analgesia, and/or major nerve blockage or continuous intra-

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articular infusion (Davies et al., 2004), all of which have associated negative side effects. Nerve blockage is associated with paresis and risk of falls, which can delay rehabilitation (Capdevila et al., 2005), while the use of opioids has several side effects (Badner et al., 1996; Niemelainen et al., 2014). In addition, intra-articular infusions have recently been associated with chondrolysis (Ong et al., 2010). The Multimodal Pain Control (Parvizi et al., 2011; Ranawat et al., 2003; Ranawat and Ranawat, 2007), established in 1988 to improve control post-operative pain in total joint replacement, targets different pain pathways through peri- or intra-articular infiltration using local anesthetic (Ranawat and Ranawat, 2007; Noyes et al., 2012; Kerr and Kohan, 2008; Kelley et al., 2013), NSAIDs (Vendittoli et al., 2006) and/or corticosteroids (Fu et al., 2010), morphine (Ritter et al., 1999) antibiotics and epinephrine (Maheshwari et al., 2009), resulting in almost 48 hours of pain control, without the complications described above (Kehlet and Andersen, 2011).

In the last two decades, there has been great interest in using toxins from plants, animals, and microorganisms in physiology studies with animal models, to search for potential clinical applications. The most notable example is the clinical use of botulin toxin type A (Botox®), which has been proven beneficial in several therapeutic approaches (Jankovic and Brin, 1991). In recent years, research on the use of biotoxins from microalgae – primary producers that serve as the base of marine and fresh water food webs, and which produce secondary metabolic products with potent biological effects – has been growing (Lagos, 1998, 2014; Manríquez et al., 2015). Biotoxins are responsible for a wide array of human illnesses, with the Paralytic Shellfish Poisoning (PSP) posing the most serious threat to public health, due to its high mortality rate in mammals (Lagos, 1998, 2003).

PSP toxins, which are water-soluble, non-protein phycotoxins, consist over 25 structurally related imidazoline guanidinium derivatives, with a low molecular weight (Oshima, 1995). The high toxicity of PSP toxins is due to their reversible binding to a site receptor on voltage-gated sodium channels of excitable cells (Catterall, 1993; Golding, 2001), thereby blocking neuronal transmission (Strichartz et al., 1995; Catterall, 2000; Andrinolo et al., 1999, 2002; Lagos and Andrinolo, 2000). Specifically, PSP toxins bind with high affinity to site 1 of the alpha unit on voltage-dependent sodium channels (Na_V channel), inhibiting channel opening. These channels play a key role in neurotransmission at neuronal synapses and neuromuscular junctions. Consequently, the main physiological effect of PSP toxins is linked to their blocking action on the axonal level, impeding both nerve impulse propagation and neuronal transmission over the neuromuscular junction. Therefore, when applied locally, two clinical activities are manifested simultaneously: (i) control of pain (anesthetic activity) and (ii) control of muscle hyperactivity (relaxant effect). One of the known PSP imidazoline guanidinium derivatives is the Gonyautoxin, an analog of Saxitoxin, the most studied toxin of this group (Lagos, 1998).

In recent publications, local infiltration of Gonyautoxin has been shown to be safe and effective in several clinical applications (Lagos, 2014). This study was designed to evaluate the clinical efficacy of Gonyautoxin 2/3 as a long-acting pain blocker in the management of pain after TKA, in order to propose a safe and effective therapeutic alternative for TKA pain control.

2. Methods

The principles of the International Ethical Guidelines for Biomedical Research Involving Human Subjects and Declaration of Helsinki (WHO, 2002) were strictly followed in the design of this study, which was approved by the Ethics Committee of the University of Chile Clinical Hospital (Acta N°70, 2015HCUCH). The purpose of the study and potential associated risks were discussed with each participant before enrollment, and their written informed consent was obtained before surgery.

2.1. Patients

This study recruited patients who required TKA due to unresponsive knee pain, with radiological evidence of osteoarthritis (with a Kellgren and Lawrence score of 2 or more).

2.2. Exclusion criteria

Patients with a mental disability that prohibited their comprehension of the study, with a body mass index (BMI) greater than 35, and/or a major neurological deficit and narcotic dependency were excluded.

2.3. Recruitment

Patients were recruited between April and May 2015, at the moment of their pre-operative appointment. During their appointment, they were presented with information about the study, the surgery, and the Gonyautoxin injection protocol, and interested participants signed the informed consent. Twenty-two patients were assessed during the study enrollment period, with five declining to participant and two excluded due to their BMI. In total, fifteen patients entered the study.

2.4. Gonyautoxin dose

Each dose of Gonyautoxin includes 40 μg of Gonyautoxin diluted in 1.0 mL of Sodium Chloride 0.9%, with pH 6.2 and isosmotic. The preparation of Gonyautoxin has been described previously (Garrido et al., 2004; Garrido et al., 2005; Garrido et al., 2007; Lattes et al., 2009; Lagos, 2014).

2.5. Surgery procedure

Standard TKA technique was followed, with the addition of the periarticular injection of Gonyautoxin during cementation. The spinal anesthetic (2.5 cc 0.5% bupivacaine), a medial parapatellar approach with posterior-stabilized implants from Vanguard BIO-MET®, minus tourniquet and without wound drainage, was performed. Thirty, intraoperative, periarticular injections of a solution containing 40 μg of Gonyautoxin in 30 cc were performed through cementation in the posterior capsule and in the retinaculum and collateral ligaments (medial and lateral), while the cement was hardening. The quadriceps tendon and subcutaneous tissue were infiltrated just before closure. Each injection had 1 cc, for a total dosage of 30 cc (Fig. 1).

Postoperatively, each patient had given a standard pain management protocol, which consisted of 100 mg of intravenous ketoprofen and 1000 mg of oral acetaminophen, every 8 hours for 3 days. All patients received venous thromboembolism prophylaxis with compression stockings, early mobilization, and 5000 iu of dalteparine subcutaneously. Physical therapy started the day after surgery and consisted of standing, partial load walking with two canes and knee range of motion exercises, performed twice a day. Patients were discharged when they achieved going up and down one flight of stairs with cane support, had a self-report VAS pain score less than 4, and had completed six doses of Cephazolin, in line with the University of Chile Clinical Hospital protocol.

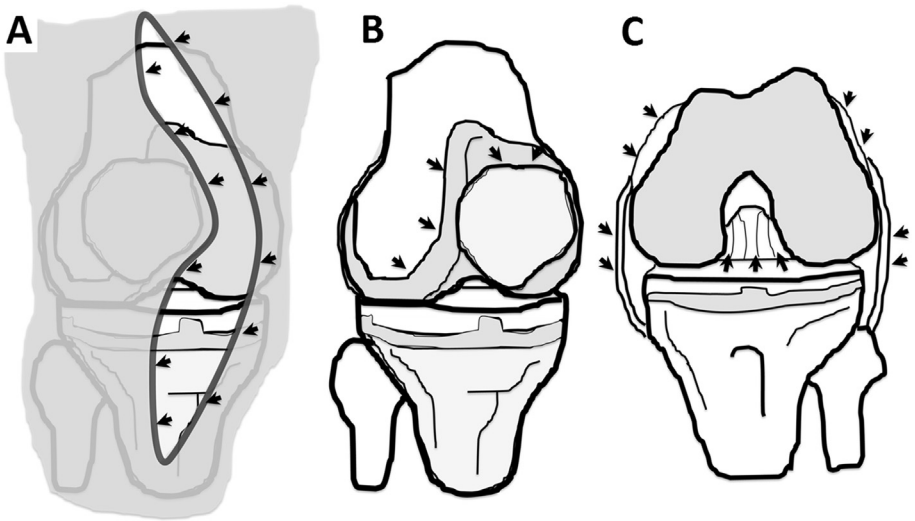

Fig. 1. Injection points in knee, from external anterior (A), interior anterior (B) and posterior (C) point of view, each arrow represents 1 cc of solution.

2.6. Variables

All patients were examined prior surgery, and their range of movement (ROM) was tested using an analog goniometer. This assessment also included the VAS, WOMAC, and the Oxford Knee Score.

Our study's primary outcome was the VAS pain score, on a scale from 0 to 10, being 0 no pain and 10 maximum pain. It was measured three times: the morning after surgery (12 hours) and over the next post-surgery days (at 36 and 60 hours). The assessments were carried out before the patient performed physical therapy, by the same orthopedic surgeon for each application (RW, MP). A VAS score of 4 or less was deemed successful. During the same assessment periods, ROM was also evaluated with analog goniometry. A successful ROM outcome was defined as patients achieving full extension (extension $= 0^{\circ}$) and full flexion (defined as flexion of 90° or higher). Complications and duration of hospital stay were recorded for each participant.

2.7. 2014 TKA patients cohort

To compare the clinical results of this study's patients, we reviewed the clinical records of 81 patients who underwent TKA in 2014 in the same hospital. All 2014 TKA patients received combined spinal anesthesia, consisting of an injection of fentanyl, epinephrine, bupivacaine, and an epidural of a continuous solution of bupivacaine and fentanyl. The same post-operation painkiller protocol followed in our study was used in the 2014 patients. Of the 81 2014 TKA patients, 12 were excluded from the comparative analysis (6 for having unicompartmental knee arthroplasty, 3 for TKA revisions, 1 due to a tumor, and 2 bilateral TKAs performed in one stage), leaving 69 patients, among which 64, 62, and 60 patients had ROM scores reported 12, 36 and 60 hours post-surgery, respectively. Data on VAS pain and length of stay was collected from all 69 patients.

2.8. Statistical analysis

Categorical variables, total, and percent frequencies were assessed. For continuous variables in which normal distribution could not be assumed, median, range, and interquartile ranges (IQ) are shown, while continuous variables with an assumed normal distribution were summarized with mean and standard deviation.

Statistical inference was performed by comparing the patients infiltrated with Gonyautoxin against the 2014 TKA patient cohort. Since the Gonyautoxin group consisted of fifteen patients, the Fisher two-sided test was implemented to compare two categorical variables: sex and success in full extension ($=0^{\circ}$), flexion ($\geq 90^{\circ}$) and pain (VAS ≤ 4). For age, the Shapiro Wilks test was performed, with a p-value higher than 0.15, assuming normal distribution. T-test for independent samples with Welch adjustment was performed. For VAS pain and ROM, a Mann-Whitney test was executed.

Binomial distribution was assumed for success in full extension, success in flexion and success in VAS pain, and a fitted repeated measurement logistic regression was estimated. Probabilities of one event per patient and odd ratios (OR) were calculated.

Significance level of 0.05 was established and the 95% confidence intervals were reported. All analyses were performed using Stata v11.2 (StataCorp LP, College Station, Texas, USA).

3. Results

The average age of the Gonyautoxin infiltrated group was 70.73 SD ± 9.61 years (Table 2). Before surgery, their WOMAC and Oxford Knee Score median values were 37.9 and 18, respectively (Table 1), and their median length of stay in the hospital was 3 days (Fig. 2). The longest stay for the Gonyautoxin patients was 10 days, corresponding to a patient who showed an acute pulmonary embolism. This was the only complication reported in this study, and we do not consider it to be a Gonyautoxin-related complication (Table 1). The 3-day length of stay of the Gonyautoxin infiltrated group was significantly less than the 2014 TKA patient cohort (Fig. 2 and Table 2).

Table 1
Summary before surgery: Womac and Oxford Score. Complications post THR are also shown.

Variable	Gonyautoxin (+)
Womac score	37.9 [31.8–42.4] (IQ 32.6–39.4)
Oxford score	18 [16–21] (IQ 17–19)
Complication	1 (06.67%)[a]

[a] Acute pulmonary embolism.

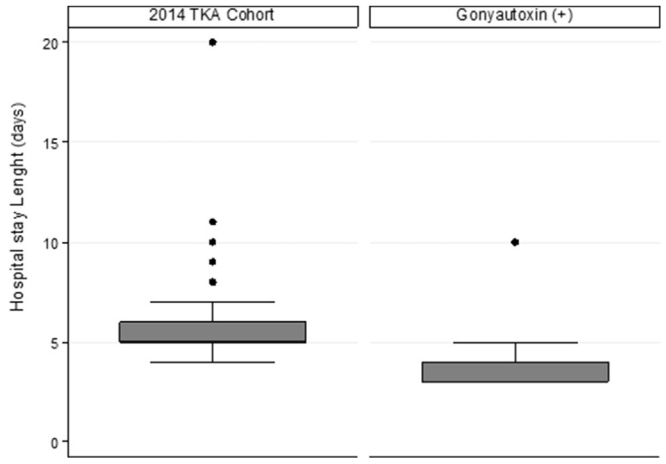

Fig. 2. Distribution of length of hospital stays (see Table 2). On the Y axis, the hospital stay length in days is shown. Graph box was estimated. The upper box shows percentile 75, bottom of the box percentile 25 and the middle line represents the median percentile 50. Dots represent outliners. The upper limit is calculated by [1.25*(Q3-Q1) + Q3] and the inferior limit is calculated by [1.25*(Q3 − Q1)] -Q2].

3.1. Range of motion (ROM)

All patients achieved full extension 12 and 36 hours post-surgery and more than 90° of flexion at 12, 36 and 60 hours after surgery. The total ROM was higher than 90° for all patients at all evaluation periods, with the median being 100°, 100° and 97° at 12, 36 and 60 hours respectively.

Comparing these data with those obtained from the 2014 TKA patient cohort, the Gonyautoxin-treated patients reached full extension and flexion higher than 90° at 12 hours. In addition, ROM was significantly higher in patients infiltrated with Gonyautoxin in all evaluations (Table 2 and Fig. 3).

3.2. Pain VAS

All patients assessed their pain as 2 or less on the VAS scale at 12 and 36 hours. Only four of them assessed a score above 4 at 60 hours post-surgery. Remarkably, 75% of the patients infiltrated with Gonyautoxin evaluated their pain as less than 2 after 36 hours (Table 2 and Fig. 4).

3.3. Repeated measured analysis

When logistic regression for repeated measured was estimated, the probability of obtaining full extension was 0.98 in the Gonyautoxin infiltrated group, and 0.66 in the 2014 TKA patients cohort. For a flexion greater than 90°, the probability was 0.99 for the Gonyautoxin infiltrated group and 0.79 in the 2014 TKA patient cohort. In the Gonyautoxin infiltrated group, the probability of having a VAS score of 4 or less was 0.9, while in the 2014 TKA patients cohort it was 0.74. The odds ratios with 95% confidence intervals are summarized in Table 3. In summary, patients infiltrated with Gonyautoxin had a higher chance of achieving better ROM without pain after surgery.

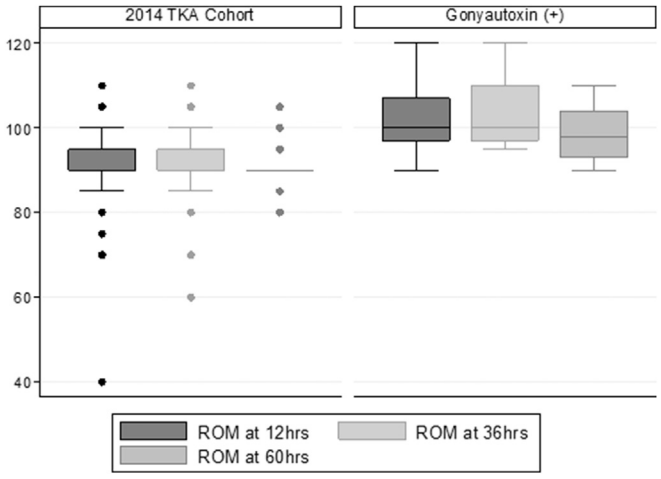

Fig. 3. Distribution of ROM by time of evaluation over cohorts. On the Y axis, the range of motion is shown, with 0° as full extension. Graph box was estimated. The upper box shows percentile 75 (Q3), bottom of the box percentile 25(Q1) and the middle line of the box is the median percentile 50(Q2). Dots represent outliners. The upper limit is calculated by [1.25*(Q3 − Q1) + Q3] and the inferior limit is calculated by [1.25*(Q3 − Q1)] -Q2.

Table 2
Summary of sex, age, knee size, ROM and pain VAS at 12, 36 and 60 hours post-surgery by the Gonyautoxin group and the 2014 TKA patients' cohort. The right column shows the significance of each test applied.

Variable	Gonyautoxin (+)	2014 TKA cohort	P (test)
Woman	6 (40.00%)	43 (62.32%)	0.15 (Fisher)
Age	70.73 (±9.61)	66.57 (±8.79)	0.14 (test,w)[a]
Right knee	8 (53.33%)	35 (51.47%)	0.99 (Fisher)
Hospital stay	3 [3–10] (IQ 3–4)	5 [4–20] (IQ 5–6)	0.00 (Mann-Whitney)
Range 12 hrs	100 [90–120] (IQ97–107)	90 [40–110] (IQ 90–95)	0.00 (Mann-Whitney)
Range 36 hrs	100 [95–120] (IQ 97–110)	90 [60–110] (IQ 90–95)	0.00 (Mann-Whitney)
Range 60 hrs	97 [90–110] (IQ 93–100)	90 [80–105] (IQ 90-90)	0.00 (Mann-Whitney)
Ext = 0° 12 hrs	15 (100%)	25 (39.06%)	0.00 (Fisher)
Ext = 0° 36 hrs	15 (100%)	44 (70.97%)	0.02 (Fisher)
Ext = 0° 60 hrs	14 (93.33%)	54 (90.00%)	0.99 (Fisher)
Flex ≥90° 12 hrs	15 (100°)	43 (67.19%)	0.01 (Fisher)
Flex ≥90° 36 hrs	15 (100°)	51 (82.26%)	0.11 (Fisher)
Flex ≥90° 60 hrs	15 (100°)	52 (86.67%)	0.35 (Fisher)
VAS pain 12 hrs	2 [1–4] (IQ 2-2)	3 [0–8] (IQ 2–5)	0.07 (Mann-Whitney)
VAS pain 36 hrs	2 [1–4] (IQ 2-2)	2 [0–10] (IQ 0–4)	0.51 (Mann-Whitney)
VAS pain 60 hrs	4 [2–5] (IQ 3–5)	1 [0–10] (IQ 0–4)	0.01 (Mann-Whitney)
VAS ≤ 4 12 hrs	15 (100%)	47 (68.12%)	0.01 (Fisher)
VAS ≤ 4 36 hrs	15 (100%)	54 (78.26%)	0.06 (Fisher)
VAS ≤ 4 60 hrs	11 (73.33%)	52 (75.36%)	0.99 (Fisher)

[a] s-wilk p > 0.15 on both cohort.

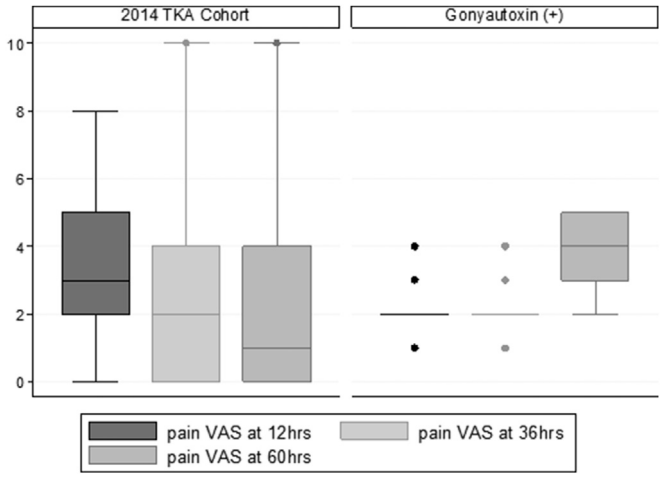

Fig. 4. Distribution of pain VAS by time of evaluation over cohorts. Graph box was estimated. The upper box shows percentile 75, bottom of the box percentile 25 and the middle line of the box is the median percentile 50. Dots represent outliners. Their upper limit is calculated by [1.25*(Q3 − Q1) +Q3] and the inferior limit is calculated by [1.25*(Q3 − Q1)] -Q2].

Table 3
Summary of the Odd Ratio (OR) estimated by a repeated measured logistic regression.

Variable	OR	95% IC
Ext = 0°	22.60	[2.68–190.45]
Flex ≥ 90°	12.02	[1.12–128.14]
VAS ≤ 4	3.62	[1.01–12.95]

4. Discussion

Pain management after surgery is an important issue. Although there are a number of treatment options for operative pain, a clear gold standard has not been established. Considering the immediate relaxant and anesthetic effects recently reported for Gonyautoxin in treating pain for other pathologies (Lagos, 2014), we decided to test the effectiveness of the toxin as a pain blocker after TKA.

The primary goal of the study was to achieve a significant decrease in the TKA patient's post-surgery VAS pain score. Specifically, we aimed for 100% of the patients treated with Gonyautoxin to have a VAS score of 4 or less 12 and 36 hours post-surgery. Our results exceeded our expectations, with 75% of the patients infiltrated with Gonyautoxin reporting a VAS pain score less than 2 at the 36 hours post-surgery evaluation.

Our secondary outcome was the number of days with a VAS score under 4, to determine a potential prophylactic effect of the Gonyautoxin infiltration. The average duration of the toxin's analgesic effect was longer than 60 hours, reducing the patients' hospital stay to an average of 3 days, with the patients reporting a median VAS score of 2 at discharge. These findings demonstrate the Gonyautoxin prophylactic effect.

The crucial component of this innovative protocol to control TKA post-surgery pain is the use of one dose of 40 μg Gonyautoxin, distributed in 30 infiltration points in the knee through cementation. Gonyautoxin exerts its effect due to the reversible binding to a site receptor on the voltage-gated sodium channel (Na_V channels) on excitable cells (Catterall, 1993; Golding, 2001), blocking neuronal transmission, resulting in total pain control without adverse side effects (Lagos, 2014).

Current scientific literature describes nine types of Na_V channels, which have amino acid identity >50% in the trans-membrane segments and extracellular loop regions, with different functions and expression profiles (Catterall et al., 2005). Of these, Na_V 1.7, Na_V 1.8 and Na_V 1.9 are predominantly expressed in peripheral damage sensing neurons and play a critical role in inflammatory pain. Furthermore, these channels are highly expressed in dorsal root ganglia of knee joint afferent and increase their expressions when there is chronic inflammation (Strickland et al., 2008; Ekberg and Adams, 2006). Although aberrant mutations in the gene SCN9A, which encodes the alpha subunit of Na_V 1.7, can cause congenital insensitivity to pain, multiple polymorphisms have been associated with the susceptibility to symptomatic knee osteoarthritis and multiple types of regional pain (Cox et al., 2006; Valdes et al., 2011). All of these Na_V channels in the knee joint are blocked by Gonyautoxin, which explains the long-acting pain blockage after TKA surgery combined with Gonyautoxin infiltration.

Study results demonstrate that Gonyautoxin periarticular infiltration yields a potent analgesic effect, with 100% of patients achieving excellent pain relief and functional recovery after TKA. Periarticular injection protocol with Gonyautoxin was shown to be a safe and effective in TKA pain control mechanism, promoting better patients' functional recovery.

The strength of this study is that, for the first time, it presents the successful and safe use of Gonyautoxin in post-surgery pain management. Our findings open up a new path for the use of this type of toxin. Although classical pain management (as used in the 2014 TKA cohort) is still greatly used, our study highlights the benefits of this innovative method of pain control, with early post-surgery toxin injections permitting early rehabilitation, faster and greater pain relief, and outstanding ROM.

As our study was only a pilot clinical case evaluation of 15 patients, a second larger clinical trial is needed to confirm these excellent early post-surgery pain management results. Another limitation is that the comparison to the 2014 cohort is insufficient, and future studies should incorporate a control group with the study design.

Nevertheless, our results indicate Gonyautoxin injections yield excellent results in pain control and ROM and perform a better solution than conventional pain control modalities (Ranawat and Ranawat, 2007; Parvataneni et al., 2007). Although there is currently no pain ideal management protocol, it is clear that patients should be offered optimum pain control after TKA for enhanced satisfaction and functioning. Our innovative method could offer TKA patients a simpler yet better pain management option.

5. Conclusions

For the first time, the effect of blocking pain neuronal transmission by Gonyautoxin local infiltration in TKA is shown. All patients successfully responded to the pain control, without negative motor effects. The Gonyautoxin infiltration was safe and effective, and patients experienced pain relief without opioid use. This protocol increased patient's ROM and reduced the average duration of their hospital stay, with total pain control achieved for at least for 60 hours. Our results are promising, and our protocol must be further explored as a pain control option for TKA patients.

Conflict of interest

We declare to have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2016.06.010.

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