Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
XX (X): X-X, XXXX
DOI: 10.9775/kvfd.2022.28380

ARTICLE IN PRESS

RESEARCH ARTICLE

Co-application of QX-314 and Lidocaine in Rabbit Brachial Plexus Block Using a Nerve Stimulator

Saghi EBADI [1] Masoumeh EZZATI GIVI [2 (*)] Hadi IMANI RASTABI [1]

[1] Shahid Chamran University of Ahvaz, Faculty of Veterinary Medicine, Department of Clinical Sciences, 61355145 Ahvaz, IRAN

[2] Shahid Chamran University of Ahvaz, Faculty of Veterinary Medicine, Department of Basic Sciences, 61355145 Ahvaz, IRAN

ORCIDs: S.E. 0000-0002-1586-4448; M.E.G. 0000-0002-5330-3350; H.I.R 0000-0002-1564-7240

Article ID: KVFD-2022-28380 Received: 02.09.2022 Accepted: 11.02.2023 Published Online: 21.02.2023

Abstract: We aimed to assess the efficacy and safety of QX-314, and lidocaine co-application (QX/Lid) in rabbit brachial plexus block (BPB) using the neurostimulation method to improve the selectivity and quality of induced regional anesthesia. Fifteen female rabbits (1.5-2 kg) randomly assigned into three groups: Lidocaine: 5 mg/kg; QX-314: 35 mM and QX/Lid: 35 mM + 5 mg/kg. The relevant anesthetic solution injected into the BP using a nerve stimulator (NS) and the BPB was evaluated by assessing sensory and motor functions. Based on the reaction to painful stimuli and the loss of bearing weight on the treated forelimb, respectively, the onset and duration of sensory and motor block were measured. The quality of sensory block assessed by using the scoring method. Blood samples collected before and at 5 min after drug injection up to180 min to measure the plasma concentration of QX-314 by HPLC. The QX/Lid resulted in a significantly faster onset of sensory and motor block, significantly decreased the duration of motor block and improved the quality of sensory block. QX-314 peak plasma concentration observed at 5 and 45 min in QX-314 and QX/Lid groups, respectively, and there was no behavioral evidence of systemic toxicity. In conclusion: QX/Lid co-application accelerate the onset of BP block with a greater quality of sensory and motor blockade separation and can be safely performed in rabbits using the NS method.

Keywords: *Brachial plexus block, Lidocaine, QX-314, Rabbits*

Bir Sinir Stimülatörü Kullanılarak Gerçekleştirilen Tavşan Brakiyal Pleksus Blokajında QX-314 ve Lidokainin Birlikte Uygulanması

Öz: Regional anestezinin seçiciliğini ve kalitesini artırmak için nörostimülasyon yöntemi kullanılarak tavşan brakiyal pleksus blokajında (BPB) QX-314 ve lidokainin birlikte uygulamasının (QX/Lid) etkinliğini ve güvenliğini değerlendirmeyi amaçladık. On beş adet dişi tavşan (1.5-2 kg); 5 mg/kg Lidokain, 35 mM QX-314 ve 35 mM + 5 mg/kg QX/Lid olmak üzere rastgele üç gruba ayrıldı. İlgili anestezik, bir sinir stimülatörü (NS) kullanılarak brakiyal pleksusa (BP) enjekte edildi ve BPB duyusal ve motor fonksiyonlar yönünden değerlendirildi. Anestezik uygulanan ön ayakta ağrı oluşturan uyaranlara verilen tepki ve üzerinde ağırlık taşıma kaybı temel alınarak, sırasıyla duyusal ve motor blokajın başlangıcı ve süresi ölçüldü. Duyusal blokajın kalitesi puanlama yöntemi ile değerlendirildi. QX-314'ün plazma konsantrasyonunun HPLC ile ölçülmesi amacıyla enjeksiyondan önce ve enjeksiyondan 5 dak. sonrasında 180. dak.'ya kadar kan örnekleri alındı. QX/Lid, duyusal ve motor blokajı anlamlı derecede daha hızlı başlattı, motor blokaj süresini anlamlı derecede kısalttı ve duyusal blokajın kalitesini artırdı. QX-314 ve QX/Lid gruplarında, QX-314'ün plazma pik konsantrasyonları sırasıyla 5 ve 45. dak.'larda gözlendi ve sistemik toksisiteye dair davranışsal bir kanıt bulunmadı. Sonuç olarak, QX-314'ün Lidokain ile birlikte uygulanması, daha yüksek kalitede duyusal ve motor blokaj ayrımı sağlayarak BP blokajının oluşmasını hızlandırmakta ve NS yöntemi ile tavşanlarda güvenle kullanılabilmektedir.

Anahtar Sözcükler: *Brakiyal pleksus bloğu, Lidokain, QX-314, Tavşan*

How to cite this article?

Ebadi S, Ezzati Givi M, Imani Rastabi H: Co-application of QX-314 and lidocaine in rabbit brachial plexus block using a nerve stimulator. *Kafkas Univ Vet Fak Derg,* 2023 (*Article in Press*).
DOI: 10.9775/kvfd.2022.28380

(*) Corresponding Author

Masoumeh EZZATI GIVI
Phone: +98 613 3226614 Cellular phone: +98 916 6513197 Fax: +98 613 3360807
E-mail: ezzatigivim@scu.ac.ir; ezzatigivim@gmail.com (M. Ezatti Givi)

Introduction

By reversibly blocking the formation and propagation of action potentials in both motor and sensory fibers, local anesthetics (LAs), as opposed to systemic analgesics, provide total analgesia and paralysis with fewer adverse effects. In the clinical use, LAs with fast onset, long action, selective effect and low local and systemic toxicities are desired. The combination of LAs is one of the methods that was used to improve the quality of induced analgesia, and decreases the toxicity effect by LAs dose reduction [1,2].

QX-314 is a membrane-impermeable quaternary derivative of lidocaine, which produces rapid onset and long-lasting nociceptive selective block. Due to its weak capacity to permeate the lipid tissues as a result of its persistent positive charge, QX-314's action is constrained. The delivery of QX-314 into nociceptors has been found to occur when transient receptor potential (TRP) channels are activated. Therefore, capsaicin, acidic solution and various LAs, such as lidocaine and bupivacaine are combined with QX-314 as TRP activators in regional nerve blocks [3-5].

Brachial plexus (BP) block is an effective method to provide anesthesia to the upper limb from the shoulder to fingertips depending on the block indication, surgery procedure, and anatomy variation [6,7]. BP block can be done with an injection of LAs around the peripheral BP nerves using different methods, including blind needle placement, ultrasound-guided, and nerve stimulator-guided. Neurostimulation is an alternative direct method to identify the peripheral location of the motor component of nerves using continuous electrical currents that produce muscular contraction and decrease required LAs for nerves block [6-9].

In rabbit, which is accepted as the BP experimental model in human and in pets, information regarding BP block by using NS method is lacking. In the present study, we aimed to evaluate the efficacy and safety of QX-314 and Lidocaine co-application (QX/Lid) in rabbit BP block using neurostimulation method to improve the selectivity and quality of induced regional anesthesia.

Material and Methods

Ethical Approval

The study was carried out based on the guidelines for the care and use of laboratory animals. All experimental procedures were approved by the Committee of Scientific Research and Institutional Animal Experimental Ethics, Shahid Chamran University of Ahvaz, Iran, (Approval no: EE/98.24.3.26578).

Animals and Study Design

Fifteen female adult New Zealand white rabbits (weighing 1.5-2 kg, 3- to 5-month-old) were purchased from Razi Vaccination, and Serum Center of Iran. Rabbits were single-housed with free access to food and tap water. The rabbits were randomly assigned into one of three groups: 1) Lidocaine [(Lid), 5 mg/kg]; 2) QX-314 [(QX), 35 mM]; and 3) QX-314 combined with lidocaine [(QX/Lid), 35 mM + 5 mg/kg]. Lidocaine and QX-314 alone injection served as controls. Drug solutions were freshly prepared before use, and the solution pH was measured by a laboratory pH meter (Crison pH meter; Basic 20$^+$, Barcelona, Spain). The rabbits fasted for 12 h before experiments, but water was available at all times. The right limb was selected for block, and the left was used as control.

Brachial Plexus Block

The rabbit's axillary region was prepared in the supine position. The location of BP nerves was determined using a nerve stimulator as described by Boogaert et al.[10]. In brief, the positive electrode was attached to the skin at a distance of 5-7 cm from the shoulder joint. A 23-gauge insulated needle (Pole Needle, Equip Medical, Holland) was connected to the other pole. Alongside the artery, the insulated needle was introduced into the skin and subcutaneous tissue. Electrical stimulations were provided until the limb's muscles began to contract, at which point the current was reduced and the needle was moved to produce the maximum twitch with the least amount of current (0.4-0.5 mA, 0.1 ms, 2 Hz). After the aspiration, a freshly prepared anesthetic solution (in total volume of 1ml) was injected slowly until the twitch disappeared. To block all branches of the BP, the needle was moved to different points and the time required to complete the procedure from the insertion of needle into the skin was recorded *(Fig. 1)*.

Assessment of Nerve Block

After the administration of drugs, the rabbits were kept in a standing position and the onset and duration of motor block was determined by the loss of bearing weight on treated forelimb. Based on the absence of response to painful stimuli, such as superficial and deep pin pricks with a 25-gauge needle and pinching of skin with a hemostat clamp closed to the first ratchet for 1-2 sec, the onset and duration of forelimb analgesia (sensory block) inside and below the elbow joint were recorded. The procedure was repeated every 10 min until a response was observed. Any obvious clinical signs related to local anesthetic toxicity, including extensor rigidity, muscle twitching, and convulsions were not monitored and recorded. The same investigator assessed analgesia in all cases and was blinded to given anesthetics.

Analgesia Maintenance Quality Score (0-3)

0- No analgesia, with obvious signs of discomfort made

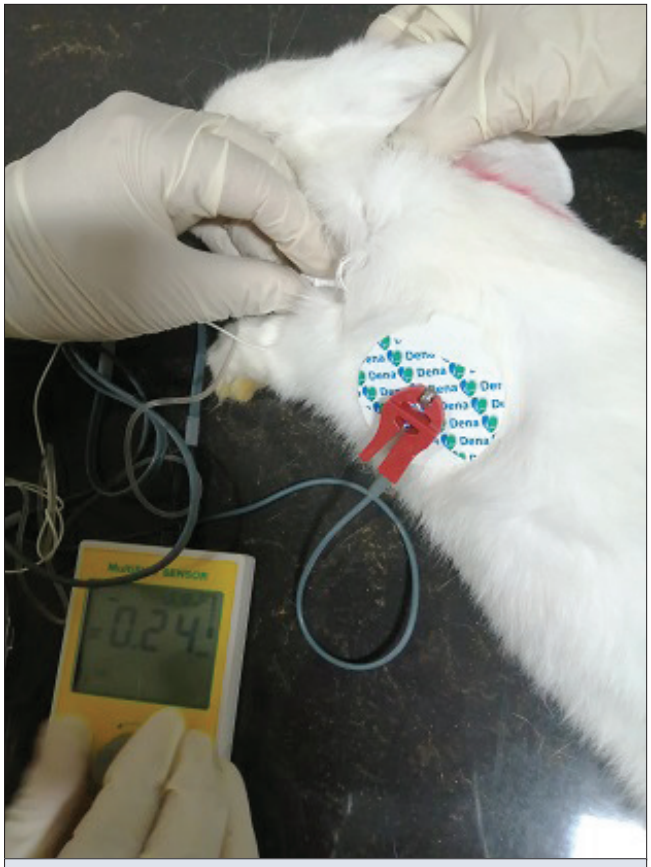

Fig 1. Brachial plexus block by using the neurostimulation method in rabbit

worst by firm pressure, the tendency to struggle and escape from their restraint.

1- Moderate analgesia, with some overt signs of the discomfort which were made worse by painful stimuli, exhibit signs of anxiety, such as whining.

2- Good analgesia, with no overt signs of discomfort but reaction to painful stimuli, teeth grinding.

3- Complete analgesia, with no overt signs of discomfort and no reaction to painful stimuli.

Measurement of QX-314 Plasma Concentration (HPLC)

Femoral artery blood samples were collected before and at 5, 10, 25, 45, 60, 90, 120, and 180 min after drug injection. The plasma concentration of QX-314 was measured by HPLC using the method that was previously described with some modifications [11]. In summary, plasma of blood samples was separated by centrifuge at 1500 rpm for 10 min at 4°C and were stored immediately at -20°C until further analysis. For sample preparation, 10 µL of perchloric acid was added to 50 µL of plasma samples and mixed using a vortex mixer for 1 min, centrifuged at 12000 rpm for 5 min at 20°C, and then filtrated. The final solution was diluted by mobile phase containing 60% of 50 mMol phosphate buffer with pH = 4, 30% of methanol, and 10% of acetonitrile with 0.16% trimethylamine. Then, 100 µL of the solution was injected into the HPLC system (Knauer HPLC system, Germany). The HPLC system consisted of an L-2100 pump, L-2300 column oven, and UV detector. The analytical column was a C18 column of 50-mm length and 4.6-mm diameter (Shiseido, Japan). The temperature was maintained at 40°C for the column and the flow rate was 0.7 mL/min. The wavelength of the detector was 210 nm and the retention time for QX-314 was 11.3 min. The detectable concentration of QX-314 was 300 ng/mL.

Statistical Analysis

Data are expressed as mean ± SEM. All statistical analyses were performed using GraphPad Prism software (Version 9.0.0) and data were analyzed for normality. The statistical significance of differences was calculated by one-way of variance (ANOVA) with Bonferroni's test, and paired t-tests for differences from the control values. Differences were considered statistically significant at ($P<0.05$).

Results

Age, body weight, American Society of Anesthesiologists (ASA) score, and the mean time of drug injection showed no significant differences among the groups. Moreover, there were no observed side effects in rabbits receiving QX-314. We assessed the effectiveness of BP block by measuring sensitivity and motor functions following QX-314 and QX/Lid injections. The results of the recorded data are presented here.

The Onset Time of Forelimb Analgesia and Paralysis

Table 1 shows the mean onset time of BP sensory and motor block. Lidocaine induced forelimb analgesia and

Table 1. *The mean onset time and duration of brachial plexus sensory and motor block*

Groups	Sensory Block		Motor Block	
	Onset Time (min)	Duration (min)	Onset Time (min)	Duration (min)
QX	-	-	-	-
Lid	7.3±2.82	26.25±3.14	2.75±1.94	25.5±3.63
QX/Lid	2.1±0.87	30.33±1.30	Less than 1 min (0.5±0.58)	14.6±0.28

QX (QX-314, 35mM), Lid (Lidocaine, 5 mg/kg), and QX/Lid (QX-314 in combination with Lidocaine (35mM+5 mg/kg)) (N = 5). Data are presented as mean ± SEM

paralysis at 7.30±2.8 and 2.7±1.94 min, respectively. QX-314 alone showed no significant effect on sensory or motor functions even at high doses (5-100 mM) over the measured time. In contrast, QX/Lid injection induced significantly faster sensory (2.1±0.87 min) (P<0.001) and motor (less than 1 min, 0.5±0.5 min) (P<0.05) block when compared to lidocaine. The onset of motor block was significantly faster than the onset of sensory block in Lid and QX/Lid groups (P<0.05) *(Fig. 2)*.

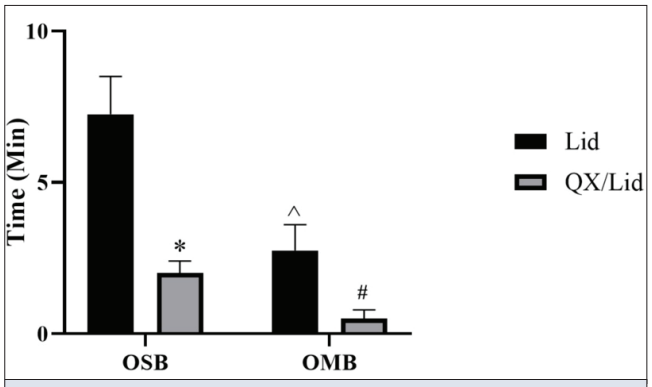

Fig 2. The mean onset time of brachial plexus sensory and motor block. Lid (Lidocaine, 5 mg/kg), and QX/Lid (QX-314 in combination with lidocaine (35 mM + 5 mg/kg)) (N=5). **OSB:** onset of sensory block; **OMB:** Onset of motor block. Data are presented as mean ± SEM. *P<0.001 vs Lid OSB, ^P< 0.05 vs Lid OSB and #P< 0.05 vs Lid OMB

Duration of Forelimb Analgesia and Paralysis

The duration of BP block for sensory and motor function is presented in *Table 1* and *Fig. 3*. QX/Lid combination significantly decreased the duration of forelimb paralysis as compared to Lid (14.6±0.28 vs 25.5±3.63 min) (P<0.05). Although there were no significant differences in the duration of forelimb analgesia among the groups, QX-314 improved the quality of induced analgesia as compared to Lid (Grade 3 vs 2/3; data not shown).

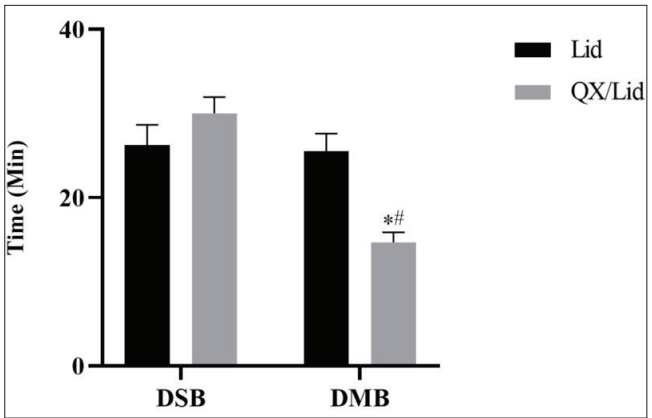

Fig 3. Duration of brachial plexus sensory and motor block. Lid (Lidocaine, 5 mg/kg), and QX/Lid (QX-314 in combination with lidocaine (35 mM + 5 mg/kg)) (N=5). **DSB:** Duration of sensory block; **DMB:** Duration of motor block. Data are presented as mean ± SEM. *P<0.05 vs Lid DMB, *P<0.001 vs QX/Lid DSB

QX-314 Plasma Concentration

The plasma concentration-time profile of QX-314 was determined by HPLC method and data are presented in *Fig. 4-A,B*. QX-314 peak plasma concentration observed at 5 and 45 min after injection in QX-314 (0.74±0.09 µg/mL) and QX/Lid (0.65±0.13 µg/mL) groups, respectively. Cmax of QX-314 showed no significant differences between two groups. However, the absorption of QX-314 was more rapid when injected alone as compared to injection in combination with lidocaine.

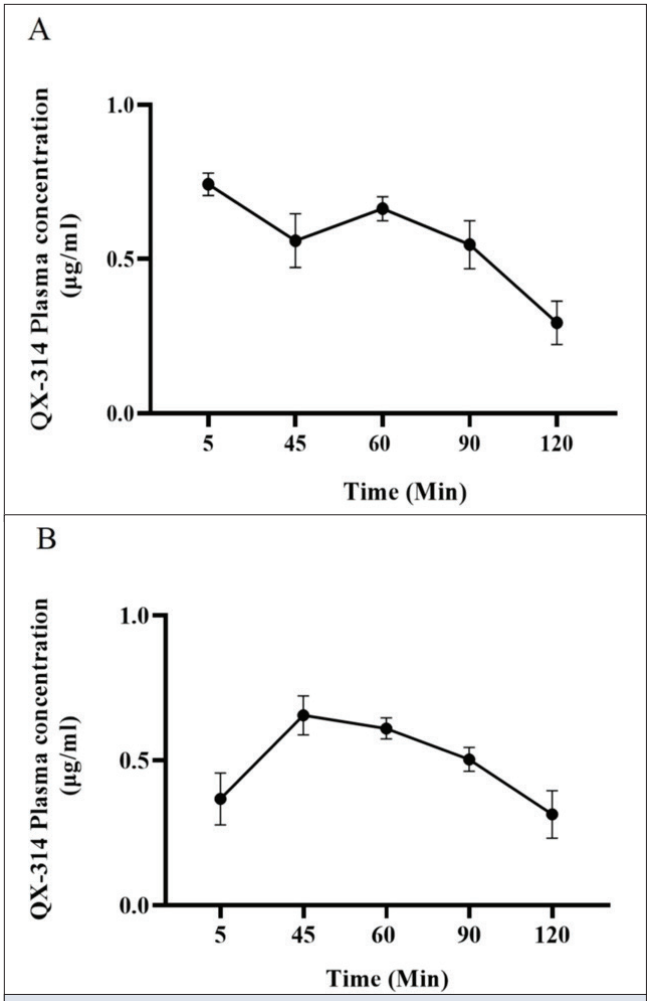

Fig 4. Mean concentration-time profile of QX-314 in plasma after brachial plexus injection. The plasma concentration of QX-314 was measured in **(A)** QX and **(B)** QX/Lid groups within 2 h after injection. N=5 per time point. Data are presented as mean ± SEM

Discussion

We evaluated the effect of QX-314 in combination with lidocaine on BP block (In both sensory and motor function) using nerve stimulator method in rabbits. The combined administration of QX-314 and Lidocaine dramatically sped up the start of BP nerve block, enhanced the quality of forelimb analgesia, and successfully separated BP

sensory from BP motor function, according to the results. Indeed, QX/Lid significantly decreased the duration of forelimb paralysis with no significant effect on sensory blockade duration, in contrast to nonselective effect of lidocaine when administrated alone.

Currently, differential nerve block is required for the surgical and non-surgical situation such as labor process, control of chronic pain and assessment of motor function during surgery. In such cases, it is necessary to provide pain relief without reducing motor function. These demands can be partially achieved with the available anesthesia methods which depend on the technique used and the types and concentration of local anesthetic.

The blockade of Brachial plexus is an effective technique to provide the surgical anesthesia and postoperative analgesia in the upper limb from the shoulder to fingertips depending on the indication and approach used [12]. BP block in rabbits can be induced by the desensitization of cervical (C5, C6, C7, and C8) and the first thoracic spinal (T1) nerves. Due to complex branching and crossing innervations, there are conflicting reports regarding rabbit BP innervation and anatomical structure; which is needed for surgical planning of experiments [12,13]. A combination of methods is needed for describing BP innervation and distinguishing between sensory and motor nerves. Despite the conflicting anatomical and innervation data, it was suggested that rabbit BP models human nerve injuries [6,7]. The BP block has been administered using a variety of techniques, including blind needle insertion, ultrasound guidance, and nerve stimulator guidance. Locating the nerve roots directly, lowering the dosage of LA, and improving the efficacy of BP block are all possible using peripheral nerve stimulator (NS). Reports indicate that the success rate of BP block using NS method is high and stable over time when compared to other methods [12-15]. In small animals LAs toxicity is common and the reducing the volume of the local anesthetic can makes it safer method. In rabbits, information regarding the clinical use of NS method for BP block is lacking, therefore, we aimed to use this method in present study.

Local anesthetics (LAs) are widely used techniques for the desensitization of a localized area of the body, which allows surgical procedures to be performed in the conscious animal. The efficacy of anesthetics can be improved by providing a faster onset time, creating selective effects, prolonging the duration of action, and decreasing side effects [16,17]. LAs vary in their ability to block sensory versus motor fibers and this differential pattern is affected by various factors, including the type of fiber, frequency of stimulation, length of nerve exposed to local anesthesia, and choice and concentration of LAs. When the nerve trunk and large nerves like the brachial plexus are targeted, the somatosensory arrangement of nerve fibers also affects the progression of the block. Therefore, combination of different LAs, the use of additive drugs, and different drug release methods are used to achieve the above-mentioned goals in LAs [17].

QX-314 is a membrane-impermeable quaternary derivative of lidocaine, which was shown to produce long-lasting regional anesthesia in animal models. It was confirmed that QX-314 is a selective sensory blocker that can induce motor block with a duration that is shorter than the sensory block [4,5,18,19]. However, due to its inadequate capacity to penetrate the plasma membrane due to its constant positive charge, its effectiveness in nerve block is restricted. Therefore, different methods and additives were used to enhance the diffusion of QX-314 across the lipid barriers. Activation of TRP channels was reported to specifically deliver QX-314 into nociceptors, so as to produce a rapid and long-lasting nociceptive-selective blockade without affecting motor function [3,20]. TRPV1 plays a critical role in peripheral nociceptor activation and management of acute and chronic pain. QX-314 has a biphasic regulatory effect on TRPV1 channels. At low concentrations (micromolar) it inhibits and at high concentrations (millimolar) activates TRPV1 channels [21]. Acidic solution, capsaicin, surfactants and various LAs, such as the lidocaine and bupivacaine, are combined with QX-314 as TRP channels activators [3,20-22]. Lidocaine, the most wildly used local anesthetic in veterinary medicine, at clinically relevant concentration is a potential non-irritative activator of TRPV channel. It can selectively deliver the QX314 to nociceptors and produce selective regional blocked [3,14,15,23,24].

Lidocaine has a variety of analgesic and anti-inflammatory properties but its application is limited due to its non-selectivity and short duration of action, especially in the postoperative pain management and induces longer duration of a motor block as compared to sensory block [14,15,23,24].

Beside the type of LAs, two items were mentioned for successful peripheral nerve block, appropriate LA concentration and injection solution volume to expose the critical length of the nerve. Injection of smaller volume and higher concentration has been suggested as critical parameters for a successful nerve block rather than the LA dose. A larger volume is suggested when nerves are poorly accessible and are not well located [16,17,25]. The total volume of LAs solution for performing the successful BP block was reported in dogs (0.25-1.0 mL/kg), cats (0.2-0.6 mL/kg), goats (0.3-0.4 mL/kg), and sheep (0.25 mL/kg). The minimum volume of LAs that is required to perform BP block in rabbits is 0.8 mL [26]. We injected 0.34±0.25 mL of lidocaine 2% in 0.65 ml QX-314 solution around the BP nerve using a nerve stimulator. Thus, the total amount

of the injectable medication solution was consistent with earlier research. The observed short duration of anesthesia is explained by the total dose of administered lidocaine, which was (7 mg/mL). Low concentration of lidocaine was just chosen as a TRP activator to accelerate the entrance of the QX-314 into nociceptive nerves to improve the BP block with fewer side effects.

The local tissue toxicity of QX-314 is concentration-dependent, and it is safe at concentrations below 35 mM. Therefore, a safe and effective concentration of QX-314 was used in combination with lidocaine, which resulted in a significantly faster sensory and motor block as compared to lidocaine. QX/Lid combination significantly decreased the duration of motor block and improved the quality of sensory block (grade 3 vs 2/3) as compared to lidocaine, which may be explained by the activation of TRP channels by lidocaine. However, the precise underlying process is yet unknown and requires more research. Increased BP block duration in TRPV1 KO mice after QX/Lidocaine administration suggests that lidocaine may aid QX-314 entrance into neurons through other major polar channels or potentially by enhancing fluidity of the neuronal membrane [20,22,27]. Surprisingly, the duration of the sensory block in our study did not differ among the groups and was lower than it was expected. This may be in terms of the low concentration of the injected drugs and/or drug-drug interactions in an aqueous solution, which reduce the effective concentration of each component. In any real solution, interactions occur among the components and reduce the effective concentration of the solution. In a solution, a rapid balance forms for lidocaine between its uncharged and charged status but QX-314 is permanently charged [23,24,28].

Injection methods may interfere with drug activity in the solution. The drug-drug interactions at the site of action or even during the injection process through microcatheters and possibly small gauge needles result in poor mixing of drugs in BP nerve roots, which affects administrated dose value and needs further studies. Although other factors such as the sensitivity of sensory and motor evaluation methods, injection technical errors, and drug absorption rate should be considered.

Furthermore, the plasma concentration of QX-314 was measured using HPLC method. Results indicated that when QX-314 was administrated alone, the peak plasma concentration occurred very fast and was observed within 5 min of injection. Because QX-314 cannot easily cross the cell membrane it may enter the blood circulation. The combination of QX-314 and lidocaine caused a slower absorption of QX-314 into blood circulation that peaked later at 45 min. In other words, lidocaine decelerated the absorption of QX-314 from the injection site into the blood. Vascularity and the binding of LAs to tissues are the factors that affect the initial rate of absorption into blood circulation. Previous studies reported that in the presence of lidocaine, QX-314 diffuses and binds to neural and also non-neural cells, which may explain the obtained results [3,30]. However, the pace of local anesthetic systemic absorption relies on a number of variables, including the drug's physicochemical qualities and formulation, the injection location, the speed of the injection, the supplied dosage, the presence of additives, and the agent's vasoactivity [28,29]. One of the main influencing factors can be the effect of LAs on local blood flow. At present, there is no referable data about the QX-314 effect on peripheral blood flow, which should be further studied. Lidocaine induces vasocontraction activity in low concentrations, but not at commonly used clinical concentrations. Therefore, future studies should investigate the vasoactivity of LAs when they use in combination forms, which affects drug concentration at the injection site.

The present study has some limitations, including the drug interaction analysis, dose optimization and the sensitivity of evaluation of sensory and motor blocks. The use of static and dynamic methods for sensory and motor block assessments is recommended.

The Combination of QX-314 and Lidocaine at the minimum effective concentration of both anesthetics, significantly accelerate the onset of BP nerve block, improved the quality of forelimb analgesia and satisfactory separate the BP sensory and motor function of. Future studies should identify the optimal ratio concentration of QX/Lid and injection volume to improve clinical success. QX-314 alone had no significant effect on the rabbit's brachial plexus block, measured by the sensory and motor performance and show a rapid absorption rate from injection site in compare to combination form. No side effects were observed in rabbits that received QX-314 alone even at high doses.

Availability of Data and Materials

The datasets analyzed during the current study are available from the corresponding author (M. Ezzati Givi) on reasonable request.

Funding Support

This study was supported by a grant from the Research Council of Shahid Chamran University of Ahvaz, Ahvaz, IRAN.

Author Contributions

ME and HI conceived and supervised the study. SE, ME and HI collected, and analyzed data. SE, ME and HI performed the anesthesia, brachial plexus function examinations and HPLC analysis. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of paper.

References

1. Enneking F, Wedel D, Horlocker T: The Lower Extremity: Somatic Blockade. 4th ed., Lippincott Williams & Wilkins: Philadelphia, PA, 2009.

2. Dillane D, Finucane BT: Local anesthetic systemic toxicity. *Can J Anaesth*, 57, 368-380, 2010. DOI: 10.1007/s12630-010-9275-7

3. Binshtok A, M Gerner, P Oh, S B, Puopolo M, Suzuki S, Roberson D, P Herbert, T Wang, C. F Kim, D Chung, G Mitani, A. A Wang, G. K Bean, B. P & Woolf C: Coapplication of lidocaine and the permanently charged sodium channel blocker QX-314 produces a long-lasting nociceptive blockade in rodents. *Anesthesiology*, 111 (1): 127-137, 2009. DOI: 10.1097/ALN.0b013e3181a915e7

4. Zhao Y, Zhou C, Liu J, Liang P, Liao D, Chen Y, Chen X: The quaternary lidocaine derivative QX-314 produces long-lasting intravenous regional anesthesia in rats. *PLoS One*, 9 (6):e99704, 2014. DOI: 10.1371/journal.pone.0099704

5. Yin Q, Li J, Zheng Q, Yang X, Lv R, Ma L, Zhang W: The quaternary lidocaine derivative QX-314 in combination with bupivacaine for long-lasting nerve block: Efficacy, toxicity, and the optimal formulation in rats. *PloS One*, 12 (3):e0174421, 2017. DOI: 10.1371/journal.pone.0174421

6. Kollitz KM, Giusti G, Friedrich PF, Bishop AT, Shin AY: Brachial plexus nerve injury and repair in a rabbit model part II: Does middle trunk injury result in loss of biceps function while repair results in recovery of biceps function. *Microsurgery*, 39 (7): 634-641, 2019. DOI: 10.1002/micr.30500

7. Kollitz KM, Giusti G, Friedrich PF, Bishop AT, Shin AY: The rabbit brachial plexus as a model for nerve injury and repair Part 1: Anatomic study of the biceps and triceps innervation. *Microsurgery*, 40 (2): 183-188, 2020. DOI: 10.1002/micr.30482

8. Mahler SP, Reece JLM: Electrical nerve stimulation to facilitate placement of an indwelling catheter for repeated brachial plexus block in a traumatized dog. *Vet Anaesth Analg*, 34, 365-370, 2007. DOI: 10.1111/j.1467-2995.2006.00335.x

9. Mosing M, Reich H, Moens Y: Clinical evaluation of the anesthetic sparing effect of brachial plexus block in cats. *Vet Anaesth Analg*, 37, 154-161, 2010. DOI: 10.1111/j.1467-2995.2009.00509.x

10. Boogaerts JG, Lafont ND, Lou H, Legros FJ: Plasma concentrations of bupivacaine after brachial plexus administration of liposome-associated and plain solutions to rabbits. *Can J Anaest*, 40 (12): 1201-1204, 1993. DOI: 10.1007/BF03009610

11. Imani Rastabi H, Khazaeel K, Naddaf H, Masroor R: Paravertebral brachial plexus block in sheep: A cadaveric and *in vivo* study. *Vet Res Forum*, 13 (3): 363-369, 2022. DOI: 10.30466/vrf.2021.138877.3088

12. Fuseya S, Yamamoto K, Minemura H, Yamaori S, Kawamata T, Kawamata M: Systemic QX-314 reduces bone cancer pain through selective inhibition of transient receptor potential vanilloid subfamily 1-expressing primary afferents in mice. *Anesthesiology*, 125, 204-218, 2016. DOI: 10.1097/ALN.0000000000001152

13. Pester JM, Varacallo M: Brachial Plexus Block Techniques. **In,** StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2022.

14. Mohiuddin M, Rahman M, Alim M, Kabir M, Kashem MA: Macro anatomical investigation of brachial plexus of the white New Zealand rabbit *(Orycotolagus cuniculus). Int J Nat Sci*, 1 (3): 74-76, 2011. DOI: 10.3329/ijns.v1i3.8825

15. Ghadirian S, Vesal N: Brachial plexus block using lidocaine/epinephrine or lidocaine/xylazine in fat-tailed sheep. *Vet Res Forum*, 4 (3): 161-167, 2013.

16. Choquette A, Del Castillo J, Moreau M, Guillot M, Alexander K, Kona-Boun JJ, Gauvin D, Tronc E: Comparison of lidocaine and lidocaine-epinephrine for the paravertebral brachial plexus block in dogs. *Vet Anaesth Analg*, 44 (2): 317-328, 2017. DOI: 10.1016/j.vaa.2016.08.002

17. Vadhanan P, Tripaty DK, Adinarayanan S: Physiological and pharmacologic aspects of peripheral nerve blocks. *J Anaesthesiol Clin Pharmacol,* 31, 384-393, 2015. DOI: 10.4103/0970-9185.161679

18. Barletta M, Reed R: Local anesthetics: Pharmacology and special preparations. *Vet Clin North Am Small Anim Pract,* 49, 1109-1125, 2019. DOI: 10.1016/j.cvsm.2019.07.004

19. Lim TK, Macleod BA, Ries CR, Schwarz SK: The quaternary lidocaine derivative, QX-314, produces long-lasting local anesthesia in animal models *in vivo. Anesthesiology*, 107 (2): 305-311, 2007. DOI: 10.1097/01.anes.0000270758.77314.b4

20. Hwang SM, Lee K, Im ST, Go EJ, Kim YH, Park CK: Co-application of eugenol and QX-314 elicits the prolonged blockade of voltage-gated sodium channels in nociceptive trigeminal ganglion neurons. *Biomolecules*, 10 (11):1513, 2020. DOI: 10.3390/biom10111513

21. Stueber T, Eberhardt MJ, Hadamitzky C, Jangra A, Schenk S, Dick F, Stoetzer C, Kistner K, Reeh PW, Binshtok AM, Leffler A: Quaternary lidocaine derivative QX-314 activates and permeates human TRPV1 and TRPA1 to produce inhibition of sodium channels and cytotoxicity. *Anesthesiology*, 124 (5): 1153-1165, 2016. DOI: 10.1097/ALN.0000000000001050

22. Rivera-Acevedo E, Pless SA, Ahern CA, Schwar SK: The quaternary lidocaine derivative, QX-314, exerts biphasic effects on transient receptor potential vanilloid subtype 1 channels *in vitro. Anesthesiology*, 114 (6): 1425-1434, 2011. DOI: 10.1097/ALN.0b013e318216ea0c

23. Zakir H.M, Masuda Y, Kitagawa J: A novel approach for detection of functional expression of TRPV1 channels on regenerated neurons following nerve injury. *J Oral Sci*, 62, 136-139, 2020. DOI: 10.2334/josnusd.19-0356

24. Zhang Y, Cui, Gong C, Tang Y, Zhou J, He Y, Liu J, Yang J: A rat model of nerve stimulator-guided brachial plexus blockade. *Lab Anim*, 53 (2): 160-168, 2019. DOI: 10.1177/0023677218779608

25. Zhang Y, Yin Q, Gong D, Kang Y, Yang J, Liu J, Zhang W: The preclinical pharmacological study of a novel long-acting local anesthetic, a fixed-dose combination of QX-OH/levobupivacaine, in rats. *Front Pharmacol*, 10:895, 2019. DOI: 10.3389/fphar.2019.00895

26. Nakamura T, Popitz-Bergez F, Birknes J, Strichartz GR: The critical role of concentration for lidocaine block of peripheral nerve *in vivo:* Studies of function and drug uptake in the rat. *Anesthesiology*, 99 (5): 1189-1197, 2003. DOI: 10.1097/00000542-200311000-00028

27. Fonseca C, Server A, Esteves M, Barastegui D, Rosal M, Fontecha CG, Soldado F: An ultrasound-guided technique for axillary brachial plexus nerve block in rabbits. *Lab Anim*, 44, 179-184, 2015. DOI: 10.1038/laban.732

28. Tsuchiya H, Ueno T, Mizogami M: Local anesthetics structure-dependently interact with anionic phospholipid membranes to modify the fluidity. *Chem Biol Interact*, 183 (1): 19-24, 2010. DOI: 10.1016/j.cbi.2009.10.006

29. Wang Q, Zhang Y, Liu J, Zhang W: Quaternary lidocaine derivatives: Past, present, and future. *Drug Des Devel Ther,* 15, 195-207, 2021. DOI: 10.2147/DDDT.S291229

30. Taylor A, McLeod G: Basic pharmacology of local anesthetics. *BJA Educ*, 20 (2): 34-41, 2020. DOI: 10.1016/j.bjae.2019.10.002