Effect of Lidocaine Solid Lipid Nano-particles on Sciatic Nerve Anesthesia Block in Rats

Wei Liu
Wei Xu

This research aimed at exploring the effect of lidocaine solid lipid nano-particles on sciatic nerve anesthesia block in rats. Firstly, we prepared liposomes encapsulated with lidocaine (nano-lidocaine).Then, we stimulated sciatic nerve of rats injected with narcotic drugs, and evaluated their sensory nerve block and motor nerve block. Finally, we observed their systemic toxic reaction and inflammatory infiltration. We found that compared with rats injected with free lidocaine, the sciatic nerve block time of those injected with nano-lidocaine was longer, and its blocking effect on sensory and motor nerve was stronger than that of those injected with free lidocaine. No systemic toxic reaction was observed in the experimental group. Histological examination manifested that there was mild or moderate inflammatory cell infiltration in the free lidocaine group, while there was no obvious cell infiltration in the nano-lidocaine group. Thus, we believe that lidocaine encapsulated with nano-liposomes has a small particle size, which can produce extra long sciatic nerve block in rats without systemic toxicity and local tissue damage. So, it has satisfactory anesthetic effect and safety.

Keywords: lidocaine, nano-liposomes, sciatic nerve block, anesthesia, safety

Tob Regul Sci.™ 2021;7(5-1):3975-3982

DOI: doi.org/10.18001/TRS.7.5.1.171

Local anesthetics are drugs that can accelerate reversible blocking of nerve transmission viasuppressing the excitationconduction process of peripheral nervous,it has a good local analgesic effect and is widely used in clinical operations[1,2]. They can interact with sodium channel in the form of ionization or non-ionization, stabilize membrane potential and finally block nerve transmission [3]. Lidocaine is a widely used amino-amide local anesthetic, which has a good analgesic effect in local nerve anesthesia. But it also has certain toxicity, which may cause adverse reactions in the nervous system or cardiovascular system [4]. However, the lipid penetration of lidocaine is weak, resulting in its limited effect. If the dosage is increased, there will be certain risks [5]. Hence, effective measures should be taken to enhance the anesthetic effect of lidocaine and reduce its toxicity.

Recently, in order to overcome these limitations, nano-particle drug carriers have been gradually applied in the medical field, especially for lipid-based systems. They could destroy and weaken highly organized intercellular lipids, thereby enhancing drug permeability and prolonging the duration of local action. Besides, they could also reduce side effects related to drug absorption by preventing systemic absorption of drugs,achieved a good response in the medical field [6,7]. Research [8] found that the delivery of gemcitabine encapsulated with nano-liposomes could effectively improve the efficacy of pancreatic ductal adenocarcinoma. Moreover, a recent study [9] has revealed that using nano-liposomes as drug carriers can not only control the size and properties of particles, but also make the drug release last longer, and nano-liposomes also have better biological stability.This confirms the role of nanoliposomes in improving the utilization of drugs.It has been proved that the drug can be released continuously in terms of local anesthetics by using liposome to encapsulate bupivacaine [10]. But the effect and safety of lidocaine encapsulated with nano-liposomes on sciatic nerve block in rats have not been studied yet.

The purpose of this research is to compare the effects of free lidocaine and lidocaine encapsulated in nano-liposomes on sciatic nerve anesthesia in rats, so as to evaluate their effects on sensory and motor functions,with a view to providing new thinking directions for improving the utilization efficiency of lidocaine.

*Wei Liu Department of Anesthesiology, Jinshan Hospital affiliated to Fudan University, Shanghai 201508,China, Wei. Xu *Department of Anesthesiology, Jinshan Hospital affiliated to Fudan University, Shanghai 201508,China *Corresponding author: DepartmentofAnesthesiology, Jinshan Hospital affiliated to FudanUniversity. Rd1508 Longhang, Jinshan District, Shanghai201508,China (E-mail: xuwei1594@sina.com)*

MATERIALS AND METHODS

Preparation of Nano-Liposomes Loaded with Lidocaine

Nano-liposomes encapsulated with lidocaine were prepared in the light of the improved ethanol injection method [11]. In brief, 175 mg phosphatidylcholine, 35 mg cholesterol and 35 ml lidocaine were dissolved in 50 μL ethanol, and then rapidly agitated and injected into 1 mL phosphate buffered saline. Thirty minutes later, nano-liposomes encapsulated with lidocaine (nano-lidocaine) was obtained. Particle size distribution and surface charge of nano-liposomes were testedthrough dynamic laser scattering (DLS) using Zetasizer (Nano ZS, Malvern Instruments Ltd, Britain), and morphology was observed by scanning electron microscope (SEM)(MIRA3 TESCAN, UK).

Establishment of Sciatic Nerve Block Rat Models

This experiment was conducted after being approved by the ethics committee of the research project in strict conformity with the nursing and use guidelines of experimental animals. All tests were carried out in male Wistar rats (280-380 g) (Experimental Animal Center, Guangdong), with 5 rats in each group. The rats were kept indoors at 21-25°C, and the relative humidity was 40%-60%. Only three rats could be kept in each cage, so as to ensure that they are mobile enough and avoid biting people, alternating regularly day and night, and getting food and water freely. In a sealed room, the animals were anesthetized with isoflurane in a 4-5% isoflurane/oxygen compound and maintained with 1% isoflurane through a nasal mask during the whole process. After that, the greater trochanter was placed on the right hindlimb by palpation, and the No. 29 nerve stimulation needle was inserted from the right femoral stalk at the sciatic nerve groove of the greater trochanter with the sciatic tubercle pointing to the sciatic bone. The ground electrode was immobilized to the animal's left ear, and the contraction of thigh muscle was searched using 0.6 mAinitial current. As the needle approached the nerve, the contraction gradually increased. Soon afterwards, the current was decreased to 0.1 mA. If the muscular reaction did not change, the needle was seen to be close to the sciatic nerve. Afterwards, 50 μL nano-lidocaine, empty liposomes (nano-Control), 0.5%(15 mmol/L) free lidocaine or normal saline (control) were injected around sciatic nerve through needles.

Sensory Function Evaluation

Sensory disturbance in rats was assessed in view of the reaction to harmful heat[12], and the specific operation was to put the feet of limbs injected with drugs gently on a metal plate at 58°C 15th, 30th and 60th min after injection. The time from the placement to the withdrawal of the soles is the withdrawal latency (PWL), which denotes sensory disturbancedegree. The baseline was about 2 s; to avoid tissue damage, the deadline was set to 14 s. PWL more than 7 s is considered to be an effective sensory nerve block.

Motor Function Evaluation

Motor function was testedthrough extensor postural thrust test [13]. The rat was holden perpendicularly, and then the injected limb could be put on the electronic balance. The value represented by the balance is the weight that the limbs can bear. Efficient motor block is equal to the percentage of suppressed body weight outnumbering 50%, counted as (baseline-test value)/baseline (%).

Toxicity Assessment

All rats were monitored for signs of systemic toxicity within two weeks after injection, including restlessness, convulsions, seizures or death. Subsequently, they were examined histologically at the end of the observation period, and euthanized by intraperitoneal injection of excessive pentobarbital. Sciatic nerves of surrounding tissues were collected, and morphological changes of tissues were assessedvia blind method under BX51 microscope system (Olympus, Tokyo, Japan). The inflammation, necrosis, degeneration and vacuoledegree in the nerve adventitia and adjacent muscles was evaluated semi-quantitatively using 0–4 scale [14]. Thereinto, 0 = normal, 1 = 0-25% of the area involved, 2 = 25%-50% of that area, 3 = 50%-75% of that area, and 4 = 75%-100%of that area.

Fatigue Behavior Test

Before the research was done, please let the animals adapt to the environment first. More specifically, the test observed them in a transparent glass box on a raised platform, so that the field of view of the rear paws was not obscured. After 15 minutes of adaptation, the rats were anesthetized in the light of the above method, and the sciatic nerve was blocked based on the research group, and then 50 μL 1% formalin was injected into the dorsal side of the right hind paw. Soon afterwards, eachwas put back into the box and the number of withdrawal was counted every 10 min over a length of 60 min. Withdrawal ranges from a simple elevation of the soles (unrelated to exercise) to violent shaking of the limbs, or exercise-related ripples in the back muscles.

Statistical Methods

SPSS20.0 was employed for statistical

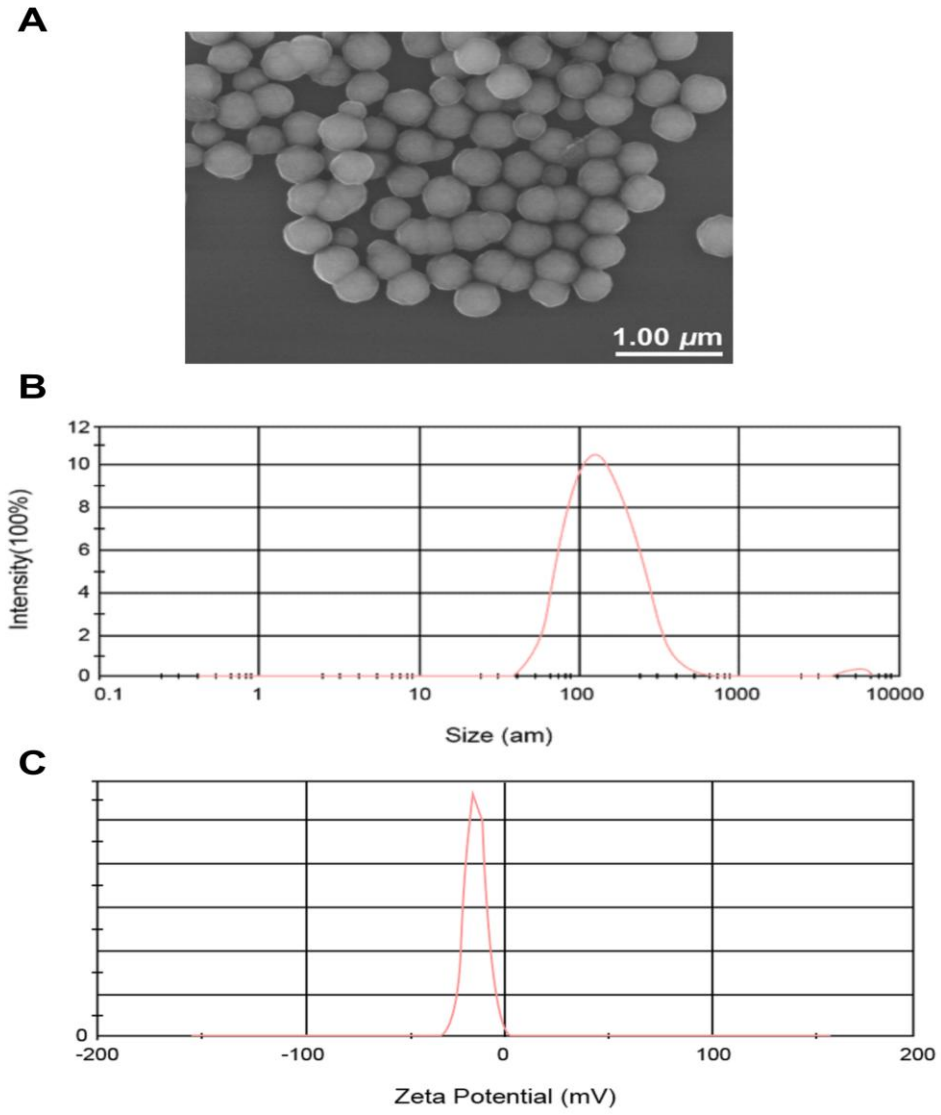
analysis.Use Graphpad prism8 to draw statistical pictures,The figures include the mean value and standard error of SEM. The data were analyzed via T test, the comparison among groups was assessed via one-way analysis of variance, and Bonferroni method was appiled to back testing. $P<0.05$ was statistically remarkable.

Characterization of Nano-Liposomes Loaded with Lidocaine

The characterization of nano-lidocaine was observed by SEM, and it has good dispersity and plump shape (Fig. 1-A). Its particle size was about 130.54±21.95 nm (Fig. 1-B), and its zeta potential was about -34.82 (Fig. 1-C).

RESULTS

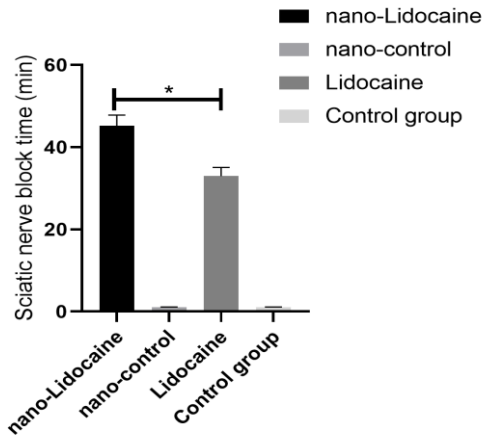
Fig. 1 Characterization of nano-liposomes loaded with lidocaine; A: characterization of nano-lidocaine by SEM observation;B: particle size; C: zeta potential value.



Comparison of Duration of Sciatic Nerve Block

We evaluated and compared the sciatic nerve block time of rats in each group. The results manifested that empty liposome and normal saline injection could not cause sciatic nerve block in rats, while nano-lidocaine and free lidocaine injection could effectively cause nerve block, with duration of 45.23±2.59 min and 32.96±2.11 min respectively. The nerve block time of nano-lidocaine rats was longer than that of free lidocaine rats, with statistically marked difference($P< 0.05$) (Figure 2).

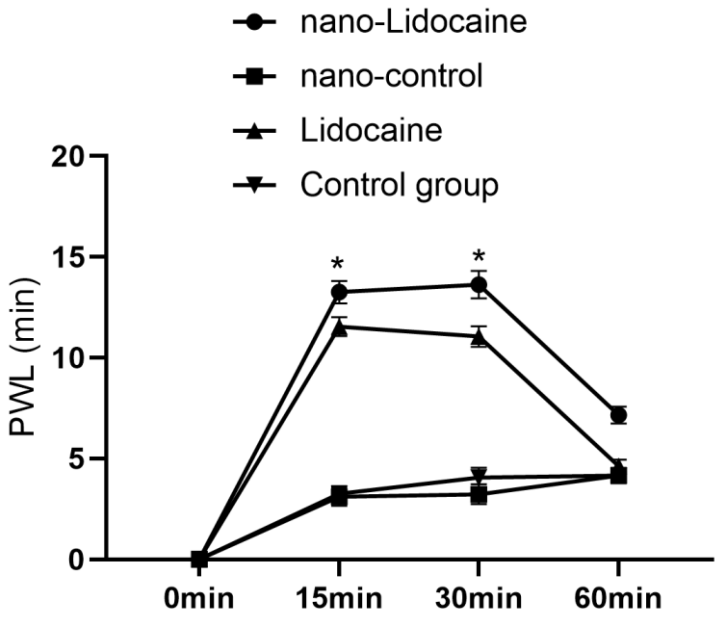
Fig. 2
Sciatic nerve block time of rats in each group;* denotes $P<0.05$.



Sensory Function Evaluation

Sensory disturbance in rats was tested by the reaction to harmful heat. The results signified that the PWL of rats injected with empty liposome and normal saline were (3.11±0.45 s, 3.23±0.48 s, 3.41±0.51 s) and (3.26±0.36 s, 4.05±0.49 s, 4.15±0.37s), respectively, with no obvious sensory nerve block; the PWL of nano-lidocaine and free lidocaine groups were (13.25±0.56 s, 13.62±0.68 s, 7.16±0.42 s) and (11.54±0.46 s, 11.05±0.51 s and 4.63±0.31 s), respectively; there was marked sensory block at 15 min and 30 min, and the PWL of nano-lidocaine was longer than that of free lidocaine, with statistically obvious difference($P< 0.05$)(Figure 3).

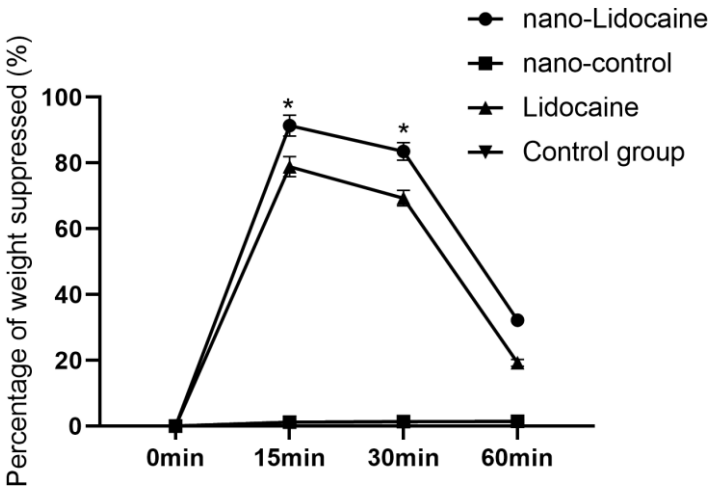
Fig. 3
Comparison of withdrawal latency of rats in each group;* denotes $P<0.05$; PWL: withdrawal latency.



Motor Function Evaluation

We measured the motor function of rats by extensor postural thrust test. There was no obvious nerve block of motor function in rats injected with empty liposome and normal saline. However, the body weight ratios of nano-lidocaine and free lidocaine groups were (91.35±3.15%, 83.54±2.69%, 32.19±1.93%) and (78.91±3.02%, 69.33±2.41%, 19.28±1.05%) respectively. The percentage of body weight of motor block in the nano-lidocaine group was higher than that in the free lidocaine group, with statistically remarkable difference($P< 0.05$) (Figure 4).

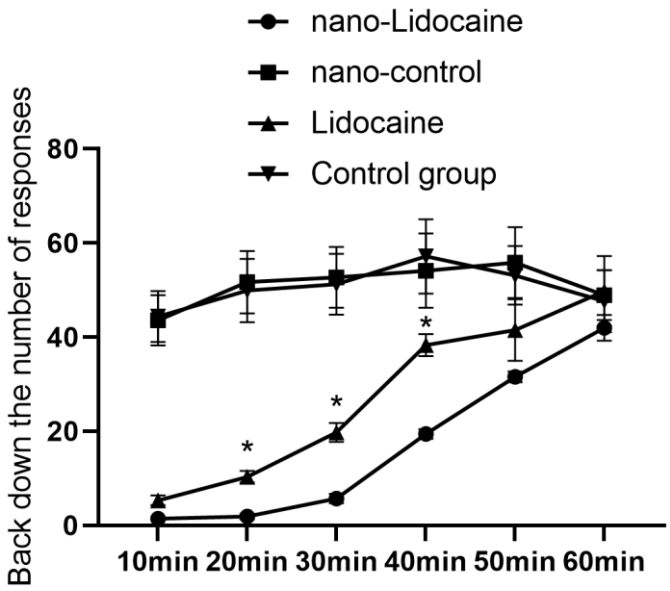
Fig. 4
Percentage of inhibitory weight of rats in each group;* denotes $P<0.05$.



Fatigue Behavior Test

Typical withdrawal behaviors were induced by formalin in all animals. The number of withdrawal responses of rats injected with empty liposomes and normal saline was (43.54±5.34, 51.67±6.62, 52.69±6.47, 54.12±7.88, 55.81±7.54, 48.93±5.27) and (44.37±5.42, 49.89±6.72,51.22±6.46, 57.14±7.92, 53.12±6.23, 47.62±6.61) respectively, with no marked difference ($P>0.05$); the number of the nano-lidocaine group was (1.43±0.45, 1.88±0.61, 5.72±1.01, 19.43±1.03, 31.55±1.14, 41.97±2.71); the number of withdrawal responses in the free lidocaine group was (5.33±1.05, 10.32±1.22, 19.79±2.01, 38.29±2.34, 41.51±6.55, 49.78±7.43), while that in the nano-lidocaine group were lower at 20th, 30th and 40th min, with statistically obvious difference($P< 0.05$)(Fig. 5).

Fig. 5
Comparison of withdrawal times of rats in each group;* denotes $P<0.05$.

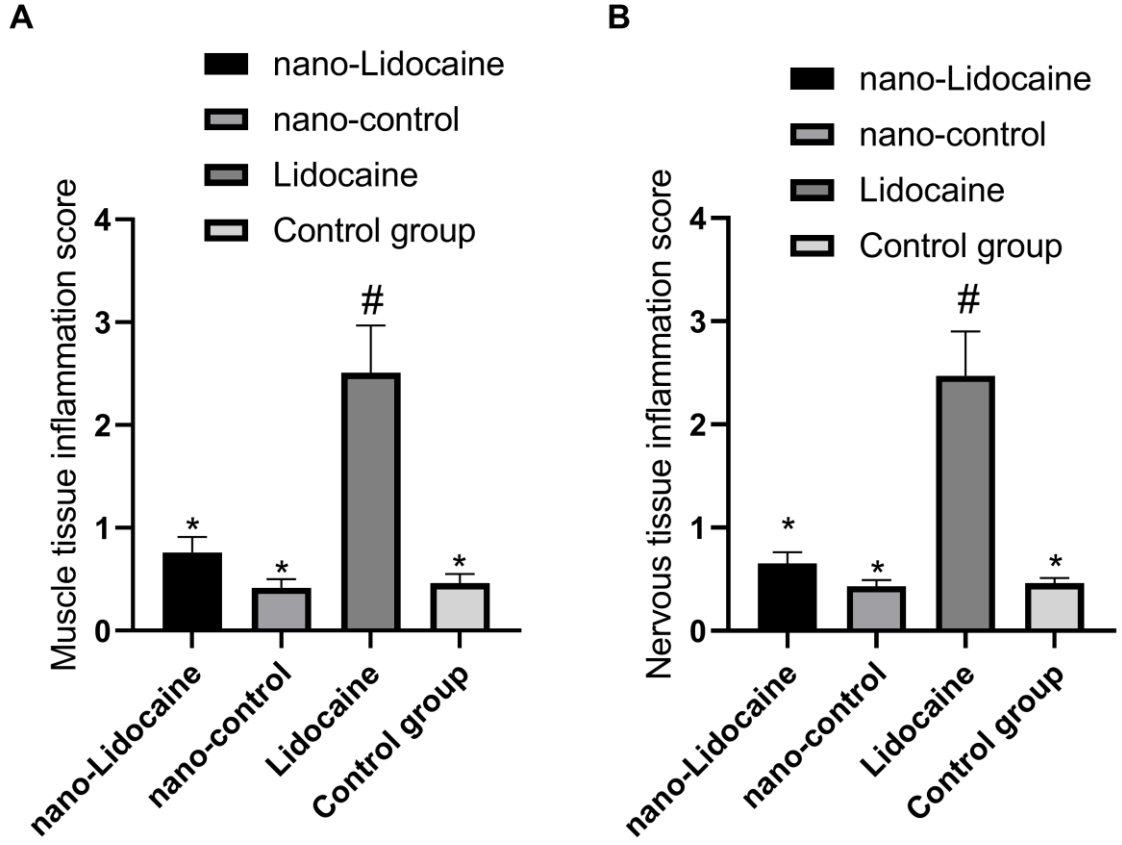


Systemic Toxicity Assessment

We evaluated the systemic toxicity of rats in each group, and found there wasno systemic toxicity or acute local stimulation. The evaluation of muscle and nerve tissue inflammation showed that there was no obvious infiltration of

inflammatory cells in muscle and nerve tissue in rats injected with empty liposome and normal saline.the scores are 0.42 ± 0.08, 0.43 ± 0.06 and 0.46 ± 0.09, 0.46 ± 0.05. The muscle (Figure 6-A) and nerve tissue inflammation scores (Figure 6-B) in the free lidocaine group were 2.51±0.46 and 2.47±0.43 respectively, higher than those in the nano-lidocaine group (0.76±0.15 and 0.65±0.11), with statistically marked differenc(P< 0.05).

Fig. 6
Tissue inflammation scores of rats in each group; A: muscle tissue inflammation scores; B: nerve tissue inflammation scores.* compared with #, P<0.05.



Discussion

At the moment, the common method to promote long-term local analgesia is continuous injection of local anesthetic. But the traditional local preparation may also create some problems. For example, due to the late barrier function and the effect of the stratum corneum of the body, if the intake dose is small, its effect is almost negligible [15,16]. To overcome the limitations of narcotic drugs, many researchers have focused on nano-particle drug carriers, and liposome is the key research direction,and it has been widely used in the medical field [17].

The effects and safety of free lidocaine and lidocaine encapsulated in nano-liposomes on sciatic nerve block were analyzed. Research has manifested that liposome-encapsulated local anesthetics are economical and less-toxic, but the only liposome anesthetic ratified by FDA is bupivacaine, which is mainly applied to trauma infiltration and has a very limited effect on nerve block [18]. Therefore, it is vital to find new nano-anesthetic agents for nerve block. We observed that the nerve block time of the nano-lidocaine group was longer than that of the free lidocaine group, which indicated that lidocaine loaded with nano-liposomes could play a more sustained nerve block effect at the same dose. It is well known that the sciatic nerve is an amalgamated nerve comprising sensory and motor fibers. Thus, we used a series of stimuli to quantify the sciatic nerve block in rats [19]. First of all, we evaluated the sensory and motor functions of rats through harmful heat response and extensor postural thrust test. It revealed that nano-lidocaine could block the nerve more effectively and continuously in both sensory and motor functions; besides, the rats in the nano-lidocaine group entered a state of deep anesthesia 15 min after drug injection, and the anesthetic effect occurred rapidly. Although the same dose of free lidocaine can also produce nerve block in rats, its efficacy and duration are not as

good as nano-lidocaine. Research [20] showed that nano-liposomes could enhance and prolong the blocking effect and duration of anesthetic agents on sensory nerves, consistent with our results.

Subsequently, we also applied formalin to induce withdrawal behavior in rats, so as to evaluate the anesthetic effects of nano-lidocaine and free lidocaine. The results manifested that the number of withdrawal responses in the nano-lidocaine group was lower than that in the free lidocaine group at 20th, 30th and 40th min, which also further confirmed our above experimental conclusions. Research [21] explained that liposomes had good affinity between cell membrane and lipid; after injection around nerve, they would carry lidocaine through lipid barrier, such as muscle and nerve adventitia, and quickly reach the action site,thereby improving the anesthetic effect and reducing the toxic side effects during the drug release. Another study [22] has shown that lidocaine was released faster and more easily from liposomes than from internal liposome chambers, so the drug flux into neurons was greater, which was essential for nerve block. At last, we observed the toxic reaction of rats in each group, and no obvious toxic reaction was found. However, after histological evaluation, we found that there was mild or moderate inflammatory cell infiltration in rats in the free lidocaine group, while there was no obvious infiltration in rats in the nano-lidocaine group,This shows that even if the rats have no obvious systemic toxicity, the injection of free lidocaine still has certain damage to the body, while the use of nano-lidocaine has less damage to the body. Research [23] has pointed out that liposomes can direct lidocaine to the site of action and avoid systemic absorption of drugs, which is ideal for delivering lidocaine, a drug that may cause systemic side effects. This is also similar to the results of our research. We also believe that it is appropriate to use liposomes to deliver lidocaine for nerve block in the body.

CONCLUSION

In general, lidocaine encapsulated with nanoliposomes has small particle size, which can produce extra long sciatic nerve block in rats free of systemic toxicity and local tissue damage, with satisfactory anesthetic effect and safety. This may be an alternative method for postoperative pain management.

REFERENCES

1. de Araujo, D.R., da Silva, D.C., Barbosa, R.M., Franz-Montan, M., Cereda, C.M., Padula, C., Santi, P. and de Paula, E., 2013. Strategies for delivering local anesthetics to the skin: focus on liposomes, solid lipid nanoparticles, hydrogels and patches. *Expert Opin Drug Deliv, 10*(11), pp.1551-1563.
2. Ginosar, Y., Haroutounian, S., Kagan, L., Naveh, M., Aharon, A. and Davidson, E.M., 2016. Proliposomal Ropivacaine Oil: Pharmacokinetic and Pharmacodynamic Data After Subcutaneous Administration in Volunteers. *Anesth Analg, 122*(5), pp.1673-1680.
3. Allard, S., Allard, P.M., Morel, I. and Gicquel, T., 2019. Application of a molecular networking approach for clinical and forensic toxicology exemplified in three cases involving 3-MeO-PCP, doxylamine, and chlormequat. *DrugTesting and Analysis, 11*(5), pp.669-677.
4. Galos, E.V., Tat, T.F., Popa, R., Efrimescu, C.I., Finnerty, D., Buggy, D.J., Ionescu, D.C. and Mihu, C.M., 2020. Neutrophil extracellular trapping and angiogenesis biomarkers after intravenous or inhalation anaesthesia with or without intravenous lidocaine for breast cancer surgery: a prospective, randomised trial.*British Journal of Anaesthesia, 125*(5), pp.712-721.
5. Aggarwal, V., Singla, M., Saatchi, M. and Hasija, M., 2020. Anaesthetic efficacy of 2% lidocaine with different concentrations of epinephrine (1:80,000 and 1:200,000) in intraligamentary injection after a failed primary inferior alveolar nerve block: a randomized double-blind study.*Acta Odontologica Scandinavica, 78*(4), pp.275-280.
6. Prow, T.W., Grice, J.E., Lin, L.L., Faye, R., Butler, M., Becker, W., Wurm, E.M., Yoong, C., Robertson, T.A., Soyer, H.P. and Roberts, M.S., 2011. Nanoparticles and microparticles for skin drug delivery.*Advanced Drug Delivery Reviews, 63*(6), pp.470-491.
7. Abidin, L., Mujeeb, M., Imam, S.S., Aqil, M. and Khurana, D., 2016. Enhanced transdermal delivery of luteolin via non-ionic surfactant-based vesicle: quality evaluation and anti-arthritic assessment.*Drug Delivery,23*(3), pp.1079-1084.
8. Deodhar, S., Dash, A.K., North, E.J. and Hulce, M., 2020. Development and In Vitro Evaluation of Long Circulating Liposomes for Targeted Delivery of Gemcitabine and Irinotecan in Pancreatic Ductal Adenocarcinoma. *AAPS PharmSciTech, 21*(6), pp.231.
9. Nogueira, E., Gomes, A.C., Preto, A. and Cavaco-Paulo, A., 2015. Design of liposomal formulations for cell targeting.*Colloids and Surfaces. B, Biointerfaces, 136*, pp.514-526.
10. Beiranvand, S., Eatemadi, A. and Karimi, A., 2016. New Updates Pertaining to Drug Delivery of Local Anesthetics in Particular Bupivacaine Using Lipid Nanoparticles. *Nanoscale Research Letters, 11*(1), pp.307.
11. Morales, J.O., Valdes, K., Morales, J. and Oyarzun-Ampuero, F., 2015. Lipid nanoparticles for the topical delivery of retinoids and derivatives. *Nanomedicine (Lond), 10*(2), pp.253-269.
12. Jeffry, J.A., Yu, S.Q., Sikand, P., Parihar, A., Evans, M.S. and Premkumar, L.S., 2009. Selective targeting of TRPV1 expressing sensory nerve terminals in the spinal cord for long lasting analgesia. *PLoS One, 4*(9),

pp.e7021.

13. Vuckovic, A. and Rijkhoff, N.J., 2004. Different pulse shapes for selective large fibre block in sacral nerve roots using a technique of anodal block: an experimental study.*Medical & Biological Engineering &Computing*, *42*(6), pp.817-824.
14. Feron, V.J., Kittel, B., Kuper, C.F., Ernst, H., Rittinghausen, S., Muhle, H., Koch, W., Gamer, A., Mallett, A.K. and Hoffmann, H.D., 2001. Chronic pulmonary effects of respirable methylene diphenyl diisocyanate (MDI) aerosol in rats: combination of findings from two bioassays.*Archives of Toxicology, 75*(3), pp.159-175.
15. Zink, W., Bohl, J.R., Hacke, N., Sinner, B., Martin, E. and Graf, B.M., 2005. The long term myotoxic effects of bupivacaine and ropivacaine after continuous peripheral nerve blocks. *Anesth Analg, 101*(2), pp.548-554, table of contents.
16. Balocco, A.L., Van Zundert, P.G.E., Gan, S.S., Gan, T.J. and Hadzic, A., 2018. Extended release bupivacaine formulations for postoperative analgesia: an update. *Current Opinion in Anaesthesiology , 31*(5), pp.636-642.
17. Pastorino, F., Brignole, C., Di Paolo, D., Perri, P., Curnis, F., Corti, A. and Ponzoni, M., 2019. Overcoming Biological Barriers in Neuroblastoma Therapy: The Vascular Targeting Approach with Liposomal Drug Nanocarriers. *Small, 15*(10), pp.e1804591.
18. Thalhammer, J.G., Vladimirova, M., Bershadsky, B. and Strichartz, G.R., 1995. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. *Anesthesiology, 82*(4), pp.1013-1025.
19. Ilfeld, B.M., Malhotra, N., Furnish, T.J., Donohue, M.C. and Madison, S.J., 2013. Liposomal bupivacaine as a single-injection peripheral nerve block: a dose-response study. *Anesth Analg, 117*(5), pp.1248-1256.
20. Sagie, I. and Kohane, D.S., 2010. Prolonged sensory-selective nerve blockade.*Proceedings of The National Academy of Sciences of The United States of America, 107*(8), pp.3740-3745.
21. Bozzuto, G. and Molinari, A., 2015. Liposomes as nanomedical devices. *International Journal of Nanomedicine, 10*, pp.975-999.
22. Becker, D.E. and Reed, K.L., 2006. Essentials of local anesthetic pharmacology.*Anesthesia Progress, 53*(3), pp.98-108; quiz 109-110.
23. Li, G., Fan, C., Li, X., Fan, Y., Wang, X., Li, M. and Liu, Y., 2012. Preparation and in vitro evaluation of tacrolimus-loaded ethosomes. *ScientificWorldJournal, 2012*, pp.874053