



Original article

Analysis of the analgesic effects of tricyclic antidepressants on neuropathic pain, diabetic neuropathic pain, and fibromyalgia in rat models

Zhenlong Qin^a, Lei Wang^b, Guoyan Li^b, Xuwen Qian^b, Jie Zhang^b, Ying Guo^b, Guokai Liu^{b,*}

^a Department of Anesthesiology, Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing 100029, China

^b Department of Anesthesiology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100700, China



ARTICLE INFO

Article history:

Received 25 March 2020
Revised 22 May 2020
Accepted 26 May 2020
Available online 1 June 2020

Keywords:

Tricyclic antidepressants
Rat model
Neuropathic pain
Diabetic neuropathic pain
Fibromyalgia

ABSTRACT

Objective: To investigate the analgesic effect of amitriptyline on neuropathic pain model rats, diabetic neuropathic pain model rats and fibromyalgia model rats.

Methods: The healthy male Sprague-Dawley (SD) rats were taken as the research object, and they were randomly divided into model group (group A), beside the sciatic nerve and injection of 5 mm amitriptyline group (group B), beside the sciatic nerve and injection of 10 mm amitriptyline group (group C), beside the sciatic nerve and injection of 15 mm amitriptyline group (group D), intraperitoneal injection of amitriptyline group (group E). Pain induced by selective injury of sciatic nerve branches in rats, pain induced by chronic compression of sciatic nerve, diabetic neuropathic pain and fibromyalgia were conducted to determine the pain threshold of mechanical stimulation in rats after drug administration.

Results: The pain threshold of mechanical stimulation in the local amitriptyline group (group B, C, D) was significantly higher than that in the group A and group E at each time point after drug treatment, and the pain threshold of mechanical stimulation gradually increased with the increase of concentration. There was no statistically significant difference in mechanical stimulation pain threshold between group A and group E at each time point after drug treatment.

Conclusion: Para-sciatic injection of amitriptyline at different concentrations has analgesic effects on neuropathic pain, diabetic neuropathic pain and fibromyalgia in rat models, and amitriptyline directly ACTS on the local sciatic nerve.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Clinically, pain lasting more than one month is called chronic pain, which seriously affects patients' work, living ability and sleep, and increases the incidence of depression, anxiety and other emotional disorders (Min et al., 2017). Neuropathic pain (NP), diabetic neuropathic pain (DNP) and fibromyalgia are common chronic pain in clinical practice. NP refers to the Pain caused by peripheral or

central nervous system lesions or injuries, which is characterized by hyperalgesia, abnormal Pain, spontaneous Pain and paresthesia (Jacob and Neal, 2017). DNP is one of the most common, serious and complex complications in patients with diabetes, which can affect any part of the peripheral nervous system including sensory nerve, motor nerve and autonomic nerve (Rao et al., 2017). Fibromyalgia is a spontaneous musculoskeletal pain syndrome characterized by generalized muscle pain and widespread tenderness points (Marques et al., 2017). Fibromyalgia is often accompanied by complications and a high disability rate, which puts a heavy burden on patients' life and economy. Depressive anxiety is a major complication of chronic pain, and depression is often associated with patients with neuropathic pain, diabetic neuropathic pain, and fibromyalgia. Therefore, Antidepressants have unique advantages in improving patients' quality of life in the treatment of neuropathic pain, diabetic neuropathic pain and fibromyalgia (Haliloglu et al., 2017). Tricyclic antidepressants (Tricyclic Antidepressive Agents, TCAs) is commonly used class of

* Corresponding author at: Department of Anesthesiology, Dongzhimen Hospital, Beijing University of Chinese Medicine, No 5 Haiyuncang Hutong, Dongcheng District, Beijing 100700, China.

E-mail address: liuguokai0729@163.com (G. Liu).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

antidepressants, clinical research shows that the analgesic effect of TCAs does not depend on its antidepressant effect. It is used to treat chronic pain for 3–7 days, obviously short than the antidepressant effect (14–21 days) (Le et al., 2018). Amitriptyline is one of the tricyclic antidepressants that inhibit and relieve pain in the central and peripheral nervous system. Amitriptyline is widely used for a variety of chronic pain, but in clinical use, with the increase of amitriptyline dose, many patients have suffered from many side effects such as dry mouth, nausea, dizziness and urine retention caused by intolerance to systemic administration (Takenoshita et al., 2017). Some studies have confirmed that amitriptyline has local anesthetic properties and analgesic effect by applying local subcutaneous, epidermal application, intrathecal and nerve block methods (Villani et al., 2017). Other studies have shown that compared with traditional local anesthetics, amitriptyline has a better intensity of analgesic action and a longer duration of action (Ryu et al., 2017). Moreover, it has been reported that local application of amitriptyline can produce analgesic effect on neuropathic pain, diabetic neuropathic pain and fibromyalgia (Hiroki et al., 2017; Abdulmajeed et al., 2015).

To sum up, evidence for the effectiveness of tricyclic antidepressants in alleviating neuropathic pain, diabetic neuropathic pain and fibromyalgia is mostly from clinical observation, and data on the effectiveness of preclinical animal models are lacking. Therefore, in this study, a variety of chronic pain models were established in healthy male SD rats to explore the analgesic effects of tricyclic antidepressants on neuropathic pain, diabetic neuropathic pain and fibromyalgia in rat models, providing reference for the treatment of chronic pain.

2. Materials and method

2.1. Experimental subject

120 healthy male SD rats (XXX animal center), 10 weeks old, weighing about 200 g. All animals were raised in cages with national standard rodent feed, 4 animals per cage, free food and water, no significant difference in body weight between groups, natural light, free diet, room temperature controlled at 20–26 °C, humidity controlled at 40–50%, and adaptive feeding for 2 weeks. The animal handling and experimental procedures comply with the national standards for experimental animals and are approved by the ethics committee.

2.2. Construction and grouping of animal models

All the rats were evenly divided into 4 groups, 30 in each group, which were respectively applied to sciatic nerve injury (SNI) induced pain in rats, sciatic nerve chronic compression injury (CCI) induced pain experiment, DNP experiment, and Fibromyalgia (FIB) experiment. Each group was randomly divided into 5 groups, with 6 in each group: the model group was given only 0.5 mL normal saline (SNI-A, CCI-A, DNP-A and FIB-A); peri sciatic nerve was injected 5 mM amitriptyline group (SNI-B, CCI-B, DNP-B and FIB-B); 10 mM amitriptyline (SNI-C, CCI-C, DNP-C and FIB-C) was injected to the sciatic nerve; the 15 mM amitriptyline group (SNI-D, CCI-D, DNP-D and FIB-D) was injected par astatic nerve; the left lower abdominal cavity was injected with 2.5 mg amitriptyline (SNI-E, CCI-E, DNP-E and FIB-E groups).

60 rats were chosen and the rats were anesthetized by intraperitoneal injection of chloral hydrate (7%, Nanjing Parsi Chemical Technology Co., LTD., China) at a dose of 5 mL/kg, and the rats were fixed on the operating table in the lateral position. The right hind limb was clipped, and the rats were sterilized with iodophor (Beijing Baioubowei biotechnology co., LTD., China). The

main sciatic nerve and three branches of the common peroneal nerve, sural nerve and tibial nerve were exposed. The common peroneal nerve and tibial nerve were ligated with 4–0 gut (Shanghai Yuyan Scientific Instrument Co., LTD., China), 30 of which were ligated and cut off to obtain SNI rat model, while the other 30 were ligated with only 4 loops at 1 mm interval to obtain CCI rat model. Topical application of penicillin powder (Shijiazhuang pharmaceutical group co., LTD., China) to prevent infection, layer by layer suture of muscle and skin.

30 rats were selected to construct DNP rat model. The blood glucose of the rats was measured with a blood glucose meter (Huanggang biotechnology co., LTD., China). After 12 h of fasting, the rats were injected with streptozotocin (Shanghai Yuanyue biotechnology co., LTD., China) intraperitoneally at a dose of 60 mg/kg to induce the diabetic model of the rats. After 7 days, venous blood from the tail of the rat was taken to measure the blood glucose value 2 h after meal. If the blood glucose value was >16 mmol/L, the modeling was successful. After continuous intraperitoneal injection of streptozotocin for 14 days, the diabetic rats showed spontaneous pain, hypersensitivity to pain and hypersensitivity to pain, which reached a significant level.

Thirty subjects were subcutaneously injected with 1 mg/kg/d reserpine for 3 consecutive days (Shanghai Yuanyue biotechnology co., LTD., China) to obtain FIB rats model.

2.3. Measurement of pain threshold for mechanical stimulation

Determination of mechanical stimulation pain threshold: before the experiment, all the rats were placed on a suspended metal scaffold and restrained in a transparent plexiglass box to adapt to the environment. The rats were operated continuously for 3 days at 30 min per day. The rats were placed in a transparent plexiglass box on a suspended metal scaffold and tested when they were quiet. Mechanical plantar induced pain instrument was used to apply vertical pressure from small to large on the skin of the middle part of the plantar of rats, with 50 g as the upper limit of stimulation value. When the time exceeded 8 s, the pressure was increased until the reaction occurred. The instrument automatically recorded the force applied to the sole of the foot during the lifting of the rat's foot. The test was conducted three times in a 10-minute interval, and the average value was taken as the mechanical stimulation pain threshold.

2.4. Methods of rat index detection in each group

SNI rat model, CCI rat model and FIB rat model were determined preoperative pain threshold of mechanical stimulation as the basic pain threshold. The drug was then treated and the pain threshold of mechanical stimulation was measured at 30 min, 60 min, 120 min and 180 min after administration.

Besides measuring the pain threshold of mechanical stimulation, thermal pain threshold should also be measured in CCI rat model. The thermal pain threshold of rats was measured at 180 min after drug treatment. Control the room temperature at about 25 °C, keep the surrounding environment quiet, put the rat into a glass partition at 30 °C at constant temperature, and fasten it in a breathable transparent plexiglass box, and let it move freely. A movable thermal radiation light source was placed under the glass partition to illuminate the plantar part on the surgical side of the rats, and the radiation intensity of the heat source was set to 45 °C. The time of foot lift reaction in rats was recorded by hot-plate pain detector (Nanjing Calvin biotechnology co., LTD., China), with 20 s as the upper limit of measurement threshold. The mean value was taken as the threshold value of thermal pain after 3 consecutive tests with an interval of 3 min.

The body weight and pain threshold of mechanical stimulation were measured on days 7, 14, 21 and 28 of DNP rat model before and after administration. Blood glucose was measured at 1, 2 and 6 w after administration.

For FIB rats model, the rod rotation experiment was conducted at 1 h and 3 h after drug treatment to evaluate the influence of amitriptyline on the motor ability of fibromyalgia model rats. The rats were placed on the stationary roller of the fatigue rod rotation instrument (TSE 337500, TSE company, Germany) with their heads turned inward to adapt to for 15 s. Turn on the fatigue swivel meter and set the rotation speed to 10 rpm/min. When the rat fell to the electric grid, the foot was electrified and an electric stimulation was given. They were trained twice a day, 5 min each time, and the rotary bar tester was wiped with ethanol (75%, Shandong fengcang chemical co., LTD., China) after each training. After 2 days of continuous training, a test was conducted, and an acceleration unit was adopted with a rotation speed of 4–40 RPM /min and a duration of 300 s. The drop latency of the rats was recorded.

2.5. Statistical method

SPSS19.0 statistical software was used for data analysis. Measurement data were expressed as mean \pm standard deviation ($x \pm s$). T test was used for comparison of the two kinds of mean and mean, and χ^2 test was used for comparison of enumeration data.

3. Results

3.1. Analgesic results of amitriptyline in SNI rats

SNI rat model was established to study the analgesic effect of different doses of amitriptyline on rats, which was shown in Fig. 1. The pain threshold of mechanical stimulation in the local administration group of amitriptyline (group SNI-B, SNI-C, SNI-D) was significantly higher than that in the group SNI-A and group SNI-E at each time point after drug treatment, with statistically significant difference ($P < 0.05$). With the increase of concentration, the pain threshold of mechanical stimulation in the locally administered amitriptyline group was gradually increased. Compared with group SNI-B, the pain threshold of mechanical stimulation

in group SNI-C was significantly increased at each time point after drug treatment, with statistically significant difference ($P < 0.05$). Compared with group SNI-C, the pain threshold of mechanical stimulation in group SNI-D was significantly increased at each time point after drug treatment, and the difference was statistically significant ($P < 0.05$). There was no significant difference in mechanical stimulation pain threshold between group SNI-A and group SNI-E at each time point after drug treatment ($P > 0.05$).

3.2. Analgesic results of amitriptyline in CCI rats

CCI rat model was established to study the analgesic effect of different doses of amitriptyline on rats, which was shown in Fig. 2. The pain threshold of mechanical stimulation in the local administration group of amitriptyline (group CCI-B, CCI-C, CCI-D) at each time point after drug treatment was significantly higher than that in the group cci-a and group CCI-E, and the difference was statistically significant ($P < 0.05$). With the increase of concentration, the pain threshold of mechanical stimulation in the locally administered amitriptyline group was gradually increased. Compared with group CCI-B, the pain threshold of mechanical stimulation in group CCI-C was significantly increased at each time point after drug treatment, with statistically significant difference ($P < 0.05$). Compared with group CCI-C, the pain threshold of mechanical stimulation in group CCI-D was significantly increased at each time point after drug treatment, and the difference was statistically significant ($P < 0.05$). There was no significant difference in mechanical stimulation pain threshold between group CCI-A and group CCI-E at each time point after drug treatment ($P > 0.05$), as shown in Fig. 2a.

Compared with group CCI-A, there was no statistically significant difference in the thermal pain threshold between group CCI-B and group CCI-E at 180 min after administration ($P > 0.05$), while the thermal pain threshold between group CCI-C and group CCI-D was significantly extended at 180 min after administration ($P < 0.05$), as shown in Fig. 2b.

3.3. Analgesic results of amitriptyline in DNP rat model

A DNP rat model was established to study the analgesic effect of different doses of amitriptyline on rats, which was shown in Fig. 3. The pain threshold of mechanical stimulation in the local administration group of amitriptyline (group DNP-B, DNP-C, DNP-D) within 28 days after drug treatment was significantly higher than that in the group DNP-A and group DNP-E, and the difference was statistically significant ($P < 0.05$). With the increase of concentration, the pain threshold of mechanical stimulation in the locally administered amitriptyline group was gradually increased. Compared with group DNP-B, the pain threshold of mechanical stimulation in group DNP-C was significantly increased within 28 days after drug treatment, with statistically significant difference ($P < 0.05$). Compared with group DNP-C, the pain threshold of mechanical stimulation in group DNP-D was significantly increased within 28 days after drug treatment, with statistically significant difference ($P < 0.05$). There was no significant difference in mechanical stimulation pain threshold between group DNP-A and group DNP-E within 28 days after drug treatment ($P > 0.05$), as shown in Fig. 3a.

At 1 week after administration, the blood glucose level of group DNP-A continued to rise, and maintained a high and stable blood glucose level from 2 weeks to 6 weeks after administration. Compared with group DNP-A, there was no significant difference in blood glucose between group DNP-B, DNP-C, DNP-D and E at 1, 2 and 6 weeks after administration ($P > 0.05$), as shown in Fig. 3b.

The weight of rats in each group after administration was lower than that before administration. Compared with group DNP-A, the

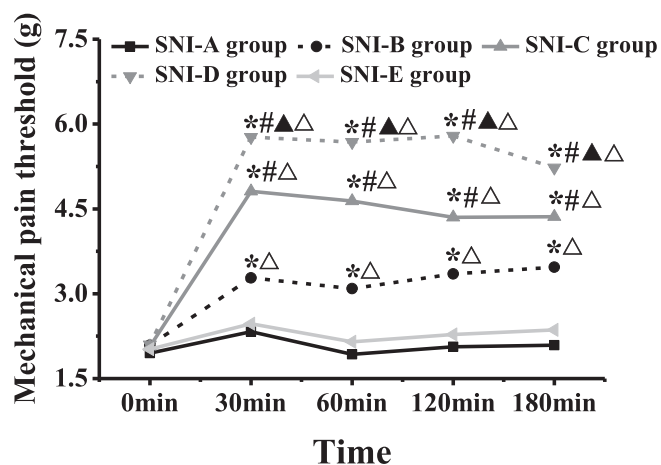


Fig. 1. SNI rats model pain results. **Note:** * indicates that the difference between SNI-A group and SNI-A group is statistically significant ($P < 0.05$). # indicates that the difference between SNI-B group and SNI-B group is statistically significant ($P < 0.05$). ▲ shows that the difference between SNI-C group and SNI-C group is statistically significant ($P < 0.05$). △ represents a statistically significant difference compared to SNI-E group, ($P < 0.05$).

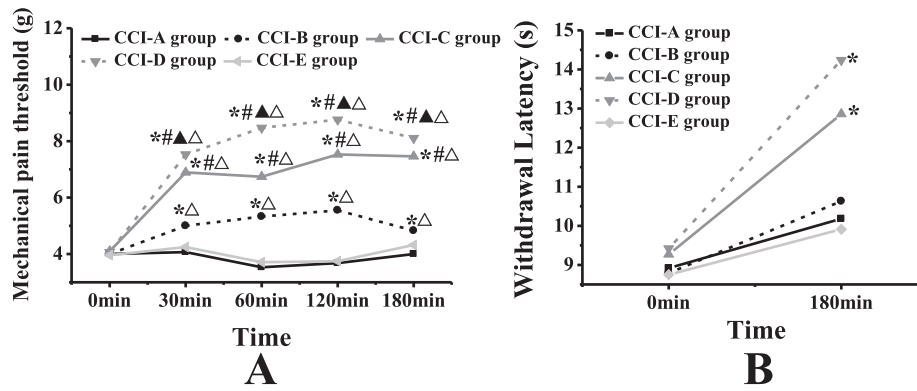


Fig. 2. Results of the pain test of CCI rats model. **Note:** A is the result of pain threshold measurement of mechanical stimulation; B is the measurement result of thermal pain threshold. * indicates that the difference between SNI-A group and SNI-A group is statistically significant ($P < 0.05$). # indicates that the difference between SNI-B group and SNI-B group is statistically significant ($P < 0.05$). Δ represents a statistically significant difference compared to SNI-E group, ($P < 0.05$).

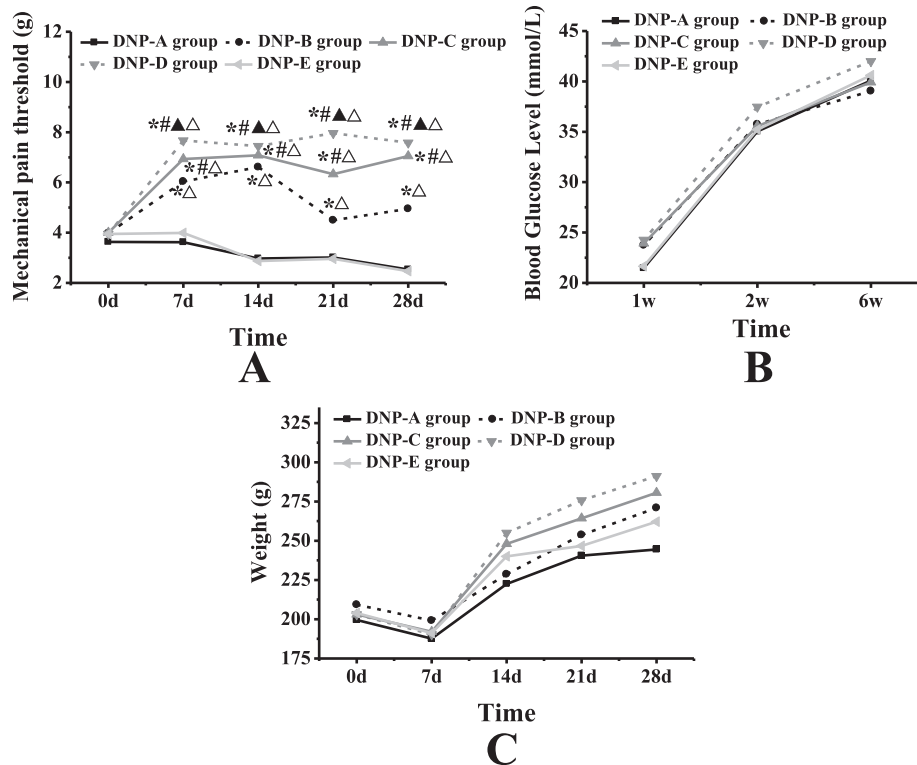


Fig. 3. Pain results of DNP rats model. **Note:** A is the result of pain threshold measurement of mechanical stimulation; B is the determination result of blood glucose; C is the result of weight change. * indicates that the difference between SNI-A group and SNI-A group is statistically significant ($P < 0.05$). # indicates that the difference between SNI-B group and SNI-B group is statistically significant ($P < 0.05$). Δ showed that the difference between SNI-C group and SNI-C group was statistically significant ($P < 0.05$). Δ represents a statistically significant difference compared to SNI-E group, ($P < 0.05$).

weight of rats in groups DNP-B, DNP-C, DNP-D and E before administration and on days 7, 14, 21 and 28 after administration showed no statistical significance ($P > 0.05$), as shown in Fig. 3c.

3.4. Analgesic results of amitriptyline on FIB rat model

FIB rat model was established to study the analgesic effect of different doses of amitriptyline on rats, which was shown in Fig. 4. The mechanical stimulation pain threshold in the local amitriptyline group (group FIB-B, FIB-C, FIB-D) at each time point after drug treatment was significantly higher than that in the group FIB-A and group FIB-E, with statistically significant differ-

ence ($P < 0.05$). With the increase of concentration, the pain threshold of mechanical stimulation in the locally administered amitriptyline group was gradually increased. Compared with group FIB-B, the pain threshold of mechanical stimulation in group FIB-C was significantly increased at each time point after drug treatment, with statistically significant difference ($P < 0.05$). Compared with group FIB-C, the pain threshold of mechanical stimulation in group FIB-D was significantly increased at each time point after drug treatment, and the difference was statistically significant ($P < 0.05$). There was no significant difference in mechanical stimulation pain threshold between group FIB-A and group FIB-E at each time point after drug treatment ($P > 0.05$), as shown in Fig. 4a.

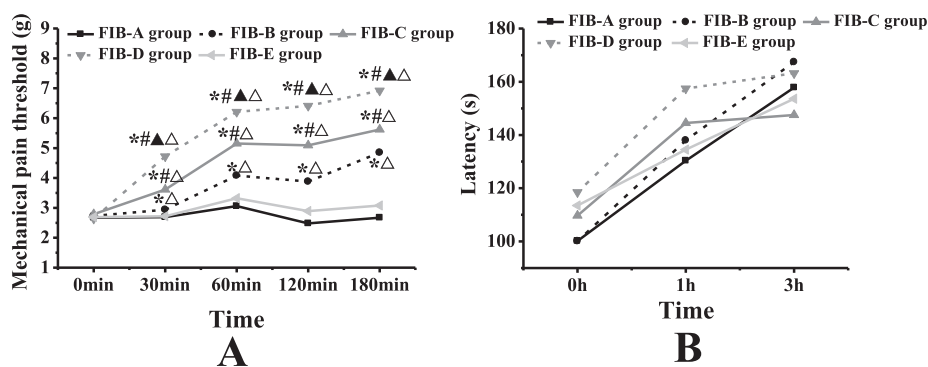


Fig. 4. Pain results of FIB rats model. **Note:** A is the result of pain threshold measurement of mechanical stimulation; B is the experimental result of rotating rod in rats. * indicates that the difference between SNI-A group and SNI-A group is statistically significant ($P < 0.05$). # indicates that the difference between SNI-B group and SNI-B group is statistically significant ($P < 0.05$). ▲ shows that the difference between SNI-C group and SNI-C group is statistically significant ($P < 0.05$). △ represents a statistically significant difference compared to SNI-E group, ($P < 0.05$).

Compared with group FIB-A, there was no statistically significant difference in the drop latency of group FIB-B, FIB-C, FIB-D, and E in the rotating rod experiment ($P > 0.05$), and amitriptyline had no effect on the motor ability of fibromyalgia model rats, as shown in Fig. 4b.

4. Discussion

Amitriptyline can play its central and peripheral analgesic role by acting on multiple links of pain conduction pathway. Studies have shown that amitriptyline can produce anti-pain hypersensitivity through intrathecal injection and intravenous injection (Maurya et al., 2017). Irina et al. reported that amitriptyline could effectively relieve the neuropathic pain caused by leprosy (Irina et al., 2018). Agarwal et al. showed that patients with neuropathic pain could obtain effective analgesic effect by taking different doses of amitriptyline orally (Agarwal and Joshi, 2017). Hiroki et al. showed that amitriptyline can enhance the analgesic effect of the drug on neuropathic pain, which may be caused by increasing the content of norepinephrine (Abdullah et al., 2012). It is speculated that the local application of amitriptyline on the injured nerve can produce analgesic effect on chronic pain such as neuropathic pain. In this research, the pain threshold of mechanical stimulation in rats was determined by SNI pain test, CCI pain test, DNP test and fibromyalgia test. According to the results, local drug delivery after the drug treatment of each point mechanical stimulation pain threshold were significantly higher than that of group A and group E. With the increase of the concentration, the pain threshold of mechanical stimulation gradually increased, indicating that para-sciatic injection of amitriptyline of different concentrations had analgesic effects on neuropathic pain, diabetic neuropathic pain and fibromyalgia in rat models, which is consistent with the results of Amin et al.'s study that the use of 3 mg/kg amitriptyline on the peripheral sciatic nerve of rats can improve the mechanical abnormal pain (Amin et al., 2017). The result achieved the expected effect. Some scholars have found that amitriptyline can significantly reduce the mechanical abnormal pain in CCI model rats, which is consistent with the results of this study (Zhou et al., 2017). In addition, there was no statistically significant difference in mechanical stimulation pain threshold between group A and group E at each time point after drug treatment. It shows that amitriptyline mainly ACTS directly on the sciatic nerve locally, but not on the whole body.

To sum up, the research studied tricyclic antidepressants model of rat rational neuropathy pain, diabetic neuropathy pain, and the analgesic effect of fibromyalgia. Para-sciatic injection of

amitriptyline at different concentrations has analgesic effects on neuropathic pain, diabetic neuropathic pain and fibromyalgia in rat models. Amitriptyline directly acts on the local sciatic nerve. It is of great clinical significance for the treatment of chronic pain to inject amitriptyline directly into the injured nerve. In this research, local injection of amitriptyline into the sciatic nerve of rats has not found any obvious side effects or abnormal behaviors, but further in-depth exploration is needed on the toxicity of amitriptyline to peripheral tissues and nerves.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

References

- Abdulmajeed, W.I., Ibrahim, R.B., Ishola, A.O., et al., 2015. Amitriptyline and phenytoin prevents memory deficit in sciatic nerve ligation model of neuropathic pain. *J. Basic Clin. Physiol. Pharmacol.* 27 (2), 101–108.
- Agarwal, N., Joshi, M., 2017. Effectiveness of amitriptyline and lamotrigine in traumatic spinal cord injury-induced neuropathic pain: a randomized longitudinal comparative study. *Spinal Cord* 55, 126–130.
- Amin, B., Hosseini, S., Hosseinzadeh, H., 2017. Enhancement of antinociceptive effect by co-administration of amitriptyline and crocus sativus in a rat model of neuropathic pain. *Iran. J. Pharmaceut. Res.* 16 (1), 187–200.
- Haliloglu, S., Ekinci, B., Uzkeser, H., et al., 2017. Fibromyalgia in patients with thyroid autoimmunity: prevalence and relationship with disease activity. *Clin. Rheumatol.* 36 (7), 1–5.
- Hiroki, T., Suto, T., Saito, S., et al., 2017. Repeated administration of amitriptyline in neuropathic pain: modulation of the noradrenergic descending inhibitory system. *Anesth. Analg.* 125 (4), 1281–1288.
- Irina, R., Patrick, R.N.A.G.S., Simone, B.H., et al., 2018. Neuropathic pain in leprosy: symptom profile characterization and comparison with neuropathic pain of other etiologies. *Pain Rep.* 3 (2), e638.
- Jacob, S., Neal, N., 2017. Management of neuropathic pain. *Neurosurg. Clin. N. Am.* 15 (2), 231–239.
- Le, Z., Zhang, Z., Zhou, M., et al., 2018. A urinary metabolomics (GC-MS) strategy to evaluate the antidepressant-like effect of chlorogenic acid in adrenocorticotrophic hormone-treated rats. *RSC Adv.* 8 (17), 9141–9151.
- Marques, A.P., Santo, A.S., Berresaneti, A.A., et al., 2017. Prevalence of fibromyalgia: literature review update. *Rev. Bras. Reumatol. Engl. Ed.* 57 (4), 356–363.
- Maurya, N., Maurya, J.K., Kumari, M., et al., 2017. Hydrogen bonding-assisted interaction between amitriptyline hydrochloride and hemoglobin: spectroscopic and molecular dynamics studies. *J. Biomol. Struct. Dyn.* 35 (6), 1367–1380.
- Min, A., Kaltwang, S., Tyler, N., et al., 2017. The automatic detection of chronic pain-related expression: requirements, challenges and a multimodal dataset. *IEEE Trans. Affect. Comput.* 7 (4), 435–451.
- Rao, S., Liu, S., Zou, L., et al., 2017. Erratum to: The effect of sinomenine in diabetic neuropathic pain mediated by the P2X₃ receptor in dorsal root ganglia. *Purinerg. Signal.* 13 (2), 1–1.

- Ryu, S., Park, S., Lee, J.H., et al., 2017. A study on CYP2C19 and CYP2D6 polymorphic effects on pharmacokinetics and pharmacodynamics of amitriptyline in healthy Koreans. *Clin. Transl. Sci.* 10 (2), 93–101.
- Takenoshita, M., Motomura, H., Toyofuku, A., 2017. Low-dose aripiprazole augmentation in amitriptyline-resistant burning mouth syndrome: results from two cases. *Pain Med.* 18 (4), 814–815.
- Villani, V., Prosperini, L., Palombini, F., et al., 2017. Single-blind, randomized, pilot study combining shiatsu and amitriptyline in refractory primary headaches. *Neurol. Sci.* 38 (6), 999–1007.
- Zhou, W.C., Jia, L., Chen, D.Y., et al., 2017. The effects of paroxetine and amitriptyline on the upper esophageal sphincter (UES) pressure and its natural history in globus pharyngeus. *Digest. Liver Dis.* 49 (7), 757.