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ORIGINAL

EFFECT OF INTRANEURAL INJECTION OF DIOSMETIN COMBINED WITH LIDOCAINE ON SCIATIC NERVE BLOCK IN RETIRED PLAYERS

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ABSTRACT

Objective: To investigate the effect and mechanism of intraneural injection of diosmetin combined with lidocaine on sciatic nerve block in retired players. **Methods:** Thirty-two retired players were randomly divided into four groups: Sham group, diosmetin group (100mg·kg^{-1}) and lidocaine group (10% lidocaine (20μL)) and lidocaine+diosmetin group (10% lidocaine (20μL) and 100mg·kg^{-1} diosmetin). Then the conduction state of sciatic nerve was detected; The sensory and motor function were assessed; the histopathological changes of sciatic nerve were observed by toluidine blue staining; the gene expression of IL-1βand TNF- α on the mRNA level was detected by RT-PCR. **Results:** Compared with lidocaine group, lidocaine+diosmetin can better enhance sensory and motor nerve block, reduce histopathological damage of sciatic nerve, and decrease the expression of inflammatory factors, such as IL-1 β and TNF- α in transcriptional level. **Conclusion:** Diosmetin has the effects of enhancing sensory and motor nerve block, anti-inflammatory and protecting nerve injury.

KEYWORDS: Diosmetin; Sciatic nerve; Nerve block; retired players

1. INTRODUCTION

Local anesthetics are usually used for peripheral nerve block and postoperative analgesia during surgery. Recently, ultrasound guided nerve block has become increasingly popular, and the position of the needle has been visualized (Andres et al., 2018; Dallakyan & Olson, 2015). However, despite the

use of ultrasound guidance, 17% of brachial plexus block and 16% of sciatic nerve block still injected local anesthetics into the brain. Intraneural administration of local anesthetics may cause mechanical and chemical damage to nerves(K Hara, Sakura, & Saito, 2010; Kaoru Hara, Sakura, Yokokawa, & Tadenuma, 2012). In most cases, the nerve damage caused by peripheral nerve block is transient, but sometimes these damages are very serious, which may lead to permanent nerve damage (Borgeat, Ekatodramis, Kalberer, & Benz, 2001; Liguori, 2004).

The duration of anesthesia and analgesia realized by peripheral nerve block can be prolonged by adding auxiliary agents to local anesthetics. Previous animal studies have shown that dexmedetomidine as an adjuvant of peripheral nerve blocker of local anesthetic can prolong the duration of sensory and motor block without causing neurotoxicity, which depends on its effective anti-inflammatory mechanism (Brummett, Norat, Palmisano, & Lydic, 2008; Zohry, 2017). Diosmetin (Dios) is a flavonoid separated from the leaves of olive (Olea europaea L) (Meirinhos et al., 2005). Diosmetin is a traditional Chinese medicine. Its pharmacological properties are anti-inflammatory, antioxidant, anti-microbial and anti-cancer (Ward et al., 2018; Yang et al., 2017).

Because of its powerful antioxidant properties, it is hypothesized that the intraneural injection of diosmetin for local anesthetics might effectively reduce or prevent nerve injury. Therefore, in this paper, we used diosmetin in the recovery treatment of nerve block induced by local anesthesia in retired players, trying to find out its efficacy and mechanism, so as to provide reference for the drug development and application of diosmetin (Khorsandi et al., 2017; Rose et al., 2012; Safran et al., 2010).

2. MATERIALS AND METHODS

2.1. Animal experiment and modeling

This study was conducted in accordance with an agreement previously approved by the Laboratory Animal Care and Use Committee of Central South University. Main reagents were 10% lidocaine was (Korean Pharmaceutical, Korea) and diosmetin (Sigma, USA). Thirty-two male Sprague-Dawley retired players weighing between 300 and 330 grams were used in this study. The constant temperature of the animal room was set at 23±1°C, and the artificial lighting was 12 hours a day. The experimental animals can freely obtain food and drinking water. The retired players were randomly divided into four groups (eight retired players in each group). In Sham group, the retired players only had the sciatic nerve exposed surgically, and there was no acupuncture into the nerve. In the diosmetin group, retired players were injected with 100mg·kg^{-1} diosmetin (Zhang, Jiang, & Lu, 2019). In the lidocaine group, retired players were injected with 10% lidocaine (20μL) (Zhao, Li, Ding, & Li, 2017), and the

hind limbs of retired players showed obvious paralysis within 30 seconds after injection. In the lidocaine+diosmetin group, they were injected with 10% lidocaine (20μL) and 100mg·kg^{-1} diosmetin.

2.2. Evaluation of Nerve Conduction State

After surgical preparation, all sciatic nerves were carefully exposed under stereomicroscope. All adipose tissue around the nerve should be removed. The nerve conduction of the sciatic nerve was measured with a Key point TM Portable electromyograph (ALPINE Biomed, Denmark). A single electric pulse was carried out to stimulate the sciatic nerve (duration of 0.1ms, intensity of 0.6-0.63mA), the compound action potential of skeletal muscle was recorded by inserting a unipolar needle (28G) into the muscle abdomen (as shown in Fig. 1A), and the amplitude of the sciatic nerve measures.

2.3. Neurobehavioral Examination

Neurobehavioral tests were performed by researchers in a double-blind setting. The neurological function of the retired players was evaluated within 30 minutes after anesthesia until they were completely recovered from the blocking state (Kim, Choi, Baek, & Lee, 2018). The sensory response was assessed using the retraction response to foot and toe clamps. The sensory function was evaluated by the retraction reflex.

The scoring criteria were as follows: 0, normal sensory function; 1, moderate retraction; 2. minimum retraction; 3, complete sensory block/no response to kneading. In addition, the motor function was scored on a scale of 0 to 3. The scoring criteria were as follows: 0, normal motor function; 1, normal dorsiflexion; 2, part dorsiflexion; 3, no dorsiflexion. The time of sensory or motor nerve recovery was defined as the time from the injection into the nerve to the time when the sensory or motor score was 0 and recorded.

2.4. RT-PCR

The left sciatic nerve was harvested after intraneural injection. The gene expression of IL-1β and TNF- α in sciatic nerve tissue in mRNA level was determined by RT-PCR. Total RNA was extracted from tissues using TRIzol. Reverse transcriptase polymerase chain reaction was performed using random primers and reverse transcriptase (Promega, Madison, Wisconsin). The PCR was cycled at 95℃ for 5 minutes, followed by 30 to 33 cycles, including 1 minute at 95℃, 1 minute at 58℃ to 62℃, and 1 minute at 72℃. The relative ratio to β-Actin was used to standardize target gene expression.

2.5. Pathological Examination of Nerve Tissue

Retired players were anesthetized by intraperitoneal injection of chloral

hydrate (350 mg/kg), then the tissue was taken out, to wash with PBS (pH 7.40, 0.02 M), and then 4% paraformaldehyde phosphate buffer was added to fix the tissue. The spinal cord segment was taken from the ligation site of the sciatic nerve, fixed in the above fixative solution for 6-8 hours, dehydrated at 4℃ with sucrose PBS buffer (0.01 M, 30%), embedded and fixed, sliced with a cryostat (5 μm), washed with PBS, dyed in an air bath or water bath at about 50 degrees for 20-30 minutes with the preheated 1% methylamine blue solution in advance, and then dehydrated in turn with gradient alcohol, observed and photographed with an Olympus microscope.

2.6. Immunological Fluorescence Staining of Tissues

Retired players were anesthetized by intraperitoneal injection of chloral hydrate (350 mg/kg), then the tissue was taken out, to wash with PBS (pH 7.40, 0.02 M), and then 4% paraformaldehyde phosphate buffer was added to fix the tissue. The spinal cord segment was taken from the ligation site of the sciatic nerve, fixed in the above stationary liquids for 6-8 hours, dehydrated at 4℃ with sucrose PBS buffer (0.01 M, 30%), embedded and fixed, sliced with a cryostat (5 μm), and washed with PBS and citrate buffer (pH 6.0) for antigen recovery; Incubated with auto fluorescence quenching agent at room temperature for 15 minutes, and sealed with 3% bovine serum albumin for 30 minutes. Goat anti Iba-1 antibody and incubate p-JNK (1:100) antibody were used, and washed with PBS for 15 minutes × 3. The slices were incubated with the appropriate second antibody at room temperature for 50 minutes. The sections were washed with PBS, fixed with fluorescent sealant, observed with fluorescent microscope, and photographed with OPLENIC software. The expressions of Iba1 and p-JNK in rat microglia were detected.

2.7. Statistical analysis

SPSS Statistics software for Windows (version 23; IBM Corp, USA) was used for statistical analysis. Single factor analysis of variance was used to measure the difference between groups. If the P value was less than 0.05, the difference was considered statistically significant.

3. RESULTS

3.1. The diosmetin can effectively reverse the sciatic nerve block induced by local anesthesia in retired players

In this experiment, we measured the nerve conduction to evaluate the nerve block or the state after the treatment with diosmetin, and used bipolar intraoperative stimulation device to stimulate the sciatic nerve. The results showed that the treatment with diosmetin could effectively reverse the sciatic nerve block induced by local anesthesia in retired players.

3.2. Effect of diosmetin on neuro-behavior in retired players

The examination of the nervous system showed that the retired players in the lidocaine group and the lidocaine+diosmetin group showed signs of sciatic nerve block after awakening from anesthesia. In terms of the time of sensory recovery and the time of motor recovery, the nerve block effect of the lidocaine+diosmetin group was significantly higher than that of the lidocaine group (Fig. 1). In addition, at 120 molecules after injection, the animals in the lidocaine+diosmetin group showed more sensory nerves (P<0.01, Fig. 2A) and motor nerve disorders (P<0.001, Fig. 2B), but no animals had sustained neurological deficits.

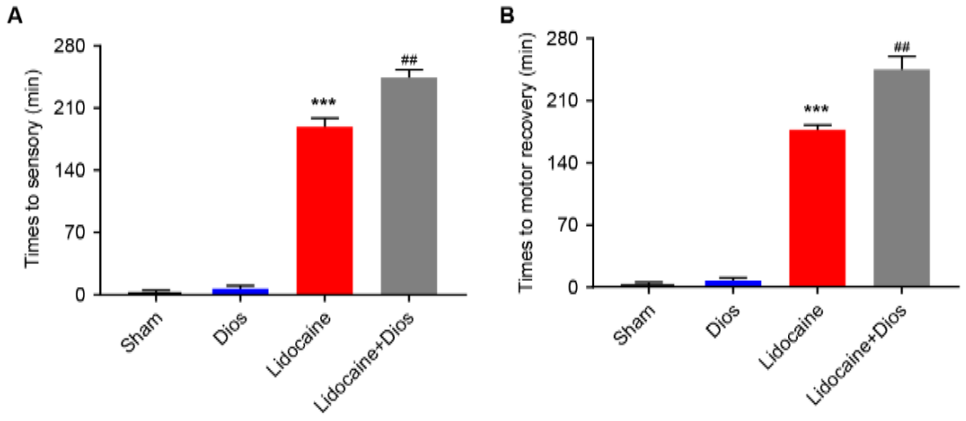

Figure 1: Time to reach sensory and motor nerves after intrasciatic injection in retired players.

*A. Time for sensory nerves. B. Time for motor nerve. Data are expressed as mean ± standard deviation, n=8. Compared with the Sham group, *** indicates P <0.001; compared with the Lidocaine group, ## indicates P <0.01.*

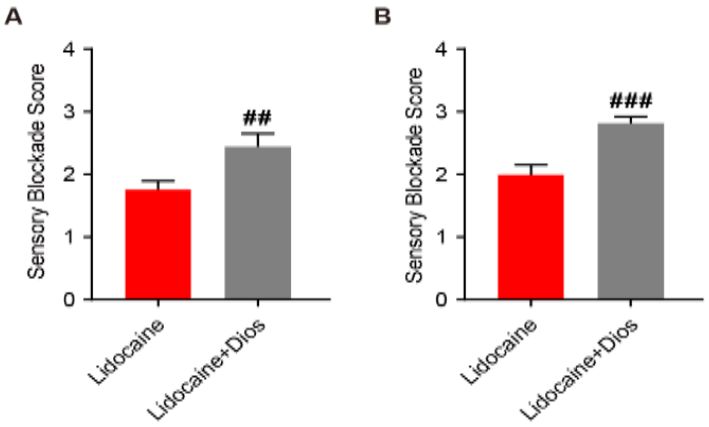

Figure 2: Obstacle scores of sensory and motor nerves after intrasciatic injection in retired players.

*A. Score of sensory nerve disorder. B. Score of motor nerve disorder. Data are expressed as mean ± standard deviation, n = 8. Compared with the Sham group, *** indicates P <0.001; compared with the Lidocaine group, ## indicates P <0.01.*

3.3. Diosmetin inhibits inflammatory damage by inhibiting the activation of rat sciatic microglia

The results of toluidine blue staining showed that 4 weeks after the injection of lidocaine into the sciatic nerve, the tissue showed overall nerve damage (Fig. 4A), in which a large number of deformed myelin sheaths were formed in the lidocaine group, which was reversed after the application of diosmetin. The level of IL-1β and TNF- α in lidocaine group was significantly higher than that of Sham group (P<0.001, Fig. 4B-C). However, compared with the lidocaine group, the level IL-6IL-1 β and TNF- α in the lidocaine+diosmetin group had decreased (P<0.01, Fig. 4B-C). At the same time, the tissue immunofluorescence results showed that the expression level of Iba1 and p-JNK protein in the sciatic nerve tissue of retired players was significantly increased after 4 weeks of lidocaine injection, while the expression level of Iba1 and p-JNK protein was significantly decreased after the application of diosmetin. The above results indicate that diosmetin can restore the level of inflammatory factors in the sciatic nerve of retired players induced by local anesthesia, and effectively reduce nerve injury.

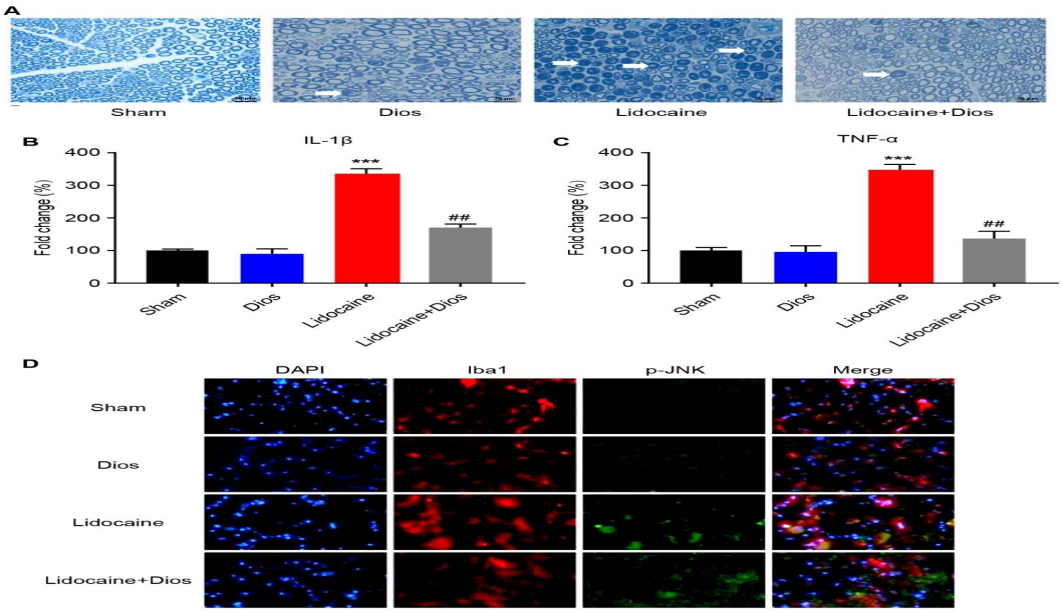

Figure 4: Effect of diosmetin on local anesthesia-induced sciatic nerve block in retired players.

*A. Semi-thin sections of the sciatic nerve stained by toluidine blue after intraneural injection for 4 weeks. White arrow indicates the degeneration of myelin sheath. The micrograph is 400 times and the scale bar is 20 μm. B. RT-PCR method is used to detect the expression level of IL-1β in sciatic nerve tissue. C. RT-PCR method is used to detect the expression level of TNF-α in sciatic nerve tissue. D. The expression and distribution of Iba1 and p-JNK protein in sciatic nerve tissue of rat are observed by immunofluorescence staining. Data are expressed as mean ± standard deviation, n = 8. Compared with the Sham group, *** indicates P <0.001; compared with the Lidocaine group, ## indicates P <0.01.*

In this paper, the effect of lidocaine combined with diosmetin on the sciatic nerve of retired players at the neurobehavioral and histopathological levels were studied. It was found that diosmetin could significantly enhance sensory and motor sciatic nerve block without clinically significant neurological damage. Compared with lidocaine alone, diosmetin can reduce the expression of IL-1 β and TNF- α in mRNA level and reduce the degree of nerve injury. However, it should be noted that the level of inflammatory mediators in the sciatic nerve block and the degree of tissue nerve damage of retired players in the lidocaine+diosmetin group were higher than those in the Sham group, indicating that diosmetin can reduce but not completely prevent local anesthetic related toxicity (Wishart et al., 2018; Zheng et al., 2020).

Compared with lidocaine alone, the intraneural administration of lidocaine+diosmetin increased the duration and recovery time of sensory and motor nerve block. The researchers found that local anesthetic injection into the sciatic nerve could cause inflammatory reaction (Kapur et al., 2007). More and more studies have reported the strong anti-inflammatory effect of diosmetin (Koosha, Mohamed, Sinniah, & Alshawsh, 2019). We found that the dose used in this study was lower than that shown in previous studies, but it also achieved the inhibitory effect of diosmetin on microglia activation and anti-inflammatory effect. In addition, we observed that local anesthetic injection could cause nerve damage, such as axonal and myelin sheath degeneration (Pabis et al., 2017). The possible mechanism of the toxic effect of lidocaine is related to the increase of inflammatory reaction, changes in the environment of the neurointima and axonal dystrophy. However, the combination of lidocaine and diosmetin can effectively reduce the degeneration of nerve myelin sheath, which suggests the neuroprotective effect of diosmetin.

In conclusion, this study shows that diosmetin can enhance the sensory and motor block inducted by lidocaine, and at the same time, diosmetin can reduce the degree of related nerve damage caused by lidocaine. This protective effect may be related to the anti-inflammatory mechanism. However, as an adjuvant of local anesthetic, the safety of diosmetin in human body needs further study.

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