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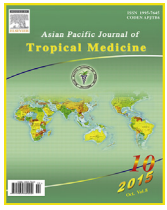

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Effect of preemptive local injection of ropivocaine with dexmedetomidine on mirror pain in rats and its mechanism

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ABSTRACT

Objective: To observe the effect of preemptive local injection of ropivocaine with dexmedetomidine on activation of glial cells and on the mirror pain in rats and its mechanism.

Methods: A total of 48 adult male Sprague-Dawley rats (weighing 180 g–220 g) were included in the study and randomized into 3 groups, Group S, Group R, and Group RD_1. A rat model of persistent postoperative pain evoked by skin/muscle incision and retraction was established in the three groups. Before procedures and nerve extraction, Group S (n = 16) was injected 0.9% saline locally; Group R (n = 16) was injected 0.5% ropivocaine locally, and Group RD_1 (n = 16) was injected 0.5% ropivocaine in combined with 1 μg dexmedetomidine locally. After the model being established in the three groups, 8 rats were used for behavior test until 28 d, and dorsal root ganglions (DRGs) of the other 8 rats were harvested on the 3rd day after surgery. Immunofluorescent and transmission electron microscopy were used to observe the activation of glial cells in DRG, and the behavior test results in the three groups were compared.

Results: The results showed that mechanical pain threshold in ipsilateral hind-paws of the Group S, Group R, Group RD_1 animals dropped to (3.640 ± 1.963) g, (5.827 ± 1.204) g, (7.482) ± 1.412 g at 3 d respectively; while in contralateral paws dropped to (7.100 ± 1.789) g, (17.687 ± 1.112) g, (16.213 ± 1.345) g on the 3 d respectively. Immunofluorescent showed that the glial cells were activated in bilateral side DRG after surgery in 3 groups, but ipsilateral paws expressed more active glial cells than contralateral paws. Transmission electron microscopy showed that mitochondria swelling/vacuolization and lysosomes were more obvious in ipsilateral paws than contralateral paws, but Group RD_1 formula could reduce glial cells activity, mitochondria swelling/vacuolization and the amount of lysosomes.

Conclusions: Local injection of ropivocaine and/or dexmedetomidine can effectively inhibit the activation of glial cells in DRG, mitigate the pathological changes of neuron in DRG and reduce mirror image pain.

1. Introduction

Open inguinal hernia repair consisting of tissue extraction is one of the most painful procedures in the clinical surgery. Skin/muscle incision and retraction (SMIR) model, akin to a common clinical procedure, is one rat model of persistent postoperative pain [1]. However, when simulating this model, we found that unilateral nerve extraction can evoke bilateral pain. A growing body of evidence indicates that unilateral nerve injury results in bilateral cellular and molecular changes in the nerve structure and pain sensitivity. This phenomenon is known as mirror image pain (MIP) [2–4]. To date, the mechanism of MIP is still unclear. Although poorly understood, a lot of researchers released findings of bilateral nociceptive-related molecular changes in the

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nervous system of unilateral pain models. It may be related to humoral immunity, central sensitization, and/or cortical downstream regulation. Surprisingly, evidence of changes in primary neurons and glial cells in regards to MIP in SMIR model is lacking [5]. It is possible that glials in the contralateral dorsal root ganglion (DRG) may play a role in primary neuronal sensitization [6,7]. They become activated and proliferate after nerve injury or inflammation [8]. Based on the close proximity of the glails cells and their ability to affect primary neurons [9], we hypothesize that glail cells activation in the contralateral DRG following unilateral peripheral nerve injury leads to increased excitability of contralateral DRG neurons and thus, MIP [10–16]. In order to observe the effect of preemptive local injection of ropivocaine with dexmedetomidine in inhibiting the activation of glial cells and reducing the mirror pain in rats and its mechanism, male SD rats were included, SMIR model was prepared, ropivocaine and dexmedetomidine were preemptively injected before model preparation. The study is reported as following.

2. Materials and methods

2.1. Experimental animals

A total of 48 SPF male SD rats, weighing (180–220) g, were included in the study. The animals were housed in a 12 h light/dark cycle and given adequate food and water. All procedures were carried out in compliance with the guidance suggestion of Animal Care Committee of Southern Medical University and the International Association for the Study of Pain [17].

2.2. Instruments and reagents

Nikon (Tokyo, Japan) fluorescence microscope, ropivacaine hydrochloride injection (Trade name: Naropin, AstraZeneca Pty Ltd, Registration No. H20100083), dexmedetomidine hydrochloride injection (produced by Jiangsu Enhua Pharmaceutical Ltd., Approval No. H20090248), goat anti-rabbit TRITC (1:1 000, Beijing Zhongshan Golden Bridge Biotechnology Co. Beijing, China) and goat-antimouse FITC (1:1 000, Beijing Zhongshan Golden Bridge Biotechnology Co. Beijing, China) were used in the study.

2.3. Model preparation

According to the method reported by Sarah J. L. Flatters [1], SMIR model was established. Rats were intraperitoneally injected with 10% chloral hydrate at a dose of 400 mg/kg. After anesthesia, a supine position was taken. Rat's back and medial thigh on one side was shaved and then swabbed with sterile alcohol wipes to sterilize the area for visualization of the saphenous vein. An incision with a length of about 7–10 mm was made in the superficial muscle. Until the muscle was separated to the adductor tendon fascia, a microscopic retractor was placed to expose the adductor tendon fascia for a persistent traction for 1 h. During the traction period, the incision was covered with sterile gauze. After traction, the muscle was sutured. Antibiotics were applied after operation for infection prevention.

2.4. Animal grouping

The experimental rats were randomly divided into 3 groups, Group S, Group R, and Group RD_1. A rat model of persistent postoperative pain evoked by SMIR was established in the three groups. Before procedures and nerve extraction, Group S (n = 16) was injected 0.9% saline locally; before procedures and nerve extraction, Group R (n = 16) was injected 0.5% ropivocaine locally; before procedures and nerve extraction, Group RD_1 (n = 16) was injected 0.5% ropivocaine in combined with 1 μg dexmedetomidine locally. After the model being established in the three groups, 8 rats were used for behavior test until 28 d, and DRGs of the other 8 rats were harvested on the 3rd day after surgery. Immunofluorescent and transmission electron microscopy were used to observe the activation of glial cells in DRG, and the behavior test results in the three groups were compared.

2.5. Observation of indicators

Behavioral testing mechanical sensitivity was assessed using the up-down method described in a previous study [18] and a set of von Frey hairs (Ugo Basile, Italy) was used to apply logarithmically increasing stiffnesses ranging from 3.61 (0.41 g) to 5.18 (15.14 g). Quick withdrawal in response to the stimulus was considered to be a positive response. Immunofluorescent and transmission electron microscopy were used to observe the activation of glial cells in DRG.

2.6. Statistical analysis

SPSS19.0 software was used for statistical analysis. The measurement data were expressed as mean ± SD, and t test was used. $P < 0.05$ was regarded as statistically significant difference.

3. Results

3.1. Changes in behavior test in rats

Unilateral SMIR model rats, Group S, exhibited noticeable bilateral pain. Compared with the basic value, the ipsilateral paw pain thresholds and mechanical hyperalgesia were significantly increased at 1–21 d following operation ($P < 0.05$) (Figure 1A). Mechanical tests of ipsilateral paws showed the paw withdrawal threshold dropped from (20.300 ± 1.204) g before surgery to (3.640 ± 1.963) g, (4.52 ± 1.89) g, (3.89 ± 1.963) g at 1 d, 3 d and 5 d after surgery. The contralateral paw pain thresholds for mechanical hyperalgesia were increased at 3–7 d following surgery as well ($P < 0.05$) (Figure 1A). Mechanical tests showed the contralateral paw withdrawal threshold dropped to (7.100 ± 1.789) g from (20.010 ± 1.412) g at 3 d post surgery.

Group R's ipsilateral side of the paw withdrawal threshold was markedly lower than contralateral side by the value of (5.827 ± 1.204) g *vs.* (17.687 ± 1.112) g at day 3 ($P < 0.05$) (Figure 1B). But from day 5 to day 7, contralateral side expressed more significant hypersensitivity than operational side. After day 14, two sides had similar trend to each other, but ipsilateral side still had lower value than contralateral side.

In Group RD_1, at day 1, both paws expressed similar value of withdrawal threshold with the value of ipsilateral (7.482 ± 1.412) g *vs.* contralateral (6.204 ± 1.963) g. At day 3 and day 5, ipsilateral side of Group R expressed lower threshold than contralateral side with the value of (11.881 ± 1.141) g *vs.* (16.213 ± 1.345) g, (7.869 ± 1.251) g *vs.* (11.549 ± 1.773) g,