Repeated intra-articular injections of acidic saline produce long-lasting joint pain and widespread hyperalgesia

N. Sugimura[1], M. Ikeuchi[1]*, M. Izumi[1], T. Kawano[2], K. Aso[1], T. Kato[1], T. Ushida[3], M. Yokoyama[2], T. Tani[1]

1 Department of Orthopaedic Surgery, Kochi Medical School, Kochi University 185-1 Oko-cho Kohasu, Nankoku 783-8505, Kochi, Japan.

2 Department of Anesthesiology and Intensive Care Medicine, Kochi Medical School, Kochi University 185-1 Oko-cho Kohasu, Nankoku 783-8505, Kochi, Japan.

3 Multidisciplinary Pain Center, Aichi Medical School, Karimata, Nagakutecho, Aichi, Japan

*Corresponding author: Masahiko Ikeuchi, MD PhD

Department of Orthopaedic Surgery

Kochi Medical School, Kochi University

185-1 Oko-cho Kohasu, Nankoku, JAPAN, 783-8505

Email: ikeuchim@kochi-u.ac.jp

Tel: +81-88-880-2386

FAX: +81-88-880-2388

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1. Introduction

Chronic joint pain is currently one of the most frequent health problems, particularly osteoarthritis and rheumatoid arthritis. It is often accompanied by referred pain and secondary hyperalgesia (Sluka 2002). For the better understanding of pain mechanisms and future progress in treatment, it is helpful to develop an animal model of chronic joint pain predominantly driven by nervous system.

Synovial fluid in inflamed joint shows a drop in pH to levels around 6.6 to 6.8. Local acidity activates nociceptors through proton-gated cation channels such as acid sensing ion channels (ASICs) and transient receptor potential vanilloid1 (TRPV1). Interestingly, repeated intra-muscular injections of acidic saline produce a bilateral, long-lasting

hyperalgesia without significant tissue damage (Sluka et al., 2001).

The purpose of this study was, 1) to develop an animal model of acid-induced chronic joint pain and 2) to clarify underlying peripheral and central pain mechanisms of this model.

2. Materials and methods

Rats were anaesthethized briefly with intraperitoneal injection of sodium pentobarbitone (30mg/kg). Rats received intra-articular injections of acidic (pH 4.0) or normal saline (pH 7.4) at day 0 and 5, and divided into 3 groups: acid-acid (n=10), acid-saline (n=5), and saline-saline groups (n=5). Pain behaviors including weight bearing asymmetry, mechanical sensitivity of the paw were compared among groups until day 35. The weight distribution was measured using a hind paw limb weight-bearing apparatus. Mechanical sensitivity of the paw was examined using von Frey filaments.

In order to characterize the involvement of proton-gated ion channels, rats with acid-induced chronic joint pain were treated with selective antagonists for ASIC1a (PcTx1), ASIC3 (APETx2), and TRPV1 (BCTC) or vehicle at day 5 or day 14. The injection of antagonists at day 5 was performed immediately before the second injection

of acidic saline. The rats were divided into 6 groups; APETx2 at day 5 (early APETx2 group), PcTx1 at day 5 (early PcTx1 group), BCTC at day 5 (early BCTC group), APETx2 at day 14 (late APETx2 group), PcTx1 at day 14 (late PcTx1 group), BCTC at day 14 (late BCTC group).

To evaluate potential tissue damage, joint histology was performed at day14. To evaluate tissue inflammation, concentrations of interleukin (IL)-1β n the knee joint (synovium) were quantified using ELISAs at day 14. To investigate the reactivity of rat knee joint afferents activity to intra-articular acidic saline injection, and its role of ASIC3, the C-fiber activities after intra-articular acidic saline injection were evaluated with or without pre-injection of APETx2. Involvement of the central mechanism of this pain model was verified using spinal phosphorylated cAMP response element-binding protein (p-CREB). Finally intra-articular pH was measured with a needle electrode.

All experiments were approved by the Animal Care and Use Committee of Kochi Medical School.

3. Results

Repeated injections of acidic saline (acid-acid group) induced significant decrease in mechanical threshold in the bilateral paws, which lasted until day 28. The ipsilateral

paw showed greater threshold decrease compared with the contralateral paw. Weight bearing asymmetry in acid-acid group was greater and lasted longer (until day 28) than acid-saline group.

Acid-induced pain behavior was significantly reduced only in rats given selective ASIC3 antagonist at day 5 (early APETx2 group). On the other hand, pain reduction was not achieved by late administration of ASIC3 antagonist (late APETx2 group). Antagonists for ASIC1a (PcTx1) and TRPV1 (BCTC) produced no significant effects regardless of the time-point of administration.

Histology of knee joints obtained at day 14 showed no evidence of cartilage damage due to acid injections. There was also no evidence of synovial inflammation such as synovial proliferation, leukocyte infiltration, or vasodilation. In addition, IL-1β concentration in acid-acid group was extremely low (mean ± SE: 0.59 ± 0.53 pg/mg) compared with carrageenan-induced inflamed joint (31.1 ± 2.4 pg/mg, $p < 0.001$).

Upon Intra-articular injection of acidic saline, the fiber activity was markedly increased, reaching peak levels at 4-6 minutes after injection, and then slowly decreased thereafter. Pretreatment with APETx2 resulted in the attenuation of acidic saline induced C-fiber activation ($p < 0.05$ vs. no APETx2 pretreated), while APETx2 alone had no influence on baseline spontaneous fiber activity.

Bilateral upregulation in p-CREB expression was demonstrated in the L3-4 superficial and deep dorsal horn 24 hours after intra-articular injection of pH 4.0 saline compared with pH 7.2 ($p < 0.05$). In contrast, there was no significant upregulation in the superficial and deep dorsal horn on day 14.

A significant drop of intra-articular pH was shown for 90 seconds following injection of pH 4.0 acidic saline, which was returned to the baseline approximately 8 minutes after the injection.

4. Discussion

Repeated injections of acidic saline into the knee joint produced a bilateral long-lasting hyperalgesia of the paw without joint damage, which was significantly reduced by early intra-articular administration of selective ASIC3 antagonist. In spite of no significant peripheral tissue damage, there was an increase of p-CREB reactivity in the bilateral spinal dorsal horn. Our results suggest that repeated activation of peripheral ASIC3 is necessary to develop the hyperalgesia, while the maintenance of hyperalgesia appears to involve the central nervous system. To the best of our knowledge, this is the first study to develop an animal model of chronic joint pain predominantly driven by nervous system.

Among proton-gated ion channels, ASIC3 is the most sensitive to such a pH change (Lingueglia 2007; Wemmie et al., 2006), abundantly expressed in dorsal root ganglia (Wemmie et al., 2006) including joint afferents (Ikeuchi et al., 2009), and strongly correlated with pain (Sluka et al., 2003; Sluka et al., 2007; Ugawa et al., 2002; Wemmie et al., 2013). In particular, peripheral ASIC3 in joint afferents is a key to the development, but not the maintenance, of chronic widespread pain in arthritis models (Ikeuchi et al., 2008; Izumi et al., 2012). In the current study, long-lasting bilateral hyperalgesia was prevented by pre-injection of APETx2 prior to second acidic saline injection. Along with the pain-related behavior tests, the C-fiber activity in primary afferent of the knee was markedly increased by intra-articular injection of acidic saline and attenuated by pre-injection of APETx2. These results are in agreement with previous reports, and activation of ASIC3 in joint afferents was responsible for initiating the bilateral, long-lasting mechanical hyperalgesia induced by repeated injections of acidic saline.

Bilateral, long-lasting hyperalgesia after unilateral intra-articular acid injections suggests that the underlying mechanisms involve changes in the central nervous system. Our study showed that p-CREB expression in the spinal dorsal horn was upregulated bilaterally on the day after second acidic saline injection. This result suggests that

involvement of cAMP pathway immediately after second acid injection is essential for widespread chronic hyperalgesia. It is likely that this change in the central nervous system underlie bilateral, long-lasting hyperalgesia in this model.

In conclusion, we developed and characterized a model of acid-induced joint pain. Repeated intra-articular injections of acidic saline produced a bilateral, long-lasting hyperalgesia along with the activation of primary afferent of the knee without joint damage. Peripheral ASIC3 and spinal p-CREB played important roles for the development of hyperalgesia. This animal model gives insights into the mechanisms of joint pain, which is helpful in developing better pain treatments.

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