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A Rat Muscle Pain Model Based on Intramuscular Formalin Injection

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ABSTRACT

Background: Musculoskeletal pain is a major clinical problem, but researchers lack viable animal models to study this phenomenon. However, formalin injections are widely used to produce tonic and neurogenic nociceptive responses in rodents. Rodents can present a potential animal model for pain.
Objectives: To develop a muscle pain model with intramuscular injection of formalin in rats.
Materials and Methods: We injected formalin intramuscularly at different concentrations (50 µl; 2%, 4% and 8%) and compared them to its plantar surface subcutaneous injection (50 µl; 2%). Pain responses were scored in the first phase (1-7 minute), interphase (8-14 minute) and the second phases of 2A (15-60 minute) and 2B (61-90 minute).
Results: After intramuscular and subcutaneous formalin injection, rats showed nociceptive behaviors for less than 10 minutes, and then nociceptive behaviors decreased significantly or stopped. Subsequently, the second phase showed ongoing peripheral activity. In the first phase subcutaneous injection was more painful and in second phase intramuscular injection with doses of 2% and 4%.
Conclusion: We developed a novel muscle pain model based on tonic nociceptive behaviors in rats; following intramuscular formalin injections. This model has great clinical relevance and can facilitate new studies on pain.

Keywords: Injections, Intramuscular; Formalin; Rats

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Introduction

Musculoskeletal injuries can produce pain in many conditions (1-4), and the mechanism of this pain might differ from acute pain in superficial tissue. Wall and Woolf, in 1984, showed that a brief C-afferent fiber input into the spinal cord can produce a prolonged excitability of muscle C-afferent fibers, which might explain differences in injuries to skin versus deep tissue (5) so they suggested that deep tissue damage might be a significant factor in the development of chronic pain. Chronic and inflammatory muscle pain has high prevalence in clinical patients and is difficult to manage pharmacologically. However, relatively little is known about the

nervous system mechanisms that mediate and modulate muscle pain, which has led to difficulties in treatment. Therefore, the development of muscle pain models that permit assessment of the mechanisms related to pain and the investigation of different pharmacological drugs to manage pain are of great clinical significance.

The aim of this study was to develop an experimental model of musculoskeletal pain by characterizing formalin-induced nociceptive behavioral responses following formalin injection into the femoral region of rats. After the injection, the nociceptive responses of rats were rated according to objective behavioral criteria.

Materials and Methods

Subjects:

This experimental study was performed with adult Sprague-Dawley rats (220-300 g). Food and water were provided and animals were housed at a temperature controlled room (22±1°C), with a 12-hour light-dark cycle. All experiments were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Research and Ethics Committee.

Formalin test:

Rats were moved to the test room at least 1 hour before starting the experiment. The formalin tests were performed in clear plastic boxes (30×30×30 cm^3) with a mirror placed underneath at a 45° angle. The rats were first acclimatized for 30 minutes in an acrylic observation box and then the formalin (50 µl; 2%) was injected subcutaneously into the plantar surface of the right hind paw or injection into the gastrocnemius muscle (50 µl; 2%, 4% and 8%) with a 30 gauge needle. Each rat was then immediately returned to the observation box, and nociceptive behaviors were scored as follows: 0, the injected paw was normal and placed its full weight on the injected foot; 1, the rat placed only a little weight on the injected paw; 2, the injected paw was elevated; and 3, the rat was licking or biting the injected paw. Recording of nociceptive behaviors began immediately after the formalin injection (time 0) and was continued for 90 minutes. The nociceptive behavior scores were measured every 3 minutes from the start of the experiment (6,7). The scores were recorded in normal rats as well as in those that received stress. In each group, the nociceptive behaviors for each rat were evaluated in phases: the first phase (1-7 minute), interphase (8-14 minute) and the second phases of 2A (15-60 minute) and 2B (61-90 minute).

Experimental protocols:

We injected formalin intramuscularly into rats at different concentrations and compared the nociceptive behaviors to those following subcutaneous injection. The nociceptive behaviors of the rats were rated according to objective behavioral criteria. Four sets of experiments in the formalin test were performed: (1) rats were given subcutaneous injections of formalin in plantar area (2%, n=7); (2) rats were given intramuscular injections of formalin (2%, n=6); (3) rats were given intramuscular injections of formalin (4%, n=6); and (4) rats were given intramuscular injection of formalin (8%, n=6).

Statistical analysis:

The mean of values were presented as Mean±SEM. The formalin pain score in all groups were analyzed by one-way analysis of variance followed by Dunnett's test for

multiple comparisons as needed. The first phase, interphase, and second phase of the formalin test were analyzed separately. The defined level for statistical significance was (p<0.05).

Results

Results of intramuscular versus subcutaneous formalin injections:

After intramuscular and subcutaneous formalin injection, rats showed nociceptive behaviors for less than 10 minutes, and then nociceptive behaviors decreased significantly or stopped. Subsequently, the second phase (15-90 minute) showed ongoing peripheral activity.

Intramuscular formalin injection (2%) induced nociceptive behaviors which were lower than the behaviors following subcutaneous formalin injection in phase 1 (p<0.01) and phase 2A (p<0.05), and more in phase 2B (p<0.05) (Fig 1).

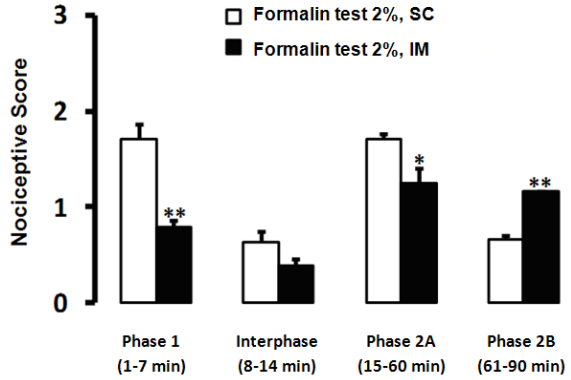

Figure1. The mean effects of subcutaneous (2%, 50 µl) and intramuscular (2%, 50 µl) injections of formalin on the nociceptive behaviors score (Mean±SEM). * p<0.05 and ** p<0.01.

Although, intramuscular formalin injection (4%) induced mean nociceptive behavior scores that were significantly lower than scores from subcutaneous formalin injection in phase1 (p<0.01) and phase 2A (p<0.01), the intramuscular injection scores were significantly higher than the subcutaneous injection scores in phase 2B (p<0.05) (Fig 2).

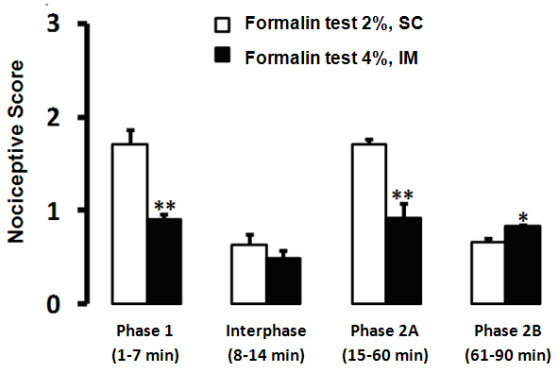

Figure 2. The mean effects of subcutaneously (2%, 50 µl) and intramuscular injections of formalin (4%, 50 µl) on nociceptive behavior scores (Mean±SEM). * p<0.05 and ** p<0.01.

Intramuscular injections with an increased formalin concentration (8%) did not change nociceptive behaviors. The nociceptive behavior scores were significantly lower than subcutaneous formalin injections in phase 1 (p<0.05) and phase 2A (p<0.01), but both groups' scores were similar in phase 2B (p>0.05) (Fig 3).

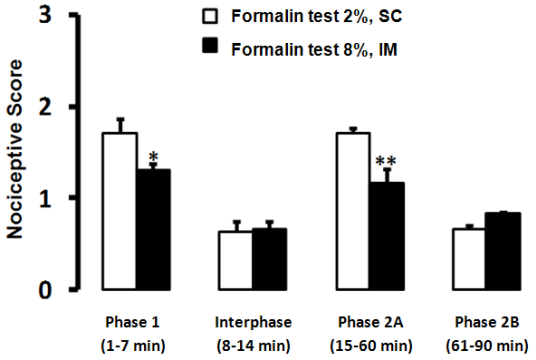

Figure 3. The effects of subcutaneous (2%, 50 µl) and intramuscular (8%, 50 µl) injections of formalin on mean nociceptive behavior scores (Mean±SEM). * p<0.05 and ** p<0.01.

Discussion

We demonstrated that, after subcutaneous plantar surface or intramuscular formalin injections, rats showed nociceptive behaviors for less than 10 minutes, and afterwards, the

nociceptive behaviors were attenuated significantly or stop. A second phase of responses began approximately 15 minutes after the formalin injection. These painful behaviors appear to be qualitatively different following subcutaneous formalin injection and intramuscular formalin injections. After intramuscular and subcutaneous formalin injection, rats showed nociceptive behaviors for less than 10 minutes, and then nociceptive behaviors decreased significantly or stopped. Subsequently, the second phase showed ongoing peripheral activity. In the first phase subcutaneous injection was more painful and in second phase intramuscular injections with doses of 2% and 4% were more painful. So pain produced by intramuscular formalin injection is monophasic with late response.

After intramuscular and subcutaneous formalin injection, rats showed nociceptive behaviors for less than 10 minutes, and then nociceptive behaviors decreased significantly or stopped. Subsequently, the second phase showed ongoing peripheral activity. In the first phase subcutaneous injection was more painful and in second phase intramuscular injection with doses of 2% and 4%.

Although some behavioral pain models are widely used to evaluate the analgesic effect of therapeutic agents, they do not clearly resemble human patients' nociceptive behaviors. The value of the formalin test is in its reliability for testing pharmacological drugs for clinical purposes. There is a lack of suitable animal pain models that reliably predict the analgesic effects of drugs on muscle pain. For example, monosodium iodoacetate injections (MIA) into joints causes cell death and can only serve as a model of pain (8). In another model, incisions can produce postoperative pain (9). In the prolonged tissue retraction model, nociceptive behaviors were investigated with hind paw stimulation (10). These models are restricted to time courses of induced nociceptive behaviors. Until now, muscle pain models have used a variety of methods that could not investigate the mechanisms involved in the generation and management of muscle pain. This study developed a muscle pain model produced by the intramuscular injection of formalin into rats. Because formalin is a chemical noxious stimulus and a widely used tool in animal behavioral models of pain, the aim of this study was to develop a muscle pain model to study nociceptive mechanisms by characterizing the formalin-induced nociceptive responses following formalin injections into the gastrocnemius muscles in rats. Formalin test is a widely used tool in animal models of tonic pain, and in basic research, it can be used to evaluate analgesic or pain inducing effects of different substances (11). Injection of an adequate amount of formalin into the hind paw of animals induces a series of nociceptive behaviors that last for more than 60 minutes (7,12-17). The formalin test consists of three phases: animals show a short period of nociceptive behaviors in phase followed by attenuation or quiescence of nociceptive responses during the interphase, but afterward, nociceptive behaviors reappear for up to 45 minutes (18,19).

In the most studies, researchers investigated muscle pain caused by an injury from intramuscular injection. Kehl *et al*., in 2000, injected Carrageenan, as a nociceptive stimulus into the muscle to produce short-lasting, acute inflammation and pain (20).

Intramuscular injection of carrageenan induced attenuation in forelimb grip force. While levorphanol decreased the Carrageenan-evoked reduction in grip force in

a dose-dependent manner, which was reversible by naltrexone. Also, other agents used clinically to treat muscle pain, blocked this effect. Forelimb grip force assessment as a measure of nociceptive behaviors is different from our study (20). Radhakrishnan *et al*., in 2003, suggested that high doses of Carrageenan (3%) injected into deep tissues produced long-lasting inflammation that (21). Carrageenan injections into muscles caused symptoms of myositis (22), and carrageenan injections into knee joints induced the synthesis and release of inflammatory mediators (23) and long-lasting elevations in glutamate and nitric oxide metabolites (24).

Sluka *et al.*, in 2001, used repeated intramuscular injections of low pH acidic saline, and induced an animal model of persistent mechanical pain that was unrelated to tissue damage and not maintained by continued primary afferent input at the site of injury (25). Dina *et al.*, in 2008, showed that repeated injections of the same agents could produce chronic pain (26). In these models, repeated injections were needed to produce mechanical pain. The formalin test, however, is easy to use and produces continuous tonic nociceptive behaviors, rather than transient responses. Therefore, the formalin test has a greater similarity to clinical pain in humans, and these properties make the formalin test a useful tool for pain research.

Pain perceptions from cutaneous tissues are typically sharp and easy to be localized, but pain perceptions from deep tissues are diffuse, dull, aching, and difficult to localize (27-29). Pain perceptions from muscles and joints are modulated differently than perceptions in cutaneous tissue (30-32). Thus, most evidence supports the hypothesis that injury to deep tissue induces different behavioral responses compared to superficial tissue injury.

Conclusion

We developed a novel muscle pain model using as tonic nociceptive behaviors produced by formalin injections, which caused muscle inflammation and nociceptive responses. Pain produced by intramuscular formalin injection is monophasic with late response. This pain model has great clinical relevance for research on muscular pain mechanisms, and could also be useful for assessing new analgesic compounds meant to treat muscular pain.

Conflict of Interest

The authors have no conflict of interest.

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