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Original article

Establishment of a novel objective and quantitative method to assess pain-related behavior in monosodium iodoacetate-induced osteoarthritis in rat knee

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ABSTRACT

Introduction: Pain in osteoarthritis (OA) patients can be present at rest but typically worsens with movement of the affected joint. However, useful assessment methods of movement-induced pain in animal models are limited. Here, we describe the reduction of spontaneous activity in a rat model of OA as an objective and quantifiable behavioral pain that can predict the analgesic activity of a variety of agents following single-dose administration. **Methods:** OA was induced in male Sprague–Dawley (SD) rats by intra-articular injection of monoiodoacetate (MIA), and the joint degeneration was assessed with histologic and radiographic analyses. Spontaneous activities were measured in nonhabituated rats using standard, photocell-based monitor systems in the dark. To investigate the potential of the OA model to predict analgesic activity, a number of nonsteroidal anti-inflammatory drugs (NSAIDs) and atypical analgesic drugs were used. **Results:** Biphasic reduction of total distance and number of rears was observed during the course of experiment after administering 1 mg and 0.3 mg of MIA, respectively. We found that number of rears was the most sensitive to MIA-induce OA and displayed the greatest percentage decrease in activity. Joint degeneration was observed with decreased bone mineral density and loss of articular cartilage 28 days post-MIA injection. Appropriate dosage of opioids reversed MIA-induced decrease of number of rears indicating that reduction of this vertical spontaneous activity reflects pain-associated behavior. As high-doses of opioids reduced spontaneous activity, the sedative effect can be distinguished from the analgesic effect. Analgesic treatment indicates the coexistence of an inflammatory pain state (early phase) sensitive to NSAIDs and a non-inflammatory pain state (late phase) resistant to NSAID treatment. **Discussion:** This study indicates that unlike standard measures of analgesia such as alteration in thermal or mechanical sensitivity, measurement of spontaneous activity is a validated method for measuring the effects of analgesics in rats with OA knee joints. Moreover, the animals require no habituation, and thus behavioral observation subjectivity is eliminated.

1. Introduction

Osteoarthritis (OA) is the most common form of joint disease, with over one-half of all people over the age of 65 demonstrating radiographic changes in the knees with pain (Hinton, Moody, Davis, & Thomas, 2002). No drug modifying OA progression is currently available, and treatment options are focused on symptomatic relief of pain and inflammation to improve the joint function. Starting with acetaminophen, treatment is then followed by non-steroidal anti-inflammatory drugs (NSAIDs), steroids and opioids in line with disease progression and increasing pain severity but none of them provide complete relief and side effects are often associated with therapeutic treatments (Hinton et al., 2002). Moreover, the response to these drugs is unsatisfactory in some patients, and the inflammatory focus is not always detected in the affected joints (Bjordal, Klovning, Ljunggren, & Slordal, 2007; Bjordal, Ljunggren, Klovning, & Slordal, 2004; Haywood et al., 2003). Since no drug modifying OA progression is currently available, development of novel and safer analgesics is highly needed.

Movement-induced pain is an early symptom characteristic of OA; however, with the progression of the disease, a continuous aching pain or pain at rest is the main clinical presentation (Creamer, Lethbridge-Cejku, & Hochberg, 1998; Sinkov & Cymet, 2003). Recent clinical studies have reported a difference in the pharmacological profile for relief of pain at rest versus movement-induced pain in OA patients (Petrella, DiSilvestro, & Hildebrand, 2002). Therefore, a thorough understanding of the mechanisms underlying the action of currently used analgesics and their utility in treating the different qualities of pain is vital.

A monoiodoacetate (MIA)-induced OA model has been well described in rats, especially in terms of the pathological progression of

Abbreviations: OA, osteoarthritis; MIA, monoiodoacetate; NSAIDs, non-steroidal anti-inflammatory drugs; BMC, bone mineral content; BMD, bone mineral density; EDTA, ethylenediaminetetraacetic acid.

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the disease, and more recently, pain behavior (Guzman, Evans, Bove, Morenko, & Kilgore, 2003; Vonsy, Ghandehari, & Dickenson, 2009). MIA injected in knee joint cavity disrupts glycolysis by inhibition of glyceraldehyde-3-phosphate dehydrogenase. It thereby targets the avascular cartilage and causes chondrocyte death, fragmentation of cartilage, and exposure of subchondral bone (Fernihough et al., 2004; Tonussi & Ferreira, 1999). These histopathologic features are similar to OA, thus providing a basis for studies on the mechanisms of pain as in OA (Clarke, Heitmeyer, Smith, & Taiwo, 1997; Guingamp et al., 1997; Guzman et al., 2003; van der Kraan, Vitters, van Beuningen, & van den Berg, 1992). The severity of MIA-induced joint pain has been evaluated by assessment of change in hind paw weight distribution and of mechanical and thermal sensitivity (Combe, Bramwell, & Field, 2004; Fernihough et al., 2004; Guingamp et al., 1997; Orito et al., 2007). However, these assays require multiple habituations of the test animals to the instruments and tend to result in false-positive outcomes due to tester subjectivity or nonspecific side effects such as sedation and muscle relaxation (Matson et al., 2007). It was reported that pain onset detected by reduction of physical activity was earlier than that by hind paw weight distribution in surgically-induced OA model (Inglis et al., 2008). In this report, it was supposed that spontaneous changes in physical activity occur with a low level of pain, but higher levels of pain are required for the detection of changes in weight distribution using an incapacitance tester. These results indicate that physical activity is more sensitive and convenient than weight distribution for the assessment of OA-associated pain.

Also reported were the effects of naloxone on the change of physical activity, however, the monitoring system used requires a 3 h period after the administration (Guingamp et al., 1997; Inglis et al., 2008). It is important to evaluate the effects of drugs at appropriate time point according to their pharmacokinetic profiles, but there is no report evaluating the effect of analgesics by measuring spontaneous activity for a short period of time. Therefore, we examined the usefulness of our novel monitoring method, which finishes within half hour, in evaluating the effects of several clinically-used analgesics in the development of chronic pain associated with the disease. Taken together, our results indicate that spontaneous vertical activity is pharmacologically well validated with clinically useful analgesic agents.

2. Materials and methods

2.1. Materials

Acetaminophen, tramadol and amitriptyline were purchased from Sigma (St. Louis, MO). Sterile physiological saline was purchased from Otsuka Pharmaceutical (Tokyo, Japan). MIA, 0.5% methyl cellulose and 10% neutral buffered formalin were purchased from Wako Pure Chemical Industries (Osaka, Japan). Celecoxib was purchased from Chemtec Lab (Tokyo, Japan). Gabapentin was purchased from Toronto Research Chemicals Inc (North York, Canada). Naproxen was synthesized in-house at Sankyo Research Laboratories (Tokyo, Japan). Morphine hydrochloride was purchased from Daiichi Sankyo Propharma (Tokyo, Japan).

2.2. Animals

All experimental procedures were performed in accordance with the in-house guideline of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. Male Sprague–Dawley (Slc:SD) rats (Japan SLC, Shizuoka, Japan), weighing 160–180 g at the start of the experiments, were used. The animals were group-housed under a 12/12 h light/dark cycle and had free access to food and water at all times. Prior to initiation of the studies, the animals were randomized and assigned to treatment groups.

2.3. MIA injection

Osteoarthritis was induced by intra-articular (*i.a.*) injection of MIA solution in the knee joint as described previously (Jones, Peters, & Shannon, 2005). Briefly, the rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL) and given a single *i.a.* administration of 0.3, 1, or 3 mg of MIA dissolved in saline through the intrapatellar ligament of both knees. MIA was administered in a volume of 50 μL with BD Low Dose (Becton, Dickinson and Company, Franklin Lakes, NJ). Basal readings were established using a group of control rats that were injected with saline.

2.4. Monitoring of spontaneous activity

Spontaneous activity was measured in a novel environment using a computerized SCANET MV-20 plus Animal Activity Monitor (MELQUEST, Toyama, Japan) equipped with 48 infrared photocell emitters and detectors that has been previously described (Mikami et al., 2002; Mikami et al., 2004). Horizontal activity (total distance) and vertical activity (number of rears) were measured. Total distance was detected by infrared sensors set at 6 cm from the floor. The sensors are arranged in a horizontal plane of a transparent Plexiglas box of 45 cm squares to make a sensor field with a 0.6-cm grid pattern. When rat interferes with the paths of the infrared rays, the coordinates of the center of the object are recorded in a computer every 0.1 s. To avoid false detection of small movement on site like grooming, gazing around, or changing its posture, we only measured the distance traveled over 3.6 cm. Number of rears was counted by another sensor frame inserted at different height level. To distinguish rears from incomplete standing actions, the upper sensor frame was adjusted to 13–17 cm from the floor according to the size of rat. Multiple test chambers were placed in a sound-isolated testing room. Total distance and number of rears were measured for 25 min in the dark. The same groups of rats were followed throughout the course of the experiment.

2.5. Radiographic analysis

Radiographic changes were assessed in rats that had received 0, 0.3, 1, or 3 mg MIA. Twenty-eight days after MIA injection, rats ($n = 6$) were euthanized with CO_2 and their knee joints were removed and fixed. High-resolution radiographs of the distal end of the femur from the epiphysial growth plate were taken using an mFX-1000 radiography system (Fujifilm Co., Ltd.). The bone mineral content (BMC) and bone mineral density (BMD) were determined using a DCS-600EX Bone Densitometry System (Aloka Co., Ltd.) (Pomonis et al., 2005).

2.6. Histological analysis

Histological changes were assessed in rats that had received 0, 0.3, 1, or 3 mg of MIA. Twenty-eight days after MIA injection, rats ($n = 6$) were euthanized with CO_2 and their knee joints were removed by sharp division at the proximal femur and distal tibia. The skin and surrounding muscles were then removed without disturbing the joint and its associated ligaments. Tissue samples were prepared for light microscopy using the standard procedures. Briefly, the samples were preserved in 10% neutral buffered formalin at 4 °C, and subsequently decalcified in 10% ethylenediaminetetraacetic acid (EDTA) (pH 7.4). The samples were dehydrated in an ethanol series and embedded in paraffin. Sections of 4 μm were obtained using a cryostat and stained with Safranin O and fast green. They were photographed with the use of a BZ-9000 BIOREVO (Keyence, Osaka, Japan).

2.7. Acute pharmacological assessments

A single dose of drug or vehicle was administered at 3 days or 28 days after the injection of 1 mg of MIA. The doses, vehicles, injection