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Efficacy of drugs with different mechanisms of action in relieving spontaneous pain at rest and during movement in a rat model of osteoarthritis

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ABSTRACT

Patients with osteoarthritis (OA) suffer from joint pain aggravated by movement, which affect their quality of life. In the present study, a weight bearing paradigm for pain at rest and a gait paradigm for pain during movement were tested in rats with unilateral knee arthritis induced by an intra-articular injection of sodium monoiodoacetate (MIA). At week 3 after MIA (1 mg/knee) injection, animals developed pain-associated, right-left imbalances of weight distribution (weight bearing) or foot print parameters (gait). Diclofenac, at doses up to 30 mg/kg orally (p.o.), did not have a significant effect on either paradigm. Morphine rectified the weight bearing and gait imbalances at 1 and 3 mg/kg subcutaneously, respectively. The weak opioid and serotonin/norepinephrine reuptake inhibitor (SNRI) tramadol also significantly corrected the indices at 10 mg/kg (weight bearing) and 100 mg/kg p.o. (gait). The SNRI duloxetine at 30 mg/kg p.o. corrected the weight bearing imbalance but not gait imbalance. We assessed the effect of different drugs on pain-induced disturbances in weight distribution and gait in MIA-induced arthritic rats. Analgesic drugs, each with different mechanisms of action, were less effective in rectifying the imbalance in gait than that in weight distribution. The assessment of the effect of analgesics on not only rest pain but pain during movement is valuable for the comprehensive examination of their therapeutic efficacies in OA.

1. Introduction

Osteoarthritis (OA) is a common condition caused by progressive destruction of joint tissues including cartilage and subchondral bone. Patients with OA suffer pain in the affected joints while at rest, with discomfort aggravated by movement (Sinkov and Cymet, 2003). Pain experienced at rest and during movement respond differently to analgesics (Petrella et al., 2002). It has suggested the existence of a pain mechanism specific to movement; for example, activation of mechanoreceptors on the afferent nerves during movement may be responsible for eliciting pain (Kelly et al., 2012). Joint pain may therefore show a differential response to analgesics depending on whether the joint is active or at rest. For this reason, the analgesic effect of drugs should be examined at rest and during movement in preclinical studies. However, evaluation of the efficacy of analgesics during both movement and at rest has not been conducted.

Monoiodoacetate (MIA)-induced arthritic rats suffer from loss of articular cartilage, subchondral bone lesions, and pain in the affected joints (Guzman et al., 2003; Kalbhen, 1987; Kobayashi et al., 2003). This animal model has been widely utilized as an animal model of knee OA (Combe et al., 2004; Fernihough et al., 2004). Given that OA pain occurs spontaneously without artificial stimulation, such as from pressure or heat, animal models of OA should also be characterized by development of spontaneous pain that is quantifiable. MIA-induced monoarthritic rats develop spontaneous pain associated with right-left weight bearing imbalance at rest as well as a gait imbalance. The weight bearing paradigm is an indicator of spontaneous pain at rest, which assesses the right-left imbalance of weight distribution of immobile animals in a test

Abbreviations: OA, osteoarthritis; NSAID, nonsteroidal anti-inflammatory drug; MIA, monoiodoacetate; SNRI, serotonin norepinephrine reuptake inhibitor

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box (Kobayashi et al., 2003). In contrast, the gait paradigm reflects spontaneous pain during movement and assesses the right-left imbalance by analyzing foot prints of animals while walking (Angeby-Moller et al., 2008; Ferland et al., 2011; Ferreira-Gomes et al., 2008; Gabriel et al., 2007; Masocha and Parvathy, 2009; Miyagi et al., 2011; Vrinten and Hamers, 2003).

Here, to assess the analgesic efficacy of drugs with different mechanisms of action in relieving spontaneous pain at rest and during movement in an animal model of OA, we administered diclofenac (a non-steroid anti-inflammatory drug [NSAID]), morphine (a strong opioid), tramadol (a weak opioid and serotonin norepinephrine reuptake inhibitor [SNRI]), and duloxetine (a SNRI) to MIA-induced arthritic rats and assessed the weight bearing and gait paradigms.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (Charles River, Kanagawa, Japan) weighing 150–185 g at the time of MIA injection were used. The animals were housed in groups of five in a polycarbonate cage with woodchip bedding and free access to food and water. The animal room was maintained at a temperature of 23 ± 2 °C and humidity of $55\% \pm 10\%$ and had a 12-h light/dark cycle (lights on at 07:30). Animals were acclimated to the housing facility for at least three days before the start of experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. (Tokyo, Japan), which has been accredited by the Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.2. Drugs

Diclofenac sodium purchased from Sigma-Aldrich (St. Louis, USA) was dissolved in distilled water and administered orally (p.o.; 3, 10, or 30 mg/kg) 2 h prior to tests. Given that diclofenac at 100 mg/kg caused severe acute gastric ulcers (Berenguer et al., 2006), 30 mg/kg was deemed the appropriate maximum test dose. Morphine hydrochloride from Takeda Pharmaceutical Co. Ltd. (Osaka, Japan) was dissolved in saline and injected subcutaneously (s.c.; 0.1, 0.3, 1, or 3 mg/kg) 1 h prior to tests. Tramadol hydrochloride purchased from Sigma-Aldrich was dissolved in distilled water and administered p.o. (3, 10, 30, or 100 mg/kg) 2 h prior to tests. Duloxetine hydrochloride prepared by Astellas Pharma Inc. was dissolved in distilled water and administered p.o. (3, 10, or 30 mg/kg) 3 h prior to tests. The volume for administration was 5 ml/kg p.o. and 1 ml/kg s.c. Lidocaine hydrochloride purchased from Sigma-Aldrich was dissolved in saline and injected into the affected knee joint cavity through the patellar ligament (1, 3, or 10 mg/knee) at a volume of 20 μL/knee 10 min prior to tests. Calculation of drug doses was based on the free base form of the drug. Individual evaluation protocols (dose and timing) for each drug were based on published literature (Chandran et al., 2009; Combe et al., 2004; Di Cesare Mannelli et al., 2013; Fernihough et al., 2004; Ferreira-Gomes et al., 2012; Ivanavicius et al., 2007; Pulichino et al., 2006; Yoshimi et al., 2010).

2.3. Induction of knee joint OA

Animals were anesthetized with 3–4% isoflurane (Mylan Inc., Canonsburg, USA). The skin of the right knee area was shaved and disinfected with 70% ethanol. Sodium MIA (Sigma-Aldrich) at a dose of 1, 1.5, or 2 mg in 50 μL of saline was injected into the joint cavity through the patellar ligament of the right knee. The dose of MIA was based on the results of our previous studies (Takeshita et al., 2011; Yoshimi et al., 2010). The sham-operated group was injected with saline instead of MIA. The animals were returned to the animal room after recovery from anesthesia. Different lots of animals were used for evaluation of each drug.

2.4. Weight bearing paradigm

Weight distribution to right and left hind limbs was measured using the incapacitance tester (Linton Instrumentation, Norfolk, UK) in accordance with a previously published method (Kobayashi et al., 2003). Briefly, animals were placed in an angled plastic test box and acclimated to the apparatus for approximately 5 min. The right hind limb of the animal was positioned on the right force plate in the box and the left hind paw was positioned on the left plate. The weight (g) distributed to each hind limb was measured for 5 s, and the difference in weight distribution between the right (MIA-injected side) and left (contralateral) limb was determined.

2.5. Gait paradigm

Experiments were based on published methods with minor modifications (Ferland et al., 2011; Ferreira-Gomes et al., 2012). The automated gait analysis apparatus (Noldus, Wageningen, Netherland) consists of a glass plate walkway equipped with light-emitting diodes (LEDs). Light from the LEDs is emitted inside the glass plate, internally reflected, and refracted to the glass plate except in the area where the animal's paw makes contact with the plate. A high-speed color camera positioned underneath the glass plate captures images of the illuminated area and sends the imaging information to a computer that runs the analysis system (CatWalk XT version 9.0 software; Noldus). The apparatus was covered with a black polyethylene sheet to block external light and installed in a sound-proof room.

All recordings of animal footprints were performed between 08:00 and 16:00. Animals were made to walk on the glass plate walkway 3 times on the day before drug evaluation. A satisfactory walk was defined as an uninterrupted walk across the walkway that left ≥ 3 footprints for each hind limb. Recordings were repeated until three satisfactory walks were obtained.

The “max contact area” and the “swing speed” parameters were determined by analyzing the footprints. The max contact area is the area of the glass plate touched by the paw. Swing speed is calculated by dividing the step length by the time that the paw is not in contact with the plate in one step cycle. The mean values of the parameters obtained from three satisfactory walks were calculated for each animal. Differences in parameter values between the right and left hind limbs were obtained by subtracting the values of the right (MIA-injected side) side from the values of the left side.

2.6. Drug evaluation

Weight bearing and gait experiments were conducted separately using different lots of animals. A total of 32 animals for weight bearing tests and 40 for gait tests were used in the evaluation of each drug. In accordance with a published protocol (Yoshimi et al., 2010), evaluation of test drugs was conducted in week 3 after the injection of 1 mg of MIA. Baseline values for weight bearing and gait measurements were obtained one day prior to drug administration. Animals were divided into 4 groups ($n=8$ per group for weight bearing evaluation and $n=10$ per group for gait evaluation) based on their baseline values and body weight. One control group received vehicle, while the remaining three groups received one particular dose of the test drug. Weight

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