Osteoarthritis and Cartilage 23 (2015) 1605–1612

Osteoarthritis and Cartilage

Deficits in spontaneous burrowing behavior in the rat bilateral monosodium iodoacetate model of osteoarthritis: an objective measure of pain-related behavior and analgesic efficacy

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ARTICLE INFO

Article history:
Received 8 October 2014
Accepted 1 May 2015

Keywords:
Burrowing
Monosodium iodoacetate
Spontaneous behavior
Pain
Rat

SUMMARY

Objective: To characterize deficits in burrowing behavior – an ethologically-relevant rodent behavior – in the monosodium iodoacetate (MIA) rat model of osteoarthritis (OA), and the sensitivity of these deficits to reversal by analgesic drugs of both prototypical and novel mechanisms of action. A second objective was to compare the burrowing assay to a spontaneous locomotor activity (sLA) assay.

Method: Male Wistar Han rats (200–220 g) received intrarticular (i.a.) injections of MIA or saline for sham animals. A deficit in the amount of sand burrowed from steel tubes filled with 2.5 kg of sand was used as a measure of pain-related behavior, and sensitivity to reversal of these deficits by analgesic drugs was assessed in bilaterally MIA-injected rats.

Results: Bilateral MIA injections induced a significant impairment of burrowing behavior, which was concentration-dependent. The temporal pattern of the deficits was biphasic: a large deficit at 3 days post-injection, resolving by day 14 and returning at the 21 and 28 day time points. At the 3 day time point ibuprofen, celecoxib and an anti-nerve growth factor (NGF) monoclonal antibody (mAb) were able to significantly reinstate burrowing behavior, whereas the fatty acid amide hydrolase (FAAH) inhibitor PF-04457845 and morphine displayed no reversal effect. Morphine impaired burrowing behavior at 3 mg/kg in sham animals. Deficits in rearing frequency in the locomotor activity assay proved irreversible by analgesics.

Conclusion: Burrowing behavior provides an objective, non-reflexive read-out for pain-related behavior in the MIA model that has predictive validity in detecting analgesic efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) and an anti-NGF mAb.

Introduction

The prevalence of osteoarthritis (OA) is estimated to be 50% in people aged 65 and older[1] and with an aging population the cost and health impact of this disease will continue to rise. The intra-articular (i.a.) injection of monosodium iodoacetate (MIA) into the rat knee joint produces histological changes representative of those seen in human OA[2]. Concomitant with the early and late histological changes in the MIA model are pain-related behaviors[3], making it a useful model for assessing the analgesic efficacy of drugs.

Pain is the main clinical manifestation of OA. The current lack of effective disease modifying agents to target the aetiology of OA[4] means the use of analgesic drugs is the mainstay treatment for alleviating the impact of the disease on daily life, namely non-steroidal anti-inflammatory drugs (NSAIDs) and weak opioids[5].

The main techniques for assessing pain-like behaviors in the MIA model are measures of mechanical hypersensitivity: shifts in weight bearing (WB) from the ipsilateral affected to contralateral unaffected knee, distal mechanical hyperalgesia and tactile allodynia[6–8]. A pressing issue in the pain field is the lack of translation between preclinical and clinical findings, which has resulted in relatively few safe and effective analgesics being developed (reviewed by Blackburn-Munro 2004[9] and Mogil, 2009[10]).

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http://dx.doi.org/10.1016/j.joca.2015.05.001
1063-4584/

Although there is evidence that current pain assays have some predictive validity from rat to human[11], it is becoming increasingly apparent that assays utilizing spontaneous rather than evoked/reflexive measures are needed to assess the global impact of pain beyond hypersensitivity[12,13].

In addition to sharp pain evoked by movement, patients with OA rank constant and aching pain among the most distressing features of the disease[14]. How well the current evoked/reflexive assays of pain-like behaviors in rodents account for this persistent pain is unclear[9]. Furthermore, clinical assessments of chronic pain are focusing increasingly on the multifaceted nature of pain, such as the effect on emotion and physical function[15], which are not assessed preclinically by reflexive assays.

Burrowing is an innate rodent behavior indicative of animal well-being that is conserved across various strains of rat[16–19] and mice[20–22]. Deficits in burrowing behavior occur in preclinical rat models of inflammatory and neuropathic pain and can be reversed by analgesics[16,18,19,23,24]. This behavior, therefore, is a useful preclinical measure of non-evoked pain. Burrowing may encompass the supraspinal mechanisms that contribute to pain phenotypes, both sensory and affective, as well as being more ethologically relevant than the commonly used assays measuring hypersensitivity[25].

Here we show deficits in burrowing behavior in the bilateral MIA model of OA and sensitivity of these deficits to reversal by prototypical and novel analgesics. To our knowledge, this is the first published article demonstrating deficits in burrowing behavior in the MIA model. Additionally, burrowing behavior was compared to another non-reflexive readout: spontaneous locomotor activity (sLA). This has been shown previously to be impaired in various preclinical animal models and to be sensitive to pharmacological modulation[26–29].

Methods

Animals

594 male Wistar Han rats (200–220 g; Charles River Laboratories, Germany) were used for all experiments and were housed in groups of four with food and water *ad libitum* with a 12 h light/dark cycle. All animal experimental protocols were authorized by the Local Animal Care and Use Committee and carried out according to the local animal care guidelines, AAALAC regulations, and the USDA Animal Welfare Act.

MIA injections

Rats were briefly anaesthetized with 5% isoflurane (Abbott Laboratories, Wiesbaden, Germany) followed by 3% maintenance, after which an i.a. injection of 3 mg of MIA (Sigma–Aldrich, Steinheim, Germany) dissolved in 50 μL of 0.9% physiological saline was performed into the femorotibial joint. Sham rats received 50 μL injections of physiological saline. All injections for pharmacology experiments were bilateral, except for one model conditions studies in which rats received either uni- or bilateral injections.

Drugs and drug administration

The analgesic drugs tested were: morphine (Caelo, Germany), ibuprofen (Sigma Aldrich, Steinheim, Germany), celecoxib (LKT Laboratories Inc., St. Paul, MN, USA) and the fatty acid amide hydrolase (FAAH) inhibitor PF-04457845 (synthesized by Boehringer Ingelheim). All drugs were administered orally (p.o.) with 0.5% (w/v) natrosol and 0.1% (v/v) tween-80 (9:1 ratio) as vehicle, except morphine which was injected subcutaneously (s.c.) in the interscapular area with 0.9% physiological saline as vehicle. All drugs were administered in a volume of 2 mL/kg except PF-04457845, which was administered in a volume of 4 mL/kg.

For the generation of the anti-nerve growth factor (NGF), monoclonal antibody (mAb) variable domains were extracted from the patent application WO 2004/058184 A2 (applicant: Rinat Neuroscience Corporation) and processed as described by Hezareh *et al.*, 2001[30]. The antibody was administered s.c., with phosphate buffered saline as vehicle.

Burrowing training and burrowing experiments

For all burrowing experiments steel tubes (32 cm in length and 10 cm in diameter) were filled with 2.5 kg of quartz sand and placed in Plexiglas cages (600 × 340 × 200 mm). The open-end of the tube was elevated 6 cm from the floor of the cage. Training for each experiment was carried in 2 phases: social facilitation (SF) and individual training (IT). For SF rats were placed in pairs in a cage for 2 h on two consecutive days. The amount of sand burrowed by each pair was measured and if a pair burrowed less than 1500 g of sand one of the pair was swapped with a rat from a pair that had burrowed greater than 1500 g for the second SF day. For IT rats were placed alone in the burrowing set-up for 30 min per day and the average amount burrowed over 3 days was calculated to attain a baseline burrowing performance value.

Before each animal was given MIA or sham intrarticular injections they were assigned to groups to ensure that each treatment group for an experiment had a comparable baseline burrowing value. For the model conditions study rats were allowed to individually burrow for 30 min 3 days after MIA or sham injections were performed and the amount of sand burrowed was recorded. For the time-course experiment burrowing performance was assessed 3, 14, 21 and 28 days post-injection. For all pharmacology experiments burrowing behavior was measured 3 days after MIA injection, and animals were placed in the burrowing set-up after the appropriate pre-treatment time.

Exclusions

During training any animal that has a baseline burrowing value of less than 1000 g or a standard deviation of burrowing (SD) greater than 450 g was excluded to ensure high and stable baselines. To ensure that all rats used in pharmacology experiments were in a comparable pain state burrowing values were also measured 1 day after MIA injection and any animal with a burrowing value greater than 1000 g was excluded from pharmacology at day 3. In total, exclusions after training and after day 1 accounted for around 5% of rats per study.

Locomotor activity

sLA was measured for 30 min with an automated monitoring system (TruScan Activity Monitor version 2.0, Coulbourn Instruments, Allentown, PA, USA). Each monitoring system was an enclosed 43 cm^2 arena with two levels of sensory photobeams; one level elevated 3 cm from the floor of the arena and the other 14 cm, measuring horizontal and vertical activity respectively. Each level of the detection system was equipped with 16 photobeams per wall of the arena, spaced 2.5 cm apart. The parameters measured by the system were: ambulatory horizontal distance moved (cm), rearing frequency and rearing time (s). Prior to testing, all rats were habituated in an annexe to the testing room.

Statistical analysis

All statistical processing was performed in GraphPad Prism version 6.0 (San Diego, CA, USA). For all analyses $P < 0.05$ was