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Gait analysis and weight bearing in pre-clinical joint pain research

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HIGHLIGHTS

- Dynamic weight bearing during locomotion gives valuable information on joint pain.
- Gait parameters add to the information on pain-related behavior in monoarthritic rats.
- Validation with naproxen shows partial normalization of weight bearing.
- Pharmacological effects of naproxen are highly reproducible.
- Translation to the clinic is improved compared to mechanical sensitivity testing.

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ABSTRACT

Background: There is a need for better joint pain treatment, but development of new medication has not been successful. Pre-clinical models with readouts that better reflect the clinical situation are needed. In patients with joint pain, pain at rest and pain at walking are two major complaints.
New method: We describe a new way of calculating results from gait analysis using the CatWalk™ setup. Rats with monoarthritis induced by injection of Complete Freund's Adjuvant (CFA) intra-articularly into the ankle joint of one hind limb were used to assess gait and dynamic weight bearing.
Results: The results show that dynamic weight bearing was markedly reduced for the injected paw. Gait parameters such as amount of normal step sequences, walking speed and duration of step placement were also affected. Treatment with naproxen (an NSAID commonly used for inflammatory pain) attenuated the CFA-induced effects. Pregabalin, which is used for neuropathic pain, had no effect.
Comparison with existing methods: Reduced dynamic weight bearing during locomotion, assessed and calculated in the way we present here, showed a dose-dependent and lasting normalization after naproxen treatment. In contrast, static weight bearing while standing (Incapacitance tester) showed a significant effect for a limited time only. Mechanical sensitivity (von Frey Optihairs) was completely normalized by naproxen, and the window for testing pharmacological effect disappeared.
Conclusions: Objective and reproducible effects, with an endpoint showing face validity compared to pain while walking in patients with joint pain, are achieved by a new way of calculating dynamic weight bearing in monoarthritic rats.

1. Introduction

Chronic pain remains a challenge, with inadequate medication for a large portion of the patients. An estimated 30–35% of the adult population reports chronic pain (Johannes et al., 2010; Wong and Fielding, 2011), and osteoarthritis (OA) is a common reason (Martel-Pelletier et al., 2016). Joint pain caused by OA as well as by e.g. rheumatoid arthritis cannot currently be treated sufficiently, and the number of sufferers increase as the population grows older. Today the recommended pain medication is mainly focused on paracetamol (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs) and weak opioids (Felson, 2009). Even though evidence for a neuropathic pain component in OA has been found (Hochman et al., 2011), drugs used for those symptoms, such as the modifiers of the $\alpha_2\delta$ subunit of calcium channels gabapentin or pregabalin, are not being recommended for OA patients. There is a need for better pain treatment, but attempts

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to develop new medication have not been successful (Hill, 2000; Wallace et al., 2002; Mogil et al., 2010). Pre-clinical models with readouts that better predict the clinical outcome are thus needed (Mogil and Crager, 2004; Berge, 2011).

In patients with joint pain, spontaneous pain at rest, disability and pain at walking are major complaints (Dieppe and Lohmander, 2005; Bijlsma et al., 2011). They are included in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale and are often used as readouts in clinical trials of OA pain (Lane et al., 2010). However, in rodent pain models spontaneous pain and pain at walking are difficult to measure. Still, evoked measures such as assessments of thresholds or reactions to mechanical and thermal stimulation are the methods most commonly used. It has been reported that 90% of 259 articles describing animal studies of neuropathic and inflammatory pain models in the journal "Pain" between 2000 and 2004 where behavioral testing was included exclusively used evoked stimuli (Mogil and Crager, 2004). Evoked measures to more specifically assess joint pain include a knee-bend test, where rats are exposed to flexion and extension of their hind limbs until a reaction appears (Ferreira-Gomes et al., 2008), and joint compression using a Randall-Selitto device until vocalization, struggle or withdrawal occurs (Ahn et al., 2011). Non-evoked measurements, like locomotor activity (Sluka et al., 2013) and rotarod analysis (Ruan et al., 2013) are used to assess function. Several systems to measure static weight bearing of the two hind limbs assess a pain-related outcome in rodents with monoarthritis, starting with subjective scoring of the guarding behavior (Coderre and Wall 1987) followed by a box equipped with force plates for the two hind paws (Schött et al., 1994), the Incapacitance tester (Linton Instrumentation) where the animals stand on force plates while the front paws are placed on a ramp (Bove et al., 2003), and the Tekscan® WB measurement system with a sensor mat to measure force (Tétreault et al., 2011; Rashid et al., 2013). Weight bearing during locomotion has proven to be more difficult to evaluate, but K Clarke built a walkway with a glass floor with light entering the long edge which when a contact was made by e.g. a paw the light was scattered and could be filmed from underneath. With this device he could study the paw contact patterns of rats, and when combining it with load measurements underneath areas in the walkway he could show that load was correlated to the light intensity of a paw print, with a coefficient of 0.92 (Clarke, 1992, 1995). Further development of this technique was reported by FPT Hamers (Hamers et al., 2001) who introduced a semi-automated version of a walkway with light entering the long edge of a glass floor and named it CatWalk. This setup was later purchased by Noldus who has since then developed it even further, and who made it commercially available as the CatWalk™. The "CatWalk test" (Ferreira-Gomes et al., 2008; Ferreira-Gomes et al., 2012) is based on the same principle but consists of a glass platform where rats are allowed to walk freely, and where paw placements are manually analyzed using the merged results from three frames with the animal walking and three frames when the animal is standing still. Attempts to measure the ongoing or spontaneous pain include using place preference (Liu et al., 2011) and burrowing (Rutten et al., 2013), but will not be discussed further here.

Several potential new drug targets have been tested in patients, and despite positive outcomes in pre-clinical models, few have been progressed to produce drugs that alleviate pain in humans. Although most of the pre-clinical targets never reach the stage of testing in humans, even those that did often failed. One example is the development of the highly potent, selective and irreversible inhibitor of fatty acid amid hydrolase-1 (FAAH) PF-04457845, which showed significant and long-lasting reversal of mechanical hypersensitivity induced by plantar injection of Complete Freund's Adjuvant (CFA), as well as induced by the model of monosodium iodoacetate (MIA) intra-articular knee injection (Ahn et al., 2011). When PF-04457845 was tested in seventy-four OA patients no evidence of pain reduction could be found in contrast to naproxen which was used as comparator (Huggins et al., 2012). Another example is the TRPV1 antagonist AZD1386, showing significant effects in rats on heat hypersensitivity induced by plantar injection of either carrageenan or CFA, or induced by the Chung model of neuropathic pain (Laird et al., 2010), but lacking effect on pain in 241 OA patients (Svensson et al., 2010; Quiding et al., 2013). Taken together, this supports the suspicion that the sensory testing which has dominated the pre-clinical testing of drug effects in animals does not mirror the pain in patients. In our previous work, using a model where joint inflammation was induced by CFA, we assessed weight bearing during locomotion and gait and showed effects by several classes of drugs that correlated well with their clinical efficacy, including the TRPV1 antagonist AZD1386 that showed no effect in these tests (Ängeby Möller et al., 2015). Here we introduce a novel method of calculating results from the commercially available CatWalk™ setup that will help to assess relevant pain-related behavior, with translational value, in rodent models of joint pain.

2. Materials and methods

2.1. Animals and housing

A total of 104 naïve male Wistar rats (Harlan, The Netherlands and United Kingdom), weighing 160–310 g at the start of testing were used. Due to delivery problems caused by infections, animals were purchased from two different breeding sites of the vendor. They were housed four per cage in Makrolon IV cages with aspen chips as bedding material and a red polycarbonate tube as cage enrichment. The rats had free access to food (SDS RM1 (E) SQC, Special Diet Services Ltd, Witham, England) and tap water throughout the studies. The temperature ($22 \pm 2\,^{\circ}C$), humidity ($55 \pm 15\%$), ventilation (12.5 ± 2.5 times/h) and lighting of the animal room (lights on from 5.30 a.m. to 5.30 p.m. including 30 min time for gradual changing in the light condition; day 300 ± 60 lx and night 5 ± 4 lx) were continuously monitored and controlled. The animals were acclimatized for at least one week before starting the experiments. All studies were carried out in accordance with the EU Directive 2010/63/EU for animal experiments and were approved by the Regional State Administrative Agency of Southern Finland (approval number ESAVI/7238/04.10.07/2014).

2.2. Induction of monoarthritis

Under deep anesthesia (5% isoflurane in oxygen/breathing air), 50 μl CFA (Sigma-Aldrich, containing 1 mg heat killed and dried *Mycobacterium tuberculosis* per ml) was injected into the left tibiotarsal joint from the dorsal side. The injection was completed in less than one minute, and rats recovered from the anesthesia within two to three minutes. Naïve rats were used for comparison.

2.3. Drugs and drug administration

Naproxen (naproxen sodium; Sigma-Aldrich) and pregabalin ((S)-Pregabalin, Toronto Research Chemicals Inc., Canada), both were dissolved in 0.9% saline and given orally via gavage (p.o.) in a volume of 3 ml/kg. The respective vehicle groups were given the same volume of 0.9% saline. Treatment started one day after monoarthritis induction. Naproxen was given twice daily, 4 h before and 2 h after the behavioral testing, while pregabalin was given once daily, 2 h before the behavioral testing.

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