Osteoarthritis and Cartilage 24 (2016) 1928–1939

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Pharmacological interrogation of a rodent forced ambulation model: leveraging gait impairment as a measure of pain behavior pre-clinically

B.L. Adams, W. Guo, R.T. Gors, K.L. Knopp*

Neuroscience Research, Lilly Research Laboratories, Indianapolis, IN 46285, USA

ARTICLE INFO

Article history:
Received 13 September 2015
Accepted 11 May 2016

Keywords:
Gait
Joint pain
Complete Freund's Adjuvant
Functional pain endpoint

SUMMARY

Objective: The aim of this study was to investigate whether inflammogen-induced temporal and spatial gait changes in a rodent forced-ambulation paradigm were sensitive to pharmacological intervention with both clinically validated and novel analgesics.

Methods: Using the GaitScan (CleverSys Inc., Reston, VA) treadmill system, we identified four functional endpoints inspired by clinical literature and sensitive to unilateral joint injury induced by intra-articular Complete Freund's Adjuvant (CFA). These endpoints included: range of motion, normalized stance distance, stance/swing ratio, and paw print size as a measure of guarding; collectively, these measures are proposed to serve as a high fidelity index of joint pain. We then examined the ability of known analgesic mechanisms to attenuate gait impairment as measured by this index.

Results: Clinically efficacious opioids, Nonsteroidal anti-inflammatory drugs (NSAIDs), and the yet unapproved anti-NGF antibody dose-dependently attenuated the CFA)-induced gait deficits, while a TNF-alpha fusion protein blocker had no effect on gait, but did produce a reduction in swelling. As well, the time course for gait impairment in the model appears to be distinct from the traditional endpoint of tactile hypersensitivity, offering the potential to assess a novel functional pain phenotype.

Conclusions: In response to the call for more functional pain measures, we submit this composite gait score as a novel endpoint to interrogate joint pain pre-clinically. As the etiology of human osteoarthritis (OA) remains unclear, this model/endpoint cannot attempt to improve construct validity, but may provide an additional dimension to interrogate pain-induced gait deficits.

Introduction

Frequent knee pain affects approximately 25% of adults and has become a leading cause of disability, due to its impact on function, mobility, and quality of life[1]. Painful joints result from various causes, but osteoarthritis (OA) is the most common cause of knee pain in people 50 years or older[2]. The assessment of joint pain clinically includes radiographic measures to identify structural abnormalities, as well as patient-rated scales of joint pain and function. However, because the relationship between structural abnormalities and pain is poor, pain and functional impairment dominate the diagnostic scale[3,4]. Indeed, the vast majority of joint pain clinical trials have used the Western Ontario and McMaster Universities Osteoarthritis Index which includes 17 items of physical function, vs five pain items[5].

Critically, these assessments of joint pain and function are subjective, patient-reported evaluations. And while pain perception cannot be standardized against an objective measure and has no easily identifiable physical correlate[6], both gait deficits and the relationship between the perception of functional deficits and measurable gait limitations can be investigated. Significantly reduced walking speed, shortened stride length, prolonged stance time, and decreased range of motion at multiple joints have been observed in joint pain patients[7]. Interestingly, the limited investigation into the degree to which a patient's description of his/her own pain and disability relates to measurable limitations implicates these same gait deficits[8].

* Address correspondence and reprint requests to: K.L. Knopp, Lilly Research Laboratories, Drop Code 0510, Lilly Corporate Center, Indianapolis, IN 46285, USA. Tel: 1-317-277-0210; Fax: 1-317-276-7600.
E-mail address: k.knopp@lilly.com (K.L. Knopp).
URL: https://www.lilly.com

http://dx.doi.org/10.1016/j.joca.2016.05.022
1063-4584/

Assessment of such gait endpoints pre-clinically is more complicated. It is not possible to dissociate changes in function from changes in pain state(s) in pre-clinical studies; these are inextricably linked in animals. Traditional pre-clinical models of pain rely on measures of evoked endpoints after some injury-producing insult. While these models are reasonable assessments of hypersensitivity and offer expediency in identifying the analgesic potential of a particular compound, they do not interrogate ongoing or movement-induced pain, nor its impact on joint function[4,9,10].

The pre-clinical pain field has recently begun supplementing the traditional evoked measures with a number of behavioral endpoints that more closely assess the impact of pain on function, including wheel running[11], weight bearing[12], locomotor activity[13,14], and limitedly, changes in gait performance[15–17]. While gait analysis has existed for several decades, leveraging gait impairment after a presumed painful insult as a reasonable surrogate of pain behavior is a relatively new trend. Systems which quantify gait on a treadmill apparatus have also shown the ability to detect impairment in rodent models of arthritis[15,16,18–25], and more importantly, a number of these studies have shown that observed gait impairments in rodent models of joint pain can be impacted pharmacologically. Thus, the aim of this study was to investigate whether Complete Freund's Adjuvant (CFA)-induced temporal and spatial gait changes in a forced-ambulation rodent gait paradigm were sensitive to pharmacological intervention with analgesics.

Methods

Animals

Female Sprague Dawley (SD) rats (Harlan, Indianapolis, IN, USA), weighing 200–240 g, were pair-housed in plexiglass cages with bedding and enrichment. Animals were maintained in temperature and humidity controlled rooms on a 12/12-h light/dark cycle and allowed *ad libitum* access to food and water until testing. Experimental protocols were reviewed and approved by the Lilly Animal Care and Use Committee. Naïve cohorts were used for each parametric and drug study due to the duration of impairment. Testing occurred between 7 a.m. and 3 p.m.

Intra-articular CFA injection

Rats were anesthetized with 4% isoflurane in oxygen and the rear right knee was shaved and sterilized with 70% ethanol. Injections were performed with a 27-gauge needle fitted with PE10 tubing such that 4 mm of the needle tip was exposed, thereby controlling injection depth. For the concentration response study, CFA (Sigma; 1 mg heat killed mycobacterium per 1 mL adjuvant) was administered between 5 and 50 μg *via* a Hamilton syringe. For the time course study, CFA was diluted in Incomplete Freund's Adjuvant (Sigma) and administered between 5 and 20 μg. For all pharmacology studies, rats received a 20 μg injection in 50 μL.

Drugs

Unless otherwise indicated, drugs were synthesized at Lilly Research Laboratories and dosed based on pharmacokinetic properties. Morphine sulfate (Sigma–Aldrich) was dissolved in saline and dosed subcutaneously (s.c.). Tramadol HCl (Teva Pharmaceuticals) tablets were sonicated in a 1% Hydroxyethylcellulose, 0.25% Tween 80% and 0.05% Dow antifoam (HEC) to form a 50 mg suspension of active drug. Rats were dosed per os (p.o.). Diclofenac sodium salt (Calbiochem) was suspended in the HEC vehicle at 5 mg/ml and administered p.o. Carprofen (Rimadyl™) was suspended in the HEC vehicle at concentrations of 5 and 15 mg/ml and dosed p.o. The EP4 receptor antagonist CJ-023,423[26] was suspended in 10% Acacia between 3.33 and 16.7 mg/ml and dosed p.o. Etanercept (Enbrel™, Immunex) was prepared at 5.5 mg/ml in saline and dosed intraperitoneally (i.p.). The anti-NGF and hIgG4 antibodies were prepared at 1 mg/ml, diluted to 0.1 mg/ml in saline and dosed i.p.

Gait analysis

The GaitScan gait analysis system was used to record and quantify gait features. The system consisted of a clear treadmill (ExerGait XL, Columbus Instruments, OH, USA) fitted with an angled mirror underneath. A high-speed camera (Basler, 100fps) beneath the treadmill belt recorded the ventral view of a moving rat. An opaque green plexiglass box with a semi-transparent viewing window was positioned above the treadmill to house the animal. Video was captured by the BCam software program at 2000 frames per animal for analysis in the GaitScan software. This color-based tracking system allowed maximal tracking of the paw, while excluding other body parts or shadows. Filters were applied to enable the analysis of multiple steps per animal. An *a priori* inclusion criterion of a minimum of four strides per limb was required for all studies.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.joca.2016.05.022.

Prior to CFA administration, rats were habituated to the treadmill for 60 s. The treadmill was slowly increased from 0 to 2 cm/s. After the animal moved forward and away from back wall twice, the speed was slowly increased to 4 cm/s. Infrequently, a cotton swab was inserted into the front of the treadmill to re-orient a rat that was excessively rearing or exploring the chamber, effectively returning the rat to a normal walking pattern. As well, a rounded 'bumper' was placed on the back wall to minimize any negative impact of contact while the rat was learning the forced ambulation paradigm. Once the animal walked consistently, trial speeds were increased in increments of 2–3 cm/s until the animal successfully ran at or near 16 cm/s. The animal was then placed back in the home cage.

For test sessions, speeds were slowly ramped from 0 to the target speed. This range was 8–24 cm/s for parametric studies, 12–16 cm/s for CFA time course and pharmacology studies, depending on the ability of the individual rat. Filters in the analysis software were implemented to exclude instances of pausing, rearing, turning, riding back, or when the rat was touching the rear of the treadmill, thereby decreasing the variability in gait measures and allowing a more accurate representation of locomotion.

Parametric gait studies

The effect of different treadmill speeds on gait parameters was assessed in order to select the speed for pharmacology studies. Animals received a habituation session to the treadmill followed by a test session 24 h later. The optimal concentration of 20 μg of intra-articular CFA was determined after administering a concentration range of 5–50 μg vs saline.

Tactile hypersensitivity

von Frey monofilaments calibrated to incremental bending forces (0.3–15 g) were used to assess tactile hypersensitivity *via* the up-down method[27]. On test days, rats were placed in elevated observation chambers with wire mesh floors and allowed a 20-min acclimation. The tactile hypersensitivity threshold was determined by applying von Frey filaments at the base of the third and fourth digits by a blinded observer.