Sensory and vascular changes in a rat monoarthritis model: prophylactic and therapeutic effects of meloxicam

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Abstract

Objective and design—The objective of this study was to determine the ability of meloxicam prophylaxis and therapy to blunt the effect of complete Freund's adjuvant (CFA) induced monoarthritis.

Materials and methods—First the validity of this animal model was established by examining joint changes at multiple levels after injecting CFA into the tibio-tarsal joint. Next, meloxicam (5 mg/kg) or vehicle was administered on days 0–7 (prophylactic) and on days 7–16 (therapeutic) in separate groups of animals.

Results—The CFA-injected joint demonstrated hallmark histological and structural changes such as pannus formation, bone remodeling, cartilage erosion and immune cell infiltration. Both prophylactic and therapeutic treatment with meloxicam effectively reduced swelling (ankle circumference), oedema and extravasation of Evans blue dye in the affected joint. Moreover, meloxicam reduced loss in range of motion and also reduced mechanical stimulus evoked pain scores. Notably, these effects persisted after discontinuing drug treatment.

Conclusion—The present study provides a unique comparison of prophylactic versus therapeutic effects of meloxicam in the CFA-induced model of monoarthritis.

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Keywords

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Introduction

Inflammatory joint disease affects approximately 1% of the adult population worldwide and is characterized by joint stiffness, deformity and pain. Disease progression in the most common type, rheumatoid arthritis, usually follows a course consisting of inflammation and proliferation of the synovial membrane. Typically, it is pain and physical disability that lead to limitations on quality of life [1–4], additional chronic health problems [5], substantial consumption of health care resources [6] and loss of productivity in the workplace [7]. Disability is primarily associated with pain [8, 9] while a radiological diagnosis of rheumatoid arthritis is not necessarily associated with disability [10, 11]. Significant resources are being applied to limit disease progression [12–16] but until recently there has been little basic scientific investigation focused on understanding the mechanisms underlying the initiation and the maintenance of inflammatory joint pain. The present study focuses on functional deficits and nociceptive scores in a rat model of joint inflammation.

In terms of possible factors underlying functional deficits, inflammatory joint disease is associated with a number of changes in the chemical balance in the joint and joint capsule [17–19], sensory neurons [20–22] and spinal cord [23–25], and with changes in nociceptive mechanisms of primary sensory neurons [26, 27] and spinal cord neurons [25]. Prostaglandins are expressed in animal models of joint inflammation, in both the periphery [17] and spinal cord [23]. Prostaglandins derive from arachidonic acid by the actions of cyclooxygenase enzymes. In addition, cyclooxygenase-2 (COX-2) is upregulated in conditions of peripheral inflammation [28]. COX-2 selective anti-inflammatory agents such as meloxicam have a better safety profile than conventional COX-1 and COX-2 nonselective anti-inflammatory agents. Meloxicam is a potent anti-inflammatory agent that is commonly prescribed to treat arthritic conditions [29, 30]. Moreover, inhibition of COX-2 has beneficial effects on nociceptive scores in animal models of inflammatory joint disease [31–33]. However, most studies have examined only the effects of acute inhibition of COX-2 [32, 33] and longer treatment regimens measured effects only during the early phase of model development and on a restricted number of functional readouts.

The aim of the present study was therefore to measure effects of COX-2 inhibition on specific functional readouts, including range of motion, ankle swelling, joint oedema, plasma extravasation, and mechanical withdrawal threshold in an animal model of monoarthritis. We hypothesized that CFA injection into the tibio-tarsal joint produces a localized inflammatory joint disease that responds to pharmacological intervention in a predictable way.

Materials and methods

Animals

Male Sprague-Dawley rats (275–325 g; Charles-River, Canada) were used for experiments. These rats were housed under controlled conditions with a 12:12 h light:dark cycle with food and water available ad libitum. All experimental procedures were done in accordance with the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care and were approved by the Animal Care Subcommittee of the University Council on Animal Care of the University of Western Ontario.

Model induction

Rats were anesthetized briefly with halothane (Abbott Laboratories, Limited, Montreal, Quebec, Canada). CFA was injected according to the method described by Butler et al. [34]. Briefly, the tarsal area of the right hind paw was grasped and the fossa distal and medial to the lateral malleolus of the fibula was palpated. A 26-gauge needle was introduced into the capsule of the tibio-tarsal joint percutaneously by directing it cephalad, mesiad and superiorly from the midpoint of the inframalleolar fossa, until a distinct loss of resistance was felt. A 25 µl volume of CFA (complete Freund's adjuvant) or the same volume of sterile saline (control for CFA) was injected into the joint. CFA was prepared by adding 60 mg of *Mycobacterium butyricum* (Difco Laboratories, Detroit, Michigan, USA) to a mixture of paraffin oil (6 ml), NaCl 0.9% (4 ml) and Tween 80 (1 ml), mixed thoroughly and then autoclaved for 20 min at 120°C to rupture the cell walls of the mycobacterium.

Characterization of the model

The effects of CFA were observed in 14 rats injected with CFA and were compared to 10 rats injected with saline. Parameters selected for characterizing the model included pre-induction and post-induction measurements of range of joint movement, ankle circumference and mechanical withdrawal threshold. The inflammation induced by CFA injection into the tibio-tarsal joint remained localized to the injected joint in 11 rats while three rats developed signs of polyarthritis and were therefore excluded from the study.

Histology

For histological analysis, rats were injected with CFA ($n = 4$). After 30 days, a lethal dose of sodium pentobarbital (50 mg/kg; Abbott Laboratories, Limited) was administered and the injected rat joints were removed. These joints were immediately skinned and fixed in 10% formalin (5–7 days) and then decalcified in 10% ethylene-diamino-tetraacetic-acid solution (5 days). The tissues were then dehydrated with alcohol and embedded in paraffin wax. Subsequently, the tissue blocks were cut in a longitudinal orientation to observe dorsoventral joint views. Next, 7 µm-thick sections were cut on a rotary microtome and mounted on glass slides, the paraffin was removed and the sections were stained with hematoxylin and eosin. An observer blinded to the treatment type assessed a minimum of 3–5 sections/joint qualitatively. Joints obtained from weight matched untreated animals were used as controls.

Micro-computed tomography

Animals were anaesthetized with sodium pentobarbital (50 mg/kg; Abbott Laboratories, Limited) and were placed in a GE Medical System, RSQ scanner. Two hundred views were acquired with X-ray strength 80 kVp and a current intensity of 450 mA. These views were then reconstructed, using an eVs beam, to a three dimensional image with approximate resolution of 46 μ m. Images were obtained from three monoarthritic rats on day 30 after induction and from weight matched untreated animals.

An observer, who was unaware of the treatment given to each rat assessed bone damage. The evaluated variables include: destruction of bone architecture, joint damage (observed as disruption of the alignment and configuration of tarsal bones) and changes in joint space due to loss of cartilage. In addition, osteoporosis was measured as decreased density of the bone, recognized as increased radiolucency relative to uninvolved adjacent bone and thinning of the cortex, particularly in the juxta-articular regions.

Range of joint motion

To determine the range of flexion to extension of the ipsilateral ankle joint the axis of the tibia was kept stationary by placing it along the *x*-axis of a protractor so that the heel of the rat paw coincided with the intersection of the *x* and *y* axes. The tarsal bones were held at their axis and the paw of the rat was dorsi-flexed and planti-flexed up to the maximum permissible range of motion. An averaged difference between the angle of maximum dorsi-flexion and the angle of maximum planti-flexion for three readings was expressed as range of motion for 11 CFA injected rats and 10 saline injected rats.

Ankle circumference

The animals were acclimatized to handling and were subsequently wrapped in a cloth. Circumference of the injected ankle was measured by wrapping it with a piece of thread, and expressed as the mean of three such measurements for 11 CFA injected rats and 10 saline injected rats.

Plasma extravasation and oedema

Plasma extravasation and oedema were measured on the seventh day after model induction in a group of CFA injected rats ($n = 5$), saline injected rats ($n = 5$) and naïve untreated rats ($n = 3$). Animals were anesthetized with sodium pentobarbital (Abbott Laboratories, Limited, Montreal, Quebec, Canada) at a dose of 50 mg/kg. Evans Blue dye (Sigma-Aldrich, Mississauga, ON, Canada) was dissolved at a concentration of 25 mg/ml in physiological saline and injected as 0.1 mg/100 g through the tail vein. Thirty minutes following Evans blue injection animals were perfused intracardially with 500 ml of 0.9% NaCl. The CFA-injected and contralateral hind paws were removed, 15 mm above the ankles. These were immediately skinned and weighed to obtain the wet weight of the remaining tissues. The joints were dried in an oven at 60°C for 24 h and then the joints were re-weighed. The difference between wet and dry weights was expressed as oedema.

To assess plasma extravasation, the dried joint was soaked in 10 ml of formamide overnight in an oven at 60°C to extract the Evans blue. Subsequently, the fluid in the vials was filtered

and evaluated by its absorbance measured by color spectrophotometry and compared to pure form-amide at wavelength 620 nm. The ratio of ipsilateral to contralateral optical densities was calculated as follows:

$$\text{Optical density} = \frac{[\text{Ipsilateral absorbance/ipsilateral weight}]}{[\text{Contralateral absorbance/contralateral weight}]}$$

Mechanical withdrawal threshold

Mechanical withdrawal threshold was determined for 11 CFA injected rats and 10 saline injected rats using a series of von Frey filaments (Stoelting, Chicago, IL, USA). Each filament had a “bending force” at a specified weight. The rat was placed on a customized platform [35] in a transparent Plexiglas® box (30 × 30 × 30 cm) and allowed 20 min to acclimatize. Each filament was then applied five times to the plantar surface of the rat ankle from beneath for about 1 s at 5 s intervals. A particular filament was applied until bending of the filament occurred. Response threshold to mechanical stimulation was defined as the lowest force that evoked a withdrawal at least three out of five times of application starting with the filament corresponding to 4.31 g. If this filament failed to elicit a response, a filament exerting greater force was applied, and if a response was observed, a filament of lesser force was used next. The bending force of each filament was regularly calibrated.

Administration of a COX-2 inhibitor

Meloxicam (Sigma-Aldrich, Mississauga, ON, Canada), was prepared in 0.5% methylcellulose and administered in a dose of 5 mg/kg intraperitoneally. In the first experiment, meloxicam ($n = 12$) or vehicle (0.5% methylcellulose; $n = 12$) was administered 20 min before injecting CFA. Subsequently the drug was administered daily, until the seventh day after model induction. In the second experiment the same dose of meloxicam ($n = 12$) or vehicle (0.5% methylcellulose; $n = 12$) was administered daily from day 7 until day 16 after model induction.

For both groups, ankle circumference, range of joint motion, and mechanical withdrawal threshold were measured before model induction and on selected days post induction. On the last day of drug administration, eight rats from each group were tested for plasma extravasation. Observations were continued on the remaining animals ($n = 4$ per group) for 3–4 weeks.

Statistical analysis

Statistical analysis was done using one way ANOVA with repeated measures along with Tukey’s test for post-hoc comparisons. Differences with p value <0.05 were considered significantly different.

Results

Histology

Compared to untreated control rats (Fig. 1a), the inflammatory process in CFA-injected rats predominantly affected synovial membranes of CFA-injected joints ($n = 4$) that were inflamed and proliferating (Fig. 1b). The tissues showed an active inflammatory and repair process macrophages, neutrophils (Fig. 1c) and with activated myofibroblasts with deeply stained elongated nuclei (Fig. 1c). These observations have confirmed what others have reported in animal models of monoarthritis and are also consistent with the human disease condition [32, 36]. However, in addition it was observed that the scar tissue extended into the synovial cavity in the form of pannus tissue, the base of which encroached upon the underlying cartilage and bone (Fig. 1d). CFA-injected joints also frequently exhibited osteophytes and proliferating chondrocytes when observed at low and at higher magnification (Fig. 1e, f), which indicated remodeling of bone. Moreover, the synovial layer showed ulceration and loss of integrity resulting in exudation of fibrin on the surface of the synovial membrane (Fig. 1g).

Micro-computed tomography

Micro-computed tomography imaging scans demonstrated that in contrast to the untreated joint (Fig. 2a, c), the CFA-injected joints (Fig. 2b, d) had a distinctly irregular surface with deformities and erosions on the CFA injected joint. Osteophytes were seen distinctly at the margin of the distal tibia and extending from the surfaces of cuboid and talus bones (Fig. 2b, d). The cross-sectional view along the sagittal plane also demonstrated osteoporosis, distortion in joint space alignment and loss of definition of the cortical surfaces of the bones of CFA-injected rats only (Fig. 2d). Images were obtained from three monoarthritic rats on day 30 after induction and from weight matched untreated animals.

Effects of meloxicam on range of motion of the joint

The CFA-injected joints of rats ($n = 11$) demonstrated a significant reduction in the range of motion ($p < 0.001$) when compared to these in saline-injected rats ($n = 10$; Fig. 3a). Moreover, when compared to pre-induction values, the range of motion of the monoarthritic joints was significantly reduced starting on day 2 ($p = 0.001$; $120^\circ \pm 4.0^\circ$) after model induction. In addition, a longitudinal assessment revealed a progressive decrease in range of motion up to day 8 after which there was no further reduction on subsequent measurements. Hence, on the 20th day after model induction, the range of motion of the inflamed ankle in the CFA-injected rats $82^\circ \pm 3.0^\circ$ was significantly lower ($p < 0.001$) than the range of motion in saline-injected rats ($140^\circ \pm 7.0^\circ$; Fig. 3a). On the other hand, saline-injected rats did not show significant alteration from baseline on any day after injection. Moreover, there was no change observed in the range of motion of the contralateral joint of CFA-injected animals at any time.

Despite prophylactic treatment with meloxicam ($n = 12$) or vehicle ($n = 12$) on days 0–7, the range of joint movement decreased in both groups for up to 3 days after CFA induction ($p < 0.05$ compared to values before model induction; Fig. 3b). However, on day 5 and onwards meloxicam-treated rats did not show any further reduction in the range of motion when

compared to day 3. On the other hand, vehicle-treated rats showed a significant ($p < 0.001$) reduction ($82^\circ \pm 2.6^\circ$) compared to range of motion measured in the same rats on day 3 ($109^\circ \pm 1.5^\circ$). Hence, the meloxicam-treated rats showed significantly greater range of motion when compared to vehicle-treated rats ($p < 0.05$) assessed on day 5 and this effect persisted until the last day of drug administration (Fig. 3b). Moreover, when drug administration was discontinued the difference between the meloxicam and vehicle-treated rats continued to be significant ($p < 0.01$; $n = 4$; see Fig. 3b). Furthermore, on the last day of observation (day 22), the range of motion of meloxicam-treated rats remained significantly less compared to pre-induction ankle circumference ($p < 0.05$; $n = 4$).

Treating the monoarthritic rats therapeutically with meloxicam on days 7–16 partially reversed the established decrease in range of motion (Fig. 3c). There was a significant reversal in comparison with vehicle treatment. Hence, after the first day of treatment with meloxicam and on the subsequent day of observation, the range of motion ($101^\circ \pm 2.4^\circ$, $n = 12$) was significantly greater ($p < 0.001$) compared to the vehicle treated group (Fig. 3c). Importantly, even after drug treatment was discontinued the range of motion of meloxicam-treated rats was greater than vehicle-treated rats, and this effect lasted for a period up to 3 weeks ($p < 0.01$; $n = 4$). Nevertheless, on day 44, the range of motion was still significantly less ($p < 0.05$) compared to the pre-induction range of motion values (Fig. 3c).

Effects of meloxicam on ankle circumference

Besides structural changes in the joint and loss of range of motion, inflammatory joint disease is also characterized by joint swelling and vascular changes. Therefore meloxicam was tested on ankle circumference and oedema of the joint. By the first day after CFA injection the circumference of the CFA-injected ankle had increased significantly from the pre-induction circumference of 28 ± 1.0 mm (Fig. 4a) to 42 ± 0.6 mm ($p < 0.001$; $n = 11$). This increase persisted and the monoarthritic ankle circumference was increased significantly from baseline throughout the period of observation ($p < 0.01$). In control rats, a transient increase in the circumference of the saline-injected ankle (32 ± 1 mm, $n = 10$) was seen for the first week ($p = 0.043$), after which mean circumference returned to baseline values (Fig. 4a). The ankle circumference of CFA-injected rats was significantly greater than that of saline-injected ankles at all time points ($p = 0.001$). The CFA-injected animals showed no change in ankle circumference in the contralateral joint throughout the study and remained at 29 ± 0.4 mm ($n = 11$; data not shown).

Despite of the prophylactic meloxicam treatment, the circumference of the CFA-injected ankle increased significantly on day 1 when compared to pre-induction ankle circumference (see Fig. 4b). On the other hand, ankle circumference of meloxicam (35 ± 0.5 mm; $n = 12$)-treated rats was significantly less ($p < 0.001$) than vehicle-treated rats (41 ± 0.8 mm; Fig. 4c; $n = 12$) on day 1.

Furthermore, the difference between the two treatment groups (meloxicam and vehicle) was significant on all days of treatment and, notably, remained significant ($p < 0.01$) even after drug treatment was discontinued ($n = 4$). Compared to pre-induction values, the ankle circumference of meloxicam-treated rats was significantly greater ($p < 0.05$) on the last day of observation (i.e. day 22; Fig. 4b).

In the group given therapeutic meloxicam treatment on days 7–16 (Fig. 4c) the ankle circumference on the first day of drug treatment (37 ± 0.5 mm, $n = 12$) was significantly less ($p < 0.01$) compared to vehicle-treated rats (41 ± 1 mm; $n = 12$). This difference was observed on all subsequent days of drug treatment. Moreover, even after discontinuing therapeutic treatment a significant difference between the meloxicam-treated ($n = 4$) and vehicle-treated ($n = 4$) groups was observed ($p < 0.05$; Fig. 4c). Compared to pre-induction ankle circumference, on day 44 the ankle circumference of meloxicam-treated was significantly greater ($p < 0.05$).

Effects of meloxicam on plasma extravasation and oedema

Plasma extravasation and oedema were studied in some rats; due to the terminal nature of these measurements, they were taken in three untreated control rats, five saline-injected rats and five CFA-injected rats. The CFA-injected joint showed higher values of plasma extravasation and oedema compared to the contralateral joint (Fig. 5a, b). The plasma extravasation and oedema values were not significantly different between untreated and saline groups. The CFA-injected group, however, showed a significantly higher level of plasma extravasation (3.41 ± 0.623 absorbance/g, $p < 0.05$, Fig. 5a) and oedema (3.06 ± 0.544 g, $p < 0.05$, Fig. 5b) when compared to saline and untreated groups.

Some rats (eight from each group) that had received meloxicam or vehicle treatment were sacrificed for assessing plasma extravasation and oedema approximately on the last day of treatment. (Note: In some rats the procedure could not be completed successfully and the number of rats in each group are stated with the results.) As shown in Fig. 5c, the rats that received prophylactic treatment on days 0–7 with vehicle (3.4 ± 0.68 absorbance/g; $p < 0.01$; $n = 5$) or with meloxicam (3.2 ± 0.42 absorbance/g; $p = 0.002$; $n = 7$) showed higher levels of plasma extravasation compared to untreated naïve rats (0.82 ± 0.07 absorbance/g; $n = 5$). Moreover, there was no difference in plasma extravasation between meloxicam-treated and vehicle-treated CFA-injected rats ($p = 0.94$; Fig. 5c).

In rats given prophylactic administration of meloxicam on days 0–7, oedema was significantly less (2.0 ± 0.02 g; $p < 0.001$; $n = 5$) when compared to oedema in rats treated with vehicle (3.19 ± 0.18 g) and was not significantly different from oedema values obtained in untreated rats (1.4 ± 0.12 g; $p > 0.05$; Fig. 5e).

In the group of animals treated therapeutically with meloxicam on days 7–16 (Fig. 5d), the plasma extravasation was significantly less (1.9 ± 0.20 absorbance/g; $p = 0.035$; $n = 7$) compared to values obtained from the vehicle-treated group (3.7 ± 0.41 absorbance/g, $n = 6$, $p < 0.01$) and was significantly greater than the untreated group (0.7 ± 0.18 absorbance/g, $n = 5$, $p < 0.05$; Fig. 5d).

When meloxicam was given therapeutically on days 7–16 it reduced oedema (Fig. 5f) in monoarthritic rats (1.66 ± 0.3 g, $n = 7$) compared to vehicle-treated monoarthritic rats (2.8 ± 0.34 g, $n = 6$, $p < 0.01$). The reduction in oedema was such that the values obtained from meloxicam-treated rats were not significantly different from naïve untreated rats (1.09 ± 0.10 g, $n = 5$).

Effects of meloxicam on mechanical withdrawal threshold

The saline-injected ankle did not display any change in threshold to mechanical stimulation throughout the 22-day test period ($n = 10$ rats; see Fig. 6a). Conversely, starting day 4 after CFA induction, the monoarthritic joint had significantly lower ($p < 0.001$; Fig. 6a) mechanical withdrawal thresholds (5.5 ± 1.20 g, $n = 11$) compared to thresholds of saline-injected rats ($p < 0.001$) and also compared to pre-induction baseline values (13.5 ± 0.10 g ($p < 0.05$)). The thresholds to mechanical stimulation dropped significantly ($p = 0.01$) on day 5 compared to day 4 after model induction. However, there was no further decrease on thresholds when observed on the following days ($p < 0.05$ compared to controls).

There was no change in withdrawal threshold for 3 days after CFA induction in rats treated with meloxicam on days 0–7 or in vehicle-treated rats (Fig. 6b). However, on day 5 the vehicle-treated rats demonstrated a significant decrease in threshold and there was a significant difference in mechanical threshold between vehicle-treated and meloxicam-treated rats. Moreover, as shown in Fig. 6b, the mechanical thresholds of meloxicam-treated rats remained at pre-induction levels and significantly higher ($p = 0.001$) than vehicle-treated rats throughout the period of drug treatment. This effect persisted after discontinuing drug treatment up to day 11. However, on day 15 and 22 (see Fig. 6b) the withdrawal threshold of the meloxicam-treated group decreased significantly. Furthermore, on day 22 the withdrawal thresholds were not significantly different between meloxicam- and vehicle-treated rats. In addition, the withdrawal thresholds of both groups on day 22 were significantly less than pre-induction levels.

In rats receiving meloxicam treatment therapeutically from days 7–16 after model induction, mechanical pain thresholds in rats treated with meloxicam (Fig. 6c; 8.9 ± 1.60 g, $n = 12$) were reversed to pre-induction values after starting treatment. Hence, mechanical pain thresholds were significantly higher ($n = 12$, $p < 0.001$) in meloxicam-treated rats compared to vehicle-treated rats on all days of drug treatment and also after drug treatment was discontinued (Fig. 6c).

Discussion

In this study, the inflammatory changes along with sensory and functional deterioration in the CFA-injected ankle joint indicate that CFA-induced monoarthritis demonstrates a symptomatic profile that resembles human joint disease, as has been reported earlier [34, 37, 38]. However, a relevant model of inflammatory joint disease must demonstrate pertinent similarities in its characteristics to the disease and at the same time the model needs to be modifiable in a practical fashion in response to rationalized intervention. Accordingly, prophylactic or therapeutic treatment with a commonly used NSAID, which shows efficacy in reducing the pain of inflammatory joint disease, produced predictable changes in this monoarthritis model. Meloxicam administration reduced symptoms when given as a prophylactic or therapeutic, thus during the induction phase and also during the maintenance phase of the monoarthritis model. This study promotes the utility of the CFA joint monoarthritis model as an effective research tool for testing new drug therapies for chronic inflammatory joint diseases.

Classically, animal models for investigating pain mechanisms of inflammatory joint disease and analgesic treatments are produced with complete Freund's adjuvant (CFA). CFA induces a chronic inflammatory process which is attributed to a T cell mediated immunological reaction [39–42]. The classical versions of the CFA model are produced by injecting CFA systemically; these procedures causes arthritis-like inflammation of all joints, or a polyarthritis [43]. However, the severity and widespread nature of the inflammatory response caused by systemically induced CFA polyarthritis is widespread and multifactorial, and imposes a range of challenges to the general physiological stability of the animal. Notably, CFA-induced polyarthritis not only accompanies febrile responses and severe weight loss, but it also induces aberrant changes in numerous body systems including the blood brain barrier [34, 44–47]. These disadvantages have resulted in a trend towards using less severe models [34, 48, 49]. Among such models, the unilateral plantar injection model is commonly used, but this model does not demonstrate long term sensory changes, and the inflammatory process spreads to other body regions within a few days, resolving to a model of systemic polyarthritis [34, 48]. Alternatively, injecting CFA directly into an intra-articular joint space has been reported to produce an inflammatory process that remains restricted to the injected ankle and persists for a period of 6 weeks or more [34, 50].

The model of monoarthritis used in this study presented physical changes, which were similar to a stable chronic inflammatory human joint disease. For instance, whereas the circumference of the joint injected with CFA increased as early as day 1, the vascular changes associated with inflammation were observed as increases in joint swelling, oedema and plasma extravasation and the inflammatory changes persisted through the 4 weeks of the present study. Similarly, the deterioration in joint range of motion and tactile hypersensitivity, analogous to tactile allodynia in humans, were observed within days of disease development, and persisted for the duration of the study. It is notable that early development of inflammatory joint disease in the model allows investigation of mechanisms involved in disease induction and hence may provide information for identifying early biomarkers of disease. Other models such as CFA-induced polyarthritis [44, 45, 51] and collagen-induced arthritis [52] models are known to require more than 2 weeks for development and are less predictable for effective experimental designs due to remissions during the course of the disease.

In addition to early development, the monoarthritic condition is stable over a prolonged period of time (i.e. up to 5 weeks) and thus provides avenues to investigate mechanisms of disease maintenance and also provides a window for studying a chronic inflammatory joint disease. Thus, the value of this CFA-induced arthritis model lies prominently in the stable and chronic disease process, with specific functional readouts.

Histological examination confirmed proliferation of synovial cells and infiltration of immune cells along with an ongoing inflammatory process in the articular and periarticular tissues, as reported by others [53]. However, in addition to what has been reported earlier, remarkably the model demonstrated formation of a pannus, a hyperplastic intimal synovial lining that invades cartilage and bone structures, with accompanying cartilage damage, an important feature associated with inflammatory joint disease such as rheumatoid arthritis. Furthermore, bone remodeling and joint damage accompanied by development of

osteophytes, as reported by Vermeirsch [37], were revealed using micro-computed tomography indicating similarities to radiographic findings of inflammatory joint disease. This novel technique offers an avenue for longitudinal observation of bone remodeling for studying disease mechanisms as well as for testing drug therapies [54, 55] on bone surfaces in addition to 3-dimensional sagittal sections.

In addition, the circumscribed nature of the disease in the joint monoarthritis model is to some extent viable from a clinical perspective. For instance, the monoarthritis model shows similarities to the monoarticular pattern in which a single joint is affected by the disease reported in a surprising 21% of 300 rheumatoid arthritis patients [56–58]. In addition, the time-course observed in the monoarthritis model is characteristic of a single cycle of disease that is long-standing with an acute and severe onset [59].

While the model behaved predictably to a commonly prescribed drug, meloxicam, this treatment failed to completely inhibit or reverse the disease, thus supporting the common clinical view that COX-2 inhibitors address mainly symptoms of the disease and are unable to cure the disease completely [60] [51]. This provides further support for future studies using this model to develop disease modifying interventions.

It was interesting to note that there was no loss of the meloxicam-mediated effects 2 weeks after discontinuation of the drug. This finding indicates that a short term treatment with meloxicam had a long lasting effect on signs of pain and inflammation, but to evoke parsimony it can be hypothesized that the long term effects may have been due to the accumulation of meloxicam during the five daily treatments with 5 mg/kg, considering the ID₅₀ for reducing joint swelling and inhibiting prostaglandin E₂ (PGE₂) (0.2–0.3 mg/kg/day) [61]. Although there is no specific evidence to support our speculation, it is however plausible that the plasma concentration of meloxicam in rats 2 weeks after discontinuation of treatment was sufficient to mediate the continued anti-inflammatory and analgesic effects.

Effects of pharmacological interventions on changes in the joint

An important earlier paper by Laird et al. [31] reported that in the same model as used in the present study daily administration of meloxicam i.p. reduced post-mortem analysis of ankle circumference and reduced the number of animals showing resistance to joint flexion and extension. Kumar et al. [33] reported that another COX-2 inhibitor, rofecoxib, reduced nociceptive scores in the dorsal flexion pain test and the compression test; Wilson et al. [38] also reported that rofecoxib and etoricoxib reduced changes in joint diameter and weight bearing in this CFA model during days 13–17. Increases in paw volume and ankle diameter have also been reported to be reduced by systemic administration of a selective prostaglandin E receptor 4 agonist given 24 h and 4 weeks after model induction [62].

In addition to the report by Laird et al. [31] that meloxicam reduced changes in stance as well as vocalization in response to successive flexions of the joint, other studies have also reported beneficial effects of pharmacological interventions. For example, Omote et al. [62] observed that intrathecal administration of a selective prostaglandin E receptor 4 agonist at 24 h and at 4 weeks reduced the decrease in withdrawal latency and threshold in the CFA-induced model of monoarthritis.

To our knowledge, this is the first study to measure changes in the range of joint movement in an animal model of monoarthritis, and it was found that there was a slow but progressive loss of the range of motion. Yet, movement of the joint has been reported to change sensory properties in this model. For example, we have reported that repetitive flexion and extension of the CFA-injected joint in the anaesthetized rat increases excitation of dorsal horn neurons to iontophoretic application of the glutamate receptor agonists, NMDA and AMPA [25].

In conclusion, the present study provides a unique comparison of prophylactic versus therapeutic effects of meloxicam in the CFA-induced model of monoarthritis. It would be interesting to see if the effects observed in this study are specific for meloxicam or if they are a class effect of all these drugs. Nevertheless, the data suggest by extrapolation, that COX-2 inhibition may be an effective therapeutic approach for symptoms of inflammatory joint disease from the earliest onset through to more advanced stages.

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Abbreviations

AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	Analysis of variance
CFA	Complete Freund's adjuvant
COX-2	Cyclooxygenase-2
CX3CR1	Chemokine receptor
ERK	Extracellular signal-regulated kinase
ID50	Infectious dose to 50 percent of exposed individuals
i.p	Intraperitoneal
NMDA	<i>N</i> -methyl-D-aspartate
NSAID	Non-steroidal anti-inflammatory drug
PGE₂	Prostaglandin E ₂

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Fig. 1.

Photomicrographs of H&E stained sagittal sections of the tibio-tarsal joint of untreated (**a**) and CFA-injected (**b**) rats. Each *scale bar* represents 100 μm . **a** Normal tibio-tarsal joint demonstrating the articulation of the distal tibia and the proximal tarsus with the synovial membrane (at *arrow 1*). **b** Synovial membrane inflamed and hyperplastic (at *arrow 1*). **c** Inflammatory cells observed in the sub synovial region showing infiltration with lymphocytes, macrophages, neutrophils and myofibroblasts indicated by *arrows 1–4* respectively. **d** Pannus extending into the joint space and infringing on articular cartilage (at *arrows 1 & 2* respectively). **e** Osteophyte forming in the tarsal bone (at *arrow 1*). **f** Osteophyte presented in **e** at a higher magnification. **g** Fibrin exudation from the synovial membrane indicating erosion of synovium

Fig. 2.

Three-dimensional reconstructions of micro-computed tomographic images obtained from the tibio-tarsal joint of control rats (**a, c**) and CFA-injected rats (**b, d**), obtained with a micro-CT scanner. CFA-induced abnormal changes in bone structure, bone remodeling with osteophytes formed extensively in CFA-treated animals. *Arrows in d* illustrate the following: *1* osteoporosis observed as increased lucency in the cortices of the bones, *2* reduction in joint space associated with loss of cartilage and changes in bone alignment, *3* osteophyte or new bone forming on the bone surfaces

Fig. 3.

Effects of meloxicam on range of motion of the CFA injected joint. **a** Effects of CFA or saline on ankle range of motion. **b, c** Effects of Meloxicam (*filled square*) or vehicle (0.5% methylcellulose; *filled diamond*) administration on days 0–7 (**b**), or on days 7–16 (**c**) on range of motion in the CFA injected joints. *Black horizontal bars* indicate the days of drug administration. Measurements from four rats from each group were obtained on given days after drug treatment was discontinued. The data shown are the mean $\pm$ SEM. * $p < 0.05$ for comparisons between CFA injected rats with untreated rats or saline-treated rats (significant at all time points). * $p < 0.05$ significant for comparisons of CFA treated rats between meloxicam and vehicle treatment. † $p < 0.05$ for comparisons with pre-induction (day-0) values

Fig. 4.

Effects of meloxicam on circumference of CFA injected joint. **a** Effects of CFA or saline on ankle circumference. **b, c** Effects of meloxicam (*filled square*) or vehicle (0.5% methylcellulose; *filled diamond*) administration on days 0–7 (**b**), or on days 7–16 (**c**) on circumference of the CFA injected joints. *Black horizontal bars* indicate the days of drug administration. Measurements from four rats from each group were obtained on given days after drug treatment was discontinued. The data shown are the $\pm$ SEM of means. $*p < 0.05$ for comparisons between CFA injected rats with untreated rats or saline-treated rats and also for comparisons of CFA treated rats between meloxicam and vehicle treatment. $^{\dagger}p < 0.05$ for comparisons with pre-induction (day-0) values

Fig. 5.

Effects of CFA or saline injections in the ankle on plasma extravasation and oedema (**a, b**). Effects of meloxicam or vehicle (0.5% methylcellulose) administration on plasma extravasation (**c, d**) and oedema (**e, f**) for treatments given on days 0–7 or 7–16 in CFA injected joints. *Black horizontal bars* indicate the days of drug administration. The data shown are the $\pm$ SEM of means. * $p < 0.05$ for comparisons between CFA injected rats with untreated rats or saline-treated rats, and also for comparisons of CFA-treated rats between meloxicam and vehicle treatment. $^{\dagger}(p < 0.05)$ for comparisons with values from naïve rats

Fig. 6.

Effects of meloxicam on mechanical withdrawal thresholds of CFA-injected joint. **a** Effects of CFA or saline on mechanical withdrawal thresholds. **b, c** Effects of meloxicam (*filled square*) or vehicle (0.5% methylcellulose; *filled diamond*) administration on days 0–7 (**b**), or on days 7–16 (**c**) on mechanical withdrawal thresholds of CFA-injected joints. *Black horizontal bars* indicate the days of drug administration. Measurements from four rats from each group were obtained on given days after drug treatment was discontinued. The data shown are the $\pm$ SEM of means. * $p < 0.05$ for comparisons between CFA-injected rats with untreated rats or saline-treated rats and also for comparisons of CFA-treated rats between meloxicam and vehicle treatment. †($p < 0.05$) for comparisons with pre-induction (day-0) values