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Gait analysis and pain response of two rodent models of osteoarthritis

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ABSTRACT

The purpose of this study was to compare the gait parameters recorded on the CatWalk and the mechanical sensitivity with von Frey filaments of two putative models of osteoarthritis over a one month period, and to evaluate the effect of celecoxib on these parameters. Animals underwent either a surgical sectioning of the anterior cruciate ligament with partial medial menisectomy (ACLT + pMMx) to create a joint instability model or received an intra-articular injection of monoiodoacetate (MIA) as a putative inflammatory joint pain model. Animals were assessed for four consecutive weeks and knee joints were then evaluated histologically. Spinal cord lumbar enlargements were harvested for selected neuropeptide analysis (substance P (SP) and calcitonin gene related peptide (CGRP)). With the MIA model, significant changes persisted in selected dynamic gait parameters throughout the study in the injured limb as well as with the von Frey filaments. The ACLT + pMMx model in contrast showed no clear differential response between both hind limb for both gait parameters and pain-related behavior with von Frey filaments occurred only on the last day of the study. Neuropeptide analysis of spinal cord lumbar enlargements revealed a significant increase in CGRP concentration in both models and an increase in SP concentration only in the MIA model. Histological evaluation confirmed the presence of articular cartilage lesions in both models, but they were much more severe in the MIA model. Celecoxib had an effect on all selected gait parameters at the very beginning of the study and had an important alleviating effect on mechanical allodynia. These results suggest that the MIA model may be more appropriate for the evaluation of short term pain studies and that celecoxib may modulate mechanical allodynia through central sensitization mechanisms.

1. Introduction

Knee osteoarthritis (OA) is a leading cause of joint pain due to cartilage degeneration and subchondral bone remodeling. These pathological events result in peripheral and central sensitization of pain pathways leading to impaired function and a diminished quality of life (Dieppe and Lohmander, 2005; Prochazkova et al., 2008). In patients, increased knee joint nociception generates a gait adaptation to minimize pain sensation by lowering biomechanical articular stress in the painful limb. However, the exact molecular events and pathways of joint pain remain incompletely understood. Gait disturbance is easily identified as a breakdown in performance and gait analysis provides insights into the underlying OA progression. Many methods have been employed to identify kinetics and kinematics of locomotor behavior in rodents (Coulthard et al., 2003; Gorska et al., 2007; Simjee et al., 2007; Vincelette et al., 2007; Johnson et al., 2008). The CatWalk, an automated quantitative gait analysis method (Hamers et al., 2001), has been employed for investigations of disturbances in locomotion in small animals. Most studies using this method have assessed rats with spinal cord (Kloos et al., 2005; Koopmans et al., 2005; Hamers et al., 2006; Couto et al., 2008) or sciatic nerve injuries (Deumens et al., 2007; Bozkurt et al., 2008). Moreover, inflammatory pain models in rats have provided detailed information of pain-induced gait adaptations with the use of the CatWalk method (Ängeby-Möller et al., 2008; Gabriel et al., 2007; Ferreira-Gomes et al., 2008).

Several animal models mimicking human OA have been developed to enhance our understanding of this condition. Animal models allow the study of structural changes and the progression of the pathology (Brandt, 2002) but also the biomechanical consequences resulting from pain associated with OA. The assessment of osteoarthritic pain and the understanding of underlying mechanisms could eventually lead to novel therapeutic strategies. It is presently difficult to identify the ideal model for the study of OA nociceptive responses. Consequently, we chose to assess two rodent OA models used in preclinical studies to evaluate gait parameters and pain-related behavior. The surgical sectioning of the anterior cruciate ligament with partial medial menisectomy (ACLT + pMMx) and the intra-articular injection of monoiodoacetate (MIA) models were evaluated. The ACLT + pMMx model has shown similarities to human OA (Hayami et al., 2006). It is a model based on surgically altered joint

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mechanics creating joint instability that modifies the magnitude and distribution of joint forces applied to the cartilage surface resulting in progressive joint degeneration (Setton et al., 1999). To our knowledge, no studies have reported gait analysis in this model. On the other hand, intra-articular injection of MIA causes the inhibition of chondrocyte glycolysis, which is mandatory to maintain the structural and functional properties of the articular cartilage (Kalbhen, 1987; Van der Kraan et al., 1989). Intra-articular injection of MIA in the rat knee joint induces pathology with similarities to OA (Bove et al., 2003; Fernihough et al., 2004; Ivanavicius et al., 2007; Piscaer et al., 2008). Although its use has been linked with the evaluation of pain behavior in rats (Vermeirsch et al., 2007; Bove et al., 2003; Fernihough et al., 2004; Pomonis et al., 2005; Ferreira-Gomes et al., 2008) a complete gait analysis of this model has not been reported.

Since the aim of the present study was to assess gait and pain-related behavior in two rodent OA models, an evaluation of both models was performed over a one month period to investigate their use to rapidly evaluate therapeutic drugs in future studies. To do so, the CatWalk system was employed in parallel to pain assessment with von Frey filaments, evaluating secondary mechanical allodynia. We hypothesized that changes in the gait pattern and in the pain-related behavioral response will be observed in both OA models, with an amplified nociceptive response in the ipsilateral affected limb leading to an asymmetry in both pain sensation and gait. To validate our findings, celecoxib a selective COX-2 inhibitor, was administered since it has been shown to relieve pain associated with OA (Stengaard-Pedersen et al., 2004; Tannenbaum et al., 2004; Pomonis et al., 2005).

Central sensitization with peripheral tissue damage leads to biochemical spinal cord changes seen in receptors, neurotransmitters and neuromodulators. Pain-related neuropeptides are localized in spinal cord central terminals of afferent nerve fibers and their release modulates the nociceptive response (Schaible and Richter, 2004). Following behavioral evaluations, selected neuropeptides (substance P (SP) and calcitonin gene-related peptide (CGRP)) known to participate in OA (Ahmed et al., 1995) were quantified in the lumbar spinal cord of ACLT + pMMx and MIA animals. We hypothesized that the spinal nociceptive neuropeptides content should be increased with OA pain.

2. Experimental procedures

2.1. Animals

Forty male Sprague–Dawley rats (Charles River, Canada) weighing 180–225 g at the beginning of the study, were used for these experiments. They were acclimatized in a standard laboratory animal environment (fresh filtered air, 15 changes/h; temperature, 21 ± 3 °C; humidity, 40% to 60%; and light–dark cycle, 12:12-h). Animals were housed in polycarbonate cages (Ancare, Bellmore, NY, U.S.A) on hardwood chip bedding (Beta chip, Northeastern Products Co., Warrenburg, NY, USA) and acclimated for 5 days prior to the initiation of the study. Rats received tap water and a standard laboratory rodent diet (Charles River Rodent chow 5075, St-Constant, QC, Canada) *ad libitum* until the start of the experiments. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine of the University of Montreal prior to animal use and is in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Experimental design

To determine if both models could induce significant gait and pain-related behavioral changes evaluated with the CatWalk and the von Frey filaments, animals were divided randomly into 6 specific experimental groups; surgical model ($n=8$), intra-articular injection of MIA model ($n=8$), sham operated group ($n=3$), intra-articular injection of saline group ($n=3$) and a negative control group i.e. animals without any insult ($n=6$). In a second study, we used the MIA model to evaluate the effect of celecoxib ($n=6$) on both behavioral tests and compared these results to animals ($n=6$) that received intra-articular MIA and saline by oral administration.

Two weeks prior to the induction of OA, animals were trained three times a week (Monday, Wednesday, and Friday) on the CatWalk runway to cross the corridor, uninterruptedly, for the gait pattern evaluation. Three complete runs per animal were analyzed. The von Frey evaluations were performed twice a week (Tuesday and Thursday). On the following week, data were collected and analyzed to obtain the baseline values of each animal for each test. Animal from different groups (MIA vs sham injected, surgical model vs sham operated and celecoxib vs saline treatments) were evaluated so that the experimenter remained blinded.

2.3. Induction of osteoarthritis

2.3.1. ACLT + pMMx surgery

Aseptic surgeries were performed with isoflurane/O_2 inhalation anesthesia. The left limb was shaved and the skin disinfected (poviodone–iodine 10%). With the leg maintained in extension, a median parapatellar skin incision of 1 cm was made. The joint capsule was cut medially and the patella was then luxated laterally. The anterior cruciate ligament was transected using a custom-made retrograde cutting hook and a partial menisectomy of the medial meniscus was performed. An anterior drawer movement was performed to confirm the ligament transection. The patella was reduced and the joint capsule sutured with vicryl 6-0 absorbable sutures. Subcuticular skin sutures were performed using 4-0 vicryl absorbable sutures. To assure a postoperative analgesia, a single subcutaneous injection of 0.05 mg/kg of buprenorphine (Temgesic®, Schering-Plough) was administered immediately after the surgery. The sham surgeries were performed under the same conditions. After the skin incision, the patella was luxated laterally and put back into place and the skin was sutured.

2.3.2. MIA injection

Prior to the MIA intra-articular injections, injections of toluidine blue were performed on dead animals to ensure proper intra-articular injection. For the MIA injection, 8 rats were anesthetized with isoflurane, the left knee area was shaved and the skin was disinfected with poviodone–iodine 10%. A single intra-articular injection of 3 mg sodium iodoacetate (SigmaUltra, 19148–5 G) in 30 μL of saline was done through the patellar ligament of the left knee using a 1 cc syringe with a 25 G needle. The dose of iodoacetate was selected based on previous studies (Beyreuther et al., 2007; Pomonis et al., 2005; Ferreira-Gomes et al., 2008; Schuelert and McDougall, 2008). The animals recovered for two days prior to the initiation of the study. For the negative control group, saline injections were performed in a similar fashion.

2.3.3. Celecoxib-treated animals

To evaluate if gait changes were related to pain and inflammation a celecoxib-treated group was used. We hypothesized that knee pain would change the gait pattern since limping, induced by the unilateral lesion, would alter gait parameters differently between the right and left limb. Since this was only observed with the MIA model, it was selected to evaluate the effect of celecoxib ($n=6$ animals). After receiving an intra-articular injection of MIA, these animals received a 30 mg/kg daily dose of celecoxib (Gentès & Bolduc, Canada) by oral administration for the complete duration of the study (28 days). Six animals with a MIA intra-articular injection received saline by gavage and were used as controls. Treatments were performed by a separate person uninformed of the substance being administered so that the behavioral evaluations were performed blinded.

2.4. Gait analysis

Gait analysis was performed using the CatWalk method, which has previously been described (Vrinten and Hamers, 2003; Hamers et al., 2006; Deumens et al., 2007). Briefly, it consists of a fluorescent light tube placed along a glass plate runway. Light enters the long edge of the glass and the rays are internally reflected except for the areas where the animal is in contact with the plate. Underneath the runway a video camera captures, records and sends the illuminated area images to a computer software. All analysis were performed with a pixel threshold value of 40 arbitrary units with a possible range between 0 and 255. To enhance animal motivation, Bacon Softies (# S3580–1, Bio-Serv, NJ) were placed at the end of the corridor in an open box joined to black PVC tubing (diameter 4 in.). Furthermore, animals were food deprived the night prior to the CatWalk tests. Following the training period, all animals crossed the walkway, uninterruptedly, and with a constant speed. Since variation in speed locomotion may affect gait parameters (Koopmans et al., 2007), it was minimized by using the mean of three quality runs per animal. Velocity was calculated by dividing the distance crossed by the animal in the corridor with the time taken to complete the distance.

2.5. Gait parameters

Mean daily reported gait parameters are from 3 corridor crossings prior and post OA induction. To measure pain-related behavioral response, the temporal phenomena of steady-state locomotion such as stepcycle phases of an individual limb were analyzed. The swing phase duration (s) is the time that the paw is not in contact with the ground in a complete stepcycle. The goals to be met in this phase of the stepcycle are to transfer and position the limb to start another stepcycle and the distance covered by the swing phase is directly related to the speed of the stepcycle. The swing speed (m/s) parameter is computed by dividing the stride length by the swing phase duration. The duty cycle (%) is the ratio between the stance duration and the complete stepcycle duration.

2.6. Assessment of pain behavior

Mechanical allodynia was assessed by measuring hind paw withdrawal thresholds in response to the application of von Frey filaments. Animals were placed in a Plexiglass chamber with a plastic floor with holes through which filaments are inserted and applied to bending at the plantar surface of the paw (Pitcher et al., 1999). A period of 10 min was allowed for the animal to acclimatize prior to the start of the daily evaluations. Mechanical sensitivity was assessed with the use of ten filaments with bending forces of 0.37 to 19.78 g applied first to the contralateral and then to the ipsilateral hind paws, beginning with the lowest force. The filaments were applied only when the animal was standing still on all four paws. Filaments were held for 3 s and this procedure was repeated 3 times with a 5 s rest in between each trial. If the animal didn't respond to the applied hair, the response was considered negative and the next larger filament was employed. If the animal flinched its paw, usually more than once, then the response was noted as positive. A positive response with a given filament for 3 trials determined the paw withdrawal threshold (PWT). If there was no response with the strongest force applied, this value was assigned as the threshold.

2.7. Histology of knee of joints

On the day following the last behavioral evaluations, rats were lightly anesthetized with isoflurane to facilitate handling and sacrificed by a section of the cervical spine. Knee joints were also collected for histological evaluations to confirm the presence of articular lesions. Knee joints were dissected free of muscle and fixed in a 10% formaldehyde solution (Fisher Scientific, NJ) for 48 h and transferred into a decalcification buffer, pH 7.3, containing 20% EDTA (ethylenediaminetetraacetic acid) (Sigma, St Louis, MO) for 14 days. Afterward, the joints were rinsed with tap water and placed in 10% formaldehyde solution for a maximum of 48 h prior to paraffin embedding. Coronal sectioning was performed and paraffin sections of 4 μm were collected and stained with safranin O-fast green (SOFG) to evaluate cartilage proteoglycans. Sections were mounted and photographed with a Nikon camera.

2.8. Neuropeptide analysis

On the same day of knee joint collection, spinal cord lumbar enlargement collection was done by flushing the vertebral canal with saline following transections of the vertebral column at the L5–L6 level as well as the cervical spine. Obtaining spinal tissue was rapidly performed (within 1 min) of placing the animal in the anesthesia induction box (isoflurane gas was used). The nervous tissue was immediately frozen (−60 °C) and stored at −80 °C pending analysis. For peptide analysis, the tissues were weighed frozen and homogenized following the addition of 0.25% TFA solution at a ratio of 1:5 (w:v). Samples were sonicated for 15 min and 200 μL of the homogenate was mixed with 200 μL of tylosine in acetonitrile. Samples were then vortexed and centrifuged at 12000 *g* for 10 min and the supernatant was kept and chromatographed using a microbore C8 10×1 mm column and a 19 min linear gradient. Selected spinal cord peptides, SP and CGRP were identified based on full scan MS/MS spectra and quantification was performed in selected reaction monitoring (SRM). The mass spectrometer was interfaced with the HPLC system using a pneumatic assisted electrospray ion source. The detailed method has been previously published (Beaudry et al., 2009).

2.9. Statistical analysis

For the analysis of gait parameters, each animal served as its own control. To normalize the data, all parameters were calculated as a percentage of respective pre-OA induction values. For each parameter, a mean of 3 assays per animal was calculated and divided by the baseline value giving a relative change in percentage ([time point value/mean baseline value]×100). This calculation allowed the parameter of each animal to be compared to its own baseline to assess group trends over time. To compare differences between both hind limbs in each group at each time point with the baseline values and to identify differences between groups at each time point, repeated measures analysis of variance (linear model) with time and hind limb or group as representative factors were performed, followed with a Bonferroni sequential correction to allow for the numerous comparisons. To analyze von Frey results statistics used are paired independent t-tests, comparing post injection or surgery values to baseline controls with or without treatments. Data are represented as mean ± standard error of the mean (SEM). SAS version 9.1 (Cary, N.C.) was used for the statistical analyses.

3. Results

3.1. Animals

Animals recovered quickly after the MIA injection and the surgeries. The wound healed normally within a few days following the ACLT + pMMx surgery. Swelling of the knee was present in both models for the first 5–6 days only. No significant difference in body weight was observed between all groups and animals had a normal weight gain during the study (data not shown).

3.2. Histological evaluation

Control animals had normal articular surfaces and underlying subchondral bones with a homogenous staining of the cartilage matrix (Fig. 1A). In the joints of ACLT + pMMx animals, there was a minimal loss of cartilage proteoglycans and chondrocytes. Variable erosion of the hyaline cartilage occurred in the lateral femoral condyle accompanied by a decrease of superficial SOFG staining on the medial tibial plateau (Fig. 1B). Formation of focal cartilage lesions represents an early event in the progressive disease process. In comparison, MIA injected joints had a much greater structural destruction and disorganization. Complete erosion of the hyaline cartilage on both the femoral and tibial surfaces was observed. Additionally, a remodeling of the subchondral bone was evident with the presence of osteophytes (Fig. 1C). In both models, no articular lesions were observed in the contralateral joints (data not shown). MIA animals that were treated with celecoxib showed a similar articular damage to the MIA animals without treatment (data not shown).

3.3. Gait analysis for both MIA and ACLT + pMMx models

3.3.1. Velocity

There were no significant differences observed between the group velocity at each time point and no significant differences between initial measurements at baseline and between each group at each time point. Means of speed of all days combined were 37 (±2.17), 38 (±2.17), 41 (±2.51) and 39.17 (±5.84) cm/s for the ACLT + pMMx, MIA, control and celecoxib groups respectively [$p = 0.47$].

3.3.2. Gait parameters

Since no differences in gait parameters were seen in different control groups, results from animals without any insult and shams (saline-injected and sham operated knee) were pooled together for comparison with OA model results. When comparing the ipsilateral to the contralateral limbs, obvious differences in strategies were observed between the two OA models (Fig. 2).

With the MIA model (Fig. 2A, top left figure), a significant progressively longer swing phase duration of the ipsilateral limb, and a shorter swing phase duration for the contralateral hind limb, was observed throughout the study [$p<0.02$], with the exception of day 8 where it is similar for both hind limbs [$p = 0.08$]. The swing phase duration of the ipsilateral hind limb significantly increased by the third week compared to the control group [$p<0.0001$]. In the ACLT + pMMx model (Fig. 2, top right figure), there were no significant differences in the swing phase duration when comparing both hind limbs. However, the swing phase duration of both limbs was greater at the beginning of the study and was shorter by the end of the study [$p<0.0001$] when comparing to controls.

With the MIA model, the swing speed of the ipsilateral limb was significantly slower when compared to the contralateral limb [$p<0.02$], with the exception of day 8 where both limb were similar [$p = 0.07$] and the contralateral hind limb swing speed was increased [$p<0.0001$] (Fig. 2, middle left figure). The ipsilateral affected limb became progressively slower suggesting a progressive degenerative process. With the ACLT + pMMx model, no statistical differences were observed between both hind limbs [$p>0.02$] (Fig. 2, middle left figure). When compared to the control group, the ACLT + pMMx model swing speed of both hind limbs was significantly greater at the end of the study [$p<0.002$].

The duty cycle was significantly smaller in the ipsilateral compared to the contralateral hind limb with the MIA model [$p<0.02$] (Fig. 2, bottom left figure) with the exceptions seen on days 8 and 12. A longer stance phase in the contralateral limb is present in this model. No significant differences were observed in the duty cycle between both hind limbs in the ACLT + pMMx model (Fig. 2. bottom right figure), with the exception of day 1 where it was significantly higher on the contralateral side [$p<0.002$].

3.4. Secondary mechanical allodynia for both MIA and ACLT + pMMx models

The MIA model showed an increased pain response in the injured limb with the von Frey filaments. The mean value of the ipsilateral hind PWT was significantly lower than the contralateral hind paw at each time point [$p<0.01$] except on day 9 (Fig. 3A). Responses in the contralateral limb remained near the baseline values throughout the study. Conversely, the ACLT + pMMx model showed no evidence of a painful response (Fig. 3B) except for the last evaluation day [$p<0.05$] when compared to the pre-operative evaluation. No significant differences were observed between the two hind paws at each time point.

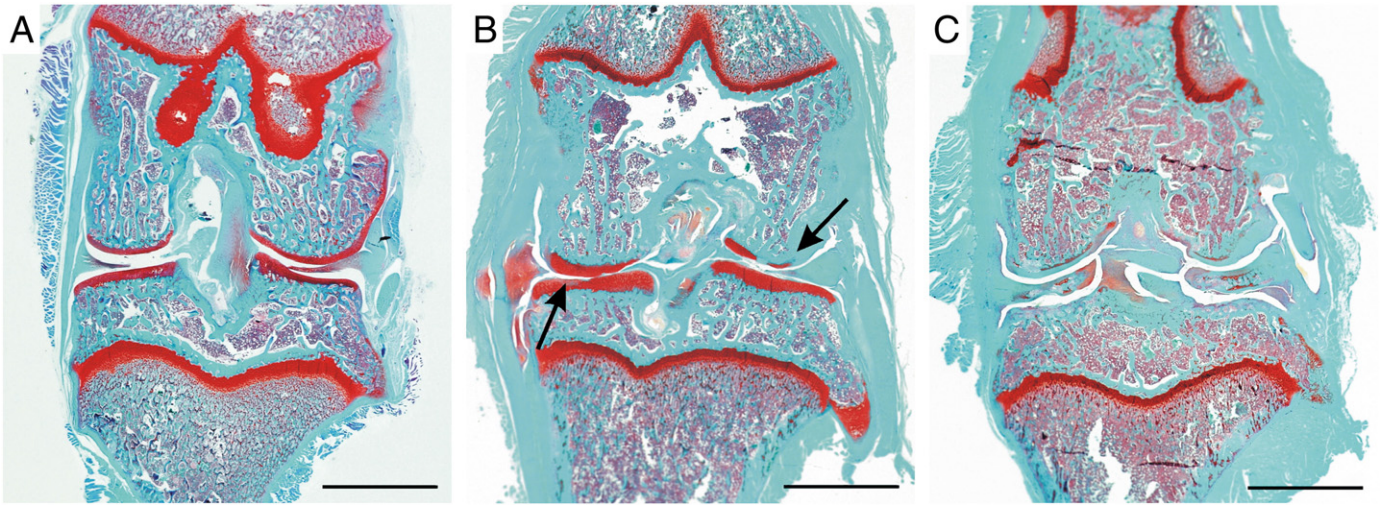

Fig. 1. Complete femorotibial joint sections stained with SOFG. Normal 16 week old femorotibial joint (A). ACLT + pMMx model 4 weeks post surgery. Ulceration of articular cartilage on the lateral femoral condyle and loss of SOFG stain on the medial tibial plateau are observed (arrows) (B). MIA model 4 weeks after injection. Complete loss of articular cartilage and extensive bone remodeling are observed (C). Scale bar = 2.5 mm.

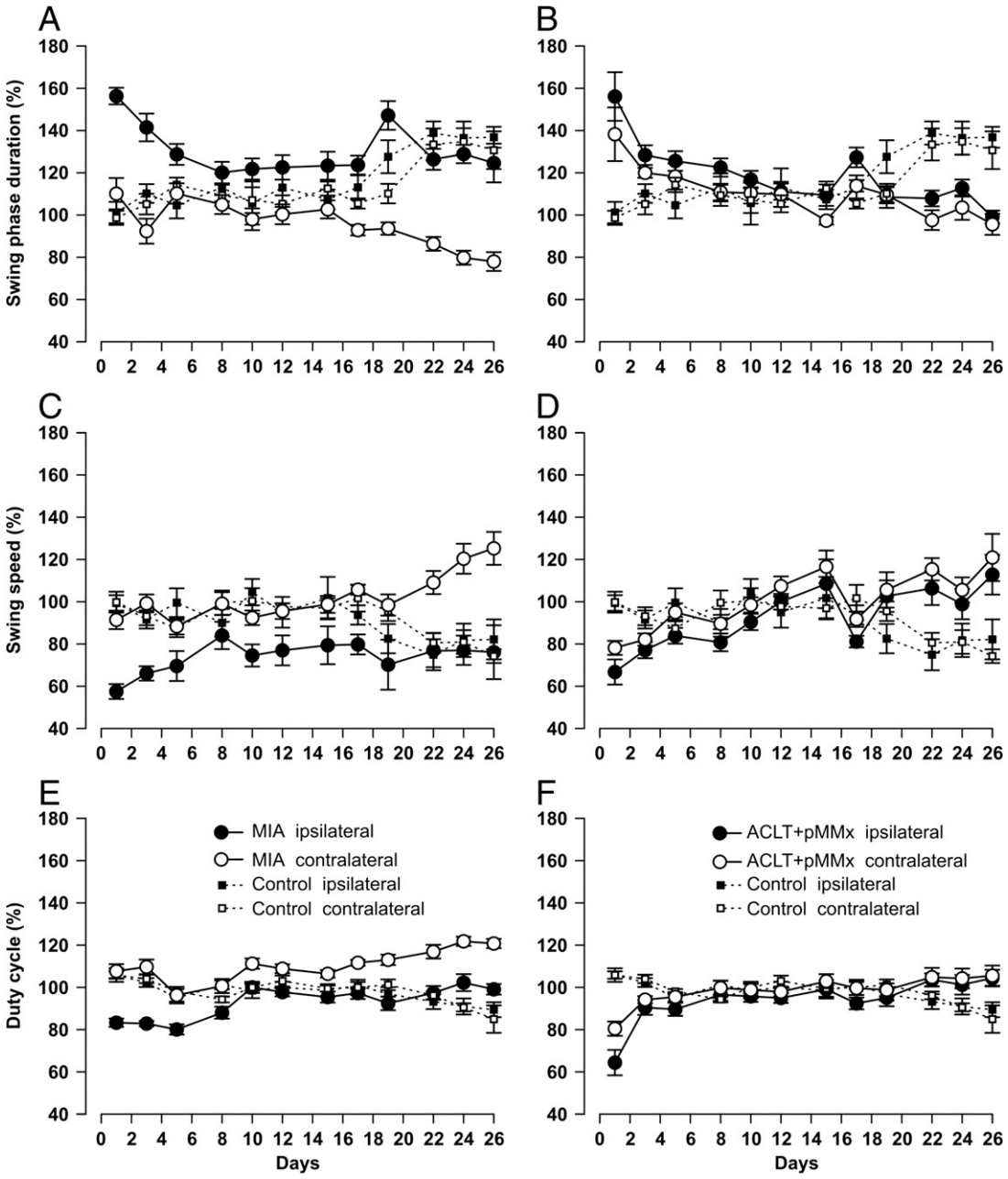

Fig. 2. Gait parameters obtained from the CatWalk analysis for both OA models. Variations in the ipsilateral and contralateral hind limbs of the swing phase duration (Top), swing speed (Middle) and duty cycle (Bottom) for both the MIA model and the ACLT + pMMx model. Results from control animals are represented as the dotted lines. All parameters are expressed as a% of the baseline values. Data are presented as Mean ± SEM. With the MIA model significant differences are present for all three gait parameters ($p<0.02$) except for the swing phase duration and swing speed on day 8 for and the duty cycle on days 8 and 12. With the ACLT + pMMx model the only significant difference was noted on day 1 of the duty cycle ($p<0.002$). Data are presented as Mean ± SEM. Statistics used are mixed linear model ANOVA and Bonferroni sequential correction.

3.5. Effect of celecoxib on gait analysis and secondary mechanical allodynia using the MIA model

Since the MIA model showed a clear differential effect between hind limbs, on gait and mechanical allodynia, this model was chosen to evaluate the effect of celecoxib treatment.

With celecoxib treatments (Fig. 4), differences were noted only on days 1 and 3 post MIA-induction for the swing speed and the duty cycle when compared to controls [$p<0.02$]. When considering all parameters, there is a trend over the first half of the study so that differences between right and left hind limbs are less pronounced for all parameters.

Celecoxib treatments relieved secondary mechanical allodynia [$p<0.001$–0.03] (Fig. 5) however no significant differences were observed on days 18 and 23 when compared to MIA animals treated with saline only.

3.6. Substance P and CGRP lumbar spinal cord concentrations

Selected peptide concentrations in the lumbar enlargement of the spinal cord are provided in Table 1. Compared to controls, SP concentrations were significantly increased in the MIA model with and without celecoxib treatment [$p<0.01$ and 0.02, respectively]. No significant difference in SP concentration occurred in the ACLT + pMMx model. The concentration of CGRP was significantly increased in both OA models in comparison to the control group [$p<0.01$] but not in the MIA animals treated with celecoxib.

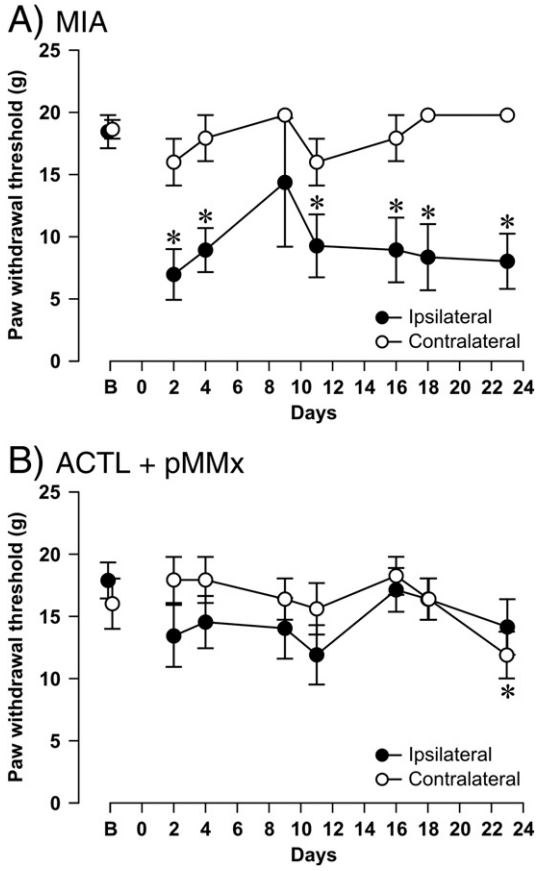

Fig. 3. Paw withdrawal threshold (g) evaluated with the von Frey hair test of the ipsilateral and contralateral hind limbs of the MIA model (A) and ACLT + pMMx model (B). Data are presented as Mean ± SEM. * = significant difference between the two hind limbs ($P<0.007$). Statistics used are paired independent *t*-test compared to controls.

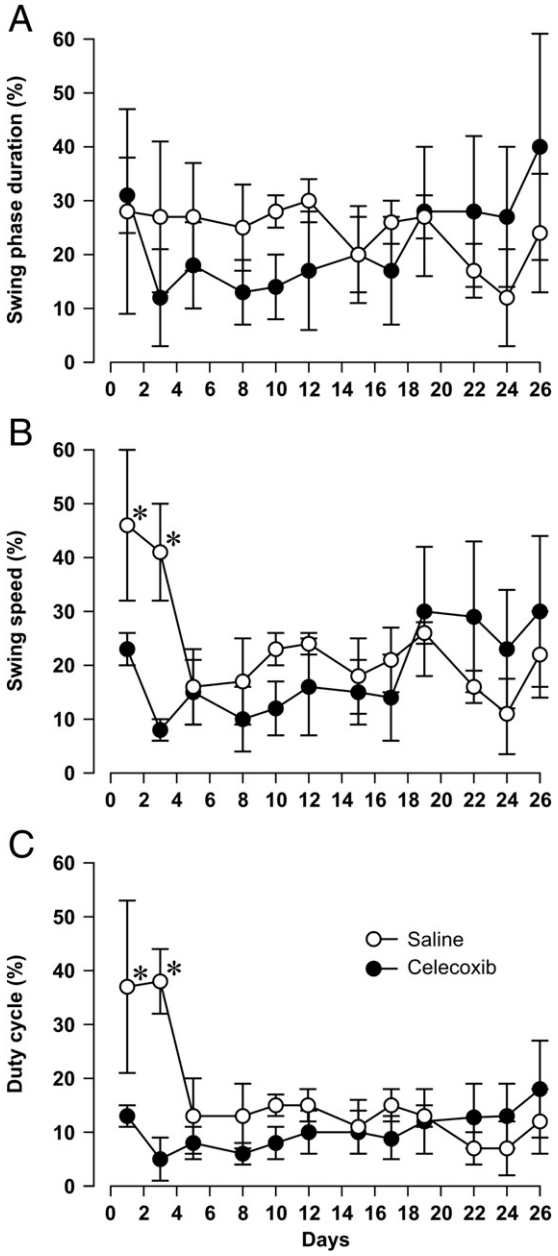

Fig. 4. Effect of celecoxib and saline treatments on gait parameters in the MIA model. Differences (%) between the right and left hind legs for swing phase duration (Top), swing speed (Middle) and duty cycle (Bottom) are presented. Differences were noted only on days 1 and 3 post MIA-induction for the swing speed and the duty cycle when compared to controls ($p<0.02$). Data are presented as Mean ± SEM. Statistics used are mixed linear model ANOVA and Bonferroni sequential correction.

4. Discussion

Two rodent models of OA were investigated to assess the gait disturbances and pain responses as they may serve as models for human joint pain response. The MIA model demonstrated clear pain-related behavioral responses in the injured limb as well as significant differences between both hind limbs in the dynamic gait parameter evaluation with the CatWalk. The gait pattern differences were compatible with unilateral knee lesion, marking clear limping gait due most probably to an unwillingness of the animals to bear weight on the ipsilateral limb, also observed in human OA compensatory gait mechanics (Kaufman et al., 2001). The flexion–extension phase of the injured knee joint, during the swing phase to move the limb forward, might be decreased because of reduced joint mobility caused by pain. This has been suggested to occur in the case of unilateral knee arthritis in humans (McGibbon and Krebs, 2002). The injured knee joints of the animals under study may have a diminished knee flexion velocity and a reduced knee torque due to a willingness to lock the joint range of motion in order to minimize their sensation of pain.

The ACLT + pMMx model revealed a much less marked pain-like behavioral response on assessment of the dynamic parameters. With an increased swing phase duration and a decreased swing speed occurring in both hind limbs in a parallel fashion, quicker and shorter steps were made in order to maintain a constant speed which was different to the strategy adopted by the MIA group. A post-surgical recuperation period was permitted to avoid data collection in the presence of surgical induced residual inflammation. Results obtained indicate an amplified pain response on day 1 that could still be related to surgical trauma. An interesting finding is that this amplified response is observed in both hind limbs. In the presence of persisting inflammation, central sensitization via peripheral nociceptor activation may occur. Inflammatory processes may alter the sensory input to the spinal cord and subsequent changes in the contralateral output may occur, inducing a bilateral pain response (Decaris et al., 1999; Schenker et al., 2003; Kelly et al., 2007).

Two published investigations of rat OA models have been conducted with the CatWalk method, with one assessing the paw print intensity only (Ferreira-Gomes et al., 2008). Ängeby-Möller et al. (2008) observed a reduced duration of the stance phase in the injected paw of the monoarthritic carrageenan rat model. It is difficult to compare their findings with the two OA models used in the study herein as the mechanisms of action underlying the method of disease induction differ. The carrageenan model produces a localized synovial inflammatory response with symptoms of joint pain that peak 3–4 h

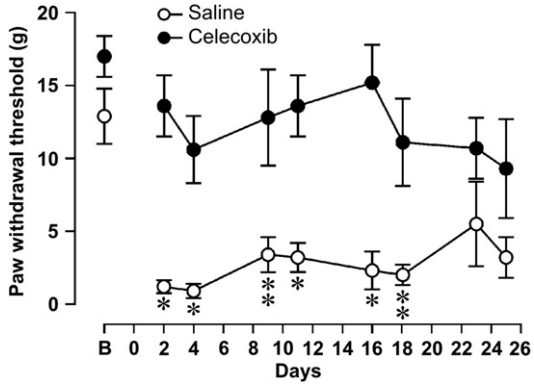

Fig. 5. Effect of celecoxib and saline treatments on von Frey paw withdrawal threshold of the ipsilateral hind limb using MIA model. Thresholds are expressed in grams. Data are presented as Mean ± SEM. Celecoxib treatments relieved secondary mechanical allodynia ($p<0.001$–0.03) except on days 18 and 23. Statistics used are paired independent t-test compared to controls.

post-injection (Tonussi and Ferreira, 1992). The MIA model in contrast, produces a pain response in the days following injection in a dose–dependent manner (Bove et al., 2003; Kobayashi et al., 2003). Furthermore, the temporal behavioral pattern of the MIA model is known to be biphasic (Guingamp et al., 1997), implying a transient synovial inflammation (Fernihough et al., 2004) post injection followed by an increase in pain-related behavioral response within the first days. The second phase has been reported to occur by day 7 post injection resulting of structural changes in the joint associated with pain-related response (Bove et al., 2003). Results shown in Figs. 1 and 2 are in accordance with the works of Bove et al. (2003), since on day 8 no differences were observed between the ipsilateral and contralateral limbs in this model. As for the ACLT + pMMx model, it reproduces progressive OA longer than the four weeks period considered in the study herein as suggested with the histological observations and CatWalk results.

Secondary mechanical allodynia measured with the von Frey filaments occurred in the ipsilateral hind paw of the MIA model with a significantly lowered threshold identified on the first day of testing through the end of the study (except for day 9) combined with an almost unchanged threshold on the contralateral side. No significant allodynia was observed in the ACLT + pMMx model except for the last evaluation at the end of the study. This also suggests that this model needs a longer time to develop as a one month study appears insufficient to show significant changes. Central sensitization may play a major role in progression to chronicity (Coutaux et al., 2005), often observed in patients with progressive OA (Poole, 2007).

Celecoxib a selective COX-2 inhibitor, was administered since it has been shown to relieve pain associated with OA (Stengaard-Pedersen et al., 2004; Tannenbaum et al., 2004; Pomonis et al., 2005). Our results show that an effect on swing speed and the duty cycle only was observed at the beginning of the study suggesting that this drug appears to work in the inflammatory phase but not in the chronic pain phase in the MIA model however the secondary mechanical allodynia was decreased during the study except for the last days of the study (18 and 23). These results would tend to suggest that central sensitization is relieved with celecoxib but not local pain occurring from the affected articulation.

The histological assessment confirmed the presence of articular structural changes in both models. Advanced degenerative articular lesions were seen only in the MIA model joints with a remodeling of the subchondral bone structure greatly associated with pain (Guzman et al., 2003) and therefore might be responsible for the generation of the greater pain response found in this model. The ACLT + pMMx model revealed early events of the OA pathology with only focal cartilage lesions identified. As hyaline cartilage is not innervated, other articular structures are responsible for generating the behavioral pain response. The instability caused by ligament damage results in greater joint displacements and more abrupt motion than would occur in a stable joint and consequently may excite spinal cord neurons responding to noxious mechanical stimuli by stimulation of deep articular tissue (Schaible et al., 2006).

Selected pain-related peptides were assessed only at the end of the study. Neuropeptide synthesis is enhanced during OA progression and spinal cord production of these peptides is an important factor modifying the process of signal transmission and modulation of central mechanisms of pain (Niissalo et al., 2002). Substance P and CGRP, coexisting in small-diameter afferent fibres, have been found to directly participate in the injury-induced neuroplasticity changes in arthritis (Ahmed et al., 1995; Garrett et al., 1995; Keeble and Brain, 2004; Bird et al., 2006). The concentrations of both neuropeptides were increased in both OA models suggesting that excitatory neurotransmission initiated long term changes at the central level. However, only CGRP was significantly increased in the ACLT-pMMx model. In this model, the SP concentration was increased but it is not significantly different from the control group. The high variability was brought about by one animal where a very low SP concentration was measured (in comparison to other animals). In the MIA model both peptides were significantly increased. Interestingly SP was still elevated with celecoxib treatment but CGRP concentrations were within normal limits (animals without pain). Since allodynia was decrease with celecoxib these results suggest that CGRP is an important peptide for central sensitization in OA. The importance of CGRP in OA pain transmission has previously been reported by Schaible et al. (2006). It would be interesting to establish a neuropeptide concentration profile at various time points in OA animal models to demonstrate variations in the time course of pain-related neuropeptide expression with progression of joint injury.

In summary, we compared the gait and pain-like behavioral responses in two different OA models in rodents frequently employed in preclinical studies. Our results suggest that the differences observed between both models could be related to distinctive knee lesions inducing different nociceptive mechanisms. Furthermore, when a relatively short study is required, the MIA model may be a better model for the evaluation of therapeutic strategies for joint pain palliation, having clear behavioral nociceptive responses in the gait parameters of the injured limb. Longer experiments need to be performed to better characterize the ACLT + pMMx model. Modifications of the nociceptive processing within the spinal cord have been identified and further investigations are required to better characterize their role in the development of pain sensitization.

Table 1
Mean (± SD) neuropeptides concentrations (pmol/g) in lumbar spinal cord enlargement at four weeks in controls, post OA induction and MIA animals with celecoxib treatment. Significant differences from the control group identified as [1] ($P<0.01$) and [2] ($P<0.02$).

	Control	ACLT + pMMx	MIA	MIA + Celecoxib
Substance P	67.6 (± 57.3)	226.3 (± 180.0)	190.2 (± 83.8)[1]	216.4 (± 109.0)[2]
CGRP	2541 (± 293)	4651 (± 1653)[1]	4561(± 1413)[1]	1850 (± 514)

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