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Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia

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Summary

Objective: In the present study, we sought to develop/characterize the pain profile of a rat model of surgically induced osteoarthritis (OA).

Methods: OA was surgically induced in male Lewis rats (200–225 g) by transection of the medial collateral ligament and medial meniscus of the femoro-tibial joint. In order to characterize the pain profile, animals were assessed for a change in hind paw weight distribution (HPWD), development of mechanical allodynia, and the presence of thermal and mechanical hyperalgesia. Rofecoxib and gabapentin were examined for their ability to decrease change in weight distribution and tactile allodynia.

Results: Transection of the medial collateral ligament and medial meniscus of male Lewis rats resulted in rapid (<3 days) changes in hind paw weight bearing and the development of tactile allodynia (secondary hyperalgesia). There was, however, no appreciable effect on thermal hyperalgesia or mechanical hyperalgesia. Treatment with a single dose of rofecoxib (10 mg/kg, PO, day 21 post surgery) or gabapentin (100 mg/kg, PO, day 21 post surgery) significantly attenuated the change in HPWD, however, only gabapentin significantly decreased tactile allodynia.

Conclusion: The rat medial meniscal tear (MMT) model mimics both nociceptive and neuropathic OA pain and is responsive to both a selective cylooxygenase-2 (COX-2) inhibitor commonly utilized for OA pain (rofecoxib) and a widely prescribed drug for neuropathic pain (gabapentin). The rat MMT model may therefore represent a predictive tool for the development of pharmacologic interventions for the treatment of the symptoms associated with OA.

Key words: Osteoarthritis, Medial meniscal tear, Secondary hyperalgesia, COX-2 inhibitor, Gabapentin, Rat, Allodynia.

Introduction

Osteoarthritis (OA) is the most common form of joint disease with over one-half of all people older than age 65 demonstrating radiographic changes of OA in the knees[1]. OA is primarily noted in the weight-bearing joints (i.e., knees, hips) and is associated with degeneration of the articular cartilage and changes to the subchondral bone at the joint margins[2]. Clinically, OA manifests itself as joint pain with concomitant loss of joint function, which may ultimately result in a substantially reduced quality of life for the patient. While a great deal is known about the symptomatic aspects of the disease, the pathophysiology behind the aforementioned structural changes is complex and poorly understood.

Currently, there are no commercially available pharmacologic approaches definitively proven clinically to alter the natural progression of this disease. The supplements, glucosamine and chondroitin sulfate, however, may have the ability to provide chondroprotective effects and improve some of the signs and symptoms associated with OA[3,4]. In the absence of disease modifying drugs, the treatment of patients with OA is often directed at relieving pain and restoring function through the use of pharmacologic therapies[5–7]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cylooxygenase-2 (COX-2) inhibitors are widely prescribed for the treatment of OA pain. These drugs have a number of issues, however, including potential undesirable side effects and variable efficacy. Therefore, the need remains for new pharmacologic approaches for the treatment of OA pain either as stand-alone or add-on therapy with existing drugs.

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While the pain associated with OA is primarily localized to the joint, it is becoming increasingly apparent that a number of patients exhibit increased nociception to adjacent or even remote areas of the body[8–10]. For example, Kosek and Ordeberg[8] found that patients with unilateral hip OA had decreased pain thresholds in the normal, contralateral hip, while DeBerry and coworkers[10] showed that patients with knee OA demonstrated increased sensitivity to pressure pain in the hip as compared to healthy, age-matched controls. This phenomenon, referred to as secondary hyperalgesia or allodynia, is thought to be the result of central sensitization[11–13]. For this reason, it is now becoming common for OA to be thought of, in part, as a neurological disease with central input in addition to the pain originating at the affected joint. Pain inhibitors that target both joint pain and secondary hyperalgesia/allodynia may therefore represent the optimal approach for reducing OA pain. As such, there exists a need for *in vivo* models that accurately mimic both the pathologic changes and pain profile (central and peripheral components) that are noted in human OA. We report here the characterization of a surgically induced rat model of OA pain that possesses both primary joint pain with concurrent secondary hyperalgesia. This model may, therefore, represent an ideal platform by which to study the nociceptive responses associated with the disease as well as be of use in the development of new pharmacologic agents for the treatment of OA symptoms.

Methods

All procedures used in this study were approved by, and in accordance with, the guidelines of the Pfizer Animal Care and Use Committee. Animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. Rats were kept in a 12:12 h light–dark cycle and housed in solid bottom cages with corncob bedding for the duration of the study, except where otherwise noted. Rats were fed standard rat chow with water available *ad libitum*.

INDUCTION OF OA

Adult male Lewis rats (Charles River, Wilmington, MA) weighing 200–225 g were allowed to acclimate for 1 week prior to surgery. OA was surgically induced as described previously[14]. Briefly, animals were anesthetized with isoflurane and the skin over the medial aspect of the right femoro-tibial joint clipped to remove the hair and surgically prepped with Nolvasan® Surgical Scrub (Fort Dodge Animal Health, Overland Park, KS) followed by 70% alcohol. The medial collateral ligament (MCL) was exposed by blunt dissection and transected to reflect the meniscus toward the femur. The joint space was visualized and the meniscus was cut through the full thickness at its narrowest point to simulate a complete tear. The skin was closed with 4.0 silk suture. For sham animals, the medial collateral ligament was exposed, but not transected. An additional group of animals, referred to as MCL sham rats, had their medial collateral ligament transected and the joint space visualized, but the meniscus was not cut. These animals served as a control for the transection of the ligament and its effect on change in hind paw weight distribution (HPWD). All surgical sites were observed daily for signs of abnormal healing including, but not limited to swelling, redness, drainage, bleeding and dehiscence.

ASSESSMENT OF CHANGE IN HPWD

The change in HPWD was utilized as an index of joint discomfort and was determined as described previously[15]. Briefly, changes in HPWD between the right (arthritic) and left (contralateral control) limbs were utilized as an index of joint pain in the surgically induced arthritic knee. An incapacitance tester (Linton Instrumentation, Norfolk, UK) was employed for the determination of HPWD. Rats ($n = 8$ per group) were placed in an angled Plexiglass chamber positioned so that each hind paw rested on a separate force plate. The force exerted by each hind limb (measured in grams) is averaged over a 5-s period. Each data point is the mean of three, 5-s readings. The change in HPWD was calculated by determining the difference in the amount of weight (g) between the left and right hind limbs. Three separate studies (72 total animals) were conducted with the data presented as the difference in weight bearing between the left (contralateral control) limb and right (arthritic) limb.

MECHANICAL ALLODYNIA

Rats (64 total animals) were housed in wire-bottom cages for the duration of the study. To assess mechanical allodynia, animals were placed in suspended wire-bottom cages and allowed to acclimate in a quiet, subdued light environment for 30 min. The mechanical withdrawal threshold of the hind paw was tested using a set of von Frey monofilament hairs (VFH). The VFH were applied to bending at the center plantar region of the ipsilateral hind paw, and the paw withdrawal threshold (PWT) was determined using the Dixon Up-Down method[16]. Briefly, six VFHs with bending forces of 2.0–28.8 g were applied for up to 6 s, beginning with the lowest force. If the rat did not withdraw its paw (flinch) in response to the force of the VFH, the next higher force was applied, etc., until recoil was observed (defined as the preliminary threshold). As a result of the flinch, the next lower VFH was then employed. Up-down force application continued for four applications post-preliminary threshold. The series of flinch/no-flinch responses were used to calculate the actual PWT. If there was no response at 28.8 g, that force was assigned.

THERMAL HYPERALGESIA

Thermal hyperalgesia was evaluated by the paw withdrawal test[17] using a Hargreaves apparatus (San Diego, CA). The device consists of a glass surface upon which the rats were placed individually in Plexiglass cubicles. The glass surface temperature is maintained at 30 °C by a feedback-controlled, under-glass, forced-air heating system. The animals were allowed to acclimate to their surroundings for 15 min. Following acclimation, a 50-W halogen light was positioned at the plantar surface of the ipsilateral paw. When the pain threshold is achieved, the animal flinches its paw, stopping the illumination and the timer. A cut-off time of 20-s is employed to avoid tissue injury. The time from the onset of the heat application to paw lifting is considered to be the paw withdrawal latency (PWL). This is then recorded and read again 5 min later so as not to produce a summation of tissue temperature. The average of the two readings is considered to be the PWL for that animal. Two separate studies were conducted with eight animals per group (32 total animals).

MECHANICAL HYPERALGESIA

Mechanical hyperalgesia was evaluated by the Randall–Selitto test[18] using a paw pressure analgesia instrument (Stoelting, Wood Dale, IL). The paw pressure pain threshold was determined by placing the ipsilateral hind paw between a flat surface and a blunt pointer and applying steadily increasing pressure (15 g/s). The threshold was determined when the rat exhibited a stereotyped flinch response and attempted to remove the foot from the apparatus. If there was no response at the maximum pressure (238.9 g), that force was assigned. Two separate studies were conducted with eight animals per group (32 total animals).

HISTOLOGIC ANALYSIS

Histologic changes were assessed in sham, MCL sham and medial meniscal tear (MMT) rats 21 days post surgery. Rats were euthanized by CO_2 and soft tissue was removed from the right (MMT, sham or MCL sham operated) leg. The patella was removed from each knee to facilitate thorough fixation of the joint. Tissue samples were prepared for light microscopy using standard procedures. Briefly, samples were preserved in 10% neutral-buffered formalin (VWR, So. Plainfield, NJ), and subsequently decalcified in 5% formic acid for 72 h. Samples were dehydrated in an ethanol series and embedded in paraffin. Sections were stained with toluidine blue according to previously published methods[19]. Two separate studies were conducted with three sham and six MMT-operated rats per group and one study with three MCL sham rats (21 total animals).

Articular cartilage damage was assessed by examining the degree of chondrophyte/osteophyte formation and cartilage loss or erosion. Subchondral bone damage was indicated by subchondral sclerosis, bone marrow fibrosis and loss of bone marrow spaces. Inflammation was characterized by expansion of the synovial membrane by proteinaceous edema fluid and fibrin with infiltrating macrophages, neutrophils, plasma cells and lymphocytes. All samples were scored by a Board-certified veterinary pathologist (RG).

PHARMACOLOGIC CHARACTERIZATION

Rofecoxib (Merk & Co., Inc., Whitehouse Station, NJ; $n = 6$ per group) was pulverized and suspended in hydroxypropylmethylcellulose (HPMC; Sigma–Aldrich, St. Louis, MO) vehicle (0.5% HPMC + 0.2% Tween 80). Gabapentin (Pfizer, Inc., Ann Arbor, MI; $n = 6$ per group) was also suspended in HPMC vehicle. A separate vehicle group ($n = 8$) in which rats were dosed with HPMC vehicle alone was run in conjunction with the drug-treated groups. Twenty-one days post surgery, changes in HPWD or mechanical allodynia were determined, as described above, to establish a baseline pain reading. The rats were then given a single dose (oral gavage) of vehicle, rofecoxib (10 mg/kg), or gabapentin (100 mg/kg). Changes in HPWD and mechanical allodynia were determined 2 h post-compound administration. The data obtained are representative of three separate studies for HPWD (eight animals per group, 72 total animals) and two separate studies for mechanical allodynia (eight each for sham and vehicle and six each for gabapentin and rofecoxib, 56 total animals).

STATISTICAL METHODS

All data are expressed as mean ± S.E.M. All statistical tests were tested at the 0.05 level of statistical significance. Time course data, post surgery, were analyzed by a repeated measures analysis of variance (RMANOVA) with terms for treatment, time, and the joint effect of treatment and time (interaction). If the treatment by time interaction was not statistically significant, the mean responses of all groups were compared in a pair-wise fashion, averaged over time, via a Tukey–Kramer multiple comparison procedure adjustment. If the treatment by time interaction was statistically significant, the effect of differing interventions could not be addressed without considering the time of the measurement; groups were then compared in a cross-sectional fashion (at each time point). A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison's procedure was performed on studies examining the efficacy of rofecoxib and gabapentin on change in HPWD and mechanical allodynia.

Results

EFFECT OF MMT SURGERY ON CHANGE IN HPWD

As early as 3 days post surgery, MMT-operated rats exhibited a significant change in HPWD when compared to sham and MCL sham-operated rats (Fig. 1). A maximal level of change in HPWD was reached 3 days post surgery and remained consistent through day 21. There was a slight increase in change in HPWD in sham and MCL sham-operated animals when compared to pre-surgery assessments; however, this increase was not statistically significant. In addition, when sham rats were compared to MCL sham rats, no significant differences were found between the two groups.

EFFECT OF MMT SURGERY ON MECHANICAL ALLODYNIA

Mechanical allodynia was assessed on the same groups of rats 1 day before surgery (day −1) and again 3, 7, 14, and 21 days post MMT or sham surgery [Fig. 2(A)]. It should be noted that only a subset of MMT-operated rats developed hypersensitivity to punctate VFH stimulation on the ipsilateral hind paw. The percentage of rats that did develop

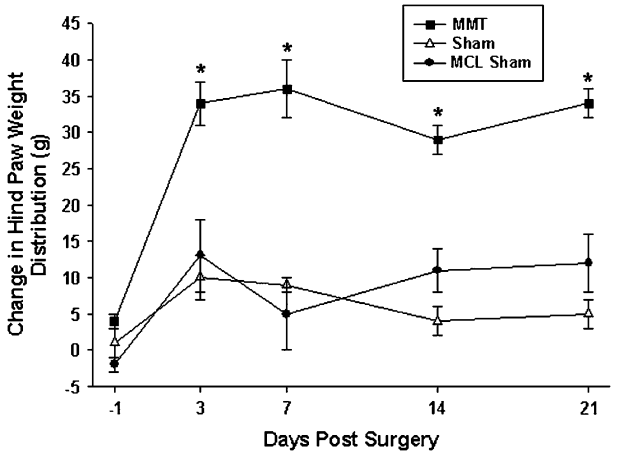

Fig. 1. Effect of MMT surgery on change in HPWD. Change in HPWD was assessed by use of an incapacitance tester 1 day before and 3, 7, 14 and 21 days post MMT, sham or MCL sham surgery. Statistically significant differences were determined by RMANOVA (*$P < 0.0001$) vs sham-operated rats. $N = 8$ rats per group. Representative of three separate experiments.

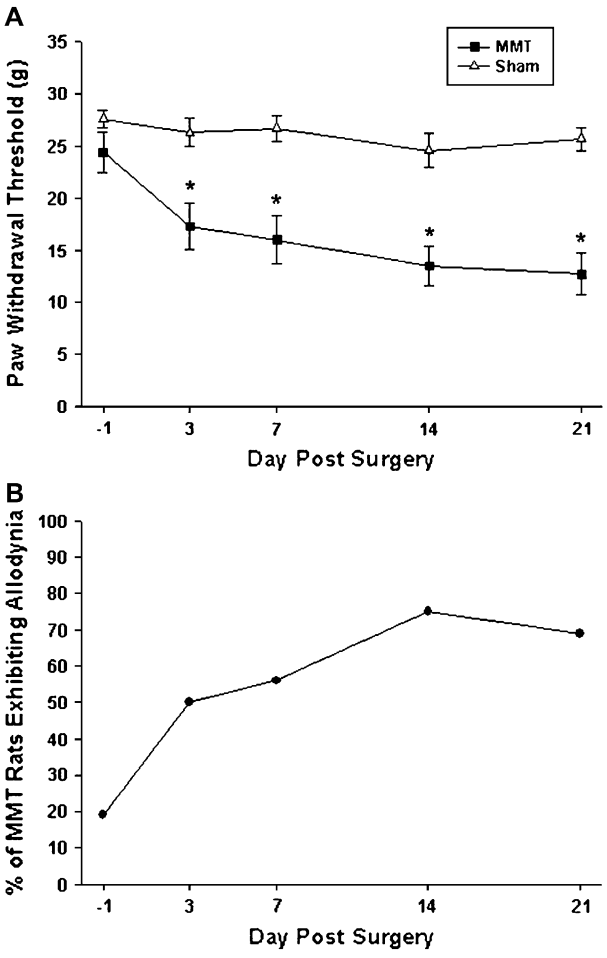

Fig. 2. Effect of MMT surgery on mechanical (punctate) allodynia. A. Mechanical allodynia was assessed using von Frey monofilaments and expressed as PWT (in grams). PWT was determined 1 day before and 3, 7, 14 and 21 days post MMT or sham surgery. Statistically significant differences were determined by RMANOVA (*$P < 0.001$) vs sham-operated rats. B. Percent of MMT rats exhibiting allodynia was calculated as follows: % incidence of rats exhibiting allodynia = ((# of rats with PWT <16 g)/(# of rats with PWT ≥16 g)) × 100. $N = 16$ rats per group. Combination of two separate experiments.

mechanical allodynia (defined, for the purpose of this study, as a PWT of less than 16 g) increased as a function of time with approximately 50% of rats exhibiting hypersensitivity 3 days post surgery and 70% by day 21 post surgery [Fig. 2(B); $N = 16$ rats per group; combination of two separate experiments]. This trend continued through day 28 post surgery (87%; data not shown). There was no decrease in PWT in sham-operated rats compared to pre-surgery assessment.

EFFECT OF MMT SURGERY ON THERMAL AND MECHANICAL HYPERALGESIA

Thermal (Fig. 3) and mechanical (Fig. 4) hyperalgesia were assessed 1 day before surgery (day −1) and again 3, 7, 14 and 21 days post MMT or sham surgery. Neither MMT nor sham surgery induced thermal or mechanical hyperalgesia when compared to pre-surgery assessments.

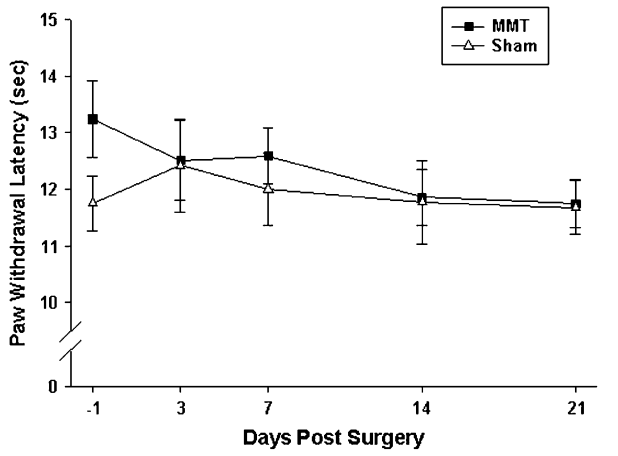

Fig. 3. Effect of MMT surgery on thermal hyperalgesia. Thermal hyperalgesia was assessed by use of a Hargreaves apparatus and expressed as PWL (in seconds). PWL was determined 1 day before and 3, 7, 14 and 21 days post MMT or sham surgery. $N = 8$ rats per group. Representative of two separate experiments.

MMT-INDUCED HISTOLOGIC CHANGES

Figure 5(A and B) represents sham and MCL sham control knees 21 days post surgery, respectively, with intact, healthy cartilage and no loss of chondrocytes or proteoglycan. No changes were noted to the subchondral bone or synovium.

Rats sacrificed 21 days post MMT surgery [Fig. 5(C)] had prominent osteophyte/chondrophytes in the medial tibial plateau immediately adjacent to the area of cartilage erosion or loss. Focally extensive areas of full thickness cartilage damage characterized by complete loss of cellular detail and some loss of proteoglycan matrix, as determined by Toluidine Blue staining, was also noted. There were extensive areas of subchondral sclerosis in the medial tibial plateau, which had markedly reduced and in some cases

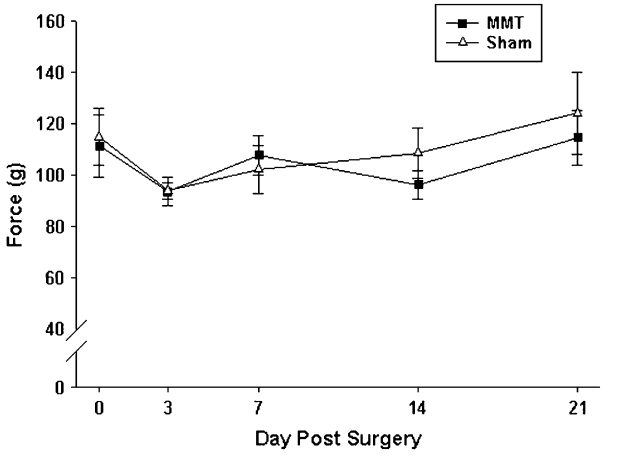

Fig. 4. Effect of MMT surgery on mechanical (blunt pressure) hyperalgesia. Mechanical hyperalgesia was evaluated by the Randall–Selitto test using a paw pressure analgesia instrument and expressed as the amount of force (g) necessary to elicit a stereotyped flinch response. Mechanical hyperalgesia was determined 1 day before and 3, 7, 14 and 21 days post MMT or sham surgery. $N = 8$ rats per group. Representative of two separate experiments.

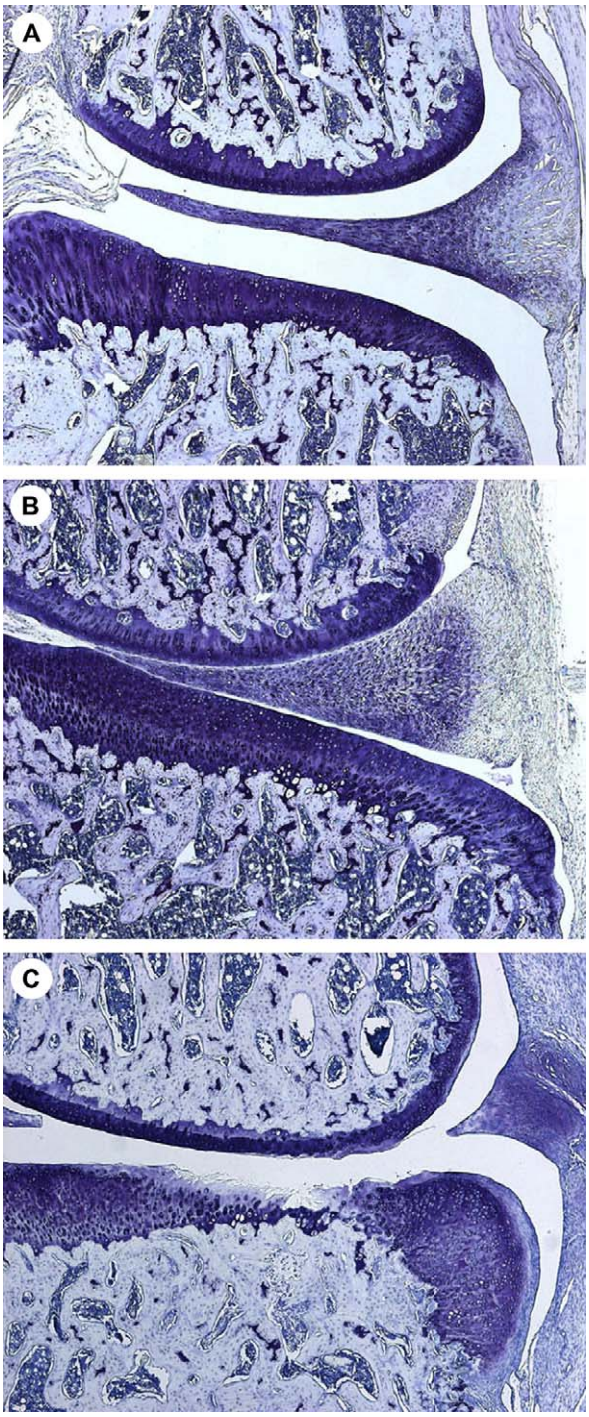

Fig. 5. Histology of sham, MCL sham- and MMT-operated rats 21 days post surgery. Frontal sections of the medial aspect of rat knee joints were stained with Toluidine Blue. (A and B) Sham and MCL sham surgery. Knees have normal healthy cartilage, subchondral bone and synovium. (C) MMT surgery. Prominent chondrophyte/osteophyte present adjacent to severe cartilage erosion. Chondrocyte loss is complete to the deep zone and significant proteoglycan loss can be detected all the way to the tidemark. Extensive subchondral sclerosis and bone marrow fibrosis is present with loss of some bone marrow spaces. No synovial inflammation is present. Magnification ×5. Representative of two separate experiments ($n = 3–6$ rats per group).

obliterated the inter-trabecular bone marrow spaces. Loosely arranged fibrous tissue or clusters of osteoblasts typically occupied remaining spaces.

EFFECT OF GABAPENTIN AND ROFECOXIB ON HPWD AND MECHANICAL ALLODYNIA

Experiments designed to test the effect of gabapentin and rofecoxib on change in HPWD and mechanical allodynia were conducted on day 21 post surgery. Gabapentin (100 mg/kg) and rofecoxib (10 mg/kg) significantly decreased change in HPWD 2 h post dose (Fig. 6; 45% and 66% decrease, respectively). A subset of allodynic rats was used to test the effect of gabapentin and rofecoxib on the mechanical allodynia. Gabapentin, but not rofecoxib, significantly increased PWT (decreased mechanical allodynia) 2 h post dose (Fig. 7; 70% and 25% inhibition of allodynia, respectively). No changes in behavior (i.e., drowsiness or agitation) were noted with either drug.

Discussion

Surgical transection of the anterior cruciate ligament (ACL), meniscectomy, or resection of the tibial plateau in experimental models has been shown to produce structural changes including cartilage lesions and changes to the subchondral bone associated with OA[20–22]. While these animal models largely mimic the structural changes noted in human OA, they have not been shown to simulate the symptomatic aspects of the disease. In the absence of pharmacologic interventions to treat the structural changes associated with OA, finding novel compounds to treat the symptoms of the disease takes on ever increasing importance. In the present study, we have sought to characterize a rat model of joint pain based on surgical transection of the medial collateral ligament and medial meniscus.

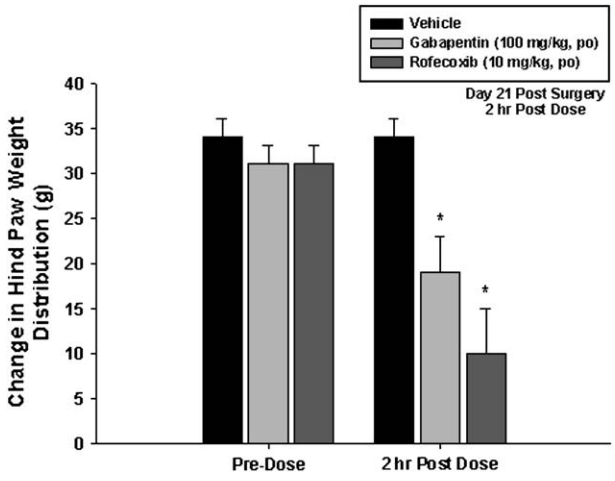

Fig. 6. Effect of rofecoxib and gabapentin on change in HPWD. MMT surgery was performed on day 0. On day 21 the rats were assessed on an incapacitance tester for change in HPWD to establish pre-dose values. Rats were then administered 10 mg/kg rofecoxib, 100 mg/kg gabapentin, or vehicle (PO) and reassessed 2 h later. Statistically significant differences were determined using one-way ANOVA followed by Dunnett's multiple comparison's procedure (*$P < 0.05$ vs vehicle at the same time point). $N = 8$ rats per group. Representative of three separate experiments.

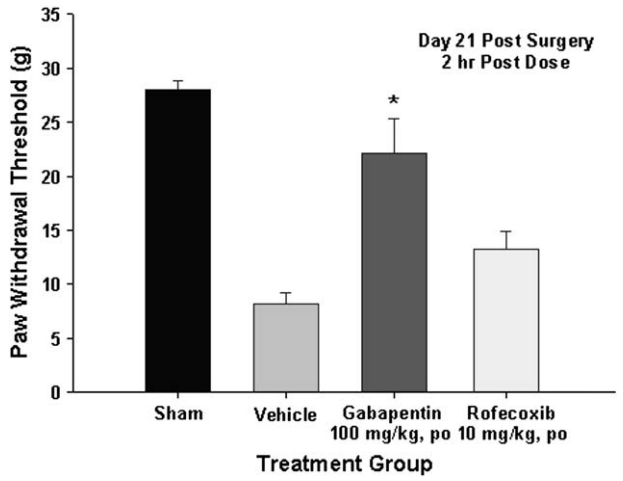

Fig. 7. Effect of rofecoxib and gabapentin on mechanical (punctate) allodynia. MMT or sham surgery was performed on day 0. On day 21, PWT was determined. The rats were then administered 10 mg/kg rofecoxib, 100 mg/kg gabapentin, or vehicle (PO) and reassessed 2 h later. Statistically significant differences were determined using one-way ANOVA followed by Dunnett's multiple comparison's procedure (*$P < 0.05$ vs vehicle). $N = 8$ (vehicle and sham) or six (rofecoxib and gabapentin) rats per group. Representative of two separate experiments.

Transection of the medial meniscus in the rat has been previously demonstrated by Bendele[14] to result in the rapid (<3 weeks) degeneration of cartilage. Histologically, the initial damage to the cartilage, as defined by fibrillation and loss of proteoglycan, is localized to the medial side. In the latter stages, there is significant cartilage damage and, in some cases, complete loss of the cartilage layer with subsequent exposure of the subchondral bone. Large medial tibial plateau osteophytes are also present by 3 weeks post surgery (Fig. 5). Alterations to the subchondral bone may be noted at approximately 7 days post surgery and continue throughout the duration of the model. The similarity of the lesions seen in the rat MMT model to those noted in the human condition provide the rationale for examining the behavioral responses in the rat following transection of the medial meniscus. These histologic similarities, coupled with the ability of commonly utilized therapeutic analgesics to inhibit changes in HPWD and mechanical allodynia (secondary hyperalgesia/allodynia), suggest that the rat MMT model has potential value for use in the identification of novel approaches designed to decrease the clinical symptoms associated with OA, as well as identifying targets to alter the associated structural changes to the cartilage and bone.

Change in HPWD was utilized as the primary index of joint pain in the present study. We and other investigators have previously demonstrated that this approach offers a direct, rapid method by which to test the ability to bear weight in a defined joint[15,23,24]. Transection of the medial collateral ligament and medial meniscus resulted in a time-dependent increase in the weight-bearing differential between the arthritic knee and the contralateral control knee (Fig. 1). The increased change in HPWD continued throughout the duration of the 3-week protocol which, coupled with histologic changes, is indicative of a chronic OA-like pain. It should be noted that sham-operated animals also demonstrated a slight increase in HPWD on days 3 and 7 post surgery, although to a much lesser degree than that noted for arthritic animals. These changes, however, were not significantly different from pre-surgery measurements and it is hypothesized that the alterations in weight distribution in sham-operated animals may be due to the effects of the surgery and a subsequent inflammatory response at the surgical site. Inflammatory cells, including neutrophils and monocytes, have been shown to possess the capacity to release a number of soluble mediators that can directly stimulate primary afferent neurons. Histologic analysis of both the arthritic and contralateral control joints showed that, at 3 days post surgery, there is a degree of inflammation present, composed primarily of granulation tissue and fibrosis with few mononuclear cell infiltrates (data not shown). These histologic changes were noted in both arthritic and sham-operated control joints and are likely associated with resolution of the surgical incision. The inflammatory response, as characterized by the lack of any cell infiltrate, had largely resolved by 7 days post surgery. MCL sham rats also exhibited a small increase in HPWD 3 days post surgery, which remained slightly elevated through day 21 and was not significantly different from sham rats. This increase may be due in part to a slight destabilization of the joint resulting from the MCL transection.

Recently, Fernihough *et al.*[25] described a model of surgically induced OA pain generated by transection of the meniscus while sparing the medial collateral ligament. In contrast to our findings, Fernihough *et al.*[25] did not demonstrate an appreciable difference in HPWD with their meniscal tear model. The possibility exists that transection of the medial collateral ligament, as was done in our studies, directly results in loss of joint stability and a subsequent change in HPWD. To address this question, we conducted a number of studies to directly examine the effect of medial collateral ligament transection on HPWD. It was found that transection of the ligament, in the absence of alterations to the meniscus (MCL sham), did not significantly alter HPWD as compared to sham animals (Fig. 1). It is also possible that in order to elicit a significant change in HPWD both the medial collateral ligament and meniscus need to be transected as was done in this study. Further, it is feasible that strain and/or gender differences may play a role in the divergence of HPWD results. A number of previous studies have demonstrated gender and strain-dependent differences in nociceptive sensitivity[26–28]. Both studies utilized male rats, however, our study used the Lewis strain while Fernihough *et al.*[25] used Wistar rats. Lewis rats have a defective HPA axis, which could potentially lead to increased pain compared to Wistar rats due to decreased levels of circulating glucocorticoids[29].

OA pain is of multifactorial origin, and is now thought to be composed of both peripheral and central nervous system components. While the pain that is associated with OA is primarily localized to the joint, it is becoming increasingly apparent that a number of patients exhibit increased nociception to adjacent or even remote areas of the body[8–10]. This secondary hyperalgesia/allodynia is primarily the result of central sensitization[11–13]. The increase in afferent signaling from the joint nociceptors to the spinal cord neurons results in increased sensitivity of the spinal cord neurons to input from the joint, rendering the spinal cord neurons hyperexcitable. Secondary hyperalgesia/allodynia occurs when the sensitized spinal cord neurons exhibit enhanced responses to stimuli from non-injured areas of the leg[30]. While weight bearing is a suitable behavioral determinant for joint pain, it does not measure all aspects of pain that can be associated with OA, such as secondary hyperalgesia/allodynia. We therefore decided to characterize the rat

MMT model using other behavioral assessments for pain in order to determine if a secondary hyperalgesia/allodynia component was present in this model. While multiple types of mechanical hyperalgesia/allodynia exist, only two types are associated with secondary hyperalgesia/allodynia: punctate hyperalgesia (mechanical allodynia; von Frey monofilament test) and stroking hyperalgesia (cotton swab test)[31–33]. In this study we tested punctuate hyperalgesia (von Frey monofilament test).

The von Frey monofilament test was used to examine the MMT model for a secondary allodynia component. Mechanical allodynia was assessed in the plantar region of the ipsilateral hind paw of MMT-operated rats using VFH monofilaments [Fig. 2(A)]. It is interesting to note that, similar to the human condition, not all of the MMT-operated rats exhibited secondary hyperalgesia [Fig. 2(B)]. The incidence of allodynic response in the MMT rats increased as a function of time, with 70–80% of the rats exhibiting secondary allodynia by 14–21 days post surgery. According to several studies conducted in which OA patients were evaluated for secondary hyperalgesia/allodynia, only a subset of patients experienced decreased pain thresholds away from the initial location of the joint pain[8,9,10,34]. Histologic analysis did not reveal appreciable differences in the cartilage, subchondral bone or the degree of synovial inflammation between those animals that exhibited secondary hyperalgesia vs those that did not. The results suggest that another underlying pathophysiologic mechanism is responsible for the presence/absence of secondary hyperalgesia.

Two additional tests were employed to examine referred pain in the ipsilateral hind paw. These tests were for thermal hyperalgesia and blunt pressure mechanical hyperalgesia. Thermal hyperalgesia was not present in any of the MMT or sham-operated rats (Fig. 3). This observation concurs with previously published literature in which secondary hyperalgesia was evaluated after capsaicin injury[35] or heat injury[36,37] to skin. In these studies it was concluded that secondary hyperalgesia is characterized by hyperalgesia to mechanical, but not heat stimuli. Similar findings in human patients have been observed by Bradley *et al.*[10] demonstrating that there was no measurable difference between OA patients and control subjects to heat stimulation at the same sites. Interestingly, MMT-operated rats did not exhibit blunt pressure (Randall–Selitto test) mechanical hyperalgesia either (Fig. 4). Our findings are in accord with Fernihough *et al.*[25] in which blunt pressure mechanical hyperalgesia was not observed in their model of surgical meniscal tear in rats. These observations are also consistent with a separate study in human hairy skin by Koltzenburg *et al.*[38] suggesting that blunt pressure hyperalgesia is not present in the zone of secondary hyperalgesia.

In order to pharmacologically validate this model, groups of animals were treated with the COX-2 specific inhibitor, rofecoxib, or the novel anticonvulsant drug, gabapentin, which has been shown to effectively reduce neuropathic pain in several animal models[39–42]. Oral administration of these compounds significantly attenuated the change in HPWD in a manner consistent with the expected time course and dose of the compound (Fig. 6). The positive responses noted with these analgesics indicate that the model responds favorably to pharmacologic intervention in a manner similar to that seen in the human. Further, these data also provide evidence that the changes in HPWD are not due exclusively to destabilization of the knee joint since one would not expect these agents to reverse destabilization-induced changes in weight distribution. In a subset of animals that were exhibiting secondary hyperalgesia, as assessed by VFH monofilaments, only gabapentin was able to alleviate mechanical allodynia (Fig. 7). It is not unexpected that a single, orally administered dose of rofecoxib did not effectively inhibit secondary allodynia as COX-2 inhibitors are considered to be generally ineffective when it comes to relieving neuropathic or centrally-mediated pain[43]. It is possible to alleviate neuropathic pain and central sensitization with a COX-2 inhibitor, but usually only by local administration or chronic administration over a period of several days[44–46]. These data suggest that the rat MMT model may have predictive value for evaluating novel pharmacologic approaches for the treatment of OA pain characterized by both primary and secondary hyperalgesia.

In summary, the MMT model of joint pain is a surgically induced model that effectively mimics both the structural and symptomatic changes associated with human OA. This close association, both morphologically and symptomatically, to the human condition, coupled with the ability of the model to respond to conventional pain-relieving therapies, indicates that the MMT model may be useful for the development of pharmacologic interventions for the treatment of the symptoms associated with OA. Additionally, the availability of such models that closely mimic the disease may provide important information as to the molecular mechanisms involved in the genesis of OA pain.

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