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Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis

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Summary

Objective: To describe an *in vivo* model in the rat in which change in weight distribution is used as a measure of disease progression and efficacy of acetaminophen and two nonsteroidal anti-inflammatory drugs (NSAIDs) in a model of monosodium iodoacetate (MIA)-induced osteoarthritis (OA).

Methods: Intra-articular injections of MIA and saline were administered to male Wistar rats (175–200 g) into the right and left knee joints, respectively. Changes in hind paw weight distribution between the right (osteoarthritic) and left (contralateral control) limbs were utilized as an index of joint discomfort. Acetaminophen and two archetypal, orally administered NSAIDs, naproxen and rofecoxib, were examined for their ability to decrease MIA-induced change in weight distribution.

Results: A concentration-dependent increase in change in hind paw weight distribution was noted after intra-articular injection of MIA. Both naproxen and rofecoxib demonstrated the capacity to significantly ($P<0.05$) decrease hind paw weight distribution in a dose-dependent fashion, indicating that the change in weight distribution associated with MIA injection is susceptible to pharmacological intervention.

Conclusion: The determination of differences in hind paw weight distribution in the rat MIA model of OA is a technically straightforward, reproducible method that is predictive of the effects of anti-inflammatory and analgesic agents. This system may be useful for the discovery of novel pharmacologic agents in human OA.

Key words: Osteoarthritis, Monosodium iodoacetate-induced arthritis, NSAIDs, Acetaminophen.

Introduction

Osteoarthritis (OA) is the most common form of joint disease with over one-half of all people older than 65 years of age demonstrating radiographic changes of OA in the knees[1]. OA is primarily noted in the weight-bearing joints (i.e., knees, hips) and involves both the degeneration of articular cartilage as well as changes to the subchondral bone[2], however, the pathophysiology behind the structural changes associated with the disease is complex and poorly understood.

Currently, there are no commercially available drugs definitively proven to alter the natural progression of this disease in the clinic. 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]. Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for the treatment of OA pain. The long-term use of these agents, however, may be accompanied by a number of undesirable effects including suppression of platelet aggregation and disruption of the gastrointestinal mucosa, the latter effect frequently leading to upper gastrointestinal erosions and ulceration[8]. Intra-articular injections of corticosteroids have been demonstrated to relieve inflammation and associated pain, but their effect is of short duration and is therefore employed infrequently[5]. For these reasons, it is apparent that the need exists for new therapeutic agents with the ability to attenuate OA-associated pain.

Studies designed to identify novel inhibitors of pain associated with OA have been hampered by the lack of a rapid, reproducible animal model that closely mimics both the pain and structural changes associated with the disease. Both surgical and spontaneous animal models of OA often require extended periods of time (months) to present with the classical features of OA including pain and changes in joint morphology[9]. An animal model, therefore, that replicates the behavioral, biochemical and pharmacological parameters of OA-associated joint discomfort would be of great value.

Injection of the metabolic inhibitor, monosodium iodoacetate (MIA; iodoacetic acid), into joints inhibits glyceraldehye-3-phosphate dehydrogenase activity in chondrocytes, resulting in disruption of glycolysis and eventual cell death[10,11]. The progressive loss of

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Fig. 1. Incapacitance tester: rats are placed in an angled plexiglass chamber and are allowed to acclimate for a period of 5–10 min before any readings are taken. When the rat is in the proper position (both hind paws on their respective force plates, both front paws on the plexiglass ramp, and facing forward) three, 5-s readings are recorded.

chondrocytes results in histologic and morphologic changes to the articular cartilage, closely resembling those seen in human OA[12]. In addition, the model has been utilized by a number of investigators to test pharmacologic agents for their ability to preserve cartilage structure[12–14].

In the present study, a rapid, reproducible rodent model of OA joint discomfort is described. Injection of MIA into the femorotibial joint of a rat resulted in a significant increase in change in hind paw weight distribution, used as an index of joint discomfort. Associated with the changes in weight distribution were histologic changes consistent with those noted in human OA, including loss of viable chondrocytes, loss of proteoglycan, and cartilage fibrillation. In addition, three commonly utilized therapies for OA pain, naproxen, acetaminophen, and the COX2 specific inhibitor, rofecoxib, significantly decreased changes in weight distribution in a dose-dependent fashion. It is concluded that the rat MIA model is a rapid, technically straightforward model that closely mimics the behavioral, pathologic and pharmacologic features associated with human OA.

Methods

INDUCTION OF OSTEOARTHRITIS

The procedures used in this study were in accordance with the guidelines of the Pfizer Animal Care and Use Committee. Male Wistar rats (175–200 g; Charles River, Wilmington, MA) were housed in solid bottom cages with corncob bedding. Animals were fed standard rat chow with water available *ad libitum*. For induction of MIA-induced arthritis, rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL) and given a single intra-articular injection of 0.1, 0.3, 1, or 3 mg MIA (Sigma, St. Louis, MO; cat #I2512) through the infrapatellar ligament of the right knee[15,16]. MIA was dissolved in physiologic saline and administered in a volume of 50 µl using a 26 gauge, 0.5 inch needle. A Hamilton PB 600-1 repeating dispenser with a 700 series luer tip microliter syringe (model 750; Hamilton Company, Reno, NV) was used for precise injection of an automated volume. The left contralateral control knee was injected with 50 µl of physiologic saline. Basal readings were established using a group of sham rats that were injected with saline in both knees.

ASSESSMENT OF CHANGE IN HIND PAW WEIGHT DISTRIBUTION

Changes in hind paw weight distribution between the right (osteoarthritic) and left (contralateral control) limbs were utilized as an index of joint discomfort in the osteoarthritic knee. An incapacitance tester (Linton Instrumentation, Norfolk, UK) was employed for determination of hind paw weight distribution. Rats were placed in an angled plexiglass chamber positioned so that each hind paw rested on a separate force plate (Fig. 1). 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 hind paw weight distribution was calculated by determining the difference in the amount of weight (g) between the left and right limbs. Results are presented as either the difference in weight bearing between the left (contralateral control) limb and right (osteoarthritic) limb or as the percent difference between baseline reading and post-treatment as calculated by the following equation:

$$(1-(\text{mean } \Delta \text{ weight of treated group}/\text{mean } \Delta \text{ weight of vehicle group}))\times(100)$$

HISTOLOGIC ANALYSIS

Histologic changes were assessed in rats that had received 1 mg of MIA. Rats were euthanized by CO_2 at the time indicated. Soft tissue was removed from the right (osteoarthritic) and left (contralateral control) legs. 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 either hematoxylin–eosin or toluidine blue according to previously published methods[17].

To assess the degree of synovial inflammation a relative scoring system was used. Each joint was scored on a scale of 0–4 as follows: 0=no inflammation, 1=minimal, 2=mild, 3=moderate, and 4=marked. 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).

COMPOUND ADMINISTRATION

Naproxen (sodium salt; Sigma–Aldrich; St. Louis, MO) was dissolved in hydroxypropylmethylcellulose (HPMC) vehicle (0.5% HPMC+0.2% Tween 80). Rofecoxib (Merk and Co., Inc., Whitehouse Station, NJ) and acetaminophen tablets (Tylenol; paracetamol; McNeil Consumer and Speciality Pharmaceuticals, Fort Washington, PA) were pulverized and suspended in HPMC vehicle. A separate vehicle group in which rats were dosed with HPMC alone was run in conjunction with the drug-treated groups. Fourteen days post-MIA injection, changes in hind paw weight distribution were determined, as described previously, to establish a baseline pain reading. The rats (N=8 per group) were then given a single dose (oral gavage) of vehicle, naproxen, acetaminophen or rofecoxib at 0.1, 1.0, 10, or 100 mg/kg. Changes in hind paw weight distribution were determined 2, 4 and 6 h post-compound administration.

STATISTICAL ANALYSIS

All data are expressed as mean±S.E.M. All statistical tests were tested at the 0.05 level of statistical significance. A standard dose response (Hill) model[18] was fit to estimate the EC_{50} of MIA for the change in hind paw weight distribution dose response. Each mean response for a concentration of MIA was compared to the mean saline response by Dunnett's test[19].

For studies examining naproxen and rofecoxib, median percent inhibitions in hind paw weight distribution for each of the doses were compared with zero percent inhibition via the Wilcoxon signed-rank test[20] adjusted for multiplicity of statistical testing with Hochberg's procedure.

Changes in hind paw weight distribution over hours 2, 4, and 6 were adjusted for pre-dose (baseline) levels and modeled as a function of treatment, time and the combined effect of treatment and time (treatment by time interaction) with a repeated measures analysis. The mean changes in hind paw weight distribution over time for the treatment groups were compared with the mean change in hind paw weight distribution over time in the vehicle controls via the Dunnett–Hsu multiple comparisons procedure[21].

Results

MIA-INDUCED JOINT DISCOMFORT

Injection of MIA into the right knee resulted in a concentration- and time-dependent increase in joint discomfort as defined by change in hind paw weight distribution (Figs. 2 and 3). All doses of MIA significantly (N=8 rats per group; representative of two separate experiments; P<0.05) increased the change in hind paw weight distribution 14 days post-injection as compared to sham-injected rats (saline in both knees; Fig. 2). The maximal degree of weight shift was noted using a concentration of 1 mg/joint. The EC_{50} was determined to be 0.54 mg using the Hill model of dose response. No further increase in weight shift was noted when the concentration was increased to 3 mg/joint, therefore, 1 mg/joint was selected as the lowest maximally effective dose for further studies involving histopathology and pharmacologic response.

The temporal pattern of MIA-induced change in weight bearing was determined using concentrations ranging from 0.1 to 3 mg/joint (Fig. 3). The same groups of rats were followed throughout the course of the experiment (1, 3, 7, and 14 days post-injection). The sham (saline-injected) hind paw weight differential was found to be 0±1 g (N=6 rats; representative of three separate experiments). A biphasic pattern of joint discomfort was noted in all of the MIA concentrations tested except for the 0.1 mg/knee. The first phase of increased change in weight bearing was achieved at a maximal level 1 day post-MIA followed by a decrease at day 3. By day 7 post-injection, the change in hind paw weight distribution had again increased to the maximal level of weight-bearing differential and remained elevated throughout the remainder of the 14-day protocol. The change in weight distribution noted at the 14-day time point was not significantly different from that noted at 7 days post-MIA injection. The degree of joint pain remained consistent even when the duration of the protocol was extended out to 6 weeks post-MIA (data not shown).

Changes in hind paw weight distribution were determined in normal rats vs saline and MIA-injected rats for the purpose of demonstrating the reproducibility of the incapacitance tester (Fig. 4). MIA-injected rats (1 mg; N=8), saline-injected (N=6) and normal (no injection; N=12) rats were measured as described previously. After acclimating the rats to the plexiglass box, three, 5-s readings were taken on the incapacitance tester, designated repeat numbers 1, 2, and 3. These three readings are normally averaged together to obtain the final change in hind paw weight distribution, however, in order to examine the variability between each repeat, all the three repeats were represented on the graph as the mean of each repeat±S.E.M. for each group of rats (MIA, saline, and normal). The three readings were consistent for all three groups of rats tested. In addition, the saline-injected rats did not demonstrate a change in weight distribution and were similar to normal rats.

MIA-INDUCED HISTOLOGIC CHANGES

The early (days 1–3) histologic changes to MIA-injected joints (1 mg) exhibited alterations to both the surrounding synovium and to the articular cartilage. As shown in Fig. 5A, the degree of inflammation was maximal at day 1 followed by a rapid decrease. By day 7, the inflammation had largely resolved. Histologically, a robust inflammatory response was noted 1 day following administration of MIA, which

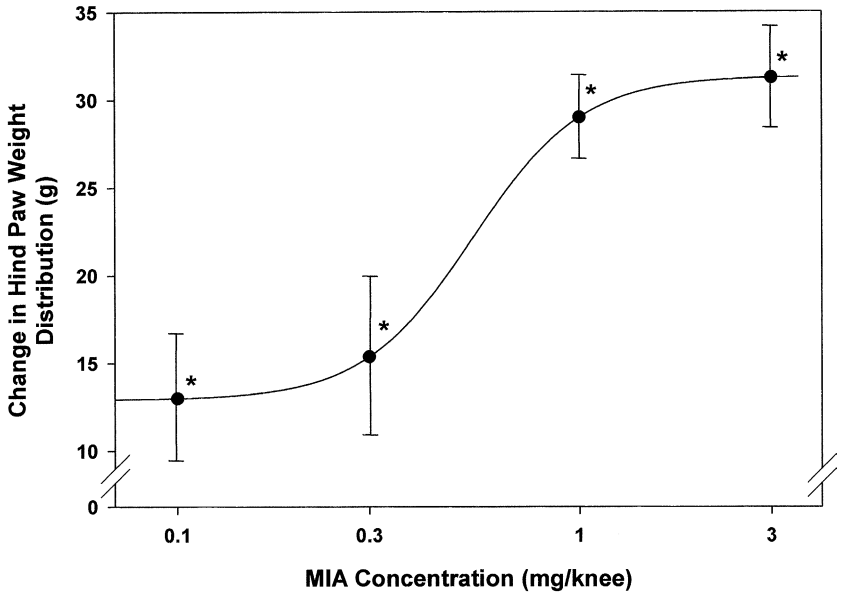

Fig. 2. MIA concentration–response relationship: effect of MIA injection into the rat knee. Rats injected with 0.1, 0.3, 1.0 or 3.0 mg of MIA in the right (osteoarthritic) knee and saline in the left (contralateral control) knee. Change in hind paw weight distribution (weight bearing) was assessed by use of an incapacitance tester 14 days after MIA injection. A fifth group of rats was injected with saline in both knees for determination of baseline weight distribution. Statistically significant differences were determined by one-way ANOVA followed by Dunnett's multiple comparison's test (*P<0.05) vs saline-injected rats. N=8 rats (MIA-injected) or 6 rats (sham) per group. Representative of two separate experiments.

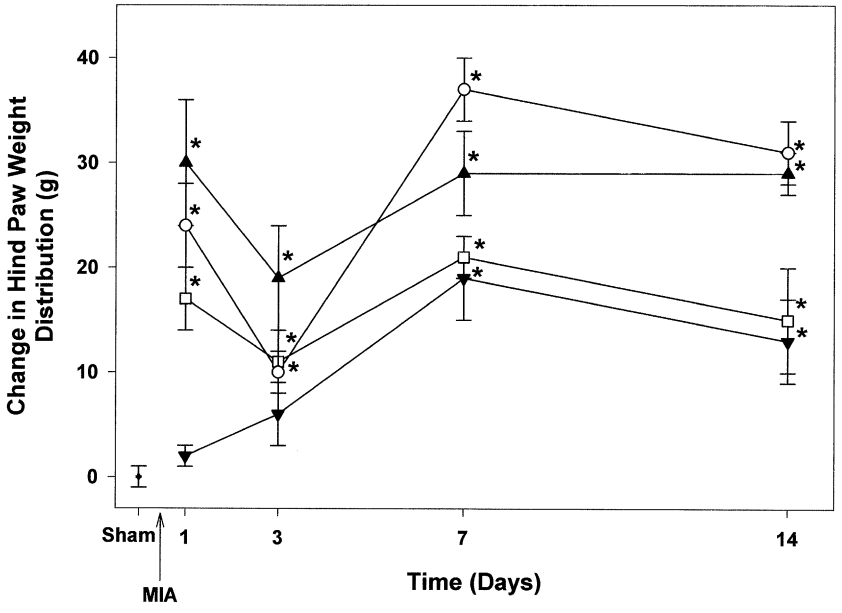

Fig. 3. Temporal pattern of MIA-induced joint pain. Rats injected with 0.1, 0.3, 1.0 or 3.0 mg of MIA in the right knee and saline in the left knee were assessed on an incapacitance tester 1, 3, 7, and 14 days after injection for change in hind paw weight distribution. Sham rats received saline in both knees. Statistically significant differences were determined using the Hochberg's procedure (*P<0.05 vs saline group). ○=3.0 mg MIA, ▲=1.0 mg MIA, □=0.3 mg MIA, and ▼=0.1 mg MIA. N=8 rats (MIA-injected) or 6 rats (sham) per group. Representative of two separate experiments.

began to subside by 3 days post-injection [Fig. 5(B)]. When present, the inflammation was characterized by expansion of the synovial membrane by proteinaceous edema fluid and fibrin with infiltrating macrophages, neutrophils, plasma cells and lymphocytes. By day 7 and for the remainder of the protocol, inflammation within the synovium and surrounding tissue had largely resolved.

Figure 6A and B represents a saline-injected contralateral control knee with intact, healthy cartilage and no loss of chondrocytes or proteoglycan. By day 7 post-MIA injection

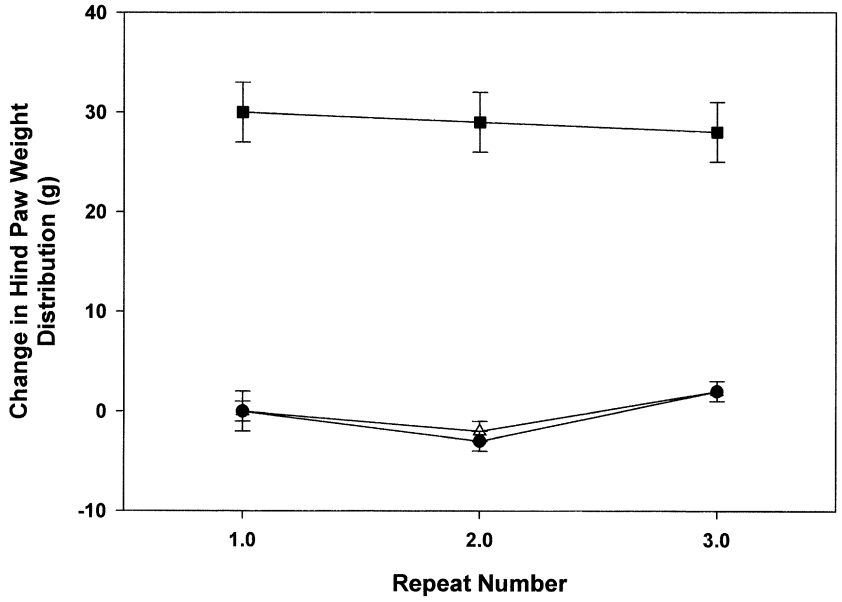

Fig. 4. Evaluation of the reproducibility of the incapacitance tester to evaluate change in hind paw weight distribution. Rats injected with either, 1 mg MIA in the right knee and saline in the left knee (MIA-injected rats; ■), saline in both knees (sham rats; △), or no injection in either knee (normal rats; ●). Change in hind paw weight distribution (weight bearing) was assessed by the use of an incapacitance tester 14 days after MIA or saline injection. Each data point is one repetition, which is the mean of a single 5-s reading (representative of three experiments). *N*=8 rats (MIA-injected), 12 rats (normal), or 6 rats (saline-injected; sham).

[Fig. 6(C and D); MIA=1 mg] there was moderate collapse of the cartilaginous matrix with complete loss of cellular detail. It should be noted that cartilage damage primarily affected only the medial tibial plateau.

Rats sacrificed 14 days post-injection [Fig. 6(E and F); MIA=1 mg] were found to have 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. In less affected areas, there was partial damage that extended approximately one third of the thickness of the cartilage. At the transition zone between damaged and viable cartilage, chondrocytes were isolated, variably sized and tightly aggregated in clusters (chondrones). Furthermore, some samples demonstrated visible marginal changes related to osteophyte growth.

EFFECT OF ARCHETYPAL ANALGESICS

Experiments designed to determine the effects of archetypal analgesics on MIA-induced weight-bearing differential utilized an MIA concentration of 1 mg/knee and were conducted on day 14 post-MIA injection. Naproxen, rofecoxib, and acetaminophen significantly decreased change in hind paw weight distribution in a dose-dependent manner (Fig. 7). Naproxen significantly decreased change in hind paw weight distribution at a dose of 1 mg/kg. In contrast, rofecoxib and acetaminophen required a dose of 10 mg/kg to achieve a significant decrease in weight differential.

The duration of action between naproxen, rofecoxib, and acetaminophen (Fig. 8) were similar. All three analgesics significantly decreased change in hind paw weight distribution for the duration of the 6-h study. No changes in physical or behavioral effects (i.e., drowsiness or agitation) were noted at any dose of the compounds. In addition, compounds did not show any effect on cartilage structure as compared to vehicle-treated rats (data not shown).

Discussion

The disease processes that lead to the structural changes and pain associated with OA are complex and poorly understood. Studies to elucidate the pathophysiology of OA and identify therapeutic interventions to treat the disease have been hampered by the lack of a rapid, reproducible animal model that mimics both the histologic changes and symptoms (i.e., pain) associated with the disease. The present study describes a rat model of OA-associated joint discomfort that allows both the rapid analysis of the mechanisms involved with OA pain and the evaluation of therapeutic interventions to treat the joint discomfort associated with the disease.

This model involves the intra-articular injection of MIA into the right femorotibial joint of Wistar rats. The MIA model is well established and has been utilized in a number of different species including horses, chickens, mice, guinea pigs and rats[11,22–24]. Intra-articular injection of MIA results in an inhibition of glyceraldehye-3-phosphate, ultimately resulting in disruption of chondrocyte metabolism and eventual cell death[10,11]. This progressive loss of chondrocytes results in histologic changes that resemble several of the salient features noted in human OA, including collapse of the cartilaginous matrix, fibrillation, and osteophyte formation. The amount of MIA utilized in this study was chosen based on both the degree of joint discomfort and the histologic changes. Interestingly, histologic examination revealed that the MIA primarily targets the articular cartilage of the medial tibial plateaus. This is supported by the fact that the rat is a medial weight-bearing animal and is also consistent with a previous study by Williams and Brandt[25] demonstrating a requirement for weight bearing on the affected joint for lesion development. Based on these findings, it is apparent that the model appears to reproduce the early lesions of OA, similar to those observed in the human disease. Due to these

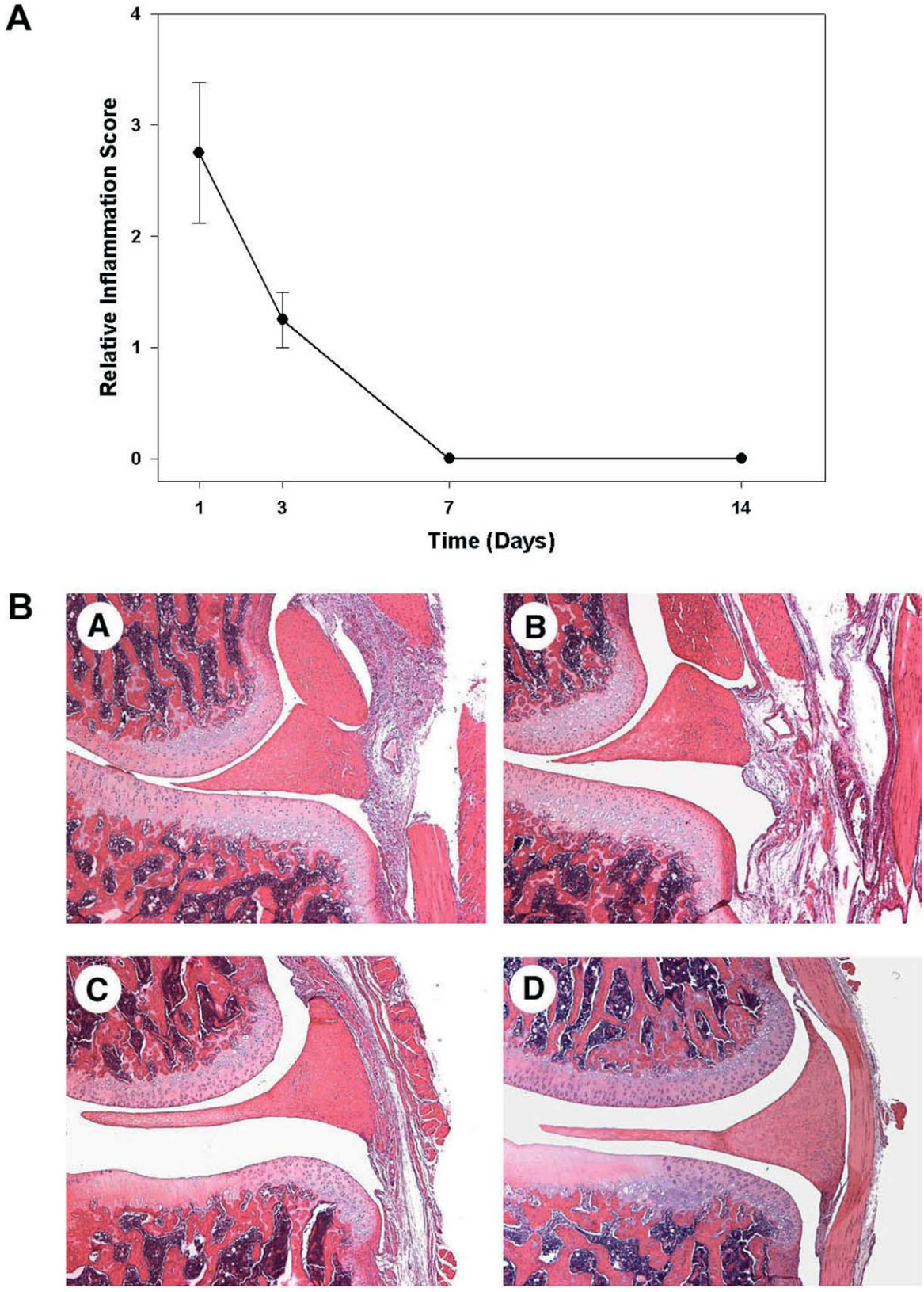

Fig. 5. Inflammation in the rat knee due to MIA-injection. **A.** Inflammation was assessed and scored 1, 3, 7, and 14 days post-MIA (1 mg; $N=4$ rats per time point). **B.** Frontal sections of the medial aspect of rat knee joints injected with 1 mg MIA; (A) 1 day post-MIA injection, showing significant expansion of the synovial membrane with a large amount of cellular infiltrate; (B) 3 days post-MIA injection, synovial membrane expansion and cellular infiltrate still present to a lesser degree; (C) 7 days post-MIA injection, little to no changes present in the synovium; (D) 14 days post-MIA injection, no presence of inflammation. The sections were stained with hematoxylin and eosin. Magnification×10.

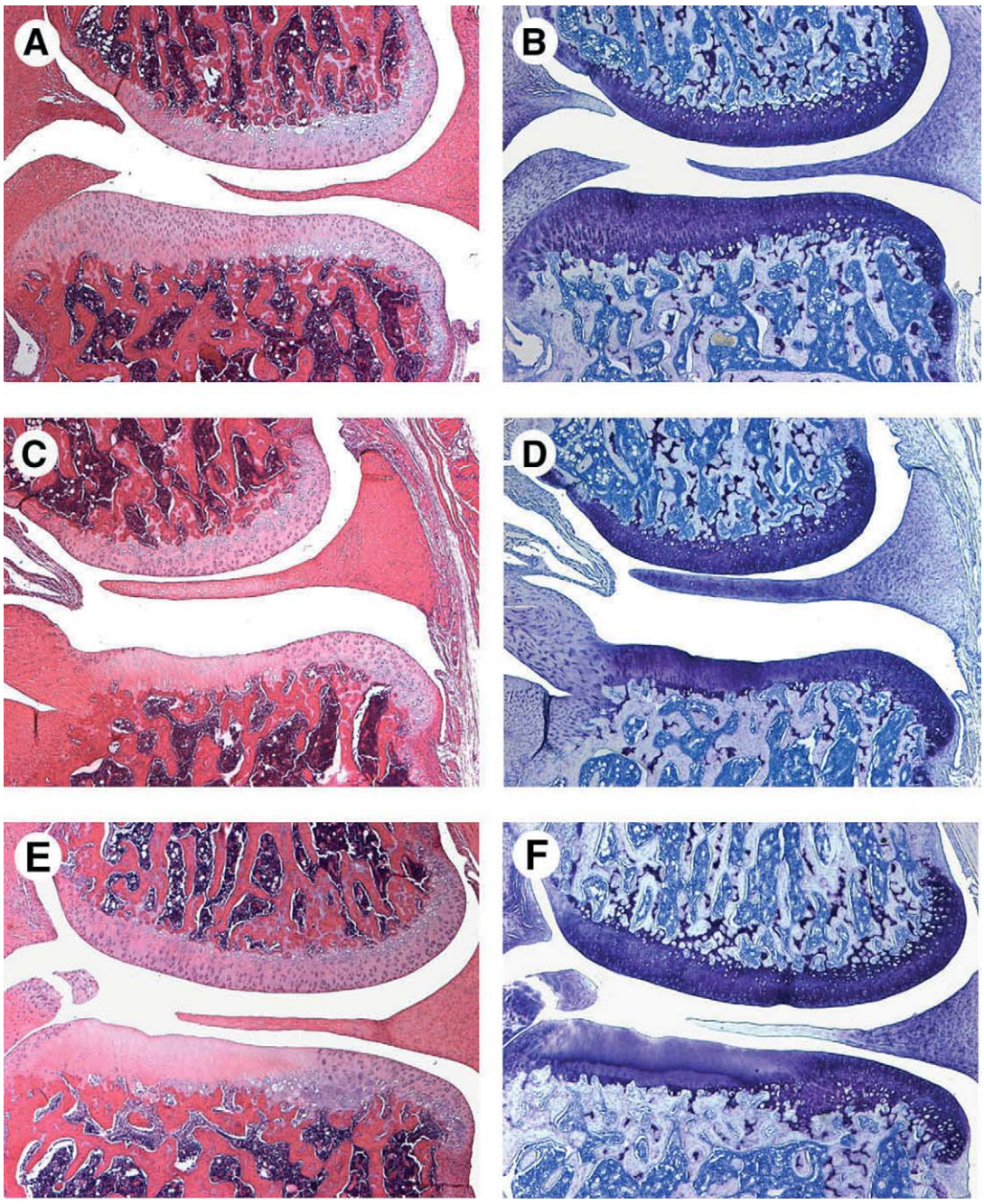

Fig. 6. Time course of histologic changes and proteoglycan loss after injection with 1 mg MIA. Frontal sections of the medial aspect of rat knee joints were stained with hematoxylin and eosin (A–E) or toluidine blue (B–F). (A and B) Saline-injected left knee demonstrating normal healthy cartilage. (C and D) 7 days post-MIA injection. A lesion covers half of the medial tibial plateau and extends well into the deep zone. Chondrocyte loss is readily apparent along with a slight loss of proteoglycan. (E and F) 14 days post-MIA injection. A significant lesion covers two third of the medial tibial plateau. Chondrocyte loss is complete to the deep zone and significant proteoglycan loss can be detected all the way to the tidemark. Magnification×10. Representative of a minimum of three separate experiments (N=8 rats per study).

histologic similarities, various adaptations of the model have been utilized to examine both the pathophysiology of OA and a number of pharmacologic agents including NSAIDs, corticosteroids and inhibitors of matrix metalloproteinases[10,12–14]. These studies, however, have relied on histologic/morphologic end points for determination of efficacy and do not address the ability of these agents to modify the symptoms associated with OA. The model outlined in the present study describes a rapid, technically straightforward model that closely mimics the pathologic and behavioral features associated with human OA.

Several previous studies have demonstrated significant changes in gait and mobility following injection of MIA[16,26]. It is apparent, however, that these behavioral paradigms do not lend themselves to the rapid analysis of a large number of animals. In the present study, we utilized the differential in weight bearing between the right (osteoarthritic) and left

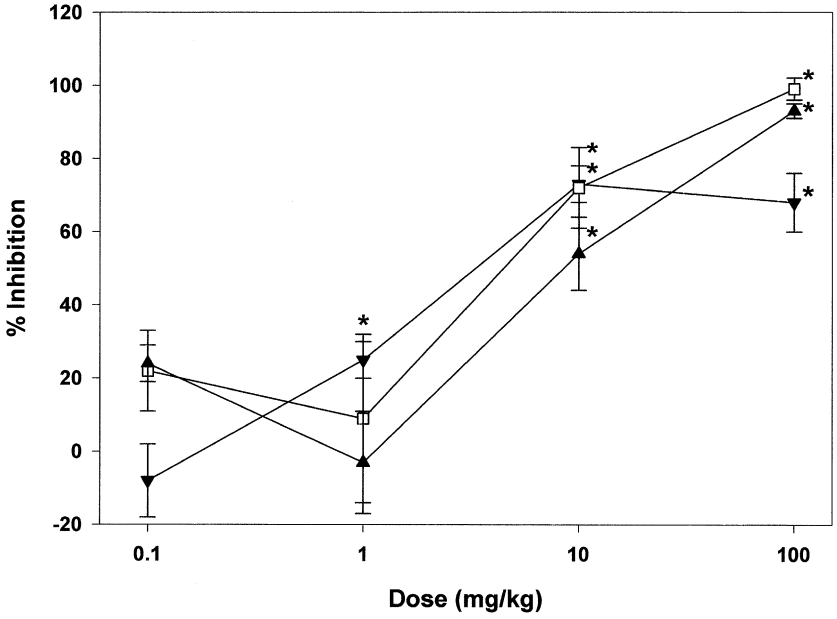

Fig. 7. Rofecoxib, naproxen, and acetaminophen dose–response: percent inhibition of change in hind paw weight distribution. Rats were injected with 1 mg of MIA in the right knee and saline in the left knee on day 0. On day 14, rats were assessed on an incapacitance tester for change in hind paw weight distribution and then administered 0.1, 1, 10, or 100 mg/kg rofecoxib, naproxen, or acetaminophen (PO). Vehicle control rats received HPMC. Two hours later, the rats were reassessed. Statistically significant differences were determined using a Wilcoxon signed-rank test followed by a Hochberg's multiple comparison procedure (*P<0.05). □=rofecoxib, ▼=naproxen, and ▲=acetaminophen. N=8 rats per group. Representative of a minimum of two separate experiments.

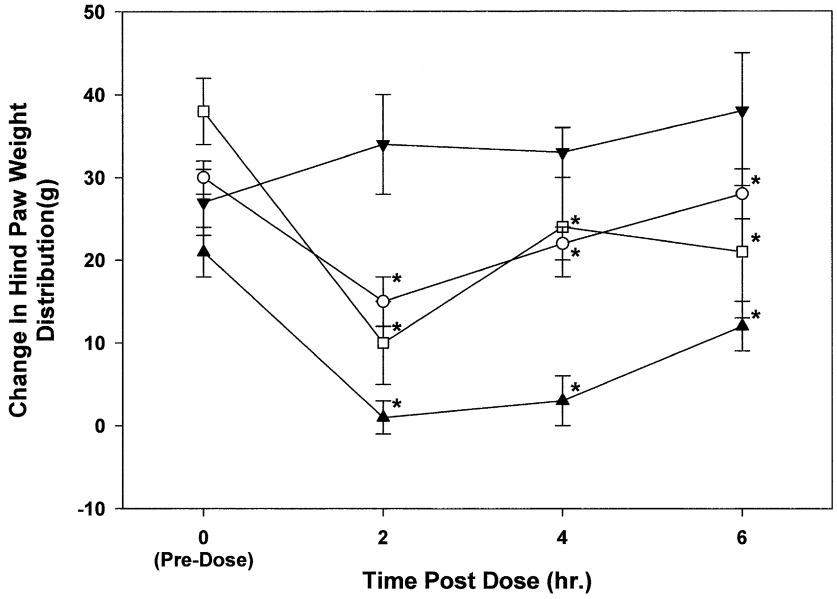

Fig. 8. Duration of effect of rofecoxib, naproxen and acetaminophen on change in hind paw weight distribution. Rats were injected with 1 mg of MIA in the right knee and saline in the left knee on day 0. On day 14 the rats were assessed on an incapacitance tester for change in hind paw weight distribution and then administered 10 mg/kg rofecoxib, naproxen, or acetaminophen (PO). The rats were reassessed 2, 4 and 6 h later. Statistically significant differences were determined using a repeated measures analysis. A statistically significant difference existed between the mean change from pre-dose pain response in the control subjects and the mean change from pre-dose pain responses in the rofecoxib, naproxen and acetaminophen groups over hours 2–6 (*P<0.05). ▼=vehicle, □=rofecoxib, ▲=naproxen, and ◯=acetaminophen. N=8 (vehicle and rofecoxib), 4 (naproxen), or 6 (acetaminophen) rats per group. Representative of a minimum of two separate experiments.

(contralateral control) limbs as an indication of joint discomfort. This approach has been shown previously to be a rapid and objective method by which to assess both arthritic and bone cancer pain[27,28]. Administration of MIA into the right femorotibial joint of Wistar rats resulted in a time- and concentration-dependent increase in joint discomfort

(Figs. 2 and 3). When administered at 1 mg per joint, change in weight distribution was found to reach a maximal level within 7 days and maintained this level for up to 6 weeks (Fig. 2; 6 week data not shown). Interestingly, the temporal changes in weight distribution noted upon addition of MIA demonstrated a biphasic pattern as described in the results (Fig. 3). These findings are in accordance with those of Guingamp *et al.* who previously noted a similar biphasic pattern using biotelemetric assessment of the spontaneous nocturnal mobility of rats injected with MIA[16]. Coincident with the first phase of increased change in hind paw weight distribution was a substantial inflammatory response within the affected joint [Fig. 5(A)]. Closely tracking the first phase of increased weight distribution was the degree of inflammation, which was maximal at day 1 and found to decrease by day 3. By day 7 the inflammatory response had largely resolved, although there were areas of extensive cartilage damage [Figs. 5(B) and 6(C and D)]. Thus, it is possible that the first phase of the joint discomfort may have been mediated by the inflammatory infiltrate within the synovium while the second phase (days 7–14) may be due to structural changes within the joint (i.e., alterations to the subchondral bone as noted previously by both Janusz *et al.* and Guzman *et al.*)[12,29].

One of the primary lines of support for the validity of the model in the setting of OA pain is provided by the ability of acetaminophen[30] and the NSAIDs naproxen[5] and rofecoxib[31] to alleviate the joint discomfort following an injection of MIA. Oral administration of acetaminophen, the COX-1/2 inhibitor, naproxen, or the COX-2 specific inhibitor, rofecoxib, decreased the change in hind paw weight distribution dose-dependently. These compounds have been demonstrated to provide a clinically significant decrease in OA-associated pain in both preclinical animal models and in the human. It should be emphasized that the respective doses of these compounds were not optimized for exposure and therefore, one should be cautioned against making a direct comparison. The ability of these compounds to attenuate joint discomfort in this model indicates that the joint discomfort observed after MIA injection is susceptible to pharmacologic intervention by a commonly utilized therapeutic agent.

Several issues pertaining to the relevance of this model to human OA warrant comment. Of importance is the anatomical origin of the MIA-induced joint discomfort. Based on the histologic findings, it is apparent that a significant degree of cartilage damage is present within 7 days post-MIA. Although cartilage loss is one of the characteristic pathologic features of clinical OA and is noted in this model, hyaline cartilage is aneural[2]. It is apparent, therefore, that other, non-cartilage-related mechanisms are responsible for mediating pain in this model. Other locations adjacent to the cartilage, however, are innervated including the synovium, meniscus and ligaments. As mentioned previously, there is a substantial inflammatory response within the synovium early in the pathogenesis of the model, but this has generally resolved by 3 days post-MIA and would therefore not be expected to play a role in mediating joint discomfort at later time points. Recent evidence has indicated that bone marrow lesions/edema may play a role in mediating the pain associated with OA[32] and may, therefore, represent a possible explanation as to the anatomic source of pain in this model. In addition, the contribution of neuropathic pain and other neurologic mechanisms to the development of pain in this model remain to be elucidated.

We conclude that the rat MIA model of OA-like joint discomfort represents a straightforward, technologically feasible *in vivo* model. The model has several advantages to existing paradigms, including rapid throughput and a high degree of reproducibility. The responsiveness of this model to conventional pain-reliving therapies indicates that it may be useful for discerning therapeutic approaches designed to treat the joint discomfort associated with OA.

References

1. Hinton R, Moody RL, Davis AW, Thomas SF. Osteoarthritis: diagnosis and therapeutic considerations. Am Fam Physician 2002;65:841–8.
2. DiPiro JT, Talbert RL, Hayes PE, Yee GC, Matzke GR, Posey LM. Pharmacotherapy: A Pathophysiologic Approach. 2nd edn. Connecticut: Appleton & Lange 1993.
3. Pavelka K, Batterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis. Arch Intern Med 2002;162:2113–23.
4. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis. JAMA 2000;283:1469–75.
5. Todd PA, Clissold SP. Naproxen: a reappraisal of its pharmacology, and therapeutic use in rheumatic diseases and pain states. Drugs 1990;40:91–137.
6. Wildy KS, Wasko MC. Current concepts regarding pharmacologic treatment of rheumatoid and osteoarthritis. Hand Clin 2001;17:321–38.
7. McColl GJ. Pharmacological therapies for the treatment of osteoarthritis. Med J Aust 2001; 175:S108–11.
8. Pavelka K. Treatment of pain in osteoarthritis. Eur J Pain 2000;4(Suppl A):23–30.
9. Bendele A, McComb J, Gould T, McAbee T, Sennello G, Chlipala E, *et al.* Animal models of arthritis: relevance to human disease. Toxicol Pathol 1999; 27:134–42.
10. Kalbhen DA. Chemical model of osteoarthritis—a pharmacological evaluation. Rheumatology 1987; 14:130–1.
11. Van der Kraan PM, Vitters EL, Van de Putte LBA, Van den Berg WB. Development of osteoarthritic lesions in mice by 'Metabolic' and 'Mechanical' alterations in the knee joints. Am J Pathol 1989;35:1001–14.
12. Janusz MJ, Hookfin EB, Heitmeyer SA, Woessner JF, Freemont AJ, Hoyland JA, *et al.* Moderation of iodoacetate-induced experimental osteoarthritis in rats by matrix metalloproteinase inhibitors. Osteoarthr Cartil 2001;9:751–60.
13. Gencosmanoglu BE, Eryavuz M, Dervisoglu S. Effects of some nonsteroidal anti-inflammatory drugs on articular cartilage of rats in an experimental model of osteoarthritis. Res Exp Med 2001;200:215–26.
14. Natchus MG, Bookland RG, De B, Almstead NG, Pikul S, Janusz MJ, *et al.* Development of new hydroxamate matrix metalloproteinase inhibitors derived from functionalized 4-aminoprolines. J Med Chem 2000; 43:4948–63.
15. Dunham J, Hoedt-Schmidt S, Kalbhen DA. Prolonged effect of iodoacetate on articular cartilage and its modification by an anti-rheumatic drug. Int J Exp Pathol 1993;74:283–9.

16. Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P. Mono-iodoacetate-induced experimental osteoarthritis: a dose–response study of loss of mobility, morphology, and biochemistry. Arthritis Rheum 1997;40:1670–9.
17. Carson FL. Histotechnology: A Self-Instructional Text Chicago: ASCP Press 1997.
18. Hill AV. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J Physiol 1910;40:iv.
19. Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 1955;50:1096–121.
20. Hollander M, Wolfe DA. Nonparametric Statistical Method. 2nd edn. Toronto: John Wiley and Sons 1999.
21. Hsu JC. The factor analytic approach to simultaneous inference in the general linear model. J Comput Graph Stat 1992;151–68.
22. Williams JM, Brandt KD. Triamcinolone hexacetonide protects against fibrillation and osteophyte formation following chemically induced articular cartilage damage. Arthritis Rheum 1985;28:1267–74.
23. Penraat JH, Allen AL, Fretz PB, Bailey JV. An evaluation of chemical arthrodesis of the proximal interphalangeal joint in the horse by using monoiodoacetate. Can J Vet Res 2000;64:212–21.
24. Hoedt-Schmidt S, Schneider B, Kalbhen DA. Histomorphological studies on the effect of recombinant human superoxide dismutase in biochemically induced osteoarthritis. Pharmacology 1993;47:252–60.
25. Williams JM, Brandt KD. Immobilization ameliorates chemically induced articular cartilage damage. Arthritis Rheum 1984;27:208–16.
26. Clarke KA, Heitmeyer SA, Smith AG, Taiwo YO. Gait analysis in a rat model of osteoarthrosis. Physiol Behav 1997;62:951–4.
27. Schott E, Berge OG, Angeby-Moller K, Hammarstrom G, Dalsgaard CJ, Brodin E. Weight bearing as an objective measure of arthritic pain in the rat. J Pharmacol Toxicol Methods 1994;31:79–83.
28. Medhurst SJ, Walker K, Bowes M, Kidd BL, Glatt M, Muller M, *et al.* A rat model of bone cancer pain. Pain 2002;96:129–40.
29. Guzman RT, Evans MG, Bove SE, Morenko BJ, Kilgore KS. Mono-iodoacetate induced histologic changes in sub-chondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol, in press.
30. Shamoon M, Hochberg MC. The role of acetaminophen in the management of patients with osteoarthritis. Am J Med 2001;110(Suppl 3A):46S.
31. Lane JM. Anti-inflammatory medications: selective COX-2 inhibitors. J Am Acad Orthop Surg 2002; 10:75–8.
32. Ghosh P, Cheras PA. Vascular mechanisms in osteoarthritis. Best Pract Res Clin Rheumatol 2001; 15:693–709.