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Pain related behaviour in two models of osteoarthritis in the rat knee

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Abstract

Osteoarthritis (OA) is a major healthcare burden, with increasing incidence. Pain is the predominant clinical feature, yet therapy is ineffective for many patients. While there are considerable insights into the mechanisms underlying tissue remodelling, there is poor understanding of the link between disease pathology and pain. This is in part owing to the lack of animal models that combine both osteoarthritic tissue remodelling and pain. Here, we provide an analysis of pain related behaviours in two models of OA in the rat: partial medial meniscectomy and iodoacetate injection. Histological studies demonstrated that in both models, progressive osteoarthritic joint pathology developed over the course of the next 28 days. In the ipsilateral hind limb in both models, changes in the percentage bodyweight borne were small, whereas marked mechanical hyperalgesia and tactile allodynia were seen. The responses in the iodoacetate treated animals were generally more robust, and these animals were tested for pharmacological reversal of pain related behaviour. Morphine was able to attenuate hyperalgesia 3, 14 and 28 days after OA induction, and reversed allodynia at days 14 and 28, providing evidence that this behaviour was pain related. Diclofenac and paracetamol were effective 3 days after arthritic induction only, coinciding with a measurable swelling of the knee. Gabapentin varied in its ability to reverse both hyperalgesia and allodynia. The iodoacetate model provides a basis for studies on the mechanisms of pain in OA, and for development of novel therapeutic analgesics.

Keywords: Gabapentin; Morphine; Diclofenac; Paracetamol

1. Introduction

The term osteoarthritis (OA) describes a range of diseases that result in a common joint pathology. Historically, X-ray examination identified the characteristic OA features of bony outgrowths (osteophytes), thickening of subchondral bone, cyst formation, joint misalignment and loss of cartilage identified by reduced joint space. With advances in MRI technology, the involvement of all tissues in the joint has been established (Guermazi et al., 2003), including modest inflammation of the synovium, ligament laxity, meniscal degradation and bone marrow oedema. Whilst these disease features impair the function of the knee joint, leading to disability, their role in the generation of chronic pain experienced by osteoarthritic patients remains unclear (reviewed by Dieppe and Lim, 2000; Felson et al., 2000a,b).

There are no current disease modifying agents available for OA; patients progressively lose joint function, until joint replacement is the only option. Osteoarthritic joint pain is local, with insidious onset, aching, with episodic stabbing pain (Creamer, 2000). Nearly all symptomatic patients have use-related pain, some 50% or less describe rest pain, and about 30% report night pain (Dieppe and Lim, 2000).

Abbreviations: OA, osteoarthritis; DRGs, dorsal root ganglia; NSAIDs, non-steroidal anti-inflammatory drugs; PWT, paw withdrawal thresholds; p.o., orally; s.c., sub cutaneously.

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Treatments recommended by the American College of Rheumatology for osteoarthritic pain include paracetamol, NSAIDs, steroids and opiates, however, these are associated with significant side effects and the most widely used drug class, NSAIDs, provide incomplete relief (Altman et al., 2000). Non-pharmacological therapies are also used (Chard and Dieppe, 2001; Creamer, 2000; Felson et al., 2000b; Moseley et al., 2002) but the limitations of current therapy are such that patients still cite pain as their worst symptom. Therefore, effective pain relief in OA is clearly a clinically important and currently unmet need (Brandt, 2002; Curatolo and Bogduk, 2001).

Although there is a correlation between pain and the extent of osteoarthritic joint damage seen by X-ray (O'Reilly et al., 1998), many patients with relatively undamaged joints report pain and vice versa (Hannan et al., 2000). Thus, the link between structural tissue changes and pain remain unclear. Animal experiments aimed at addressing knee joint pain have focussed on describing the fibre type and neurotransmitter expression patterns for primary sensory afferents (Salo and Theriault, 1997; Tamura et al., 1998).

To date, no animal model has addressed the issue of pain in OA (Brandt, 2002), although models that involve surgical or chemical induction of OA have demonstrated clear parallels with the human condition in terms of articular joint destruction (Brandt, 2002; van den Berg, 2001). Two such models are the injection of iodoacetate into the joint space (Guingamp et al., 1997; Janusz et al., 2001; Kalbhen, 1987) and partial meniscectomy (Janusz et al., 2002; Karahan et al., 2001; Pastoureau et al., 2003). This study aimed to identify an animal model of pain in OA, by examining these two models, comparing pathology progression with pain behaviour. Histologically defined pathology developed in the knee joints over the 28 days following surgery or iodoacetate injection, characteristic of changes seen in OA. In the iodoacetate model, the rats showed robust hyperalgesia and allodynia in the hind paw within 1 week of iodoacetate injection. After partial medial meniscectomy the animals showed allodynia in the hind paw. Morphine, and to varying extents, diclofenac, paracetamol and gabapentin attenuated hyperalgesia and allodynia in the iodoacetate model.

2. Methods

All surgery was performed according to UK Home Office approved procedures (Animals Scientific procedures Act 1986) after in-house ethical review. Adult male Wistar rats (Charles River) weighing approximately 280+/−60 g were used for these studies. Surgery was carried out under enflurane/O_2 inhalation anaesthesia. Three animals in each treatment group were used for histological investigation and six for behavioural studies.

2.1. Partial meniscectomy

Under anaesthesia, the left leg of the rat was shaved and the skin cleaned with suitable skin antiseptic. With the leg held in an extended position a medial para-patellar incision (approx 5 mm) and arthrotomy were performed. The patella was dislocated laterally and the knee joint fully flexed exposing the medial collateral ligament. An incision was then made anterior to the medial collateral ligament to separate the synovial tissues. Approximately 3 mm of the medial meniscus was freed from its attachments to the margin of the medial tibial plateau. The meniscus was then grasped with fine tipped haemostatic forceps, retracted and transected with fine-tipped scissors. When released, the anterior half of the meniscus retracts anteriorly and the posterior half retracts posteriorly. The incision was closed with a wound closure clip. Sham operated animals were treated identically except that transection of the meniscus was omitted.

2.2. Iodoacetate injection

Joint damage was induced by a single intra-articular injection of 2 mg of sodium iodoacetate into the left knee joint of anaesthetised rats in a total volume of 25 μl. The dose of iodoacetate was chosen based on previous literature (Guingamp et al., 1997) and in-house dose response data using 0.5, 1 and 2 mg. At all these doses, a robust hyperalgesia was observed at day 28: mean paw withdrawal thresholds (PWT) (and SEM values) for the ipsilateral paw dosed with 0.5, 1.0 and 2.0 mg, respectively, were 58.3 (1.83 g), 63.3 (3.92 g) and 55.0 (1.41 g) compared to the vehicle control of 100 (1.41 g). However, only the 2.0 mg dose produced pronounced allodynia in all animals within the group, with a mean PWT of 2.97 (SEM 0.92 g), compared to the 0.5 mg (mean, 6.33, SEM 0.67 g); 1.0 mg dose (mean, 4.26 g, SEM 2.47 g); and vehicle control (mean, 12.33 g, SEM 2.03 g).

Iodoacetate causes joint pathology via the inhibition of glycolysis, thereby targeting the avascular cartilage and causing chondrocyte death (Janusz et al., 2002). The 2 mg dose produced robust, reproducible hyperalgesia and allodynia with no effect on the general health of the animals. With the left leg flexed at a 90° angle at the knee, sodium iodoacetate solution was injected through the patellar ligament using a 26G×3/8 needle. Control rats received an intra-articular injection of sterile saline (25 μl) alone. After recovery from either procedure, the animals were returned to cages in groups of three, with 12 h light/dark cycle and food and water ad libitum.

2.3. Identification of cell bodies of joint afferents

In a separate set of experiments in three naïve animals, 10 μl of 1% w/v Fast blue in saline was injected into each left knee under the same protocol as that for iodoacetate

injection. Over 5 days, the dye was retrogradely transported to the DRGs where it could be visualised under UV light to identify the cell bodies of neurons innervating the knee. Lumbar DRGs at levels L2–L6, both contra and ipsilateral to the injection, were dissected from the surrounding tissue after pentobarbitone anaesthesia (60 mg/kg) and cardiac perfusion with phosphate buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. After cryopreservation in 15% sucrose in 0.1 M phosphate buffer, embedding in cryoembed tissue-tek and freezing at −80 °C, the DRGs were cut on a Bright cryostat in 10 μm sections. To avoid counting the same cell body twice, every tenth section in each DRG was viewed under a Nikon E800 microscope with appropriate UV fluorescence filters and the number of labelled cell bodies noted. The contralateral DRG was processed in the same way to determine systemic spread of the Fast blue dye. The number of labelled cell bodies was then expressed as a percentage of those counted across all DRGs removed from that animal, i.e. the sum of cells counted in L2–L6.

2.4. Tissue preparation for articular joint histology

Joint histology was performed on a separate group of animals to those presented in the behavioural analysis. However, all animals on which a histological assessment was made were first assessed behaviourally to ensure they were hyperalgesic/allodynic to the same extent as those undergoing behavioural testing. 7, 14, 21 or 28 days after iodoacetate injection or partial medial meniscectomy, animals were killed by cervical dislocation, and the knee joints dissected out and surrounding muscle trimmed. Tissues were fixed overnight with 10× volume of 4% paraformaldehyde in physiological saline and then decalcified for a maximum of 16 h in water containing 7% w/v $AlCl_3.6H_2O$, 5% Formic acid and 8.5% HCl. This rapid decalcification buffer has been described previously as capable of maintaining histological integrity of joint tissue (Schwab et al., 1997). The decalcified knee joints were washed overnight in 0.1 M phosphate buffer pH7.4 and then incubated in 15% sucrose in 0.1 M phosphate buffer as a cryopreservative, for a further 24 h. The tissue was embedded in cryoembed tissue-tek, noting the orientation of the knee, and then frozen at −80 °C.

2.5. Histological staining and scoring of pathology

Each knee was cut using a Bright cryostat (Huntingdon, Cambridgeshire, UK) to provide serial coronal 20 μm sections, which were thaw mounted onto Vectabond (Vector Laboratories, Peterborough, UK) coated slides, with three sections per slide. After air drying, slides were viewed to identify those that spanned the central load bearing region of the knee. These sections were dipped in 0.1% toluidine blue for 1 min, and then sequentially dehydrated through 70, 90 and 100% ethanol. Finally, sections were cleared in Histoclear, and mounted in Histomount, before photographing on a Nikon Eclipse E800 microscope with a JVC colour digital camera attached. Approximately 15 sections from each animal were individually scored for pathology, comprising representative images from a 2 mm region across the femoro-tibial joint surface. The score, calculated as indicated below, was averaged for these sections.

Cartilage damage was scored according to previously published scales (Janusz et al., 2002) as 0, normal; 1, minimal, damage in the superficial zone only; 2, mild, damage extending from the surface into the upper mid zone; 3, moderate, damage reaching well into mid-zone; 4, marked, damage extending into the deep zone but not to tidemark; and 5, severe, full thickness degeneration to tidemark. The resulting score was then multiplied by 1, 2 or 3 depending on the extent of degradation in thirds across the medial articular surface; e.g. full depth degeneration to the tidemark across the entire (three thirds) medial articular surface would give a maximum score of 15.

2.6. Joint swelling

Knee diameter was measured using calibrated digital calipers (World Precision Instruments, Stevenage, UK) adapted by reinforcing the tips for knee diameter measure.

2.7. Behavioural testing

2.7.1. Weight bearing

The difference in weight borne by the contralateral compared to the ipsilateral limb was measured using a Dual Channel Weight Averager (Linton Instrumentation, Diss, Norfolk, UK). Rats were placed in a Perspex chamber designed so that each hind paw was resting on a separate transducer pad, which records over three seconds the distribution of the animal's body weight on each paw. Results are presented as weight bearing of the ipsilateral paw as a percentage of the bodyweight of the rat.

2.7.2. Mechanical hyperalgesia

Mechanical hyperalgesia was assessed by measuring PWT to an increasing pressure stimulus placed on the dorsal surface of the hind paw (Randall and Selitto, 1957) using an analgesymeter (7200, Ugo-Basile, Milan, Italy), employing a wedge-shaped probe (area 1.75 mm^2) and a cut-off of 250 g. Withdrawal thresholds were measured on ipsilateral (left) and contralateral (right) paws, prior to and then at regular intervals following the induction of OA. The data are expressed as withdrawal thresholds in grams, and drug effects as percentage reversal of hyperalgesia, defined as:

$$\text{percentage reversal} = 100 - \left(\frac{\text{right predose PWT} - \text{left post dose PWT}}{\text{right predose PWT} - \text{left predose PWT}} \times 100 \right)$$

2.7.3. Tactile allodynia (Von Frey hairs)

Tactile allodynia was assessed by measuring withdrawal thresholds to calibrated von Frey hairs. Hairs exerting a weight higher than 15 g can lift the paw as well as eliciting a response, thus 15 g represents the cut off point in these studies. Animals were placed into a Perspex chamber with a metal grid floor giving access to the underside of their paws and allowed to acclimatise prior to the start of the experiment. Tactile allodynia was tested by touching the plantar surface of the animals hind paw with von Frey hairs in ascending order of force for up to 6 s. A positive response was noted if the paw was sharply withdrawn or there was flinching upon removal of the hair. Once a positive withdrawal response was established, the paw was re-tested, starting with the next descending von Frey hair until no response occurred. The lowest amount of force required to elicit a response was recorded as the PWT (in g). Data for drug effects were expressed as mean grams threshold, calculated as postdose–predose value.

2.8. Pharmacological testing

Sodium diclofenac (30 mg/kg) and paracetamol (acetaminophen, 300 mg/kg) were obtained from Sigma Chemical Co. Ltd. and made up in 0.9% (v/v) saline or 0.5% methyl cellulose respectively. Morphine sulphate (6 mg/kg) was obtained from MacFarlane Smith Ltd (Edinburgh, UK) and was dissolved in 0.9% saline. Gabapentin (100 mg/kg) was supplied by Novartis Pharma, Basel, Switzerland and was made up in 0.5% methyl cellulose (v/v). Drugs were administered either orally (p.o.: gabapentin and paracetamol) or subcutaneously (s.c: diclofenac and morphine) in a volume of 1 ml. The doses of morphine and gabapentin were chosen to minimise sedative effects seen at higher doses. Diclofenac was administered s.c. to minimise direct irritant effects on the stomach that are seen when it is administered orally. Paracetamol at the dose and route chosen has no side effects in rats. On the day of testing, pre-dose behavioural readings were taken and the animals dosed following a set group randomisation procedure. One hour after dosing a further behavioural reading was taken. In-house data (not shown) has previously shown that optimal reversal of pain behaviour is seen at this time point with the administration routes chosen. On each subsequent day of testing the same set randomisation was used.

2.9. Statistical analysis

Statistical analysis for behavioural experiments was carried out on raw data using repeated measures of ANOVA followed by Tukey's post-hoc test ($P<0.05$ was set as the level of statistical significance).

3. Results

3.1. General health

No changes in body weight gain compared to age matched naïve control animals were noted over a four week period after either iodoacetate injection or partial medial meniscectomy (data not shown). The general health of the animals was good with no signs of spontaneous nociceptive behaviour, impaired locomotion or distress.

3.2. Osteoarthritic histopathology

Joint pathology was assessed at days 7, 14, 21 and 28 days following intra-articular iodoacetate injection. Fig. 1a shows a normal knee from an untreated animal with even thickness articular cartilage, which can be divided into surface, mid and deep zone according to cell shape and matrix architecture. At the molecular level, this is structurally undamaged, as demonstrated by uniform and intense dark purple/blue toluidine blue staining of intact cartilage proteoglycans. The underlying normal, subchondral, trabecular bone architecture shows thin struts of bone running predominantly perpendicular to the articular surface. These bone trabeculae adjoin the tidemark, that is the junction between cartilage and bone. Fig. 1b shows loss of proteoglycan from the extracellular matrix of the articular cartilage as indicated by the reduced, paler toluidine blue staining (⇗) in the surface zone compared to the deep zone. The articular surface is still intact at this time point, with no overt structural damage to the extra cellular matrix. Fig. 1c shows that at day 14, proteoglycan loss, again by toluidine staining, is extensive throughout the depth of the cartilage (⇗). Additionally, the medial articular cartilage surface has been damaged through shearing stress (↘), shown by the horizontal fracture. In Fig. 1d, after 21 days, the loss of proteoglycan staining has progressed to almost full depth cartilage thinning (⇘). Fissuring of the articular surface has extended to the deep zone (↘) and thickening of the subchondral bone has occurred immediately below the tidemark (*). Fig. 1e shows that the pathology 28 days after the injection of iodoacetate is very similar to that at day 21, with severe proteoglycan loss and cartilage thinning to the deep zone (⇘), clefts through the articular surface to the deep zone (↙) and thickened subchondral bone (*).

A time-course for the development of joint damage in the meniscectomy model has been published elsewhere (Janusz et al., 2002), and so in the current study, a quantitative assessment was made at day 28 only, Fig. 1f. This shows severe focal damage to the medial compartment (↘) with full depth cartilage loss through the tidemark to the thickened subchondral bone (*).

Osteophytes (bony outgrowths at the joint margins) were observed at day 21 and 28 in the iodoacetate model (two out of three animals) and at day 28 in the meniscectomised animals (two out of three animals). High power

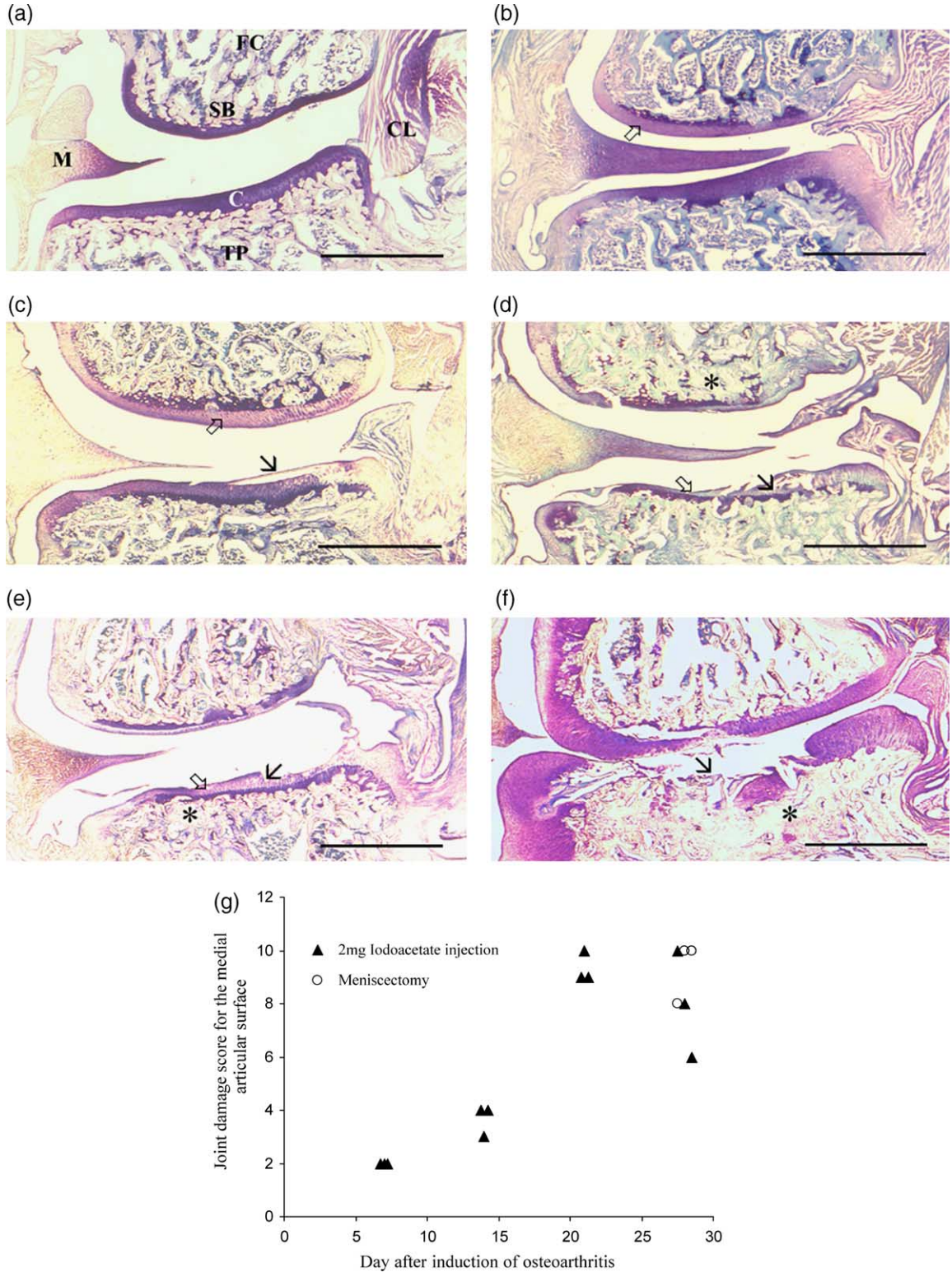

Fig. 1. Progression of osteoarthritic pathology in the partial meniscectomy and iodoacetate models of OA in the rat knee joint (a–f) Representative 20 μm thick sections through the femoro-tibial joint space stained with toluidine blue, with meniscus (M) to the left; cruciate ligament (CL) to the right; femoral condyle (FC) at the top; and tibial plateau (TP) at the bottom. (a) naive full depth normal cartilage (C) and normal subchondral bone architecture (SB). (b) day 7 after iodoacetate, slight reduction of proteoglycan staining (⇗), but normal structure. (c) day 14 after iodoacetate visible cartilage damage (↘) and loss of proteoglycan staining (⇗) in both femoral and tibial cartilage. (d) day 21 after iodoacetate injection: fibrillation (↘) and marked thinning of the whole articular surface, loss of proteoglycan staining (⇘) and thickening of subchondral bone (*). (e) day 28 after iodoacetate very similar histology, with the same features as day 21. (f) day 28 after partial medial meniscectomy. Focal full depth loss of cartilage (↘) to thickened subchondral bone (*). Scale bar: 1 mm g: quantitative scoring of osteoarthritic joint pathology.

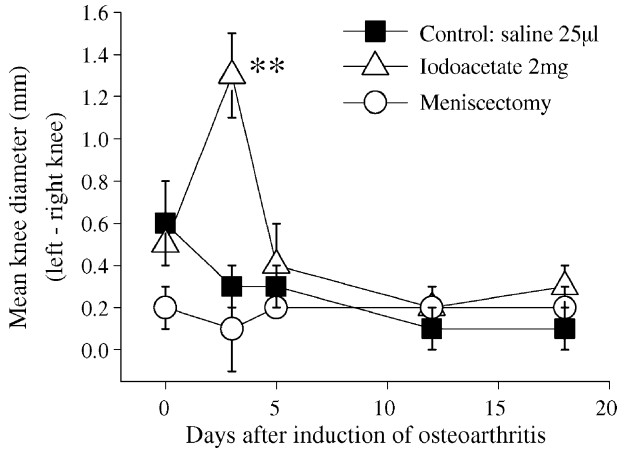

Fig. 2. The effect of OA model induction on knee diameter/swelling. ** $P<0.001$ compared to control group, six rats in each group.

magnification of both models showed chondrocytic cloning (chondrocyte cell division in the deep layer of the cartilage) in all animals at day 28, although this was more marked in the meniscectomy model.

In order to compare both models directly, the medial compartment was scored according to previously published scales (Bendele and Hulman, 1991; Janusz et al., 2002) and this is shown in Fig. 1 g. This demonstrates that both models showed comparable scores for their eventual joint destruction and that for the iodoacetate model, this level of damage was achieved by day 21. Damage in the iodoacetate model was characterised by cartilage thinning, loss of proteoglycan staining and cleft formation across the entire articular cartilage surface; whereas the full depth loss of cartilage in the meniscectomy model was localised to the central weight bearing region of the medial compartment.

3.3. Knee diameter

Knee diameters were measured to determine the amount of tissue swelling as an index of inflammation that occurred as result of the surgery or intra-articular injection of iodoacetate. A normal knee diameter was 11.4+/−0.1 mm. A brief period of inflammation was noted after iodoacetate injection, characterised by an increase in knee diameter to 13.0 mm ($P=0.0007$ for difference from vehicle controls) at day three post injection, which was reduced to normal levels by day five. Diclofenac (30 mg/kg s.c.) at day three did not reduce the knee swelling (data not

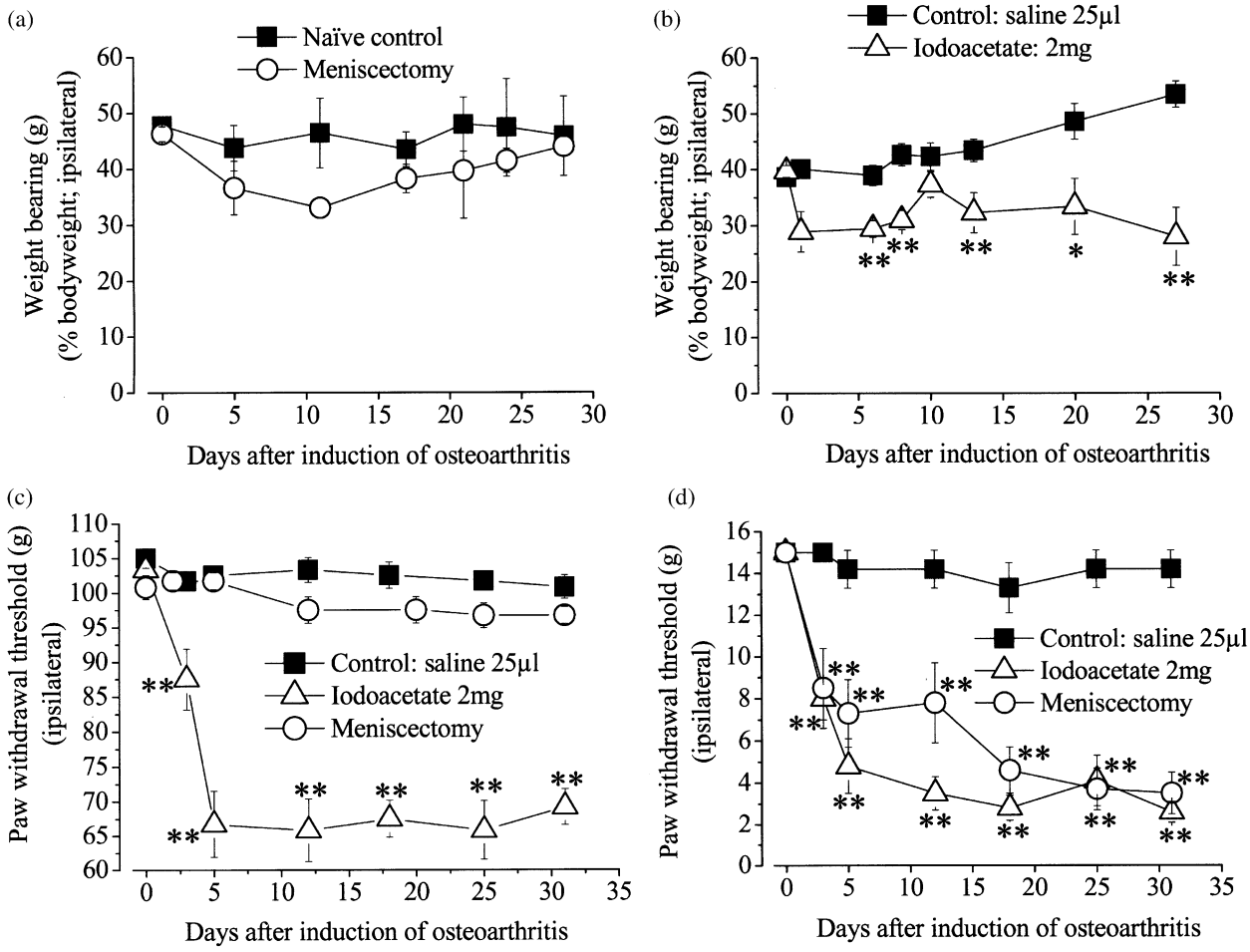

Fig. 3. The effect of OA model induction on behaviour. The effects on weight-bearing following meniscectomy (a) or iodoacetate injection (b), mechanical hyperalgesia (c) and tactile allodynia (d) are shown. Each point represents the mean value ± SEM of six rats. *$P<0.05$, **$P<0.01$ compared to control group.

shown). No change in knee diameter was seen in the meniscectomy model (Fig. 2).

3.4. Behaviour

At all time points, and for all behavioural tests, the contralateral paws of both controls and treated animals were not statistically different from each other. All P values expressed are calculated by Tukey's post hoc test following ANOVA to show differences between groups.

3.5. Weight bearing

Initial experiments examined the effects of partial medial meniscectomy or iodoacetate injection on weight bearing as a potential measure of pain. As can be seen in Fig. 3a, a small reduction in weight-bearing of the affected limb was observed following meniscectomy, but this effect was not significant and diminished with time. The intra-articular injection of 2 mg iodoacetate also induced small, but statistically significant reductions in the weight bearing of the ipsilateral limb throughout the 28 day study (Fig. 3b). At day 0 the mean weight borne on the ipsilateral limb by either the iodoacetate treated animals or the vehicle controls was 39% of the rat's bodyweight. This is not 50% as might be expected as some weight is also distributed over the tail and forelimbs. The maximum change from vehicle controls was seen at day 6 and 28 when the mean percentage weight on the ipsilateral limb dropped to 28% ($P=0.00023$ and 0.00019 respectively for day 6 and 28).

3.6. Mechanical hyperalgesia

Fig. 3c shows that mechanical hyperalgesia in the iodoacetate injected animals reached a maximum at day five (mean PWT of 66.7 g, $P=0.00018$) and was maintained for the duration of the study. No mechanical hyperalgesia was observed in menisectomised rats. Saline injected and sham operated animals maintained a PWT of at least 100 g throughout the 31 day study.

3.7. Tactile allodynia

Allodynia, which is a painful response to a normally innocuous stimulus, was measured by withdrawal responses to von Frey hairs. A threshold of 6 g was chosen to represent the generation of pronounced allodynia. Fig. 3d demonstrates that this pronounced level of allodynia, was seen in the hind paw of all 6 animals following intra-articular iodoacetate injection, from 5 days onwards ($P=0.0003$ for day 5). The onset of allodynia following meniscectomy, was evident 18 days following surgery ($P=0.00019$). From day 18 onwards the allodynia recorded in the 2 groups of OA rats was similar. Saline injected and sham operated animals did not respond to the maximum weight tested (15 g) throughout the study.

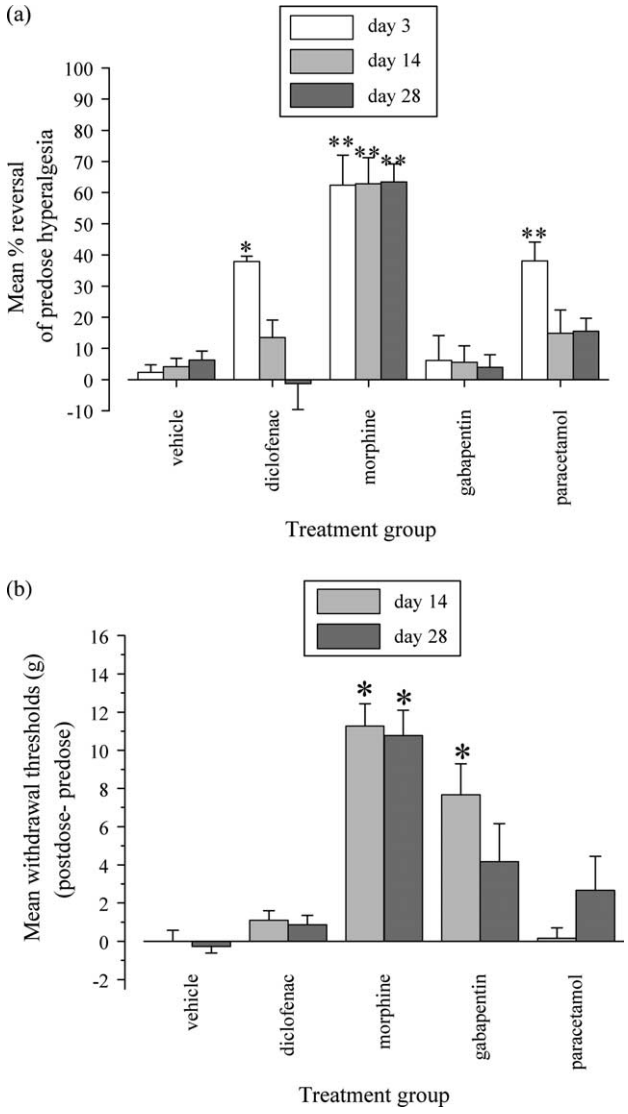

Fig. 4. The effect of analgesic agents on mechanical hyperalgesia (a) and tactile allodynia (b) following the induction of OA model in the knee joint of the rat. No drug effects on allodynia were recorded on day 3 since at this time, pronounced allodynia was not observed in every one of the group of animals. Each point represents the mean value ± SEM of six rats. $*P<0.05$, $**P<0.01$ compared to control group. Statistical analysis performed on raw data (predose–postdose values).

3.8. Pharmacological testing

The iodoacetate model was chosen to evaluate the activity of analgesic drugs as this induced the more reproducible behavioural changes. In addition, since the magnitude of the weight bearing change was not as robust a signal as the induced hyperalgesia and allodynia, analysis was restricted to the latter behaviours. Reversal of mechanical hyperalgesia was performed at days 3, 14 and 28 to represent the early/inflammatory, established and late stage of the disease respectively. Reversal of allodynia was performed at days 14 and 28 only, as definitive allodynia was not observed at day 3 in all animals of the group tested.

Morphine at a non-sedative dose of 6 mg/kg s.c. produced around 60% reversal of mechanical hyperalgesia at all time points tested ($P=0.00016$ day 3, 0.00027 day 14 and 0.00016 day 28). Near complete inhibition of allodynia was achieved 14 ($P=0.00014$) or 28 ($P=0.00034$) days following iodoacetate injection. These data are represented graphically in Figs. 4a and b. Diclofenac (30 mg/kg s.c.) at day 3 reduced pre-dose mechanical hyperalgesia by 37.9% ($P=0.0175$). Paracetamol (300 mg/kg p.o.) was also statistically significantly effective at this time point, reducing pre-dose hyperalgesia by 38.1% ($P=0.005$). These two drugs were ineffective at other time points and against allodynia (Fig. 4b). Gabapentin (100 mg/kg p.o.) was ineffective against hyperalgesia (Fig. 4a) but reversed tactile allodynia at day 14 by 76.4% of the predose value ($P=0.0003$) (Fig. 4b).

3.9. Identification of cell bodies of joint afferents

Retrograde backlabelling with Fast blue injected into the knee joint space enabled the primary knee afferents innervating the knee to be traced to their cell bodies in the DRGs. Nearly half (48.9, SD 1.69%) of the Fast blue labelled cell bodies, derived from primary afferents from the knee, were seen in the L4 DRG and the remaining half were predominantly split between L3 (28.6, SD 2.31%) and L5 DRGs (18.1 SD 2.82%). Minimal numbers of labelled cells were found in L2 (3.8, SD 1.39%) or L6 (0.6 SD 0.6%).

4. Discussion

Both iodoacetate injection and partial medial meniscectomy in the knee joint of the rat induced histological changes and pain related behaviours characteristic of human OA. Although the behavioural changes and histology both worsened over time, the majority of the pain responses were apparent within one week of surgery or iodoacetate injection, whereas gross joint damage was not evident until around day 21. This mirrors the clinical situation, where the extent of joint damage and the percentage of patients reporting pain are statistically correlated, but there remain many patients who report no pain with severe joint damage, and vice versa (Hannan et al., 2000). The reasons for this disparity are unknown, but raise the possibility that pain is induced by more subtle changes or biochemical events without structural correlates. Cartilage degradation is perhaps the best-studied feature of these two models; however, mechanical activation of nerves after cartilage degradation will be via aberrant loading of nerves in bone as cartilage itself is aneural.

Patient studies that have attempted to identify structural links with pain are only now beginning to emerge as advances in imaging techniques such as MRI are made. The anatomical features that show an association with joint pain are osteophytes (Creamer et al., 1999), bone oedema (Felson et al., 2001) and synovitis (Bollet, 2001; Felson et al., 2001; Hill et al., 2001). However, these findings have not all been confirmed by other investigators (Garnero et al., 2002; Link et al., 2003; Sowers et al., 2003). Whilst both models at the end of the study period showed the presence of osteophytes, at a time point when both models also presented allodynia, further quantitative studies would be needed to confirm any direct correlation between osteophyte formation and pain onset. The initial period of knee swelling in the iodoacetate induced model implied a transient synovial inflammation, which may have clinical correlates with early synovial inflammation that predicts development of OA in the knee (Saxne et al., 2003). It is currently unknown why this resolved for the remaining duration of the study, whereas in the human disease, recurrent inflammatory phases are noted. Inflammatory osteoarthritic pain in this model has been studied previously, using weight bearing alone as the behavioural measure (Bove et al., 2003; Kobayashi et al., 2003; Pomonis et al., 2003). Although Bove et al described the ability of NSAIDs to reverse these weight bearing changes, the mechanism behind iodoacetate induced synovial inflammation and any consequent relationship to pain sensation remain unknown. Free nerve endings have been identified in rat (Buma et al., 2000; Schwab et al., 1997) and human (Saito and Koshino, 2000) synovium and as such, this tissue probably plays an important role in joint pain sensation.

After day 5 following iodoacetate induced OA, behaviour was characterised by profound referred hyperalgesia and allodynia measured at the ipsilateral hind paw. Patients with OA also show hyperalgesic responses to noxious stimuli applied around an osteoarthritic knee (Bajaj et al., 2001; Wessel, 1995) and commonly report referred allodynia in other areas adjacent to the affected joint. Both referred hyperalgesia and allodynia are alleviated by joint replacement (Kosek and Ordeberg, 2000a,b). In animals, the mechanism by which referred pain is generated from the knee has been studied in inflammatory arthritis, and is characterised by an increase in afferent activity; lowered threshold of spinal nerves to innocuous stimulation; and an increase in the receptive field of spinal neurones to include anatomical regions adjacent to that affected by the initial tissue damage (Schaible et al., 2002). The precise mechanisms behind the central sensitisation responsible for the generation of pain responses in human OA are currently unknown. Backlabelling from the joint space in this study confirmed that the nerves supplying the knee have their cell bodies distributed in DRGs in segments between L2–L6. These five DRGs form the dorsal root origins for the saphenous (L2–3) and sciatic (L4–6) nerves that innervate the whole of the lower rat limb (Decosterd and Woolf, 2000). Therefore, cell bodies for the nerves from the knee co-localise in DRGs containing cell bodies from nerves supplying the paw. This would allow cross talk at the level of the spinal cord to generate the referred pain measured in the hyperalgesia and allodynia tests. This suggests that the development of OA in the knee may cause the same increase

in receptive field size or recruitment of central neurones that occurs as a result of inflammation in the knee (Schaible and Grubb, 1993; Schaible et al., 2002).

After induction by the mechanically induced model of partial medial meniscectomy, animals demonstrated less severe behavioural changes than iodoacetate injected animals. The reasons behind this difference are unclear, but are consistent with MRI studies that have shown that although meniscal lesions in humans are common, they are also rarely associated with pain (Guermazi et al., 2003; Bhattacharyya et al., 2003). Although the severity of joint damage by quantitative scoring is similar to that in the iodoacetate model, the damage is focal, and it may be that the type of damage rather than the absolute extent is important in generating a behavioural pain response.

Aside from the similarities between these induced models and clinical features of human OA mentioned above, it is important to note that no induced rodent model can reproduce accurately the full complexity of the human disease. In the human condition, the initiating factors and/or tissue are not fully known, and mechanical factors differ considerably between a small quadruped and man. Although human OA is defined as a non-inflammatory disease, there are clearly molecular and clinical inflammatory indices that are linked with pathology/pain (Pelletier et al., 2001; Hedbom and Hauselmann, 2002); yet this aspect is perhaps most difficult to reproduce in a predominantly non-inflammatory model that duplicates OA rather than RA histology.

The ability of a non-sedative dose of morphine to reverse both the hyperalgesia and allodynia in iodoacetate induced OA at all time points studied, produces strong evidence that both these behaviours were pain related. Pharmacological tests with other agents gave more variable results. The NSAID, diclofenac reduced hyperalgesia at day 3 following iodoacetate injection, when there is measurable knee joint swelling, suggesting an early component of inflammatory pain in this chemically induced model. The lack of effect of diclofenac at days 14 and 28 argues that non-inflammatory mechanisms are responsible for the pain behaviours at later time points. Paracetamol, although not classed as an anti-inflammatory drug, had a similar effect to diclofenac in the iodoacetate model. Its effect in the treatment of osteoarthritic pain is variable and it has been described as both more or less effective than NSAIDs. For a meta-analysis of controlled trials involving paracetamol, see (Zhang et al., 2004). There is currently no way to predict in which patient an NSAID or paracetamol will be more effective (Brandt and Bradley, 2001). It is effective as an add-on therapy to NSAIDs or selective Cox-2 inhibitors for the treatment of OA flare pain (Silverfield et al., 2002) supporting an alternative mechanism of action. Its precise site of action is unknown, but is has been reported as effective both peripherally (Ouellet and Percival, 2001) and centrally (reviewed by Muth-Selbach et al., 1999).

Although gabapentin has been used extensively to treat neuropathic pain in patients (Mellick and Mellicy, 1995; Rose and Kam, 2002; Rosenberg et al., 1997; Rosner et al., 1996) and has well established efficacy in animals models of neuropathy (Fox et al., 2003; Hwang and Yaksh, 1997; Rose and Kam, 2002), this is the first demonstration of activity in a model of OA, where it effectively reversed allodynia at day 14. This drug acts directly on neurons, probably through its ability to bind $\alpha_2\delta$ subunits of voltage dependant calcium channels (Gee et al., 1996), although the precise mechanism of analgesia is not known. Likely mechanisms include dampening of neuronal, ectopic discharge and effects on neurotransmitter release in the spinal cord. In the kaolin/carrageenan model of inflammatory arthritis, gabapentin is effective both centrally (Lu and Westlund, 1999) against thermal hyperalgesia, and peripherally (Hanesch et al., 2003) against mechanical sensitivity. At the dose used in this study (100 mg/kg p.o.) it may be working centrally and/or peripherally.

Clearly, the causes of pain in patients with OA are complex and in addition to the widely studied peripheral mechanisms, the importance of a central aspect of pain processing is now being recognised within a clinical setting (Melton, 2003). Dissecting out the factors that contribute to changes in patients' nociceptive capabilities, and those that are related to the communication of their pain as a central/cognitive perception, will be an essential focus for future clinical research. At the same time, in the poorly understood field of musculoskeletal pain, more basic research is needed to understand which molecules are involved in the induction, sensation and maintenance of chronic arthritic joint pain. The model of intra-articular iodoacetate injection described here provides such a pre-clinical tool in which a consistent pain readout is observed that is modulated by clinically relevant analgesics. The relationship between structural changes in the knee and pain remains unclear, as does the precise mechanism of pain generation. Further studies examining changes in the nociceptive pathway in this model may help to delineate some of the mechanisms underlying osteoarthritic pain. This model, therefore, provides a means of evaluating drugs with novel mechanisms of action, and may be more predictive for clinical efficacy than other chronic or acute pain models.

References

Altman RD, Hochberg MC, Moskowitz RW, Schnitzer TJ. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis GuidelinesAmerican College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Arthritis Rheum 2000;43:1905–15.

Bajaj P, Bajaj P, Graven-Nielsen T, Arendt-Nielsen L. Osteoarthritis and its association with muscle hyperalgesia: an experimental controlled study. Pain 2001;93:107–14.

Bendele AM, Hulman JF. Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea pigs. Arthritis Rheum 1991;34:1180–4.

Bhattacharyya T, Gale D, Dewire P, Totterman S, Gale ME, McLaughlin S, Einhorn TA, Felson DT. The clinical importance of meniscal tears demonstrated by magnetic resonance imaging in osteoarthritis of the knee. J Bone Joint Surg Am 2003;85:4–9.

Bollet AJ. Edema of the bone marrow can cause pain in osteoarthritis and other diseases of bone and joints. Ann Intern Med 2001;134:591–3.

Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, Schrier DJ. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage 2003;11:821–30.

Brandt KD. Animal models of osteoarthritis. Biorheology 2002;39:221–35.

Brandt KD, Bradley JD. Should the initial drug be used to treat osteoarthritis pain be a nonsteroidal antiinflammatory drug? J Rheumatol 2001;28:467–73.

Buma P, Elmans L, van den Berg WB, Schrama LH. Neurovascular plasticity in the knee joint of an arthritic mouse model. Anat Rec 2000; 260:51–61.

Chard J, Dieppe P. The case for nonpharmacologic therapy of osteoarthritis. Curr Rheumatol Rep 2001;3:251–7.

Creamer P. Osteoarthritis pain and its treatment. Curr Opin Rheumatol 2000;12:450–5.

Creamer P, Lethbridge-Cejku M, Hochberg MC. Determinants of pain severity in knee osteoarthritis: effect of demographic and psychosocial variables using 3 pain measures. J Rheumatol 1999;26:1785–92.

Curatolo M, Bogduk N. Pharmacologic pain treatment of musculoskeletal disorders: current perspectives and future prospects. Clin J Pain 2001; 17:25–32.

Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 2000;87:149–58.

Dieppe PA, Lim K. Osteoarthritis and related disorders; clinical features and diagnostic problems. In: Dequeker J, Dieppe PA, editors. Rheumatology, 2nd ed. London: Mosby; 2000. p. 831–6.

Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, Kington RS, Lane NE, Nevitt MC, Zhang Y, Sowers M, McAlindon T, Spector TD, Poole AR, Yanovski SZ, Ateshian G, Sharma L, Buckwalter JA, Brandt KD, Fries JF. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000a;133:635–46.

Felson DT, Lawrence RC, Hochberg MC, McAlindon T, Dieppe PA, Minor MA, Blair SN, Berman BM, Fries JF, Weinberger M, Lorig KR, Jacobs JJ, Goldberg V. Osteoarthritis: new insights. Part 2: treatment approaches. Ann Intern Med 2000b;133:726–37.

Felson DT, Chaisson CE, Hill CL, Totterman SM, Gale ME, Skinner KM, Kazis L, Gale DR. The association of bone marrow lesions with pain in knee osteoarthritis. Ann Intern Med 2001;134:541–9.

Fox A, Gentry C, Patel S, Kesingland A, Bevan S. Comparative activity of the anti-convulsants oxcarbazepine, carbamazepine, lamotrigine and gabapentin in a model of neuropathic pain in the rat and guinea-pig. Pain 2003;105:355–62.

Garnero P, Ayral X, Rousseau JC, Christgau S, Sandell LJ, Dougados M, Delmas PD. Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. Arthritis Rheum 2002;46:2613–24.

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem 1996;271:5768–76.

Guermazi A, Zaim S, Taouli B, Miaux Y, Peterfy CG, Genant HG. MR findings in knee osteoarthritis. Eur Radiol 2003;13:1370–86.

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.

Hanesch U, Pawlak M, McDougall JJ. Gabapentin reduces the mechanosensitivity of fine afferent nerve fibres in normal and inflamed rat knee joints. Pain 2003;104:363–6.

Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. J Rheumatol 2000;27:1513–7.

Hedbom E, Hauselmann HJ. Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation. Cell Mol Life Sci 2002;59: 45–53.

Hill CL, Gale DG, Chaisson CE, Skinner K, Kazis L, Gale ME, Felson DT. Knee effusions, popliteal cysts, and synovial thickening: association with knee pain in osteoarthritis. J Rheumatol 2001;28:1330–7.

Hwang JH, Yaksh TL. Effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. Reg Anesth 1997;22:249–56.

Janusz MJ, Hookfin EB, Heitmeyer SA, Woessner JF, Freemont AJ, Hoyland JA, Brown KK, Hsieh LC, Almstead NG, De B, Natchus MG, Pikul S, Taiwo YO. Moderation of iodoacetate-induced experimental osteoarthritis in rats by matrix metalloproteinase inhibitors. Osteoarthritis Cartilage 2001;9:751–60.

Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L, Heitmeyer SA. Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. Osteoarthritis Cartilage 2002;10:785–91.

Kalbhen DA. Chemical model of osteoarthritis—a pharmacological evaluation. J Rheumatol 1987;14:130–1.

Karahan S, Kincaid SA, Kammermann JR, Wright JC. Evaluation of the rat stifle joint after transection of the cranial cruciate ligament and partial medial meniscectomy. Comp Med 2001;51:504–12.

Kobayashi K, Imaizumi R, Sumichika H, Tanaka H, Goda M, Fukunari A, Komatsu H. Sodium iodoacetate-induced experimental osteoarthritis and associated pain model in rats. J Vet Med Sci 2003;65:1195–9.

Kosek E, Ordeberg G. Abnormalities of somatosensory perception in patients with painful osteoarthritis normalize following successful treatment. Eur J Pain 2000a;4:229–38.

Kosek E, Ordeberg G. Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. Pain 2000b;88:69–78.

Link TM, Steinbach LS, Ghosh S, Ries M, Lu Y, Lane N, Majumdar S. Osteoarthritis: MR imaging findings in different stages of disease and correlation with clinical findings. Radiology 2003;226:373–81.

Lu Y, Westlund KN. Gabapentin attenuates nociceptive behaviors in an acute arthritis model in rats. J Pharmacol Exp Ther 1999;290:214–9.

Mellick GA, Mellicy LB. Gabapentin in the management of reflex sympathetic dystrophy. J Pain Symptom Manage 1995;10:265–6.

Melton L. Osteoarthritis pain goes central. Lancet Neurol 2003;2:524.

Moseley JB, O'Malley K, Petersen NJ, Menke TJ, Brody BA, Kuykendall DH, Hollingsworth JC, Ashton CM, Wray NP. A controlled trial of arthroscopic surgery for osteoarthritis of the knee. N Engl J Med 2002;347:81–8.

Muth-Selbach US, Tegeder I, Brune K, Geisslinger G. Acetaminophen inhibits spinal prostaglandin E2 release after peripheral noxious stimulation. Anesthesiology 1999;91:231–9.

O'Reilly SC, Jones A, Muir KR, Doherty M. Quadriceps weakness in knee osteoarthritis: the effect on pain and disability. Ann Rheum Dis 1998; 57:588–94.

Ouellet M, Percival MD. Mechanism of acetaminophen inhibition of cyclooxygenase isoforms. Arch Biochem Biophys 2001;387:273–80.

Pastoureau P, Leduc S, Chomel A, De Ceuninck F. Quantitative assessment of articular cartilage and subchondral bone histology in the meniscectomized guinea pig model of osteoarthritis. Osteoarthritis Cartilage 2003;11:412–23.

Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis Rheum 2001;44:1237–47.

Pomonis J, Boulet J, Walker K, Cranbury LP. Development of a Rat Model of Osteoarthritis Pain. poster number 774. 20-3-2003. 22nd Annual Scientific Meeting American Pain Society 2003.

Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Intern Pharmacodyn 1957;111:409–19.

Rose MA, Kam PC. Gabapentin: pharmacology and its use in pain management. Anaesthesia 2002;57:451–62.

Rosenberg JM, Harrell C, Ristic H, Werner RA, de Rosayro AM. The effect of gabapentin on neuropathic pain. Clin J Pain 1997;13:251–5.

Rosner H, Rubin L, Kestenbaum A. Gabapentin adjunctive therapy in neuropathic pain states. Clin J Pain 1996;12:56–8.

Saito T, Koshino T. Distribution of neuropeptides in synovium of the knee with osteoarthritis. Clin Orthop 2000;172–82.

Salo PT, Theriault E. Number, distribution and neuropeptide content of rat knee joint afferents. J Anat 1997;190:515–22.

Saxne T, Lindell M, Mansson B, Petersson IF, Heinegard D. Inflammation is a feature of the disease process in early knee joint osteoarthritis. Rheumatology (Oxford) 2003;42:903–4.

Schaible HG, Grubb BD. Afferent and spinal mechanisms of joint pain. Pain 1993;55:5–54.

Schaible HG, Ebersberger A, Von Banchet GS. Ann N Y Acad Sci 2002; 966(3):43–54.

Schwab W, Bilgicyildirim A, Funk RHW. Microtopography of the autonomic nerves in the rat knee: a fluorescence microscopic study. Anatomical Record 1997;247:109–18.

Silverfield JC, Kamin M, Wu SC, Rosenthal N. Tramadol/acetaminophen combination tablets for the treatment of osteoarthritis flare pain: a multicenter, outpatient, randomized, double-blind, placebo-controlled, parallel-group, add-on study. Clin Ther 2002;24:282–97.

Sowers MF, Hayes C, Jamadar D, Capul D, Lachance L, Jannausch M, Welch G. Magnetic resonance-detected subchondral bone marrow and cartilage defect characteristics associated with pain and X-ray-defined knee osteoarthritis. Osteoarthritis Cartilage 2003;11:387–93.

Tamura R, Hanesch U, Schmidt RF, Kumazawa T, Mizumura K. Examination of colocalization of calcitonin gene-related peptide-and substance P-like immunoreactivity in the knee joint of the dog. Neurosci Lett 1998;254(1):53–6.

van den Berg WB. Lessons from animal models of osteoarthritis. Curr Opin Rheumatol 2001;13:452–6.

Wessel J. The reliability and validity of pain threshold measurements in osteoarthritis of the knee. Scand J Rheumatol 1995;24: 238–42.

Zhang W, Jones A, Doherty M. Does paracetamol (acetaminophen) reduce the pain of osteoarthritis? A meta-analysis of randomised controlled trials Ann Rheum Dis 2004;63(8):901–7.