Osteoarthritis and Cartilage 21 (2013) 1336–1345

Osteoarthritis and Cartilage

Differences in structural and pain phenotypes in the sodium monoiodoacetate and meniscal transection models of osteoarthritis

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ARTICLE INFO

Article history:
Received 16 January 2013
Accepted 19 June 2013

Keywords:
Osteoarthritis
Animal models
Pain
Inflammation
Glucocorticosteroids

SUMMARY

Objectives: To characterize differences in joint pathology and pain behavior between two rat models of osteoarthritis (OA) in order to inform selection of animal models for interventional studies.
Method: Knee OA was induced in Sprague Dawley rats by either meniscal transection (MNX) or intra-articular injection of monosodium iodoacetate (MIA). Controls were subjected to sham surgery or saline-injection. In a separate experiment, a single intra-articular injection of triamcinolone acetonide was administered 14 days after MNX or MIA arthritis induction. Pain behavior and joint pathology were quantified.
Results: Both models displayed synovial inflammation, chondropathy and osteophytosis. Chondropathy scores increased with time similarly in the two models. Inflammation and osteophyte scores were greater in MNX model compared to the MIA model. At day 49, the MNX model exhibited a greater number of channels crossing the osteochondral junction compared to all other groups. The MNX model exhibited greater weight bearing asymmetry compared to the MIA model, whereas the MIA model displayed more consistent hindpaw allodynia. Triamcinolone attenuated weight bearing asymmetry and distal allodynia to control levels in the MNX model, but distal allodynia was unaltered in the MIA model.
Conclusions: The comparison of the two models of OA in rats, using identical assessment tools has demonstrated that although both models display features of OA, there are differences between the models which may represent different aspects of human OA. Thus, model selection should be based on the pathological aspects of OA under investigation.

Introduction

The presenting symptoms of osteoarthritis (OA) are chronic pain and disability. However, the associations between OA structural changes and pain are often weak, and are incompletely understood[1,2]. Current drug treatments for OA are often limited by adverse events and incomplete efficacy. Intra-articular injection of corticosteroids and the oral or topical application of non-steroidal anti-inflammatory drugs[3] may be helpful for some patients. This, together with imaging and pathological evidence, suggests an important contribution from inflammation to OA pain[4]. Back-translation of these findings to animal models should permit the development of more specific and effective treatments for OA.

It is currently unknown to what extent models of OA reflect relationships between structure (including synovitis) and symptoms observed in human disease. OA models have often been developed to explore mechanisms of cartilage damage, and reports of synovitis and pain behavior are often limited. Furthermore, few studies report on more than one model, and it is difficult to identify studies that make direct comparisons between models undertaken concurrently within the same experiment, with animals randomized between models and assessed using identical methods. As such, OA model selection is typically a matter of model experience or following precedence, rather than based on the most relevant pathophysiological phenotype for the question in hand.

We have compared structural and pain phenotypes between two commonly used animal OA models induced by meniscal transection (MNX) and intra-articular injection of sodium

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1063-4584/$ – see front matter
http://dx.doi.org/10.1016/j.joca.2013.06.031

monoiodoacetate (MIA). The MNX model is induced by the transection of the medial collateral ligament and subsequent full thickness cut made through the meniscus[5–7]. Pathological changes similar to post-traumatic human OA are believed to result from instability and incongruity between joint surfaces. The monosodium iodoacetate (MIA) model[8–10] is induced by intra-articular injection of the metabolic inhibitor MIA, which inhibits the enzyme glyceraldehyde-3-phosphate dehydrogenase and disrupts glycolysis, on which articular chondrocytes are obligately dependant, leading to cell death[11,12].

We monitored pain behavior by two methods. Firstly, weight distribution, which has been shown to be reduced on the ipsilateral hind limb[1,11]. Secondly, by changes in mechanical paw withdrawal thresholds at a site distal to the injured joint, the foot pad[9,13]. Pain on weight bearing, and reduced pain pressure thresholds, are both features of human OA[14]. Distal reduced pain pressure thresholds may represent abnormal central pain processing.

To investigate the contribution of inflammation to pain behavior we have employed a commonly prescribed drug used in human OA, triamcinalone acetonide, which we administered by intra-articular injection in both models once pathological changes had become established.

We hypothesized that the two different rat models of OA mimic different aspects of the relationship between pain and joint structure that have been demonstrated in human OA, and aimed to characterize any such differences in order to permit the appropriate selection of animal models for interventional studies.

Methods

Animal models

Experiments were approved by the University of Nottingham Local Ethics Review Committee and performed under United Kingdom Home Office licence, using male Sprague Dawley rats of approximately 180 g (Charles River, Kent, UK). Rats were housed on a 12 h light/dark cycle with food and water *ad libitum*. Joint swelling was measured with digital electronic calipers (Mitutoyo, UK), with values representing difference in knee diameters (mm) between the experimental (left) and contralateral (right) knee joints.

Study design

84 rats were randomly assigned to the following groups: MNX, Sham surgery, MIA and intra-articular injection of saline and eight animals per group ($n = 4$ for saline) were sacrificed for the investigation of pathological changes at 14, 35 and 49 days after model induction. In a separate experiment, rats received a single intra-articular injection of triamcinalone acetonide Kenalog 40®, 1 mg/25 μl (E.R Squibb & Sons Ltd, Uxbridge, UK) or vehicle control, 14 days after model induction. 48 rats ($n = 8$ in each group) were randomly assigned to the following: MIA/vehicle, MIA/triamcinalone acetonide, saline/triamcinalone acetonide, MNX/vehicle, MNX/triamcinalone acetonide and Sham/triamcinalone acetonide. Vehicle control for the triamcinalone acetonide injection comprised of 10 ml sterile 0.9% saline containing 0.075 g sodium carboxymethyl cellulose, 90 μl benzoyl peroxide and 4 μl Tween 80 (all Sigma UK). In each experiment, rats from all experimental groups were from a single batch of rats and studied concurrently by the same observers blinded to experimental group. Each experimental measurement, e.g., weight bearing or paw withdrawal threshold, was made by the same observer in both models. Rats were sacrificed at 21 days after model induction.

Induction of MNX model

The MNX model of arthritis was induced as previously described[5]. Rats were anaesthetized using 2.5% Isoflurane (Abbott, Maidenhead, UK) in oxygen with a flow rate of 1 L per minute. The left leg was shaved and surgically prepared. The medial collateral ligament was exposed and a section of it was removed to expose the meniscus. The meniscus was cut through its full thickness at the narrowest point. The connective tissue layer and skin were closed with coated Vicryl 8-0 and 4-0 sutures, respectively (Ethicon, Livingstone, UK). No post-operative analgesic drug was administered as pain behavior was an outcome measure of the experiment. Sham operated animals underwent an identical procedure with the exception that the meniscus was not transected.

Induction of MIA model

The MIA model of arthritis was induced as previously described[5,15]. Rats received a single intra-articular injection of monosodium iodoacetate (1 mg in 50 μl sterile saline, Sigma UK) through the infra-patella ligament of the left knee. Control rats received intra-articular injection of saline (50 μl).

Triamcinalone acetonide intervention study

OA pathology and pain behavior were allowed to develop for a period of 14 days after model induction, a time point at which the pathological features of inflammation, chondropathy and osteophyte formation are established. A single injection of 1 mg triamcinalone acetonide was then given into the left knee, the equivalent dose in mg/kg as that used in human treatment.

Baseline behavioral pain measurements were made prior to model induction and then at day 7, 14 (prior to steroid injection), 15, and 21 days. Animals were sacrificed 21 days after arthritis induction (7 days after intra-articular triamcinalone acetonide administration).

In both studies, the animals were sacrificed by exposure to a slowly rising concentration of carbon dioxide.

Histology

For each rat, skin was removed and the tibiofemoral joints were isolated by cutting mid-femur and tibia. The intact joints were preserved in neutral buffered formalin (containing 4% formaldehyde) for 48–72 h and subsequently decalcified in neutral buffered formalin containing 10% formic acid for approximately 10 days. Each joint was split by frontal sectioning and embedded to give an anterior and posterior block. Coronal sections (5 μm), were taken through each of the wax blocks and stained with either hematoxylin and eosin, or safranin O. Sections were selected to give an approximate spacing of 200 μm as recommended by the OARSI histopathology initiative[16].

For the triamcinalone acetonide interventional experiment, the synovium was removed prior to fixation and snap frozen before subsequent storage at −80°C.

Histomorphometry and pathology scoring

The pathology scoring was performed on H/E sections with the exception of chondropathy which was done on Safranin O stained sections.

Osteophytosis and chondropathy were evaluated by the method of Janusz[5]. Osteophytosis was scored on a scale of 0–3, follows: 0. No osteophyte present; 1. Mild, <50 μm; 2. Moderate, 50–150 μm; and 3. Severe, >150 μm. Chondropathy was scored on a scale of 0–5 as follows: 0. Cartilage of normal appearance; 1. Minimal fibrillation, superficial zone only; 2. Mild, extends to the upper middle