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Relationship between structural pathology and pain behaviour in a model of osteoarthritis (OA)

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SUMMARY

Objectives: To address the hypothesis that different types of established osteoarthritis (OA) pain behaviours have associations with different aspects of articular pathology, we investigated the relationship between structural knee joint pathology and pain behaviour following injection of a low vs a high dose of monosodium iodoacetate (MIA) in the rat.
Methods: Rats received a single intra-articular injection of 0.1 mg or 1 mg MIA or saline (control). Pain behaviour (hind limb weight bearing asymmetry (WB) and hindpaw withdrawal threshold (PWT) to punctate stimulation) was assessed. Cartilage and synovium were examined by macroscopic visualisation of articular surfaces and histopathology.
Results: Both doses of MIA lowered PWTs, 1 mg MIA also resulted in WB asymmetry. Both doses were associated with cartilage macroscopic appearance, proteoglycan loss, abnormal chondrocyte morphology, increased numbers of vessels crossing the osteochondral junction, synovitis and macrophage infiltration into the synovium. PWTs were more strongly associated with chondrocyte morphology, synovitis and macrophage infiltration than with loss of cartilage surface integrity.
Conclusions: Both pain behaviours were associated with OA structural severity and synovitis. Differences in pain phenotype following low vs higher dose of MIA were identified despite similar structural pathology. OA structural pathology as traditionally measured only partially explains the MIA-induced pain phenotype.

Introduction

Osteoarthritis (OA) is a chronic debilitating disease affecting around 8.8 million people in the UK[1]. Pain is the commonest clinical symptom that leads OA sufferers to seek medical care. OA pain contributes to loss of joint function, disability and reduced quality of life in the ageing population[2], and is an important unmet clinical need. Our incomplete knowledge of the sources of OA pain, and the relationship between pain phenotypes and pathology hinders progress in the identification of better targets for treatments which improve OA pain.

The classification of radiographic OA depends on the presence of osteophytes and joint space narrowing (JSN). Radiographic evidence of OA is associated with pain[3,4], but this association is often only weak[5,6]. Some patients report pain despite minimal radiographic changes[7], whilst others with abnormal knee radiographs report no pain[8]. Magnetic resonance imaging (MRI) has revealed associations of bone marrow lesions or synovitis with pain[9–11] although their contributions as direct sources of OA pain remain uncertain[5]. Central sensitization can moderate the link between joint damage and pain, through complex pain-amplifying neuroplastic alterations to the central nervous system[12].

Preclinical models of knee OA have potential to extend understanding of OA pain mechanisms, and the contributions of specific structural features to OA pain. Intra-articular injection of the glycolysis inhibitor monosodium iodoacetate (MIA) into the rat tibiofemoral joint produces cartilage and subchondral bone pathology that are similar to those seen in human OA knees[4,5]. Intra-articular MIA injection also leads to pain-related behaviours

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(weight bearing (WB) asymmetry and reduced hindpaw withdrawal thresholds (PWTs) to punctate stimulation)[6,7] that resemble pain on WB and more widespread reduced pain thresholds[13] observed in human OA. Lowered PWTs implicates a contribution of central sensitisation[14], whereas WB asymmetry likely reflects a combination of peripheral and central sensitisation.

Identifying specific aspects of joint pathology that contribute to different OA pain phenotypes might help identify pain phenotype-specific peripheral treatment targets. We hypothesised that different types of established OA pain behaviours may have associations with different aspects of articular pathology. To address this question, we have used two doses of MIA that result in two different pain profiles in the rat and then identified associations between WB asymmetry and lowered PWTs and a range of macroscopic and histopathological changes in the knee. A secondary question addressed is whether there is delayed progression of structural pathology in the lower dose MIA model.

Materials and methods

Animals

Studies used male Sprague–Dawley rats (Charles River, Kent, UK) ($n = 64$) weighing 250–300 g at time of intra-articular injection. Studies were conducted in accordance with UK Home Office regulations and followed the guidelines of the International Association for the Study of Pain. Rats were housed in groups of four per cage under standard conditions with a 12 h light/dark cycle, with unlimited access to food and water. Rats, anaesthetised with isoflurane (2% in O_2), received a single intra-articular injection of MIA (0.1 mg/50 μl or 1 mg/50 μl, based on previous studies[14–16]) in sterile 0.9% normal saline through the infrapatellar ligament of one knee. In two separate experiments (experiment 1 = 24 rats, experiment 2 = 40 rats), rats were randomly assigned to the experimental (MIA) and control groups (saline) and results from both studies combined. The groups included 1 mg MIA; $n = 18$, 0.1 mg MIA; $n = 18$ and saline-injected rats; $n = 8$ stopped at day 20 and 0.1 mg MIA; $n = 10$ and saline-injected rats; $n = 10$, stopped at day 42 (Supplementary Fig. 1). Rats were killed by an overdose of carbon dioxide and tissues harvested at 20 days (1 mg; $n = 18$ and 0.1 mg MIA; $n = 18$) or 42 days (0.1 mg MIA; $n = 10$) post-injection. Previous studies indicated that OA pathology and pain behaviour were fully developed by 20 days after intra-articular injection of 1 mg MIA[17]. All outcome measurements were carried out by an experimenter blinded to intra-articular injections.

Behavioural measurements of OA pain

Pain behaviours were measured as withdrawal thresholds (g) to punctuate stimulation of the hind paw[18] and as hind limb weight-bearing asymmetry. Weight-bearing asymmetry was assessed as difference between hind limbs as a percentage of total weight borne through both hind limbs[19]. Measurements were obtained immediately prior to intra-articular injection (day 0) and at regular intervals from day 3–20 (1 mg or 0.1 mg MIA) or day 42 (saline and 0.1 mg MIA).

Joint pathology

Synovia with patellae from both knees were harvested at sacrifice and immediately embedded in optimal cutting temperature (OCT) and snap frozen over melting isopentane. Tibiofemoral joints were then isolated and dissected to assess the severity of damage to the chondral surfaces. Macroscopic lesions were graded using the method of Guingamp[15]; grade 0 = normal appearance, 1 = slight yellowish discolouration of the chondral surface, 2 = little cartilage erosion in load bearing areas, 3 = large erosions extending down to the subchondral bone and 4 = large erosions with large areas of subchondral bone exposure. Five chondral compartments of the knee: femoral groove, medial and lateral femoral condyles and medial and lateral tibia plateaus were scored then summated to give a maximum possible score of 20.

Following macroscopic scoring, joints were fixed in neutral buffered formalin for 48 h, and then decalcified in 10% formic acid-formalin for 7 days at room temperature, split into anterior and posterior blocks, and embedded in paraffin. Six frontal sections per rat (three anterior and three posterior) were stained with haematoxylin and eosin (H&E) and corresponding consecutive sections for Safranin-O-Fast green[20]. Chondropathy and chondrocyte morphology were scored on H&E sections whereas proteoglycan content of the cartilage was scored on Safranin-O Fast green stained sections. Chondropathy was scored using the Janusz method as previously described[21]. It was evaluated from 1 (minimal superficial damage) to 5 (severe full thickness degeneration to tidemark). This score was multiplied by the extent of cartilage area involved (1/3, 2/3 or 3/3). Chondrocyte morphology and proteoglycan content of the cartilage were evaluated using the modified Mankin score as previously described using a Zeiss Axioscop-50 microscope (Carl Zeiss Ltd, Welwyn Garden City, UK) at 4× objective lens[22]. Chondrocyte morphology was scored from 0 (normal) to 3 (complete chondrocyte death or hypocellularity) and proteoglycan content from 0 (no loss) to 4 (complete loss of proteoglycan). Other histological assessments used a 20× objective lens. Osteochondral junction integrity was assessed as the number of vascular channels present in the articular cartilage per length of tibial plateau section (number per mm) on H&E sections[23].

Inflammation was assessed as joint swelling (knee diameter) (mm) using digital callipers (Miyutoyo UK Ltd., Andover, UK)[24], synovitis score and macrophage infiltration into the synovium. Synovial sections (5 μm) were either stained with H&E to assess lining thickness and cellularity from a scale of 0 (lining layer, 1–2 cells thick) to 3 (lining layer >9 cells thick and/or severe increase in cellularity)[23]. Macrophage infiltration was visualised by immunohistochemistry using the monoclonal antibody ED1 directed to CD68[25], avidin–biotin–peroxidase conjugate (ABC) and developed with diaminobenzidine using the glucose oxidase/nickel-enhanced method[26]. Macrophage fractional area was the percentage of synovial section area immunoreactive for CD68 from four fields view on one section per rat analysed using a Zeiss Axioscop-50 microscope (Carl Zeiss Ltd, Welwyn Garden City, UK) and a KS300 image analysis system (Image Associates, Thame, UK)[26].

Reagents

Monoclonal antibody to CD68 (clone ED1) was from Serotec (Oxford, UK). Biotinylated rat-adsorbed horse anti-mouse antibody and avidin–biotin complexes (Vectastain® Elite ABC Kits) from Vector laboratories (Peterborough, UK). Other reagents used were from Sigma–Aldrich UK.

Statistical analysis

Data were analysed using Prism v6 (GraphPad, San Diego, California, USA). Tests for normal distribution were made using the Kolmogorov–Smirnov test and were found to be non-parametric. Therefore, groups were compared using the Kruskal–Wallis test followed by *post hoc* Dunn's tests. Pain behaviours were analysed using area under the curve (AUC) for the comparison data between arthritic and non-arthritic. Associations were evaluated between