Osteoarthritis and Cartilage 26 (2018) 84–94

Osteoarthritis and Cartilage

Role of TrkA signalling and mast cells in the initiation of osteoarthritis pain in the monoiodoacetate model

J. Sousa-Valente [†], L. Calvo [‡ §], V. Vacca [† ‖], R. Simeoli [†], J.C. Arévalo [‡ §], M. Malcangio [† *]

† *Wolfson CARD, King's College London, SE1 1UL, UK*
‡ *Department of Cell Biology and Pathology, Institute of Neurosciences Castilla y León, University of Salamanca, Salamanca, 37007, Spain*
§ *Institute of Biomedical Research of Salamanca, Salamanca, 37007, Spain*
‖ *Institute of Cell Biology and Neurobiology, National Research Council, IRCCS Fondazione Santa Lucia, Rome, 00143, Italy*

ARTICLE INFO

Article history:
Received 18 April 2017
Accepted 17 August 2017

Keywords:
Prostaglandin D_2
NGF
TrkA
Osteoarthritis
Mast cells

SUMMARY

Objective: Aiming to delineate novel neuro-immune mechanisms for NGF/TrkA signalling in osteoarthritis (OA) pain, we evaluated inflammatory changes in the knee joints following injection of monoiodoacetate (MIA) in mice carrying a TrkA receptor mutation (P782S; TrkA KI mice).

Method: In behavioural studies we monitored mechanical hypersensitivity following intra-articular MIA and oral prostaglandin D_2 (PGD_2) synthase inhibitor treatments. In immunohistochemical studies we quantified joint mast cell numbers, calcitonin gene-related peptide expression in synovia and dorsal root ganglia, spinal cord neuron activation and microgliosis. We quantified joint leukocyte infiltration by flow cytometry analysis, and PGD_2 generation and cyclooxygenase-2 (COX-2) expression in mast cell lines by ELISA and Western blot.

Results: In TrkA KI mice we observed rapid development of mechanical hypersensitivity and amplification of dorsal horn neurons and microglia activation 7 days after MIA. In TrkA KI knee joints we detected significant leukocyte infiltration and mast cells located in the vicinity of synovial nociceptive fibres. We demonstrated that mast cells exposure to NGF results in up-regulation of COX-2 and increase of PGD_2 production. Finally, we observed that a PGD_2 synthase inhibitor prevented MIA-mechanical hypersensitivity in TrkA KI, at doses which were ineffective in wild type (WT) mice.

Conclusion: Using the TrkA KI mouse model, we delineated a novel neuro-immune pathway and suggest that NGF-induced production of PGD_2 in joint mast cells is critical for referred mechanical hypersensitivity in OA, probably through the activation of PGD_2 receptor 1 in nociceptors: TrkA blockade in mast cells constitutes a potential target for OA pain.

Introduction

The neurotrophin nerve growth factor (NGF) is constitutively produced and released by synovial fibroblasts in the joint and sensitizes nociceptive neurons that express TrkA receptors and innervate the joints[1]. Under inflammatory conditions, NGF expression is up-regulated by cytokines, and inflammatory cells such as mast cells release NGF[2]. Indeed, extracellular NGF levels are higher in inflamed joints and NGF blockade with both TrkA-IgG fusion protein and NGF monoclonal antibodies produces analgesic effects in preclinical settings[3,4]. Relevantly, humanised monoclonal anti-NGF antibodies are effective analgesics in people with osteoarthritis joint pain[5]. However, unexpected side effects such as increased incidence of bone necrosis indicate that more studies are needed to understand NGF-mediated regulation of osteoarthritis (OA) pain.

In this preclinical study we use intra-articular monoiodoacetate (MIA) injection as this OA model is associated with dose-dependent and rapid pain-like responses in the ipsilateral limb, which persists for several weeks. The ipsilateral joints display morphological changes of the articular cartilage and bone disruption, which are reflective of some aspects of patient pathology, as well as synovitis

* Address correspondence and reprint requests to: M. Malcangio, Wolfson Centre for Age-Related Diseases, Kings College London, Wolfson Wing, Hodgkin Building, Guys Campus, London, SE1 1UL, Italy.
E-mail addresses: joao.de_sousa_valente@kcl.ac.uk (J. Sousa-Valente), lau@usal.es (L. Calvo), valentina.vacca@ibcn.cnr.it (V. Vacca), raffaele.simeoli@kcl.ac.uk (R. Simeoli), arevalojc@usal.es (J.C. Arévalo), marzia.malcangio@kcl.ac.uk (M. Malcangio).

http://dx.doi.org/10.1016/j.joca.2017.08.006
1063-4584/

and macrophage infiltration[3,6]. Furthermore, this model of OA reflects specific changes in the NGF/TrkA system as follows: 1) the TrkA antagonist AR786 shows both prophylactic and therapeutic anti-nociceptive efficacy in MIA rats[3]; 2) local intra-articular injection of NGF in MIA-knee joints facilitates the responses of spinal cord neurons to extension of the knee joint[7].

The most frequent types of immune cells found in people with OA joints are macrophages, T cells and mast cells[8] TrkA receptors are expressed by mast cells and macrophages and their activation by NGF leads to up-regulation and release of inflammatory mediators, which sensitize joint nociceptors[2].

With the aim to investigate neuro-immune mechanisms mediated by NGF/TrkA signalling in OA pain, we have examined the development of MIA-referred mechanical hypersensitivity and joint pathology in wild type (WT) and mice carrying a mutation in the TrkA receptor in which Proline 782 is mutated to Serine. This TrkA mutation confers a defect on ubiquitination by Nedd4-2 (E3 ubiquitin ligase) which leads to 1) an increase in NGF-mediated signalling; 2) approximately 30% increase of dorsal root ganglia neurons; 3) increased sensitivity to noxious hot and cold, but not mechanical, stimuli; 4) enhanced behavioural response to intraplantar formalin and 5) increased activation of spinal cord neurons after formalin[9,10].

Method

Animals

Studies were conducted in accordance with UK Home Office regulations, International Association for the Study of Pain and ARRIVE guidelines[11]. Male and female TrkAP782S (TrkA KI) and WT littermates of C57BL/6 background, aged 3–6 months old (25–35 g) were housed under a 12-h light/dark cycle, with food and water available *ad libitum*. In all studies, experimental groups were randomized and an equal number of 3–6 months old-female and -male transgenic mice was used and data combined. Experiments were performed blind.

Behavioural testing

Mechanical withdrawal thresholds were assessed by calibrated von Frey monofilaments (0.02–1 g, North Coast Medical Inc.) application to the plantar surface of the hind paw. 50% paw withdrawal threshold (PWT) was determined according to the "up–-down" method[12].

In the monoiodoacetate model mice received an intra-articular injection of either saline or 0.7 mg MIA (Sigma–Aldrich) in 10 μL of sterile saline[6].

Drug administration

A Tanezumab-like antibody (Levicept) or antibody control (IgG1, kappa Sigma–Aldrich, I5154), dissolved in sterile saline were administered subcutaneously at 5 mg/kg, every 5 days, starting from the day before MIA injections. HQL-79 (Tocris) was dissolved in 0.5% methylcellulose. HQL-79 (3 and 10 mg/kg) or vehicle was administered by oral gavage.

Immunohistochemistry

Seven days after MIA injection, mice were perfuse-fixed under terminal anaesthesia through the ascending aorta with heparinised saline followed by 4% paraformaldehyde fixative solution with 1.5% picric acid in phosphate buffer (0.1 M, pH 7.4, PB). Sections from knee joints (30 μm), lumbar spinal cord (20 μm) and L3-L5 DRG (10 μm) were incubated overnight with sheep anti–calcitonin gene-related peptide (CGRP, 1:500, Enzo Life Sciences). Spinal cord sections were incubated as follows: rabbit anti-c-Fos (1:1000, Cell Signaling Technology) followed by Alexa Fluor 488- or 546-conjugated antibodies (1:1000; Molecular Probes); rabbit anti– p-p38 (1:100, Cell Signaling Technology) and biotinylated secondary antibody (1:400 biotin donkey anti-rabbit, Jackson ImmunoResearch Labs); peroxidase containing avidin-biotin complex (1:200, Vector Laboratories) and biotinylated tyramide (NEN Life Science Products) which was detected with ExtrAvidin-FITC (1:500, Sigma–Aldrich); rabbit anti-Iba-1 (1:100 Wako Pure Chemical Industries Ltd.) followed by Alexa Fluor 546-conjugated antibody (1:1000; Molecular Probes). Knee joint sections were incubated with anti-cluster of differentiation 117 (CD117, 1:1000 R&D Systems) or anti–calcitonin gene-related peptide (CGRP). In control experiments, no staining was visible when joint, spinal cord or DRG sections were processed for immunohistochemistry with omission of the primary antibody. All slides were mounted with Vectashield Mounting Medium containing nuclear marker 49,6-diamidino-2-phenylindole·2HCl (DAPI; Vector Laboratories), and visualized using a Zeiss Axioplan 2 fluorescent microscope (Zeiss). Acquisition parameters remained constant and unprocessed images were used for analysis and quantification.

Quantitative assessment of fluorescent intensity

DRG neurons were identified and cell bodies were selected as regions of interest (ROI) using ImageJ software (NIH). At least 200 neurons were sampled, in serial sections at a distance of at least 10 sections (i.e., 100 μm) apart. c-Fos immunoreactivity was determined by counting number of positive profiles within a region defined as laminae I and II, using Axiovision LE 4.8 software (Zeiss). Iba-1 and p-p38 immunoreactivity was determined by counting number of positive profiles within three 2.25×10^4 μm^2 boxes in laminae I–III. Knee joint sections were examined at 10× magnification to identify areas with the highest nerve fibre or cell density in the synovium. The length of nerve fibres was determined by manually tracing them from Z-stack images using Axiovision LE 4.8 software (Zeiss) and reported as density of fibres per volume of synovium – area of synovium × thickness (30 μm) – as described previously[13]. $CD117^+$ mast cells were counted and data was expressed as density of cells per volume of synovium[13].

Western blotting

RBL-2H3 cells were homogenized in lysis buffer and 30 mg/ml of protein was loaded on a 10% SDS-PAGE gel. Proteins were wet-transferred using the Bio-Rad system (Bio-Rad Laboratories) and blots were probed overnight with rabbit anti-cyclooxygenase-2 (COX-2) (1:5000; Abcam), incubated with HRP–conjugated anti-rabbit immunoglobulin (Dako) and visualised with BioSpectrum System (Ultra-Violet Products Ltd). Bands were analysed with Quantity One (Bio-Rad Laboratories). β-actin (1:1000; Cell Signaling) was used as loading control.

Flow cytometry

Seven days after OA induction, the skin and muscle were removed and the knee joint isolated with care not to damage the bone and release bone marrow content. Knee joints were digested in serum-free RPMI containing collagenase D (0.5 μg/ml) and DNAse (40 μg/ml). Isolated cells were incubated with Fc block anti-mouse CD16/CD32 (Clone 2.4G2, BD Biosciences) followed by fluorochrome-conjugated anti-mouse antibodies: CD45.1-Pacific Blue™ (Clone 30-F11, BioLegend), F4/80-PE (Clone BM8, eBioscience), CD11b-APC (Clone M1/70, eBioscience), CD117-PeCy7