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Spinal neuropeptide modulation, functional assessment and cartilage lesions in a monosodium iodoacetate rat model of osteoarthritis

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ABSTRACT

Background and aims: Characterising the temporal evolution of changes observed in pain functional assessment, spinal neuropeptides and cartilage lesions of the joint after chemical osteoarthritis (OA) induction in rats.
Methods and results: On day (D) 0, OA was induced by an IA injection of monosodium iodoacetate (MIA). Rats receiving 2 mg MIA were temporally assessed at D3, D7, D14 and D21 for the total spinal cord concentration of substance P (SP), calcitonin gene related-peptide (CGRP), bradykinin (BK) and somatostatin (STT), and for severity of cartilage lesions. At D21, the same outcomes were compared with the IA 1 mg MIA, IA 2 mg MIA associated with punctual IA injection of lidocaine at D7, D14 and D21, sham (sterile saline) and naïve groups. Tactile allodynia was sequentially assessed using a von Frey anaesthesiometer. Non-parametric and mixed models were applied for statistical analysis. Tactile allodynia developed in the 2 mg MIA group as soon as D3 and was maintained up to D21. Punctual IA treatment with lidocaine counteracted it at D7 and D14. Compared to naïve, [STT], [BK] and [CGRP] reached a maximum as early as D7, which plateaued up to D21. For [SP], the increase was delayed up to D14 and maintained at D21. No difference in levels of neuropeptides was observed between MIA doses, except for higher [STT] in the 2 mg MIA group ($P = 0.029$). Neuropeptides SP and BK were responsive to lidocaine treatment. The increase in severity of cartilage lesions was significant only in the 2 mg MIA groups ($P = 0.01$).
Conclusion: In the MIA OA pain model, neuropeptide modulation appears early, and confirms the central nervous system to be an attractive target for OA pain quantification. The relationship of neuropeptide release with severity of cartilage lesions and functional assessment are promising and need further validation.

1. Introduction

Osteoarthritis (OA) is a complex degenerative disease of the whole joint characterised by progressive loss of articular cartilage, sclerosis of the underlying subchondral bone, formation of osteophytes, synovial inflammation, and loss of normal joint function (Loeser and Treede, 2008; Loeser et al., 2012). As a major OA symptom, pain often combines hyper-sensitivity and continuous pain at rest, and generally involves the processes of both peripheral and central sensitisation. The joint capsule, subchondral bone, periosteum, synovium and ligaments are deeply innervated and could be an important source of nociceptive input in OA (Kidd et al., 1990). The interaction of inflammatory cytokines within stifle joint afferents and the regulation of the transcription nuclear factor (NF)-κB activity have been well documented (Bowles et al., 2014).

Central sensitisation mechanisms include various biochemical processes such as increased spinal release of neuro-transmitters/-modulators and accentuated (in time and intensity) excitability of postsynaptic neurons (Woolf, 1996). There is expanding indication that the cartilage destruction in OA is the result of multiple biochemical activities implicating cytokines, inflammatory mediators and enzymes, with a close intrication to nociceptive stimulation and sensitisation, for which

Abbreviations: OA, osteoarthritis; D, day; IA, intra-articular; MIA, monosodium iodoacetate; SP, substance P; CGRP, calcitonin gene related-peptide; BK, bradykinin; STT, somatostatin; (NF)-κB, nuclear factor-kappa B; L, lidocaine; PWT, paw withdrawal threshold; n, number of animals; w/v, weight/volume; v/v, volume/volume; p, probability; adj-*P*, adjusted *P* value; RR, relative ratio; w/v, weight/volume; SD, standard deviation; SEM, standard error of the mean; ATF-3, activating transcription factor-3

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targeting peptide receptors offer new attractive therapeutic strategies for OA pain (Malfait and Miller, 2016). In pain transmission, peptidergic neurons and central neuronal synapses involve neuropeptides expression (Malavolta and Cabral, 2011). For example in rodent spinal cord, tachykinin such as substance P (SP) (Saidi et al., 2016), calcitonin gene-related peptide (CGRP) (Le Greves et al., 1997) and somatostatin (STT) (Prasoon et al., 2015) are well recognised to participate in the modulation of central pain sensitisation (Latremoliere and Woolf, 2009). Moreover, the peptide bradykinin (BK) is also known to potentiate synaptic transmission in pain hypersensitivity (Kohno et al., 2008). However, the contribution of such spinal neuro-mediators in OA chronic pain establishment has been poorly described.

Recently, the reliability and concurrent validity of different pain assessment methods were tested in a chemically-induced OA model in rats (Otis et al., 2016). Interestingly, the behavioural responses, being semi-subjective, were validated in comparison to objective pain neuropeptidomic measurements that were sensitive to chemical OA induction as well as to analgesic treatment responsiveness.

The chemical monosodium iodoacetate (MIA) OA model is considered a standard OA model for pain assessment. Injection of the irreversible glyceraldehyde-3-phosphate dehydrogenase inhibitor into the stifle (equivalent to the human knee) joint space reproduces cartilage lesions similar to human OA (Marker and Pomonis, 2012) in a dose-dependent manner (Guingamp et al., 1997; Janusz et al., 2001). In parallel to degenerative changes within the joint in the inflammatory early (first 7 days) phase, a second pain phenotype rapidly progresses in the ipsilateral hind limb, less sensible to nonsteroidal anti-inflammatory drugs (Ivanavicius et al., 2007). However, blocking the transient receptor potential cation channel subfamily V member 1, with systemic antagonists, attenuated OA pain behaviour (Kelly et al., 2015), suggesting the presence of peripheral/central sensitisation (Guingamp et al., 1997). Lidocaine is widely used for regional anaesthesia in order to block impulses in peripheral nerves by inhibiting voltage-gated sodium channels (Catterall, 2000). Many other mechanisms regarding the site of action have been proposed for lidocaine in nerve fibers, such as interaction with membrane receptors, proteins, phospholipids, modulation of multiple calcium and potassium channels and kinases (Zhang et al., 2014). Use of intra-articular (IA) local anaesthetic as analgesic presents the advantage of avoiding false positives to behavioural assessment from drugs (such as systemic opioids and gabapentinoids) that cause motor impairment or sedative effects. The involvement of IA lidocaine injection in a MIA OA model with simultaneous spinal neuropeptide regulation remained relevant to understand mechanism underlying OA progression.

Our hypothesis is that the modulation of spinal neuropeptides in the MIA OA model can be detected, quantified and related with the extent of behavioural functional assessment, and of cartilage lesions in the affected stifle with or without lidocaine treatment. Consequently, over a period of 21 days (D) and with temporal sacrifice, the aim of this prospective, randomised, blinded and controlled study was to characterise the modulation of four different spinal pain neuropeptides (SP, CGRP, BK and STT) and to determine their potential expression as biomarkers of pain in comparison with the functional assessment of nociception, and the severity of cartilage lesions in the ipsilateral stifle joint.

2. Materials and methods

2.1. Animals and induction of OA

Procedures were carried out in accordance with the Canadian Council on Animal Care guidelines and approved by the Institutional Animal Care and Use Committee (CIPA #H11005ETr). A total of 61 female Sprague-Dawley rats (230–250 g) (Charles River Laboratories International, Inc., Saint-Constant, QC, Canada) were housed in groups of four, with food and water *ad libitum*, and kept at a constant temperature of 22 °C in a 12-hour light-dark cycle. At day (D) 0, rats were mask-anaesthetised with 2% isoflurane in O_2 to receive a single blinded IA MIA injection through the infrapatellar ligament of the right stifle as previously described (Guingamp et al., 1997). Rats were randomly distributed in groups of $n = 8$: (1) MIA (Sigma-Aldrich, Inc., St-Louis, MO, USA) at a dose of 1 mg (sacrifice at D21); (2 to 5) MIA 2 mg ($n = 32$, with progressive sacrifice of 8 rats at each time point: D3, D7, D14 and D21); (6) MIA 2 mg with a punctual lidocaine (L) injection (2 mg MIA-L group) in the right stifle on days 7, 14 and 21 post-MIA injection, 25 min before functional assessment. Rats from the 2 mg MIA-L group were again similarly mask-anaesthetised with a single IA injection of lidocaine through the infrapatellar ligament of the right stifle. Lidocaine Neat® (2%, Zoetis Canada, Kirkland, QC, Canada) was injected at a volume of 50 µL using a 26-gauge, 0.5-inch needle mounted on a 0.5-mL syringe; (7) 0.9% sterile saline (50 µL) for a sham group; in addition an eighth group ($n = 5$) of naïve rats were kept in the same conditions and sacrificed at the end of the experiments. All MIA doses were dissolved in 50 µL of 0.9% sterile saline.

2.2. Functional assessment of nociception

Testing was performed during the daylight phase. All animals were allowed to acclimate to testing conditions according to an acclimation protocol using five occurrences of exposure over 2 weeks before the OA induction, as previously published (Otis et al., 2016). Functional assessments were performed at one day before OA induction for baseline values, and at D3, D7, D14 and D21 post-induction.

2.2.1. Sensory-reflexive evaluation

Tactile sensitivity was assessed using an Electronic von Frey Anesthesiometer® (IITC Life Science Inc., Woodland Hills, CA, USA) applied to the plantar surface of the hind paw, and by measuring the paw withdrawal threshold to von Frey ascending mechanical stimuli as previously described (Otis et al., 2016). Gradually increasing pressure was applied with a mechanical von Frey polypropylene probe (0.7 mm^2, Rigid Tip®, IITC Life Science Inc., Woodland Hills, CA, USA) fitted to a handheld force transducer. The stimulus was continued until the hind paw was withdrawn and considered indicative of the paw withdrawal threshold (PWT). For each animal, triplicates of each hind paw were taken with a 60-s interval between each stimulus.

2.3. Proteomic analysis

2.3.1. Spinal cord sample preparation

Rat spinal cords were rapidly collected at sacrifice; snap frozen and homogenised as previously published (Otis et al., 2016). Briefly, at the end of each experimentation day, the entire spinal cord tissue of rats was rapidly collected by a flush of saline within the lumbar spinal canal following deep anaesthesia with isoflurane and sacrifice by transection of the cervical spine. Samples were snap-frozen in liquid nitrogen and stored at − 80 °C pending analysis. Each spinal cord was weighed accurately and homogenised using a tissue tear and following the addition of phosphate-buffered saline solution (PBS) 0.01 M at a ratio of 1:5 (w/v) and protease inhibitor cocktail (Sigma-Aldrich Inc., Oakville, ON, Canada, number PP8340) at the same ratio (v/v). The samples were then sonicated and the homogenate was mixed with acetonitrile at a ratio of 1:1 (v/v) to remove larger proteins. The samples were vortexed and centrifuged for 10 min (× 12,000*g*) and the supernatant was transferred into an injection vial then spiked with the internal standard solution at a ratio of 1:1 (v/v). The spinal cords from the naïve group were also collected to obtain a baseline value from normal rats to normalise values obtained from the 1 or 2 mg MIA, 2 mg MIA-L, and sham groups.

2.3.2. Neuropeptidomic analysis

Neuropeptide quantification in spinal cord homogenate was