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Central Sensitization and Neuropathic Features of Ongoing Pain in a Rat Model of Advanced Osteoarthritis

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Abstract: **Osteoarthritis (OA) pain is most commonly characterized by movement-triggered joint pain. However, in advanced disease, OA pain becomes persistent, ongoing and resistant to treatment with nonsteroidal anti-inflammatory drugs (NSAIDs). The mechanisms underlying ongoing pain in advanced OA are poorly understood. We recently showed that intra-articular (i.a.) injection of monosodium iodoacetate (MIA) into the rat knee joint produces concentration-dependent outcomes. Thus, a low dose of i.a. MIA produces NSAID-sensitive weight asymmetry without evidence of ongoing pain and a high i.a. MIA dose produces weight asymmetry and NSAID-resistant ongoing pain. In the present study, palpation of the ipsilateral hind limb of rats treated 14 days previously with high, but not low, doses of i.a. MIA produced expression of the early oncogene, FOS, in the spinal dorsal horn. Inactivation of descending pain facilitatory pathways using a microinjection of lidocaine within the rostral ventromedial medulla induced conditioned place preference selectively in rats treated with the high dose of MIA. Conditioned place preference to intra-articular lidocaine was blocked by pretreatment with duloxetine (30 mg/kg, intraperitoneally at −30 minutes). These observations are consistent with the likelihood of a neuropathic component of OA that elicits ongoing, NSAID-resistant pain and central sensitization that is mediated, in part, by descending modulatory mechanisms. This model provides a basis for exploration of underlying mechanisms promoting neuropathic components of OA pain and for the identification of mechanisms that might guide drug discovery for treatment of advanced OA pain without the need for joint replacement.**

***Perspective:** Difficulty in managing advanced OA pain often results in joint replacement therapy in these patients. Improved understanding of mechanisms driving NSAID-resistant ongoing OA pain might facilitate development of alternatives to joint replacement therapy. Our findings suggest that central sensitization and neuropathic features contribute to NSAID-resistant ongoing OA joint pain.*

Key words: *Advanced osteoarthritis, neuropathic pain, duloxetine, central sensitization, descending facilitation.*

Osteoarthritis (OA) pain is one of the most frequent causes of chronic pain, with symptomatic OA characterized by joint stiffness and pain with movement and joint loading.[12,13,33] Some patients develop advanced OA pain characterized as ongoing pain that persists during rest and is resistant to nonsteroidal anti-inflammatory drugs (NSAIDs).[12,13,33] In patients with OA pain, joint pathology does not correspond to the degree of pain reported, and the mechanisms driving OA pain are not understood.[12,13,33] Because chronic OA pain is often inadequately treated, many patients undergo joint replacement therapy to alleviate pain.[12,13,33] This highlights a need for improved understanding of mechanisms driving NSAID-resistant OA pain to guide development of improved therapies.

Persistent ongoing pain is an important aspect of advanced OA pain that has not been possible to capture in preclinical OA models until recently. Using high-dose

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intra-articular monosodium iodoacetate (MIA) administration to model advanced OA, we showed the presence of weight asymmetry and ongoing pain.[21,26] Notably, diclofenac, an NSAID, failed to block the ongoing component of joint pain at a dose that blocked weight asymmetry, consistent with ineffectiveness of NSAIDs on advanced OA pain in patients.[26] We used this preclinical model to explore mechanisms driving NSAID-resistant ongoing pain in advanced OA.

Patients who reported moderate to severe pain showed signs of central sensitization such as referred pain and enhanced temporal summation.[2,10,14,19,22,25,33,41] Expression of the early oncogene, FOS, within the spinal dorsal horn in response to normally non-noxious stimulation such as touch[24,44] or non-noxious palpation[16,34] has been used as a marker of the development of spinal sensitization. Another key component of central sensitization is descending pain facilitatory pathways from the rostral ventromedial medulla (RVM).[28] Transient inactivation of the RVM by administration of lidocaine reverses evoked hypersensitivity and ongoing pain in animals with nerve injury–induced pain, indicating that evoked hypersensitivity and persistent ongoing or spontaneous pain are dependent on descending pain facilitatory pathways from the RVM.[18,29,32,40] We therefore examined whether animals in this model of advanced OA pain develop central sensitization.

It has also been proposed that OA patients with moderate to severe ongoing pain might have a neuropathic pain component, leading to suggestions of individualized treatment strategies for these different populations of patients.[33,37,38] Recently, duloxetine, a serotonin, norepinephrine reuptake inhibitor (SNRI) was approved by the U.S. Food and Drug Administration for treatment of OA pain. Antidepressants such as the monoaminergic reuptake inhibitors duloxetine and milnacipran, are part of the first line of therapies for patients with neuropathic pain.[11] Therefore, we determined whether duloxetine effectively blocks NSAID-resistant ongoing pain associated with advanced OA joint pain in this model.

Using a rat model of advanced OA pain, we tested the following hypotheses: 1) central sensitization is observed selectively in the context of ongoing joint pain associated with advanced OA pain, and 2) duloxetine, an SNRI prescribed for neuropathic pain,[8,23,39] blocks NSAID-resistant ongoing joint pain.

Methods

Subjects

Male Sprague Dawley rats weighing 225 to 300 g at the start of the experiments were housed in an animal care facility at the University of New England, with a 12-hour light/dark cycle. Food and water were available ad libitum. All testing was performed in accordance with policies and recommendations of the International Association for the Study of Pain and the National Institutes of Health guidelines for the handling and use of laboratory animals. All experimental protocols received approval from the Institutional Animal Care and Use Committee of the University of New England.

Bilateral RVM Cannula Implantation

Animals were anesthetized with an injection of ketamine and xylazine (100 mg/kg ketamine, 10 mg/kg xylazine, intraperitoneally [i.p.]) and placed in a stereotaxic apparatus. The skull was exposed and leveled, and bilateral 26-gauge guide cannulas, separated by 1.2 mm, were directed toward the lateral portions of the RVM (anteroposterior, 11.0 mm from bregma; lateral, ± .6 mm; dorsoventral, 8.5 mm from the skull according to Paxinos and Watson[30]). These coordinates were determined on the basis of previous studies.[18,32,40] The guide cannulas were cemented in place and secured to the skull by small stainless steel machine screws. Animals were allowed to recover 5 to 7 days after surgery before intra-articular injections of MIA or saline. Microinjections into the RVM were administered in a volume of .5 μL injected through a 33-gauge injector that protruded 1 mm beyond the end of the guide cannula and into fresh tissue to prevent backflow. Injections occurred over a period of 1 minute. Cannula placement was verified at the end of the study by microinjection (.5 μL) of Evans blue dye (50 mg/mL; Sigma-Aldrich, Saint Louis, MO). Animals with incorrect cannula placement were used as off-site control animals.

Intra-Articular Injection

Rats were anaesthetized with a 2% isoflurane O_2 mixture and given a single "low" (3 mg) or "high" (4.8 mg) dose of MIA (Sigma-Aldrich) through the infrapatella ligament of the left knee in 60 μL saline, corresponding to concentrations of 50 and 80 mg/mL MIA, respectively. Control animals received equivolume sterile saline. Evaluation of pain behaviors occurred 14 days after intra-articular injection of MIA or saline (control).

Weight Asymmetry

Changes in hind paw weight distribution between the left (MIA) and right (contralateral) limbs were used as an index of joint discomfort in the MIA-treated knee.[21,26] An incapacitance tester (Stoelting Co, Wood Dale, IL) was used for determination of hind paw weight distribution. Rats were placed in an angled plexiglass chamber positioned so that each hind paw rested on a separate force plate. The force exerted by each hind limb (measured in grams) was determined over a 5-second period. Each data point is the mean of 3 readings. As previously described,[21,26] data were normalized as percentage of injured and noninjured weight-bearing, such that sensitivity on the injured side is indicated by values <100%; equal weight distribution is indicated by 100%.

Conditioned Place Preference Testing

Ongoing pain was assessed using conditioned place preference (CPP) to a chamber paired with