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Screening the Role of Pronociceptive Molecules in a Rodent Model of Endometriosis Pain

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Abstract: **Chronic pain is a major symptom in patients with endometriosis, a common gynecologic condition affecting women in their reproductive years. Although many proalgesic substances are produced by endometriosis lesions, experimental evidence supporting their relative roles is still lacking. Furthermore, it is unclear whether these proalgesic agents directly activate nociceptors to induce endometriosis pain. To determine their relative contribution to pain associated with endometriosis, we evaluated the intrathecal administration of oligodeoxynucleotides (ODNs) antisense to messenger RNA for receptors for 3 pronociceptive mediators known to be produced by the ectopic endometrium. Two weeks after the implant of autologous uterine tissue onto the gastrocnemius muscle, local mechanical hyperalgesia was observed in operated rats. Intrathecal antisense ODN targeting messenger RNA for the interleukin 6 receptor–signaling complex subunit glycoprotein 130 and the nerve growth factor tyrosine kinase receptor A, but not their mismatch ODNs, reversibly attenuated mechanical hyperalgesia at the implant site. In contrast, intrathecal antisense ODN targeting the tumor necrosis factor receptor 1, at a dose that markedly inhibited intramuscularly injected tumor necrosis factor alpha, had only a small antihyperalgesic effect in this model. These results indicate the relative contribution of pronociceptive mediators produced by ectopic endometrial tissue to endometriosis pain. The experimental approach presented here provides a novel method to evaluate for the differential contribution of mediators produced by other painful lesions as well as endometriosis lesions as targets for novel treatment of pain syndromes.**

***Perspective:** This article presents evidence for the relative contribution of proalgesic mediators to primary hyperalgesia displayed by rats submitted to a model of endometriosis pain. This approach can be used to identify potential targets for the treatment of endometriosis pain.*

Key words: *Antisense, hyperalgesia, chronic pain models, nociceptor.*

Endometriosis is a common and very disabling clinical condition affecting women in their reproductive years that is characterized by the presence of endometrial tissue and glands outside of the uterine cavity.[24] Chronic pelvic pain is the main symptom reported by women with endometriosis, typically evoked by mechanical stimuli (eg, dysmenorrhea, dyschezia, dysuria, and dyspareunia).[24,54] This pain is often resistant to available analgesic treatments, presumably because they are not directed to specific mediators that induce endometriosis pain.

Given the complex pathophysiology of endometriosis, several mediators appear as possible candidates underlying endometriosis pain.[24] Although many attempts have been made to unveil the role of these proalgesic mediators in endometriosis pain, the available studies are correlative, and direct evidence for the involvement of candidate mediators as well as side-by-side comparison of their contribution is still lacking. Furthermore, it is unclear whether these pronociceptive mediators directly activate nociceptors innervating endometriosis lesions or act indirectly on other cell types to induce endometriosis pain. For instance, proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), and monocyte chemoattractant protein 1 have been observed in the plasma and peritoneal fluid of

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patients suffering endometriosis pain,[42,50,56] and all of them also induce primary mechanical hyperalgesia after local injection.[12,18,19,47] Neurotrophins such as nerve growth factor (NGF) are also produced in endometriosis lesions,[4,6-8] and NGF putative receptors, namely tyrosine kinase receptor A (TrkA) and p75, are expressed in nerve fibers innervating endometriosis lesions.[62] The TrkA receptor plays a well-established role in mechanical inflammatory and neuropathic hyperalgesia.[39]

Reversible knockdown of receptors in nociceptors by intrathecal administration of antisense (AS) oligodeoxynucleotides (ODNs) has been shown to be a reliable tool to assess their role in the processing of nociceptive information.[57] Injected intrathecally, AS ODNs reach the soma of sensory neurons[11,31,32,57] and selectively inhibit the expression of proteins in these cells[28,30,59] and peripheral fibers.[29,30,59] Taking advantage of this approach, we assessed the contribution of IL-6, TNFα, and NGF, which are pronociceptive mediators produced by endometriosis lesions,[4,6-8] and surgical rat models of endometriosis[68] by intrathecally administering to rats previously submitted to a model of endometriosis pain AS ODNs targeting the mRNA of the TNF receptor 1 (TNFR1), the IL-6 receptor–signaling complex subunit glycoprotein 130 (gp130), and TrkA. Because the putative ligands for these receptors have been reported to induce a local mechanical hyperalgesia that is sensitive to intrathecal AS treatment,[18,19,39,47] we evaluated their contribution to mechanical hyperalgesia observed in a surgical model of endometriosis by activating nociceptors innervating the endometriosis-like lesion.

Methods

Animals

Adult female Sprague Dawley rats (220–240 g; Charles River, Hollister, CA) were used in these experiments. They were housed in the Animal Care Facility at the University of California, San Francisco, under environmentally controlled conditions (lights on 0700–1900 hours; room temperature 21–23°C) with food and water available ad libitum. On completion of experiments, rats were euthanized by carbon dioxide asphyxiation followed by bilateral thoracotomy. Animal care and use conformed to National Institutes of Health guidelines (National Institutes of Health Guide for the Care and Use of Laboratory Animals). The University of California, San Francisco Committee on Animal Research approved all experimental protocols. Concerted effort was made to minimize the number and suffering of experimental animals, in accordance with the principle of the minimal sample size stated in the Ethical Guidelines for investigations of Experimental Pain in Conscious Animals.[69]

Surgical Induction of Endometriosis

Details of the model of surgically induced muscle endometriosis used here have been previously described.[2] We have previously provided evidence that the implant of autologous uterine tissue onto the gastrocnemius muscle, but not control surgical procedures, induces long-lasting mechanical hyperalgesia.[1,2] We used the same surgical procedure to implant the ectopic uterine tissue and behavioral nociceptive evaluation here. We[2] and others[10,41] have provided evidence that lesions developed from ectopic uterine implants are innervated by nociceptors arising from the receptor tissue. Furthermore, these nociceptors display markers typically observed in nociceptors of the receptor tissue, indicating a neo-innervation of the ectopic tissue by these primary nociceptive afferents.[2,10,41] Briefly, female rats were premedicated with a mixture of ketamine hydrochloride and xylazine (80 and 6 mg/kg, subcutaneously, respectively), and anesthesia was maintained with isoflurane (1–1.5% in 99–98.5% oxygen). The right dorsal paravertebral area was infiltrated with .25% bupivacaine (Marcaine; Hospira, Lake Forest, IL), and under aseptic conditions, an incision approximately 2 cm in length was performed to expose and isolate the right uterine horn. After ligature of uterine blood vessels, a 1-cm segment was removed and immediately placed in a Petri dish containing .9% NaCl. The musculature of the dorsal abdominal wall was closed with single crossed stitches and the skin incision closed with horizontal mattress stitches. The excised uterine tissue was measured with a millimeter scale and opened longitudinally; a full-thickness 3 $\times$ 3-mm square of uterine tissue was then removed and kept in physiologic saline. The implant was performed through an incision in the biceps femoris muscle allowing exposure of the underlying gastrocnemius muscle. The square of uterine tissue was sutured to the surface of the gastrocnemius muscle applying 3 or 4 single stitches using 5-0 nylon, with the endometrial portion of the uterine tissue contacting the gastrocnemius muscle. After checking for hemostasis, the biceps femoris muscle and the skin incisions were sutured separately with single stitches. The sham surgical procedure was similar but the uterus was left intact and not implanted on the surface of the gastrocnemius. Surgical procedures were performed regardless of the estrous cycle status of the rats.

Determination of Estrous Cycle Phases

The phase of the estrous cycle was assessed preoperatively, as reported previously.[2] Immediately after the induction of anesthesia for surgical implant of ectopic endometrium, 30 μL of NaCl .9% was flushed 3 or 4 times into the vaginal cavity. The resulting fluid was then placed onto a slide and observed unstained at $\times$100 magnification. The diagnostic criteria used to determine the phase of the estrous cycle were based on cellular type predominance, as previously described.[40]

Measurement of Hyperalgesia at the Site of Endometriosis Lesion

Mechanical nociceptive threshold in the site of surgical intervention was quantified using a digital force transducer (Chatillon DFI2; Amtek Inc, Largo, FL) with a