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Analgesic effect of minocycline in rat model of inflammation-induced visceral pain

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ABSTRACT

The present study investigates the analgesic effect of minocycline, a semi-synthetic tetracycline antibiotic, in a rat model of inflammation-induced visceral pain. Inflammation was induced in male rats by intracolonic administration of tri-nitrobenzenesulphonic acid (TNBS). Visceral hyperalgesia was assessed by comparing the viscero-motor response (VMR) to graded colorectal distension (CRD) prior and post 7 days after TNBS treatment. Electrophysiology recordings from CRD-sensitive pelvic nerve afferents (PNA) and lumbo-sacral (LS) spinal neurons were performed in naïve and inflamed rats. Colonic inflammation produced visceral hyperalgesia characterized by increase in the VMRs to CRD accompanied with simultaneous activation of microglia in the spinal cord and satellite glial cells (SGCs) in the dorsal root ganglions (DRGs). Selectively inhibiting the glial activation following inflammation by araC (Arabinofuranosyl Cytidine) prevented the development of visceral hyperalgesia. Intrathecal minocycline significantly attenuated the VMR to CRD in inflamed rats, whereas systemic minocycline produced a delayed effect. In electrophysiology experiments, minocycline significantly attenuated the mechano-transduction of CRD-sensitive PNAs and the responses of CRD-sensitive LS spinal neurons in TNBS-treated rats. While the spinal effect of minocycline was observed within 5 min of administration, systemic injection of the drug produced a delayed effect (60 min) in inflamed rats. Interestingly, minocycline did not exhibit analgesic effect in naïve, non-inflamed rats. The results demonstrate that intrathecal injection of minocycline can effectively attenuate inflammation-induced visceral hyperalgesia. Minocycline might as well act on neuronal targets in the spinal cord of inflamed rats, in addition to the widely reported glial inhibitory action to produce analgesia.

1. Introduction

Chronic visceral pain, observed in several gastrointestinal (GI) disorders, is a multifaceted problem and remains poorly understood (Smith, 2010). Despite conventional belief that visceral pain is a variant of somatic pain, it significantly differs in neurological mechanisms and transmission pathways (Cervero and Laird, 1999). Unfortunately, there are very few specific analgesics for visceral pain and therapies commonly used are extensions of those used for general pain management (Cervero and Laird, 1999). Currently available treatments for visceral pain are unsatisfactory due to their adverse effects like altered GI mucosal homeostasis, motility, nausea, constipation, irritation and ulceration. Activation of glial cells and neuro-glial interactions are emerging as key mechanisms underlying chronic pain (DeLeo and Yezierski, 2001; Suter et al., 2007). Accumulating evidence has implicated activation of glial cells in the development and maintenance of chronic pain: microglia and astrocytes of the central nervous system (CNS) and satellite glial cells (SGCs) of the dorsal root ganglia (Takeda et al., 2009; Ji et al., 2013). Robust glial activation mediated pain has been reported in several models of pain including sciatic inflammatory neuropathy (Ledeboer et al., 2005), chronic constriction nerve injury (Stuesse et al., 2000), partial sciatic nerve ligation (Coyle, 1998), spinal nerve ligation (Jin et al., 2003), spinal nerve transection (Raghavendra et al., 2003) and peripheral inflammation (Cho et al., 2006). Given their involvement in several pathological conditions, activated glial cells are being considered as a potential pharmacological target for treating various forms of pain.

Minocycline, a second-generation, broad spectrum, semi-synthetic tetracycline antibiotic is of particular interest as an analgesic, in addition to its anti-microbial effect. Minocycline effectively crosses the blood-brain barrier (Aronson, 1980) and has a proven safety record in humans (Thomas and Le, 2004).

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Minocycline mediated inhibition of microglial activation has been reported to reduce nociception in inflammation-evoked pain (Hua et al., 2005), spinal cord contusion injury (Hains and Waxman, 2006) and spinal nerve ligation (Lin et al., 2007). While the majority of these studies attribute the analgesic effect of minocycline to inhibition of microglial activation (Tikka et al., 2001; Garrido-Mesa et al., 2013), some studies have also reported on its action on other targets like astrocytes (Zhang et al., 2012) and neurons (González et al., 2007). Extensive literature published in the last decade indicates an essential role of glial cells in the development and maintenance of hyperalgesia. However, very little is known about the involvement of activated glial cells in visceral pain. Further, there is no information on the efficacy of minocycline as a potential analgesic for inflammation-induced visceral pain. The objective of this study was to determine (1) whether visceral hyperalgesia caused by inflammation of the colon is due to activation of the microglia and SGCs and (2) whether this visceral hyperalgesia can be attenuated by administration of minocycline. The study evaluates the effect of minocycline on both the DRG neurons and lumbar spinal cord neurons using a combined approach involving behavioral and electrophysiology experiments.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (Taconic, Indianapolis, IN, USA) with an average weight of 400 g (range: 350–450 g) were used for this study. Rats were kept in controlled conditions with a 12 h light/dark schedule and had access to both food and water ad libitum. Twenty-four hours before behavioral and electrophysiology studies, the animals were placed in a wire-bottom cage and access to food, but not water, was denied in order to empty the colon. All experiments were performed according to the approved guidelines of the Institutional Animal Care and Use committee (AUA # 355) at the Medical College of Wisconsin and The International Association for the Study of Pain (IASP).

2.2. Drugs and chemicals

TNBS (tri-nitrobenzene sulfonic acid) was purchased from Sigma-Aldrich, USA. Minocycline (Sigma-Aldrich, USA) was freshly dissolved in sterile distilled water before administration and heated briefly in a water bath until completely dissolved and the solution was clear. Minocycline was administered intraperitoneally (i.p.) at a dose of 50 mg/kg, intrathecally (i.t.) at dose of 50 μg/animal and intravenously (i.v.) at dose of 25 mg/kg based on previously published reports (Cho et al., 2012; Liu et al., 2012). AraC (Arabinofuranosyl Cytidine; Sigma-Aldrich, USA) dissolved in saline was administered at a dose of 10 μg/animal (i.t.) for 7 days (van der Kogel and Sissingh, 1985). Intrathecal (i.t) drugs were administered in a volume of 5 μL followed by 5–10 μL saline flush depending on the length of the catheter.

2.2.1. Induction of inflammation-induced visceral hyperalgesia

TNBS (tri-nitrobenzene sulfonic acid)-induced colonic inflammation was used as a model for visceral hyperalgesia and performed as previously described (Morris et al., 1989). Briefly, overnight fasted rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and 0.5 mL of 50% TNBS (dissolved in ethanol, 1:1) was slowly injected into the descending colon using a 16 gauge gavage needle. Intracolonic TNBS-induced colon inflammation is considered a model for Crohn's disease (Kruschewski et al., 2001). Onset of inflammation in TNBS-treated rats were confirmed biochemically by measuring the myeloperoxidase (MPO) activity in the colon and pathologically by staining the colon tissue with hematoxylin and eosin (*H* and *E*) stain using previously standardized procedures (Banerjee et al., 2009).

2.3. Recording of viscero-motor response (VMR)

2.3.1. Surgical procedure

Rats were initially anesthetized by injecting pentobarbital sodium (50 mg/kg, ip). Teflon coated electrodes (Cooner Wire, Part No. A5631, Chatsworth, CA, USA) were implanted in the external oblique muscle of the abdomen for electromyography (EMG) recordings. For intrathecal (i.t.) drug administration, the rats were placed in a stereotactic head holder under pentobarbital anesthesia and a polyethylene catheter (PE-10) was inserted through an incision in the atlanto-occipital membrane. The catheter was advanced caudally 7 or 7.5 cm from the incision site to the lumbar enlargement of the spinal cord. The external end of the catheter (PE-50) was tunneled subcutaneously to exit at the top of the head. The electrode and the catheter were externalized dorsally near the neck and secured in place with silastic tubing and the skin was closed using 3–0 silk suture. The position of the catheter was confirmed via infusion of 20 μL sterile 2% lidocaine. Animals that exhibited transient hind limb paralysis due to the lidocaine injection alone were used for the study. The position of the catheter was also checked visually during laminectomy. Post-surgery, the rats were closely monitored and euthanized (Beuthanasia-D, Schering-Plough Animal Health Corp, NJ, USA) if they showed any signs motor abnormalities or paralysis.

2.3.2. VMR recordings

No less than 72 h after surgery, rats were placed inside the plexiglass restraining tubes for 2 h/day for three consecutive days in order to acclimatize them to experimental conditions. On the day of VMR recordings (6–7 days after electrode/catheter implantation), rats were placed in the restraining tube and a highly compliant, flaccid latex balloon (6 cm long and 3.5 cm OD) coated with non-reactive bacteriostatic lubricant (Surgilube, Savage Laboratories, Melville, USA) was inserted into the descending colon and taped to the tail. Rats were allowed to rest inside the tube for at least 30 min before testing the VMR to colorectal distension (CRD). The EMG signal was amplified using the amplifier (A-M System, model 1700, Carlsborg, WA, USA). A stimulus-response function (SRF) to graded CRD (10, 20, 30, 40, 60 mmHg) was recorded. The duration of distension was 30 s with a 180 s inter-stimulus interval between the distension. Data were recorded real-time using the Spike 4/CED 1401 data acquisition program (CED 1401; Cambridge Electronic Design, Cambridge, UK). Following a baseline SRF, rats were lightly anesthetized with pentobarbital sodium and TNBS was administered as described above. Rats were then allowed to recover for 7 days prior to repeating the VMR.

2.4. Electrophysiology

2.4.1. Recording from CRD-sensitive pelvic nerve afferent (PNA) fiber

PNA fiber recordings were performed both in naïve and TNBS-treated rats. For PNA fiber recordings, a 3–4 cm long incision was made in the lower abdomen after anesthesia and the prostate lobe was reflected laterally to access the major pelvic ganglion (MPG), pelvic, and hypogastric nerves. The pelvic nerve was isolated from the surrounding fatty tissues and a pair of Teflon-coated stainless wire was placed around it proximal to the MPG to deliver electrical stimulation to pelvic nerve in order to confirm that recordings were made from the S1 sacral dorsal root. The electrodes were secured in place by