A Rat Model of Chronic Postinflammatory Visceral Pain Induced by Deoxycholic Acid

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Background & Aims: **Chronic visceral hyperalgesia is considered an important pathophysiologic symptom in irritable bowel syndrome (IBS); previous gastrointestinal inflammation is a potent etiologic factor for developing IBS. Although there are several animal models of adult visceral hypersensitivity after neonatal perturbation or acute colonic irritation/inflammation, current models of postinflammatory chronic visceral hyperalgesia are unsatisfactory. The aim of this study was to establish a model of chronic visceral hyperalgesia after colonic inflammation in the rat.** ***Methods:*** **Deoxycholic acid (DCA) was instilled into the rat colon daily for 3 days and animals were tested for up to 4 weeks.** ***Results:*** **DCA induced mild, transient colonic inflammation within 3 days that resolved within 3 weeks. An exaggerated visceromotor response, referred pain to mechanical stimulation, increased spinal Fos expression, and colonic afferent and dorsal horn neuron activity were apparent by 1 week and persisted for at least 4 weeks, indicating chronic dorsal horn hyperexcitability and visceral hyperalgesia. There was no spontaneous pain, based on open field behavior. There was a significant increase in opioid-receptor activity.** ***Conclusions:*** **DCA induces mild, transient colitis, resulting in persistent visceral hyperalgesia and referred pain in rats, modeling some aspects of postinflammatory IBS.**

Irritable bowel syndrome (IBS) is a functional, multifactorial disorder characterized by abdominal pain and altered bowel habits. Population-based surveys documented that IBS affects between 8% and 22% of the general population.[1] In general, psychosocial factors, abnormal gastrointestinal motility and secretion, and visceral hypersensitivity are thought to contribute to the symptoms of IBS. Visceral hypersensitivity currently is considered to be the most important pathophysiologic factor in IBS and may partially result from the sensitization of primary afferent fibers innervating the gastrointestinal tract.[2–4] However, central sensitization also contributes to the development of abdominal pain.[2] As many as 26% of patients suffer from acute gastroenteritis that resolves before developing IBS.[5,6] Therefore, establishing an appropriate chronic visceral hyperalgesia model that mimics characteristics of postinflammatory IBS is of great clinical significance.

Several chemical irritants have been used to produce colonic inflammation resulting in visceral hyperalgesia. Acetic acid,[7] mustard oil,[8–12] and zymosan[13,14] evoke short-term hyperalgesia associated with transmural tissue damage/colonic inflammation. Dextran sulfate sodium induces colonic inflammation without visceral hypersensitivity.[15–17] Intracolonic trinitrobenzene sulfonic acid induces histologic changes in the distal colon 24 hours after injection including severe colonic inflammation, necrosis, loss of myenteric neurons, and a reduction of sensory terminals in the colon wall,[18,19] and is considered a model for Crohn's disease.[20,21] This is accompanied by hyposensitivity to colorectal distention (CRD) within 2–3 days (unpublished observations) and hyperalgesia starting at 4–5 days, with all symptoms diminishing by 14 days.[22,23] However, hyperalgesia reappeared at later time points in a subset of animals.[8,24] Given the severe tissue damage and pattern of changes in visceral sensitivity, it cannot be ruled out that the delayed hypersensitivity is a form of neuropathic pain. Most recently, butyrate enemas were reported to induce a sustained, concentration-dependent, colonic hypersensitivity.[25] However, there were no signs of macroscopic or histologic tissue damage. Therefore, it cannot be used as a model of postinflammatory IBS.

Bile acids are derivatives of cholesterol synthesized in the hepatocytes. Deoxycholic acid (DCA), an unconjugated secondary bile acid, is produced in the colon from the salts of glycocholic and taurocholic acid converted by an action of anaerobic bacterial enzymes in the cecum/proximal colon. DCA acts as a detergent to solubilize fats for intestinal absorption and is cytotoxic to colonic epithelial cells, inducing inflammatory changes within 12–48 hours after treatment at higher concentrations.[21,26] DCA, at a dose of 4 mmol/L, increases colonic mucosal perme-

Abbreviations used in this paper: CRD, colorectal distention; DCA, deoxycholic acid; IBS, irritable bowel syndrome; LS, lumbosacral; MPO, myeloperoxidase; RM, repeated measures; TL, thoracolumbar; vmr, visceromotor response.

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ability,[27–29] causes colonic epithelial proliferation,[30] and plays a role as a colon cancer promoter.[31,32] Bile salt malabsorption underlies some forms of postinfectious IBS.[6] However, it has never been determined if colonic inflammation with DCA results in chronic visceral hyperalgesia.

The aim of this study was to establish an animal model of chronic visceral hyperalgesia after colonic inflammation. Our results suggest that DCA induces mild transient colitis and long-term visceral hyperalgesia in rats, which may provide a suitable model for examining mechanisms underlying chronic visceral pain. Some of these data have been presented in abstract form.[33]

Methods and Materials

Animals

Adult male Sprague Dawley rats (240–320 g; Harlan Sprague Dawley, Indianapolis, IN) were used in this study. Animals were housed under a 12-hour/12-hour light/dark cycle. Experiments were performed under approval of the University of Maryland Dental School Animal Care and Use Committee and conformed to the ethical treatment of animals published by the International Association for the Study of Pain.

Induction of Colonic Inflammation

Rats were anesthetized with Nembutal (Ovation Pharmaceuticals, Deerfield, IL) (45 mg/kg intraperitoneally [IP]). A gavage needle was inserted through the anus approximately 6 cm into the colon and 1 mL of 4 mmol/L DCA in Kreb's solution (in mmoles: NaCl, 122; KCl, 3.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2$, 1.2; pH 7.4) was injected while the needle slowly was withdrawn. Rats were left on a mound of bedding in a head-down position to prevent leakage of DCA. Rats were injected once daily on 3 consecutive days, the first injection counting as day 1. Rats in the control group received 1 mL 0.9% saline instead of DCA.

Plasma Extravasation

Plasma extravasation (PE) in the colon was determined with Evan's blue dye. Rats injected with DCA or saline (1 and 4 weeks) were distended to 60 mm Hg (Visceromotor Response study). Subsequently, rats were anesthetized with Nembutal and injected with Evan's blue dye (50 mg/kg intravenously [IV]) for 10 minutes followed by perfusion with 150 mL saline. A 5-cm length of colon proximal to the level of the pubic symphysis was removed and placed in 5 mL dimethyl sulfoxide for 48 hours.[34] The concentration of extracted Evan's blue dye was detected by spectrophotometer at 620 nm absorbance. Plasma extravasation was expressed as micrograms of Evan's blue dye per gram of dry weight colon.

Myeloperoxidase Assay

Rats injected with DCA or saline (1, 2, 3, 4 wk) were distended (60 mm Hg) and euthanized. Five centimeters of fresh colon tissue proximal to the pubic symphysis was removed. The distal half of each colon was rinsed briefly with saline, cut into smaller pieces, snap-frozen, and stored at −80°C until use. Myeloperoxidase (MPO) activity was measured 48 hours later. The proximal half was placed in 4% paraformaldehyde and used for histologic examination (see later).

For the MPO assay the tissue was weighed and placed in 0.5 mL of 0.5% hexadecyltrimethylammonium bromide in 50 mmol/L phosphate buffer (pH 6.0). The tissue was minced with scissors for 20 seconds on an ice-cold plate and then homogenized for 20 seconds. Another 0.5 mL of hexadecyltrimethylammonium bromide buffer was added to the homogenate, vortexed, and centrifuged at 14,000 rpm for 2 minutes. The supernatant (50 μL) was assayed for MPO activity by adding 1.45 mL O-dianisidine (0.167 mg/mL in 5 mmol/L phosphate buffer [pH 6.0], with 0.0005% H_2O_2 added) and the absorbance was taken at 460 nm 4 times at 30-second intervals. The MPO units (1 unit of MPO is defined as 1 μmole of H_2O_2 split in 1 minute, which is equal to 1.13×10^{-2} changes in absorbance) were normalized to the colon wet weight and expressed as MPO units/mg.[35]

Histologic Examination of Colonic Samples

The proximal colon samples described earlier were fixed in 4% paraformaldehyde for 5 days and transferred to 30% sucrose. Sixteen micron transverse sections were mounted on glass slides, dehydrated in a graded ethanol series, and stained with H&E. A pathologist blinded to treatment counted 3 random fields at 400× and recorded the numbers of neutrophils located within the mucosa, submucosa, and density within capillaries for each field.

Paraffin sections (6 μm) from the proximal half of the sample were cut and stained with toluidine blue. Mast cells were counted in 3 random sections at 200×.

Visceromotor Response

The visceromotor response is a pseudoaffective reflex contraction of the abdominal muscles in response to CRD. Five days before starting the experiment, Teflon-coated, 32-gauge stainless steel wire (Cooner Wire Company, Chatsworth, CA) made into electromyography electrodes were implanted into the lateral abdominal muscle wall. The electrode leads were exteriorized at the back of the neck.[36] Rats were housed singly after surgery.

Rats were fasted overnight (with free access to water) to facilitate balloon placement. On the day of the experiment, rats were sedated briefly with isoflurane and a 5- to 6-cm balloon (made from the finger of a latex glove) attached to Tygon tubing (VWR International, West Chester, PA) was inserted through the anus into the rectum and descending colon, with the distal end 1 cm