Acupuncture at GV01 Relieves Somatic Pain Referred by Colitis in Rats

Hee-Young Kim1,2, Dae-Hyun Hahn4, Younbyoung Chae2, Kyungeh An5, Kwang-Ho Pyun3, Hyejung Lee2, and Insop Shim3

1Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX 77555-1069, USA; 2College of Oriental Medicine, Kyung Hee University, Seoul 130-701, Korea; 4Department of Integrative Medicine, College of Medicine, The Catholic University of Korea, Seoul 137-701, Korea; 5Acupuncture & Meridian Science Research Center, Kyung Hee University, Seoul 130-701, Korea; and 6School of Nursing, University of Texas Medical Branch, Galveston, TX 77555-1069, USA

The present study aimed to expand our previous findings regarding the therapeutic effects and underlying mechanisms of acupuncture at GV01 in colitis. Our results showed that acupuncture at GV01 has antinociceptive effects on referred somatic pain induced by experimental colitis, and that endogenous opioid pathways may mediate these effects.

Key words: colitis, GV01, referred pain, acupuncture.

Referred somatic pain is a characteristic feature of visceral pain. In contrast to true visceral pain, which is usually felt as a dull, vague, and poorly defined sensation, referred pain is sharper, much better defined, and localized, often with allodynia or secondary hyperalgesia (tenderness) characterized by an increased sensitivity to pain stimuli and a decreased pain threshold [1]. Patients with visceral pain often report unpleasant tenderness known as visceral pain. In contrast to true visceral pain, which is usually referred pain is sharper, much better defined, and locally felt as a deep, dull, vague, and poorly defined sensation, visceral pain causing referred somatic pain, only a few recent studies have tried to alleviate these cutaneous symptoms [6, 7]. However, drugs such as lidocaine [6] and morphine [7], used in previous studies, may produce undesirable side effects.

Acupuncture has been used successfully without side effects to manage both somatic pain and visceral pain [8, 9]. Although it has been shown to be effective in treating various types of pain, including visceral pain [9, 10], little is known about its effect on somatic pain referred from visceral pain. In our recent studies, we reported the anticolitic effects of acupoint GV01 and its possible mechanisms [11–13]. GV01 (Changqiang, Governing Vessel 1) is a single acupoint in the depression ventral to the base of the tail and dorsal to the anus. It is one of the most effective acupoints for treating diarrhea in humans and animals [14–18]. We have shown that acupuncture at GV01 depressed proximal colonic motility by decreasing the total duration and frequency of contractile states in conscious dogs [12], and it also had anti-inflammatory effects on colitis rats [13]. Furthermore, we suggested that the effects of acupuncture at GV01 in colitis involve endogenous opioid pathways [13]. Based on these findings, we hypothesized that acupuncture at GV01 mediates endogenous opioid pathways, whereby it can relieve somatic pain referred by colitis.

To assess the antinociceptive effects of acupuncture at GV01 on somatic pain referred by colitis and its mediation of opioid pathways, the present study explored whether (i) acupuncture at GV01 suppressed referred perineal somatic pain in colitic rats using the von Frey test, (ii) pretreatment with naloxone, a nonspecific opioid antagonist, affected its effects, and (iii) acupuncture at GV01 affected Fos expression in the periaqueductal gray (PAG), a brain area known to be critically involved in acupuncture-analgesia and opioid pathways [19, 20].

Materials and methods

Animals. Male Sprague-Dawley rats, weighing about 250 g, were used. The rats, housed in individual cages, were kept in a restricted access room with a controlled temperature (23°C) and a light/dark cycle (12 h/12 h). Food and water were provided ad libitum. All procedures involving the use of animals conformed to the guidelines set by the Kyung Hee University Institutional Animal Care and Use Committee.

Experimental schedule. Starting one day after the induction of colitis, the acupuncture groups received acupuncture for 20 min once daily for 7 days. A behavioral test for tactile cutaneous hypersensitivity was performed 30 min after needle insertion (i.e., 10 min after needle insertion).

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Correspondence should be addressed to: Insop Shim, Department of Integrative Medicine, College of Medicine, The Catholic University of Korea, Seoul 137-701, Korea. Tel: +82-2-590-2971, Fax: +82-2-592-6359, E-mail: ishim@catholic.ac.kr
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in our preliminary study [13]. Briefly, for needle insertion each rat was placed in a plastic holder with tail and hip protruding. In groups 2, 4, and 6 (GV01 acupuncture groups), an acupuncture needle (30 mm long, 0.25 mm in diameter) was inserted 2 cm deep perpendicular to the skin at GV01 between the anus and the tail base. The needle’s handle was attached to the tail base with a bandage. The insertion and bandaging procedures did not exceed 2 min. Each rat was allowed to move freely in the cage, and the needle was withdrawn after 20 min. In the nonacupoint group, a needle of the same diameter was inserted 2 cm deep obliquely into the gluteal muscle of the right hip between the greater trochanter and the iliac crest and left in place there for 20 min. Acupuncture treatment. We applied the acupuncture method used in our previous study [13]. Briefly, for needle insertion each rat was placed in a plastic holder with tail and hip protruding. In groups 2, 4, and 6 (GV01 acupuncture groups), an acupuncture needle (30 mm long, 0.25 mm in diameter) was inserted 2 cm deep perpendicular to the skin at GV01 between the anus and the tail base. The needle’s handle was attached to the tail base with a bandage. The insertion and bandaging procedures did not exceed 2 min. Each rat was allowed to move freely in the cage, and the needle was withdrawn after 20 min. In the nonacupoint group, a needle of the same diameter was inserted 2 cm deep obliquely into the gluteal muscle of the right hip between the greater trochanter and the iliac crest and left in place there for 20 min. Assessment of referred somatic pain (tactile cutaneous hypersensitivity test). In our preliminary study, mechanically sensitive areas in colitic rats were observed in the caudal part of the body, including the tail, hip, perineum, and scrotum regions (data not shown). Of the sensitive areas, the left perineum (an area of about 20 mm in diameter) between the ischiatic tuberosity and the scrotal attachment at the tail base was chosen for the cutaneous hypersensitivity test (Fig. 1B). The selected region was easy to access and was well defined when each animal was placed on a metal mesh floor under a custom-made transparent plastic dome (8 x 8 x 18 cm). Referred somatic pain (tactile cutaneous hypersensitivity) was measured by applying von Frey filaments, using a method modified from Millecamps et al. [7]. Briefly, the rats were completely shaved on the caudal part of body before the test. After a withdrawal of the acupuncture needle, each animal was placed on a metal mesh floor under a transparent plastic dome and was then acclimatized to the test cage for 10 min before testing. The threshold of cutaneous hypersensitivity corresponded to the gram force of the von Frey filament that induced avoidance behavior in the animal (an abrupt withdrawal of the perineal area, crumpling of the skin, change of position, or escape). Then von Frey filaments, ranging from 0.2 to 26 g (cut off), were applied five times for 1 min. Avoidance behaviors of at least three withdrawal). To explore the involvement of endogenous opioid pathways, naloxone or vehicle was administered 30 min before acupuncture. All rats were sacrificed after the last treatment to examine Fos expression in the PAG of the brain (Fig. 1A).

Experimental design. The rats were randomly assigned to six groups: (1) untreated normal (n = 5), i.e., normal untreated rats; (2) normal-GV01 (n = 5), i.e., normal rats given manual acupuncture at GV01; (3) colitis control (n = 6), i.e., rats with untreated experimental colitis; (4) colitis-GV01 (n = 6), i.e., rats with experimental colitis given manual acupuncture at GV01; (5) nonacupoint (n = 6), i.e., rats with experimental colitis given manual acupuncture at a nonacupoint; (6) colitis-Nal-GV01 (n = 6), i.e., rats with experimental colitis that received naloxone (3 mg/ml/kg) subcutaneously at 30 min before manual acupuncture at GV01.

Rats in groups 1 to 5 received a normal saline solution subcutaneously at the same volume (1 ml/kg) as the solution used to administer the naloxone dose to rats in the group 6.

Colitis induction. Experimental colitis was induced, using TNBS/ethanol as described previously [21]. Rats fasted for 12 h were placed in an induction chamber and received a combination of oxygen (1 l/min) and 3.5% isoflurane. We carefully observed the loss of righting reflex and decreased respiratory rate. When deep and slow respiration was achieved, the rat was removed from the chamber. An intragastric tube was inserted rectally into the colon so that the tip was 8 cm proximal to the anus. Thereafter, 0.60 ml of 50 g trinitrobenzene sulphonic acid/l (TNBS 30 mg, Sigma Chemical Co.) in 0.25 ml of 50% ethanol (v/v), resulting in a total volume of 0.85 ml, was instilled into the lumen of the colon, and the tube was flushed with 0.5 ml of air. A similar volume of physiological saline was injected into the colon of rats in groups 1 and 2 (normal and normal-GV01 groups). All rats completely recovered within 90–120 s after removal from the chamber.

Acupuncture treatment. We applied the acupuncture method used in our previous study [13]. Briefly, for needle insertion each rat was placed in a plastic holder with tail and hip protruding. In groups 2, 4, and 6 (GV01 acupuncture groups), an acupuncture needle (30 mm long, 0.25 mm in diameter) was inserted 2 cm deep perpendicular to the skin at GV01 between the anus and the tail base. The needle’s handle was attached to the tail base with a bandage. The insertion and bandaging procedures did not exceed 2 min. Each rat was allowed to move freely in the cage, and the needle was withdrawn after 20 min. In the nonacupoint group, a needle of the same diameter was inserted 2 cm deep obliquely into the gluteal muscle of the right hip between the greater trochanter and the iliac crest and left in place there for 20 min.

Assessment of referred somatic pain (tactile cutaneous hypersensitivity test). In our preliminary study, mechanically sensitive areas in colitic rats were observed in the caudal part of the body, including the tail, hip, perineum, and scrotum regions (data not shown). Of the sensitive areas, the left perineum (an area of about 20 mm in diameter) between the ischiatic tuberosity and the scrotal attachment at the tail base was chosen for the cutaneous hypersensitivity test (Fig. 1B). The selected region was easy to access and was well defined when each animal was placed on a metal mesh floor under a custom-made transparent plastic dome (8 x 8 x 18 cm). Referred somatic pain (tactile cutaneous hypersensitivity) was measured by applying von Frey filaments, using a method modified from Millecamps et al. [7]. Briefly, the rats were completely shaved on the caudal part of body before the test. After a withdrawal of the acupuncture needle, each animal was placed on a metal mesh floor under a transparent plastic dome and was then acclimatized to the test cage for 10 min before testing. The threshold of cutaneous hypersensitivity corresponded to the gram force of the von Frey filament that induced avoidance behavior in the animal (an abrupt withdrawal of the perineal area, crumpling of the skin, change of position, or escape). Then von Frey filaments, ranging from 0.2 to 26 g (cut off), were applied five times for 1 min. Avoidance behaviors of at least three
times in five applications were considered positive; an absence of avoidance response was considered negative. Depending on the positive or negative response, subsequent filaments were applied in the order of descending and ascending intensity, respectively. The von Frey test was performed once daily to avoid the influences of tactile (von Frey) stimulation to the perineum [22].

**Fos expression in the PAG.** After the last treatment, all rats were transcardially perfused with 100 ml 0.9% saline followed by 400 ml cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 under an overdose of pentobarbital (60 mg/kg, i.p.). Their brains were removed and postfixed for 2 h, immersed in 30% sucrose overnight at 4°C, and cut into 30 µm transverse sections with a freezing microtome (Leica 1900). Incubations were performed in 5% normal goat serum/0.2% Triton X-100. The primary antibody was a rabbit anti-Fos polyclonal antibody (1:2,000, Santa Cruz Biotechnology, USA). The secondary antibody was a biotinylated goat antirabbit IgG (Vector, 1:200). Fos-like immunoreactivity was visualized by diaminobenzine and ammonium nickel sulfate in the presence of 0.01% hydrogen peroxide. For each animal, Fos-positive neurons were counted on both sides of the ventrolateral periaqueductal gray (vlPAG) of brain sections located about 8.5 mm caudally from bregma under a Leica DMRXA microscope. This level corresponded to plates 53 and 54 of the rat brain atlas [23].

**Statistical analysis.** All values are expressed as mean ± SE. The statistical significance among groups was evaluated by an analysis of variance (ANOVA) followed by Tukey’s post hoc tests. *P* values < 0.05 were regarded as significant.

**Results**

Compared with the normal group, the colitic control group exhibited a marked and prolonged threshold reduction in tactile cutaneous hypersensitivity after an induction of colitis (Fig. 2A). Compared with the colitis control or nonacupoint group, acupuncture at GV01 significantly increased the threshold of tactile cutaneous hypersensitivity in colitic rats on every test day, except the 2nd one. In the colitis-GV01 group on that day, three of the six rats showed an alleviation of referred pain after acupuncture, and three did not. Taken together, on the 2nd test day the colitis-GV01 group showed no statistically significant differences from the colitis-control group. In the colitis-Nal-GV01 group, naloxone pretreatment blocked the inhibitory effect of acupuncture at GV01 on the tactile cutaneous hypersensitivity from the 3rd test day. The values from the 1st and 2nd days in the colitis-Nal-GV01 group were decreased slightly, but were not significantly different from the values in the colitis-GV01 group (Fig. 2B).

Compared with normal rats (normal group), colitic rats had more Fos-labeled neurons in the vlPAG. Compared with untreated normal rats (normal group), acupuncture at GV01 elicited no Fos expression in normal rats (normal-GV01 group). However, compared with the nonacupoint and control groups, acupuncture at GV01 significantly increased Fos-labeled neurons in the vlPAG in colitic rats (Fig. 3).

**Discussion**

One of the most important features of visceral nociception is that pain is referred to certain somatic regions remote from the lesion [4]. The referral area is generally segmental and superficial to skin or muscle, or to both, innervated by the same spinal nerves as the affected viscus; the referral area also shows hyperalgesia. Such tenderness develops slowly, taking many minutes, even hours, to manifest, and the tenderness persists for prolonged periods [2]. Colonic pain or colitis generates referred hyper-
sensitive pain at the level of the lumbosacral dermatomes, which include the perineal, tail, hind limb, hip, GV01 area, and perianal regions [7, 11, 22, 24]. In the present study, colitic rats exhibited marked and prolonged referred somatic pain (decreased mechanical threshold) to the perineal region in comparison with normal rats.

In our previous study, we showed that acupuncture at GV01 inhibited colonic motility in normal dogs [12] and colitic rats [13] and had anti-inflammatory effects in colitis. Acupuncture at GV01 decreased the scores of adhesion, macroscopic damage, the myeloperoxidase activity, and colonic motility in colitic rats. Naloxone, a nonspecific opioid antagonist, completely abolished these therapeutic effects on colitis [13]. That study suggested the involvement of opioid pathways [13]. The present results showed that acupuncture at GV01 was effective in reducing the referred somatic pain from colitis and that naloxone pretreatment inhibited the acupuncture effect (except on days 1 and 2). These results may support the current hypothesis and our previous findings [13] that the therapeutic effects of acupuncture at GV01 in colitis involve endogenous opioid pathways. However, we cannot exclude the possibility that the alleviation of colitis by acupuncture at GV01 in the acupuncture groups may have been associated with a decrease of referred visceral pain.

TNBS-treated rats generally exhibit severe watery diarrhea and hemorrhaging accompanied by weakness and anorexia, and their clinical symptoms and weight loss peak on about day 2 [25]. Like days 1 and 2 of our present results, it is not very likely to cause any acupuncture responses during these severe events because acupuncture is generally much less effective on extremely sick patients. It is possible that these rats had more severe colonic injury at the onset of treatment than the other rats treated with acupuncture.

The PAG contains μ- and δ-opioid receptors [26] and plays an important role in stimulation-produced analgesia [27]. Previous studies suggest that PAG neurons are involved in the influence of acupuncture and play an analgesic role in acupuncture analgesia [27, 28]. Fos, the protein product of the early gene c-fos, is a useful marker of neuronal activation by short-term nociceptive or nonnociceptive stimuli [29]. Acupuncture can evoke c-fos–labeled cells in the PAG [19, 30]. De Medeiros et al. [19] showed that electroacupuncture at ST36 elevated the levels of analgesia and c-fos expression, specifically in the ventrolateral PAG (vLPAG). Using c-fos immunohistochemistry to analyze the effects of acupuncture, we paid careful attention to the fact that noxious stimuli such as acupuncture can elicit Fos expression by itself. We found it interesting
that acupuncture at GV01 itself (normal-GV01 group) induced Fos expression at a low level in the vPAG, and there was no significance between normal and normal-GV01 groups, indicating that the intensity of acupuncture at GV01 was in the nonnoxious stimulus range. Also, as shown in one study of colonic pain [31], colitis similarly increased Fos expression in the PAG, and acupuncture at GV01 in colitic rats augmented Fos expression in the vPAG, as compared with animal groups subjected only to colitis or to acupuncture at a nonacupoint in colitis. Previous studies suggest that acupuncture at acupuncture points induces an inhibitory control over pain signals from particular parts of the body, which may explain why stimulation at a body site produces analgesia in the same or in a distant segment [33]. From our limited data, it is possible that a GV01 acupoint area projects to the PAG, and its stimulation inhibited referred somatic pain in the perianal region in the same segment. In contrast to acupuncture at GV01, a minor response by a nonacupoint stimulation may be more closely related to a response by stress [34]. Stimuli applied to the vPAG have been reported to evoke opioid-dependent analgesia, hyperpermeability, and sympathetic inhibition and to suppress spinal reflex by colocalized distortion [27]. Guo et al. [20] reported that Fos-positive cells are present in close apposition to the neuronal process containing enkephalin or β-endorphin in the PAG, particularly the vPAG after electroacupuncture. Fos-positive neurons are considered to interact with opioid-containing axons and/or dendrites presynaptically and postsynaptically [35–38]. Based on previous findings [20, 35–38], acupuncture at GV01 may decrease the referred somatic pain by affecting inhibitory neuronal pathways in the vPAG.

Combined with our previous studies [12, 13], our results show that by reducing colonic inflammation, motility, and referred somatic pain, acupuncture at GV01 had therapeutic effects on colitis. The present study may support our previous findings that these effects on colitis involve endogenous opioid pathways.

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REFERENCES

15. de Medeiros MA, Canteras NS, Suchecki D, Mello LE. Analgesia and c-Fos expression in the periaqueductal gray induced by electroacupuncture at the GV01 acupoint in colitic rats augmented Fos expression in the rostral ventrolateral medulla and periaqueductal gray in cats: relation to opioid containing neurons. Brain Res, 2006;100:210-15.