

Electroacupuncture ameliorates experimental colitis induced by acetic acid in rat

Jeoung-Woo Kang¹, Tae-Wan Kim², Jun-Ho La¹, Tae-Sik Sung¹, Hyun-Ju Kim¹, Young-Bae Kwon³, Jeum-Yong Kim³, Il-Suk Yang^{1,*}

¹Department of Physiology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

²Department of Physiology, College of Veterinary Medicine, Kyungpuk National University, Daegu 712-715, Korea

³Institute of Bioscience and Biotechnology, Daewoong Pharm Co. LTD., Yongin 449-814, Korea

The effect of electroacupuncture (EA) on experimental colitis was investigated in Sprague-Dawley rats. Colitis was induced by intracolonic instillation of 4% acetic acid. EA (2 Hz, 0.05 ms, 2 V for 20 min) was applied to bilateral Hoku (LI-4) and Zusanli (ST-36) on 12 hrs and 36 hrs after induction of colitis. EA-treatment significantly reduced the macroscopic damage and the myeloperoxidase activity of colonic samples at 3 days post-induction of colitis. Colitic colon showed a decreased *in vitro* motility. However, colonic motility of EA-treated group was not significantly different from that of normal group. The anti-inflammatory effect of EA was not inhibited by a glucocorticoid receptor antagonist, RU-486, but suppressed by a β -adrenoceptor antagonist, propranolol. These results suggest that EA-treatment has a beneficial effect on colitis, and its anti-inflammatory effect is mediated by β -adrenoceptor activation but not by endogenous glucocorticoid-dependent mechanism.

Key words: colitis, electroacupuncture, glucocorticoid, β -adrenoceptor

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder with unknown etiology and pathogenesis. In patients with IBD, gut inflammation is associated with intestinal muscle dysfunction [25,34]. These observations have been confirmed in a variety of animal models of experimental intestinal inflammation [9,18], showing that smooth muscle dysfunction is linked to the inflammatory reaction.

Aminosalicylic acid and corticosteroids are the drugs most

commonly used in treatment of IBD, but long-term use of these drugs may give rise to adverse effects, such as nephrotoxicity, pulmonary toxicity and male infertility [5,11]. Therefore, many researchers are recently interested in an alternative medical treatment such as acupuncture.

Acupuncture therapy has been utilized to relieve and treat various inflammatory diseases [3,13,38]. However, few studies have evaluated the effect of acupuncture on IBD, and moreover, its therapeutic mechanism is still unclear. Electroacupuncture (EA) has been reported to activate hypothalamic-pituitary-adrenal (HPA) axis and consequently release glucocorticoids that have potent anti-inflammatory properties [15,16]. EA was also reported to modulate the secretion rates of catecholamines from adrenal medulla by influencing sympathetic activity [20,23]. Catecholamines are known to induce anti-inflammatory responses through β -adrenoceptor activation [36]. Therefore, we hypothesized that EA has therapeutic effect on IBD and the anti-inflammatory effect of EA is mediated by glucocorticoids and/or catecholamines acting on β -adrenoceptor. The present study was designed to examine this hypothesis using a widely used animal model of colitis, the rat model of acetic acid induced-colitis [6,24]. In this model, we investigated (1) whether the EA treatment would reduce the tissue inflammatory responses and the smooth muscle dysfunction, and (2) whether an antagonist of either glucocorticoids receptor or β -adrenoceptor could modulate the effect of EA on colitis.

Materials and Methods

Animal preparation and experimental groups

Male Sprague-Dawley rats, weighing 250–300 g were used. The rats were housed in stainless steel hanging cages in colony room maintained under a 12 h light/dark cycle with a room temperature of $22 \pm 1^\circ\text{C}$ and humidity of 65–70%. Water and food were available *ad libitum*.

*Corresponding author

Tel: 82-2-880-1261; Fax: 82-2-885-2732

E-mail: isyang@snu.ac.kr

Induction of experimental colitis

All experimental animals were fasted for 24 hrs before induction of colitis. Each rat was lightly anesthetized with ether, and a polyethylene cannula (PE-60) was inserted into the lumen of the colon via the anus. The tip of the cannula was positioned at 8 cm proximal to the anus. Either 1 ml of acetic acid (4% vol/vol in 0.9% NaCl) or saline as the sham control was slowly infused into the distal colon. After 30 seconds exposure, 1 ml of saline (0.9%) was instilled in order to withdraw the previous solution from colon.

Treatment of electroacupuncture

Two acupoints, bilateral Zusanli (ST-36), located at the lateral upper tibia, and bilateral Hoku (LI-4), located at the junction of the first and the second metacarpal bones, were selected for the experiments. Stimulation of these two points is known to have therapeutic effect on gastrointestinal diseases [7,14]. Animals were anesthetized with ketamine. An acupuncture needle (Φ 0.18 mm, length 15 mm) was soldered to a flexible electrical wire, and the needle was inserted about 3 mm deep into the muscle layer at the acupoint. The second identical needle, as a positive pole, was inserted into the other point approximately 5–10 mm from the first one. An electric current of square wave pulses (2 Hz, 0.05 ms, 2 V for 20 min) were applied from stimulator (S88, Grass-telefactor, West Warwick, RI, USA) through a stimulus isolation unit (SIU5B, Grass-telefactor, West Warwick, RI, USA) on 12 hrs and 36 hrs after the induction of experimental colitis.

Measurement of myeloperoxidase (MPO) activity

At 3 days post-induction of colitis, rats were sacrificed by cervical dislocation. MPO activity was estimated in the whole colonic tissue obtained from the rats with and without colitis [2]. A segment of colon was minced finely with scissors in 5 ml of 50 mmol/L potassium phosphate buffer, pH 6.0 containing 14 mmol/L hexadecyl-trimethylammonium bromide and homogenized for 3 min. The sample were frozen in liquid nitrogen and thawed three times and centrifuged for 20 min in cold at 20000 g using microcentrifuge. Aliquots of supernatants (20 μ l) were mixed with 980 μ l of *o*-dianisidine solution which was made of 16.5 mg of *o*-dianisidine-HCl (Sigma, St louis, MI, USA), 90 ml of distilled water, 10 ml of potassium phosphate buffer, pH 6.0 and 50 μ l of 1% H₂O₂ (Sigma, St louis, MI, USA). Absorbance was measured at 450 nm every 1 min over a period of 10 min. MPO activity was expressed as units/g of tissue. The enzyme unit was defined as the conversion of 1 μ mol of H₂O₂ per min at 25°C.

Measurement of colonic motility

At 3 days post-induction of colitis, rats were sacrificed by cervical dislocation, and a 2 cm distal colonic segment was removed. The segments were suspended in a 20 mL organ

bath containing oxygenated (95% O₂, 5% CO₂) Krebs solution at 37°C. The distal end of the colonic segment was tied around the mouth of J-tube and this was connected via a 3-way connector to a syringe and to a pressure transducer (RP-1500, Narco Bio-systems Inc, Houston, TX, USA). The ligated proximal end was secured with a silk thread to an isometric force displacement transducer (FT-03, Grass-telefactor, West Warwick, RI, USA). The signals from both transducers were processed through Powerlab/400 and Chart 4.2 (AD Instruments, Castle Hill, NSW, Australia). The motilities of the colonic segments were detected as both longitudinal muscle contraction (isometric tension) and intraluminal pressure, which has been reported to reflect the contractile activity of circular muscle [4].

For calculation of spontaneous motility, we measured the mean longitudinal contraction and mean intraluminal pressure in steady states for 5 min. Mean longitudinal contraction or mean intraluminal pressure were calculated by the area under tension curve or pressure curve for 5-min period divided by duration of periods (5 \times 60 sec) and expressed per gram wet weight of the colonic segment.

In order to determine the effects of carbachol (CCh) and N^o-nitro-L-arginine methyl ester (L-NAME), we measured mean longitudinal contraction and mean intraluminal pressure at the end of the equilibration time and when the new stable level reached after each drug administration. The concentration-response curves to CCh (0.1–10 mM) were obtained cumulatively by adding each concentration to the bath.

Involvement of glucocorticoid and β -adrenoceptor in the action of electroacupuncture

To investigate the mechanisms of EA, the corticosteroid receptor antagonist (RU 486 in DMSO: 20 mg/kg) or β -adrenoceptor antagonist (propranolol in saline: 10 mg/kg) was intraperitoneally administrated at 2 hrs before EA stimulation.

Solutions and drugs

The Krebs-solution contained (in mM) 118.5 NaCl, 4.75 KCl, 2.54 CaCl₂, 1.19 MgSO₄, 25 NaHCO₃, 1.19 NaH₂PO₄, and 11.0 dextrose. The solution was continuously gassed with 95% O₂ and 5% CO₂(v/v), and the pH ranged from 7.3 to 7.4. Carbamylcholine chloride (CCh), acetic acid, RU 486, propranolol, N^o-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma Chemical Co. All drugs were added to the baths in volumes less than 1% of the total bath volume.

Statistical analysis

Data are expressed as means \pm S.E.M. with *n*, the number of animals. The responses were statistically tested using ANOVA followed by the Newman-Keuls multiple comparison test, or using Student's *t*-test. The value of

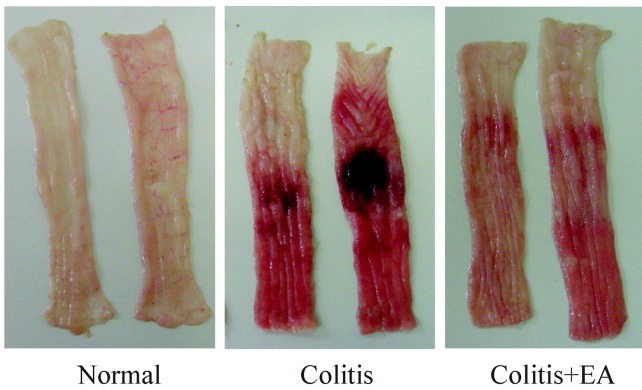


Fig. 1. The macroscopic features of colonic tissue of normal, colitis and colitis + EA group.

$p < 0.05$ considered to be significantly different.

Results

Macroscopic observation

Rats developed diarrhea 2-3 days after colitis induction. The colitis + EA group showed less severe diarrhea than the colitis group. However, the normal group did not develop diarrhea (Data not shown). The colonic tissue of colitis group showed prominent congestion and swelling, while the macroscopic inflammatory features of colon in the colitis+EA group were moderate (Fig. 1).

MPO activity

MPO activity in the colitis group was significantly higher than that in the normal group (0.93 ± 0.17 Unit/g, $n = 8$ vs 0.15 ± 0.02 Unit/g, $n = 5$, $p < 0.01$). But, MPO activity of the colitis + EA group was significantly lower than that of the colitis group (0.37 ± 0.09 Unit/g, $n = 7$ vs 0.93 ± 0.17 Unit/g, $n = 8$, $p < 0.01$). There was no statistical difference in MPO activity between the normal group and the colitis + EA group, implying that EA has an anti-inflammatory effect on the acetic acid-induced experimental colitis (Fig. 2).

Colonic smooth muscle motility

All colonic segments from normal group exhibited a spontaneous and highly synchronized rhythmic longitudinal phasic contractions and intraluminal pressure waves. However, most colonic muscles from the colitis group showed spontaneous motility with small amplitude and irregular pattern. But, colonic segments from the colitis + EA group showed spontaneous and regular motility with considerable amplitude (Fig. 3A).

The mean weight of the colonic segments from the normal, colitis and colitis + EA group were 365 ± 37 , 410 ± 35 and 378 ± 31 mg, respectively. There was no significant difference between them ($n = 11$, $p > 0.05$).

The mean longitudinal contraction of colitic colonic

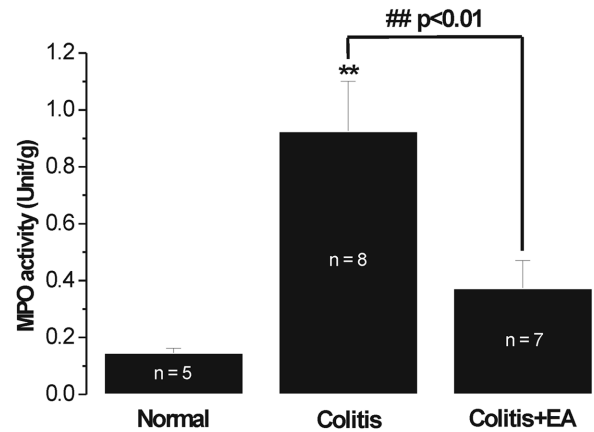


Fig. 2. MPO activity of each experimental group. $**p < 0.01$ as compared with normal group, $##p < 0.01$ as compared with colitis group.

segments (25.6 ± 3.6 mN/g wet segment wt; $n = 11$) was significantly less than that of normal colonic segments (10.5 ± 3.5 mN/g wet segment wt; $n = 11$; $p < 0.05$). In contrast, the mean longitudinal contraction of colonic segments in the colitis + EA group (24.7 ± 4.8 mN/g wet segment wt; $n = 11$) was significantly higher than that in the colitis group (10.5 ± 3.5 mN/g wet segment wt; $n = 11$; $p < 0.05$). The mean intraluminal pressure of colonic segments from normal, colitis, colitis + EA group were 4.2 ± 0.8 , 1.4 ± 0.2 and 3.5 ± 0.9 mmHg/g wet segment wt; $n = 11$), respectively (Fig. 3B).

CCh (0.1-10 μ M), a potent cholinergic agonist, increased both mean longitudinal contraction and mean intraluminal pressure of all groups in a concentration-dependent manner. The increases of mean longitudinal contraction and intraluminal pressure by CCh in the normal and the colitis+EA group were higher than that in colitis group ($n = 6$, Fig. 4).

L-NAME (100 μ M), a nitric oxide synthase inhibitor, significantly increased both mean longitudinal contraction and mean intraluminal pressure in the normal and the colitis + EA group. However, the colonic segments of colitis group did not respond to L-NAME ($n = 5$, Fig. 5).

Effects of RU486 and propranolol

To determine whether glucocorticoid, a pivotal mediator of HPA axis, was involved in the anti-inflammatory effect of EA, a corticosteroid receptor antagonist, RU486, was pretreated 2 hrs before the EA treatment. RU486 did not affect the EA induced anti-inflammatory effect (Fig. 6). But, pretreatment with β -adrenoreceptor antagonist, propranolol, significantly suppressed the effect of EA (Fig. 7).

Discussion

The present study demonstrates that EA stimulation at

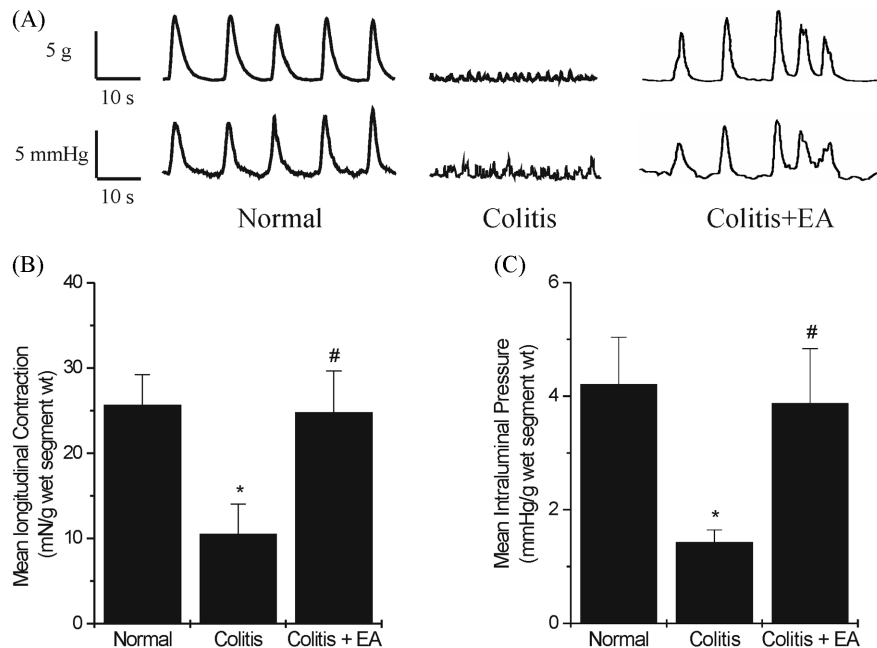


Fig. 3. Typical recordings showing the spontaneous mechanical activity of colonic segments in normal, colitis and colitis + EA group, detected as isometric tension (upper trace) and intraluminal pressure (lower trace) (A). B and C are statistical graphs for mean longitudinal contraction and mean intraluminal pressure, respectively. * $p < 0.05$ as compared with normal group, # $p < 0.05$ as compared with colitis group.

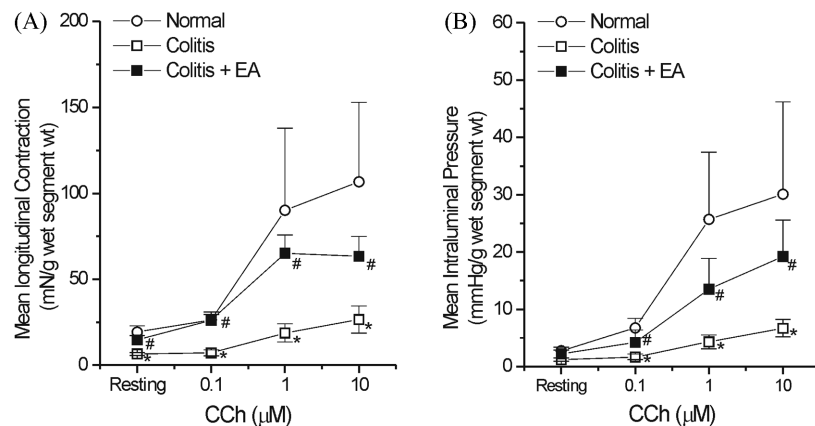


Fig. 4. Effects of CCh on mean longitudinal contraction and mean intraluminal pressure of colonic segments in normal, colitis and colitis + EA group. * $p < 0.05$ as compared with normal group, # $p < 0.05$ as compared with colitis group.

Zusanli (ST-36) and Hoku (LI-4) has therapeutic effect on experimental colitis. The colitis+EA group showed milder macroscopic lesion in colon than the colitis group, implying that EA treatment can effectively improve the colonic mucosal lesions. More convincingly, tissue MPO activity in the colitis + EA group was significantly less than that of the colitis group. The MPO activity was known to be a marker for tissue neutrophil content and be useful to quantify the extent of inflammation [2]. It has been reported that the accumulation of neutrophil is a characteristic feature of such gastrointestinal inflammatory disease as colitis [2]. Therefore, the decrease of the MPO activity in the colitis

+ EA group evidences that EA indeed reduced the inflammation in colitic tissue.

The decreased colonic motility is generally observed in IBD patients [12,25,34] and in the animal models of experimental colitis [17]. In the current study, colonic segments of colitis group also showed significantly decreased spontaneous longitudinal and circular motilities, compared with those of normal group. However, the spontaneous colonic contractile activities of colitis + EA group were significantly higher than those of colitis group. These findings suggest that EA treatment suppresses the inflammatory response and restores the ability of the colonic

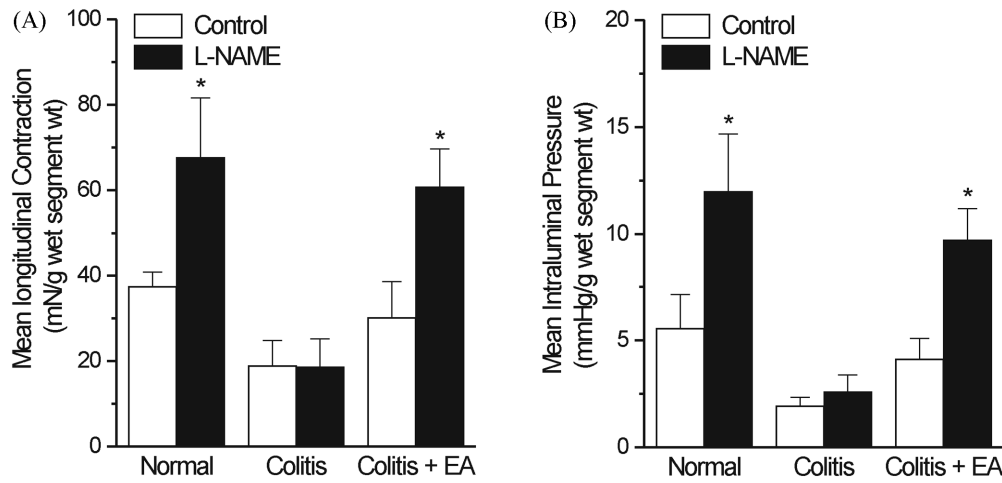


Fig. 5. Effects of L-NAME on the spontaneous mechanical activity of colonic segments in normal, colitis and colitis + EA group, monitored as isometric tension and intraluminal pressure. * $p < 0.05$ as compared with control.

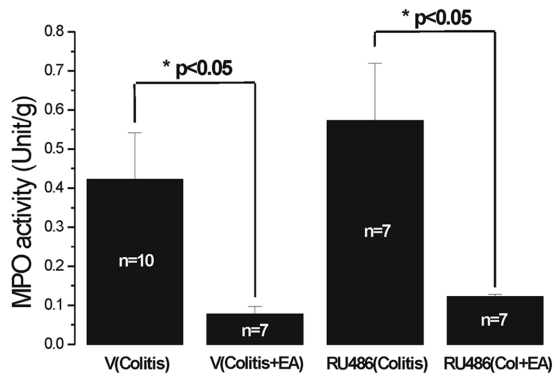


Fig. 6. Effect of RU486, a glucocorticoid receptor antagonist on the lowering MPO activity by EA. Vehicle: DMSO, n = animal number, * $p < 0.05$.

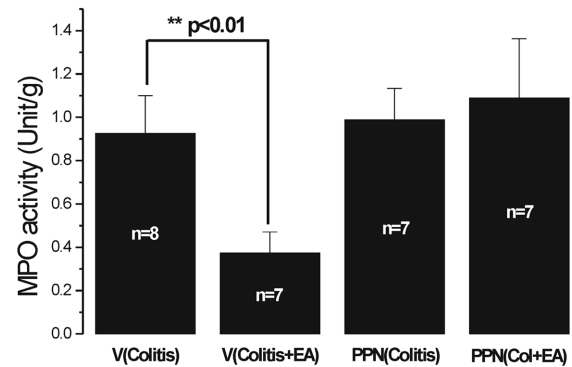


Fig. 7. Effect of propranolol (PPN), β -adrenoceptor antagonist, on the lowering MPO activity by EA. Vehicle: saline, n = animal number, ** $p < 0.01$.

muscle to develop spontaneous motility.

In acetic acid-induced colitis, it was reported that CCh-induced contraction was significantly decreased, compared with that of normal group [9]. In the present study, the CCh-induced increases of longitudinal and circular motilities in the colitis group were significantly less than those in the normal and in the colitis + EA groups. These results indicate that EA treatment improves the colitis-induced damage in the colonic contractile function.

Because NO has been shown to act as a major nonadrenergic, noncholinergic (NANC) inhibitory neurotransmitter in the gut, the changes in the gastrointestinal motility have been attributed to an impairment of NO function in the various dysfunctional condition [21,30]. It was also reported that nitrgenic neurons were impaired in the rat model of experimental colitis [19]. The damage of nitrgenic neural function was also observed in the present study. In the colitis group, L-NAME, a nitric oxide synthase inhibitor, failed to further increase the amplitude of the spontaneous motility.

On the other hand, in the normal and the colitis + EA group, L-NAME increased the spontaneous longitudinal and circular mechanical activity, implying that tonic nitrgenic neural function was maintained in the colitis + EA group as in the normal group. Taken together, these data support that EA treatment can suppress intestinal inflammation and reverses intestinal smooth muscle dysfunction caused by colitis.

IBD is a chronic relapsing inflammation of the intestine mediated by the activation of immune cells and the release of inflammatory mediators. It is well established that neuroendocrine and immune systems communicate bidirectionally [28]. Increased tissue production of interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)- α has been found during the episodes of active IBD in patients with ulcerative colitis or Crohn's disease [10]. Cytokines produced by immune cells during inflammation can stimulate the HPA axis to release corticosteroids, which are important immunoregulators. The corticosteroids are

known to effectively shut off the immune response [27,33].

In addition to the HPA axis activation, pro-inflammatory cytokines (e.g., IL-1 β) can also enhance the sympathetic activity, including the release of catecholamines from sympathetic terminals and adrenal medulla. It has been proposed that catecholamines function as endogenous anti-inflammatory agents [1,29].

Although the hypotheses on mechanisms of acupuncture are various, it is often proposed that EA activates the HPA axis [15,16,22] or sympathetic nervous system [20,23]. In the present study, a glucocorticoid receptor antagonist, RU486, did not alter the anti-inflammatory effect of EA on colitis. This indicates that glucocorticoids do not participate in the EA-induced anti-inflammation on colitis, at least in this experimental condition. However, the possibility cannot be excluded that the released glucocorticoids by EA was not enough to reduce the acetic acid-induced colitis.

We found that pretreatment with a β -adrenoceptor antagonist, propranolol, blocked the anti-inflammatory effect of EA. This result implies that the anti-inflammatory effect of EA on colitis is mediated by catecholamines acting through β -adrenoceptor. The mechanisms involving β -adrenoceptor in the anti-inflammatory effect of EA remain to be elucidated. It is noteworthy that immune cells can bind different neurotransmitters [29]. For example, catecholamines are known to act on macrophages and monocytes through binding to the cell surface β -adrenergic receptors. β -adrenoceptors are coupled to the GTP-binding protein of the adenylate cyclase complex for increasing intracellular cAMP levels and activating protein kinase A upon stimulation [35]. In this way, catecholamines reduce the production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , and enhance the secretion of anti-inflammatory cytokines such as IL-10 [36]. Indeed, it was reported that EA greatly inhibited the expression of IL-1 β and IL-6 mRNA in the rat model of ulcerative colitis [32,37]. Oral administration of enteric-coated recombinant human IL-11 (rhIL-11), a potent anti-inflammatory cytokine, suppresses intestinal inflammation and restores the ability of the smooth muscle to develop active tension in both jejunum and colon in HLA-B27 transgenic rats with chronic intestinal inflammation [8].

It should be mentioned that opioid receptors are suggested to be involved in the anti-inflammatory action of acupuncture [3,26] and opioids have anti-inflammatory effects on synovitis in rheumatoid arthritis [31]. Therefore, it will be necessary to test whether endogenous opioid system is also involved in the EA-induced anti-inflammation on experimental colitis. Future experiments will attempt to elucidate the relationship between opioid receptors and the anti-inflammatory effect of EA.

In conclusion, EA therapy ameliorates intestinal inflammation and reverses intestinal smooth muscle dysfunction in experimental colitis induced by acetic acid in

rat. The anti-inflammatory effect of EA does not involve the endogenous glucocorticoid-dependent mechanism but requires the β -adrenoceptor activation. Further studies are needed to elucidate the exact mechanism of EA action on experimental colitis.

Acknowledgment

This work was supported by the Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University.

References

1. **Bhattacharya SK, Das N, Sarkar MK.** Inhibition of carrageenin-induced pedal oedema in rats by immobilisation stress. *Res Exp Med (Berl)* 1987, **187**, 303-313.
2. **Bradley PP, Priebat DA, Christensen RD, Rothstein G.** Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982, **78**, 206-209.
3. **Ceccherelli F, Gagliardi G, Visentin R, Sandona F, Casale R, Giron G.** The effects of parachlorophenylalanine and naloxone on acupuncture and electroacupuncture modulation of capsaicin-induced neurogenic edema in the rat hind paw. A controlled blind study. *Clin Exp Rheumatol* 1999, **17**, 655-662.
4. **Coupar IM, Liu L.** A simple method for measuring the effects of drugs on intestinal longitudinal and circular muscle. *J Pharmacol Toxicol Methods* 1996, **36**, 147-154.
5. **Di Paolo MC, Paoluzi OA, Pica R, Iacopini F, Crispino P, Rivera M, Spera G, Paoluzi P.** Sulphasalazine and 5-aminosalicylic acid in long-term treatment of ulcerative colitis: report on tolerance and side-effects. *Dig Liver Dis* 2001, **33**, 563-569.
6. **Elson CO, Sartor RB, Tennyson GS, Riddell RH.** Experimental models of inflammatory bowel disease. *Gastroenterology* 1995, **109**, 1344-1367.
7. **Fireman Z, Segal A, Kopelman Y, Sternberg A, Carasso R.** Acupuncture treatment for irritable bowel syndrome. A double-blind controlled study. *Digestion* 2001, **64**, 100-103.
8. **Greenwood-Van Meerveld B, Venkova K, Keith JC Jr.** Recombinant human interleukin-11 restores smooth muscle function in the jejunum and colon of human leukocyte antigen-B27 rats with intestinal inflammation. *J Pharmacol Exp Ther* 2001, **299**, 58-66.
9. **Grossi L, McHugh K, Collins SM.** On the specificity of altered muscle function in experimental colitis in rats. *Gastroenterology* 1993, **104**, 1049-1056.
10. **Isaacs KL, Sartor RB, Haskill S.** Cytokine messenger RNA profiles in inflammatory bowel disease mucosa detected by polymerase chain reaction amplification. *Gastroenterology* 1992, **103**, 1587-1595.
11. **Jankauskiene A, Druskis V, Laurinavicius A.** Cyclosporine nephrotoxicity: associated allograft dysfunction at low trough concentration. *Clin Nephrol* 2001, **56**, S27-29.
12. **Koch TR, Carney JA, Go VL, Szurszewski JH.**

- Spontaneous contractions and some electrophysiologic properties of circular muscle from normal sigmoid colon and ulcerative colitis. *Gastroenterology* 1988, **95**, 77-84.
13. **Kumar AM, Wen XL.** Acupuncture treatment for osteoarthritic pain and inflammation of the knee. *Altern Ther Health Med* 2002, **8**, 128.
 14. **Li Y, Tougas G, Chiverton SG, Hunt RH.** The effect of acupuncture on gastrointestinal function and disorders. *Am J Gastroenterol* 1992, **87**, 1372-1381.
 15. **Liao YY, Seto K, Saito H, Fujita M, Kawakami M.** Effect of acupuncture on adrenocortical hormone production: I. Variation in the ability for adrenocortical hormone production in relation to the duration of acupuncture stimulation. *Am J Chin Med* 1979, **7**, 362-371.
 16. **Liao YY, Seto K, Saitoh H, Kawakami M.** Effect of acupuncture on adrenocortical hormone production in rabbits with a central lesion. *Am J Chin Med* 1981, **9**, 61-73.
 17. **Lu G, Qian X, Berezin I, Telford GL, Huizinga JD, Sarna SK.** Inflammation modulates in vitro colonic myoelectric and contractile activity and interstitial cells of Cajal. *Am J Physiol* 1997, **273**(6 Pt 1), G1233-45.
 18. **Martinolle JP, Garcia-Villar R, Fioramonti J, Bueno L.** Altered contractility of circular and longitudinal muscle in TNBS-inflamed guinea pig ileum. *Am J Physiol* 1997, **272**(5 Pt 1), G1258-67.
 19. **Mizuta Y, Isomoto H, Takahashi T.** Impaired nitrergic innervation in rat colitis induced by dextran sulfate sodium. *Gastroenterology* 2000, **118**, 714-723.
 20. **Mori H, Uchida S, Ohsawa H, Noguchi E, Kimura T, Nishijo K.** Electro-acupuncture stimulation to a hindpaw and a hind leg produces different reflex responses in sympathoadrenal medullary function in anesthetized rats. *J Auton Nerv Syst* 2000, **79**, 93-98.
 21. **Mule F, Serio R.** Spontaneous mechanical activity and evoked responses in isolated gastric preparations from normal and dystrophic (*mdx*) mice. *Neurogastroenterol Mot* 2002, **14**, 667-675.
 22. **Pan B, Castro-Lopes JM, Coimbra A.** Activation of anterior lobe corticotrophs by electroacupuncture or noxious stimulation in the anaesthetized rat, as shown by colocalization of Fos protein with ACTH and beta-endorphin and increased hormone release. *Brain Res Bull* 1996, **40**, 175-182.
 23. **Sato A, Sato Y, Suzuki A, Uchida S.** Reflex modulation of catecholamine secretion and adrenal sympathetic nerve activity by acupuncture-like stimulation in anesthetized rat. *Jpn J Physiol* 1996, **46**, 411-421.
 24. **Singh VP, Patil CS, Jain NK, Singh A, Kulkarni SK.** Effect of nimesulide on acetic acid- and leukotriene-induced inflammatory bowel disease in rats. *Prostaglandins Other Lipid Mediat* 2003, **71**, 163-175.
 25. **Snape WJ Jr, Williams R, Hyman PE.** Defect in colonic smooth muscle contraction in patients with ulcerative colitis. *Am J Physiol* 1991, **261**(6 Pt 1), G987-G991.
 26. **Son YS, Park HJ, Kwon OB, Jung SC, Shin HC, Lim S.** Antipyretic effects of acupuncture on the lipopolysaccharide-induced fever and expression of interleukin-6 and interleukin-beta mRNAs in the hypothalamus of rats. *Neurosci Lett* 2002, **319**, 45-48.
 27. **Sternberg EM.** Neuroendocrine factors in susceptibility to inflammatory disease: focus on the hypothalamic-pituitary-adrenal axis. *Horm Res* 1995, **43**, 159-161.
 28. **Straub RH, Herfarth H, Falk W, Andus T, Scholmerich J.** Uncoupling of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis in inflammatory bowel disease? *J Neuroimmunol* 2002, **126**, 116-125.
 29. **Straub RH, Westermann J, Scholmerich J, Falk W.** Dialogue between the CNS and the immune system in lymphoid organs. *Immunol Today* 1998, **19**, 409-413.
 30. **Takahashi T.** Pathophysiological significance of neuronal nitric oxide synthase in the gastrointestinal tract. *J Gastroenterol* 2003, **38**, 421-430.
 31. **Takeba Y, Suzuki N, Kaneko A, Asai T, Sakane T.** Endorphin and enkephalin ameliorate excessive synovial cell functions in patients with rheumatoid arthritis. *J Rheumatol* 2001, **28**, 2176-2183.
 32. **Tian L, Huang YX, Tian M, Gao W, Chang Q.** Downregulation of electroacupuncture at ST36 on TNF- α in rats with ulcerative colitis. *World J Gastroenterol* 2003, **9**, 1028-1033.
 33. **Turnbull AV, Rivier CL.** Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 1999, **79**, 1-71.
 34. **Vermillion DL, Huizinga JD, Riddell RH, Collins SM.** Altered small intestinal smooth muscle function in Crohn's disease. *Gastroenterology* 1993, **104**, 1692-1699.
 35. **Woiciechowsky C, Asadullah K, Nestler D, Eberhardt B, Platzer C, Schoning B, Glockner F, Lanksch WR, Volk HD, Docke WD.** Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. *Nat Med* 1998, **4**, 808-813.
 36. **Woiciechowsky C, Schoning B, Lanksch WR, Volk HD, Docke WD.** Mechanisms of brain-mediated systemic anti-inflammatory syndrome causing immunodepression. *J Mol Med* 1999, **77**, 769-780.
 37. **Wu HG, Zhou LB, Pan YY, Huang C, Chen HP, Shi Z, Hua XG.** Study of the mechanisms of acupuncture and moxibustion treatment for ulcerative colitis rats in view of the gene expression of cytokines. *World J Gastroenterol* 1999, **5**, 515-517.
 38. **Zijlstra FJ, van den BeLI, Huygen FJ, Klein J.** Anti-inflammatory actions of acupuncture. *Mediators Inflamm* 2003, **12**, 59-69.