

## Effects of Traditional Acupuncture on Colonic Motility in the Rat with Colitis

Hee-Young Kim<sup>1</sup>, In-Sop Shim, Hye-Jung Lee, Seong Mok Jeong\*,  
Sun Young Kim\* and Tchi-Chou Nam\*

Graduate School of East-West Medical Science, Kyung Hee University

\*College of Veterinary Medicine, Seoul National University

**Abstract :** The aims of this study were to investigate the efficacy of acupuncture on myoelectrical activity of inflamed or normal colon in the rat, and whether the effect of acupuncture on colonic motility was related to endogenous opioids. Twenty-two male Sprague-Dawley rats were divided into three groups. Experimental groups were normal group (n = 8), colitis group (n = 6) and naloxone group (n = 8). Stainless steel bipolar electrodes were implanted on the serosal layer of the proximal colon of rats. Colitis was induced 7 days after electrode implantation using trinitrobenzene sulphonic acid (TNBS) and ethanol. Electromyograms (EMG) were recorded by using polygraph 11 days after implantation of electrodes. In normal group, normal colonic motility was recorded for 60 min, and then traditional acupuncture at GV-1 was applied for 20 min and EMG was recorded for further 60 min in untreated rats. In colitis group, after recording of basal colonic motility for 60 min, 20 min of acupuncture treatment and further EMG recording was performed for 60 min in TNBS/ethanol treated rats. In naloxone group, following subcutaneous administration of naloxone (3 mg/kg), recording of EMG and acupuncture treatment were performed in TNBS/ethanol treated rats. In the normal group, acupuncture at GV-1 did not induce significant changes in colonic motility. TNBS/ethanol treatment had no significant effect on the frequency of colonic motility. And in colitis group, GV-1 acupuncture significantly decreased colonic motility ( $p < 0.01$ ). In naloxone group, after injection of naloxone, acupuncture at GV-1 did not change colonic motility in TNBS/ethanol treated rats. On the inflamed colon, naloxone blocked the effect of acupuncture. The present results suggested that endogenous opioids released by acupuncture at GV-1 decrease the motility of inflamed colon in rats, but not normal colon.

**Key words :** acupuncture, GV-1, colonic motility, endogenous opioids, rat

### Introduction

Stimulation of several acupoints release the endogenous opioids which are well known to be contributed to the acupuncture analgesia<sup>2,5,6,14,15,24</sup>. It has been reported that endogenous opioids are involved in the control of motility, secretion, and absorption in the gastrointestinal tract<sup>4,10,16</sup>. Acupuncture in oriental medicine and opioids in western have been used for the treatment of diarrhea<sup>1,4,7,9,18,22,23,25</sup>. Acupuncture and opioids affect a variety of intestinal functions, such as blood flow, gut motility, absorption and secretion<sup>4,8,10-12,16,17,20</sup>. But, there are few reports that the endogenous opioids released by acupuncture are related to the control of motility of intestine.

The purposes of this study were to investigate the efficacy of acupuncture on myoelectrical activity of inflamed or normal colon in the rat, and whether the effect of acupuncture on colonic motility was related to endogenous opioids.

### Materials and Methods

#### Animals

Male Sprague-Dawley rats (Samyuk Experimental Centers, Korea) weighing approximately 250 g were used. The ani-

mals were kept in a restricted access room with controlled temperature (23°C) and 12 hour light/dark cycle. Rats were housed in individual cages. Food and water were provided *ad libidum*.

#### Induction of colitis

Colitis was induced 7 days after implantation of electrodes using trinitrobenzene sulphonic acid (TNBS, Sigma Chemical Co, USA) and ethanol. Rats were fasted for 12 hrs and slightly anesthetized using isoflurane chamber. Intra-gastric tube was inserted into the colon via rectum, and the tip of the tube was positioned 8 cm cranial to the anus. Thereafter, mixed 0.60 ml of 5% w/v TNBS and 0.25 ml of 50% ethanol solution, 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.

#### Experimental groups

Twenty-two male Sprague-Dawley rats were divided into three groups. Experimental groups were normal group (n=8), colitis group (n=6) and naloxone group (n=8). In normal group, traditional acupuncture at GV-1 was performed in normal rats. In colitis group, acupuncture and electromyogram (EMG) recording was performed in colitis- induced rats. Finally, in naloxone group, following subcutaneous administration of naloxone (Sigma Chemical Co, USA) at a dose of 3 mg/kg, acupuncture and EMG recording was per-

<sup>1</sup>Corresponding author.

E-mail : vet20@hanmail.net

formed in colitis induced rats.

### Implantation of electrode

Anesthesia was maintained with 2% isoflurane (Aerane®, Ilisung Co, Korea) in oxygen. Stainless steel bipolar electrodes were implanted on the serosal layer of the proximal colon at 2 cm from the ileocolic junction, under aseptic condition. During surgical procedure body temperature was maintained using thermostatic heating pad. Subcutaneous tunnel was made to exit the electrodes at the back of the animal's neck. After surgery, the animals were housed individually for 7 days at least for recovery.

### Acupuncture

GV-1 (*Hou Hai*) acupoint is located at the depression ventral to the base of the tail and dorsal to the anus. This is a single point. Deep to the point, external anal sphincter and rectococcygeus muscles exist. Acupuncture needle (0.25 mm diameter, 30 mm length) was inserted 2 deep and maintained for 20 min.

### Recording of electromyogram (EMG)

Before the first recording of EMG, animals were habituated to the 2 hour sessions of recording procedure for 3 days. The EMG was recorded by using polygraph (Model 79, Grass, USA) 11 days after implantation of electrodes. During EMG recordings, rats were placed in the rat holder. All measurements of colonic myoelectric activity were obtained one hour after rats had been placed in the rat holder.

In normal group, after recording of normal colonic motility for 60 min, traditional acupuncture at GV-1 was applied for 20 min and recordings were continued for further 60 min in untreated rats. In colitis group, after recording of basal colonic motility for 60 min, 20 min of acupuncture treatment and further EMG recording was performed for 60 min in TNBS/ethanol treated rats. Finally, in naloxone group, after subcutaneous administration of naloxone at a dose of 3 mg/kg, basal EMG recording, acupuncture and further EMG recording were performed in TNBS/ethanol treated rats.

The number of colonic spike bursts (phasic contractions)

during 60 min before and after acupuncture was compared in each group. The motility index (MI) was calculated by dividing the number of total spike bursts recorded for 60 min after acupuncture by the number of basal spike burst. The motility index was expressed as the percentage.

### Statistical analysis

All values are expressed as the mean  $\pm$  SE. Test of statistical significance were performed with paired *t*-test or Tukey's multiple comparison test. *P* values < 0.01 was regarded as significant.

## Results

Diarrhea was evident in all the rats treated with TNBS/ethanol. Intracolonic instillation of TNBS/ethanol resulted in inflammatory response by extensive mucosal disruption. Linear and deep ulcers and hemorrhage were shown in post-mortem macroscopic examination.

In the normal colon, acupuncture at GV-1 had no significant effect on colonic motility. The motility index (MI) of normal group was  $95.9 \pm 5.9\%$  (Table 1 and Fig 1). TNBS/ethanol treatment had no significant effect on the frequency of colonic motility. The frequencies of basal colonic spike bursts were  $65.1 \pm 9/\text{hour}$  and  $71.2 \pm 13/\text{hour}$  in normal and colitis group, respectively. In colitis group, colonic motility was significantly decreased after acupuncture at GV-1 ( $p <$



Fig 1. Electromyogram of the colon following traditional acupuncture at GV-1 in normal rat; basal recording (A) and after GV-1 acupoint stimulation (B).

**Table 1.** Effect of traditional acupuncture at GV-1 on colonic motility in normal and TNBS/ethanol treated rats

Group	Treatment	Number of spike bursts on EMG (/h)		Motility Index (%) ****
		Before acupuncture	After acupuncture ***	
Normal	None	65.1 $\pm$ 9	62.1 $\pm$ 6	95.9 $\pm$ 5.9
Colitis	TNBS/ethanol *	71.2 $\pm$ 13	60.0 $\pm$ 10 <sup>a</sup>	84.6 $\pm$ 6.2
Naloxone	TNBS/ethanol, Naloxone **	75.9 $\pm$ 17	75.8 $\pm$ 15	100.8 $\pm$ 11.2

Data are expressed as mean  $\pm$  SE. <sup>a</sup>significantly different from before acupuncture ( $p < 0.01$ )

\*administered 4 days before experiment

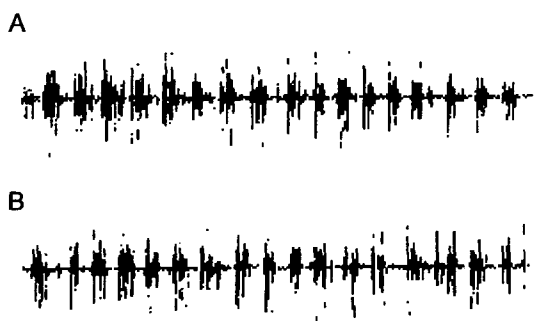
\*\*subcutaneously administered (3 mg/kg) 60 min before acupuncture at GV-1

\*\*\*acupuncture at GV-1 was maintained for 20 min

\*\*\*\*number of spike bursts after acupuncture / before acupuncture  $\times$  100



**Fig 2.** Electromyogram of the colon following traditional acupuncture at GV-1 in TNBS/ethanol treated rat; basal recording (A) and after GV-1 acupoint stimulation (B).



**Fig 3.** Electromyogram of the colon following traditional acupuncture at GV-1 in TNBS/ethanol treated rat; basal recording after naloxone administration (A), and after GV-1 acupoint stimulation (B). Naloxone was administered 60 min before acupuncture stimulation.

0.01, MI:  $84.6 \pm 6.2\%$ ) (Table 1 and Fig 2). In TNBS/ethanol treated rats, naloxone treatment did not affect basal colonic motility (Table 1). In naloxone group, the colonic motility was not changed significantly after acupuncture at GV-1 (MI:  $100.8 \pm 11.2\%$ ) (Table 1 and Fig 3).

## Discussions

It has been known that opioids have antidiarrheal effects by blocking intestinal propulsion and inhibiting intestinal fluid secretion. Many studies have shown that endogenous opioids are involved in the control of motility, secretion and absorption in the gastrointestinal tract<sup>4,10,16</sup>.

GV-1 acupoint has been used for diarrhea in the oriental medicine. Choi *et al*<sup>3</sup> reported that acupuncture at GV-1 was very effective on the watery diarrhea in calves. Aqua-acupuncture at GV-1 with 0.2 ml of 3% saline significantly reduced the duration of illness in piglets with preweaning diarrhea<sup>7</sup>.

It has been reported that the endogenous opioids such as  $\beta$ -endorphine, enkephaline and dynorphine etc. could be

released by acupuncture and reversed by naloxone, non-selective opioids antagonist<sup>5,6,15,21</sup>.

In the present study, pretreatment with naloxone blocked the effect of the acupuncture at GV-1 on colonic motility in rats with colitis. This suggests that GV-1 acupoint stimulation release the endogenous opioids in the presence of chronic colonic inflammation.

Several studies revealed that opioids exhibit increased potency under conditions of inflammation. In fact, it has been reported that  $\kappa$ -opioid receptor agonists has antinociceptive effect only during inflammation. In long term inflammation of colon (4 days after TNBS instillation into the colon), the ED<sub>50</sub> of the EMD61753, peripherally restricted  $\kappa$ -opioid receptor agonist, was reduced significantly<sup>13,19</sup>. Our results showed that acupuncture at GV-1 decreased the colonic motility in inflammation (colitis), not normal colon.

## References

1. Awouters F, Niemegeers CJE, Janssen PAJ. Pharmacology of antidiarrheal drugs. *Annu Rev Pharmacol Toxicol* 1983; 23: 279-301.
2. Chang HT. Neurophysiological interpretation of acupuncture analgesia. *Endeavor* 1980; 4: 92-96.
3. Choi HI, Lee KK, Yun YM, Park SJ. Acupuncture therapeutics for the treatment of the watery diarrhea in calves. *Kor J Vet Acupunct Mox* 1993; 3: 34-46.
4. de Luca A, Coupar IM. Insights into opioid action in the intestinal tract. *Pharmacol Ther* 1996; 69: 103-115.
5. Han JS, Terenius L. Neurochemical basis of acupuncture analgesia. *Ann Rev Pharmacol Toxicol* 1982; 22: 193-220.
6. He LF. Review article: involvement of endogenous opioid peptides in acupuncture analgesia. *Pain* 1987; 31: 99-121.
7. Hwang YC, Jenkins EM. Effect of acupuncture on young pigs with induced enteropathogenic *Escherichia coli* diarrhea. *Am J Vet Res* 1988; 49: 1641-1643.
8. Jang JH. Ear acupuncture for hypotonia in gastrointestinal examination. *Am J Res* 1986; 147: 862.
9. Khoe WH. Chronic ulcerative and spastic colitis treated with acupuncture and nutrition. *Am J Acupunct* 1975; 3: 211-214.
10. Kromer W. Endogenous and exogenous opioids in the control of gastrointestinal motility and secretion. *Pharmacol Rev* 1988; 40: 123-151.
11. Lee GTC. A study of electrical stimulation of acupuncture locus tsusanli (ST-36) on mesenteric microcirculation. *Am J Chi Med* 1974; 2: 53-66.
12. Matsumoto T, Hayes MF. Acupuncture, electric phenomenon of the skin and postvagotomy gastrointestinal atony. *Am J Surg* 1973; 125: 176-180.
13. Maureen BB, Gebhart GF. Effects of kappa opioids receptor agonists on responses to colorectal distension in rats with and without acute colonic inflammation. *J Pham Exp Ther* 1998; 285: 707-715.
14. Pert A. Mechanisms of opiate analgesia and role of endorphins in pain suppression. *Adv Neurol* 1982; 33: 107-122.

15. Pomeranz B, Chiu D. Naloxone blockade of acupuncture analgesia: endorphine implicated. *Life Sci* 1976; 19: 1757-1762.
16. Porcher C, Julè Y, Henry M. A qualitative and quantitative study on the enkephalinergic innervation of the pig gastrointestinal tract. *J Histochem Cytochem* 2000; 48: 333-343.
17. Ren Z. The effect of auricular-plaster theory on gall stone expulsion and on expansion-contraction function of the biliary system. *J Trad Chin Med* 1986; 6: 263-266.
18. Requena Y. Ulcerative colitis treated by traditional Chinese acupuncture. *Am J Acupunct* 1981; 9: 341-346.
19. Sengupta JN, Snider A, Su X, Gebhart GF. Effects of kappa opioids in the inflamed rat colon. *Pain* 1999; 9: 175-185.
20. Sodipo JOA, Falaiye JM. Acupuncture and gastric acid studies. *Am J Chin Med* 1979; 7: 356-361.
21. Wang YJ, Wang SK. Effect of electroacupuncture on brain enkephalins content at different times in rats. *J Trad Chin Med* 1989; 9: 53-56.
22. Wenling F. Acupuncture treatment for 30 cases of infantile chronic diarrhea. *J Trad Chin Med* 1989; 9: 106-107.
23. Yingchun L. Observation of therapeutic effects of acupuncture treatment in 170 cases of infant diarrhea. *J Trad Chin Med* 1987; 7: 203-204.
24. Zhong XH. Correlation between endogenous opiate-like peptides and serotonin in laserpuncture analgesia. *Am J Acupuncture* 1989; 17: 39-43.
25. Zhonzxin X. Clinical observation of 500 cases with pediatric diarrhea treated by acupuncture. *Chin Acupunct Mox* 1989; 9: 10.

## 결장염 유발 Rat의 결장운동성에 침술이 미치는 영향

김희영<sup>1</sup> · 심인섭 · 이해정 · 정성목\* · 김순영\* · 남치주\*

경희대학교 동서의학대학원

\*서울대학교 수의과대학

**초 록 :** 본 연구는 정상 및 결장염을 유발한 rat에서 침술이 결장운동성에 미치는 영향을 알아보고, 이에 내인성 opioid가 관여하는지의 여부를 알아보기 위하여 수행하였다. 22마리의 Sprague-Dawley rat을 정상군(n=8), 결장염군(n=6) 및 naloxone군(n=8)의 세군으로 나누어 실험을 실시하였다. Rat의 근위 결장의 장막하에 스테인레스 전극을 장착하고, 전극장착 7일 후에 trinitrobenzene sulphonie acid(TNBS)과 ethanol을 이용하여 결장염을 유발하였다. 결장의 근전도는 전극장착 11일 후에 polygraph를 이용하여 측정하였다. 정상군에서는 정상 결장의 기초 근전도를 60분간 기록한 후에 GV-1에 20분간 전통 침술을 실시하고, 다시 60분간 근전도를 기록하였으며, 결장염군에서는 TNBS/ethanol이 미리 투여된 rat에 정상군에서와 같은 방법으로 근전도 기록 및 침술을 실시하였다. Naloxone군에서는 결장염군과 같은 방법으로 TNBS/ethanol이 미리 투여된 rat에 침술을 실시하기전 naloxone(3 mg/kg, SC)을 투여하고 다른 군에서와 마찬가지로 근전도와 침술을 실시하였다. 정상군에서는 침술이 결장의 운동성에 영향을 미치지 않았다. 그러나 TNBS/ethanol을 투여하여 결장염이 유발된 결장염군에서는 침술에 의해서 결장의 운동성이 유의적으로 저하되었다(p<0.01). Naloxone군에서는 침술에 의한 결장운동성의 저하는 관찰할 수 없었다. 이상의 결과로 보아 rat에서 GV-1 혈위의 침술에 의해 유발된 내인성 opioid는 염증상태의 결장 운동성을 감소시킨 것으로 사료된다.

**주요어 :** 침술, GV-1, 결장 운동성, 내인성 opioid, rat