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돼지의 세균성 위장관 질환에 따른 평활근 기능 이상에 대한 침술의 효과

김태완, 나준호, 성태식, 강정우, 남치주, 최민철, 윤여성, 양일석*
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The Effect of Acupuncture on the Intestinal Smooth Muscle Dysfunction Caused by Enteric Bacterial Infection in Weaning Piglet

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Abstract: It is now generally accepted that acupuncture is effective in diarrhea caused by bacterial infection. However, its effect on the intestinal smooth muscle dysfunction is not clear. Therefore, we investigated the effect of acupuncture therapy at Jiao-chao (GV-1) on the intestinal muscle dysfunction in weaning piglets orally infected by *Escherichia coli*.

The animals are divided into four groups; 1) *E. coli* + no-treatment, 2) *E. coli* + antibiotic, 3) *E. coli* + acupuncture, 4) normal group. In the three *E. coli* infected groups, low frequency electrical field stimulation (EFS, 1 Hz) provoked triphasic responses composed of initial relaxation followed by on-contraction and off-contraction. While in the normal group, EFS (1Hz) induced biphasic responses composed of relaxation during the stimulation and off-contraction. At the high frequency (16Hz) EFS, both on-contraction and off-contraction of the *E. coli* + antibiotic, *E. coli* + acupuncture and the normal group were larger than those of the *E. coli* + no-treatment group. In the non-adrenergic non-cholinergic (NANC) condition, only biphasic responses occurred to EFS in all experimental groups and the off-contraction of *E. coli* + antibiotic, *E. coli* + acupuncture and the normal group were larger than those of the *E. coli* + no-treatment group. The response to carbachol of those three groups was also significantly greater than that of the *E. coli* + no-treatment group. These results suggest that acupuncture is as effective as antibiotic in the dysfunction of colonic circular muscle caused by *E. coli* infection. The maintenance of contractile neuromuscular transmission seems to be involved in the mechanism of the acupuncture effects on diarrhea.

Key words: acupuncture, Jiao-chao (GV-1), piglet, *E. coli* infection, colon

Introduction

Acupuncture has been used for various conditions in Chinese traditional medicine [3, 24], and recently, it is re-evaluated as an alternative medicine for diseases [9, 13]. Especially, acupuncture is suggested as the alternative to

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antibiotics on the infectious gastrointestinal diseases because acupuncture is known to affect gastrointestinal function including motility and secretion.

In veterinary medicine, it was reported that acupuncture had a clinical therapeutic effect on diarrhea [1], but there are limited available reports about its anti-diarrheal effect on the basis of smooth muscle function. Therefore, in the present research, we purposed to study whether acupuncture is effective in the enteric bacterial infection-induced smooth muscle dysfunction. We selected jiao-chao (GV-1) located at the indentation between the base of tail and the anus as acupoint, because this acupoint has been used for treatment of diarrhea in Chinese traditional medicine[9]. In this experiment, we investigated the effect of acupuncture therapy at GV-1 on the intestinal muscle dysfunction in weaning piglets orally infected by *Escherichia coli* (*E. coli*).

Materials and Methods

Experimental protocol

We purchased 32 weaning piglets of either sex (Landrace, 21 days old) from a local pig farm. After 3 days adaptation period, these piglets were divided into 4 groups; 1) *E. coli* infection without treatment (*E. coli* + no treatment), 2) *E. coli* infection with antibiotic treatment (*E. coli* + antibiotic), 3) *E. coli* infection with acupuncture treatment (*E. coli* + acupuncture), 4) normal group. All piglets were administered cultured *E. coli* (6K987P) solution 100 ml through stomach tube except the piglets of the normal group. Preliminary experiments had shown that this dosage of *E. coli* was enough to induce diarrhea. During the experiment, food and water were available ad libitum. Piglets of acupuncture treatment group were stimulated at GV-1 for 20 minute once a day for consecutive 3 days, while those of antibiotic treatment group were administered enrofloxacin (1.5 ml/10 kg) by intramuscular injection once a day for 2 days.

Tissue preparations

All piglets were sacrificed 3 days after administration of *E. coli*. Then distal colon was quickly removed. The colon was opened along the mesenteric border, cleared of remaining fecal matter, and the mucosal layer was removed in a dissecting dish containing oxygenated (95% O_2 and 5% CO_2) Kreb's solution. The cleaned segment was then pinned flat to the Sylgard bottom of a dissecting dish filled with continuously oxygenated Kreb's solution. After removal of the mucosal layer, muscle strips (2×10 mm) were cut parallel to the circular muscle fiber.

Organ bath experiment

The strips were suspended to the tissue holder and placed between two platinum plates, which were located in 20 ml chambers filled with warmed (37 ± 0.5℃) and oxygenated (95% O_2-5% CO_2) Kreb's solution. The strip was connected to the isometric force transducer (FT-03, Grass-Telefactor, West Warwick, RI, USA). The output of transducer was processed through MacLab/2e (AD Instruments, Castlehill, Australia) and recorded on a Macintosh computer. Electrical field stimulation (EFS) was performed by means of two platinum plates which were connected to the stimulator (Grass-Telefactor, S88). Square wave pulses (120V, 0.5 ms pulse width) were delivered at a frequency of 1 or 16 Hz for 20 s. These parameters were selected in preliminary experiments to produce consistent and reproducible responses of the circular muscle. Preliminary experiments had shown that all the mechanical responses produced in this way are blocked by 1 μM tetrodotoxin, indicating their neuronal origin. An initial 0.5 g of tension was loaded onto each muscle strips during a 60 min equilibration period with rinsing every 15 min during this period.

Drugs

Atropine sulfate, guanethidine, carbamylcholine chloride (CCh) were purchased from Sigma (St. Louis, MO, USA). Enfloxacin (Baytril) was purchased from Bayer Korea (Seoul, Korea).

The composition (in mM) of the Kreb's solution was 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 11 glucose. The solution was titrated to pH 7.4 with HCl.

Analysis of data

Muscle tension was usually represented as force per unit muscle area (N/cm^2) to compare the contraction force among the strips of each group. However, sometimes the tension was represented as % of maximal response (in contractile response) or % of pre-stimulated tension (in relaxant response). Statistical analysis was performed by

means of Student's *t*-test for paired data or by analysis of variance (ANOVA), when applicable. The value of $p<0.05$ considered to be significantly different.

Results

Electrical field stimulation (EFS) provoked multiphasic responses in the colonic circular muscle strips of each group. The different pattern of responses were observed at low frequency EFS (1 Hz, 0.5 ms pulse duration, 120 V, 20 s train) between normal and three *E. coli* treated groups. In the normal group, colonic circular muscle showed biphasic response at low frequency EFS (1 Hz). The muscle strips of normal group were relaxed during the stimulation and contracted after cessation. However, in the three *E. coli*- treated groups, the muscle strips showed triphasic responses composed of initial relaxation then late contraction during the stimulation (on-contraction) and post-EFS rebound contraction (off-contracton) at low frequency EFS (1 Hz) (Fig. 1). On the other hand, the muscle strips of all experimental groups showed the triphasic responses at high frequency (16 Hz) stimulation (Fig. 2).

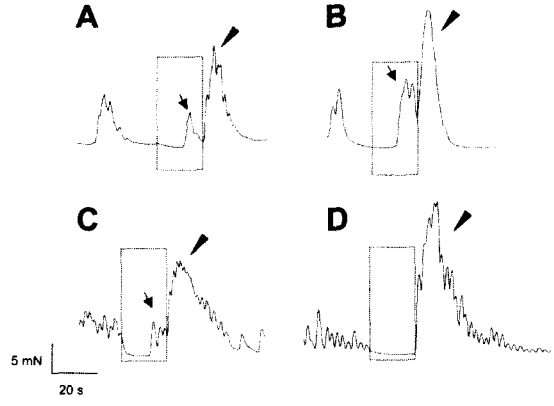

Fig. 1. Representative traces showing the multiphasic responses induced by EFS (1 Hz, 0.5ms pulse, 120 V for 20 s) in colonic circular muscle strip of each experimental group. (A) *E. coli* + no-treatment, (B) *E. coli* + antibiotic, (C) *E. coli* + acupuncture, and (D) normal group. Box indicates the period of EFS application (20 s). Note the small contraction during the EFS application in all inoculated groups (arrow). Arrow head indicates the post-EFS contraction.

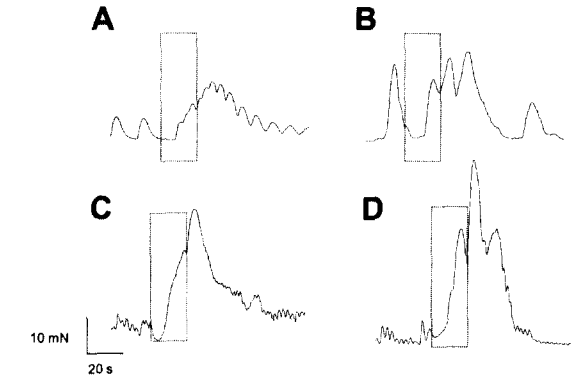

Fig. 2. Representative traces showing the triphasic responses induced by EFS (16Hz, 0.5ms pulse, 120V for 20s) in colonic circular muscle strip of each experimental group. (A) *E. coli* + no-treatment, (B) *E. coli* + antibiotic, (C) *E. coli* + acupuncture, and (D) normal group. Box indicates the period of EFS application (20s). Contraction appeared during the EFS application in all groups.

At low frequencies of EFS (1 Hz), the normal group showed only relaxation during EFS application, while other groups showed contraction in the late phase of EFS (on-contraction). There were no significant differences in the amplitude of relaxation and off-contraction of each experimental group to the low frequency EFS (1 Hz). The on-contraction of the *E. coli* + antibiotic and the *E. coli* + acupuncture group to the low frequency EFS (1 Hz) did not show significantly difference with that of the *E. coli* + no-treatment group (Fig. 3A). And in the high frequency (16Hz) induced relaxation, there was no significant difference among the groups. However, at the high frequency (16 Hz), both on-contraction and off-contraction of the *E. coli* + antibiotic and the normal group were significantly larger than those of the *E. coli* + no-treatment group. Although it was not significant, the strips of the *E. coli* + acupuncture group showed a tendency to produce bigger on-contraction and off-contraction than those of the *E. coli* + acupuncture group at the high frequency (16 Hz) stimulation (Fig. 3B).

An exogenous muscarinic agonist carbachol (CCh, 10 μM) and an adrenergic transmission blocker guanethidine (5 μM) were administered for the NANC condition. In this condition, the strips of all experimental group showed biphasic responses composed of relaxation during the stimulation and off-contraction. At the low frequency (1 Hz)

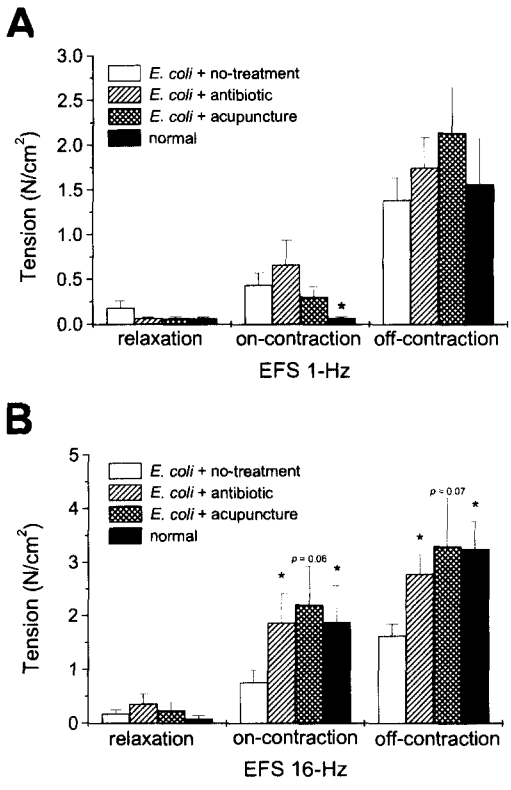

Fig. 3. Summarized graphs showing the EFS-induced multiphasic response in colonic circular muscle strip of each experimental group. At low frequencies of EFS (1 Hz), the normal group showed only relaxation during EFS application, while other groups showed contraction in the late phase of EFS (A and B, "on-contraction"). At the high frequency (16 Hz), contractile response of the acupuncture-treatment, the antibiotic-treatment and the normal group was larger than that of the no-treatment group (B).
* $p<0.05$ as compared with *E. coli* + no treatment group.

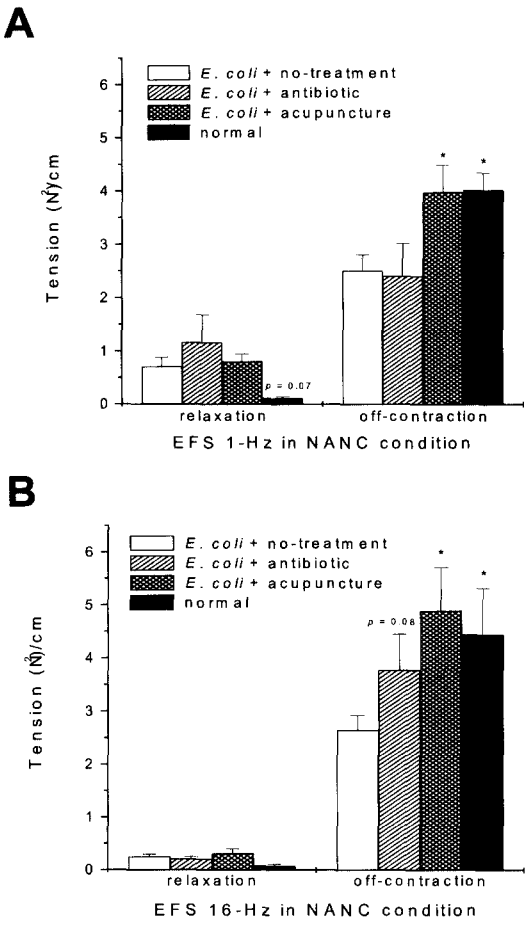

Fig. 4. EFS-induced nonadrenergic-noncholinergic (NANC) biphasic response in colonic circular muscle of weaning piglet. An exogenous muscarinic agonist carbachol (10 μ M) and an adrenergic transmission blocker guanethidine (5 mM) were administered for the NANC condition. At the low frequency (1 Hz) of EFS, the off-contraction of the acupuncture-treatment and the normal group was significantly different from that of the no-treatment group (A). At the high frequency (16 Hz), the contractile response of the acupuncture-treatment, the antibiotics-treatment and the normal group was larger than that of the no-treatment group (B). * $p<0.05$ 05 as compared with *E. coli* + no treatment group.

stimulation, the off-contraction of the *E. coli* + acupuncture and the normal group was significantly larger than that of the *E. coli* + no reatment group. At the high frequency (16 Hz), the contractile response of the *E. coli* + acupuncture and the normal group was significantly larger than that of the *E. coli* + no-treatment group. And the strips of the *E. coli* + antibiotic group showed a tendency to produce bigger off-contraction at the high frequency (16 Hz) EFS (Fig. 4).

There were no significant differences among the experimental groups in the low and high EFS-induced relaxation (Fig. 4).

Application of CCh produced contraction in the colonic circular muscle of all experimental groups in a dose-dependent manner. The contractile response of the *E. coli* + acupuncture, the *E. coli* + antibiotic and the normal group

was significantly larger than that of the *E. coli* + no-treatment at 3 μM CCh (Fig. 5).

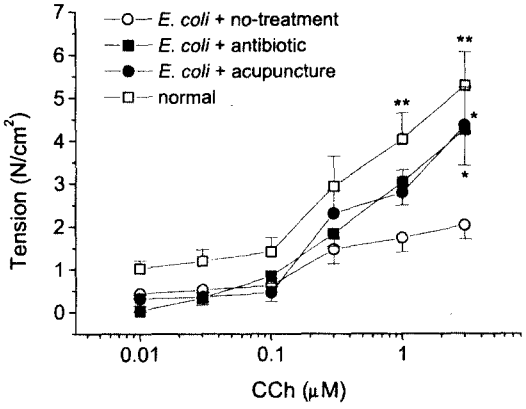

Fig. 5. Carbachol (CCh)-induced contraction in colonic circular muscle strip of each group. The contractile response of the acupuncture-treatment, the antibiotics-treatment and the normal group was larger than the no-treatment group at 3 μM CCh. * $p<0.05$, ** $p<0.01$ 05 as compared with *E. coli* + no treatment group.

Discussion

The main observation of this experiment is that acupuncture at GV-1 has the effect on the colonic motility dysfunction induced by *E. coli* infection.

Diarrhea is still a major pediatric health problem worldwide. *E. coli* is an important cause of diarrhea faced by human infants [5] and young animals including piglets [4, 10, 15, 21]. It was reported that traditional acupuncture at several acupoints including GV-1 was effective in *E. coli* infection-induced diarrhea in preweaning pigs [9]. However they did not mention whether its anti-diarrhea effect was related to colonic muscle function. Although investigation for acupuncture induced alteration of gastrointestinal motility has been performed *in vivo* in various species using various methods [2, 12, 17, 22], the information about the piglet colon *in vitro* is scanty. Therefore we purposed to study whether acupuncture is effective in the *E. coli* infection-induced colonic muscle dysfunction.

In this experiment, in the three *E. coli*-infected groups, low frequency EFS (1 Hz) provoked triphasic responses composed of initial relaxation followed by on-contraction and off-contraction. While in the normal group, EFS (1 Hz) induced biphasic responses composed of relaxation during the stimulation and off-contraction. This finding implicates that the relaxant response was reduced or the contractile response was increased after *E. coli* infection. However, the contractile response of the *E. coli* + no-treatment group at high frequency EFS was significantly smaller than that of normal group. These results suggest that both contractile and relaxant function of colon were injured by *E. coli* inoculation. It was reported that the nitrergic neuron was impaired [14], and the responses for various contractile neurotransmitter were reduced in the rat model of experimental colitis [8, 16].

In the acupuncture treatment group, the strips still showed on-contraction in the later part of the low frequency stimulation (1 Hz). But both on-contraction and off-contraction of acupuncture group were significantly larger than those of the no-treatment group, but not significantly different from those of normal group. The EFS-induced responses of antibiotic group were very similar with those of acupuncture treatment group.

Besides neurogenic responses, myogenic responses of two treatment groups to cholinergic agonist, carbachol, were significantly larger than those of no treatment group at high concentration of CCh. There was no significant difference between acupuncture treatment group and antibiotic treatment group. These results imply that acupuncture treatment at GV-1 has similar level of effect with antibiotic treatment on impaired contractile neuromuscular transmission of colonic smooth muscle induced by *E. coli* infection. This is consistent with previous in vivo experiment that the recovery rates of acupuncture treated group was similar with those of antibiotic treated group in the piglets with induced *E. coli* infection-induced diarrhea [9]. In addition, it was reported that combination of acupuncture and antibiotics showed a synergistic effect capable of empowering the anti-infectious therapy in canine otitis [20].

Diarrhoeagenic *E. coli* are classified into named pathotypes, including enterotoxigenic, enteroinvasive, enterohemorrhagic, enteroaggregative and enteropathogenic *E. coli*. Each pathotype is defined by characteristic set of virulence-associated determinants that act in concert to determine the clinical, pathological and epidemiological features of the disease they cause [19]. Enteropathogenic *E. coli* (EPEC) is primarily associated with infantile diarrhea and is known to induce epithelial inflammatory response [7]. And it was

reported that inflammation decreased intestinal smooth muscle contractility [16] and inhibited neur-omuscular transmission [11].

On the other hand, it was reported that acupuncture had a beneficial effect on inflammatory disease including intestinal inflammation [23, 25]. But the exact mechanisms of acupuncture action are still unknown. However, experimental research has recently shown that there is close correlation between acupuncture effect on immune system and endogenous opioid pathway. It was reported that acupuncture simultaneously increased the β-endorphin level, percentage of NK cells and monocyte phagocytosis [18]. And it was supposed that opioid activation by acupuncture leads to the processing of opioid peptides from their precursor, proenkephalin, and the simultaneous release of antibacterial peptides contained within the precursor as well, which may be coupled to immune enhancement [6].

In conclusion, acupuncture at jiao-chao (GV-1) has beneficial effects on colonic muscle dysfunction induced by *E. coli* infection. It is suggest that the acupuncture effect is related with recovery of contractile neuro-muscular transmission. Acupuncture may be useful treatment for bacterial colonic dysfunction as a alternative medicine instead of antibiotics.

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