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Original Article

Acupuncture on Siguan Points (LI4 and LR3) Restores Loperamide / Scopolamine-induced Intestinal Immotility in Mice

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Objectives : Siguan points (LI4, LR3) have been most frequently applied for various diseases, especially different digestive disorders such as constipation, abdominal pain or various intestinal inflammatory diseases. The fact that gastrointestinal movement is closely connected with physiologic functions or pathologic process of alimentary canal led us to ask the question if Siguan points affects on intestinal motility.

Design: To investigate the effect of Siguan acupuncture on the intestinal movement in both physiologic and pathologic conditions, we divided the experimental animals into 12 groups. Six groups were pre-treated with loperamide (0.5 mg/kg, sc) or scoploamine (0.5 mg/kg, sc) to suppress the intestinal movement and another three groups were pretreated with carbachol (0.5 mg/kg, po) to activate it, whereas the rest three groups didn't receive any pretreatment to be kept in the physiological condition. After the administration with charcoal meal, mice were acupunctured bilaterally on sham point or Siguan points as the manner of tap-stimulation, with the exception of no acupuncture groups.

Methods : Mice were scarified in twenty minutes after the administration of charcoal to measure the distance of charcoal passage from stomach-duodenal junction. The effect on intestinal movement was presented by calculating the relative distance where charcoal arrived to total length of small intestine.

Results : In physiological state, charcoal meal passed around 53%, and there was no significant difference between Siguan points and sham points groups. On the other hand, Siguan points-stimulation significantly ameliorated loperamide or scoplolamine-induced suppressed travel rate of 17.3 % and 18.6 % in sham point into 26 % and 26.3 % respectively ($p<0.05$). In carbachol-induced accelerated condition, Siguan points-stimulation didn't affect intestinal motility comparing to sham point group passed about 97.6 %.

Conclusions : These results postulate that acupuncture at Siguan points have a therapeutic effect by restoring cholinergic activity on pathogenically suppressed intestinal peristalsis, but does not affect the gastrointestinal motility in the normal or accelerated condition.

Key Words: Siguan; four gates; LI4; LR3; gastrointestinal movement; loperamide; scopolamine; carbachol

Introduction

Every meridian has only one side distribution of yin or yang on the body surface except Large Intestine meridian of Hand-Yangming (LI) and Liver Meridian of Foot-Jueyin (LR). LI starts from the yin side of index finger and runs along yang area after passing Hegu (LI 4), whereas LR originates from the yang side of great toe and goes upward along yang distribution after passing Taichong (LR3). Therefore, the combination of the four acupoints, bilateral Hegu (LI 4)

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and Taichong (LR3), is called as Siguan, meaning "four gates"[1]. Since ancient times, Siguan points have been most frequently applied for the patients complaining pain syndromes, both hot and cold, hypertension, headaches, hiccups, and especially many gastrointestinal disorders by opening the "four gates"[2,3].

Many research groups have demonstrated the effects of acupuncture on LI4 or Liv-3 separately on mainly clinical symptoms. LI4 was shown as working positively on postpartum hemorrhage4, dental pain5, dysmenorrhea6 and nausea7, and Liv-3 on hypertension[8,9] and ocular hypertension10. One research group performed a study on the thermographic change of human body after the acupuncture on Siguan points[11].

On the other hand, acupuncture on Siguan points as been adapted clinically for treating gastrointestinal problems such as indigestion, nausea, vomiting, stomach pains, abdominal distention, constipation according to the traditional oriental medical theory of "blocking of Qi leads to pain" particularly in digestive track.12 Also, it is well known fact that intestinal motility is closely connected with not only physiological functions but also pathological state of digestive system.[13]

Hence, it can be anticipated that Siguan points play a role on modulating the movement of alimentary canal, and that to prove this will be helpful to study Siguan's therapeutic applications. Previously, several studies have been done on the effect of acupuncture at different acupoints on gastric emptying,14 duodenal or intestinal motility,[15,16] gastric or intestinal myoelectrical activity[18,19,20] and relation of Oddi-sphincter,[21] but there hasn't been on Siguan points yet.

Therefore, we here aimed at examining the effect of Siguan points on intestinal motility using mice models in both normal and pathologic conditions.

Materials and Methods

1. Chemical preparation

Loperamide, scopolamine and Carbachol were purchased from Sigma (USA). Loperamide was dissolved in normal saline (0.1 mg/ml) containing 0.05% tween 80. Both scopolamine and Carbachol was dissolved in normal saline (0.1 mg/ml), respectively. Charcoal (Wako, Japan) meal was made by suspension of 5% charcoal in 10% Gum Arabic solution.

2. Animals and treatment

Specific pathogen-free seven-week old male ICR mice, around 25±3 g of body weight, were purchased from Daehan-Biolink (Yeumsung, Korea). Eighty-four mice were randomly divided into 12 groups of 7 animals in each cage. They were housed in an environmentally controlled room at 22±2℃, in 55± 10% of relative humidity, with 12-hour light/dark cycle, and fed with commercial pellets (Samtako, Korea) and tap water *ad libitum*. After one -week acclimation, these mice were used for this experiment in state of 24 hour fasting.

For inducing suppressed intestinal motility model, each mouse of six groups was treated with loperamide (0.5 mg/kg, sc) or scopolamine (0.5 mg/kg, sc) accurately 15 min before the charcoal meal administration (10 ml/kg) by serial manners. Whereas carbachol (0.5 mg/kg, po) was given to three groups of mice for excessive intestinal motility, the mice in the physiologic state model were used without any pretreatment.

3. Acupuncture and measuring the effects

15 minutes after the drug treatments, each mouse was orally administrated with 0.2 ml of charcoal meal, whereas mice for physiologic state model were done without any pretreatment. After the charcoal meal

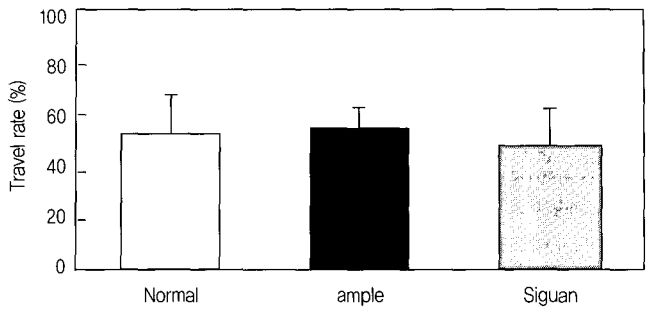

Fig. 1. *Effect of Siguan-acupuncture on intestinal motility at normal state.*

Mice were tap-acupunctured twice on bilateral Siguan-correspondent points or fake points for sham control groups immediately after administration of charcoal meal. Intestinal motility was determined at 20 min after administration of charcoal meal by calculating the percentage of passage distance to whole length of small intestine. The data were expressed as mean ± SD.

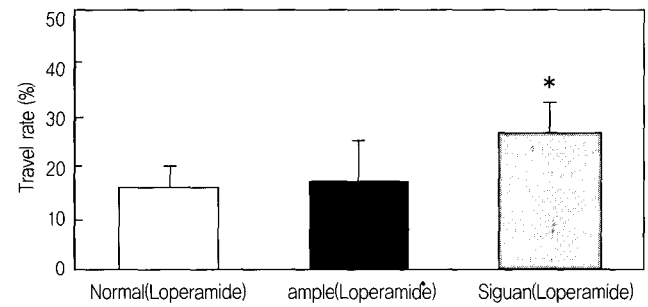

Fig. 2. Effect of Siguan-acupuncture on intestinal motility at suppressed state by loperamide treatment.

Mice were injected subcutaneously with loperamide (0.5 mg/kg) 15min before acupuncture. Mice were tap-acupunctured twice on bilateral Siguan-correspondent points or fake points for sham control groups immediately after administration of charcoal meal. Intestinal motility was determined at 20 min after administration of charcoal meal by calculating the percentage of passage distance to whole length of small intestine. The data were expressed as mean ± SD. An asterisk denotes response that is significantly different from the sham acupuncture group as determined by Student's t-test at $p<0.05$ (*)

administration, mice were tap-acupunctured in the order of Rt. LR3, Lt. LR3, Rt. LI4 and Lt. LI4, in the depth of 2mm, twice with 10 minutes interval by trained-oriental medical doctor on Siguan-correspondent points or sham points on the middle of sole using sterile disposable acupuncture needles with 0.3mm diameter (Sindongban Co, Korea). For none-acupuncture groups, we have grasped mice for a moment to give them stress without acupuncture same as when other groups have been acupunctured. Finally, mice were scarified 20 minute after the charcoal administration by cervical dislocation, and the travel rates of charcoal were calculated as the percentage of passage distance to full small intestine.

Our animal experiments have been conducted in

accordance with the use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

4. Statistical analysis

Results were marked as the mean±SD. Statistical analysis of the data was carried out by Student's *t*-test. A difference from the respective control data at the levels of $p<0.05$ were regarded as statistically significant.

Results

1. Effect of siguan points on intestinal motility at physiological state

First, we adapted an experimental model to investigate the role of on intestinal motility at normal condition. To know whether stimulation itself on any points can affect the charcoal passage rate, we compared a none-stimulated group with shame point group. At time point of 20 min, charcoal meal passed very similarly like as 52.6 % and 55.1 % for none-acupunctured and shame groups respectively, indicating being acceptable of this study. We next asked that acupuncture on Siguan points might suppress or activate the intestinal motility comparing to sham point. The result showed slightly reduced travel rate of charcoal meal by Siguan points-stimulation as 48.9 %, but there wasn't any statistical significance by comparing to no treatment or sham points (Fig. 1).

2. Effect of Siguan points on intestinal motility at loperamide-induced suppressed state

Next, in other to explore Siguan's affect on pathological condition, we made two differentially changed models; drug-induced suppressed or activated states of intestinal motility.

Foremost, to examine the Siguan's effect at suppressed motility model, three groups of mice were pre-treated subcutaneously with 0.5 mg/kg of loperamide, an antimotility agent, before charcoal administration. The loperamide treatment repressed the charcoal meal passage as 15.9 % and 17.3 % in none-stimulated and sham groups respectively. On the other hand, the group of Siguan acupuncture improved passage rate by 26 % comparing to 17.3 % in sham point group. This result revealed the effect of Siguan points on ameliorating the pathologically suppressed motility by presenting the statistical significance comparing to sham point as p-value less than 0.05 (Fig. 2).

3. Effect of Siguan points on intestinal motility at scopolamine-induced suppressed state

The above result let us further investigate Siguan's effect, anticipating that Siguan points might play a activating role on intestinal movement through stimulating parasympathetic nerve system. For answering this question, we pre-treated three groups of mice subcutaneously with 0.5 mg/kg of scopolamine, a cholinergic antagonist. This treatment reduced the intestinal motility as like 22.9 % of charcoal meal passage rate in none-stimulated group. Likewise, sham point group didn't much change the movement rate of charcoal like 18.6 %. However, Siguan acupuncture improved the repressed passage rate up to 26.3 % with statistically significant difference as p-value less than 0.05 (Fig. 3).

4. Effect of Siguan points on intestinal motility at carbachol-induced activated state

For exploring if Siguan points could give any influence at activated condition, three groups of mice were orally pre-treated with 0.5 mg/kg of carbachol, a cholinergic agonist, before charcoal administration. Carbachol treatment accelerated the intestinal motility likely rapid passage rate of 95.2 % in none stimulated group. Because some of mice showed over-passed

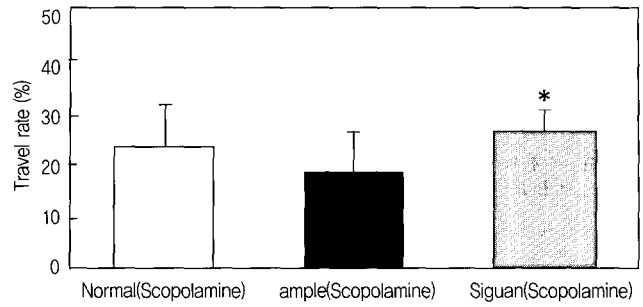

Fig. 3 Effect of Siguan-acupuncture on intestinal motility at suppressed state by scopolamine treatment.
Mice were injected subcutaneously with scopolamine (0.5 mg/kg) 15min before acupuncture. Mice were tap-acupunctured twice on bilateral Siguan-correspondent points or fake points for sham control groups immediately after administration of charcoal meal. Intestinal motility was determined at 20 min after administration of charcoal meal by calculating the percentage of passage distance to whole length of small intestine. The data were expressed as mean ± SD. An asterisk denotes response that is significantly different from the sham acupuncture group as determined by Student's t-test at $p<0.05$ (*)

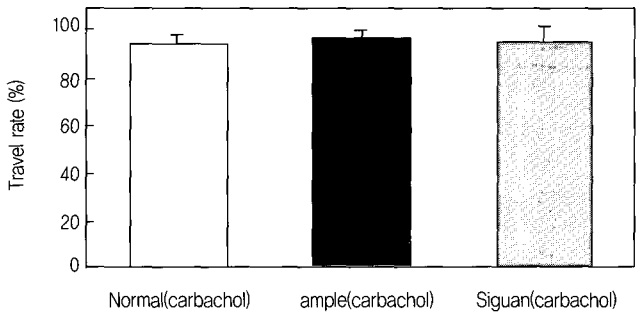

Fig. 4. Effect of Siguan-acupuncture on intestinal motility at activated state by carbachol treatment.
Carbachol (0.5 mg/kg, po) was given to three groups of mice for excessive intestinal motility model 15min before acupuncture. Mice were tap-acupunctured twice on bilateral Siguan-correspondent points or fake points for sham control groups immediately after administration of charcoal meal. Intestinal motility was determined at 20 min after administration of charcoal meal by calculating the percentage of passage distance to whole length of small intestine. The data were expressed as mean ± SD.

charcoal meal by entire small intestine in all of three groups in this model, there was a little difficult to calculate the accurate change of charcoal arrival distance. However, both groups of Siguan and shame points generally didn't show any difference in passage rate as 96.4 % and 97.6 % respectively (Fig. 4).

Discussion

Siguan points are the most often selected acupoints for patients with various disorders in digestive system. This clinical application is based on the Siguan's

meridian theory, which the Siguan's points are placed at yin-yang crossover position like as four gates.1 In oriental theory, digestive passageway (usually presented as spleen in oriental medicine) is closely connected with four limbs and they are belonged to yang organs doing their works as empting contrast to yin organs doing as storing. Accordingly, various digestive problems are frequently described as locking at meridian gates, and Siguan point has been frequently applied for these pathologic situations. Also, it is presumed that Siguan, even without any scientific proof for that at all, may affect the gastrointestinal movement by adjusting autonomic nerve functions.

In the present day, although increasing population is using the oriental therapeutics, highly controlled studies are required for providing scientific data.22, 23 There are some clinical studies for acupuncture's effects on gastrointestinal disorders such as constipation,24, 25, 26 irritable colon syndrome or diarrhea27, 28 by treating various acupoints, but not Siguan.

Therefore, in order to obtain evidence for theoretical application and traditional meridian practice and to partially characterize the Siguan's therapeutic roles in digestive disorders, we here engaged mice intestinal motility models; normal, loperamide or scopolamine-induced suppressed, and carbacol-induced activated states. Although it is indeed doubtful whether animal can be suitable for acupuncture study, animal study sometimes may be inevitable to search the mechanism of acupuncture using artificial disease models such as present study.[29] We top-acupunctured the mice at Siguan-correspondent points as do for pediatric patients.

First, we investigated if Siguan points could alter the gastrointestinal movement in normal condition by measuring the speed of charcoal meal passage after acupuncture treatment. As shown in Fig.1, Sawhan didn't affect the passage rate of charcoal meal comparing with sham points, even it showed slightly reduced-rate as 48.9 % comparing to 52.6 % and 55.1 % in none-acupunctured and shame group, respectively. This result indicates that that Siguan points doesn't affect the intestinal motility in the physiological state.

Next, above result let us explore the effects of Siguan points on intestinal motility at pathologically suppressed or activated states similarly to clinical symptoms. Relying on the fact that mainly acetylcholine and its muscarinic receptor direct the gastrointestinal muscle,30 we imitated simply pathologic models with cholinergic drugs. Also, we expected that maybe acupuncture on Siguan points be able to correct the choline-related unbalanced state for its therapeutic properties because some studies showed the linkage of acupuncture and acetylcholine synthesis or its activity. [31,32]

A synthetic antidiarrheal agent, loperamide known as interfering with acetylcoline release,33 significantly suppressed intestinal muscle movement like as charcoal travel rate of 15.9 %. But in this model, Siguan acupuncture ameliorated the suppressed motility by improving as 26% as shown in Fig.2. In addition to this result, we found that Siguan points could overcome scopolamine-caused immortality within certain limits such like Fig. 3. Scopolamine has been well known to inhibit the contractile muscle activity by blocking muscarinic receptor on intestinal muscle.[34]

Subsequently, we investigated if Siguan points could influence the intestinal motility at accelerated condition induced by carbachol, a synthetic parasympathomimetic drug. Carbachol strongly speeded the passage of charcoal meal by almost 95.2 % in 20 min no treatment at all. And, either sham or Siguan acupuncture didn't almost change the passage rate as 96.4 % and 97.6 % respectively (Fig. 2).

From these results, we speculate that acupuncture on Siguan points may activate the intestinal movement, when it's suppressed, by way of improving acetylcoline release and possibly improving the activity of

muscarinic receptors in the intestinal muscles, but the involvement of muscarinic receptors needs to be studied more. In addition, acupuncture on Siguan points affected the intestinal motility not in the accelerated condition, but in the suppressed condition. This is considered to coincide with the oriental medical theory that Siguan(four gates) opens the gates of vital energy and regulate the system.

Oriental medical therapies are expected to adjust the imbalanced status, be more powerful when the system is abnormal than normal, therefore, has less side effects[35]. In the present study, acupuncture on Siguan points did not make any considerable change in the normal condition, which seems to correspond to the theory.

In conclusion, our results demonstrated an experimental evidence of Siguan's positive role in improving intestinal peristalsis by especially alteration of suppressed motility. To our knowledge, our study first shows scientific observation for underlying mechanism of Siguan's clinical applications.

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