

Full Length Research Paper

Study on stomach physiological functions by electroacupuncture at zusanli points under effects of atropine and cimetidine

Yong Peng^{1*}, Man Sun¹, Zhe Liu¹, Jianxin Wang², Yingjie Liu¹, Yanxia Zhang¹ and Bin Jiang¹

¹Department of Biomedicine Engineering, College of Electrical Engineering, Yanshan University, Qinhuangdao, China.

²The First Hospital of Qinhuangdao City, Qinhuangdao, China.

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To explore the effect and mechanism on the physiological functions of stomach by electroacupuncture at zusanli (STOMACH-36), the changes of the gastric electrical frequency and amplitude, and the flux of gastric juice secretion were observed with modern apparatus, when electroacupuncture at zusanli was administered on rabbits, before and after injecting atropine and cimetidine. The experimental results show that the gastric electrical frequency reduced ($P < 0.01$, $P < 0.05$), while, the gastric electrical amplitude increased ($P < 0.05$), and the flux of gastric juice secretion increased ($P < 0.05$) when acupuncture at zusanli was administered in the control group (the no drug group). Compared with the control group, the gastric electrical frequency increased ($P < 0.05$), while the gastric amplitude reduced ($P < 0.05$), and the flux of gastric juice secretion reduced ($P < 0.05$) when acupuncture at zusanli was applied, after treatment with atropine. Compared with the control group, the gastric electricity frequency increased ($P < 0.05$), while the gastric amplitude reduced ($P < 0.05$), and the flux of gastric juice secretion reduced ($P < 0.05$) when acupuncture at zusanli was applied after treatment with cimetidine. Therefore, our study shows that when electroacupuncture at zusanli is applied, the gastric electrical frequency increased and gastric electrical amplitude reduced, while the flux of gastric juice secretion increased. Compared with the control group, the gastric electrical frequency increased and gastric electrical amplitude reduced, and the flux of gastric juice secretion reduced when electroacupuncture at zusanli was applied after treatment with atropine and cimetidine, respectively. Thus, we supposed that the mechanism of regulating gastric electrical activity and gastric juice secretion might become true by the two pathways of gastric H₂ and M-receptors.

Key words: Electroacupuncture, zusanli (ST-36), gastric electrophysiological signal, gastric juice secretion, atropine, cimetidine.

INTRODUCTION

Zusanli (ST-36) is the lower he-sea point of stomach meridian of foot yang brightness. The traditional Chinese medicine is of the view that it has the ability of regulating the immune system of body, enhancing the resistance to disease, invigorating spleen-stomach and replenishing 'qi', which is the first choice acupoint for treating

gastrointestinal diseases in traditional Chinese medicine (Cabyoglu et al., 2006; Chou et al., 2003). Zusanli have been widely used for a long time by traditional Chinese medicine to treat many diseases such as gastritis, stomach pain, stomach cramps, gastroptosis etc. Acupuncture at zusanli has a certain regulatory role on the physiological function of gaster-intestine. There is also the good effect in clinical practice by the way of massage, acupuncture and moxibustion with this acupuncture point (Gervais et al., 1992).

At present, some researches on the effects of zusanli

*Corresponding author. E-mail: py81@sina.com. Tel: 0335-8074715. Fax: 0335-8074715.

on humoral, nervous and immune systems are in progress all over the world. It has been confirmed that the physiological functions of stomach can be altered by stimulating zusanli. In turn, the change of physiology signal of stomach can also show obviously how drugs work on stomach. However, no research on the changes of the gastric electrophysiological signal and gastric juice secretion by electroacupuncture at zusanli with two sorts of receptor antagonists (H_2 -receptor and M-receptor antagonists, respectively) at the same time to probe the effective mechanism has been reported. Our study aimed to explore the effect and mechanism, by observing the fluctuation of gastric electrophysiological signal and the flux of gastric juice secretion, when electroacupuncture at zusanli is applied, before and after treatment with atropine and cimetidine, respectively, which provide experimental evidence for the treatment of stomach diseases by electroacupuncture at zusanli.

MATERIALS AND METHODS

Experimental animals

30 healthy adult rabbits (2.0 to 3.0 kg), which were ordinary experimental animals, were provided by Department of Biomedicine Engineering, Yanshan University. Rabbits were kept in a clean room with appropriate temperature ($20 \pm 2^\circ\text{C}$), and provided 7 to 8 h illumination every day. They were fed with a common diet and water *ad libitum*. The animals were treated according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and their experimental use was approved by the animal Ethics Committee of the University.

Experimental materials

Sumianxin (II) injection (Military Veterinary Institute in Academy of Military Medical Sciences in China, product batches number was 0507111), atropine injection (Tianjin jinyao Amino acid Company Limited in Tianjin in China, product batches number was 0703121) and cimetidine injection (Fangming Pharmacy Limited in Shandong in China, product batches number was 0511071) were used. The acupoint orientation apparatus was 401A-Type LCR for automatic measure apparatus (Aoshun Technology Company in Tianjin in China). The electrical stimulation apparatus was WQ1002 Han's Multifunctional electrical treatment apparatus (Anlong Photoelectric Technology Company in Beijing, China). The recording system of gastric electrophysiological signal and gastric juice secretion flow was BL-420E Biological Function Experimental System (Taimeng Science and Technology Limited in Chengdu, China).

Experimental methods

The operations on 30 rabbits were done after they were fed for 24 h and allowed to drink water freely. The rabbits were injected with Sumianxin (II) injection (0.1 ml/kg) by the pathway of the ear vein of rabbits for anesthesia, respectively. They were operated through the center line of the abdomen about 5 cm length. The rabbits were divided into three groups, which were: the non-drug treatment group (the control group), the atropine treatment group and the cimetidine treatment group. The non-drug treatment group was divided into three steps, in accordance with the sequence of time: N (I) step (before acupuncture for 10 min), N (II) step (during acupuncture for

10 min), and N (III) step (stopping acupuncture for 10 min). The atropine treatment group was divided into three steps, in accordance with the sequence of time: A (I) step (before acupuncture for 10 min), A (II) step (during acupuncture for 10 min), A (III) step (stopping acupuncture for 10 min). The cimetidine treatment group was also divided into three steps in accordance with the sequence of time: C (I) step (before acupuncture for 10 min), C (II) step (during acupuncture for 10 min), C (III) step (stopping acupuncture for 10 min).

The frequency (Hz) and amplitude (mv/min) of gastric electrical activity, as well as flux of gastric juice secretion (drop/min) were consecutively recorded with the BL-420E Biological Function Experimental System.

The calculation of the acupuncture effect was defined in the light of the literature (Zhou, 1983) and its experimental content. The effects of acupuncture on the frequency and amplitude of gastric electrical activities were calculated, with the percent of frequency or amplitude of the no acupuncture group for 10 min taken as 100%. The calculating method is shown in Equation 1:

$$\rho = \frac{A_1(A_2) - A_0}{A_0} \times 100\%$$

Where, ρ = acupuncture effect; A_0 = electrical frequency or amplitude of no acupuncture; A_1 = electrical frequency or amplitude of during acupuncture and; A_2 = electrical frequency or amplitude of stopping acupuncture.

The effect of acupuncture on gastric juice secretion was calculated with the percent of the flow of gastric juice secretion, and the flow of gastric juice secretion of the no acupuncture group for 10 min was taken as 100%. The calculating method is shown in Equation 2:

$$\eta = \frac{f_1(f_2) - f_0}{f_0} \times 100\%$$

Where, η = acupuncture effect; f_0 = gastric juice secretion flow of no acupuncture; f_1 = gastric juice secretion flow of during acupuncture and; f_2 = gastric juice secretion flow of stopping acupuncture.

Determination of position of zusanli acupoints

The precise position of zusanli was 1.2 cm below the capitulum fibulae, posterolateral to the hind limb knee joint of a rabbit (Yu, 1984). The depth of acupuncture stimulation was about 0.8 cm. The position of zusanli was determined by the LCR Automatic measuring apparatus. The measuring format was set into soft error rates (SER) and the frequency into 100 Hz after the apparatus was operated. The position of zusanli was made sure by the minimum value of resistance, and the maximum value of capacitance measured by the apparatus. Both side positions of 0.2 cm near to zusanli were taken, respectively as the control groups (the left control group and the right control group) to determine the acupoints, by comparing the resistance and capacitance value of acupoints with those of the control groups.

Stimulation of zusanli acupoints

The WQ1002 Multifunction electrical treatment apparatus of Han's was applied in this experiment. The electroacupuncture parameters of the apparatus were set as follows: the type of electroacupuncture was ACU, the waveform was the continuous wave, the intensity was

Table 1. Skin resistance and capacitance values on rabbits.

Parameter	Left control group	Zusanli group	Right control group
Resistance (K Ω)	12.17 \pm 1.02	6.56 \pm 0.32*	10.02 \pm 0.73
Capacitance (nF)	11.44 \pm 1.03	21.16 \pm 1.91*	13.18 \pm 1.01

*P < 0.01 as compared with left and right control group (mean \pm S.D., n = 10).

2 V, and the frequency was 100 Hz. Zusanli of both legs of a rabbit were stimulated for 10 min at the same time by the WQ1002 Multifunction electrical treatment apparatus of Han's. Then, the signal of gastric electrophysiology activity and the flow of gastric juice secretion on rabbits were continuously recorded with the BL-420E Biological Function Experiment System.

Recording experimental data

The experimental data were consecutively recorded with the BL-420E Biological Function Experiment System for the date of the first step (before acupuncture group for 10 min), the second step (during acupuncture group for 10 min) and the third step (stopping acupuncture group for 10 min). The gastric electrophysiological signal and the gastric juice secretion flow were detected continuously to contrast, among the different groups. The way of acupoints injection was chosen in both of the drug treatment groups in light of scientific literature (Zhu et al., 2005).

Statistical analysis

The experimental data were analyzed with the statistical software SPSS.11.5 and presented in the form of "mean \pm standard deviation" (mean \pm S.D.). The t-test statistical way was used in the prominence test of acupuncture effect, and P < 0.05 was taken as the statistical significance. When the consequence of before acupuncture was contrasted to one of during acupuncture and one of stopping acupuncture, respectively, the prominence test way was used in the mean differences in the same sample, before and after disposal, and the prominence test way with the mean difference of two samples was used in comparison among these different groups.

RESULTS

Determination of position of zusanli acupoints

The experimental results show that the skin resistance values of the zusanli group were lower than those of both sides of the control groups (P < 0.01), while the skin capacitance values of the zusanli group were higher than those of both sides of control groups (P < 0.01). The skin resistance value and the capacitance value of the same rabbit were detected, respectively to determine the precise position of zusanli (Table 1).

Effects of stimulating zusanli acupoints

Comparing the data of the N (II) step with ones of the N (I) step, the gastric electrical frequency decreased (P < 0.01), while the gastric electrical amplitude increased (P < 0.01),

and the flux of gastric juice secretion increased (P < 0.05). Comparing the data of the N (III) step with ones of the N (I) step, the gastric electrical frequency decreased (P < 0.05), while the gastric electrical amplitude increased (P < 0.05), and the flux of gastric juice secretion also increased (P < 0.05), but the N (II) step was inferior to the N (III) step in the aspect of the change intensity (P < 0.05, P > 0.05) (Tables 2 and 3).

The results show that the gastric electrical frequency decreased when zusanli were stimulated, and the gastric electrical amplitude and the flux of gastric juice secretion increased. Furthermore, these effects were able to still last for a period time, after stopping stimulating zusanli. That is, acupuncture at zusanli had some residual effects.

Electroacupuncture at zusanli after treatment with atropine

Comparing the data of A (II) step, with ones of A (I) step, after atropine injection by the pathway of zusanli, the gastric electrical frequency increased (P < 0.05), while the gastric electrical amplitude decreased (P < 0.05), and the flux of gastric juice secretion also increased (P < 0.05). Comparing the data of A (III) step with ones of A (I) step, the gastric electrical frequency increased (P < 0.05), while the gastric electrical amplitude decreased (P < 0.05), and the flux of gastric juice secretion also increased (P < 0.05). However, the acupuncture effects of atropine injection groups were still lower than those of stimulating zusanli groups (Tables 3, 4 and 5).

The results reflect that comparing the data of A (II) step with N (II) step, the gastric electrical frequency was promoted and the gastric electrical amplitude was inhibited (Tables 2 and 4), while the gastric juice secretion was inhibited after treatment with atropine (Tables 3 and 5).

So, we inferred that one of the effect mechanism might be that the change of gastric electrophysiological signal and gastric juice secretion when electroacupuncture at zusanli could be realized by the pathway of gastric M-receptor.

Electroacupuncture at zusanli after treated with cimetidine

Comparing the data of C (II) step with ones of C (I) step

Table 2. Fluctuation of gastric electrophysiological frequency and amplitude on stimulating zusanli.

Parameter	No acupuncture group	During acupuncture group	Stopping acupuncture group
Gastric electrical frequency (Hz)	56.21±5.05	41.73±2.18**	47.23±4.09*#
Effect (%)	-	-25.76	-15.97
Gastric electrical amplitude (mV)	0.11±0.02	0.17±0.01**	0.15±0.02*#
Effect (%)	-	54.55	36.36

(Mean ± S.D., n = 10) during acupuncture group versus no acupuncture group, **P < 0.01; stopping acupuncture group versus no acupuncture group, *P < 0.05; stopping acupuncture group versus during acupuncture group, #P < 0.05.

Table 3. Flux of gastric juice secretion on stimulating zusanli.

Parameter	No acupuncture group	During acupuncture group	Stopping acupuncture group
Flux (drop/min)	8.14±0.72	12.94±0.97**	10.91±0.97*#
Effect (%)	-	58.97	34.03

(Mean ± S.D., n = 10) during acupuncture group versus no acupuncture group, **P < 0.05; stopping acupuncture group versus no acupuncture group, *P < 0.05; stopping acupuncture group versus during acupuncture group, #P > 0.05.

Table 4. Fluctuation of gastric electrical frequency and amplitude on stimulating zusanli after atropine injection.

Parameter	No acupuncture group	During acupuncture group	Stopping acupuncture group
Gastric electrical frequency (Hz)	56.21±9.05	71.24±2.25**	76.21±2.57*#
Effect (%)	-	26.74	35.58
Gastric electrical amplitude (mV)	0.11±0.03	0.10±0.04**	0.09±0.03*#
Effect (%)	-	-9.09	-18.18

(Mean ± S.D., n = 10) during acupuncture group versus no acupuncture group, **P < 0.05; stopping acupuncture group versus no acupuncture group, *P < 0.05; stopping acupuncture group versus during acupuncture group, #P > 0.05.

Table 5. Flux of gastric juice secretion on stimulating zusanli after atropine injection.

Parameter	No acupuncture group	During acupuncture group	Stopping acupuncture group
Flux (drop/min)	8.65±0.71	10.21±0.84**	9.77±0.71*#
Effect (%)	-	18.03	12.95

(Mean ± S.D., n = 10) during acupuncture group versus no acupuncture group, **P < 0.05; stopping acupuncture group versus no acupuncture group, *P < 0.05; stopping acupuncture group versus during acupuncture group, #P > 0.05.

after cimetidine injection by the pathway of zusanli, the gastric electrical frequency increased ($P < 0.05$), while the gastric electrical amplitude decreased ($P < 0.05$), and the flow of gastric juice secretion also increased ($P < 0.05$). Comparing the data of C (III) step with ones of C (I) step, the gastric electrical frequency increased ($P < 0.05$), while the gastric electrical amplitude decreased ($P < 0.05$), and the flux of gastric juice secretion also increased ($P < 0.05$), yet the acupuncture effects of cimetidine injection groups were lower than those of stimulating zusanli groups (Tables 3, 6 and 7).

The results indicate that, comparing the data of C (II) step with N (II) step, the gastric electrical frequency was promoted and the gastric electrical amplitude was

inhibited (Tables 2 and 6), while the gastric juice secretion was inhibited after treatment with cimetidine (Tables 3 and 7).

For this, we conclude that one of the action mechanism might be that the change of gastric electrophysiological signal and gastric juice secretion when electroacupuncture at zusanli could be realized by the path of gastric H₂-receptor.

DISCUSSION

Acupuncture is a treasure of traditional Chinese medicine, which is the wisdom crystallization of the long-term

Table 6. Fluctuation of gastric electrical frequency and amplitude on stimulating zusanli after cimetidine injection.

Parameter	No acupuncture group	During acupuncture group	Stopping acupuncture group
Gastric electrical frequency (Hz)	56.21±9.05	78.09±7.00**	80.94±3.53 [#]
Effect (%)	-	38.93	44.00
Gastric electrical amplitude (mV)	0.11±0.03	0.10±0.04**	0.09±0.03 [#]
Effect (%)	-	-9.09	-18.18

(Mean ± S.D., n = 10) during acupuncture group versus no acupuncture group, **P < 0.05; stopping acupuncture group versus no acupuncture group, *P < 0.05; stopping acupuncture group versus during acupuncture group, [#]P > 0.05.

Table 7. Flux of gastric juice secretion on stimulating zusanli after cimetidine injection.

Parameter	No acupuncture group	During acupuncture group	Stopping acupuncture group
Flux (drop/min)	7.64±0.73	9.15±0.91**	8.89±0.93 [#]
Effect (%)	-	19.76	16.36

(Mean ± S.D., n = 10) during acupuncture group versus no acupuncture group, **P < 0.05; stopping acupuncture group versus no acupuncture group, *P < 0.05; stopping acupuncture group versus during acupuncture group, [#]P > 0.05.

experience accumulation of the Chinese family by disease prevention and disease therapy, and which is an important part of Chinese medicine. Zusanli is one of the most important acupoints in clinical practice, and it has been confirmed to be effective in clinical practice for thousands of years. Zusanli has been taken as the preferred acupuncture to treat gastrointestinal diseases in Chinese traditional medicine for a long time. Acupuncturing at zusanli can be used to treat digestive, respiratory, circulatory, urinary, neurological and even other systems of dozens of diseases (Toku, 2006; Gollub et al., 1999). Above all, acupuncture can regulate gastrointestinal motility. The researches of Chinese medicine shows that acupuncture may exert two kinds of influence on gastrointestinal motility: promoting and suppressing influences.

In this study, the resistance and capacitance of skin were determined by modern biomedicine apparatus. The research consequence shows that the resistance value of the zusanli group was lower and the capacitance value higher, while the resistance values of both sides control groups were higher and the capacitance values lower. According to the characteristics of the lower resistance value of the skin of acupoints and the higher capacitance value, the precise positions of zusanli were further determined at the same time by the two indexes above, based on the relationship of the resistance value and capacitance value being reverse. The modern apparatus were applied in detecting the two indexes of resistance and capacitance to ensure the veracity of acupoint orientation. This method of acupoint orientation was superior to the tradition method by the single indexes of detecting resistance. Based on the fact that the two indicators can be confirmed with each other, this method was more accurate and reliable than the tradition method.

Our study explores the action and mechanisms of acupuncture at zusanli on the physiological functions of stomach. Two kinds of receptor antagonists were used in our experiment in order to know which receptors have the relationship with the acupuncture effects. The experiment results show that acupuncturing at zusanli make a positive effect at stomach activity. Compared with the control group, the two drugs (Atropine and Cimetidine) treatment groups could all play the role in increasing the gastric electrical frequency, reducing gastric electrical amplitude and gastric juice secretion flux. So, atropine and cimetidine may play the same effect. However, compared with the action of the two drugs, the effect of cimetidine was more obvious than that of atropine.

As we studied, the regulation mechanisms of gastrointestinal motility are complicated, which may come true by two pass ways: nerves (adrenergic nerve, cholinergic nerve, and nonadrenergic non-cholinergic nerve by neurotransmitter) and body fluids (hormone and neuroendocrine such as motilin and gastric inhibition polypeptide). The changes of physiological signal such as gastric electrical frequency and amplitude, as well as gastric juice secretion flux were studied in this experiment. Atropine is a specific inhibitor of M-receptor which can reduce gastric acid secretion by blocking the path of M-receptor on gastric cell membrane combined with acetylcholine (GAO et al., 2007), and cimetidine is also an inhibitor of H₂-receptor, which can reduce gastric acid secretion, by blocking the path of H₂-receptor on gastric cell membrane selectively (Robert and Mitchell, 1993). Thus, we inferred that the action mechanism of stomach activity by acupuncture at zusanli might become true by the two pathways of gastric H₂ and M-receptors, that is to say, cimetidine and atropine might be all valid in the aspect of treating gastric disease, caused by excessive

gastric acid secretion.

On the whole, we supposed that stimulating zusanli with electroacupuncture might play a good role in regulating stomach activity, and the effective mechanism might be implemented by the two pathways of gastric H₂ and M-receptors. This experimental results also indicate that electroacupuncture at zusanli could be used to treat some diseases of digestive system. Electroacupuncture acupoints may have more advantages than drugs such as few side-effects and two-way modulation effects. Our study has provided some experimental basis for the electro-acupuncture treatment of stomach trouble and the application of acupoint injection in clinical practice. However, there are lots of problems that still need deep studies.

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