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Special Topic for 973 Program

Effect of electroacupuncture on electrogastrogram and gastric antrum ghrelin in rats with diabetic gastroparesis

电针对糖尿病胃轻瘫模型大鼠胃电及胃窦 ghrelin 的影响

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Abstract
Objective: To observe the effect of electroacupuncture (EA) on the electrogastrogram and gastric antrum ghrelin in rats with diabetic gastroparesis (DGP).
Methods: Fifty Sprague-Dawley (SD) rats were randomly divided into group A, group B, group C, group D and group E, 10 rats in each group. Group A was the blank control group without intervention. Group B, Group C, Group D and Group E were treated with single dose intraperitoneal injection of 2% streptozotocin (STZ), combined with 8-week high glucose and high fat diet to establish DGP rat models. Group B was the model group without treatment. Group C was the EA at acupoint group, was treated with EA at Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6). Group D was the EA at non-acupoint group, was treated by EA at the control points of Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6). Rats in the metoclopramide control group received 1.7% metoclopramide solution [10 mL/(kg·bw)] by gavage. Rat's blood glucose level was measured by blood glucose meter; gastric emptying rate was detected using phenol red as a marker; the electrogastrogram was detected by BL-420F biological function system; the protein level of ghrelin was detected by enzyme-linked immunosorbent assay (ELISA); the expression of ghrelin mRNA was detected by real-time polymerase chain reaction (RT-PCR).
Results: Compared with group A, the blood glucose of group B, C, D and E were significantly increased before and after the treatment (all $P<0.01$); after treatment, the gastric emptying rate of group B was significantly decreased ($P<0.01$), the migration rates of small intestine in group B, C, D and E were significantly lower (all $P<0.01$), and the protein content of ghrelin in group C was significantly decreased ($P<0.01$); the expressions of ghrelin mRNA were significantly increased in group B, C, D and E (all $P<0.01$), the mean amplitudes of electrogastrogram in group B and D were significantly decreased (both $P<0.01$). After treatment, compared with group B, the blood glucose of group C was significantly decreased ($P<0.05$), the gastric emptying rate and small intestine migration rate were significantly increased in group C and E ($P<0.05$, $P<0.01$), the small intestinal migration rate was significantly increased in group D ($P<0.05$), the expression of ghrelin in protein and mRNA in group C was significantly lower ($P<0.01$), the expression of ghrelin mRNA in group E was significantly lower ($P<0.05$), and the mean amplitude of electrogastrogram in group C was significantly increased ($P<0.05$). After treatment, compared with group D, the protein and mRNA expressions of ghrelin in group C were significantly decreased ($P<0.01$). After treatment, compared with group E, the protein expression of ghrelin in group C was significantly decreased ($P<0.01$).
Conclusion: EA at Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6) could regulate the blood glucose level of DGP model rats, enhance electrogastrogram activity, promote gastric emptying, and regulate ghrelin expression in protein and mRNA.
Keywords: Acupuncture Therapy; Electroacupuncture; Specificity of Acupoints; Point Selection; Diabetes Complications; Gastroparesis; Rats

【摘要】目的：观察电针对糖尿病胃轻瘫(DGP)模型大鼠胃电及胃窦ghrelin的影响。**方法**：将50只Sprague-Dawley (SD)大鼠随机分为A组、B组、C组、D组和E组，每组10 只，A 组为空白对照组，不接受任何干预；B组、C组、D组和E组采用单次腹腔注射2%的链脲佐菌素(STZ)并配合8星期高糖高脂饮食建立DGP大鼠模型。B组为模型组，

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不接受治疗; C组为电针穴位组, 采用电针足三里、梁门和三阴交治疗; D组为电针非穴位组, 采用电针足三里、梁门及三阴交的对照点治疗; 胃复安对照组大鼠接受1.7%胃复安药液[10 mL/(kg•bw)]灌胃。用血糖仪检测大鼠血糖值; 以酚红为标记物, 检测大鼠胃排空率, BL-420F生物机能实验系统检测胃电, 酶联免疫吸附测定法(ELISA)检测胃窦ghrelin含量, 实时荧光定量聚合酶链反应(RT-PCR)检测ghrelin mRNA的表达。**结果**: 与A组比较, 治疗前和治疗后B、C、D和E组的血糖均显著增高(均P＜0.01); 治疗后B组大鼠胃排空率显著降低(P＜0.01), 治疗后B、C、D和E组小肠推进率显著降低(均P＜0.01), 治疗后C组胃窦ghrelin含量显著降低(P＜0.01), 治疗后B、C、D和E组胃窦ghrelin mRNA表达显著升高(均P＜0.01), 治疗后B组和D组胃电平均振幅显著降低(均P＜0.01); 与B组比较, C组治疗后血糖明显下降(P＜0.05), 治疗后C、E组胃排空率及小肠推进率明显升高(P＜0.05, P＜0.01), D组小肠推进率明显升高(P＜0.05), 治疗后C组胃窦ghrelin含量及mRNA表达显著降低(P＜0.01), E组ghrelin mRNA表达显著降低(P＜0.01), 治疗后C组胃电平均振幅明显增加(P＜0.05); 与D组比较, 治疗后C组胃窦ghrelin含量及mRNA表达显著降低(P＜0.01); 与E组比较, 治疗后C组胃窦ghrelin含量显著降低(P＜0.01)。**结论**: 电针足三里、梁门及三阴交可以调节DGP模型大鼠的血糖水平, 增强胃电活动, 促进胃排空, 调节胃窦ghrelin水平及下调ghrelin mRNA的表达。

【关键词】针刺疗法; 电针; 穴位特异性; 选穴; 糖尿病并发症; 胃轻瘫; 大鼠

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Diabetic gastroparesis (DGP) is a common complication of diabetes mellitus, mainly manifested as delayed gastric emptying without mechanical obstruction. The main symptoms are nausea, vomiting, postprandial fullness and abdominal pain[1]. Studies have shown that gastric dysmotility is closely related to gastric rhythm disorders. The effective mechanical contraction cannot be produced when the slow wave in electrogastrogram appears rhythm disturbance, which will lead to significantly decreased gastric antrum motility index and contractility, delayed gastric emptying, and even disappeared gastric motility[2]. Recent studies have suggested that the gastrointestinal tract can secrete a variety of peptides, which play important roles in the regulation of gastrointestinal motility, such as ghrelin[3], motilin (MTL)[4-5], chorcystokinin (CCK), gastrin (GAS), somatostatin (SS)[6-7], glucagon-like peptide-1 (GLP-1)[8-9] and substance P[10]. All these substances show effects on gastric emptying. Our previous studies[11-13] showed that electro-acupuncture (EA) could promote gastric emptying in DGP rats, regulate blood glucose levels, increase serum insulin (INS) and growth hormone (GH) levels, reduce gastric antrum CCK levels, upregulate ghrelin mRNA and growth hormone secretagogue receptor (GHSR) mRNA expressions. This study aimed to observe the effect of EA on electrogastrogram and gastric antrum ghrelin in DGP model rats, and to explore the therapeutic effect and possible mechanism of EA for DGP.

1 Materials and Methods

1.1 Experimental animals

A total of 50 adult Sprague-Dawley (SD) rats (SPF grade) include half male and half female, weighing 200-220 g. Animal quality certificate number: 43004700021526. Rats were fed at 20-24 ℃, relative humidity of 50%-70%, with natural light and dark cycle. During the experiment, operation and disposal of animals were strictly complied with the relevant provisions of the *Guiding Directives for Humane Treatment of Laboratory Animals* issued by the Ministry of Science and Technology of the People's Republic of China in 2006.

1.2 Main reagents and instruments

Streptozotocin (STZ, lot number: S0130-1G, Sigma, USA); metoclopramide (batch number: H14020782, Shanxi Yunpeng Pharmaceutical Co., Ltd., China); rat ghrelin ELISA assay kit (Nanjing Jiancheng Bioengineering Institute, China); TRIzol (Invitrogen, USA); citric acid, sodium citrate, trichloroacetic acid and sodium hydroxide (Sinopharm Group Chemical Reagent Co., Ltd., China); phenol red (Tianjin Guangfu Fine Chemical Research Institute, China); stable and easy blood glucose meter and blood glucose test paper (Johnson & Johnson, USA); Hwato Brand SDZ-V model EA instrument and acupuncture needles (0.30 mm in diameter and 13 mm in length, Suzhou Medical Products Co., Ltd., China); UV-2450 UV spectrophotometer (Shimadzu, Japan); DG5033A microplate reader (Nanjing East China Electronics Group Medical Equipment Co., Ltd., China); fluorescence Quantitative PCR Cycler (Da An Gene Co., Ltd. of Sun Yat-Sen University, China); BL-420F biological function experimental system (Chengdu Tai Meng Technology Co., Ltd., China).

1.3 DGP modeling and grouping

According to the random number table, the rats were randomly divided into a blank control group (group A, n＝10) and a DGP model group (n＝40). DGP model was established according to the literature[14]: briefly, rats were fasted for 12 h with water deprivation for 2 h. Single dose of 2% STZ solution, which was prepared with 0.1 mmol/L (pH 4.2, 4 ℃) citric acid-sodium citrate buffer before use, was injected into the left lower abdomen [55 mg/(kg·bw)]. Rats in the blank control group were injected intraperitoneally with equal volume of citric acid-sodium citrate buffer. Blood glucose was tested in the tail blood 72 h later, and rats with immediate blood glucose ≥ 16.7 mmol/L were used as diabetic models. Rats in the blank control group

were regularly fed with ordinary diet; in the DGP model group were fed with irregular (morning in odd days and afternoon in even days) high-glucose and high-fat diet. During the 8-week feeding, rats with blood glucose ＜16.7 mmol/L were excluded from the following experiments. Markers of successful DGP models: blood glucose ≥16.7 mmol/L, gastric emptying rate and/or electrogastrogram amplitude were significantly different compared with those in the blank control group. DGP rats were randomly divided into a DGP model group (group B), an EA at acupoint group (group C), an EA at non-acupoint group (group D) and a metoclopramide group (group E), 10 rats in each group.

1.4 Intervention methods in each group

1.4.1 Acupoint locations

Acupoint locations were determined by *Experimental Acupuncture Science*[15].

Zusanli (ST 36): Posterior and lateral to the knee joint, approximately 5 mm inferior to the small head of the fibula.

Sanyinjiao (SP 6): 10 mm directly above the tip of the medial malleolus.

Liangmen (ST 21): The junction between the upper 3/4 and lower 1/4 of the line connecting the upper border of the manubrium and pubis is regarded as the umbilicus. This point is located at the junction between the midclavicular line and the line connecting the xiphisternal joint and umbilicus.

Positions of non-acupoint control points were referred to the above 3 points. Control point of Zusanli (ST 36): 5 mm lateral to Zusanli (ST 36); control point of Liangmen (ST 21): 5 mm lateral to Liangmen (ST 21); control point of Sanyinjiao (SP 6): 5 mm interior to Sanyinjiao (SP 6).

1.4.2 Treatment for each group

Rats in group A and group B received binding for 20 min and intragastric administration of saline [1 mL/(kg·bw)], once a day for 15 d.

Rats in group C received acupuncture at unilateral Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6) by a depth of 3 mm. Then points from 2 mm vertically below Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6) along the meridian were respectively selected as the control points, by an acupuncture depth of 2 mm. After needling, the 3-group output leads of the Hwato Brand SDZ-V-model EA treatment instrument were connected to the needles at the 3 acupoints and their control points, with cathode to acupoints and anode to the control points, using alternate sparse wave and dense wave (10 Hz/50 Hz), with an intensity of 1 mA. EA was conducted for 20 min each time, once a day, with alternate use of points on both sides for a continuous treatment of 15 d, and intragastric administration of saline after acupuncture.

Rats in group D accepted acupuncture at unilateral control points of Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6). Other procedures were same as those used in group C.

1.5 Testing items and methods

1.5.1 Blood glucose

Blood was collected by cutting the rat tails, and the blood glucose level was measured with a stable and easy blood glucose meter and blood glucose test papers.

1.5.2 Gastric emptying rate[16]

Rats were fasted for 24 h with water deprivation for 2 h. The experimental rats were treated with intragastric administration of 2 mL (500 mg/L) phenol red solution, and sacrificed 20 min later.

Rat belly was opened; the cardia and pylorus were ligated; the whole rat stomach was isolated and cut along the curvatura gastrica major, then washed with saline to collect stomach contents with a total volume of 20 mL; 20 mL 0.5 mol/L NaOH was added into 20 mL of the collected stomach contents and stirred for 1 h. 5 mL supernatant was collected after standing for 1 h, mixed with 0.5 mL of 20% trichloroacetic acid for deproteinization, and then centrifuged at 3500 r/min for 10 min. The optical density (OD) of the supernatant was measured with an ultraviolet spectrophotometer at a wavelength of 560 nm. 18 mL of distilled water, 20 mL of 0.5 mol/L NaOH, and 4 mL of 20% trichloroacetic acid were added into 2 mL of phenol red solution and mixed by stirring, and then the OD value was used as the standard OD value of phenol red. Rat's gastric emptying rate ＝ (1 － Measured OD value of phenol red ÷ Standard OD value of phenol red) × 100%.

1.5.3 Migration rates of rats' small intestines[12]

The isolated small intestines were straightly put on the ice and cut a small mouth with the eye scissors at the red end after macroscopic observation; a small volume of 0.5 mmol/L NaOH solution was dropped on the small mouth, which would change into purple if this site had phenol red; after that, small volume of NaOH solution was further dropped on small intestine, in the front and behind the purple area, to determine the actual position of phenol red arriving.

Migration rate of small intestines ＝ [Distance of small intestine end stained with phenol red to the end of the pylorus ÷ Total length of the small intestine (i.e. the distance from the pylorus to the ileocecal valve)] × 100%.

1.5.4 Electrogastrogram

Rats were fasted for 24 h with water deprivation for 2 h before experiment. Rats were anesthetized and fixed on the rat plate with a supine position. Removed the upper abdominal hair, and defatted the skin with

75% alcohol. Cut the abdomen along the median line of the upper abdomen. Three electrodes of Ag/AgCl were respectively horizontally inserted into (about 1 cm) the gastric placenta percreta. The junction between gastric antrum and gastric corpus was connected to the positive pole; about 0.5 cm left to the gastric corpus was connected to the negative pole; and about 1 cm right was connected to the relevant electrode. The electrogastrogram was recorded by BL-420F biological function system.

Experimental parameters: Gain (G)＝1 mV, time constant (T)＝1 s, filter (F)＝1 Hz, recording speed ＝5 s/div, recording time＝30 min. The amplitude and frequency of the continuous 5 waves were observed for 10 min when the electrogastrogram signal was stable, and the mean value was analyzed.

1.5.5 Ghrelin protein level

The ghrelin protein level in gastric antrum tissues was detected by ELISA. The OD value was measured strictly according to the requirements of the kit.

1.5.6 Ghrelin mRNA expression in gastric antrum

Ghrelin mRNA expression in gastric antrum tissues was detected by real-time polymerase chain reaction (RT-PCR).

RNA extraction: Total RNA was extracted from gastric antrum tissues, using TRIzol kit according to the instruction. RNA samples were diluted with 1 × TE Buffer to 100 times or appropriate multiple, and the absorbances at 260 nm and 280 nm were measured to determine the RNA quality. RNA concentration ＝ OD260 × dilution multiple × 0.04 μg/μL. Cuvette diameter was 1 cm, and the measured OD260/280 was between 1.8 to 2.1, indicating high RNA purity.

cDNA synthesis: cDNA was prepared using the Fermentas reverse transcription kit according to the instruction manual.

PCR amplification: GAPDH was used as the internal reference, and primers are showed in Table 1. The amplification conditions were as follows: pre-denaturation at 95 ℃ for 5 min, denaturation at 95 ℃ for 15 s, annealing/extension at 60 ℃ for 20 s, with a total of 40 cycles. After completion of amplification reactions, the dissolution curve of PCR product was established by the following: 95 ℃ for 10 s, 60 ℃ for 60 s, 95 ℃ for 15 s and slowly heating from 60 ℃ to 99 ℃.

Analysis results of relative quantitation $2^{-\Delta\Delta Ct}$: ΔCt ＝ ΔCt value of target gene in each sample Ct value of GAPDH in each sample. ΔΔCt ＝ ΔCt － Mean value of the Ct value in the blank control group.

Table 1. Primer sequences and amplification lengths

Primer	Primer sequences	Amplification lengths (bp)
GAPDH	F: 5'-TGTTGCCATCAACGACCCCTT-3' R: 5'- CTCCACGACATACTCAGCA-3'	202
Ghrelin	F: 5'-ACCTGGTGTCCTTTGTCCTC-3' R: 5'-TGCTGTCATGTGTTGATGCT-3'	193

1.6 Statistical methods

All data were analyzed by SPSS 17.0 version statistical software. Normal distribution test and homogeneity test for variance of the measurement data were determined first. Data with homogeneity of variance were presented as mean ± standard deviation ($\bar{x} \pm s$); one-way ANOVA was used to compare the differences between groups; the least significant difference (LSD) method was used for data with homogeneity of variance; Dunnett T2 method was used for data with heterogeneity of variance. Data with skewed distribution were presented by the mean ± quartile range (M±QR), and compared using the rank sum test. Paired *t*-test was used to analyze the intra-group difference. $P<0.05$ indicated a statistically significant difference.

2 Results

2.1 Comparison of blood glucose in rats of each group

Before treatment, blood glucose in group B, C, D and E were significantly higher versus that in group A (all $P<0.01$). Differences of blood glucose among group B, C, D and E were not statistically significant ($P>0.05$). After treatment, blood glucose levels in group B, C, D and E were still significantly higher versus that in group A (all $P<0.01$), indicating that the blood glucose was still at a high level (>16.7 mmol/L). After treatment, the blood glucose of group C was significantly decreased, and there was a significant difference between group B and group C ($P<0.05$). The results suggested that EA at Zusanli (ST 36), Liangmen (ST 21) and Sanyinjiao (SP 6) could reduce blood glucose in DGP rats (Table 2).

Table 2. Comparison of blood glucose levels among groups before and after treatment ($\bar{x}$ ±*s*, mol/L)

Group	*n*	Before-treatment	Post-treatment	*t*-value	*P*-value
A	10	5.29±0.50	5.61±1.24	0.749	0.473
B	10	25.53±5.23[1)]	25.72±3.33[1)]	0.092	0.929
C	10	25.37±5.90[1)]	22.62±4.09[1)2)]	–1.211	0.241
D	10	24.59±3.69[1)]	24.37±3.55[1)]	–0.139	0.892
E	10	24.05±3.89[1)]	23.21±3.16[1)]	–0.464	0.653
F-value		42.249	66.458		
P-value		0.000	0.000		

Note: Compared with group A, 1) $P<0.01$; compared with group B, 2) $P<0.05$

2.2 Comparison of rat gastric emptying rate and migration rate of small intestines among groups

Compared with group A, rat gastric emptying rate in group B was significantly lower ($P<0.01$); migration rates of small intestines in group B, C, D and E were also significantly lower than that in group A (all $P<0.01$). This indicated that, after modeling in rats, the gastric emptying was delayed, small intestine migration was slow, and gastric motility was weakened. Compared with group B, the gastric emptying rate and small intestine migration rate were significantly increased in group C and E ($P<0.05$, $P<0.01$). The small intestine migration rate in group D was higher than that in group B ($P<0.05$). These results suggested that EA could improve the gastric emptying delay, promote the small intestine migration and enhance the gastric motility of rats. The therapeutic efficacy of EA at acupoint group was better than EA at non-acupoint group and the metoclopramide group (Table 3).

Table 3. Comparison of rat gastric emptying rate and migration rate of small intestines after treatment among groups ($\bar{x}$ ±*s*, %)

Group	*n*	Gastric emptying rate	Migration rate of small intestines
A	10	70.14±15.50	66.71±12.38
B	10	47.10±14.46[1)]	37.53±7.25[1)]
C	10	64.63±18.28[3)]	52.65±9.84[1)2)]
D	10	58.65±15.64	48.84±6.41[1)3)]
E	10	63.00±12.15[3)]	52.41±11.09[1)2)]
F-value		3.179	11.669
P-value		0.022	0.000

Note: Compared with group A, 1) $P<0.01$; compared with group B, 2) $P<0.01$, 3) $P<0.05$

2.3 Comparison of frequency and amplitude of electrogastrogram among groups

There were no statistically significant differences in the average frequency of electrogastrogram among groups (all $P>0.05$). Compared with group A, the mean amplitudes of group B and group D were significantly decreased (all $P<0.01$). Compared with group B, the mean amplitude of group C was significantly increased ($P<0.05$). The above results suggested that EA at acupoints could increase the amplitude of electrogastrogram and enhance the contraction of gastric smooth muscle (Table 4).

Table 4. Comparison of frequency and amplitude of rat electrogastrogram after treatment among groups ($\bar{x}$ ±*s*)

Group	*n*	Average frequency (cpm)	Average amplitude (mv)
A	10	4.78±1.26	0.65±0.15
B	10	4.49±1.03	0.37±0.05[1)]
C	10	4.67±0.76	0.54±0.13[2)]
D	10	5.24±0.79	0.39±0.06[1)]
E	10	5.01±0.97	0.52±0.14
F-value		0.866	9.969
P-value		0.492	0.000

Note: Compared with group A, 1) $P<0.01$; compared with group B, 2) $P<0.05$

2.4 Comparison of ghrelin protein level and mRNA expression in rat gastric antrum among groups

Compared with group A, the ghrelin protein level in gastric antrum was significantly decreased, while the expression of ghrelin mRNA was significantly increased in group C (all $P<0.01$). The expressions of ghrelin mRNA in group B, D and E were higher than that in group A ($P<0.01$). Compared with group B, the expressions of ghrelin protein and mRNA in group C were significantly decreased ($P<0.01$), and the expression of ghrelin mRNA in group E was significantly lower ($P<0.01$); compared with group D, the ghrelin protein level and mRNA expression of rat gastric antrum in group C were significantly decreased (all $P<0.01$). Compared with group E, ghrelin protein level of rat gastric antrum in group C was significantly decreased ($P<0.01$). These results indicated that EA at acupoints could regulate the protein level of ghrelin in gastric

antrum, down-regulate ghrelin mRNA expression in the gastric antrum, thereby promoting gastric emptying and gastric motility (Table 5).

Table 5. Comparing expressions of ghrelin protein and mRNA in rat gastric antrum among groups (M ±QR)

Group	*n*	Ghrelin protein (pg/mL)	Ghrelin mRNA
A	10	1835.24±264.63	1.24±1.38
B	10	1879.33±261.13	6.78±3.23[1)]
C	10	1648.28±144.69[1)2)3)4)]	2.57±0.93[1)2)3)]
D	10	1776.15±79.88	5.44±3.26[1)]
E	10	1808.87±274.19	3.15±2.31[1)2)]
F-value		15.828	64.012
P-value		0.003	0.000

Note: Compared with group A, 1) $P<0.01$; compared with group B, 2) $P<0.01$; compared with group D, 3) $P<0.01$; compared with group E, 4) $P<0.01$

3 Discussion

According to the International Diabetes Federation (IDF), the global prevalence of diabetes mellitus (DM) in 2000 was about 2.8%, and reached 8.3% by 2014[17]. There are 92.4-113.9 million adult DM patients in china, ranking first in the world[18]. DM has become the third most serious chronic non-infectious disease, after tumor and cardiovascular disease, to threaten the global human health. DGP is one of the most common chronic complications of diabetes, with the characteristics of delayed gastric emptying. Studies have shown that about 50% -76% of DM patients have gastric paralysis[19]. The pathogenesis of gastric emptying disorders due to diabetes mellitus is complex and has not yet been fully elucidated. It is currently believed that it may be related such factors as gastrointestinal hormonal disorders, hyperglycemia, impaired Cajal interstitial cells, gastrointestinal microangiopathy, autonomic neuropathy and Helicobacter pylori infection. The stomach is a complex electrochemical organ. Gastric motility is achieved by coordination of the electrical activity and mechanical activity, periodically produced by the smooth muscles of each part, which is regulated by the intestinal nervous system and gastrointestinal hormones[20]. The electrical activity in the gastrointestinal tract determines the form of gastrointestinal smooth muscle movement. The amplitude and frequency of the electrogastrogram reflect the contraction intensity and peristalsis rhythm of the gastric smooth muscle. The basic status of gastric motility can be measured by observing the frequency and mean amplitude of electrogastrogram slow wave. Decrease in the amplitude of the electrogastrogram can cause a delayed gastric emptying[21]. It can be seen that normal gastric emptying requires normal slow wave activity of electrogastrogram, gastric electromyography and mechanical contraction coupling, as well as coordinated movement of normal gastric antrum, pylorus and duodenum. The direct power of gastric emptying is the pressure difference between stomach and duodenum, and the prime mover is the contraction of gastric smooth muscle[22]. Disorder of slow wave rhythm of electrogastrogram cannot produce effective mechanical contraction. This will cause decreased gastric motility and gastric emptying. Studies have shown that disorder of electrogastrogram rhythm in DGP patients is closely related to the symptoms during eating. After treatment, the symptoms of gastroparesis were alleviated with the improvement of gastric rhythm disorder[22].

Ghrelin is an endogenous ligand for growth hormone secretagogue receptor[23], and a brain-gut peptide composed by 28 amino acid residues, mainly synthesized and secreted into blood by the X/A cells from mucosal oxyntic glands in the gastric fundus. Octanoylation of the third serine is its main active form, free to pass through the blood-brain barrier. Ghrelin is widely distributed in a variety of tissues and organs, including gastrointestinal tract, heart, pancreas, kidney, pituitary and hypothalamus, highest in the gastric tissues, accounting for 20% of total amount in the body[24]. Ghrelin can regulate growth hormone secretion, energy metabolism, ingestion, gastrointestinal function, endocrine and other biological functions[25-26]. The expressions of ghrelin, both protein and mRNA in plasma and stomach may be affected by diabetic duration, hyperglycemia, insulin, insulin-like growth factor-1 (IGF-1) and other gastrointestinal hormones. Literature[27] showed that plasma ghrelin level was increased significantly during early DM, which could cause over-eating and increased gastrointestinal motility; during late DM, plasma ghrelin level was decreased, which may be associated with anorexia, gastric emptying delay and even gastroparesis. A study showed that the expression and release of ghrelin in gastric mucosa of DGP patients, were mainly related to blood glucose level[28]. Short-term elevated blood glucose may be involved in gastric emptying delay, by inhibiting ghrelin mRNA expression in gastric tissues. Long-term hyperglycemia may increase ingestion and maintain energy balance, by promoting ghrelin mRNA expression and release. Increased blood glucose level and gastric emptying delay have a certain relationship. Blood glucose increase can delay the gastric emptying rate; gastric emptying delay in turn causes poor blood glucose control. It has been reported that there is a significant negative correlation between ghrelin and insulin. Insulin inhibits the synthesis of ghrelin[29]. A related study showed that in the DM rats, STZ induced increase of serum ghrelin protein level, decease of ghrelin mRNA level in gastric tissues, significantly

decreased ghrelin level and number of ghrelin immunocompetent cells in gastric fundus[30].

Our results showed that after modeling, the blood glucose was significantly higher, the gastric emptying rate and small intestine migration rate were significantly decreased, the mean amplitude of electrogastrogram was decreased, the expressions of both ghrelin protein and mRNA in gastric antrum were increased significantly in group B. This indicated that paralysis may be related to gastric antrum ghrelin both in protein and mRNA; and blood glucose level may also have a certain impact on gastric motility. After intervention treatment, the gastric emptying rate in group C and group E, and the small intestine migration rate in group C, D and E were increased significantly; the mean amplitude of electrogastrogram was increased and the protein level of ghrelin was decreased in group C; the expressions of ghrelin mRNA in gastric antrum of group C and E were significantly decreased.

These results suggested that EA could enhance the contraction of gastric smooth muscle in DGP rats, increase gastric emptying and promote small intestine migration. Treatment using EA at acupoints was superior to metoclopramide and EA at non-acupoint therapy, indicating that EA has a unique effect on DGP and showing the specificity of acupoints. This may be related to the regulation of blood glucose levels and gastric anthum ghrelin levels, and downregulation of ghrelin mRNA expression. Gastric antrum ghrelin level may exist a certain internal connection with electrogastrogram and gastric motility, however, the specifically involved pathway still needs to be further explored.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

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