EVALUATION OF THE INFLUENCE OF ACUPOINTS SELECTION ON GHRELIN AND GHSR EXPRESSION IN GASTRIC FUNDUS TISSUE OF DGP RATS

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ABSTRACT

Objective: *To investigate the influence of acupoint selection on ghrelin and GHSR expressions in the gastric fundus of rats with diabetic gastroparesis (DGP).*

Methods: *Sixty male SPF SD rats were given adaptive feeding for one week and were randomly divided into a blank control group, a model group, Zusanli and Zhongwan (ZZ) group, Zusanli and Neiguan group (ZN), as well as Zusanli and non-meridian acupoint (ZNA) group, with 12 rats in each group. Except for the blank group, the other 48 rats were used to establish a DGP model by using streptozotocin. Acupuncture therapy was performed from the end of the tenth week for an additional four weeks. Then the rats were sacrificed and the sera and gastric fundus tissues were collected. Serum ghrelin content was assayed with ELISA kits. The expression of ghrelin positive cells in the gastric fundus tissue was determined by immunohistochemistry, while the expression of ghrelin and GHSR mRNA in the gastric fundus tissue was determined by real-time polymerase chain reaction (RT-PCR).*

Results: *Compared with the control group, the feed intake and the expression of ghrelin and GHSR mRNA in the gastric fundus tissue in the model group was significantly increased, while small intestinal propulsion rate, serum ghrelin content, and the gray value of the gastric fundus were significantly decreased ($p < 0.05$). Compared with the model group, the expression of ghrelin and GHSR mRNA in the gastric fundus tissue, small intestinal propulsion rate, serum ghrelin content, and gray value of the gastric fundus were significantly increased ($p < 0.05$). Feed intake was significantly higher in the ZN and the ZNA groups ($p < 0.05$), and the expressions of ghrelin and GHSR mRNA in the gastric fundus tissue were significantly increased ($p < 0.05$) but the serum ghrelin content in ZN group was significantly decreased ($p < 0.05$).*

Conclusions: *These results suggest that improvement of gastrointestinal motility in DGP by acupuncture therapy is associated with serum ghrelin content and GHSR expression in the gastric fundus. They also indicate that the effect of treatment with local acupoints compatibility is better than that of distal acupoints compatibility, and that acupoint selection according to location or outcome is a key factor that influences acupoints compatibility.*

Keywords: *Acupoints compatibility, Acupoints selection, Diabetic gastroparesis, Acupuncture therapy.*

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Introduction

Acupoints compatibility is a method of choosing two or more acupoints with the same effect to exert a synergistic effect, achieve a certain therapeutic effect, and improve clinical efficacy. This is based on traditional Chinese meridian theory, guided by the acupoint selection principle and combined with clinical and acupoints characteristics. However, there are three possibilities for acupoints compatibility of two or more acupoints. These are efficiency, inefficiency, and invalidity. Therefore, the appropriateness of acupoints compatibility is a key factor that affects clinical efficacy. Thus, mastering the factors that influence acupoints compatibility is an important part of treatment[1-3]. In this study, acupoint selection according to location was considered as one of the influencing factors of acupoints compatibility and was held as the hypothesis, with diabetic gastroparesis (DGP) as the disease model, and the difference between acupoints selection according to the meridian and acupoints selection according to the location were compared. The aim was to ascertain if acupoint selection according to location influences acupoints compatibility.

Diabetic gastroparesis (DGP), the most common chronic complications of diabetes, refers to diabetic patients with clinical syndromes characterized by low gastric motility such as anorexia, early satiety, nausea, and vomiting(4). At present, the pathogenesis of DGP has not been fully understood, although it is believed to be caused by a combination of multiple factors, and is associated with abnormal secretion of gastrointestinal hormone (intestinal peptide or brain gut peptide)(5, 6), decreased number of pacemaker cells(7), autonomic neuropathy(8), or hyperglycemia(9). Currently, ghrelin and growth hormone secretagogue receptor (GHSR) have been the molecular entities targeted in studies on the pathogenesis of DGP. The main functions of ghrelin are stimulation of growth hormone secretion and regulation of energy balance

In addition, loss of weight caused by low-medium caloric diet, exercise, anorexia nervosa, organ (heart, lung, kidney and liver) failure, or malignant tumor cachexia could lead to increased level of ghrelin(10, 11). It has been suggested that in addition to being a short-term signal for regulation of food intake, ghrelin is also a long-term signal for nutritional status and it promotes gastric acid secretion and gastrointestinal motility and protects the digestive mucosa(12). Besides, ghrelin plays a role in gastrointestinal motility, gastrointestinal tract digestive enzyme secretion, and intestinal cell proliferation(13). Therefore, this study was focused on the effect of acupoint selection according to location on the expression of serum ghrelin and gastric fundus tissue of diabetic rats with DGP.

Materials and methods

Animals and grouping

Sixty SPF adult male Sprague Dawley (SD) rats weighing 200-220 g were provided by Hunan SJA Laboratory Animal Co., Ltd. (Certificate No.: SYXK2013 (Hunan) -0005). Rats with normal blood glucose were selected. They were maintained in the SPF laboratory animal room of experimental animal center in Hunan University of Chinese Medicine, at a temperature of 22-25 oC, and humidity of 40 - 60 %. After adaptive feeding for 1 week, the rats were randomly divided into a blank control group, a model group, Zusanli and Zhongwan (ZZ) group, Zusanli and Neiguan (ZN) group, as well as Zusanli and non-meridian acupoint (ZNA) group, with 12 rats in each group. Ethical approval for the study was given by the medical ethics committee of Hunan University of Chinese Medicine (approval number: 2016004). During the experiment, the treatment of the animals and disposal of their carcass complied with the guidelines and regulations for handling experimental animals promulgated by the Ministry of Science and Technology.

Reagents and instruments

The reagents and instruments used, and their sources were: streptozotocin (STZ) (Sigma, Cat.No.015H1492, U.S.A.); Trizol kit (Invitrogen, U.S.A.); blood glucose meter and blood sugar test paper (OneTouch SelectSimple, Johnson & Johnson, U.S.A.); Acupuncture needle (0.30 mm×25 mm, Hua Tuo brand, Suzhou),Violet spectrophotometer (UV-1800, Shimazduo, Japan) ; Electrophoresis apparatus (DDY-5, Beijing Liuyi Instrument Factory); Gel Document System (Bio-Red, U.S.A.); Centrifuge (Thermo Legend Micro 17 R, U.S.A.); PCR Instrument for Fluorescence Detection (Applied Biosystems, 7900 HT Fast, U.S.A.); Rabbit anti-mouse ghrelin antibody (1:2500) and Rabbit anti-human GHS-R antibody (1:100), and primary and secondary antibodies (Phoenix Biological Technology Co., Ltd.).

Modeling methods and evaluation

All the study rats were fasted for 12 h, and STZ was diluted in citric acid/ sodium citrate buffer (pH 4.5, 4 oC) to a concentration of 2 % before use and then administered once by injection in the left lower abdominal cavity at a dose of 60 mmol/kg body weight. Random blood glucose was determined 72 h after the injection. Rats with random blood glucose level ≤16.7 mmol/L were excluded. The rats in the blank control group were injected with an equivalent volume of 0.1 mmol/L citric acid/ sodium citrate buffer. The rats were fed at 10:00 am daily. During the 13 weeks of the experiment, rats with random blood glucose level ≤16.7mmol/L were removed from the experiment.

Inclusion criteria of rats in the DGP model were as follows(14): blood glucose level must be ≥16.7mmol/L; the general conditions and stool characteristics of the rats should be significantly different from those of the blank group; the rate of gastric emptying and small intestinal propulsion rate in rats should also to be significantly different from those of the blank group.

Intervention methods

The location of acupoints: according to the Atlas of animal acupuncture and moxibustion[15], the acupoints of the rats were located as follows: Zhongwan: located in the linea alba, about 20 mm above the umbilicus; Zusanli: located in the underside of the knee joint, 5 mm away from the margo inferior of fibulae capitulum, with one on each side; Neiguan: located in the gap between the ulna and radius in the inside of the forelimbs, about 3 mm away from the wrist joint of the rat; and non-meridian acupoint: located in the medial elbow, midpoint of the connecting line between elbow and axillary.

In the blank and model groups, the rats were fastened to the rat board, 30 min each time, and once a day for 4 weeks. In the ZZ, ZN, and the ZNA groups: the corresponding points on both sides of the rats were selected and acupunctured to the depth of 0.3-0.5 cm following the conventional needle-insertion method with the needle retained for 30 min; the hand-manipulation of the needle (lifting and thrusting, twirling, mild reinforcing, and attenuating) was performed every 10 min. The range of lifting and thrusting was 0.3-0.5 cm, and the frequency was 60-90 times/min. The range and frequency of twirling were equal to those of lifting and thrusting. The needle was withdrawn 30 min at the end of the hand-manipulation procedure. This experiment was performed for 4 weeks.

Observation indices and detection methods

Feed intake: 300 g ordinary feed were provided to the rats at 10:00 am daily. On the next day, the weight of the remaining feed was recorded before fresh feeding. Daily feed intake in each cage of the rats was calculated by difference.

Blood glucose measurement: Blood samples were collected from the vena caudalis per week for determination of blood glucose using blood glucose meter and blood glucose test paper.

Determination of small intestine propulsion rate: Rats were fasted for 24 h after the last injection. Intragastric administration of black ink was performed in each group of the rats at a dose of 1mL/l00 g body weight. Then the rats were sacrificed and the abdominal cavities were opened 20 min after the intragastric administration. The small intestine (from the pylorus to the ileocecal bowel) was gently extracted with tweezers and placed on a tray.

The small intestine was gently pulled into a straight line and the gastrointestinal propulsion index was measured with ruler. The gastrointestinal propulsion index was calculated by the following formula: Small intestinal propulsion rate = the distance from the front of ink mark to the front end of the range of the x 100% (cm) /h sphincter to the small intestine end distance (cm).

ELISA: Four milliliters of blood from the rat heart was collected after anesthesia and placed in the centrifuge tube without anticoagulant (for determination of serum ghrelin). Whole blood samples were placed at 4 °C overnight and then centrifuged at 1000 revolutions/min for 20 min. The supernatants were separated and stored at -20 °C or -80 °C; repeated freezing and thawing was avoided. The ELISA was performed strictly according to the kit instructions.

Immunohistochemistry: The gastric tissues were excised and cut into 4 mm pieces, then, post-fixed in paraformaldehyde buffer for 8 h and washed with tap water, followed by 20% to 30% sucrose gradient, dehydrated, embedded and thereafter frozen in liquid nitrogen. Then, 10 μm serial sections were prepared by using the cryostat freezing microtome, and placed on slides with poly-lysine. The immunohistochemical staining of ghrelin and GHS-R was performed by using the SABC method. Rabbit anti-mouse ghrelin antibody (1:2500) and rabbit anti-human GHS-R antibody (1:100) were used as primary antibodies. The expression of ghrelin and GHS-R were observed by means of microscope under high magnification after DAB development and neutral gum sealing. Ten fields of view were selected in each slice under high magnification (400×), and the average value was considered as the average gray value of the slice.

Real-time fluorescent quantitative PCR for determination of ghrelin and GHSR mRNA expression levels in the gastric fundus: The tissues of the gastric fundus were collected and stored at -80 O C for subsequent biochemical determinations. Total RNA of the rat gastric fundus was extracted by Trizol extraction kit according to the manufacturer's instructions and analyzed using 1.5 % agarose gel electrophoresis. The concentration and purity of extracted RNA were calculated by measuring the OD 260 and OD 280. The concentration of RNA as determined in this study was above 1.5μg/μL, and the purity was between 1.8 and 2.1, which indicated that the quality of the extracted RNA was acceptably high. Reverse transcription was used to synthesize cDNA by using SuperScript III reverse transcription reagent kit. PCR amplification was performed using β-actin as internal control and the primers used are shown in Table 1. The amplification conditions were as follows: 10 min initial denaturation at 95 °C, 40 cycles each of 10 s; denaturation at 95 °C, 60 s annealing/extension at 60 °C (fluorescence collection). After the amplification reaction, the conditions were used for establishing the dissolution curve of PCR products: 10 s at 95 °C, 60 s at 60 °C, 15 s at 95 °C, then slow heating from 60 °C to 99 °C. Relative expression level was quantified by using the relative quantitative $2^{-\Delta\Delta Ct}$ method:

$\Delta Ct = Ct_{simple\ target\ gene} - Ct_{simple\ \beta\text{-}actin\ gene}$, $\Delta\Delta Ct = \Delta Ct$ - averaged $Ct_{blank\ control\ group}$.

Primer	Primer sequences	Length of amplified products (bp)
β-actin	F: 5'-CCTGTACGC-CAACACAGTG-3'	211
	R: 5'-ATACTCCTGCTTGCTGATC-3'	
Ghrelin	F: 5'-ATCCAA-GAAGCCACCAGC-TA-3'	121
	R: 5'-GAAGGGAG-CATTGAACCTGA-3'	
GHSR	F: 5'-CTCTA-CACCCGAAGCCG-TAG-3'	118
	R: 5'-CTGCC-CATCTGGCTC-TACTC-3'	

Table 1: Primer sequences and the length of amplified products.

Statistical analysis

SPSS 17.0 statistical software was used for data analysis. Data following normal distribution were represented as $\bar{x}$±s. If the data followed normal distribution and the variance was homogeneous, one way ANOVA and LSD test were used for multi-group comparison and the post-hoc test, respectively. Tamhane T^2 test was used if there was heterogeneity of variance. Data with skewed distribution were represented as median (M) and interquartile range (QR), and the rank-sum test was used for comparison among groups. A value of < 0.05 was considered significant.

Results

Comparison of general signs in the study rats

During the experiment, the rats in the blank group showed good mental state, normal locomotor behavior, sensitive response, healthy eating, milky white and glossy fur, and normal cacation and emiction. Polydipsia, polyphagia, and polyuria appeared in the rats of the ZZ, ZN, and the ZNA groups three days after STZ injection. The DGP rats showed poor mental state[16], slow behavioral activity, slow growth of body mass and even reduced body mass from the third week; unpolished fur, changed stool characteristics, unpleasant odor, and abdominal swelling in the sixth week. Mortality status: one rat in the blank group, three rats in the model group, two rats each in in the ZZ and the ZN group, and three rats in the ZN group, died.

The results presented in Table 2 revealed that compared with the blank group, the feed intake was significantly increased in the model group ($p < 0.05$). Feed intake was significantly increased in the ZNA group (< 0.05) but not in the ZZ or the ZN groups ($p > 0.05$). Relative to the ZZ group, the feed intake was significantly increased in the ZN and ZNA groups ($p < 0.05$). These results showed that local acupoints compatibility was capable of reducing feed intake of DGP rats, while distal acupoints compatibility showed no significant changes in feed intake. The non-meridian acupoint also increased feed intake of DGP rats.

Comparison of the effect of acupoint selection according to location on food intake in rats

Group	n	Food intake (g/day)
Blank	11	104.73±13.57
Model	9	230.86±19.10*
Zusanli and Zhongwan	10	213.65±29.58
Zusanli and Neiguan	10	240.04±15.99▲
Zusanli and non-meridian acupoint	9	250.41±20.89△▲

Table 2: Comparison of the effect of acupoint selection according to location on food intake in rats ($\bar{x}$±s).
**p < 0.05, value significantly different compared with blank group;< 0.05 ; ▲p < 0.05, value significantly different compared with model group, △p < 0.05, value significantly different compared with Zusanli and Zhongwan groups.*

Comparison of the effect of acupoint selection according to location, on small intestinal propulsion rate in rats

The results on Table 3 reveal that compared with the blank group, the small intestinal propulsion rate was significantly decreased in the model group ($p < 0.05$), which is evidence of successful preparation of the DGP disease model. Small intestinal propulsion rate was significantly increased in the ZZ, ZN, and the ZNA groups when compared with the model group ($p < 0.05$). However, there was no significant difference in small intestinal propulsion rate among the three treatment groups ($p > 0.05$). These results indicate that the small intestinal propulsion rate of DGP rats were improved in the three treatment groups; the effect of local acupoints compatibility was better than that of distal acupoints compatibility, and the effect of the latter was better than that of non-meridian acupoint.

Group	n	Propulsion rate
Blank	11	0.73±0.05
Model	9	$0.60 \pm 0.05^{*}$
Zusanli and Zhongwan	10	$0.73 \pm 0.05^{\triangle}$
Zusanli and Neiguan	10	$0.70 \pm 0.06^{\triangle}$
Zusanli and non-meridian acupoint	9	$0.69 \pm 0.05^{\triangle}$

Table 3: Comparison of the effect of acupoint selection according to location on small intestinal propulsion rate in rats ($\bar{x}$±s).

*Note: compared with blank group, *P < 0.05; compared with model group, △P < 0.05.*

Comparison of the effect of acupoint selection according to location on ghrelin content in the serum of rats

Group	n	Concentration (ng/ml)
Blank	11	2380.79±176.02
Model	9	$1795.74 \pm 65.48^{*}$
Zusanli and Zhongwan	10	2019.51±210.72△
Zusanli and Neiguan	10	1836.65±170.67▲
Zusanli and non-meridian acupoint	9	$1978.10 \pm 147.81^{\triangle}$

Table 4: Comparison of the effect of acupoint selection according to location on serum ghrelin content of rats ($\bar{x}$ ±s).

*p < 0.05, compared with blank group; $^{\triangle}$p < 0.05, compared with model group; $^{\blacktriangle}$P < 0.05, compared with Zusanli and Zhongwan groups,

The results shown in Table 4 suggest that compared with the blank group, the ghrelin content in the serum was significantly decreased in the model group ($p < 0.05$), which is indicative the successful preparation of the rat model of DGP. Compared with the model group, the ghrelin content was significantly increased in the ZZ and the ZNA groups ($p < 0.05$) but not in the ZN group ($p > 0.05$). The ghrelin content was significantly decreased in the ZN group, relative to the ZZ group ($p < 0.05$). These results show that the effect of local acupoints compatibility and non-meridian acupoint were better than those of distal acupoints compatibility with respect to regulation of serum ghrelin content.

Comparison of the effect of acupoint selection according to location on gray value of ghrelin in the gastric fundus of the study rats

The immunohistochemical changes associated with ghrelin expression in the gastric fundus of rats in the various groups are evident in Figure 1.

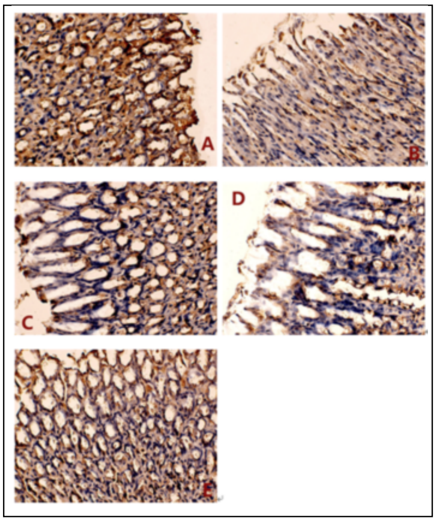

Fig 1: Immunohistochemical images of ghrelin expression in the gastric fundus. A: Blank group, B: Model group, C: *Zusanli* and *Zhongwan* group, D: *Zusanli* and group, E: *Zusanli* and non-meridian acupoint group

Group	N	Gray value
Blank	11	161.23±0.89
Model	9	$71.29 \pm 35.43^{*}$
Zusanli and Zhongwan	10	$158.40 \pm 15.55^{\triangle}$
Zusanli and Neiguan	10	$157.47 \pm 37.97^{\triangle}$
Zusanli and non-meridian acupoint	9	$129.43 \pm 18.56^{\triangle\blacktriangle\#}$

The results in Figure 1 and Table 5 show that, compared with the blank group, the gray value of ghrelin in the gastric fundus was significantly decreased in the model group ($p < 0.05$); compared with the model group, the gray value of ghrelin was significantly increased in the ZZ, ZN, and the ZNA groups ($p < 0.05$); and that compared with the ZZ group, the gray value of ghrelin was significantly decreased in the ZNA group ($p < 0.05$), but not in the

ZN group ($p > 0.05$). These results reveal that the effects of the treatments on local and distal acupoints compatibility were better than the effects of the non-meridian acupoint.

Comparison of the effect of acupoint selection according to location on ghrelin and GHSR mRNA expression in the gastric fundus of the study rats

Results in Table 6 show that the expression of GHSR mRNA in the gastric fundus tissue was significantly increased in the model group, when co pared with the control group ($p < 0.05$); compared with the model group, both ghrelin and GHSR mRNA expressions in the gastric fundus tissue were significantly decreased in the ZZ and ZN groups, and the GHSR mRNA expression was also significantly decreased in the ZNA group ($p < 0.05$). Relative to the ZZ group, the expressions of ghrelin and GHSR mRNA in the gastric fundus tissue were significantly increased in the ZN group ($p < 0.05$); and compared with the ZN group, GHSR mRNA expression in the gastric fundus tissue was decreased significantly in the ZNA group ($p < 0.05$).

Discussion

In this study, acupoint selection according to location was hypothesized as one of the factors influencing acupoints compatibility, and the correctness of this hypothesis was evaluated by using DGP as the disease model. Acupoints selection according to meridian and local acupoints selection according to locations were the treatment groups. Distal distal acupoints selection according to the locations and non-meridian acupoint were used as controls for comparison. DGP-induced syndromes such as nausea, vomiting and abdominal distension belong to the category of traditional Chinese medicine that explores how conditions such as "vomit" and "nausea" can be managed.

In Traditional Chinese medicine the DGP syndrome is attributed to dysfunction in the ascending and descending movements of the spleen and stomach. If the spleen is unable to ascend and the stomach is unable to descend, it will lead to mental and visual confusion. A review of relevant clinical literature available in the last five years on acupuncture treatment of DGP revealed that the most frequently used acupoint was the Zusanli which belongs to the Yangming Stomach Meridian of Foot (the percentage of the total frequency was 15.53%). So Zusanli was chosen for the meridian.

Acupoint selection according to location includes two kinds of acupoints: the local and the distal acupoints. A total of 41 acupoints which are commonly used in the treatment of DGP were used in this study. They were divided into two groups. The most frequently used acupoint in each group was selected: Zhongwan (local) and Neiguan (distal). Therefore, a comparative study was performed in this research by using healthy SPF rats as the blank group, the DGP model rats as the model group, ZZ group as the treatment group, and the ZN and ZNA groups as the control groups. The treatment effect among local acupoints, distal acupoints and non-meridian acupoint were compared to guide the clinical work.

The main manifestations of patients with DGP were disappearance of MMCIII period, pylorospasm, uncoordinated contraction among sinuses ventriculi, pylorus and duodenum, and delayed gastric emptying. Therefore, gastrointestinal propulsion rate is commonly used as a standard to evaluate the DGP model. Intestinal propulsive rate was decreased significantly in the model group, but it was increased significantly in the ZZ, ZN, and the ZNA groups, which indicated that the therapy in the three treatment groups had a promoting effect on the gastrointestinal propulsion rate.

Ghrelin is a newly discovered endogenous ligand of the growth hormone secretagogue receptor (GHSR), which is secreted into the blood by the gastric fundus. Since this brain gut peptide was isolated in mice and human gastric endocrine cells and the hypothalamic arcuate nucleus, several studies have been carried out and the findings reveal that ghrelin plays an important role in a variety of tissues which border on immunity[17, 18]. A large number of studies[19, 20] showed that acupuncture and other traditional Chinese medicine treatments could improve gastrointestinal function by regulating the expression of ghrelin. The effects of acupoint selection according to location on the expression and levels of ghrelin and GHSR in serum and the gastric fundus of DGP rats suggest an effect of the local acupoint (Zhongwan) on regulation of feed intake, since serum ghrelin content, and ghrelin and GHSR mRNA expressions in the gastric fundus of DGP rats were better than those of the distal acupoint (Neiguan); and the effect of the local acupoint (Zhongwan) on regulation of small intestine propulsion rate and expression of ghrelin-positive cells in the gastric fundus were better than those of the distal acupoint (Neiguan).

This confirms that acupoint selection according to location is one of the factors influencing acupoints compatibility. In this study, spleen and stomach disease were used as the carrier, Zhongwan as the research object, and Neiguan and non-meridian acupoint as the controls in order to be able to reach this conclusion with reasonable degree of certitude. Whether the acupoint selection according to location has the same advantages in the treatment of the lesions in other organs or parts thereof are yet to be verified.

Studies have found that in the early stage of DM, a large amount of ghrelin is secreted into blood from the gastric fundus, which results in increased contraction frequency of the pylorus and the frequency of gastric emptying[21]. In the late stage of diabetes, plasma ghrelin is decreased, which might be associated with anorexia, muscle atrophy, decreased body mass, or even gastric paralysis. Clinical studies have shown that patients with DGP have decreased serum ghrelin content[22]. The results obtained in this study showed that the content of ghrelin in the sera of model group rats was lower than that in the blank group. These findings are consistent with the previous reports.

The results of this study showed that the protein content and gene expression of ghrelin and GHSR in the gastric fundus of the DGP model were higher than those of the blank group. A previous study showed that the protein content and gene expression of ghrelin and GHSR in the gastric fundus of DGP model were decreased, which is not consistent with the findings in the present study. For now, no generally accepted conclusion has been reached about the time taken for the establishment of the DGP model, so studies use different modeling methods. The time for the establishment of the DGP model varied in previous studies which mostly focused on the time from 4 weeks to 12 weeks[24-26].

Therefore, the increased expression of ghrelin in the model group in this study does not agree with extant information in the literature. This might be due to the differences in methods and time used for the establishment of the DGP model. In addition, as one of the gastrointestinal hormones, the level of ghrelin is completely consistent with gastric motility in the progress of the DGP model although such has not been reported yet.

Further experimental studies are needed to demonstrate and confirm this phenomenon.

Conclusion

The improvement of gastrointestinal motility in DGP by acupuncture therapy seems to be associated with the ghrelin content in the serum and GHSR expression in the gastric fundus. Local acupoints compatibility was better than that of the distal acupoints compatibility for acupoint selection. Furthermore, the results of this study confirm that acupoint selection according to location is an important factor that influences acupoints compatibility.

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