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**Basic Study**

# Effects of electroacupuncture of different frequencies on SP and VIP expression levels in colon of rats with slow transit constipation

## 不同频率电针对慢传输型便秘大鼠结肠SP、VIP水平的影响

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**Abstract**

**Objective:** To observe the effects of electroacupuncture (EA) of different frequencies on the expression levels of substance P (SP) and vasoactive intestinal peptide (VIP) in the colon of rats with slow transit constipation (STC).

**Methods:** One hundred healthy male Sprague-Dawley (SD) rats were randomly divided into a normal group, a model group, a low-frequency EA group, a high-frequency EA group and a variable-frequency EA group, with 20 rats in each group. The rats in the normal group were fed with normal diet. The rats in the other groups were fed with phenethylpiperidine in the diet at a dose of 8 mg/(kg·bw) per day, for 120 d, to establish the STC model. Rats in the normal group and the model group did not receive any treatment; rats in the low-frequency EA group were treated with 2 Hz continuous wave EA, rats in the high-frequency EA group were treated with 100 Hz continuous wave EA, and rats in the variable-frequency EA group were treated with 2 Hz/100 Hz sparse-dense EA. The current intensity of the EA was determined by the slight vibration of the rat limbs without painful screaming. The intervention was performed once a day, 15 min/time for continuous 15 d. After treatment, the intestinal transit function and the expression levels of SP and VIP in the colon of the rats in each group were determined.

**Results:** After treatment, the defecation duration of the first dark stool in the model group was significantly longer than that in the normal group ($P$<0.05); the defecation durations of the first dark stool in the low-frequency EA group, high-frequency EA group and variable-frequency EA group were significantly shorter than the duration in the model group (all $P$<0.05); compared with the low-frequency EA group, the first dark stool defecation duration of rats in the variable-frequency EA group was significantly shorter ($P$<0.05); compared with the normal group, the SP and VIP expression levels in the colon of the model group were significantly decreased (both $P$<0.01); the SP and VIP expression levels in the colon of the low-frequency EA group, the high-frequency EA group and the variable-frequency EA group were significantly higher than those in the model group (all $P$<0.05); compared with the high-frequency EA group, the SP expression levels in the colon in the low-frequency EA group and the variable-frequency EA group were significantly increased (both $P$<0.05); compared with the low-frequency EA group, the VIP expression levels in the colon in the high-frequency EA group and the variable-frequency EA group were significantly increased (both $P$<0.05).

**Conclusion:** EA improves the intestinal function of STC model rats by regulating the expression levels of SP and VIP in rat colon. The EA stimulation with 100 Hz continuous wave, 2 Hz/100 Hz sparse-dense wave shows a better improvement in the colonic transit function in STC rats, followed by 2 Hz continuous wave.

**Keywords:** Acupuncture Therapy; Electroacupuncture; Constipation; Substance P; Vasoactive Intestinal Peptide; Colon; Rats

**【摘要】目的**：观察不同频率电针对慢传输型便秘(STC)模型大鼠结肠P物质(SP)和血管活性肠肽(VIP)水平的影响。**方法**：将健康雄性Sprague-Dawley (SD)大鼠100只随机分为正常组、模型组、低频电针组、高频电针组和变频电针组，每组20只。正常组大鼠以普通饲料喂养，其余组大鼠在饲料中添加复方苯乙哌啶，每日8 mg/(kg·bw)，连续给药120 d，建立STC模型。正常组和模型组不进行任何治疗；低频电针组给予2 Hz连续波电针治疗，高频电针组给予100 Hz连续波电针治疗，变频电针组给予2 Hz/100 Hz疏密波电针治疗。电针电流强度以大鼠肢体微微颤动而不痛苦尖叫为度。每日1次，每次治疗15 min，共治疗15 d。治疗后，测定各组大鼠肠道传输功能和结肠中SP和VIP的含量水平。**结果**：治疗后，与正常组比较，模型组大鼠首粒黑便排出时间明显延长($P$＜0.05)；低频电针组、高电针组和变频电针组大鼠首粒黑便排出时间均较模型组明显缩短(均$P$＜0.05)；变频电针组大鼠首粒黑便排出时间

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较低频电针组明显缩短($P$＜0.05)。与正常组比较，模型组大鼠结肠SP和VIP含量明显降低(均$P$＜0.01)；低频电针组、高频电针组和变频电针组大鼠的结肠SP和VIP含量均较模型组明显升高(均$P$＜0.05)。与高频电针组比较，低频电针组和变频电针组大鼠的结肠SP含量明显升高(均$P$＜0.05)；与低频电针组比较，高频电针组和变频电针组大鼠的结肠VIP含量明显升高(均$P$＜0.05)。**结论**：电针疗法可通过调节大鼠结肠中SP和VIP的含量改善STC模型大鼠的肠道功能；100 Hz连续波和2 Hz/100 Hz疏密波电针刺激对改善STC大鼠的结肠传输功能相对较好，2 Hz连续波的作用次之。

**【关键词】**针刺疗法；电针；便秘；P 物质；血管活性肠肽；结肠；大鼠

**【中图分类号】**R2-03　　**【文献标志码】**A

With the aging population and the changes in modern life rhythms and dietary habits, constipation is becoming a common problem. Constipation not only reduces the quality of life in patients, but also brings negative emotions such as anxiety and restlessness[1]. Functional constipation (FC) does not have an organic cause, structural abnormalities or metabolic disorders, and excludes irritable bowel syndrome. Main symptoms of FC are difficulty in defecation, hard stools, incomplete sense of evacuation or reduced stools[2]. According to the characteristics of colon dynamics, FC can be divided into slow transit constipation (STC), functional outlet obstruction constipation (FOOC) and combination of STC and FOOC (CSOC). The intestinal contents are retained in the colon, or the colon passing is slow in STC due to the colonic motility transit disorder. Propulsion rate of the colonic contents was slowed due to the reduced colonic motility, resulting in delayed emptying. At the same time, STC may also be accompanied by other gastrointestinal dysfunction caused by abnormal autonomic dysfunction, such as delayed gastric emptying and intestinal dyskinesia[3]. Patients with STC are mainly characterized by decreased defecation frequency, a hard stool mass or no awareness of defecation. Its pathogenesis and etiology are still unclear. A large number of studies in recent years have shown that the pathogenesis of STC is related to the gastrointestinal regulatory peptides, such as colonic substance P (SP) and vasoactive intestinal peptide (VIP). Abnormal changes in the expression levels of these substances can affect intestinal movement, reduce intestinal excitement and colonic motility, leading to the occurrence of STC[4-6].

Chinese medicine therapies, such as acupuncture, warm needling moxibustion, electroacupuncture (EA) and acupoint application, have good curative effects in the treatment of constipation. In recent years, many studies on acupuncture treatment of constipation have emerged[7-9]. In clinical practice, EA treatment of STC has also achieved good results[10-12], but the selected frequencies of EA are different, and there is no common application standard and scientific comparative study. This study aimed to observe the effects of EA of different frequencies on colonic transit function and expression levels of SP and VIP in STC model rats, to select an appropriate frequency in EA treatment of STC, thus to provide scientific bases for the frequency selection in EA treatment of STC.

## 1 Materials and Methods

### 1.1 Laboratory animals and grouping

A total of 100 healthy, mature and clean grade male Sprague-Dawley (SD) rats, weighing 200-250 g, were purchased from the Experimental Animal Center of Hebei Medical University [certificate number: SCXK (Ji) 2013-1-003]. The rats were housed in separated cages (5 per cage) and kept in the quiet and ventilated environment with daily light for 12 h and free access to food. The room temperature was 16-25 ℃ with relative humidity of 50%-70%. The rats were randomly divided into 5 groups by random number table method, including a normal group, a model group, a low-frequency EA group, a high-frequency EA group and a variable-frequency EA group, with 20 rats in each group.

### 1.2 Instruments and reagents

HANS-200 EA instrument (Beijing University of Traditional Chinese Medicine Saile Electronics Co., Ltd., China); acupuncture needle (Suzhou Medical Products Factory Co., Ltd., China); compound phenethyl piperidine (Changle Pharmaceutical Factory, Xinxiang City, Henan Province, China); radioimmunoassay kit for iodine ($^{125}$I) SP, radioimmunoassay kit for iodine ($^{125}$I) VIP (Beijing Huayi Lite Biotechnology Research Institute, China).

### 1.3 Model preparation

Rats in the normal group were fed with normal diet, which was prepared by the Institute of Zoology, Hebei Medical University. Rats in the other groups were used to prepare the STC model with the modeling method developed by Liu HF, *et al*[13]: compound phenethidine was added into the rat diet at a dose of 8 mg/(kg·bw) every day for 120 d. After the designated diet, the model rats showed decreased numbers of defecation and fecal pellets, and also dry and hard fecal matter. The defecation duration of the first dark stool was significantly longer than that of the normal group ($P$<0.05).

### 1.4 Intervention groups

Rats in the normal group and the model group were only placed on the device for binding without any intervention, once a day, 15 min/time, for continuous 15 d.

Bilateral Tianshu (ST 25), Zhigou (TE 6) and Zusanli

(ST 36) in the EA treatment groups were selected according to the principle of acupoint selection in humans and located by referring the *Veterinary Acupuncture*[14] and *Experimental Acupuncture Science*[15].

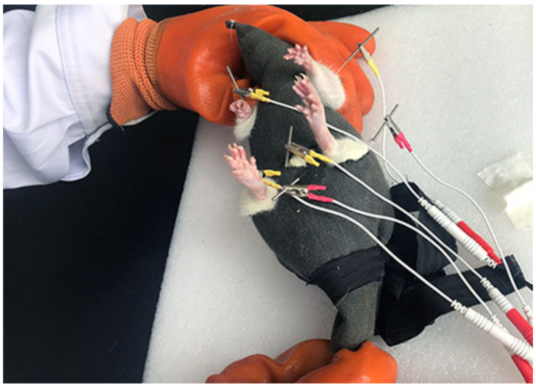

**Figure 1. EA points**

Tianshu (ST 25) is on the abdomen of the rats and about 5 mm away from the umbilicus.

Zhigou (TE 6) is on the lateral forelimbs and about 4 mm from the wrist joint between the ulna and the radius.

Zusanli (ST 36) is on the posterolateral side of the knee joints, about 5 mm below the capitulum fibulae.

The rats were placed on a self-made mouse binding device when the modeling was completed. After being routinely sterilized, the acupoints were acupunctured with needles (0.35 mm in diameter and 25 mm in length). Tianshu (ST 25) was acupunctured for about 5 mm, and Zhigou (TE 6) and Zusanli (ST 36) were acupunctured about 3 mm. The EA device was connected to the needle after needle insertion. The low-frequency EA group selected a continuous wave of 2 Hz; the high-frequency EA group selected a continuous wave of 100 Hz; the variable-frequency EA group selected a sparse-dense wave of 2 Hz/100 Hz. The current intensity was determined by the slight trembling of the rat limbs without painful screaming. Once a day, 15 min/time, for continuous 15 d.

### 1.5 Observation items

1.5.1 Intestinal transit function measurement

After the treatment was completed, the rats in each group were fasted for 24 h, and 2 mL of 100 g/L activated carbon suspension was orally administered. The time from the completion of activated carbon administration to the first dark stool defecation was recorded.

1.5.2 SP and VIP expression levels in colon

Rats in each group were sacrificed by bloodletting, and the middle section (about 2 cm) of the colon was quickly removed by opening the belly. About 2 mL of physiological saline was added after the contaminated blood was removed to obtain a homogenate by grinding. The supernatant was isolated by centrifuge at 3 000 r/min and 4 ℃ for 15 min and stored in a refrigerator at −20 ℃. Radioimmunoassay was used to determine the expression level of SP in the colon using the iodine ($^{125}$I) SP radioimmunoassay kit, and the expression level of VIP in the colon was determined by the iodine ($^{125}$I) VIP radioimmunoassay kit.

### 1.6 Statistical methods

All data were statistically analyzed using SPSS version 13.0 software. Measurement data that conformed to the normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$), and analyzed using one-way analysis of variance. The difference was statistically significant at $P$<0.05.

## 2 Results

### 2.1 Comparison of the first dark stool defecation duration

Compared with the normal group, the first dark stool defecation duration in the model group was significantly prolonged, and the difference between the two groups was statistically significant ($P$<0.05); compared with the model group, the first dark stool defecation durations of the low-frequency EA group, the high-frequency EA group and the variable-frequency EA group were significantly shortened ($P$<0.05); among them, the first dark stool defecation duration in the variable-frequency EA group was significantly shorter than that in the 2 Hz EA group. The difference between the two groups was statistically significant ($P$<0.05). The details were shown in Table 1.

**Table 1. Comparison of the first dark stool defecation duration of rats in each group ($\bar{x} \pm s$, minute)**

| Group | $n$ | Defecation duration of the first dark stool |
|---|---|---|
| Normal | 20 | 391.63±53.81 |
| Model | 20 | 620.57±103.44$^{1)}$ |
| Low-frequency EA | 20 | 505.03±82.11$^{2)}$ |
| High-frequency EA | 20 | 464.69±73.17$^{2)}$ |
| Variable-frequency EA | 20 | 417.07±68.75$^{2)3)}$ |

Note: Compared with the normal group, 1) $P$<0.05; compared with the model group, 2) $P$<0.05; compared with the low-frequency EA group, 3) $P$<0.05

### 2.2 Comparison of the SP and VIP expression levels in colon

Compared with the normal group, the SP and VIP expression levels in colon of the model group were significantly decreased, and the differences between the two groups were statistically significant ($P$<0.01). The SP and VIP expression levels in colon of the low-frequency EA group, the high-frequency EA group and the variable-frequency EA group were significantly higher than those in the model group (all $P<0.05$); compared with the high-frequency EA group, the SP expression levels in colon of the low-frequency EA group and the variable-frequency EA group were significantly increased (both $P<0.05$); compared with the low-frequency EA group, the VIP expression levels in the colon of the high-frequency EA group and the variable-frequency EA group were significantly increased (both $P<0.05$). The details were shown in Table 2.

**Table 2. Comparison of the SP and VIP expression levels in colon of each group ($\bar{x}\pm s$, pg/mL)**

| Group | $n$ | SP | VIP |
|---|---|---|---|
| Normal | 20 | 39.21±4.15 | 229.51±37.12 |
| Model | 20 | 18.53±2.63 1) | 159.88±29.63 1) |
| Low-frequency EA | 20 | 34.19±3.92 2)3) | 180.53±27.77 2) |
| High-frequency EA | 20 | 28.73±3.44 2) | 202.18±31.06 2)4) |
| Variable-frequency EA | 20 | 35.66±4.01 2)3) | 208.42±36.44 2)4) |

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the high-frequency EA group, 3) $P<0.05$; compared with the low-frequency EA group, 4) $P<0.05$

## 3 Discussion

Chinese medicine believes that the human body is an organic whole, and the coordination of various organs is the basis of health. The occurrence of constipation is related to dysfunctions of the human body. Zhang JP, *et al*[16] believe that its etiology is closely related to the liver, lung, spleen and kidney; the liver failing to maintain the normal flow of qi, impairment of purifying and descending function of the lung, dysfunction of the spleen in transportation, and loss of warmth in the kidney would cause weakness in large intestinal transit, thus leading to the occurrence of STC.

STC, also known as colonic weakness, is characterized by decreased colonic motility and slow colonic propagation[17]. STC is more common in the elderly, and its symptoms are similar to the deficient constipation in traditional Chinese medicine. Its pathogenesis is related to the lack of qi-blood and body fluid.

EA therapy refers to the connection of the EA instrument after the needle is inserted into the acupoints. The pulse current of the EA device acts on the needle to vibrate the needle body to strengthen the needle sense, enhance the acupoint stimulation and promote intestinal peristalsis. Tianshu (ST 25) is the Front-Mu point of the large intestine. It is an acupoint where Yangming meridian qi deeply gathers in the intestines and the abdomen; Zusanli (ST 36) is the He-Sea point of the Stomach Meridian and the Lower He-Sea point of the stomach. Acupuncture at Zusanli (ST 36) can smooth the organs and promote qi circulation. Zhigou (TE 6) is the best to regulate the qi movement of three jiao, which can help regulate the intestinal organ, dredge the stagnation and pass stools. A large number of clinical cases show that the above acupoints are the main points for treatment of constipation, especially the STC[18-20].

The pathogenesis of STC remains to be further clarified. Recent studies have shown that the occurrence of STC is associated with abnormalities in certain gastrointestinal regulatory peptides[4]. There are dozens of gastrointestinal regulatory peptides[21], among which, the excitatory neurotransmitters mainly include SP and the inhibitory neurotransmitters mainly include VIP. SP strongly stimulates the smooth muscle to contract, which can promote intestinal movement, reduce intestinal mucosal electrolyte and water transportation. VIP is an inhibitory neurotransmitter that inhibits intestinal movement and regulates intestinal water metabolism to increase intestinal secretion. Therefore, the reduction of both can affect the effective propulsion of the colon, leading to the occurrence of STC[22]. There have occurred many studies on EA for constipation in recent years[5,7,10-12,19,23-27]. The frequencies of EA selected by doctors are different, including 2 Hz, 100 Hz, 2 Hz/15 Hz, 2 Hz/100 Hz, etc., and each doctor uses a single frequency, lacking a comparison between different frequencies. In this experiment, to compare the effects of different EA frequencies, a representative low-frequency of 2 Hz, high-frequency of 100 Hz and variable-frequency of 2 Hz/100 Hz were selected for comparative study, thus to guide the EA frequency selection for the treatment of STC.

The results of this experiment showed that EA treatment at three different frequencies can effectively improve the intestinal transit function of STC model rats, thus to treat STC. The comparison of intestinal transit function showed that the defecation duration of the first dark stool in the variable-frequency EA group was significantly shorter than that in the low-frequency EA group ($P<0.05$). The SP and VIP expression levels in colon of each group indicated that the 100 Hz high-frequency and 2 Hz/100 Hz variable-frequency EA stimulation had better effect on improving colonic transit function in STC rats.

There are many factors causing STC. In this experiment, only the intestinal transit time, and SP and VIP expression levels in the colon have been studied. Further research is needed in the future to observe the effects of different EA frequencies on other factors causing STC. This will provide reliable theoretical bases for selecting the most suitable EA frequency in clinical practice.

**Conflict of Interest**

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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## References

[1] Kang C. Negative emotions and quality of life of patients with chronic slow transit constipation and the correlations. Jiefangjun Yufang Yixue Zazhi, 2017, 35(10): 1317-1319.

[2] Fang XC. Therapeutic strategy of chronic constipation based on diagnosis and subtyping. Wei Chang Bing Xue, 2012, 18(10): 577-579.

[3] Wang P, Zhang YC. Review of the etiology and mechanisms of slow transit constipation. Xiandai Shengwu Yixue Jinzhan, 2012, 12(30): 5994-5996, 5984.

[4] Zhang D, Yuan XX, Wang BY, Zhang YL. Observation on effects of moxibustion at abdominal acupoints for slow transit constipation due to yang deficiency of the spleen and kidney. J Acupunct Tuina Sci, 2017, 15(6): 426-433.

[5] Ruan YD, Zhao ZY. Study of the neuroendocrine mechanism of the action of electroacupuncture on colonic motility in a mouse of slow transit constipation. Shanghai Zhenjiu Zazhi, 2016, 35(11): 1358-1361.

[6] Zhou YX, Wang YJ, Zhang H, Yan SG, Wang B, Xie P. Effect of vasoactive intestinal peptide on defecation and VIP-cAMP-PKA-AQP3 signaling pathway in rats with constipation. Zhongnan Daxue Xuebao (Yixue Ban), 2016, 41(11): 1175-1180.

[7] Zhang X, Zhang XP, Du YZ, Xing HJ, Jian R, Pan LJ, Xu J, Jia CS. Effect of manual acupuncture, electroacupuncture and moxibustion on intestinal motility and expression of enteric nervous activity related proteins in functional constipation rats. Zhen Ci Yan Jiu, 2017, 42(5): 407-412.

[8] Bai DY, Han WW, Wang BJ, Xie GD, Wang ZR. Effect of acupoint application of Fuzi Dingxiang powder on serum vasoactive intestinal peptide and substance P in rats with slow transit constipation. Shandong Yiyao, 2018, 58(3): 42-44.

[9] Quan NN, Guo XH, Wang AP, He JJ, Tang XG. Research progress in electroacupuncture therapy for functional constipation. Jiceng Yixue Luntan, 2018, 22(1): 109-111.

[10] Peng SF, Shi T, Shi ZH, Yang JY, Wu YF, Hu W, Zhou XL. The efficacy of electroacupuncture at the oppilative symptoms, colonic transit and anxiety and depression state in slow transit constipation patients. Weichangbingxue He Ganbingxue Zazhi, 2013, 22(3): 250-253.

[11] Shen YF, Fang JQ, Chen LF, Shen Z, Fang F, Sun J. Electroacupuncture in treating severe functional constipation: a randomized controlled trial. Shanghai Zhenjiu Zazhi, 2016, 35(12): 1393-1396.

[12] Duan JX, Peng WN, Liu ZS, Yang DL, Guo J, Cai HJ. Clinical study on deep insertion at Tianshu (ST 25) for colonic slow transit constipation. J Acupunct Tuina Sci, 2011, 9(1): 46-50.

[13] Liu HF, He JT, Wang XW, Jiang LG. Changes of colonic myoelectric activity in a rat model of slow transit constipation. Wujing Yixue, 2004, 15(12): 887-891.

[14] Yang Y. Veterinary Acupuncture. Beijing: Higher Education Press, 2006: 195-207.

[15] Li ZR. Experimental Acupuncture Science. Beijing: China Press of Traditional Chinese Medicine, 2007: 327.

[16] Zhang JP, Wang CR. Relationship between liver spleen and kidney dysfunction and constipation. Huanqiu Zhongyiyao, 2015, 8(6): 713-715.

[17] Wald A. Slow transit constipation. Curr Treat Options Gastroenterol, 2002, 5(4): 279-283.

[18] Xu MH, He HY, Gao RR. Clinical observation on acupuncture treatment for constipation due to intestinal qi stagnation. J Acupunct Tuina Sci, 2016, 14(6): 401-406.

[19] Zhao YW, Ge ZX. A clinical research on acupuncture point embedding therapy combined with intermediate frequency electrotherapy in treatment of slow transit constipation. Linchuang Yixue Gongcheng, 2009, 16(8): 98-99.

[20] Chen L, Sun JH, Han GH. Clinical study on deep needling at Tianshu (ST 25) and Fujie (SP 14) plus electro-acupuncture for functional constipation. Shanghai Zhenjiu Zazhi, 2016, 35(3): 287-290.

[21] Jiang JZ, Yi YL, Wang LL. Effect of acupuncture on colonic motility in patients with slow transit constipation. Zhongyiyao Linchuang Zazhi, 2013, 25(9): 787-788.

[22] Li Y, Wang J, Li YP, Hu Y, Tang J, Yu LF. Effect of Qilang decoction on substance P and vasoactive intestine polypeptide in the mucous enteric nervous system. Shijie Huaren Xiaohua Zazhi, 2008, 16(3): 272-276.

[23] Liu HF, He JT, Wang XW, Jiang LG. Colonic myoelectric activity of rats with slow transit constipation and its pathogenesis. Disi Junyi Daxue Xuebao, 2005, 26(7): 603-606.

[24] Sun JH, Guo H, Chen L, Wu XL, Li H, Pei LX, Peng YJ, Lu B. Effect of electroacupuncture at Tianshu (ST 25) on colonic electromyography in slow transit constipation rats. Zhonghua Zhongyiyao Zazhi, 2011, 26(9): 2077-2079.

[25] Jia F, Li GD. Observation of curative effect of electroacupuncture at Baliao points and Chengshan (BL 57) point on slow transit constipation. Xiandai Zhongxiyi Jiehe Zazhi, 2015, 24(10): 1055-1057.

[26] Jia F, Li GD. Clinical research of electroacupuncture combined with dispensing granule decoction of enriching the blood and moisturizing the intestine granules in the treatment of slow transit constipation. Zhongguo Yiyao Daobao, 2015, 12(7): 114-118.

[27] Peng WN, Wang L, Liu ZS, Guo J, Cai HJ, Ni JN, Duan JX, Yang DL. Analysis on follow-up and safety of slow transit constipation treated with individualized deep puncture at Tianshu (ST 25): a multi-central randomized controlled trial. Zhongguo Zhen Jiu, 2013, 33(10): 865-869.

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