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Special Topic Study

# Moxibustion at Front-Mu Point of Abdomen for Intestinal Dysbacteriosis in Rats

## 艾灸腹部募穴调整大鼠肠道菌群失调的实验研究

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【**Abstract**】**Objective**: To study the moxibustion at Guanyuan (CV 4) and Tianshu (ST 25) on intestinal dysbacteriosis in rats. **Methods**: Fifty Wistar rats, clearing grade, were randomly divided into 5 groups, a normal group, a model group, a medical group, and a Guanyuan (CV 4) group and a Tianshu (ST 25) group, with 10 rats in each group. The rats were treated with Lincomycin Hydrochloride via intragastric administration for developing models. The model rats were treated with medicine and moxibustion respectively. 0.1 g fresh rat feces in each group were cultured on the selective culture medium of bifidobacterium species (BS), lactobacillus (LBS), enterobacteriaceae (EB) and enterococcus (EC). The growth and quantity of the bacterial colony were tested by biochemical identification tubes and turbidimetry. **Results**: Moxibustion at Guanyuan (CV 4) increased BS and LBS while moxibustion at Tianshu (ST 25) improved EB and EC. **Conclusion**: Moxibustion at Front-Mu points of different body parts selectively regulated advantaged probiotics for treating intestinal dysbacteriosis.

【**Key Words**】Moxibustion Therapy; Moxa Stick Moxibustion; Points, Front-Mu; Bifidobacterium; Lactobacillus; Enterobacteriaceae; Enterococcus

【**摘要**】**目的**：观察艾灸关元、天枢对实验性大鼠肠道菌群失调的影响。**方法**：将 50 只清洁级Wistar大鼠随机分为正常组(Normal group)、模型组(Model group)、药物组(Medical group)、关元组(CV 4 group )、天枢组(ST 25 group ),每组 10 只。用大量盐酸林可霉素灌胃造模,造模成功后,分别进行药物治疗和艾灸治疗。1 个疗程后，采取各组大鼠新鲜粪便 0.1 g，应用双歧杆菌(Bifidobacterium Species，BS)、乳酸杆菌(Lactobacillus，LBS)、肠杆菌(Enterobacteriaceae，EB)、肠球菌(Enterococcus，EC)选择性培养基进行细菌培养,生化鉴定管和比浊法检测不同菌落生长情况和各组菌落数量。**结果**：艾灸关元穴使BS、LBS数量有所增加；艾灸天枢穴使EB、EC数量有所增加。**结论**：艾灸不同部位的募穴可以选择性调整肠道优势益生菌群，从而治疗肠道菌群失调症。

【**关键词**】灸法；艾条灸；募穴；双歧杆菌；乳酸杆菌；肠杆菌；肠球菌
【**中图分类号**】R2-03　　【**文献标志码**】A

Microorganisms that inhabit in the gastrointestinal tract of healthy human are called intestinal flora. Intestinal flora is an integral part of the internal environment to benefit the organism without harm, and form a large and complex microecosystem in the gastrointestinal tract, bringing about balance in quantity and quality. The homeostasis of quality and quantity for intestinal flora would be broken if there were changes in the internal and external environment, resulting in clinical symptoms, which is known as intestinal dysbacteriosis. Antibiotics abuse was one of the key causes for the intestinal dysbacteriosis. In this study, the alteration resulted from the abuse of antibiotics was treated with moxibustion at the Front-Mu point of the abdomen.

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## 1 Materials

### 1.1 Animals

Fifty Wistar rats (25 males and 25 females) weighed (200±20) g, clearing grade, were supplied by

the Experimental Animal Center of Liaoning University of Traditional Chinese Medicine and raised in the Lab of the Acupuncture and Tuina College in the university. The rats were free to eat and drink. The lab was good in lighting and not draughty, clean and quiet, with the temperature of around 22℃

### 1.2 Reagents and medicine

Lincomycin Hydrochloride of Lingyan Brand (H32021365, batch number 070308) was purchased from Suzhou No. 6 Pharmaceutical Factory, Jiangsu Wuzhong Medicine Group. Live Bifidobacterium Preparation (capsule) was from Lizhu Pharmaceutical Factory, Lizhu Group, stored at 4℃ for use.

### 1.3 Agar

TPY agar (batch number 20071023) was used for selective culture of bifidobacterium species (BS). MRS agar (batch number 20080321) was for selective culture of lactobacillus (LBS). Sorbitol-MacConkey agar (batch number 20080324-1) was for enterobacteriaceae (EB) while Pfizer enterococcus selective agar (batch number 20080428) was for enterococcus (EC). All the agars above were purchased from Hangzhou Baisi Biotechnology Company for modulation.

### 1.4 Equipments

The Biochemical Technology Experiment Center for Basic Immunology in the Liaoning University of Traditional Chinese Medicine supplied the equipments as follows: Anthos 2010 ELIASA (Austria), Jinghong electrothermal constant temperature blast drying oven (DHG-9070A, Shanghai), Hengyu electrothermal constant temperature incubator (HH.B11.500, Shanghai Yuejin Medical Apparatus and Instruments Factory), the imaging system of BX51 system microscope (Japan), DP70 digital microscope camera (Japan), and Sanyo autoclave (SANYOMLS-3780, Japan).

Anaerobic bag, oxygen indicator and 2.5 L sealed culture tank made by Mitsubishi Gas Chemistry Co., Ltd. were purchased from Beijing Bingyang Science and Technology Co., Ltd.

## 2 Methods

### 2.1 Groups

Wistar rats were normally raised for a week, freely able to eat and drink. The rats were randomly divided into 5 groups, normal group, model group, medical group, Guangyuan (CV 4) group and Tianshu (ST 25) group, with 10 rats in each group.

### 2.2 Model

The rats in each group, except the normal group, were treated with intragastric administration of Lincomycin Hydrochloride at 5 000 mg/kg in the consecutive 5 d. The rats suffered diarrhea, significant increase in frequency of defecation, and watery feces, indicating the degree of dysbacteriosis caused by antibiotics decontamination.

### 2.3 Animal therapy

The rats in the medical group were washed with physiological saline solution of live bifidobacterium preparation on the quantity of 0.5 mL/d one day per week, starting from the 6 d after the model reduced.

The rats in the Guanyuan (CV 4) group and Tianshu (ST 25) group were treated with smokeless moxa stick for warm moxibustion by copper moxibustion gun. The treatment was carried out once a day and 20 min per time per week for a treatment course. The acupoints of Guanyuan (CV 4) point and Tianshu (ST 25) point were located according to experimental acupuncture science[1].

### 2.4 Media preparations

MRS agar was weighed for 66.24 g and dissolved in 1 000 mL distilled water with 1.32 mL glacial acetic acid. The antigrading mixture was heated and agitated then cooled to 50℃ to pour into a sterile culture dish for use. TPY agar was weighed for 46.4 g, dissolved in 1 000 mL distilled water, subpackaged into a triangular flask, autoclaved at 121℃ for 15 min and poured into sterile culture dish for use. The procedure for Pfizer agar was 58 g dissolving in 1 000 mL distilled water, subpackage, autoclaving, and pouring into culture dish. Sorbitol-MacConkey agar was weighed for 55 g and dissolved in 1 000 mL distilled water, subpackaged into triangular flask, autoclaved at 121℃ for 15 min and cooled to 50℃, added 1 mL of 0.05% sterile potassium nitrite solution per 20 mL agar solution, and poured into sterile culture dish for use.

### 2.5 Animal sampling, bacterial culture and measurement

#### 2.5.1 Animal sampling

The fresh feces of each rat in every group were weighed at 0.1 g and diluted with 0.9 mL physiological saline (10% W/V) in the sterile bottles with beadings at the end of a treatment course. The bottles were oscillated at 200 times per minute for 15 min.

#### 2.5.2 Bacterial culture

The 40 μL of each oscillated samples were dropped to the MRS agar medium for LBS, TPY agar medium, Pfizer enterococcus selective agar medium for EC as well as Sorbitol-MacConkey agar medium for EB and pushed by L-shaped rod. The inoculations were carried out from high dilution to low dilution (10 times to $10^{-6}$ times). Two drops of the diluents at $10^{-4}$ to $10^{-6}$ dilution were inoculated to the corresponding media with the fluid volume of 1/47 mL. The inoculated media were standing for 20 min and the EB and EC were incubated in 37℃ incubator for 24h while BS and LBS were blocked in an anaerobic jar with an anaerobic bag and an oxygen indicator and anaerobically incubated in 37℃ incubator for 48-72 h.

#### 2.5.3 Measurement

The probiotics in each media were dissolved in 4 mL distilled water to examine the OD value of the bacterial colony in each group by ELIASA in turbidimetry for comparison of the intergroup difference. Meanwhile, the bacteria in each group were identified in grams stain and biochemical method[2, 3]. The typical bacterial colonies were picked by aseptic oese and inoculated into corresponding assessor to incubate at 37℃ in baking oven for 48-72 h. Then the colors of different identification tubes were observed. The anaerobic bacteria in the assessor were sealed by glycerin.

### 2.6 Statistics

Data were expressed as the mean ± standard deviation ($\bar{x}\pm s$). The data were analyzed with SPSS 11.5. Analysis of variance and $q$ test were used for group mean-comparison. Significance was defined by $P<0.05$.

## 3 Results

### 3.1 Moxibustion influencing typical probiotics in intestinal tract of experimental rats

#### 3.1.1 Moxibustion at Guanyuan (CV 4) point influencing BS and LBS in intestinal tract

The comparisons of the quantities of BS and LBS in intestinal tract of the rats in the Guanyuan (CV 4) group and medical group had statistical significance ($P<0.05$), indicating that the moxibustion at Guanyuan (CV 4) point increased the quantities of BS and LBS (table 1).

**Table 1. Comparison of BS and LBS in each group ($\bar{x}\pm s$)**

| Groups | $n$ | BS | LBS |
|---|---|---|---|
| Normal | 10 | 0.791±0.108[1)] | 2.117±0.164[1)] |
| Model | 10 | 0.507±0.159 | 1.609±0.108 |
| Medical | 10 | 0.561±0.0831) | 2.125±0.249[1)] |
| CV 4 | 10 | 0.714±0.167[1)2)] | 2.187±0.192[1)2)] |

Note: Compared with the model group, 1) $P<0.05$; compared with the medical group, 2) $P<0.05$

#### 3.1.2 Moxibustion at Tianshu (ST 25) point influencing EB and EC in intestinal tract

Table 2 showed the comparisons of the quantities of BS and LBS in intestinal tract of the rats in Guanyuan (CV 4) group and medical group with statistical significance ($P<0.05$), indicating that the moxibustion at Tianshu (ST 25) point increased the quantities of EB and EC.

**Table 2. Comparison of EB and EC in each group ($\bar{x}\pm s$)**

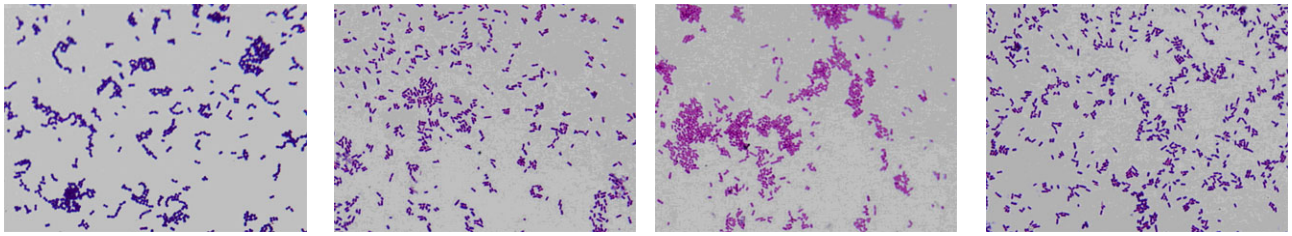
| Groups | $n$ | EB | EC |
|---|---|---|---|
| Normal | 10 | 2.230±0.197[1)] | 0.429±0.074[1)] |
| Model | 10 | 1.758±0.351 | 0.196±0.098 |
| Medical | 10 | 2.109±0.310[1)] | 0.402±0.092[1)] |
| ST 25 | 10 | 2.259±0.33[1)2)] | 0.570±0.110[1)2)] |

Note: Compared with the model group, 1) $P<0.05$; compared with the medical group, 2) $P<0.05$

### 3.2 Stain of typical bacterial colony from intestinal tract of experimental rats

Fig.1 showed the stain of typical bacterial colony from intestinal tract of experimental rats.



BS LBS EB EC

Fig.1. Typical bacterial colony stain (Grams stain, ×1 000)

### 3.3 Colonial morphology and biochemical identification

The features of colonial morphology on MRS agar media were medium size, smooth surface, whitish, round and irregular margins. The colonial morphology on TPY agar media was small, transparent, regular margins, milk white, soft and delicate. For Pfizer enterococcus selective agar media, the colonial morphology was gray, opaque, smooth and small. The colonial morphology on Sorbitol-MacConkey agar media was in different sizes, untidy margins, asperity, white and opaque. The colors of vaccinated biochemical identification tube and non vaccinated biochemical identification tubes were compared after 24-48 h. The changed color indicated positive while unchanged color was for negative, concluding to the results as identification tubes instruction. The biochemical identification was performed according to the *Manual of Common Determinative Bacteriology*[2] and the *Experimental Techniques in Microbiology*[3].

## 4 Discussion

Normally, the relative equilibrium between gut flora and human internal/external environment secures human health. Many physical chemical and biological factors, such as antiauxin, radioactive substances, non-green food, surgery, examine, hormone, immunosuppressive agents and cytotoxic drug[4], can result in the microflora imbalance. Stressful work and a fast paced life enhanced the possibility of intestinal dysbacteriosis. The intestinal dysbacteriosis may result in plenty of diseases such as diarrhea, constipation, liver disease, decreased immunity, progeria, and tumor, and then induce diseases of other tissues and organics, being one of the principal diseases for health threats. In the study, the relation of intestinal dysbactriosis and chronic diarrhea was discussed depending on animal experiment. The intestinal dysbacteriosis in rats was induced by feeding them large doses of antibiotics for a long time. The results showed that the change in quantity of enterobacteria and imbalance of ratio diminished the immuneityand clearance function of normal flora, leading to a large population of pathogenic bacteria to stimulate the intestinal mucosa and diarrhea occured as a result. Chronic diarrhea may induce and improve the intestinal dysbacteriosis. Chronic diarrhea and alteration of intestinal flora were reciprocal causation[5].

Tianshu (ST 25), the Front-Mu point of the large intestine, and Guanyuan (CV 4), the Front-Mu point of the small intestine, were investigated in the study. "Shu" means the key for ascending lucidity and descending turbidity, playing a role in invigorating spleen to resolve dampness. "Guan" means hiding and "Yuan" means the qi of nephroyin and nephroyang. Guanyuan (CV 4) point relates with the uterus and scrotum, which holds the qi of nephroyin and nephroyang with the function of adjusting the Thoroughware and Conception Vessels, warming kidney, fastening essence, and regulating qi and blood. Moxibustion at the two Front-Mu points regulated the functional disorders of the large intestine and small intestine induced by intestinal flora[6-8].

The warm moxibustion at Guanyuan (CV 4) and Tianshu (ST 25) improved the diarrhea and regulated the intestinal flora. Guanyuan (CV 4) effectively increased the quantity of anaerobic bacteria flora (the BS and the LBS) while Tianshu (ST 25) significantly improved the quantity of aerobic bacteria flora (the BS and the LBS), balancing the intestinal flora to the normal range. The Front-Mu points, with a close relationship to intestinal flora, were in every organ.

Intestinal flora formed large and complex microecosystem. Different Front-Mu points in different organs selectively regulated probiotics groups and inhibited pathogenic bacteria with targeting features that different Front-Mu points specifically regulated some kinds of bacteria flora from different intestinal segments when they were heated. The warm moxibustion at the Front-Mu points [Guanyuan (CV 4) point and Tianshu (ST 25) point] selectively adjusted probiotics group for treating intestinal dysbacteriosis. The warm moxibustion at Front-Mu points of the abdomen had a positive effect on intestinal mucosa protection, mucus secretion and gastrointestinal motility to recover the homeostasis of the quantity and quality of various flora and improve the growth of probiotics in the intestines[9,10]. Moxibustion is a safe and effective treatment for intestinal dysbacteriosis.

## References


[1] Li ZR. Experimental acupuncture science. Beijing: China Press of Traditional Chinese Medicine, 2007: 1.

[2] Dong XZ, Cai MY. Manual of common determinative bacteriology. Beijing: Science Press, 2001: 2.

[3] Guan YZ, Wang AL, Li J. Experimental techniques in microbiology. Beijing: Chemical Industry Press, 2006: 1.

[4] Jia WX. Medical microbiology. Beijing: People's Medical Publishing House, 2005: 7.

[5] Zhuang GL, Mou ZQ, Liu XM. Observation of clinical effects of acupuncture on postoperative alteration of intestinal flora. Shan Xi Lin Chuang Yi Yao, 2001, 10(8): 623-624.

[6] Tang XS. Thinking about needling Zusanli (ST 36) and moxibustion at Guanyuan (CV 4). Shan Dong Zhong Yi Za Zhi, 2002, 21(4): 245-246.

[7] Ju XD, Sui H. Treatment of 100 cases of functional diarrhea with acupuncture. Nei Meng Gu Yi Yao, 2010, 29(2): 32-33.

[8] Li F, Cui XM. Treatment of chronic diarrhea with acupuncture. Xian Dai Zhong Xi Yi Jie He Za Zhi, 2009, 18(30): 3685-3686.

[9] Xu YQ, Dong Y. Clinical function and application of bifidobacterium. Zhong Guo Xian Dai Yao Wu Ying Yong, 2007, 1(7): 61-62.

[10] Wang XM, Shi Z, Ma XP, Zhou CL, Qin XD, Liu HR, Wu HG. Thinking and methods of moxibustion regulation of pattern recognition receptors and signal transduction in inflammatory bowel disease. Shang Hai Zhen Jiu Za Zhi, 2010, 29(3): 136-139.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