Electro-acupuncture on Lower He-sea Points: How Does it Affect Serum HMGB1 and Tissue α7 nAChR in Ulcerative Colitis Rats

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Abstract

Objective: To observe the expression levels of serum high mobility group box 1 (HMGB1) , nicotinic acetylcholine receptor α7 (α7 nAChR) and nuclear factor kappa-B mRNA(NF-κB mRNA) in colon tissue after electroacupuncture (EA) on Shangjuxu, Zusanli, Xiajuxu,Yanglingquan and Chengjin points, by comparison of the different effects we could thus explore the relative specificity of Shangjuxu on treating its corresponding viscera' s disease.

Methods: Seventy SD rats were randomly assigned to seven groups labeled by blank control (A), Model (B), Shang Ju Xu (C), Zu San Li (D), Xia Ju Xu (E), Yang Ling Quan (F), Cheng Jin (G), ten (half males and half females) in each group. Except for the blank control group, animals in other groups were modeled to ulcerative colitis (UC) by lavation of 2-4-6trinitro-benzene-sulfonic acid (TNBS)/ethanol solution. After successful modeling animals in the five treatment groups were respectively treated with electroaccupuncture at corresponding acupoint for 10 consecutive days. Then histological changes in the colon were observed by 1ight microscopy. ELISA method was used to check models'serum HMGB1 content, and α7 nAChR in the colon were detected by western blot , RT-PCR was used to detecte NF-κB mRNA.

Results: ①Compared with the model group, the tissue injury changes in 5 treatment groups improved to a certain extent, and the colon tissue NF-κB mRNA expression of them decreasd signifcantly（P<0.01),as well, the α7 nAChR expression of Shang Ju Xu group, Zu San Li group and Xia Ju Xu group increased signifcantly (P<0.01 or P<0.05),and their serum HMGB1 content made obvious decrease(P<0.01 or P<0.05), the α7 nAChR expression in Yang Ling Quan group was also increased significantly（P<0.01).②Compared with Shang Ju Xu group, the tissue injury changes of groups including Xia Ju Xu,Yang Ling Quan and Cheng Jin are much severe under light microscope, and the α7 nAChR expression of them decreased with significance（P<0.01),both serum HMGB1 content and colon tissue NF-κB mRNA expression increased significantly as well（P<0.01),the serum HMGB1 content of Zu San Li group also increased significantly（P<0.01).

Conclusion: ①Electroacupuncture on all five different acupoints can improve the UC through regulating HMGB1,α7 nAChR and NF-κB mRNA, and effectively inhibiting the immune response, reducing inflammatory response of colon and improving pathological changes of colonic mucosa.②The effect of treating UC inflammation in rats when electroacupuncture at Shang Ju Xu was better than Zu San Li,Xiajuxu,Yang Ling Quan and Cheng Jin,which demonstrated that Shangjuxu treating UC has some relative specificity.

Keywords: electroacupuncture, lower He-sea point, UC model rats, HMGB1, α7 nAChR, NF-κB mRNA, curing viscera diseases by He-sea points

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Lower He-sea points are specific points that Qi flows of six-Fu organs converge at the three foot Yang meridians- each Fu organ has its specific He-sea point. *Pure questioning of TCM·Discussion on Coughing recorded*, "to treat Fu-organ diseases, He-sea points should be considered first", that means, He-sea points are commonly used to treat corresponding inner Fu-organ diseases. Our previous studies [1,2] have demonstrated that compared with Upper He-sea points the lower He-sea points have better specificity on treating Fu-organ diseases, so the above-mentioned He-sea points should mean the lower He-sea points. Shang Ju Xu (ST37) is the lower He-sea point of large intestine, according to the above-mentioned principle, it should be the major point to treat large intestinal diseases. Some clinical studies[3,4] also indicated that Shang Ju Xu (ST37) plays an important role on treating diarrhea, abdominal pain, etc. Studies also found that Zu San Li (ST36) and Xia Ju Xu (ST39) had certain effects for large intestineal diseases[5,6]. Similarly, Zu San Li (ST36) and Xia Ju Xu are also lower He-sea points (of stomach and small intestine respectively), which are located together with Shang Ju Xu on the stomach meridian. Can other lower He-sea points on the same meridian or around also treat the large intestinal diseases? We know that Yang Ling Quan (GB34, the lower He-sea point of gall bladder) and Cheng Jin (BL56, the lower He-sea point of urinary bladder) are located under the knee joint, having the same nerve segmental control with Shang Ju Xu. Further, Yang Ling Quan (GB34) is also tightly related to the digestive function. We have reason to ask, can all the lower He-sea points effectively treat large intestinal diseases, or Shang Ju Xu (ST37) has relative specificity on treating those diseases? To answer this question, we established an ulcerative colitis (UC) rat model, to observe the serum high mobility group box chromosomal protein 1 (HMGB1), alpha 7 nicotinic acetylcholine receptor (α7nAChR) and nuclear factor kappa-B mRNA (NF-κB mRNA) expression changes after electro-acupuncture (EA) stimulation on above- mentioned lower He-sea points, by comparison of the effects we aimed at exploring the specificity of lower He-sea points on treating inner Fu-organ diseases and its underlying mechanism.

1 Materials and methods

1.1 Animal grouping

Seventy healthy SD rats, SPF grade, half male and half female, weighing (230±15)g, fed at temperature 20~25℃ and humidity 50%~70% were provided by the Experimental Animal Center, Hunan University of Traditional Chinese Medicine (animal certificate no. 43004700003928). After adaptive feeding for ten days, the animals were fasted but drinking free for 24 hrs before experiment, then were randomly assigned to seven groups by sex: blank control (A), model (B), Shang Ju Xu group (C), Zu San Li group (D), Xia Ju Xu group (E), Yang Ling Quan group (F) and Cheng Jin group (F) , 10 in each group, half males and half females.

1.2 Major reagents and instruments

1.2.1 5% 2-4-6 trinitrobenzene sulfonic acid (TNBS) was from sigma company, USA; absolute alcohol was from Shanghai Sinopharm Bio; Ethylurethanm was from Shanghai Shan Pu Chemical Company; Rat HMGB1 ELISA kit was from Wuhan Huamei Biological Engineering Co.,Ltd.; Protein enzyme-inhibitor was from Merck company; BCA protein assay kit was from Wellbio company, HRP goat anti-mouse IgG and HRP goat anti-rabbit IgG were from Protein Tech company; Reverse transcription kit was from Fermentas company; Tris, EDTA, E.B.Sybgreen PCR Mix and DEPC were from Sigma company, USA, Trizol was from Invitrogen company; DL2000DAN marker, dNTP and Taq polymerase were from Genstar Corporation; Primer was from Shanghai Biological Engineering Co. Ltd.

1.2.2 Major instruments: the full set of animal surgery apparatus and CBV-1500A aseptic table were from Shanghai Rui Yang purification equipment Co. Ltd.; needles (size 0.3mm*25mm) and SDZ-V electronic acupuncture device were from Suzhou Medical Supplies Co. Ltd.; TGL-16 Benchtop High Speed Refrigerated Centrifuge was from Xiang Yi centrifuge machines Co., Ltd.; Automatic microplate washer PW-812 and MB-530 microplate reader were from

HMGB1:high mobility group box 1; α7 nAChR :alpha7 nicotinic acetylcholine receptor; NF-κB :nuclear factor kappa-B; EA:electro-acupuncture; UC :ulcerative colitis; TNBS :2-4-6trinitro-benzene-sulfonic acid ; ECL :chemiluminescence; RT-PCR :reverse transcription polymerase chain reaction

Shen Zhen Hui Song Technology Development Co. Ltd.; THZ-C constant temperature oscillator was from Taicang Qiang Le Experimental Equipment Co.Ltd.; TS-92-type shaker was from Qi Lin Bei Er Instrument Manufacturing Co., Ltd.; Electrophoresis apparatus (Cat. no.164-5050) was from Bio-rad company; DYCZ-24EN type electrophoresis tank and DYCZ-40A Transfer apparatus were from Beijing Liuyi Instrument Factory); 5810R Refrigerated Micro Centrifuge was from Eppendorf company; Fluorescence quantitative PCR machine and constant temperature water bath machine were from Thermo Corporation; another electrophoresis apparatus, horizontal agar sugar electrophoresis tank and ultra-low temperature freezer were from Zhong Ke Meiling; M199-1 slicer was from Leica company, Germany; CX41-72C02 microscope was from Olympus Corporation, Japan.

1.3 Modelling method

By improvement of a modelling method from literature[7] we made this model: fasting but drinking free 24 hrs before modelling, then intraperitoneal injection of 25% urethane was given to the animal for anesthesia purpose (0.5mL/100g), afterwards, an intragastric administration needle was inserted gently into 8cm depth of the intestine from the anus, through which 0.85mL of 50% alcohol solution (it contains 30mg trinitrobenzene sulfonic acid (2.5% TNBS 0.6mL)) was injected; right after, the anus was clenched quickly to prevent the drug overflow. After 5min the rat was sent back, lying supinely in cage. After waking up it was fed normally. Then, during the normal feeding any abnormal activities such as lazy move, humping up back, decreased appetite, increased defecation, soft or loose stool, mucus at the end of defecation, were recorded and marked with 1 (having such activity) or 0 (having not such activity) point. The total point equal or more than 2 were regarded as successful modelling.

1.4 Therapeutic method

1.4.1Process of animals

Animals in the blank control were administered saline instead of TNBS/ethanol solution during the modelling stage, with nothing else "treatment". 24 hrs after modelling, the rats in model group were fixed on special rat board for 30 min, with nothing else "treatment"; similarly, rats in other groups were also fixed on special rat board for 30 min but treated with EA on corresponding acupoints on double sides, for consecutive 10 days. The process of animals was rigidly in accordance with *Ethical Issues in Animal Experimentation 2009*[8].

1.4.2Location of acupoints and EA method

Methods of acupoints location refer to *Experimental Acupuncture*[9] (by Zhong-Ren LI) and *Acupoints Map for Experimental Animals*[10] (by Xing-Bang HUA) and were based on anthropomorphism. Shang Ju Xu (ST37) :located 5mm right under the "Hou San Li" point on the hind legs, EA vertically piercing into 7mm; Zu San Li(ST36): posterolateral side of knee, around 5mm under the fibular head, EA vertically piercing into 5mm; Xia Ju Xu(ST39): 15mm under tibial lateral condyle on the outside of the knee, EA vertically piercing into 5mm; Yang Ling Quan (GB34): 5mm of superolateral side of Hou San Li, EA vertically piercing into 7mm; Cheng Jin (BL56): in the middle of Gastrocnemius muscle belly on the back side of hind legs, EA vertically piercing into 7mm. The EA apparatus was SDZ-V type, Hua Tuo brand, by being adjusted the parameters to alternate wave (10/50Hz), positive left and negative right, current strength regulated between 1~3mA, until mild shaking seen in limbs. Needles were kept for 30min, once per day.

1.5 Sampling and test

1.5.1 Sample collection and process: after the intervention, on the eleventh day, the rats were anesthetized by 25% urethane (0.5ml/100g), then were laparotomized immediately and were taken blood through abdominal aortic to EP tubes. The EP tubes were then placed in benchtop high-speed refrigerated centrifuge at 4 ℃, 4000 r/min for 15 min. The supernatant was stored in refrigerator at -70 ℃. Soon after the blood sampling an eight cm length of intestinal tissue was taken from the proximal to the distal end. The intestinal tract was cut open via the longitudinal axis of mesenteric, washed by icy saline once and divided into three parts. The weight for each

part was around 150mg. One part was fixed by 4% paraformaldehyde and the other two were kept in refrigerator at -70 ℃.

1.5.2Observation under light microscopy: Specimens, after being dehydrated, embedded, sliced and H&E Staining, were observed under light microscopy.

1.5.3 Serum HMGB1 assay: ELISA method was employed to measure the value of serum HMGB1 as follows:① adding sample after establishment of the standard curve. Adding 100μL serum to each well, then sealing plate and putting in incubator at 37 ℃ for 120min; ② washing the plate for five times and then adding 50μL of the first antibody solution, after vortex mixing sealing plate and putting in incubator at 37 ℃ for 60min; ③ washing the plate for five times and then adding 100μL of HRP working solution to each well, mixing and sealing plate and putting in incubator at 37 ℃ for 60min; ④ washing the plate for five times and then adding substrate solution 100μL to each well, mixing and sealing plate and putting in incubator at 37 ℃ for 10min; ⑤ adding 50μL of stop solution to each well , mixing, and measuring the absorbance value at the wavelength of 450nm. HMGB1 content of the sample was calculated by the standard curve.

1.5.4 α7 nAchR test: The Western Blot method was employed to detect the expression of α7 nAchR in tissue cells, by steps as follows: took around 0.1g tissue sample, after adding lysate solution, mashing, homogenizing, cracking, centrifugation and deposit we got the protein. According to the instruction of BCA protein assay kit (Wellbio), protein concentration was determined. The process was as follows: took total protein 50 to 100ug for each sample, mixed with 5*loading buffer, quickly cooled down after being cooked in boiling water for 5min, added samples, did SDS-PAGE electrophoresis (concentrated gel voltage 80v and separation gel voltage 120v), did transmembrane about 2hr, blocked with 5% nonfat dry milk (prepared withTBS-T); added the primary antibody and incubated under 4 ℃ overnight, washed by TBS-T for three times (15min for each time). Diluted the secondary antibody 1:3000 in antibody buffer and shaking incubated with the membrane at 37 ℃ for 60 min, washed by TBS-T for three times (15min for each time). Incubated the membrane with chemiluminescence (ECL) for 3min, X film exposed, after development, photographic fixing and scanning the results were recorded. The target bands on scanned images were analyzed by Image-Pro-Plus software. The grayscale ratio of α7 nAchR and NA/K ATP enzyme was regarded as the quantitative values of ralative expression of α7 nAchR.

Table 1 Primer sequences used for NF-kB and β-actin in RT-PCR

Primer		Sequence	Amplified fragment size
NF-κB	NF-κB-F	5’- CCCATCGGGTTCCCATAAAG -3’	146bp
	NF-κB-R	5’- GCCTGAAGCAAATGTTGGCGTA -3’	
β-actin	β actin-F	5’- CATCCTGCGTCTGGACCTGG-3’	107bp
	β actin-R	5’-TAATGTCACGCACGATTTCC-3’	

1.5.5 Test of colon NF-kB mRNA: RT-qPCR method was employed to detect the NF-kB mRNA expression in colonic samples by the following steps: the total RNA was extracted according to the instructions of TRIzol reagent kit; the purity of total RNA was tested by agarose gel electrophoresis (AGE); the complementary DNA (cDNA) was produced from the total RNA template via reverse transcription according to the kit instructions. The first strand cDNA was synthesized via reverse transcription in the ordinary PCR instrument. The total reaction volume was 20μL, then 1μL of reaction products was used as template, through SYBR method, to prepare 30μL of PRC system on ice according to the quantitative real-time PCR kit instructions. The conditions of RT-PCR were:95 ℃ initial denaturation 10min, 95 ℃ denaturation 10s, 59 ℃ annealing / extension 50s; amplification for 40 cycles and melting curve acquisition at 60 ℃ -95 ℃,identification of products purity according to the average melting temperature in

the melting curve. If the products were specific, then there were no impure peaks in the melting curve, the average melting temperature was consistent. The Ct value of β-actin was used for calibration of Ct value of target gene. The difference of target gene mRNA transcription levels was evaluated by $2^{-\Delta\Delta C}$ method. The β-actin was used as an internal control, to search the target gene sequence in NCBI. The primer 5 software was used for design the primer sequences of NF-kB and β-actin by Nanjing GenScript company. The amplification primers were listed in table 1.

1.6 Statistics

The data were expressed by ($\bar{X}$ ±s) format and tested normal distribution first. Serum HMGB1, α7 nAChR and NF-kB mRNA were in normal distribution and had homogeneity of variance. One-way ANOVA (LSD method) was used for comparison of data in multiple groups. All data were processed by SPSS 17.0 statistical package. $P<0.05$ was set as the criterion of significant difference.

2 Results

During the experiment, two rats in the model group (B) died due to perforation; one rat in the Yang Ling Quan group (F) died due to exacerbation, so total 67 rats were included in the final analysis. Each group ten in group A, C, D, E and G, 8 in group B and 9 in group F.

2.1 H&E Staining results

The HE staining results for rat colonic histopathology showed that: the blank control group had intact mucosa, normal submucosal lamina propria and no obvious abnormalities in muscular layer; the model group had mucosal degeneration and necrosis, as well as submucosal lamina propria damage and diffuse infiltration by a lot of inflammatory cells; the Shang Ju Xu group relatively recovered well: basically intact mucosa, no obvious damage in submucosa, and a very small amount of inflammatory cell infiltration; The Zu San Li, Xia Ju Xu and Yang Ling Quan groups recovered moderately: mild or moderate degeneration of mucosa, little damage in submucosa, as well as small amount of inflammatory cell infiltration; the Cheng Jin group had relatively poor recovery: severe mucosal degeneration, irregular arrangement and damage in submucosa, and more inflammatory cell infiltration.

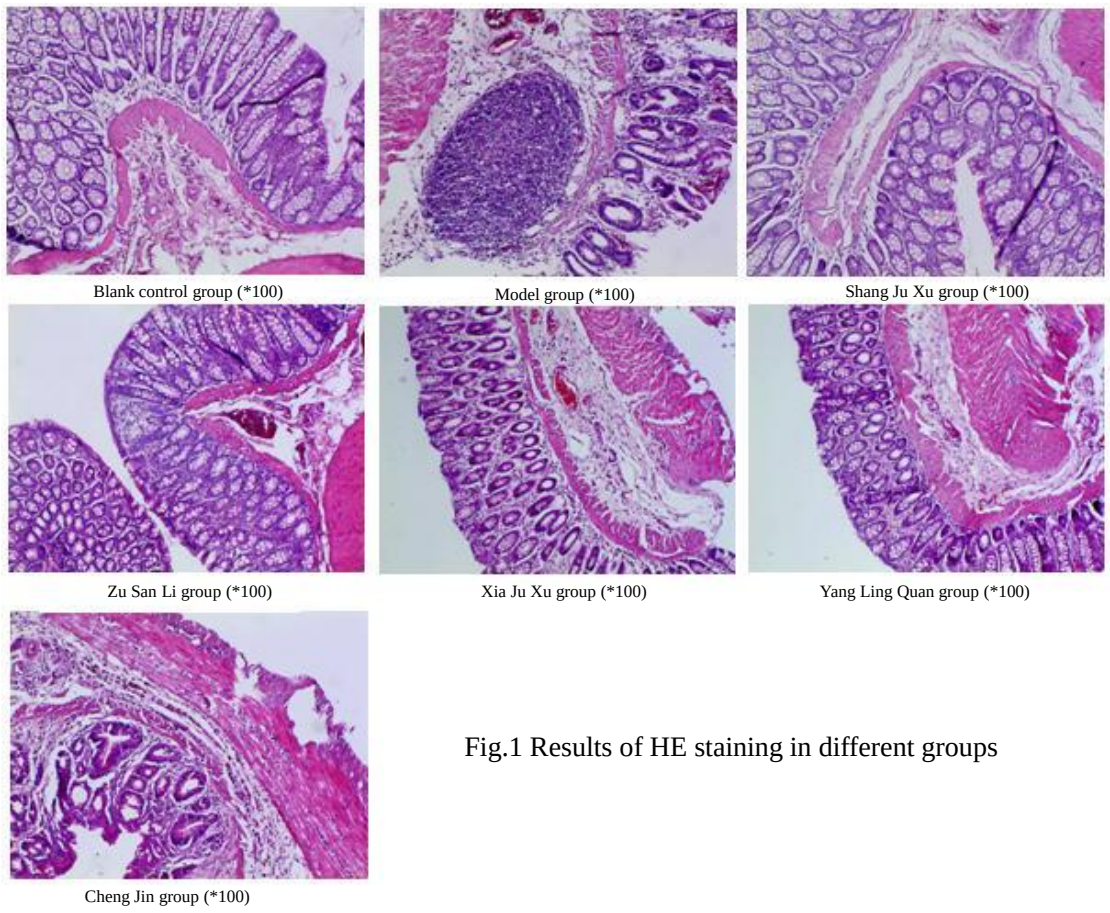

Fig.1 Results of HE staining in different groups

2.2 Comparison of HMGB1 expression in different groups

As shown in table 2, compared with group A, HMGB1 values in other groups were much higher, with statistical significance（P<0.05or P<0.01）; compared with group B, the values of HMGB1 in group C, D and E were lower, with statistical significance（P<0.05or P<0.01）, those values in group F and G were also lower, but had no statistical significance（P>0.05）; compared with group C, values of HMGB1 in other groups were higher, with statistical significance（P < 0.05or P<0.01）; compared with group D, only those in group G got higher, with statistical significance（P<0.01）, those in group E and F had no statistical difference (P>0.05).

Table 2 Comparison of HMGB1 expression in different groups ($\bar{x}$ ±s)

Group	n	HMGB1（pg/ml）
Blank control (A)	10	210.57±23.50
Model(B)	8	326.70±41.26★★
Shang Ju Xu(C)	10	241.35±37.16★▲▲
Zu Shang Li(D)	10	275.66±30.43★★▲▲•
Xia Ju Xu(E)	10	294.61±38.94★★▲••
Yang Ling Quan(F)	9	297.32±28.98★★••
Cheng Jin(G)	10	315.39±29.14★★••■

Note: compared with group A, ★P<0.05,★★P<0.01; compared with group B,▲P<0.05,▲▲P<0.01; compared with group C,•P<0.05,••P<0.01; compared with group D,■P < 0.05.

2.3 Comparison of α7 nAChR expression in different groups

As shown in table 3, compared with group A, except for group C, the values of α7 nAChR in other five groups were lower, with statistical significance（P < 0.01）, the α7 nAChR value in group C also lower but had no statistical significance（P>0.05）; compared with group B, the α7 nAChR values in the four lower He-sea point groups were higher, with statistical significance（P < 0.01）, α7 nAChR value in group G also increased, but had no statistical significance（P>0.05）; compared with group C, α7 nAChR values in group E, G and F had decreased, with statistical significance（P < 0.01）, group D had no statistical significance（P>0.05）; compared with group D, E and F, α7 nAChR value in group G was lower, with statistical significance（P<0.05or P < 0.01）.

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Table 3 Comparison of α7 nAChR expression in different groups ($\bar{x}$ ±s)

Group	n	α7 nAChR（gray value）
Blank control (A)	10	0.75±0.16
Model(B)	8	0.31±0.07★★
Shang Ju Xu(C)	10	0.70±0.19▲▲
Zu Shang Li(D)	10	0.59±0.16★★▲▲
Xia Ju Xu(E)	10	0.50±0.15★★▲▲••
Yang Ling Quan(F)	9	0.50±0.09★★▲▲••
Cheng Jin(G)	10	0.36±0.07★★••■■◆#

Note: compared with group A, ★★P<0.01; compared with group B,▲▲P<0.01; compared with group C, ••P<0.01; compared with group D, ■■P < 0.01; compared with group E, ◆P < 0.05; compared with group F,#P < 0.05.

2.4 Comparison of NF-kB mRNA expression in different groups

Table 4 Comparison of NF-kB mRNA expression in different groups ($\bar{\chi} \pm s$)

Group	n	NF-κB mRNA
Blank control (A)	10	1.09±0.19
Model(B)	8	3.47±0.26★★
Shang Ju Xu(C)	10	1.80±0.33★★▲▲
Zu Shang Li(D)	10	1.92±0.40★★▲▲
Xia Ju Xu(E)	10	2.56±0.25★★▲▲●●■■
Yang Ling Quan(F)	9	2.45±0.17★★▲▲●●■■
Cheng Jin(G)	10	2.67±0.33★★▲▲●●■■

Note: compared with group A,★★P<0.01; compared with group B,▲▲P<0.01; compared with group C, ●●P<0.01; compared with group D, ■■P < 0.01.

As shown in table 4, compared with group A, the NF-κB mRNA values in other groups were higher, with statistical significance（P<0.01）; compared with group B, the NF-κB mRNA values in other "treatment groups" were lower, with statistical significance（P<0.01）; compared with group C, the NF-κB mRNA values in group E, F and G were higher, with statistical significance（P < 0.01）, but group D had no statistical difference（P>0.05）; compared with group D, the NF-κB mRNA values in group E, F and G were higher, with statistical significance（P < 0.01）.

3 Discussion

Ulcerative colitis (UC) belongs to the Li Ji disease in Chinese medicine. It is a common large intestinal Fu-disease. According to the "curing viscera diseases by He-sea points", He-sea points should be taken as a routine to treat inner Fu-disease (compared to outer Jing-disease). Shang Ju Xu (ST37) is the lower He-sea point of large intestine, so the ancient practitioners attached more importance to take Shang Ju Xu to treat colonic diseases, and it has been proved the point had relatively specificity for inflammatory bowel disease[1,11]. In this study, we selected some lower He-sea points, which belong to either the same nerve segment, or the same meridian, as the control to Shang Ju Xu, by using electro-acupuncture, to observe their effects to UC, so that to understand if Shang Ju Xu had specific effects on large intestinal disease. This would be an interesting effort to test the scientific connotation of the classic theory that "curing viscera diseases by He-sea points".

It has been repeatedly proved that acupuncture could treat diseases due to immune disorders by regulation of the Neuroendocrine Immune Network[12]. Some studies also pointed out that the effects of acupuncture on inflammatory diseases might be realized by activation of the cholinergic anti-inflammatory pathway[13]. Alpha 7 nAChR plays a crucial role in regulating the production of inflammatory factors during the inflammatory reaction[14], it gradually becomes the pharmaceutical target for regulation of the secretion of High mobility group protein B1 (HMGB1) as well as cholinergic anti-inflammatory effect[15], serving as one of the "cross" molecule between the cholinergic nerve and immune system[16,17]. As one of the important advanced inflammatory mediators, HMGB1 has double regulation effects on both inflammation and immune system[18]. Studies have shown that HMGB1 could upregulate the α7 nAChR expression in PC 12 cells by a time and dose-dependent way, thus could influence expression of some important functional receptors in nerve cells[19]. NF-kB activation is the common passage behind action mechanism of many inflammatory mediators. During a disease process, NF-kB is activated quickly, then enters the cell nucleus and combines with the promoter or enhanser on the target gene, which can further regulate genetic transcription, activate immune response. NF-kB mRNA can partially reflect the activation extent of NF-kB. Based on above-mentioned, we selected those indexes for comparison study so that to explore the specificity of lower He-sea point of large intestine on

ulcerative colitis.

In this study, we found that after modeling the serum HMGB1, tissue NF-kB mRNA expressions were higher, but α7 nAChR was lower than those in blank control group, besides, some damage changes had been observed in colonic tissue under light microscope, suggesting the model has been successfully made (initiated inflammatory reaction, active immune response). In this study, EA on all the five points could repair the colonic tissue and down-regulate the NF-kB mRNA expression on certain extent; Shang Ju Xu (ST37) , Zu San Li (ST36) and Xia Ju Xu (ST39) could promote the expression of α7 nAChR and down-regulate the HMGB1 content; Yang Ling Quan (GB34) could also promote the expression of α7 nAChR, indicating that EA could more or less treat UC by suppressing immune response and reducing inflammatory reactions. By comparison we know, Shang Ju Xu had better effects on lowering down HMGB1 contents, down-regulating the expression of NF-kB mRNA, promoting the expression of α7 nAChR and repairing colonic tissue injuries than Xia Ju Xu, Yang Ling Quan and Cheng Jin; also the HMGB1 values in Shang Ju Xu group were significantly lower than that in Zu San Li group. Those indicated that Shang Ju Xu had the best and specific effects on treating UC. Further, Zu San Li had relatively better effects than Xia Ju Xu, Yang Ling Quan and Cheng Jin.

"Large intestine and small intestine belong to stomach the Zu Yang Ming", this provided the theoretical basis for Zu San Li on treating large intestinal diseases. In the *Soul Box for Acupuncture ·Four seasons* it recorded, "when the evil Qi is in large intestine, Qihai, Shang Ju Xu and Zu San Li should be selected for acupuncture", showing that Shang Ju Xu and Zu San Li are a common acupoint set for treating large intestine diseases. By literature review[20], Zu San Li and Shang Ju Xu are on the list of top five most frequently selected points for treating inflammatory bowel disease (IBD). There are also numerous reports on the combination use of Zu San Li and Shang Ju Xu for treating large intestine diseases[21]. We therefore could easily understand why Zu San Li in this study also had good effects on UC.

To conclude: ①EA on all digestion-related lower He-sea points, as well as Cheng Jin point could effectively treat UC, based on the regulation of HMGB1, α7 nAChR and NF-kB mRNA and subsequent suppression of immune response and reduction of inflammatory reactions; ② Shang Ju Xu had better effects than other points, suggesting that Shang Ju Xu had relative specificity on UC, which provides a good example on the specific relation between lower He-sea point and Fu-organ.

Competing interests

The authors declare that they have no competing interests.

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