Supplement 1

This supplement contains the following items:

1. Original protocol
2. Final protocol
3. Updates on original protocol
4. Final statistical analysis plan

ORIGINAL PROTOCOL

Electro-acupuncture Versus Prucalopride for Severe Chronic Constipation: A Non-inferiority, Multicenter, Randomized Controlled Trial

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1. Guang'anmen Hospital, China Academy of Chinese Medical Sciences;
2. Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine;
3. West China Hospital of Sichuan University;
4. The Third Affiliated Hospital of Zhejiang Chinese Medical University;
5. Hengyang Hospital Affiliated to Hunan University of Chinese Medicine;
6. Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University;
7. The Affiliated Hospital of Shandong University of Traditional Chinese Medicine;
8. The First Hospital of Hunan University of Chinese Medicine;
9. Hubei Provincial Hospital of Traditional Chinese Medicine;
10. Jiangsu Province Hospital of Traditional Chinese Medicine;
11. Shaanxi Province Hospital of Traditional Chinese Medicine;
12. Qingdao Hiser Medical Group;
13. Guangdong Province Hospital of Traditional Chinese Medicine;
14. Wuhan Hospital of Traditional Chinese and Western Medicine.

Data Coordinating and Statistical Centers:

Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences

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2. Study Design

2.1 Study Overview

The objective of this trial is to compare the effect of Electro-acupuncture (EA) versus prucalopride for severe chronic constipation (SCC). The efficacy and safety of EA will be simultaneously evaluated.

2.2 Background

Constipation affects approximately 12% of people around the world[1] with the prevalence of 4-6% in China[2,3]. Constipation occurs more commonly in women than men and its incidence increases with age[4,5]. Severe chronic constipation (SCC) is defined as two or fewer complete, spontaneous bowel movements (CSBMs) per week, hard stool and a sensation of straining during defecation[6]. Laxatives are used for constipated patients; however, a high level of dissatisfaction has been reported for laxatives[6]; and only 1/4~2/3 patients with chronic constipation was satisfied with laxatives[7,8]. It is generally believed that western medicine lacks sustained effect, that is, the symptoms of constipation often reappears after the treatment is stopped. One trial was searched reporting the sustained effects by using PEG 3350[9]. After stopping the PEG 3350 for 38.4±14.1 days, 61.7% patients regained the symptoms of constipation and reapplied laxatives[9]. One European survey of chronic constipation revealed that almost half were using alternative treatments (homeopathy, massage and acupuncture), and nearly 90% of respondents expressed interests in new therapies [9]. Acupuncture were used for chronic constipation widely for thousands of years. A systematic review for constipation manifested that acupuncture was safe for chronic functional constipation and might improve weekly spontaneous bowel movements (SBMs), the quality of life, and relevant symptoms. However, the evidence was limited by the small sample size and the methodological quality[10].

A pilot study (n = 60) we have finished showed that 31.67% patients had 3 or more weekly complete spontaneous bowel movements (CSBMs) over 8 weeks of treatment, and 40% patients had 3 or more weekly CSBMs over 12 weeks of follow-up, which reflected the sustained effect of acupuncture (to be published). This is a protocol of a phase-II trial aiming to compare the efficacy and safety of electroacupuncture (EA) versus prucalopride for severe chronic constipation. Prucalopride was a high-selected 5-HT_4 receptor agonist, which

could shorten the colonic transit time, and was recommended by the World Gastroenterology Organization for patients with SCC for whom previous laxative use failed to provide satisfactory relief[11]. It was also considered effective and safe in a randomized controlled trial conducted in the Asia-Pacific region[12]. Thus, the use of prucalopride as the control drug in this trial is reasonable.

2.3 Study Objective and Hypothesis

The objective of this trial is to evaluate if the effect of EA is non-inferior to prucalopride for SCC. In addition, If the therapeutic efficacy of EA could sustain for 3 months or 6 months? The safety and acceptance of EA will be simultaneously evaluated.

Hypothesis: EA is non-inferior to prucalopride for severe chronic constipation over weeks 3-8.

2.4 Methodology

2.4.1 Trial Design

This is a prospective, non-inferior, randomized, controlled trial conducted in 14 sites: Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, West China Hospital of Sichuan University, The Third Affiliated Hospital of Zhejiang Chinese Medical University, Hengyang Hospital Affiliated to Hunan University of Chinese Medicine, Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, The First Hospital of Hunan University of Chinese Medicine, Hubei Provincial Hospital of Traditional Chinese Medicine, Jiangsu Province Hospital of Traditional Chinese Medicine, Shaanxi Province Hospital of Traditional Chinese Medicine, Qingdao Hiser Medical Group, Guangdong Province Hospital of Traditional Chinese Medicine, Wuhan Hospital of Traditional Chinese and Western Medicine. All the acupuncturists in each site are required to have an official license and clinical work experience no less than 2 years.

2.4.2 Sample Size Calculation

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3-8. According to our previous 60-size pilot study, the proportion was 31.67% by EA; and the proportion by prucalopride was 30.9%[6]. The non-inferiority sample size calculation formula is listed as below:

$$n = \frac{(Z_{1-\alpha} + Z_{1-\beta})^2[p_1(1-p_1) + p_2(1-p_2)]}{(\varepsilon - \delta)^2}$$

$$= \frac{(1.645 + 0.845)^2[0.3167 \times (1 - 0.3167) + 0.309 \times (1 - 0.309)]}{[0.3167 - 0.309 - (-0.1)]^2} = 230$$

To assess the non-inferiority between the treatment and control groups, a sample size of 276 for each group will be sufficient, with a non-inferior margin of -10%, one-sided 5% level of significance, a power of 80% and allowing for a 20% dropout. In this trial, we aim to recruit 560 participants considering the balance of 14 centers, with 40 participants of each center.

2.4.3 Randomization

After the baseline assessment has been carried out and the informed consent has been obtained, eligible participants will be randomized, stratified by centers, to the EA group or the prucalopride group with the ratio of 1:1. The random numbers will be obtained by the acupuncturists through the Central Randomization System by phone or website. Randomization will be performed centrally with varied blocks by the Clinical Evaluation Centre of the China Academy of Chinese Medical Sciences in Beijing using the PROC PLAN program of SAS 9.3 software, and the random scheme generator will not take any part in the statistical analysis. The randomizing scheme and related parameters was called the blind codes, which will be sealed by the generator and stored by a special researcher. Except for the most senior system administrator, no one has the access to the random scheme in the central random system.

2.4.4 Blinding

The statisticians, outcome assessors, and data managers will be blinded to the allocation. Nurse or postgraduate (outcome assessors) from each site who know nothing about the assignment will take charge of the outcome assessment. And the statistician who knows nothing about assignment from the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences will be responsible for the statistical analysis.

2.4.5 Participants

Particinants with SCC met the Rome III criteria[13] are planned to be recruited via advertisements in newspapers, television, websites and posters in hospitals.

2.4.5.1 Inclusion Criteria:

1) Primary chronic constipation meeting the Rome III criteria[13], with ≤2 mean weekly CSBMs (CSBM refers to the bowel movement without the help of drug or hands within the past 24 hours with the feeling of complete evacuation. The bowel movement beyond 24 hours after the use of medicine/hands also could be considered an SBM) in the past three months. They should conform to at least one of the following items at the same time: a) a feeling of straining during evacuation (at least once per 4 bowel movements); b) lumpy or hard stools (at least once per 4 bowel movements); c) a feeling of incomplete evacuation after a bowel movement (at least once per 4 bowel movements); d) a feeling of obstruction in the anus/rectum (at least once per 4 bowel movements); e) needing the help of hands for evacuation (at least once per 4 bowel movements).
2) Conditions for the diagnosis of irritable bowel syndrome (IBS) are inadequate.
3) Previous laxative use failed to provide satisfactory relief.
4) Participants aged 18 to 75 years old.
5) Two or fewer CSBMs per week during the 2-week long baseline assessment (run-in) period.
6) No use of medicine for constipation during the 2 weeks before enrolment (except rescue medication usage); no acupuncture treatment for constipation in the previous 1 month; no participation in any other on-going trial.
7) Volunteered to join this trial and signed the informed consent.

2.4.5.2 Exclusion Criteria:

1) Irritable bowel syndrome, organic constipation or secondary constipation caused by endocrine, metabolic, nervous, postoperative diseases, or drugs;
2) More than one mushy or watery stool during baseline while not taking laxatives (Bristol stool type 6 or 7);
3) A history of pelvic floor dysfunction;
4) Taking medicine that can influence the intestinal function or induce constipation (However, during the trial, participants are allowed to take drugs for other diseases which may affect the evaluation of the efficacy, the usage of the drugs should be recorded in detail)
5) Severe haemorrhoid or anal fissure;
6) Participants with severe cardiovascular, hepatic or renal diseases, cognitive impairment, abdominal aortic aneurysm or hepatosplenomegaly, aphasia, mental disorders, or illness that will influence the examination or treatment;
7) Women in the gestation or lactation period;
8) Participants with blood coagulation disorders or taking anticoagulants regularly, such as
Warfarin and Heparin;
9) Participants with a cardiac pacemaker carrier.

*Notes for the screening and baseline evaluation (run-in) period: ① Medical history and physical examination were needed. Secondary constipation should be noticed if any symptom or sign of medical history suggests that constipation may be secondary; ② Colonoscopy is recommended for participants ≥50 years; ③ Colonoscopy may also be needed if any of the following alarming signs or/and symptoms occurs in any age: gastrointestinal bleeding, anemia, progressive weight loss, abdominal mass, significant abdominal pain, history of colon polyps especially in participants with family history of colorectal cancer; ④ The stool routine test and occult blood test must be done in each prospective case; ⑤ Baseline assessment (run-in) for 2 weeks: participants should discontinue laxatives or other medications and measures for constipation (except for rescue medicine) and maintain the usual diet and lifestyle. During baseline assessment (run-in) period, participant's gender, age, course of constipation, previous treatment for constipation, bowel movements, spontaneous defecation or not, complete evacuation or not, stool consistency, degree of straining, rescue medicine usage will be recorded

2.4.5.3 Subject Withdrawals

The Subjects may leave the study at their own discretion, or the experts from the gastrointestinal or anorectal department in each site may determine, for the sake of participants' health, to discontinue participation (due to worsening of symptoms, or the occurrence of a serious adverse event).

2.4.6 Interventions

2.4.6.1 Acupuncturists and Apparatus

Acupuncturists in each site need to have an official license and at least two-year clinical experience. Before conducting the trial, acupuncturists will be trained by principle investigator.
Huatuo disposable needles (size 0.30 mm×40mm, 0.30 mm×50mm, 0.30mm×75mm, Suzhou Medical Appliance, Suzhou, Jiangsu Province, China), and Huatuo electro-acupuncture apparatus (type SDZ-V, Suzhou

Medical Appliance, Suzhou, Jiangsu Province, China) will be used.

2.4.6.2 Electro-acupuncture Group

Acupoints:

Bilateral Tianshu (ST 25), Fujie (SP 14), Shangjuxu (ST 37). Moreover, BL33, DU20 and DU24 could be used according to the individual situation; BL33 were used for severe straining if any, DU20 and DU24 could be used for participants accompanied with the symptoms of anxiety and depression.

Location:

According to the WHO Standardized Acupuncture Points Location[14].

Manipulation:

After sterilizing the skin in participants with supine position, needles of the size of 0.30mm×50mm, or 0.35mm×75mm will be inserted into ST25 and SP14 slowly and vertically, without manipulation, for approximately 30-70mm until they pierce into the muscle layer of the abdominal wall. Needles of the size of 0.30mm×40mm will be inserted in to ST37 vertically for about 25mm. If BL33 is needed, all points could be simultaneously needled when participants are in the lateral position. The site 0.5-1cm outside and above the 3rd posterior sacral foramina will serve as the inserting site. The needle is inserted at the inserting site inwardly and downwardly at an angle of 30-45 degree to a depth of 50–60mm. For DU20 and DU24, the needle is obliquely inserted back towards the galea aponeurotica to a depth of 40mm.

Paired alligator clips of the EA apparatus will be attached transversely to the needle holders of the bilateral ST25 and SP14 (and BL33 if needed). EA stimulation will last for 30 minutes with a dilatational wave of 10/50 Hz and current intensity of 0.1-1.0mA (for BL33, the intensity is 0.5-2.0 mA). The skin around the acupoints shivering mildly indicates the proper dose. For ST37 (and DU20, DU24 if needed), three small equal manipulations of twirling, lifting and thrusting will be performed. The participants' feeling of sore and distention show the proper manipulation of deqi. The twirling, lifting and thrusting manipulation should be performed every 10 minutes, three times in 30 minutes.

Course of treatment:

Each participant will accept an 8-continuous-week treatment with 28 sessions in total. The participants will be treated 30 minutes once, once a day, five times per week in the first two weeks, and three times per week in the latter 6 weeks.

2.4.6.3 Prucalopride Group

Prucalopride Succinate (Janssen S.P.A) will be taken orally at a dose of 2 mg/day before breakfast for 8 weeks. Participants will undergo an electrocardiogram (ECG) at the 8th
week, and the drug will be taken for another 24 weeks in the absence of QT interval elongation or other severe cardiac adverse events (like myocardial infarction, severe arrhythmia, etc.). The use of other medicine for constipation will not be allowed during the trial. Prucalopride will be distributed to the participants beforehand every 2 weeks in the treatment period, and every 4 weeks in the follow-up period. The untaken pills will be recycled on the day of next distribution.

2.4.6.4 Rescue Medicine

Bisacodyl (5–10 mg) and glycerin enema (110 ml) can be used for participants who fail to have a bowel movement for 3 or more consecutive days. Furthermore, the date, specific time, dose of bisacodyl or glycerin enema using should be recorded in the defecation diary and case report form (CRF).

Other drugs or measurements for constipation are not allowed; However, details of the date, specific time, dose should also be recorded in the defecation diary and CRF if used. Rescue medicine is available during the whole study period (baseline assessment [run-in], treatment, and follow-up); nevertheless, it is not allowed 48 hours before and after the first treatment, no matter EA or prucalopride, in order to evaluate the time to the first spontaneous defecation. Rescue medicine will be distributed to the participants beforehand every 2 weeks in the treatment period, and every 4 weeks in the follow-up period.

2.4.6.5 Research Period

The whole research period is 34 weeks, including 2-week baseline assessment（run-in） period (week -2, week -1), 8-week treatment period (weeks 1–8), and 24-week follow-up period (weeks 9–32). The time frame of the trial is presented in Figure 1.

Outcome assessing points: 1) baseline assessment (run-in) period: the last day of week -2 and week -1 (baseline evaluation); at week 0, the randomization and the first treatment will be carried out in one day. 2) Treatment period: the last day of week 2, week 4, week 6, week 8. 3) Follow-up period: the last day of week 12, week 16, week 20, week 32.

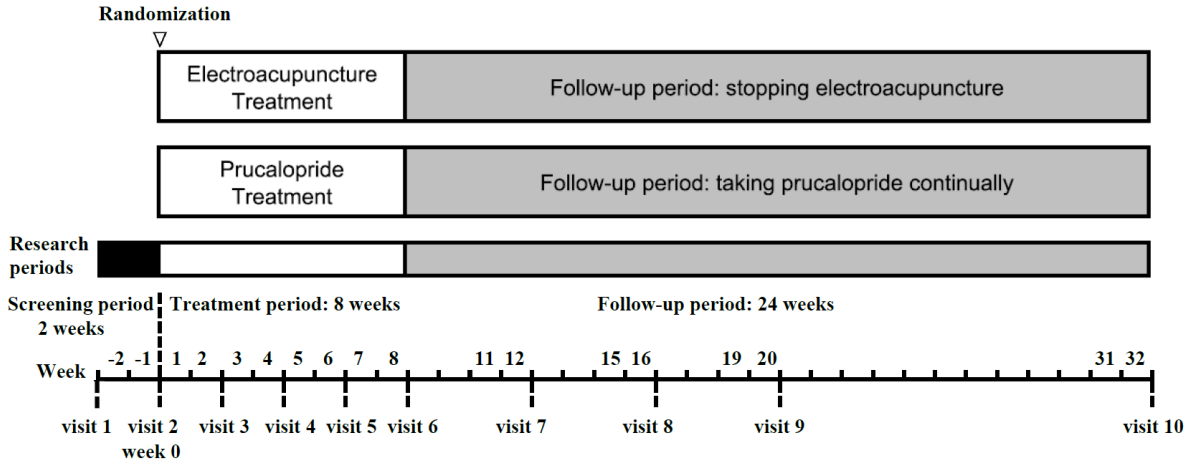


Figure 1 Research period and assessing points

2.4.7 Subject Evaluations and Outcomes

2.4.7.1 Baseline Evaluations

Outcomes contain the complete spontaneous bowel movements (CSBMs), spontaneous bowel movements (SBMs), stool consistency (according to Bristol Stool Scale), straining during the defecation, rescue medicine used, and the form of Chinese-version Patient Assessment of Constipation Quality of Life (PAC-QOL). Participants will record their bowel movements, constipation-related symptoms, medicine used for constipation every day through week -2, week -1, weeks 1-8, weeks 11-12, weeks 15-16, weeks 19-20, and weeks 31-32. In both groups, participants without bowel movements (BMs) for 3 or more consecutive days were allowed to use bisacodyl (5-10mg a day before bedtime, Boehringer Ingelheim) or 110 ml glycerol enema as rescue medicine with documentation (time, dosage, and frequency of use) in the stool diary.

The SBMs are the bowel movements that occur in the absence of rescue medicine, or other assistant methods (laxatives, enemas, suppositories usage, or digging out by fingers) within the preceding 24 hours, which include the CSBMs. When participants used rescue medicine, or other measures for constipation, the defecation within 24 hours was considered a non-spontaneous bowel movement, whereas defecation exceeding 24 hours was deemed an SBM. CSBMs were the SBMs with the sensation of complete evacuation.

Outcome record time point: Baseline (week 0), week 2, week 4, week 6, week 8, week 12, week 16, week 20, and week 32 (Figure 1).

Outcome assessing time frame: baseline period (weeks -2 to week -1), the latter 6-week treatment (weeks 3-8), the first 2-week treatment (weeks 1-2), and the follow-up periods (weeks 9-32).

2.4.7.2 Primary Outcome

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3–8. Assessing time frame: the latter 6 weeks of treatment (weeks 3-8).

2.4.7.3 Secondary Outcomes

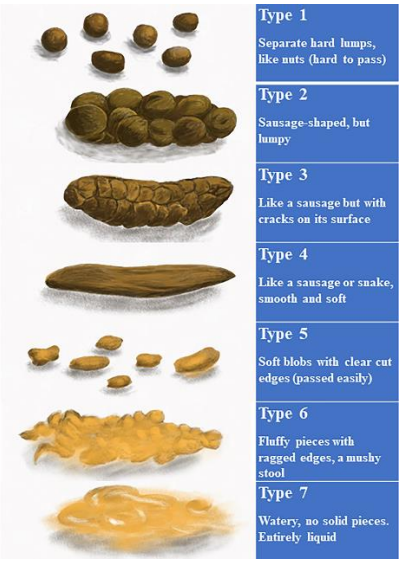
1) The proportion of participants with 3 or more mean weekly CSBMs over weeks 1-2, 9-12, 9-16, 9-20, 9-32. The EA group was discontinued after 8 weeks of treatment, while the drug group continued to take prucalopride for 24 weeks in the absence of QT interval elongation or other severe cardiac adverse events (like myocardial infarction, severe arrhythmia, etc.). The comparison between the two groups can fully illustrate the sustained effect of EA, which might be the superiority of EA. Assessing time frame: weeks 1-2, weeks 9-12, 9-16, 9-20, 9-32.

2) The proportion of participants with ≥1 increase of mean weekly CSBMs from baseline over weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32. Assessing time frame: baseline, weeks 1-2, weeks 3-8, weeks 9-12, 9-16, 9-20, 9-32.

3) the mean weekly CSBMs and the change from baseline in mean weekly CSBMs over weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32. Assessing time frame: baseline, weeks 1-2, weeks 3-8, weeks 9-12, 9-16, 9-20, 9-32.

4) the mean weekly SBMs and the change from baseline in mean weekly SBMs over weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32. Assessing time: baseline, weeks 1-2, weeks 3-8, weeks 9-12, 9-16, 9-20, 9-32.

5) The change from baseline in the mean score of stool consistency of each SBM over weeks 1-2 and weeks 3-8. Assessing time: baseline, weeks 1-2 and weeks 3-8. Participants will self-report their stool consistency of each SBM according to the 7-type BSFS[15] (scored by 1 to 7 respectively).



- Type 1: Separate hard lumps, like nuts (hard to pass)
- Type 2: Sausage-shaped, but lumpy
- Type 3: Like a sausage but with cracks on its surface
- Type 4: Like a sausage or snake, smooth and soft
- Type 5: Soft blobs with clear cut edges (passed easily)
- Type 6: Fluffy pieces with ragged edges, a mushy stool
- Type 7: Watery, no solid pieces. Entirely liquid

6) The change from baseline in the mean score of straining of each SBM over weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32. Assessing time: baseline, weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32. Participants will self-report their straining degree of each SBM in the defecation diaries according to the following scale.

0 = not difficult;
1 = a little difficult, need some straining to defecate;
2 = difficult, need straining to defecate;
3 = very difficult, need hard straining to defecate.

7) Time to the first CSBMs, which was the time from the first treatment to the first CSBMs counting by days. Rescue medicines are not allowed to be used during 48 hours before and after the first treatment. If the first CSBM had not occurred yet, and rescue medicines were used in the condition of no BM in a succession of three or more days, the BM occurs within 24 hours after the use of rescue medicine will not be regarded as CSBM, and the days-counting will continue till the occur of the first CSBM.

8) The change from baseline of the score of PAC-QOL[16] at week 4 and week 8. PAC-QOL is a self-report questionnaire to evaluate the quality of life in participants with constipation, which was distributed by Mapi Research Trust in France. This questionnaire contains 28 items including 4 basic parts of physical discomfort, worries and concerns, psychosocial discomfort, and satisfaction. We use the Chinese version in our trial[16]. Assessing point: baseline, week 4 and week 8.

9) The proportion of participants using rescue medicine (bisacodyl or glycerol enema) and the mean weekly dosage of rescue medicine used ((bisacodyl or glycerol enema) over weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32. Assessing time frame: baseline, weeks 1-2, 3-8, 9-12, 9-16, 9-20, 9-32.

2.4.7.4 Defecation Diaries

Defecation diaries during the treatment period (weeks 1-8):

Participants will record their bowel movements (BMs), spontaneous defecation or not, complete evacuation or not, stool consistency, degree of straining, the time, dosage, and frequency of rescue medicine or other assistant methods used every day from the baseline to the end of week 8. Distribution of the defecation diaries: the diary of week 1 will be distributed at the first treatment of week 1, and the diary of week 2 will be distributed at the last treatment of week 1; the diary of week 1 will be taken back at the first treatment of week 2, and the diary of week 3 will be distributed at the last treatment of week 2; and so on. The diary of weeks 11-12 (follow-up period) will be distributed at the last treatment of week 8.

Defecation diaries during the follow-up period (weeks 11-12/15-16/19-20/31-32):

Participants will record their bowel movements (BMs), spontaneous defecation or not, complete evacuation or not, degree of straining, and the time, dosage, frequency of rescue medicine or other assistant methods used on the diaries every day of week 11-12/15-16/19-20/31-32. Distribution of the defecation diaries: The diaries of weeks 11-12 will be distributed at the last treatment of week 8. Within week 11, participants will get the diaries of weeks 15-16; within week 15, participants should return the diaries of weeks 11-12, and get the diaries of weeks 19-20; within week 19, participants should return the diaries of weeks 15-16, and get the diaries of weeks 31-32; within one week after week 32, participants should return the diaries of weeks 31-32.

For the participants who do not abide by our treatment protocol strictly, the outcome evaluators should still follow them up through phone, text or a visit to record the related items.

2.4.8 Safety Assessment

2.4.8.1 Adverse Events

The adverse events are some unintended symptoms, physical signs, or health conditions that show up during the whole trial. They may have no causality with the interventions. A health condition or an illness existing before the trial can be deemed as an adverse event (AE) if the situation becomes worse within the study period. An abnormal result of the laboratory examination can be deemed as an AE only if it causes some clinical symptoms and need related therapies. We observe the adverse events by asking the participants non-inducing questions at each assessing point. We also observe the adverse events through participants' self-report, or through physical examination, or by laboratory examination. All the adverse events must be record at length on the CRF. If an AE was observed, the following information should be offered:

1. Order of severity (mild, moderate, severe);
2. If it is related to the interventions (affirmably, very likely, possibly, possibly not, affirmably not);
3. Duration of the AE (start and end dates, whether still exists at the end of the trial);
4. Severe adverse effect (SAE) or not.

SAE is defined as any of the following:

1. Fatal or life threatening;
2. Permanent or conspicuous loss of function or disability;
3. Need hospitalization or need prolonged hospitalization;
4. Severe medical accidents, which need medical or surgical interventions to prevent any of the above occurs.

Solution for controlling the adverse events:

Further observing, without any treatment;
Adjusting or stopping the treatment;
Aborting the trial permanently;
Adding combined drugs therapy;
Giving non-pharmacologic therapy;
Hospitalizing or prolonging hospitalization.

All the treatment measures for the adverse events should be recorded in detail on the CRF. We will follow up the adverse event through the whole trial until it is solved. We will confirm that if it is permanent. At every assessing point, we will evaluate the adverse event's severity, relation to our interventions, and the treatment measures. The common adverse events related to the interventions are listed in the investigator's brochure. During the updating of the brochure, related adverse events will be announced by the form of investigator's note (IN). We will also discuss with the participants about the adverse events that might occur during the trial.

2.4.8.2 Report of the Severe Adverse Events

For participants' safety, from the day they sign the informed consent until within 30 days after the end of the study, any SAE no matter if it is related to our interventions should be reported to Rui Ma (Project management office, Acupuncture Department, Guang'anmen Hospital of China Academy of Chinese Medical Sciences; +86 18001252056)、Zhishun Liu (Acupuncture Department, Guang'anmen Hospital of China Academy of Chinese Medical Sciences; +86 13651016313) within 24 hours. SAE occurs over 30 days after the end of the study will not require to be reported, unless it is related to our interventions. The reoccurrence, complication, or the progression of a reported SAE will be reported as the follow-up information of that SAE within 24 hours. All the information of the SAE will be collected and recorded on the form of SAE. Researchers should assess the relation of the SAE with our interventions and fax the finished SAE form to the department of Integrated Medical Safety (IMS) of our project in Guang'anmen Hospital within 24 hours. The SAE form and the fax will be kept together with the CRF in the research center.

Safety Evaluation:

1. Safe, no adverse event, no abnormal examination of the safety index;
2. Relatively safe, mild adverse event with no treatment needed, no abnormal examination of the safety index;
3. Having safety problems, moderate adverse event which needs relative treatment, mildly abnormal examination of the safety index;
4. Withdrawal from the study due to the severe adverse event, or obviously abnormal examination of the safety index.

2.4.8.3 Safety and Acceptance Evaluation

The adverse events should be documented in detail over the treatment and follow-up periods. Participants and acupuncturists will be responsible for the adverse events recording.

Electro-acupuncture Related Adverse Events

Adverse events will be recorded and measured both by participants themselves and acupuncturists. Acupuncturists are responsible for the recording of the EA-related adverse events on the CRF. The adverse

events relating to acupuncture include broken needle, nausea during acupuncture, faint caused by needling during the treatment, unbearable pricking (VAS≥7) caused by needling during the treatment, sharp pain lasting more than one hour (VAS≥4) after acupuncture, hematoma or bleeding around the site of needling, numbness or infection around the site of needling, sleeplessness after acupuncture, dizziness after acupuncture, other discomforts (including palpitation, headache, loss of appetite, drowsiness, or aggravation of existing symptoms) after acupuncture. Acupuncturists will record the symptoms, frequency, degree, and the duration of each AE. The pricking caused by needling will be assessed through 10-point visual analog scale (VAS).

Prucalopride Related Adverse Events

Diarrhea, nausea, abdominal pain, abdominal distention, vomiting, dyspepsia, inappetence, flatulence, abnormal bowel sound, hemoproctia, dizziness, headache, tremor, palpitation, myocardial ischemia/infarction, prolonged QT interval, frequent micturition, fatigue, fever.

Acceptance evaluation of EA:

We will evaluate the acceptability of acupuncture for the EA group. The acceptability of acupuncture will be evaluated through a point system as following:

0= hard to accept;
1= a little hard to accept;
2= acceptable;
3= easy to accept;
4= very easy to accept.

The scores of acceptability will be recorded after 5 minutes of the first and tenth treatment (the score will be summed up and divided by 2). If the participant drops out after the first treatment, then the score recorded of the first treatment will be used.

2.4.9 Compliance Assessment

The adherence of participants towards treatments were counted via the EA sessions or prucalopride tablets they have received. Prucalopride tablets were recycled back from the participants every two weeks in treatment period and every four weeks in follow-up period, and the number of tablets were documents in CRF by outcome assessors.

2.5 Reference

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3. Flowchart

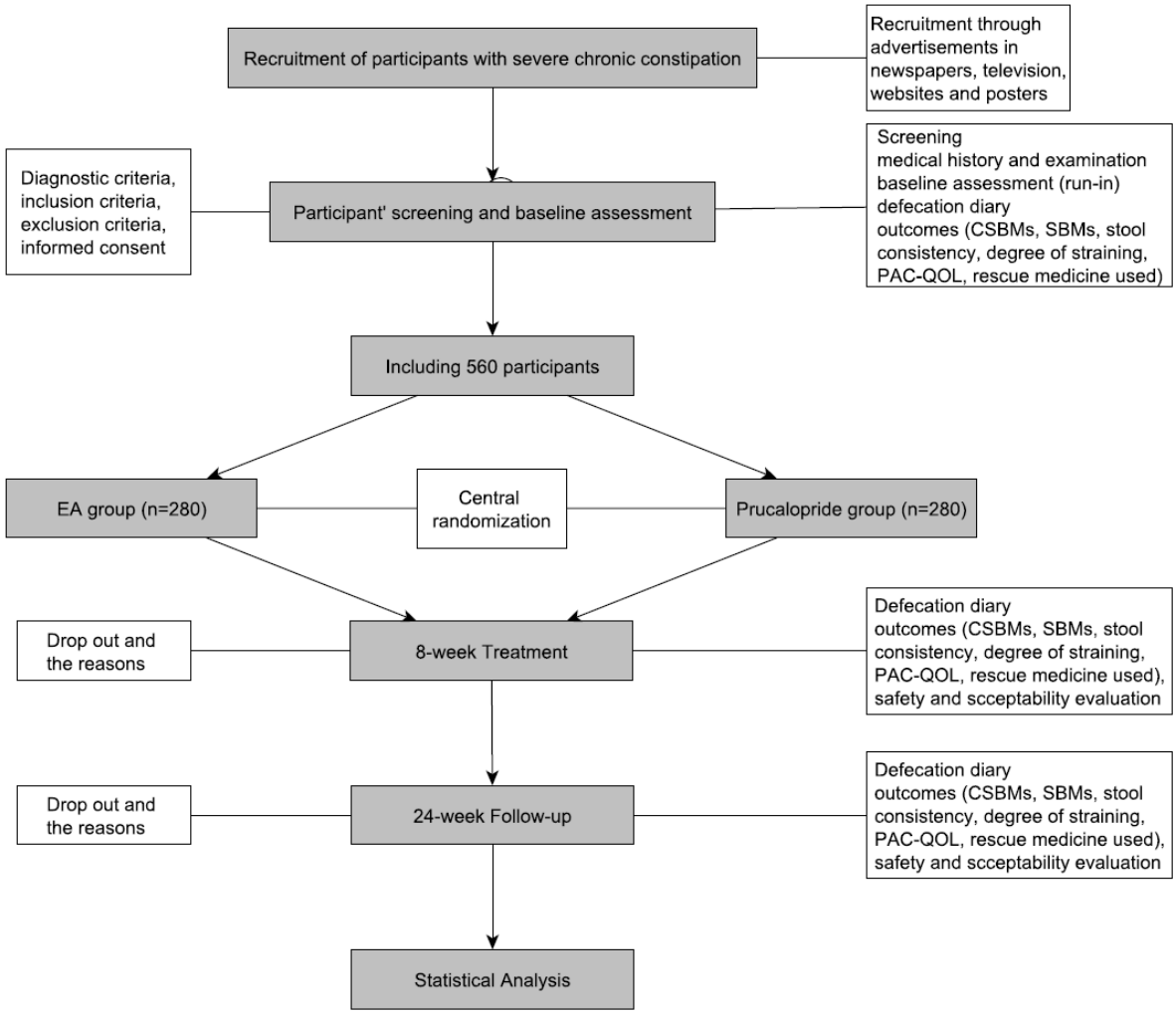

FIGURE 2 FLOWCHART

4. Informed Consent

Informed Consent: Study Introduction

Dear participant:

We will invite you to participate in a clinical study comparing the efficacy of electro-acupuncture and prucalopride (positive medicine recommended by the constipation guide) for severe chronic constipation. This study is supported by the 12th five-year national science and technology project. It plans to use two kinds of clinically effective treatment methods to treat severe chronic constipation. Through the comparison of efficacy and safety evaluation of the two methods, the treatment of severe chronic constipation with electroacupuncture will be scientifically evaluated. Please read the following paragraphs carefully, which can help you understand the details of the study. If you wish, you can discuss it with your relatives and friends, or ask your doctor for explanation to help you make a decision.

Introduction

I. Background

Constipation affects around 12% of the general population. Severe chronic constipation is characterized by complete, spontaneous bowel movements no more than twice per week, hard stools and/or straining with the course no less than 6 months, which have a big influence on participants' quality of life. Laxatives are always used for constipation; however, it lacks sustained effect. Constipation often re-appear when discontinuation the medication. Electro-acupuncture could promote intestinal motility and improve intestinal function. It may increase the spontaneous bowel movements, improve constipation related symptoms, and improve the quality of life. Our previous study using electro-acupuncture revealed that electro-acupuncture could significantly increase spontaneous bowel movements, promote the stool consistency and straining with the sustained effect for 6 months. In order to evaluate the curative effect and clinical application value of electroacupuncture in treating severe chronic constipation, Guang'anmen hospital affiliated to Chinese Academy of Chinese Medical Sciences carried out this study together with 13 hospitals in China.

II. Who should not participate in the study

1) Irritable bowel syndrome, organic constipation or secondary constipation caused by endocrine, metabolic, nervous, postoperative diseases, or drugs;
2) More than one mushy or watery stool during baseline while not taking laxatives (Bristol stool type 6 or 7);
3) A history of pelvic floor dysfunction;
4) Taking medicine that can influence the intestinal function or induce constipation;
5) Severe haemorrhoid or anal fissure;
6) Participants with severe cardiovascular, hepatic or renal diseases, cognitive impairment, abdominal aortic aneurysm or hepatosplenomegaly, aphasia, mental disorders, or illness that will influence the examination or treatment;
7) Women in the gestation or lactation period;
8) Participants with blood coagulation disorders or taking anticoagulants regularly, such as Warfarin and Heparin;
9) Participants with a cardiac pacemaker carrier.

III. What to do next, if you decide to participate?

1. Before your enrollment in the study, you will receive the following exams to determine whether you are eligible to participate in the study:

The doctor will inquire and record your medical history and physical examination as well as stool routine examination and occult blood test. If necessary, colonoscopy may be required to confirm the diagnosis.

2. If the results of the above screening examinations meet the inclusion criteria and you are willing to participate in this study, you will be invited to continue study participation in the following steps:

1) Based on the random number generated from the computer, the doctor will assign you to either the electro-acupuncture (EA) or prucalopride group. Prucalopride is a new highly selective 5-HT_4 receptor agonist that can shorten the colonic transit time. It is recommended by the constipation guidelines of world gastrointestinal organization as grade A for severe chronic constipation.
2) The duration of this study is 34 weeks, including a baseline period of 2 weeks, a treatment period of 8 weeks, and a follow-up period of 24 weeks. Frequency and duration of EA treatment: five sessions each week for the first 2 weeks, followed by 3 sessions per week for the latter 6 weeks. The participants will receive 28 sessions of treatment in total. Prucalopride usage: one tablet per day for at least 8 weeks and was generally taken for 24-week follow-up period with the same dose.
3) During the study period, you need to record defecation diary faithfully (the bowel movements, defecation time, stool consistency, time and dosage of drug use if any, etc.). You need to hand in your

diary to the doctor timely, and the doctor will record your signs and symptoms in detail.

3. Other requirements for your cooperation

As a participant of this study, you will have some relevant responsibilities, such as adherence to the schedule for examination, treatment, and outpatient follow-up. Additionally, you are also responsible for reporting any changes in your physical and mental status to your doctor during the study process regardless of whether you think these changes are related to the study or not.

During the study, you are not allowed to use other medications for constipation. However, if you do not have a bowel movement for three or more consecutive days, you are permitted to take bisacodyl (5–10 mg) or glycerin enema (110 ml). The use of bisacodyl and glycerin enema should be recorded in the defecation diary timely. If laxatives are used, you will need record the name, dosage, time of drug use faithfully in time. Rescue medicine is also available during screening period; however, **rescue medicine or other measurements for constipation is not allowed to be used 48 hours before and after the first treatment.** The purpose is to evaluate the time to the first complete spontaneous bowel movement, it can help us to evaluate the state of your illness better.

You should follow the scheduled appointments with the doctor to come to the hospital for treatment (during follow-up, the doctor may get to know your conditions by phone or visiting your home). Your follow-up is very important because the doctor will determine whether the treatment that you are receiving really works, and the doctor will be able to guide the prevention and management of your symptoms timely.

IV. Potential benefits of study participation

You may benefit from this study. The benefits may include improvement of symptoms, no matter by electro-acupuncture or by prucalopride. If you decide to participate in the study, you will get free electro-acupuncture treatment for 8 weeks or free prucalopride treatment for 32 weeks. You will receive comprehensive medical education of constipation. If you decide to participant in the study, you will also receive free stool routine test; if necessary, you can also receive free colonoscopy and other examinations.

V. Potential side effects, risks, discomforts, and inconveniences

The doctors will make every effort to prevent and treat any side effects brought on by this study.

During acupuncture treatment, you may feel soreness, numbness, heavy, distension sensation, etc., which are normal reactions to acupuncture. Acupuncture treatment may have some adverse effects, but it is rare and mild. You may feel fainting due to your individual physique or emotional stress when receive acupuncture needling. Your symptoms should be relieved after the cessation of acupuncture treatment and rest. Bleeding, hematoma, and other phenomena may occur after acupuncture treatment, and these phenomena should disappear after applying local pressure. If infection occurs in the needle site, your doctor will handle it timely.

A few participants taking prucalopride will have the side effects such as diarrhea, abdominal pain, headache, palpitations etc. Most of these side effects are mild. And the side effects will be relieved when taking prucalopride for a while，or the medicine dosage reduced, or the medicine is discontinued.

When you accept the treatment according to the study protocol, if any adverse reactions or adverse events occur during the treatment, you shall promptly notify your doctor, and he/she will evaluate the condition and give you appropriate medical treatment.

VI. Payments/Compensation for participation

If you participate in the study, during the study, you will get relevant physical and biochemical examination, acupuncture treatment, and prucalopride for free. If adverse events occur during the study, medical experts committee will identify whether they are related to the study. If trial related damage happened, the study group will deal with it appropriately in accordance with relevant provisions. The treatment and examination required for your concomitant diseases will not be free of charge.

VII. Confidentiality of personal information

All the information related to your participation in this study will be kept confidential by the institute where your participation takes place. Only the institutes responsible for the study, clinical research institutes, and ethics committees may have access to your medical records. Your name will not appear in any publications or reports related to this study.

We will make every effort to protect the privacy of your personal medical information as per legal requirements and laws.

VIII. How to acquire extra information?

You can ask any questions about the study at any time and will get answers timely. If we notice any new information that may affect your willingness and decision to continue participating in the study, the doctor will keep you informed.

IX. Can you voluntarily choose to participate in or withdraw from the study?

Whether to participate in this study or not entirely depends on your desire. You can refuse to participate in the study or withdraw from the study at any time during the study, which will not affect the relationship between you and your doctor and will not affect your medical interests or interests in other areas.

For the consideration of your best interests, doctors or researchers may terminate your participation in this study at any time.

If you withdraw from the study for any reason, you may be asked for information related of acupuncture and prucalopride treatments, or the use of other medications during your participation of the study. If the doctor considers it necessary, you may also be asked to have some laboratory tests and physical examinations performed.

Telephone of the ethics committee office of Guang 'anmen hospital, China academy of Chinese medical sciences: +86 010 88001552. If you have any complaints during the test, please contact them.

X. What you need to do now?

Decide whether to participate in this study or not.

Before you make the decision to participate in the study, please ask your doctor if you have any concerns.

Thank you for reading the above information. If you decide to participate in this study, please tell your doctor, he/she will help you make arrangement for the study.

Please keep this document for your own record.

Informed Consent: Signature Page

Study Title: Electroacupuncture versus prucalopride for severe chronic constipation: a multicenter, randomized, controlled, non-inferiority trial

Study Organizer: Guang'anmen Hospital, China Academy of Chinese Medical Sciences

Collaborative Institute: Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine; West China Hospital of Sichuan University; The Third Affiliated Hospital of Zhejiang Chinese Medical University; Hengyang Hospital Affiliated to Hunan University of Chinese Medicine; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University; The Affiliated Hospital of Shandong University of Traditional Chinese Medicine; The First Hospital of Hunan University of Chinese Medicine; Hubei Provincial Hospital of Traditional Chinese Medicine; Jiangsu Province Hospital of Traditional Chinese Medicine; Shaanxi Province Hospital of Traditional Chinese Medicine; Qingdao Hiser Medical Group; Guangdong Province Hospital of Traditional Chinese Medicine; Wuhan Hospital of Traditional Chinese and Western Medicine.

Statement of agreement

I have read the above information about this study and have the opportunity to discuss this study with my doctor and ask questions. All my questions were answered satisfactorily. I understand the potential risks and benefits from participation in this study. I understand the participation of the study is voluntary and I confirm that I was given sufficient time for consideration of study participation. I confirm that I understand that:

I can always ask the doctor for additional/more information.

I can withdraw from the study at any time without discrimination or retaliation and my medical treatment and interests will not be affected.

I agree to allow the research institute, collaborative institutes, and ethics committees to inspect the data relevant to my study participation. I will receive a signed and dated copy of the informed consent form. Finally, I decide and agree to participate in this study and ensure the adherence to doctor's orders to the best I can.

Signature of participant: _____ _____Year _____month_____day

Telephone of participant: _____

Doctor Statement

I confirm that I have explained this study in detail to the participant, including participant's rights as well as the potential benefits and risks, and have given the participant a signed copy of the informed consent form.

Signature of doctor: _____ _____Year _____month_____day

Telephone of doctor: _____

5. Quality Control and Quality Guarantee

5.1 Quality Control

5.1.1 Selection of the Trial Design

This study adopted a randomized controlled design, which is the most effective measure to avoid the selective bias. This trial adopts the central randomization, which is undertaken by the Chinese Academy of Chinese Medical Sciences. Central randomization with varied blocks can ensure the implement of allocation concealment; therefore, the researchers cannot anticipate the next group. The specific randomization and operation were showed in the handbook.

5.1.2 Restriction of the study subject

Strict limitation of inclusion criteria is one important method to control bias. Setting specific inclusion and exclusion criteria makes the study subject strictly limit to a particular range, and reduces the differences between subjects, which is conducive to make objective conclusions on the observed factors.

5.1.3 Blinding

The statisticians, outcome assessors will be blinded to the allocation. Outcome assessors who know nothing about the assignment will take charge of the outcome assessment. And the statistician who knows nothing about assignment from the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences will be responsible for the statistical analysis.

5.1.4 Quality Control Level

A 3-level monitoring system will be established to periodically assess the performance of the trial: Level 1, Inspection; Level 2, Supervision; Level 3, Audit. Inspection: The investigator of each center will designate at least one researcher who take no part in the intervention manipulation to conduct a quality review of the center; Supervision: for each center, the study organizer (Guang'anmen Hospital, China Academy of Chinese Medical Sciences) will designate at least three researchers who take no part in the intervention manipulation to monitor the quality of the studies of that center; Audit: the Clinical Pharmacological Center of Xuanwu Hospital of Traditional Chinese Medicine will designate at least five quality control personnel to conduct a quality audit of the research.

5.2 Quality Guarantee

Before the start of the clinical trial, we will have a clinical training conference for all the researchers. After the training, the researchers should familiar with the trial protocol, and the standard operation procedure (SOP) of the trial's performance.

5.3 Compliance Improvement

The formula of the compliance evaluation is as following:

$$\text{Rate of Compliance} = \frac{\text{The times of the treatment accepted by the patient actually}}{\text{The times of the treatment according to the protocol}} \times 100\%$$

The rate of compliance no less than 80% will be considered as a good compliance. For getting the rate of compliance no less than 80%, we will take measures as following:

1) Participants should participate the trial of their own accord, and sign the informed consent; 2) Researchers should communicate with the participants frequently, patiently, and with concern for getting a better physician-participant relationship, and explain the related examination, interventions, and form filling at length; 3) Researchers should record the participants' contact information in detail for the convenience of the follow-up; 4) Before the randomization, researchers should inform the participants that all the cost referring to the examination and treatment would be exempted; 5) The defecation diaries of the screening and treatment periods will be distributed weekly; 6) The diaries of the follow-up period will be distributed every 4 weeks; 7) Researchers should take back the completed diaries in time and verify the related items with the participants. We will afford rescue medicines for the participants for severe constipation; 8) We will remind the participants to fill in their defecation diaries in time by affording them brochures, or by texting; 9) For participants who have less compliance, we will still follow them up for recording the related outcomes through phone or message; 10) rescue medicine (bisacridine/glycerin enema) should be provided in time.

6. Data Management

6.1 Flowchart of the Data Management

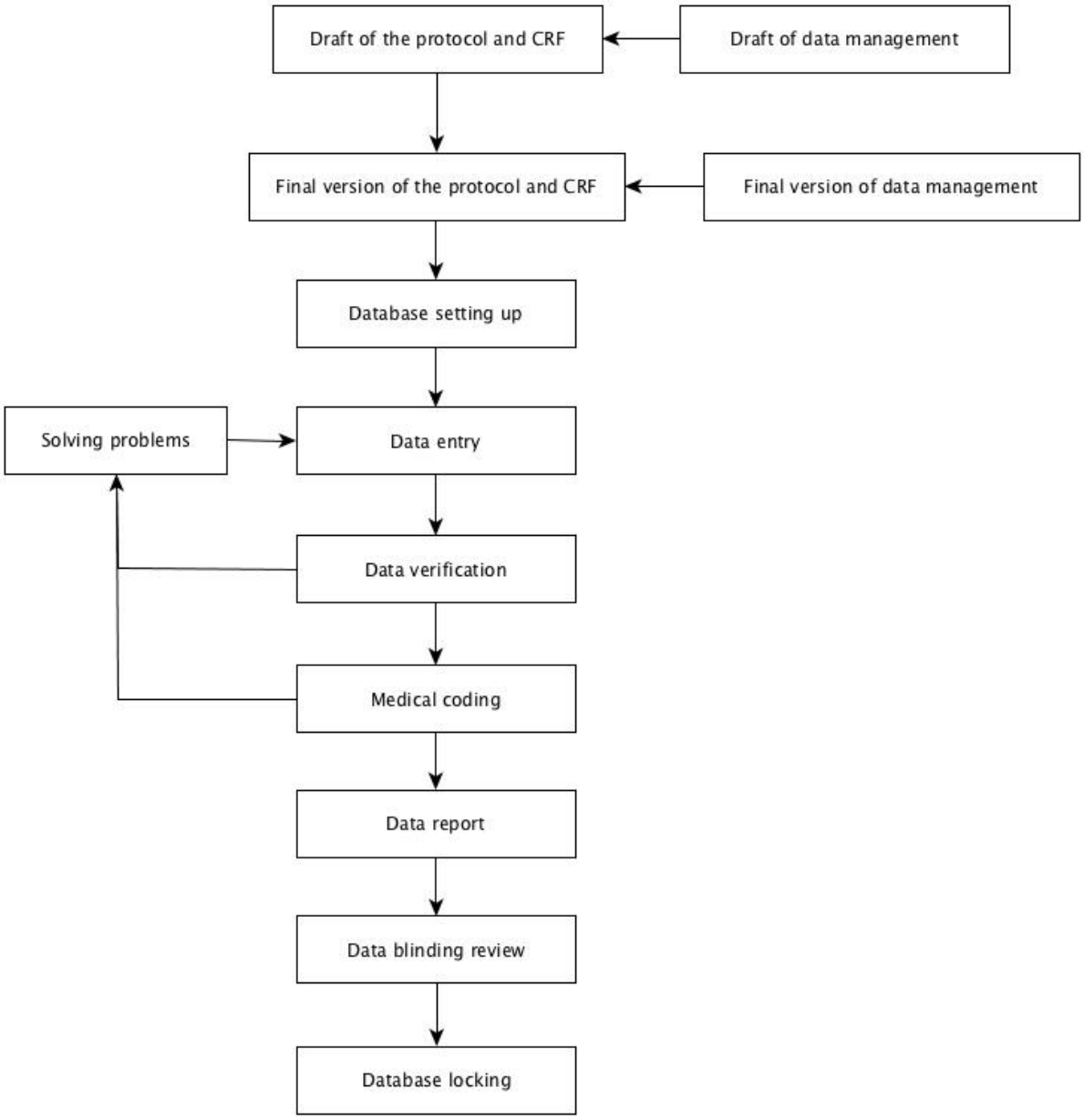

FIGURE 3 FLOWCHART OF THE DATA MANAGEMENT

6.2 The Raw Data Management and Archiving

We use Remote Data Capture (RDC) system to perform data entry. The research assistants will fill out all the electrical CRF through RDC system. Researchers will inspect the eCRF and signed electrically for the eCRF going into effect. The eCRF and the trace of eCRF revising will be left in the Oracle database.

6.3 Data Entry and Storage

6.3.1 Database Building and Testing, Data Entry Interface

The eCRF will be noted through CDISC SDTM standard, and the data entry interface will be generated through the Oracle Clinical software. The data entry interface should be in accordance with the paper-version CRF as far as possible. The inputted data will be stored in the Oracle database. After preliminarily setting up the database, the entry clerks will input some analog data according to the CRF to test the database. The testing contains: (1) the agreement of the data entry interface and the paper-version CRF; (2) the agreement of the exported data from the database and the analog data; (3) the agreement of the structure of the exported database and the paper-version CRF.

After the testing, data administrators should revise the database and make a testing report. Then they electrically signed on the approval page of the database to indicate that the testing is completed.

During database testing, the following documents should be stored in the project folder: 1) simulated CRF; 2) annotated CRF and CRF annotation plan; 3) screen capture database entry interface; 4) database test report; 5) database approval signature page.
If the database needs to be updated during the process of the trial, the corresponding document above should also be updated.

6.3.2 Data Entry and Inspection

The research assistants take charge of the data entry for our trial. Before the entry, all the research assistants will accept the related training according to the data entry handbook. Researchers will inspect the database, and then sign electrically to let the data go into effect.

6.4 Data Verification and Problems Solving

Researchers will verify the data through Data Verification Plan (DVP) approved by the data administrator and the statisticians. Data queries will be inputted to a data query database and form the DCF. After being inspected, the DCF will then be handed back to the original site, and the researchers of the site should answer the queries. Any revision of the database will be recorded through the RDC software.

6.5 Medical Coding

A data administrator who has the background in medicine will take charge of the medical coding. The contents of the coding are the clinical history, adverse events, and combined medication. The clinical history and adverse events will be coded through MedDRA dictionary (Version 13.0), and the combined medication will be coded via WHO DD dictionary (Version 2007.03). The lead researchers will verify the coded e-files.

6.6 Data Report

Data report contains the aspects as followed: (1) members of the project; (2) disagreement from the primary data management plan; (3) actual finish time of every project; (4) problems and the solution during the data management (if have any); (5) reconstruction of the database (if have any); (6) distribution of the participants; (7) participants who disobey the trial protocol; (7) classifying plan of the statistical analysis population.
Data report will be performed monthly since the first entry of the eCRF.

6.7 Data Blinding Review

We will hold a blinding review meeting. On the meeting, the data managers, statisticians, principal investigator, clinical monitors, and other related members would have a discussion on the following items according to the data management report and the data lists:

- Distribution of the participants;
- Protocol disobeying or not;
- Possible outlier;
- Baseline characteristics;
- Outcomes;
- Statistical analysis plan.

Participants will be classified to their suitable statistical analysis sets. No participant can be excluded from the analysis, unless getting the permission of the meeting participants. All the meeting participants should sign the data locking consent and the statistical analysis set division plan.

6.8 Database Locking

The database will be locked if it fulfills all the aspects as followed:

All the queries have been solved, and the database has been updated;
No query has been found through the data blinding review;
The medical coding has been completed;
The statistical analysis set division has been approved;
The final draft of the SAP has been made and approved by the project leader.

The data managers will sign the data locking form, and then the database will be locked. The locked database will be sent to the statisticians for further statistical analysis. The data cannot have any change after database locking.

7. Statistical Analysis

7.1 Objective and Hypothesis

<u>Objective</u>

Through comparing the efficacy of EA and prucalopride, we aim to evaluate if the effect of EA will be non-inferior to that of prucalopride. We also will evaluate if the therapeutic effect of EA could sustain for

3~6months.

Hypothesis

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3-8. The non-inferior margin of the primary outcome is set as -10%.

$$H0: A_{EA}\text{-}B_{Prucalopride} \leq -10\%;$$
$$H1: A_{EA}\text{-}B_{Prucalopride} > -10\%.$$

7.2 Statistical Analysis Set

Intention-to-treat (ITT) set will be defined as all the participants who accept randomization with baseline data. Per-protocol (PP) set will be composed of all randomly assigned participants without major protocol violations, which defined as the participants in EA group who were unable to complete 23 or more of the EA sessions, or the participants in control group who take prucalopride less than 180 days; or the participants who use prohibited medications and others which will be deemed to affect the interpretation of the primary outcome.

7.3 Statistical Methods

The data from the 14 centers will be pooled, and the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences in Beijing will conduct the statistical analysis using the SAS 9.1.3 (SAS Institute, Cary, NC, US) software. All statistical analyses will be two-sided tests except for the primary outcome (one-sided). The level of significance will be established at 0.05. Continuous data will be represented by the mean (standard deviation), mean [95% confidence interval (CI)], or median (interquartile range); categorical data will be represented by percentages. To compare two independent samples, a t-test or nonparametric test will be used for continuous data, and a chi-square test/the Fisher exact test/non-parametric tests will be used for categorical data. For the primary outcome, the CMH test will be used to avoid the center effect.

8. Ethical Principles

The trial protocol is in accordance with the principles of the Declaration of Helsinki and has been approved by the review board and ethics committee of the participating hospitals.

FINAL PROTOCOL

Electro-acupuncture Versus Prucalopride for Severe Chronic Constipation: A Non-inferiority, Multicenter, Randomized Controlled Trial

Clinical Sites:

1. Guang'anmen Hospital, China Academy of Chinese Medical Sciences;
2. Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine;
3. West China Hospital of Sichuan University;
4. The Third Affiliated Hospital of Zhejiang Chinese Medical University;
5. Hengyang Hospital Affiliated to Hunan University of Chinese Medicine;
6. Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University;
7. The Affiliated Hospital of Shandong University of Traditional Chinese Medicine;
8. The First Hospital of Hunan University of Chinese Medicine;
9. Hubei Provincial Hospital of Traditional Chinese Medicine;
10. Jiangsu Province Hospital of Traditional Chinese Medicine;
11. Shaanxi Province Hospital of Traditional Chinese Medicine;
12. Qingdao Hiser Medical Group;
13. Guangdong Province Hospital of Traditional Chinese Medicine;
14. Wuhan Hospital of Traditional Chinese and Western Medicine.

Data Coordinating and Statistical Centers:

Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences

Table of Contents

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2. Study Design

2.1 Study Overview

The objective of this trial is to compare the effect of electro-acupuncture (EA) versus prucalopride for severe chronic constipation (SCC). The efficacy and safety of EA will be simultaneously evaluated.

2.2 Background

Constipation affects approximately 12% of people around the world[1] with the prevalence of 4-6% in China[2,3]. Constipation occurs more commonly in women than men and its incidence increases with age[4,5]. Severe chronic constipation (SCC) is defined as two or fewer complete, spontaneous bowel movements (CSBMs) per week, hard stool and a sensation of straining during defecation[6]. Laxatives are used for constipated patients; however, a high level of dissatisfaction has been reported for laxatives[6]; and only 1/4~2/3 patients with chronic constipation were satisfied with laxatives[7,8]. It is generally believed that western medicine lacks sustained effect, that is, symptoms of constipation often reappears after the treatment is stopped. One trial reported after stopping PEG 3350 for 38.4±14.1 days, 61.7% patients regained the symptoms of constipation and reapplied laxatives[9]. One European survey of chronic constipation revealed that almost half were using alternative treatments (homeopathy, massage and acupuncture), and nearly 90% of respondents expressed interests in new therapies [9]. Acupuncture were widely used for chronic constipation in east Asian countries for thousands of years. A systematic review for constipation found that acupuncture was safe for chronic functional constipation and might improve weekly spontaneous bowel movements (SBMs), quality of life, and relevant symptoms. However, the evidence was limited by the small sample size and the relatively poor methodological quality of the published trials[10].

A pilot study (n = 60) showed that 31.67% patients had 3 or more weekly complete spontaneous bowel movements (CSBMs) over 8 weeks of acupuncture treatment, and 40% patients had 3 or more weekly CSBMs

over 12 weeks of follow-up, which reflected the sustained effect of acupuncture (to be published). This is a multi-center, randomized, controlled study protocol aiming to compare the efficacy and safety of electroacupuncture (EA) versus prucalopride for severe chronic constipation. Prucalopride is a highly selected 5-HT_4 receptor agonist, which could shorten the colonic transit time, and was recommended by the World Gastroenterology Organization for patients with SCC for whom previous laxative use failed to provide satisfactory relief[11]. It was also considered effective and safe in a randomized controlled trial conducted in the Asia-Pacific region[12]. Thus, the use of prucalopride as the control drug in this trial is reasonable.

2.3 Study Objective and Hypothesis

The objective of this trial is to evaluate if the effect of EA is non-inferior to prucalopride for SCC. In addition, we also would like to evaluate whether the therapeutic efficacy of EA could sustain for 3 months or 6 months? The safety and acceptance of EA will be simultaneously evaluated.

Hypothesis: EA is non-inferior to prucalopride for severe chronic constipation over weeks 3-8.

2.4 Methodology

2.4.1 Trial Design

This is a prospective, non-inferior, randomized, controlled trial conducted in 14 sites: Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, West China Hospital of Sichuan University, The Third Affiliated Hospital of Zhejiang Chinese Medical University, Hengyang Hospital Affiliated to Hunan University of Chinese Medicine, Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, The First Hospital of Hunan University of Chinese Medicine, Hubei Provincial Hospital of Traditional Chinese Medicine, Jiangsu Province Hospital of Traditional Chinese Medicine, Shaanxi Province Hospital of Traditional Chinese Medicine, Qingdao Hiser Medical Group, Guangdong Province Hospital of Traditional Chinese Medicine, Wuhan Hospital of Traditional Chinese and Western Medicine. All the acupuncturists in each site are required to have an official license and clinical work experience of no less than 2 years.

2.4.2 Sample Size Calculation

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3-8. According to our previous 60-size pilot study, the proportion was 31.67% by EA; and the proportion by prucalopride was 30.9%[6]. The non-inferiority sample size calculation formula is listed as below:

$$n = \frac{(Z_{1-\alpha} + Z_{1-\beta})^2[p_1(1-p_1) + p_2(1-p_2)]}{(\varepsilon - \delta)^2}$$

$$= \frac{(1.645 + 0.845)^2[0.3167 \times (1 - 0.3167) + 0.309 \times (1 - 0.309)]}{[0.3167 - 0.309 - (-0.1)]^2} = 230$$

To assess the non-inferiority between the treatment and control groups, a sample size of 276 for each group will be sufficient, with a non-inferior margin of -10%, one-sided significance level of 5% and a power of 80%, allowing for a 20% dropout. In this trial, we aim to recruit 560 participants considering the balance of 14 centers, with 40 participants of each center.

2.4.3 Randomization

After baseline assessment and informed consent, eligible participants will be randomized, stratified by enrollment centers, to the EA or prucalopride group with a ratio of 1:1. The random numbers will be obtained by the acupuncturists through the Central Randomization System by phone or website. Randomization will be performed centrally with varied blocks by the Clinical Evaluation Centre of the China Academy of Chinese Medical Sciences in Beijing using the PROC PLAN program of SAS 9.3 software, and the random scheme generator will not take any part in the statistical analysis. The randomizing scheme and related parameters was called the blind codes, which will be sealed by the generator and stored by a special researcher. Except for the most senior system administrator, no one will have access to the random scheme in the central random system.

2.4.4 Blinding

The statisticians, outcome assessors, and data manager who take charge of the diagnosis and differential diagnosis of SCC will be blinded to group allocation. Nurses or postgraduates (outcome assessors) from each site who know nothing about the assignment will be in charge of outcome assessment. And the statistician who knows nothing about assignment from the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences will be responsible for the statistical analyses.

2.4.5 Participants

Participants with SCC met the Rome III criteria[13] are planned to be recruited via advertisements in newspapers, television, websites and posters in hospitals.

2.4.5.1 Inclusion Criteria:

1) Primary chronic constipation meeting the Rome III criteria[13], with ≤2 mean weekly CSBMs (CSBM refers to the bowel movement without the help of drug or hands within the past 24 hours with the feeling of complete evacuation. A bowel movement beyond 24 hours after the use of medicine/hands also could be considered an SBM) in the past three months. Participants should also meet at least one of the following criteria: a) a feeling of straining during evacuation (at least once 4 bowel movements); b) lumpy or hard stools (at least once per 4 bowel movements); c) a feeling of incomplete evacuation after a bowel movement (at least once per 4 bowel movements); d) a feeling of obstruction in the anus/rectum (at least once per 4 bowel movements); e) needing the help of medication or hands for evacuation (at least once per 4 bowel movements).
2) Does not meet the diagnostic criteria for irritable bowel syndrome (IBS).
3) Previous laxative use failed to provide satisfactory relief.
4) Participants aged 18 to 75 years old.
5) Two or fewer CSBMs per week during the 2-week baseline assessment (run-in) period.
6) No use of medicine for constipation during the 2 weeks before enrolment (except rescue medication usage); no acupuncture treatment for constipation in the previous 1 month; no participation in any other on-going trial.
7) Volunteered to join this trial and signed informed consent.

2.4.5.2 Exclusion Criteria:

1) Irritable bowel syndrome, organic constipation or secondary constipation caused by endocrine, metabolic, nervous, postoperative diseases, or drugs;
2) More than one mushy or watery stool during baseline while not taking laxatives (Bristol stool type 6 or 7);
3) A history of pelvic floor dysfunction;
4) Taking medicine that can influence the intestinal function or induce constipation (However, during the trial, participants are allowed to take drugs for other diseases which may affect the evaluation of the efficacy, the usage of the drugs should be recorded in detail)
5) Severe haemorrhoid or anal fissure;
6) Participants with severe cardiovascular, hepatic or renal diseases, cognitive impairment, abdominal aortic aneurysm or hepatosplenomegaly, aphasia, mental disorders, or illness that will influence the examination or treatment;
7) Women in the gestation or lactation period;
8) Participants with blood coagulation disorders or taking anticoagulants regularly, such as Warfarin and Heparin ;
9) Participants with a cardiac pacemaker.

*Notes for the screening and baseline evaluation (run-in) period: ① Medical history and physical examination were needed. Secondary constipation should be noticed if any symptom or sign of medical history suggests that constipation may be secondary; ② Colonoscopy is recommended for participants ≥50 years; ③ Colonoscopy may also be needed if any of the following alarming signs or/and symptoms occurs in any age: gastrointestinal bleeding, anemia, progressive weight loss, abdominal mass, significant abdominal pain, history of colon polyps especially in participants with family history of colorectal cancer; ④ The stool routine test and occult blood test must be done in each prospective case; ⑤ Baseline assessment (run-in) for 2 weeks: participants should discontinue laxatives or other medications and measures for constipation (except for rescue medicine) and maintain the usual diet and lifestyle. During baseline assessment (run-in) period, participant's gender, age, course of constipation, previous treatment for constipation, bowel movements, spontaneous defecation or not, complete evacuation or not, stool consistency, degree of straining, rescue medicine usage will be recorded

2.4.5.3 Subject Withdrawals

Subjects may leave the study at their own discretion at any time. Experts from the gastrointestinal or anorectal department in each site may also decide, for the sake of participants' health, to discontinue participation (due to worsening of symptoms, or the occurrence of a serious adverse event).

2.4.6 Interventions

2.4.6.1 Acupuncturists and Apparatus

Acupuncturists in each site need to have an official license and at least two-year clinical experience. Before

conducting the trial, acupuncturists will be trained by principle investigator.
Huatuo disposable needles (size 0.30 mm×40mm, 0.30 mm×50mm, 0.30mm×75mm, Suzhou Medical Appliance, Suzhou, Jiangsu Province, China), and Huatuo electro-acupuncture apparatus (type SDZ-V, Suzhou Medical Appliance, Suzhou, Jiangsu Province, China) will be used.

2.4.6.2 Electro-acupuncture Group

Acupoints:

Bilateral Tianshu (ST 25), Fujie (SP 14), Shangjuxu (ST 37) will be used in all participants. Additionally, BL33, DU20 and DU24 could be used according to the individual situation: BL33 could be used for severe straining if any, DU20 and DU24 could be used for participants accompanied with the symptoms of anxiety and depression.

Location:

According to the WHO Standardized Acupuncture Points Location[14].

Manipulation:

After sterilizing the skin in supine position, needles with the size of 0.30mm×50mm, or 0.35mm×75mm will be inserted into ST25 and SP14 slowly and vertically, without manipulation, for approximately 30-70mm until they pierce into the muscle layer of the abdominal wall. Needles with the size of 0.30mm×40mm will be inserted into ST37 vertically for about 25mm. If BL33 is needed, all points could be simultaneously needled when participants are in the lateral decubitus position. The needle is inserted at BL33 inwardly and downwardly at an angle of 30-45 degree to a depth of 50–60mm. For DU20 and DU24, the needle is obliquely inserted backwards into the galea aponeurotica to a depth of 40mm.
Paired alligator clips of the EA apparatus will be attached transversely to the needle holders of bilateral ST25 and SP14 (and BL33 if needed). EA stimulation will last for 30 minutes with a dilatational wave of 10/50 Hz and current intensity of 0.5-2.0mA. The skin around the acupoints shivering mildly indicates the proper dose. For ST37 (and DU20, DU24 if needed), three small equal manipulations of twirling, lifting and thrusting will be performed. The participants' feeling of soreness and/or distention indicates the proper manipulation of deqi. The twirling, lifting and thrusting manipulation should be performed every 10 minutes, three times, in 30 minutes.

Course of treatment:

Each participant will accept an 8-continuous-week treatment with 28 sessions in total. The participants will be treated 30 minutes for each session, once a day, five times per week in the first two weeks, and three times per week in the latter 6 weeks.

2.4.6.3 Prucalopride Group

Participants will take Prucalopride Succinate (Janssen S.P.A) orally at a dose of 2 mg/day before breakfast for 8 weeks. Participants will undergo an electrocardiogram (ECG) at the 8th week, and the drug will be taken for another 24 weeks if no QT interval elongation or other severe cardiac adverse events (like myocardial infarction, severe arrhythmia, etc.). The use of other medicine for constipation will not be allowed during the trial. Prucalopride will be distributed to the participants beforehand every 2 weeks in the treatment period, and every 4 weeks in the follow-up period. The untaken pills will be recycled on the day of next distribution.

2.4.6.4 Rescue Medicine

Bisacodyl (5–10 mg) and glycerin enema (110 ml) can be used for participants who fail to have a bowel movement for 3 or more consecutive days. Furthermore, the date, specific time, dose of bisacodyl or glycerin enema use should be recorded in the stool diary and case report form (CRF).
Other drugs or measurements for constipation are not allowed; however, if used, details of the date, specific time, dose should also be recorded in the stool diary and CRF. Rescue medicine is available during the whole study period (baseline assessment [run-in], treatment, and follow-up); nevertheless, it is not allowed 48 hours before and after the first treatment, no matter EA or prucalopride, in order to evaluate the time to the first spontaneous defecation. Rescue medicine will be distributed to the participants beforehand every 2 weeks in the treatment period, and every 4 weeks in the follow-up period.

2.4.6.5 Research Period

The whole research period is 34 weeks, including 2-week baseline assessment（run-in） period (week -2, week -1), 8-week treatment period (weeks 1–8), and 24-week follow-up period (weeks 9–32). The time frame of the trial is presented in Figure 1.
Outcome assessing points: 1) baseline assessment（run-in） period: the last day of week -2 and week -1 (baseline evaluation); at week 0, the randomization and the first treatment will be carried out in one day. 2) Treatment period: the last day of week 2, week 4, week 6, week 8. 3) Follow-up period: the last day of week 12, week 16, week 20, week 32.

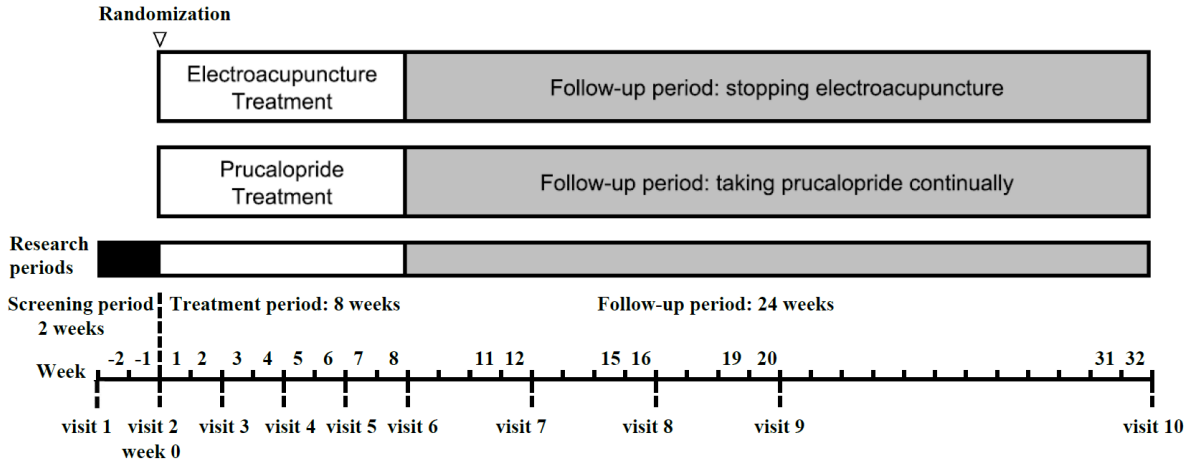


FIGURE 1 RESEARCH PERIOD AND ASSESSING POINTS

2.4.7 Subject Evaluations and Outcomes

2.4.7.1 Baseline Evaluations

Outcomes contain the complete spontaneous bowel movements (CSBMs), spontaneous bowel movements (SBMs), stool consistency (according to Bristol Stool Scale), straining during defecation, rescue medicine used, and the form of Chinese-version Patient Assessment of Constipation Quality of Life (PAC-QOL). Participants will record their bowel movements, constipation-related symptoms, medicine used for constipation every day during week -2, week -1, weeks 1-8, weeks 11-12, weeks 15-16, weeks 19-20, and weeks 31-32. In both groups, participants without bowel movements (BMs) for 3 or more consecutive days were allowed to use oral bisacodyl (5-10mg a day before bedtime, Boehringer Ingelheim) or 110 ml glycerol enema as rescue medicine with documentation (time, dosage, and frequency of use) in the stool diary.

The SBMs are the bowel movements that occur in the absence of rescue medicine, or other assistant methods (laxatives, enemas, suppositories usage, or digging out by fingers) within the preceding 24 hours. When participants used rescue medicine, or other measures for constipation, the defecation within 24 hours was considered a non-spontaneous bowel movement, whereas defecation exceeding 24 hours was deemed an SBM. CSBMs were the SBMs with the sensation of complete evacuation.

Outcome record time point: Baseline (week 0), week 2, week 4, week 6, week 8, week 12, week 16, week 20, and week 32 (Figure 1).

Outcome assessing time frame: baseline period (weeks -2 to week -1), the first 2 weeks of treatment (weeks 1-2), the latter 6 weeks of treatment (weeks 3-8), and the follow-up periods (weeks 11-12, weeks 15-16, weeks 19-20, weeks 31-32).

2.4.7.2 Primary Outcome

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3–8. Assessing time frame: the latter 6 weeks of treatment (weeks 3-8).

2.4.7.3 Secondary Outcomes

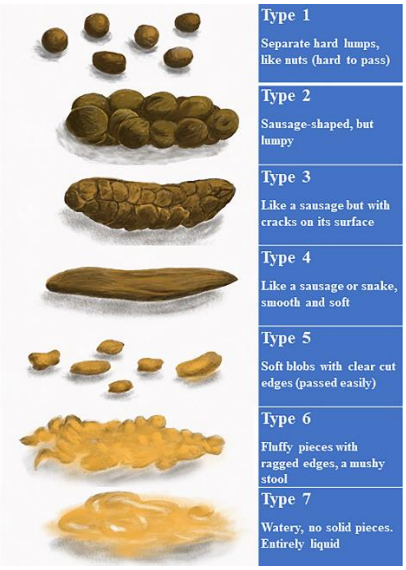
1) The proportion of participants with 3 or more mean weekly CSBMs over weeks 1-2, 11-12, 15-16, 19-20, 31-32. EA was discontinued after 8 weeks of treatment, while participants in the prucalopride group continues to take prucalopride for 24 weeks if no QT interval elongation or other severe cardiac adverse events (like myocardial infarction, severe arrhythmia, etc.). Assessing time frame: weeks1-2, weeks 11-12, 15-16, 19-20, 31-32.

2) The proportion of participants with ≥1 increase of mean weekly CSBMs from baseline over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time frame: baseline, weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32.

3) The change from baseline in mean weekly CSBMs over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time frame: baseline, weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32.

4) The change from baseline in mean weekly SBMs over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time: baseline, weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32.

5) The change from baseline in the mean score of stool consistency of each SBM over weeks 1-2 and weeks 3-8. Assessing time: baseline, weeks 1-2 and weeks 3-8. Participants will self-report their stool consistency of each SBM according to the 7-type BSFS[15] (scored by 1 to 7 respectively).



- Type 1: Separate hard lumps, like nuts (hard to pass)
- Type 2: Sausage-shaped, but lumpy
- Type 3: Like a sausage but with cracks on its surface
- Type 4: Like a sausage or snake, smooth and soft
- Type 5: Soft blobs with clear cut edges (passed easily)
- Type 6: Fluffy pieces with ragged edges, a mushy stool
- Type 7: Watery, no solid pieces. Entirely liquid

6) The change from baseline in the mean score of straining of each SBM over weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32. Assessing time: baseline, weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Participants will self-report their straining degree of each SBM in the defecation diaries according to the following scale.

0 = not difficult;
1 = a little difficult, need some straining to defecate;
2 = difficult, need straining to defecate;
3 = very difficult, need hard straining to defecate.

7) Time to the first CSBMs, which was the time from the first treatment to the first CSBMs counting by days. Rescue medicines are not allowed to be used during 48 hours before and after the first treatment. If the first CSBM had not occurred yet, and rescue medicines were used in the condition of no BM in a succession of three or more days, the BM occurs within 24 hours after the use of rescue medicine will not be regarded as CSBM, and the days-counting will continue till the occur of the first CSBM.

8) The change from baseline in the score of PAC-QOL[16] at week 4 and week 8. PAC-QOL is a self-report questionnaire to evaluate the quality of life in participants with constipation, which was distributed by Mapi Research Trust in France. This questionnaire contains 28 items including 4 basic parts of physical discomfort, worries and concerns, psychosocial discomfort, and satisfaction. We use the Chinese version in our trial[16]. Assessing point: baseline, week 4 and week 8.

9) The proportion of participants using rescue medicine (bisacodyl or glycerol enema) and the mean weekly dosage of rescue medicine used ((bisacodyl or glycerol enema) over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time frame: baseline, weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

2.4.7.4 Defecation Diaries

Defecation diaries during the treatment period (week 1 – week 8):

Participants will record their bowel movements (BMs), spontaneous defecation or not, complete evacuation or not, stool consistency, degree of straining, the time, dosage, and frequency of rescue medicine or other assistant methods used every day from the baseline to the end of week 8. Distribution of the defecation diaries: the diary of week 1 will be distributed at the first treatment of week 1, and the diary of week 2 will be distributed at the last treatment of week 1; the diary of week 1 will be taken back at the first treatment of week 2, and the diary of week 3 will be distributed at the last treatment of week 2; and so on. The diary of weeks 11-12 (follow-up period) will be distributed at the last treatment of week 8.

Defecation diaries during the follow-up period (weeks 11-12/15-16/19-20/31-32):

Participants will record their bowel movements (BMs), spontaneous defecation or not, complete evacuation or not, degree of straining, and the time, dosage, frequency of rescue medicine or other assistant methods used on the diaries every day of week 11-12/15-16/19-20/31-32. Distribution of the defecation diaries: The diaries of weeks 11-12 will be distributed at the last treatment of week 8. Within week 11, participants will get the diaries of weeks 15-16; within week 15, participants should return the diaries of weeks 11-12, and get the diaries of weeks 19-20; within week 19, participants should return the diaries of weeks 15-16, and get the diaries of weeks 31-32; within one week after week 32, participants should return the diaries of weeks 31-32.

For the participants who do not abide by our treatment protocol strictly, the outcome evaluators should still follow them up through phone, text or a visit to record the related items.

2.4.8 Safety Assessment

2.4.8.1 Adverse Events

The adverse events are some unintended symptoms, physical signs, or health conditions that show up during the whole trial. They may or may not have causality with the interventions. A health condition or an illness existing before the trial can be deemed as an adverse event (AE) if the situation becomes worse within the study period. An abnormal result of the laboratory examination can be deemed as an AE only if it causes some clinical symptoms and/or require related treatments. We will look for adverse events by asking the participants non-inducing questions at each assessing point. We will also look for the adverse events through participants' self-report, or through physical examination, or by laboratory examination. All the adverse events must be recorded at length on the CRF. If an AE was observed, the following information should be acquired:

1. Degree of severity (mild, moderate, severe);
2. If it is related to the interventions (affirmably, very likely, possibly, possibly not, affirmably not);
3. Duration of the AE (start and end dates, whether still exists at the end of the trial);
4. Severe adverse effect (SAE) or not.

SAE is defined as any of the following:

1. Fatal or life threatening;
2. Permanent or loss of function or disability;
3. Need hospitalization or need prolonged hospitalization;
4. Severe medical accidents, which need medical or surgical interventions to prevent any of the above occurs.

Solution for controlling the adverse events:

Observation, without any treatment;
Adjusting or stopping the treatment;
Aborting the trial permanently;
Adding combined drugs therapy;
Giving non-pharmacologic therapy;
Hospitalizing or prolonging hospitalization.

All the treatment measures for the adverse events should be recorded in detail on the CRF. We will follow up with the adverse event through the whole trial until its resolution. We will confirm if it is permanent. At every assessing point, we will evaluate the adverse event's severity, relation to our interventions, and arrange appropriate the treatments. The common adverse events related to the interventions are listed in the investigator's brochure. To update the brochure, related adverse events will be announced by the form of investigator's note (IN). We will also discuss with the participants about the adverse events that might occur during the trial.

2.4.8.2 Report of the Severe Adverse Events

For participants' safety, from the day they sign the informed consent until 30 days after the end of the study, any SAE no matter if it is related to our interventions or not should be reported to Rui Ma (Project management office, Acupuncture Department, Guang'anmen Hospital of China Academy of Chinese Medical Sciences; +86 18001252056) or Zhishun Liu (Acupuncture Department, Guang'anmen Hospital of China Academy of Chinese Medical Sciences; +86 13651016313) within 24 hours. SAE occurs over 30 days after the end of the study is not required to be reported, unless it is related to our interventions. The reoccurrence, complication, or the progression of a reported SAE will be reported as the follow-up information of that SAE within 24 hours. All information of the SAE will be collected and recorded on the form of SAE. Researchers should assess the relation of the SAE with our interventions and fax the finished SAE form to the department of Integrated Medical Safety (IMS) of our project in Guang'anmen Hospital within 24 hours. The SAE form and the fax will be kept together with the CRF in the research center. Acupuncturists and gastroenterologist will make the final decision regarding whether the AEs were TEAEs.

Safety Evaluation:

1. Safe, no adverse event, no abnormal examination of the safety index;
2. Relatively safe, mild adverse event with no treatment needed, no abnormal examination of the safety index;
3. Having safety problems, moderate adverse event which needs relative treatment, mildly abnormal examination of the safety index;
4. Withdrawal from the study due to the severe adverse event, or obviously abnormal examination of the safety index.

2.4.8.3 Safety and Acceptance Evaluation

The adverse events should be documented in detail over the treatment and follow-up periods. Participants and

acupuncturists will be responsible for recording adverse events.

Electro-acupuncture Related Adverse Events

Adverse events will be recorded and measured both by participants themselves and acupuncturists. Acupuncturists are responsible for the recording of the EA-related adverse events on the CRF. The adverse events relating to acupuncture include broken needle, nausea during acupuncture, faint caused by needling during the treatment, unbearable pricking (VAS≥7) caused by needling during the treatment, sharp pain lasting more than one hour (VAS≥4) after acupuncture, hematoma or bleeding around the site of needling, numbness or infection around the site of needling, sleeplessness after acupuncture, dizziness after acupuncture, other discomforts (including palpitation, headache, loss of appetite, drowsiness, or aggravation of existing symptoms) after acupuncture. Acupuncturists will record the symptoms, frequency, degree, and the duration of each AE. Pricking caused by needling will be assessed through 10-point visual analog scale (VAS).

Prucalopride Related Adverse Events

Diarrhea, nausea, abdominal pain, abdominal distention, vomiting, dyspepsia, inappetence, flatulence, abnormal bowel sound, hemoproctia, dizziness, headache, tremor, palpitation, myocardial ischemia/infarction, prolonged QT interval, frequent micturition, fatigue, fever.

Acceptance evaluation of EA:

We will evaluate the acceptability of acupuncture for the EA group. The acceptability of acupuncture will be evaluated through a point system as following:

0= hard to accept;
1= a little hard to accept;
2= acceptable;
3= easy to accept;
4= very easy to accept.

The scores of acceptability will be recorded after 5 minutes of the first and tenth treatment (the score will be summed up and divided by 2). If the participant drops out after the first treatment, then the score recorded of the first treatment will be used.

2.4.9 Compliance Assessment

The adherence of participants towards treatments were counted via the EA sessions or prucalopride tablets they have received. Prucalopride tablets were recycled back from the participants every two weeks in treatment period and every four weeks in follow-up period, and the number of tablets were documents in CRF by outcome assessors.

2.5 Reference

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3. Flowchart

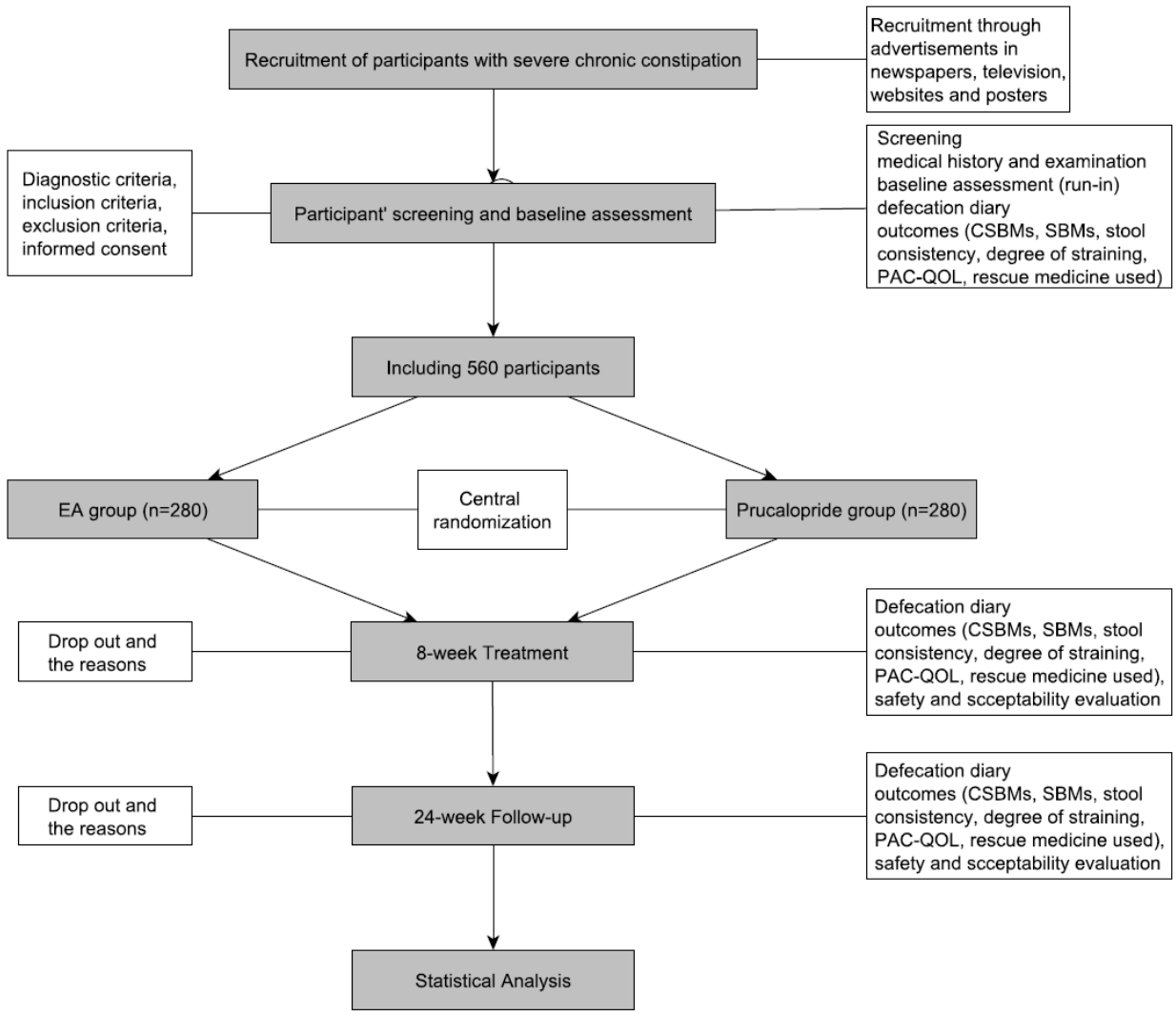


FIGURE 2 FLOWCHART

4. Informed Consent

Informed Consent: Study Introduction

Dear participant:

We invite you to participate in a clinical study comparing the efficacy of electro-acupuncture and prucalopride (a medication used in constipation) for severe chronic constipation. This study is supported by the 12th five-year national science and technology project. It plans to use two kinds of clinically effective treatment methods to treat severe chronic constipation. Through the comparison of efficacy and safety evaluation of the two methods, the treatment of severe chronic constipation with electroacupuncture will be scientifically evaluated. Please read the following paragraphs carefully, which can help you understand the details of the study. If you wish, you can discuss it with your relatives and friends, or ask your doctor for explanation to help you make a decision.

Introduction

I. Background

Constipation affects around 12% of the general population. Severe chronic constipation is characterized by complete, spontaneous bowel movements no more than twice per week, hard stools and/or straining with the course no less than 6 months, which have a big influence on participants' quality of life. Laxatives are always used for constipation; however, it lacks sustained effect. Constipation often re-appear when discontinuation the medication. Electro-acupuncture could promote intestinal motility and improve intestinal function. It may increase the spontaneous bowel movements, improve constipation related symptoms, and improve the quality of life. Our previous study using electro-acupuncture revealed that electro-acupuncture could significantly increase complete, spontaneous bowel movements, promote the stool consistency and straining with the sustained effect for 6 months. In order to evaluate the clinical application value of electroacupuncture in treating severe chronic constipation, Guang 'anmen hospital affiliated to Chinese Academy of Chinese Medical Sciences planned to perform this study together with 13 hospitals in China.

II. Who should not participate in the study

1) Irritable bowel syndrome, organic constipation or secondary constipation caused by endocrine, metabolic, nervous, postoperative diseases, or drugs;
2) More than one mushy or watery stool during baseline while not taking laxatives (Bristol stool type 6 or 7);
3) A history of pelvic floor dysfunction;
4) Taking medicine that can influence the intestinal function or induce constipation;
5) Severe haemorrhoid or anal fissure;
6) Participants with severe cardiovascular, hepatic or renal diseases, cognitive impairment, abdominal aortic aneurysm or hepatosplenomegaly, aphasia, mental disorders, or illness that will influence the examination or treatment;
7) Women in the gestation or lactation period;
8) Participants with blood coagulation disorders or taking anticoagulants regularly, such as Warfarin and enoxaparin;
9) Participants with a cardiac pacemaker.

III. What to do next, if you decide to participate?

1. Before your enrollment in the study, you will receive the following exams to determine whether you are eligible to participate in the study:

The doctor will inquire and record your medical history and physical examination as well as stool routine examination and occult blood test. If necessary, colonoscopy may be required to confirm the diagnosis.

2. If the results of the above screening examinations meet the inclusion criteria and you are willing to participate in this study, you will be invited to continue study participation in the following steps:

1) Based on the random number generated from the computer, the doctor will assign you to either the electro-acupuncture (EA) or prucalopride group. Prucalopride is a new highly selective 5-HT_4 receptor agonist that can shorten the colonic transit time. It is recommended by the constipation guidelines of world gastrointestinal organization as grade A evidence for severe chronic constipation.
2) The duration of this study is 34 weeks, including a baseline period of 2 weeks, a treatment period of 8 weeks, and a follow-up period of 24 weeks. Frequency and duration of EA treatment: five sessions each week for the first 2 weeks, followed by 3 sessions per week for the latter 6 weeks. The participants will receive 28 sessions of treatment in total. Prucalopride usage: one tablet per day for at least 8 weeks and was generally taken for 24-week follow-up period with the same dose.
3) During the study period, you need to record defecation diary faithfully (the bowel movements, defecation time, stool consistency, time and dosage of drug use if any, etc.). You need to hand in your diary to the doctor timely, and the doctor will record your signs and symptoms in detail.

3. Other requirements for your cooperation

As a participant of this study, you will have some relevant responsibilities, such as adherence to the schedule for examination, treatment, and outpatient follow-up. Additionally, you are also responsible for reporting any changes in your physical and mental status to your doctor during the study process regardless of whether you think these changes are related to the study or not.

During the study, you are not allowed to use other medications for constipation. However, if you do not have a bowel movement for three or more consecutive days, you are permitted to take bisacodyl (5–10 mg) or use glycerin enema (110 ml). The use of bisacodyl and glycerin enema should be recorded in the defecation diary timely. If laxatives are used, you will need record the name, dosage, time of drug use faithfully in time. Rescue medicine is also available during screening period; however, **rescue medicine or other measurements for constipation is not allowed to be used 48 hours before and after the first treatment.** The purpose is to evaluate the time to the first complete spontaneous bowel movement, it can help us to evaluate the state of your illness better.

You should follow the scheduled appointments with the doctor to come to the hospital for treatment (during follow-up, the doctor may get to know your conditions by phone or visiting your home). Your follow-up is very important because the doctor will determine whether the treatment that you are receiving really works, and the doctor will be able to guide the prevention and management of your symptoms timely.

IV. Potential benefits of study participation

You may benefit from this study. The benefits may include improvement of symptoms, no matter by electro-acupuncture or by prucalopride. If you decide to participate in the study, you will get free electro-acupuncture treatment for 8 weeks or free prucalopride treatment for 32 weeks. You will receive comprehensive medical education of constipation. If you decide to participant in the study, you will also receive free stool routine test; if necessary, you can also receive free colonoscopy and other examinations.

V. Potential side effects, risks, discomforts, and inconveniences

The doctors will make every effort to prevent and treat any side effects brought on by this study.

During acupuncture treatment, you may feel soreness, numbness, heavy, distension sensation, etc., which are normal reactions to acupuncture. Acupuncture treatment may have some adverse effects, but it is rare and mild. You may feel fainting due to your individual physique or emotional stress when receive acupuncture needling. Your symptoms should be relieved after the cessation of acupuncture treatment and rest. Bleeding, hematoma, and other phenomena may occur after acupuncture treatment, and these phenomena should disappear after applying local pressure. If infection occurs in the needle site, your doctor will handle it timely.

A few participants taking prucalopride will have the side effects such as diarrhea, abdominal pain, headache, palpitations etc. Most of these side effects are mild. And the side effects will be relieved when taking prucalopride for a while，or the medicine dosage reduced, or the medicine is discontinued.

When you accept the treatment according to the study protocol, if any adverse reactions or adverse events occur during the treatment, you shall promptly notify your doctor, and he/she will evaluate the condition and give you appropriate medical treatment.

VI. Payments/Compensation for participation

If you participate in the study, during the study, you will get relevant physical and biochemical examination, acupuncture treatment, and prucalopride for free. If adverse events occur during the study, medical experts committee will identify whether they are related to the study. If trial related impairment happened, the study group will treat it appropriately in accordance with relevant provisions. The treatment and examination required for your concomitant diseases will not be free of charge.

VII. Confidentiality of personal information

All the information related to your participation in this study will be kept confidential by the institute where your participation takes place. Only the institutes responsible for the study, clinical research institutes, and ethics committees may have access to your medical records. Your name will not appear in any publications or reports related to this study.

We will make every effort to protect the privacy of your personal medical information as per legal requirements and laws.

VIII. How to acquire extra information?

You can ask any questions about the study at any time. If we notice any new information that may affect your willingness and decision to continue participating in the study, the doctor will keep you informed.

IX. Can you voluntarily choose to participate in or withdraw from the study?

Whether to participate in this study or not entirely depends on your desire. You can refuse to participate in the study or withdraw from the study at any time during the study; this will not affect the relationship between you and your doctor and will not affect your medical interests or interests in other areas.

To protect your best interests, doctors or researchers may terminate your participation in this study at any time.

If you withdraw from the study for any reason, you may be asked for information related of acupuncture and prucalopride treatments, or the use of other medications during your participation of the study. If the doctor considers it necessary, you may also be asked to have some laboratory tests and physical examinations performed.

Telephone of the ethics committee office of Guang 'anmen hospital, China academy of Chinese medical sciences: +86 010 88001552. If you have any complaints during the test, please contact them.

X. What you need to do now?

Decide whether to participate in this study or not.

Before you make the decision to participate in the study, please ask your doctor if you have any concerns.

Thank you for reading the above information. If you decide to participate in this study, please tell your doctor, he/she will help you make arrangement for the study.

Please keep this document for your own record.

Informed Consent: Signature Page

Study Title: Electroacupuncture versus prucalopride for severe chronic constipation: a multicenter, randomized, controlled, non-inferiority trial

Study Organizer: Guang'anmen Hospital, China Academy of Chinese Medical Sciences

Collaborative Institute: Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine; West China Hospital of Sichuan University; The Third Affiliated Hospital of Zhejiang Chinese Medical University; Hengyang Hospital Affiliated to Hunan University of Chinese Medicine; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University; The Affiliated Hospital of Shandong University of Traditional Chinese Medicine; The First Hospital of Hunan University of Chinese Medicine; Hubei Provincial Hospital of Traditional Chinese Medicine; Jiangsu Province Hospital of Traditional Chinese Medicine; Shaanxi Province Hospital of Traditional Chinese Medicine; Qingdao Hiser Medical Group; Guangdong Province Hospital of Traditional Chinese Medicine; Wuhan Hospital of Traditional Chinese and Western Medicine.

Statement of agreement

I have read the above information about this study and have the opportunity to discuss this study with my doctor and ask questions. All my questions were answered satisfactorily. I understand the potential risks and benefits from participation in this study. I understand the participation of the study is voluntary and I confirm that I was given sufficient time for consideration of study participation. I confirm that I understand that:

I can always ask the doctor for additional/more information.

I can withdraw from the study at any time without discrimination or retaliation and my medical treatment and interests will not be affected.

I agree to allow the research institute, collaborative institutes, and ethics committees to inspect the data relevant to my study participation. I will receive a signed and dated copy of the informed consent form. Finally, I decide and agree to participate in this study and ensure the adherence to doctor's orders to the best I can.

Signature of participant: _____ _____Year _____month_____day

Telephone of participant: _____

Doctor Statement

I confirm that I have explained this study in detail to the participant, including participant's rights as well as the potential benefits and risks, and have given the participant a signed copy of the informed consent form.

Signature of doctor: _____ _____Year _____month_____day

Telephone of doctor: _____

5. Quality Control and Quality Guarantee

5.1 Quality Control

5.1.1 Selection of the Trial Design

This study adopted a randomized controlled design, which is the most effective measure to avoid the selective bias. This trial adopts the central randomization, which is undertaken by the Chinese Academy of Chinese Medical Sciences. Central randomization with varied blocks can ensure the implement of allocation concealment; therefore, the researchers cannot anticipate the next group. The specific randomization and operation were showed in the handbook.

5.1.2 Restriction of the study subject

Strict inclusion criteria is one important method to control bias. Setting specific inclusion and exclusion criteria makes the study subject strictly limited to a particular range, and reduces the differences between subjects, which is conducive to make objective conclusions on the observed factors.

5.1.3 Blinding

The statisticians, outcome assessors will be blinded to the allocation. Outcome assessors who know nothing about the assignment will take charge of the outcome assessment. And the statistician who knows nothing about assignment from the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences will be responsible for the statistical analysis.

5.1.4 Quality Control Level

A 3-level monitoring system will be established to periodically assess the performance of the trial: Level 1, Inspection; Level 2, Supervision; Level 3, Audit. Inspection: The investigator of each center will designate at least one researcher who take no part in the intervention manipulation to conduct a quality review of the center; Supervision: for each center, the study organizer (Guang'anmen Hospital, China Academy of Chinese Medical Sciences) will designate at least three researchers who take no part in the intervention manipulation to monitor the quality of the studies of that center; Audit: the Clinical Pharmacological Center of Xuanwu Hospital of Traditional Chinese Medicine will designate at least five quality control personnel to conduct a quality audit of the research.

5.2 Quality Guarantee

Before the start of the clinical trial, we will have a clinical training conference for all the researchers. After the training, the researchers should familiar with the trial protocol, and the standard operation procedure (SOP) of the trial's performance.

5.3 Compliance Improvement

The formula of the compliance evaluation is as following:

$$\text{Rate of Compliance} = \frac{\text{The times of the treatment accepted by the patient actually}}{\text{The times of the treatment according to the protocol}} \times 100\%$$

The rate of compliance no less than 80% will be considered as a good compliance. For getting the rate of compliance no less than 80%, we will take measures as following:

1) Participants will participate the trial of their own accord, and sign the informed consent; 2) Researchers will communicate with the participants frequently, patiently, and with concern for getting a better physician-participant relationship, and explain the related examination, interventions, and form filling at length; 3) Researchers will record the participants' contact information in detail for the convenience of the follow-up; 4) Before the randomization, researchers will inform the participants that all the cost referring to the examination and treatment would be exempted; 5) The defecation diaries of the screening and treatment periods will be distributed weekly; 6) The diaries of the follow-up period will be distributed every 4 weeks; 7) Researchers will take back the completed diaries in time and verify the related items with the participants. We will afford rescue medicines for the participants for severe constipation; 8) We will remind the participants to fill in their defecation diaries in time by affording them brochures, or by texting; 9) For participants who have less compliance, we will still follow them up for recording the related outcomes through phone or message; 10) rescue medicine (bisacridine/glycerin enema) should be provided in time.

6. Data Management

6.1 Flowchart of the Data Management

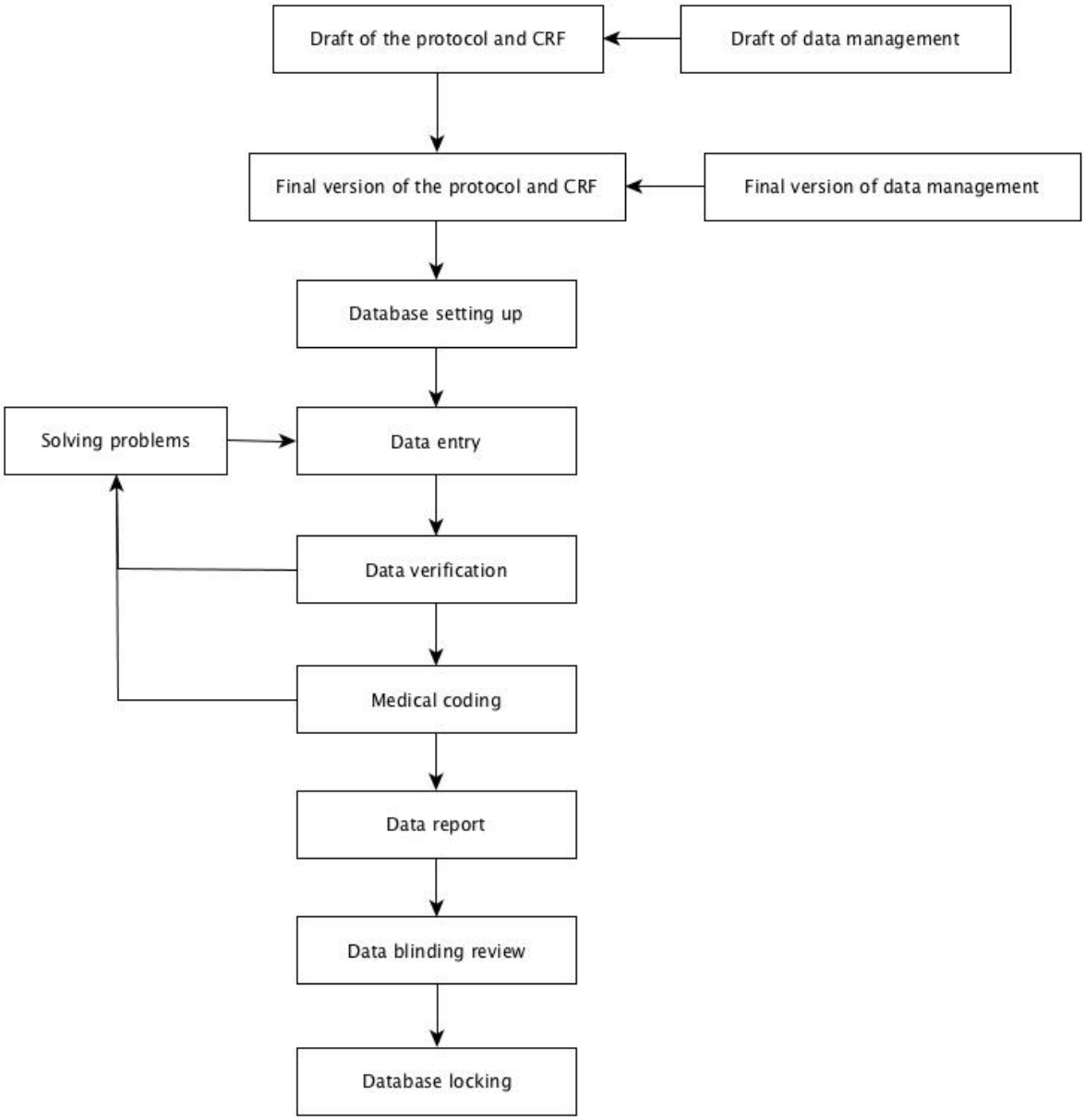


FIGURE 3 FLOWCHART OF THE DATA MANAGEMENT

6.2 The Raw Data Management and Archiving

We use Remote Data Capture (RDC) system to perform data entry. The research assistants will fill out all the electrical CRF through RDC system. Researchers will inspect the eCRF and signed electrically for the eCRF going into effect. The eCRF and the trace of eCRF revising will be left in the Oracle database.

6.3 Data Entry and Storage

6.3.1 Database Building and Testing, Data Entry Interface

The eCRF will be noted according to the CDISC SDTM standard, and the data entry interface will be generated through the Oracle Clinical software. The data entry interface should be in accordance with the paper-version CRF as far as possible. The inputted data will be stored in the Oracle database. After preliminarily setting up the database, the entry clerks will input some analog data according to the CRF to test the database. The testing contains: (1) the agreement of the data entry interface and the paper-version CRF; (2) the agreement of the exported data from the database and the analog data; (3) the agreement of the structure of the exported database and the paper-version CRF.

After the testing, data administrators should revise the database and make a testing report. Then they electrically signed on the approval page of the database to indicate that the testing is completed.

During database testing, the following documents should be stored in the project folder: 1) simulated CRF; 2) annotated CRF and CRF annotation plan; 3) screen capture database entry interface; 4) database test report; 5) database approval signature page.
If the database needs to be updated during the process of the trial, the corresponding document above should also be updated.

6.3.2 Data Entry and Inspection
Research assistants take charge of the data entry for our trial. Before the entry, all the research assistants will accept the related training according to the data entry handbook. Researchers will inspect the database, and then sign electrically to let the data go into effect.

6.4 Data Verification and Problems Solving
Researchers will verify the data through Data Verification Plan (DVP) approved by the data administrator and the statisticians. Data queries will be inputted to a data query database and form the DCF. After being inspected, the DCF will then be handed back to the original site, and the researchers of the site should answer the queries. Any revision of the database will be recorded through the RDC software.

6.5 Medical Coding
A data administrator who has the background in medicine will take charge of the medical coding. The contents of the coding include clinical history, adverse events, and medication use. The clinical history and adverse events will be coded through MedDRA dictionary (Version 13.0), and the combined medication will be coded via WHO DD dictionary (Version 2007.03). The lead researchers will verify the coded e-files.

6.6 Data Report
Data report contains the aspects as followed: (1) members of the project; (2) disagreement from the primary data management plan; (3) actual finish time of every project; (4) problems and the solution during the data management (if have any); (5) reconstruction of the database (if have any); (6) distribution of the participants; (7) participants who disobey the trial protocol; (7) classifying plan of the statistical analysis population.
Data report will be performed monthly since the first entry of the eCRF.

6.7 Data Blinding Review
We will hold a blinding review meeting. On the meeting, the data managers, statisticians, principal investigator, clinical monitors, and other related members would have a discussion on the following items according to the data management report and the data lists:
- Distribution of the participants;
- Protocol disobeying or not;
- Possible outlier;
- Baseline characteristics;
- Outcomes;
- Statistical analysis plan.

Participants will be classified into their suitable statistical analysis sets. No participant will be excluded from the analysis, unless getting the permission of the meeting participants. All the meeting participants should sign the data locking consent and the statistical analysis set division plan.

6.8 Database Locking
The database will be locked if it fulfills all the aspects as followed:
All the queries have been solved, and the database has been updated;
No query has been found through the data blinding review;
The medical coding has been completed;
The statistical analysis set division has been approved;
The final draft of the SAP has been made and approved by the project leader.
The data managers will sign the data locking form, and then the database will be locked. The locked database will be sent to the statisticians for further statistical analysis. The data cannot have any change after database locking.

7. Statistical Analysis
7.1 Objective and Hypothesis
Objective
Through comparing the efficacy of EA and prucalopride, we aim to evaluate if the effect of EA will be non-inferior to that of prucalopride. We also will evaluate if the therapeutic effect of EA could sustain for

3~6months.

Hypothesis

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3-8. The non-inferior margin of the primary outcome is set as -10%.

$$H0: A_{EA}\text{-}B_{Prucalopride} \leq -10\%;$$
$$H1: A_{EA}\text{-}B_{Prucalopride} > -10\%.$$

7.2 Statistical Analysis Set

Modified intention-to-treat (mITT) set will be defined as all the participants who accept randomization with baseline data. Per-protocol (PP) set will be composed of all randomly assigned participants without major protocol violations. Major protocol violations are defined as participants in EA group who were unable to complete 23 or more of the EA sessions, or the participants in control group who take prucalopride less than 180 days; or the participants who use prohibited medications and others which will be deemed to affect the interpretation of the primary outcome.

7.3 Statistical Methods

The data from the 14 centers will be pooled, and the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences in Beijing will conduct the statistical analysis using the SAS 9.4 (SAS Institute, Cary, NC, US) software. All statistical analyses will be two-sided tests except for the primary outcome (one-sided). The level of significance will be established at 0.05. Continuous data will be represented by the mean (standard deviation), mean [95% confidence interval (CI)], or median (interquartile range); categorical data will be represented by percentages. To compare two independent samples, a t-test or nonparametric test will be used for continuous data, and a chi-square test/the Fisher exact test/non-parametric tests will be used for categorical data. For the primary outcome, the CMH test will be used to avoid the center effect.

8. Ethical Principles

The trial protocol is in accordance with the principles of the Declaration of Helsinki and has been approved by the review board and ethics committee of the participating hospitals.

UPDATES ON ORIGINAL PROTOCOL

As compared to the original protocol (published: Liu B, Wang Y, Wu J, et al. Effect of electroacupuncture versus prucalopride for severe chronic constipation: protocol of a multi-center, non-inferiority, randomized controlled trial. BMC Complement Altern Med. 2014 Jul 23; 14: 260), the present finalized study protocol had made a few amendments. The major updates were provided in Table 1.

TABLE 1. MAJOR UPDATES OF THE ORIGINAL PROTOCOL

No.	Item	Original Version	Final Version
1	Assessing timeframe of the follow-up period	weeks 9-12, weeks 9-16, weeks 9-20, weeks 9-32	weeks 11-12, weeks 15-16, weeks 19-20, and weeks 31-32
2	Current parameter	An electric stimulator will be placed on the pair of ST25 and SP14 points with a spare-dense wave, 10/50 Hz, 0.1–1.0 mA (for BL33, the intensity is 0.5-2.0 mA).	Paired alligator clips of the EA apparatus will be attached transversely to the needle holders of the bilateral ST25 and SP14 (and BL33 if needed). EA stimulation will last for 30 minutes with a dilatational wave of 10/50 Hz and current intensity of 0.5-2.0mA.

FINAL STATISTICAL ANALYSIS PLAN

Electro-acupuncture Versus Prucalopride for Severe Chronic Constipation: A Non-inferior, Multicenter, Randomized Controlled Trial

Clinical Sites:

1. Guang'anmen Hospital, China Academy of Chinese Medical Sciences;
2. Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine;
3. West China Hospital of Sichuan University;
4. The Third Affiliated Hospital of Zhejiang Chinese Medical University;
5. Hengyang Hospital Affiliated to Hunan University of Chinese Medicine;
6. Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University;
7. The Affiliated Hospital of Shandong University of Traditional Chinese Medicine;
8. The First Hospital of Hunan University of Chinese Medicine;
9. Hubei Provincial Hospital of Traditional Chinese Medicine;
10. Jiangsu Province Hospital of Traditional Chinese Medicine;
11. Shaanxi Province Hospital of Traditional Chinese Medicine;
12. Qingdao Hiser Medical Group;
13. Guangdong Province Hospital of Traditional Chinese Medicine;
14. Wuhan Hospital of Traditional Chinese and Western Medicine.

Statistical Centers:

Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences

Sep 01, 2017

Table of Contents

1. Study Objective

The objective of this trial is to evaluate if the effect of EA is non-inferior to prucalopride for SCC. In addition, If the therapeutic efficacy of EA could sustain for 3 months or 6 months? The safety and acceptance of EA will be simultaneously evaluated.

2. Background

Constipation affects approximately 12% of people around the world[1] with the prevalence of 4-6% in China[2,3]. Constipation occurs more commonly in women than men and its incidence increases with age[4,5]. Severe chronic constipation (SCC) is defined as two or fewer complete, spontaneous bowel movements (CSBMs) per week, hard stool and a sensation of straining during defecation6. Laxatives are used for constipated patients; however, a high level of dissatisfaction has been reported for laxatives[6]; and only 1/4~2/3 patients with chronic constipation was satisfied with laxatives[7,8]. It is generally believed that western medicine lacks sustained effect, that is, the symptoms of constipation often reappears after the treatment is stopped. One trial was searched reporting the sustained effects by using PEG 3350[9]. After stopping the PEG 3350 for 38.4±14.1 days, 61.7% patients regained the symptoms of constipation and reapplied laxatives[9]. One European survey of chronic constipation revealed that almost half were using alternative treatments (homeopathy, massage and acupuncture), and nearly 90% of respondents expressed interests in new therapies[9]. Acupuncture were used for chronic constipation widely for thousands of years. A systematic review for constipation manifested that acupuncture was safe for chronic functional constipation and might improve weekly spontaneous bowel movements (SBMs), the quality of life, and relevant symptoms. However, the evidence was limited by the small sample size and the methodological quality[10].

A pilot study (n = 60) we have finished showed that 31.67% patients had 3 or more mean weekly complete spontaneous bowel movements (CSBMs) over 8 weeks of treatment, and 40% patients had 3 or more mean weekly CSBMs over 12 weeks of follow-up, which reflected the sustained effect of acupuncture (to be published). This is a protocol of a phase-II trial aiming to compare the efficacy and safety of electro-acupuncture (EA) versus prucalopride for severe chronic constipation. Prucalopride was a high-selected 5-HT4 receptor agonist, which could shorten the colonic transit time, and was recommended by the World Gastroenterology Organization for patients with SCC for whom previous laxative use failed to provide satisfactory relief[11]. It was also considered effective and safe in a randomized controlled trial conducted in the Asia-Pacific region[12]. Thus, the use of prucalopride as the control drug in this trial is reasonable.

3. Study Design

This is a prospective, non-inferior, randomized, controlled trial conducted in 14 sites: Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, West China Hospital of Sichuan University, The Third Affiliated Hospital of Zhejiang Chinese Medical University, Hengyang Hospital Affiliated to Hunan University of Chinese Medicine, Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, The First Hospital of Hunan University of Chinese Medicine, Hubei Provincial Hospital of Traditional Chinese Medicine, Jiangsu Province Hospital of Traditional Chinese Medicine, Shaanxi Province Hospital of Traditional Chinese Medicine, Qingdao Hiser Medical Group, Guangdong Province Hospital of Traditional Chinese Medicine, Wuhan Hospital of Traditional Chinese and Western Medicine. All the acupuncturists in each site are required to have an official license and clinical work experience no less than 2 years.

4. Randomization

After the baseline assessment has been carried out and the informed consent has been obtained, eligible participants will be randomized，stratified by enrollment centers, to the EA group or the prucalopride group with the ratio of 1:1. The random numbers will be obtained by the acupuncturists through the Central Randomization System by phone or website. Randomization will be performed centrally with varied blocks by the Clinical Evaluation Centre of the China Academy of Chinese Medical Sciences in Beijing using the PROC PLAN program of SAS 9.3 software, and the random scheme generator will not take any part in the statistical analysis. The randomizing scheme and related parameters was called the blind codes, which will be sealed by the generator and stored by a special researcher. Except for the most senior system administrator, no one has the access to the random scheme in the central random system.

5. Blinding

The statisticians, outcome assessors, and data managers will be blinded to the allocation. Nurse or postgraduate (outcome assessors) from each site who know nothing about the assignment will take charge of the outcome assessment. And the statistician who knows nothing about assignment from the Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences will be responsible for the statistical analysis.

6. Participants

Participants with SCC met the Rome III criteria[13]are planned to be recruited via advertisements in newspapers, television, websites and posters in hospitals.

6.1 Inclusion Criteria

1) Primary chronic constipation meeting the Rome III criteria[13] with ≤2 mean weekly CSBMs (CSBM refers to the bowel movement without the help of drug or hands within the past 24 hours with the feeling of complete evacuation. The bowel movement beyond 24 hours after the use of medicine/hands also could be considered an SBM) in the past three months. They should conform to at least one of the following items at the same time: a) a feeling of straining during evacuation (at least once per 4 bowel movements); b) lumpy or hard stools (at least once per 4 bowel movements); c) a feeling of incomplete evacuation after a bowel movement (at least once per 4 bowel movements); d) a feeling of obstruction in the anus/rectum (at least once per 4 bowel movements); e) needing the help of hands for evacuation (at least once per 4 bowel movements).
2) Conditions for the diagnosis of irritable bowel syndrome (IBS) are inadequate.
3) Previous laxative use failed to provide satisfactory relief.
4) Participants aged 18 to 75 years old.
5) Two or fewer CSBMs per week during the 2-week baseline assessment (run-in) period.
6) No use of medicine for constipation during the 2 weeks before enrolment (except rescue medication usage); no acupuncture treatment for constipation in the previous 1 month; no participation in any other on-going trial.
7) Volunteered to join this trial and signed the informed consent.

6.2 Exclusion Criteria

1) Irritable bowel syndrome, organic constipation or secondary constipation caused by endocrine, metabolic, nervous, postoperative diseases, or drugs;
2) More than one mushy or watery stool during baseline while not taking laxatives (Bristol stool type 6 or 7);
3) A history of pelvic floor dysfunction;
4) Taking medicine that can influence the intestinal function or induce constipation (However, during the trial, participants are allowed to take drugs for other diseases which may affect the evaluation of the efficacy, the usage of the drugs should be recorded in detail)
5) Severe haemorrhoid or anal fissure;
6) Participants with severe cardiovascular, hepatic or renal diseases, cognitive impairment, abdominal aortic aneurysm or hepatosplenomegaly, aphasia, mental disorders, or illness that will influence the examination or treatment;
7) Women in the gestation or lactation period;
8) Participants with blood coagulation disorders or taking anticoagulants regularly, such as
Warfarin and Heparin;
9) Participants with a cardiac pacemaker carrier.

6.3 Subject Withdrawals

The participants may leave the study at their own discretion, or the experts from the gastrointestinal or anorectal department in each site may determine that it is the best for the subject to discontinue participation (due to worsening of symptoms, or the occurrence of a serious adverse event).

7. Interventions

7.1 Apparatus

Huatuo disposable needles (size 0.30 mm×40mm, 0.30 mm×50mm, 0.30mm×75mm, Suzhou Medical Appliance, Suzhou, Jiangsu Province, China), and Huatuo EA apparatus (type SDZ-V, Suzhou Medical Appliance, Suzhou, Jiangsu Province, China) will be used.

7.2 EA Group

Acupoints:

Bilateral Tianshu (ST 25), Fujie (SP 14), Shangjuxu (ST 37). Moreover, BL33, DU20 and DU24 could be used according to the individual situation; BL33 were used for severe straining if any, DU20 and DU24 could be used for participants accompanied with the symptoms of anxiety and depression.

Location:

According to the WHO Standardized Acupuncture Points Location[14].

Manipulation:

After sterilizing the skin in participants with supine position, needles of the size of 0.30mm×50mm, or 0.35mm×75mm will be inserted into ST25 and SP14 slowly and vertically, without manipulation, for

approximately 30-70mm until they pierce into the muscle layer of the abdominal wall. Needles of the size of 0.30mm×40mm will be inserted in to ST37 vertically for about 25mm. If BL33 is needed, all points could be simultaneously needled when participants are in the lateral position. The site 0.5-1cm outside and above the 3rd posterior sacral foramina will serve as the inserting site. The needle is inserted at the inserting site inwardly and downwardly at an angle of 30-45 degree to a depth of 50–60mm. For DU20 and DU24, the needle is obliquely inserted back towards the galea aponeurotica to a depth of 40mm.
Paired alligator clips of the EA apparatus will be attached transversely to the needle holders of the bilateral ST25 and SP14 (and BL33 if needed). EA stimulation will last for 30 minutes with a dilatational wave of 10/50 Hz and current intensity of 0.5-2.0mA. The skin around the acupoints shivering mildly indicates the proper dose. For ST37 (and DU20, DU24 if needed), three small equal manipulations of twirling, lifting and thrusting will be performed. The participants' feeling of sore and distention show the proper manipulation of deqi. The twirling, lifting and thrusting manipulation should be performed every 10 minutes, three times in 30 minutes.
Course of treatment:
Each participant will accept an 8-continuous-week treatment with 28 sessions in total. The participants will be treated 30 minutes once, once a day, five times per week in the first two weeks, and three times per week in the latter 6 weeks.

7.3 Prucalopride Group
Prucalopride Succinate (Janssen S.P.A) will be taken orally at a dose of 2 mg/day before breakfast for 8 weeks. Participants will undergo an electrocardiogram (ECG) at the 8th week, and the drug will be taken for another 24 weeks in the absence of QT interval elongation or other severe cardiac adverse events (like myocardial infarction, severe arrhythmia, etc.). The use of other medicine for constipation will not be allowed during the trial. Prucalopride will be distributed to the participants beforehand every 2 weeks in the treatment period, and every 4 weeks in the follow-up period. The untaken pills will be recycled on the day of next distribution.

7.4 Rescue Medicine
Bisacodyl (5–10 mg) and glycerin enema (110 ml) can be used for participants who fail to have a bowel movement for 3 or more consecutive days. Furthermore, the date, specific time, dose of bisacodyl or glycerin enema using should be recorded in the defecation diary and case report form (CRF).
Other drugs or measurements for constipation are not allowed; However, details of the date, specific time, dose should also be recorded in the defecation diary and CRF if used. Rescue medicine is available during the whole study period (baseline assessment [run-in], treatment, and follow-up); nevertheless, it is not allowed 48 hours before and after the first treatment, no matter EA or prucalopride, in order to evaluate the time to the first spontaneous defecation. Rescue medicine will be distributed to the participants beforehand every 2 weeks in the treatment period, and every 4 weeks in the follow-up period.

8. Research Period
The whole research period is 34 weeks, including 2-week baseline assessment（run-in） period (week -2, week -1), 8-week treatment period (weeks 1–8), and 24-week follow-up period (weeks 9–32). The time frame of the trial is presented in Figure 1.
Outcome assessing points: 1) baseline assessment（run-in） period: the last day of week -2 and week -1 (baseline evaluation); at week 0, the randomization and the first treatment will be carried out in one day. 2) Treatment period: the last day of week 2, week 4, week 6, week 8. 3) Follow-up period: the last day of week 12, week 16, week 20, week 32.

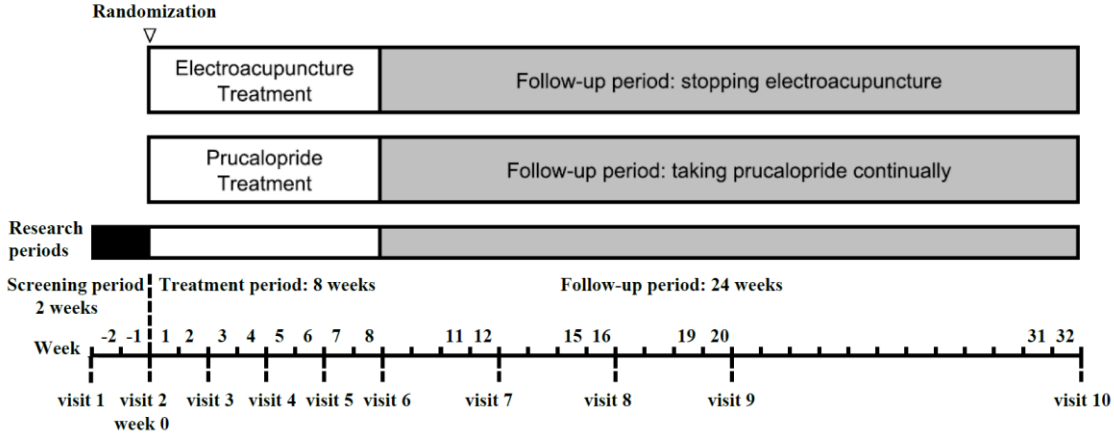


Figure 1 Research period and assessing point

9. Sample Size Calculation

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3-8. According to our previous 60-size pilot study, the proportion was 31.67% by EA; and the proportion by prucalopride was 30.9%[6]. The non-inferiority sample size calculation formula is listed as below:

$$n = \frac{(Z_{1-\alpha} + Z_{1-\beta})^2[p_1(1-p_1) + p_2(1-p_2)]}{(\varepsilon - \delta)^2}$$

$$= \frac{(1.645 + 0.845)^2[0.3167 \times (1-0.3167) + 0.309 \times (1-0.309)]}{[0.3167 - 0.309 - (-0.1)]^2} = 230$$

To assess the non-inferiority between the treatment and control groups, a sample size of 276 for each group will be sufficient, with a non-inferior margin of -10%, one-sided 5% level of significance, a power of 80% and allowing for a 20% dropout. In this trial, we aim to recruit 560 participants considering the balance of 14 centers, with 40 participants of each center.

10. Subject Evaluations and Outcomes

10.1 Baseline Evaluations

Outcomes contain the complete spontaneous bowel movements (CSBMs), spontaneous bowel movements (SBMs), stool consistency (according to Bristol Stool Scale), straining during the defecation, rescue medicine used, and the form of Chinese-version Patient Assessment of Constipation Quality of Life (PAC-QOL). Participants will record their bowel movements, constipation-related symptoms, medicine used for constipation every day through week -2, week -1, weeks 1-8, weeks 11-12, weeks 15-16, weeks 19-20, and weeks 31-32. In both groups, participants without bowel movements (BMs) for 3 or more consecutive days were allowed to use bisacodyl (5-10mg a day before bedtime, Boehringer Ingelheim) or 110 ml glycerol enema as rescue medicine with documentation (time, dosage, and frequency of use) in the stool diary.

The SBMs are the bowel movements that occur in the absence of rescue medicine, or other assistant methods (laxatives, enemas, suppositories usage, or digging out by fingers) within the preceding 24 hours, which include the CSBMs. When participants used rescue medicine, or other measures for constipation, the defecation within 24 hours was considered a non-spontaneous bowel movement, whereas defecation exceeding 24 hours was deemed an SBM. CSBMs were the SBMs with the sensation of complete evacuation.

Outcome record time point: Baseline (the last day of week -2 and week -1), week 2, week 4, week 6, week 8, week 12, week 16, week 20, and week 32 (Figure 1).

Outcome assessing time frame: baseline period (weeks -2 to week -1), the latter 6-week treatment (weeks 3-8), the first 2-week treatment (weeks 1-2), and the follow-up periods (weeks 11-12, weeks 15-16, weeks 19-20, weeks 31-32).

10.2 Primary Outcome

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs over weeks 3–8. Assessing time frame: the latter 6 weeks of treatment (weeks 3-8).

10.3 Secondary Outcomes

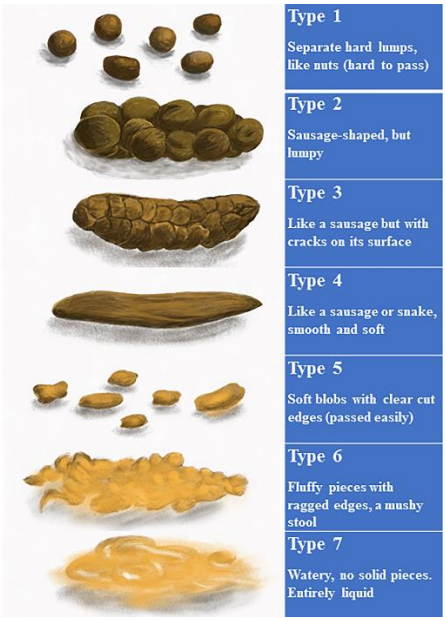
1) The proportion of participants with 3 or more mean weekly CSBMs over weeks 1-2, 11-12, 15-16, 19-20, 31-32. The EA group was discontinued after 8 weeks of treatment, while the drug group continued to take prucalopride for 24 weeks in the absence of QT interval elongation or other severe cardiac adverse events (like myocardial infarction, severe arrhythmia, etc.). Assessing time frame: weeks 1-2, weeks 11-12, 15-16, 19-20, 31-32.

2) The proportion of participants with ≥1 increase of mean weekly CSBMs from baseline over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time frame: baseline, weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32.

3) The change from baseline in mean weekly CSBMs over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time frame: baseline, weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32.

4) The change from baseline in mean weekly SBMs over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time: baseline, weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32.

5) The change from baseline in the mean score of stool consistency of each SBM over weeks 1-2 and weeks 3-8. Assessing time: baseline, weeks 1-2 and weeks 3-8. Participants will self-report their stool consistency of each SBM according to the 7-type BSFS[15] (scored by 1 to 7 respectively).



- Type 1: Separate hard lumps, like nuts (hard to pass)
- Type 2: Sausage-shaped, but lumpy
- Type 3: Like a sausage but with cracks on its surface
- Type 4: Like a sausage or snake, smooth and soft
- Type 5: Soft blobs with clear cut edges (passed easily)
- Type 6: Fluffy pieces with ragged edges, a mushy stool
- Type 7: Watery, no solid pieces. Entirely liquidz

6) The change from baseline in the mean score of straining of each SBM over weeks 1-2, weeks 3-8, weeks 11-12, 15-16, 19-20, 31-32. Assessing time: baseline, weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Participants will self-report their straining degree of each SBM in the defecation diaries according to the following scale:

0 = not difficult;
1 = a little difficult, need some straining to defecate;
2 = difficult, need straining to defecate;
3 = very difficult, need hard straining to defecate.

7) Time to the first CSBMs, which was the time from the first treatment to the first CSBMs counting by days. Rescue medicines are not allowed to be used during 48 hours before and after the first treatment. If the first CSBM had not occurred yet, and rescue medicines were used in the condition of no BM in a succession of three or more days, the BM occurs within 24 hours after the use of rescue medicine will not be regarded as CSBM, and the days-counting will continue till the occur of the first CSBM.

8) The change from baseline of the score of PAC-QOL[16] at week 4 and week 8. PAC-QOL is a self-report questionnaire to evaluate the quality of life in participants with constipation, which was distributed by Mapi Research Trust in France. This questionnaire contains 28 items including 4 basic parts of physical discomfort, worries and concerns, psychosocial discomfort, and satisfaction. We use the Chinese version in our trial16. Assessing point: baseline, week 4 and week 8.

9) The proportion of participants using rescue medicine (bisacodyl or glycerol enema) and the mean weekly dosage of rescue medicine used ((bisacodyl or glycerol enema) over weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32. Assessing time frame: baseline, weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

11. Safety and Acceptance Evaluation

11.1 Adverse Events

The adverse events are some unintended symptoms, physical signs, or health conditions that show up during the whole trial. They may have no causality with the interventions. A health condition or an illness existing before the trial can be deemed as an adverse event (AE) if the situation becomes worse within the study period. An abnormal result of the laboratory examination can be deemed as an AE only if it causes some clinical symptoms and need related therapies. We observe the adverse events by asking the participants non-inducing questions at each assessing point. We also observe the adverse events through participants' self-report, or through physical examination, or by laboratory examination. All the adverse events must be record at length on the CRF.

<u>EA Related Adverse Events</u>

Adverse events will be recorded and measured both by participants themselves and acupuncturists. Acupuncturists are responsible for the recording of the EA-related adverse events on the CRF. The adverse events relating to acupuncture include broken needle, nausea during acupuncture, faint caused by needling during the treatment, unbearable pricking (VAS≥7) caused by needling during the treatment, Sharp pain lasting more than one hour (VAS≥4) after acupuncture, hematoma or bleeding around the site of needling, numbness or infection around the site of needling, sleeplessness after acupuncture, dizziness after acupuncture, other discomforts (including palpitation, headache, loss of appetite, drowsiness, or aggravation of existing symptoms) after acupuncture. Acupuncturists will record the symptoms, frequency, degree, and the duration of

each AE. The pricking caused by needling will be assessed through 10-point visual analog scale (VAS).

Prucalopride Related Adverse Events

Diarrhea, nausea, abdominal pain, abdominal distention, vomiting, dyspepsia, inappetence, flatulence, abnormal bowel sound, hemoproctia, dizziness, headache, tremor, palpitation, myocardial ischemia/infarction, prolonged QT interval, frequent micturition, fatigue, fever.

11.2 Acceptance Evaluation of EA

We will evaluate the acceptability of acupuncture for the EA group. The acceptability of acupuncture will be evaluated through a point system as following:

0= hard to accept;
1= a little hard to accept;
2= acceptable;
3= easy to accept;
4= very easy to accept.

The scores of acceptability will be recorded after 5 minutes of the first and tenth treatment (the score will be summed up and divided by 2). If the participant drops out after the first treatment, then the score recorded of the first treatment will be used.

12. Compliance Assessment

The adherence of participants towards treatments were counted via the EA sessions or prucalopride tablets they have received. Prucalopride tablets were recycled back from the participants every two weeks in treatment period and every four weeks in follow-up period, and the number of tablets were documents in CRF by outcome assessors.

13. Statistical Analysis

13.1 Objective and Hypothesis

Objective

Through comparing the efficacy of EA and prucalopride, we aim to evaluate if the effect of EA will be non-inferior to that of prucalopride. We also will evaluate if the therapeutic effect of EA could sustain for 3~6 months.

Hypothesis

The primary outcome is the proportion of participants with 3 or more mean weekly CSBMs. The non-inferior margin of the primary outcome is set as -10%.

$$H0: A_{EA}-B_{Prucalopride} \leq -10\%;$$
$$H1: A_{EA}-B_{Prucalopride} > -10\%.$$

13.2 Statistical Analysis Set

Modified intention-to-treat (mITT) set will be defined as all the participants who accept randomization with baseline data. Per-protocol (PP) set will be composed of all randomly assigned participants without major protocol violations, which defined as the participants in EA group who were unable to complete 23 or more of the EA sessions, or the participants in control group who take prucalopride less than 180 days; or the participants who use prohibited medications and others which will be deemed to affect the interpretation of the primary outcome.

13.3 General Principles of Statistical Analysis

The primary outcome was one-tailed test at significance levels of 0.025. All the other statistical analyses were two-tailed tests, at a significant level of 0.05, using SAS software, version 9.4 (SAS Institute Inc, Cary, North Carolina, USA). For measurement data, mean±standard deviations (M±SD), median, minimum, maximum, were presented. Differences between groups were compared using group t tests or nonparametric tests. Comparisons with the baseline used paired t tests or nonparametric tests. For counting data, cases and percentages were presented, and differences between groups were compared using Chi-squared tests, Fisher's exact probabilities or non-parameters tests. For abnormal distributed data, differences between groups were compared using nonparametric tests. Missing data for the primary outcome were imputed using the multiple imputation (MI) method under the missing at random assumption for the mITT population. The results for the PP set were used as the sensitivity analysis for the multiple imputation. For secondary outcomes, no imputation was used and observed data were used for analyzing.

13.4 Participants Distribution and Demographic Data

The distribution of participants among centers after randomization and the demographic data and outcome values at baseline were described.

13.5 Primary Outcome

The primary outcome was the proportion of participants with 3 or more mean weekly CSBMs over weeks 3-8. The Cochran-Mantel-Haenszel (CMH) test, stratified by center, was used to test a hierarchical comparison between two groups.

13.6 Secondary Outcomes

(1) For the proportion of participants with 3 or more mean weekly CSBMs, the Cochran-Mantel-Haenszel (CMH) test, stratified by center, was used to test a hierarchical comparison between two groups. Assessing timeframe: weeks 1-2, 11-12, 15-16, 19-20, 31-32.

(2) For the proportion of participants with ≥1 increase of mean weekly CSBMs from baseline, the Cochran-Mantel-Haenszel (CMH) test, stratified by center, was used to test a hierarchical comparison between two groups. Assessing timeframe, weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

(3) For the change from baseline in mean weekly CSBMs, a repeated measures analysis of variance was used, setting group, time, and the interaction of group and time as the fixed effects; besides, the baseline CSBMs data and usage of rescue medicine were set as the covariate. Assessing timeframe: weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

(4) For the change from baseline in mean weekly SBMs, the same statistical analysis was conducted in accordance with the change from baseline in mean weekly CSBMs. Assessing timeframe: weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

(5) For the mean scores of straining, the Wilcoxon rank-sum test was used. Assessing timeframe: weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

(6) For the mean scores of stool consistency, the Wilcoxon rank-sum test was used. Assessing timeframe: weeks 1-2, 3-8.

(7) For the time to the first CSBM (counting by days), the Wilcoxon rank-sum test was used.

(8) For the change in mean scores of PAC-QOL between two groups, t test was used. Assessing time point: week 4 and week 8.

(9) Rescue medicine usage: For the proportion of participants using rescue medicine (bisacodyl or glycerine enema), the Chi-square test was used. For the average dosage of bisacodyl or glycerine enema used per week, the Wilcoxon rank-sum test was used. Assessing timeframe: weeks 1-2, 3-8, 11-12, 15-16, 19-20, 31-32.

(10) Post-hoc analyses: A weekly CSBM responder was defined as a patient who had ≥3 CSBMs for a given week and an increase from baseline of ≥1 CSBM for that same week. An overall CSBM responder was a patient who was a weekly CSBM responder for at least 6 of the 8 treatment weeks (75%). A sustained CSBM responder met the overall responder criteria, and also met the responder criteria for at least 3 of the last 4 treatment weeks. For proportion of the overall CSBM responder and sustained CSBM responder over weeks 1-8, Chi-square tests were used. For the weekly CSBMs and its changes from baseline of each week for data collection (week -2, week -1, weeks 1-8, weeks 11-12, weeks 15-16, weeks 19-20, weeks 31-32), a repeated measures analysis of variance was used, setting group, time, and the interaction of group and time as the fixed effects; besides, the baseline CSBMs data and usage of rescue medicine were set as the covariate.

(11) For the acceptability of EA, the number and proportion of participants who accepted the treatment of electroacupuncture was describe.

13.7 Safety Assessment

The number and proportion of participants with adverse events in two groups were described and were evaluated by using Chi-squared test or Fisher's exact probabilities. The number and proportion of participants with intervention-related adversre events were described.

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