The role of Berberine in the prevention for colorectal adenoma recurrence: Rationale and design of a randomized controlled trial

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Registered on 23th August 2014, at www.clinicaltrials.gov
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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide and in recent years its incidence is increasing rapidly in China, which constitutes a major public health burden [1, 2]. Almost 90% of CRC cases develop from precursor adenomatous polyps, through a series of genetic changes known as adenoma-carcinoma sequence during at least 10 years [3]. Detection and removal of colorectal adenoma (CRA) could reduce CRC mortality risk by colonoscopy [4], but the recurrence rate is high [5, 6]. Chemoprevention of adenoma and cancer remains its importance in both aspects of public health and cost effectiveness.

Our previous clinical trial has shown that supplementation of 1mg folic acid per day decreases significantly incidence of sporadic CRA in elder healthy people [7]. However, the role of folic acid in the prevention for CRA recurrence has kept controversial to date [8]. Favorable effects of several kinds of medicine on the secondary prevention of CRA have been reported in observational studies and randomized controlled trials (RCTs), including aspirin, vitamin D, etc. A latest meta-analysis summarized that regular intake of aspirin or COX-2 inhibitors seemed to be effective in preventing relapse of adenomas, but meanwhile a dose-related increase of gastrointestinal complications could be observed [9]. To evaluate the protective potential of vitamin D3 and/or calcium, a large-scale randomized trial conducted in 2259 participants with a recent history of CRA showed unfortunately no significant effect of any group [10]. Thus looking for suitable prophylactic drugs for CRA recurrence is urgent needed and is always of our interest.

In these years, Traditional Medicine has attracted more and more attention all over the world [11]. Chinese herbs and their derivatives have wide biological properties and are well-accepted in the Chinese population because of their magic medicinal effect, high safety, and low cost [12]. Recently, Berberine (BBR) hydrochloride, a natural isoquinoline alkaloid extracted from the Chinese herb Coptis chinensis, has come into our eyes. BBR hydrochloride has been widely used in China to cure diarrhea and enteritis for centuries with minimal side effects [13], and accumulating evidences have showed its anticancer activity, but its molecular mechanism has not been quite
clear. Our previous study presented that BBR could block the colorectal adenoma-carcinoma sequence in mice by changing microbiota structures [14]. In order to further evaluate its clinical potential for CRA recurrence, we have initiated this clinical trial of pharmacological intervention in persons after a recent polypectomy. This is a prospective, double-blind, randomized, placebo-controlled, multicenter clinical trial conducted at 7 hospitalized centres in China. We target 1000 individuals for enrollment (approximately 150 individuals per centre). We hypothesize that BBR supplementation reduce the risk of adenoma in Chinese people. Our secondary hypotheses indicate the relationship between BBR and the risk of all types of polyps or advanced adenoma or even CRC, as well as the relationship between changes in fecal microflora under long-term BBR medication and the incidence of adenoma.

METHODS

Study setting
The trial involved 7 hospitals (Ren-Ji Hospital, Shanghai Jiao-Tong University School of Medicine; Shanghai 10st Hospital, Shanghai Tong-Ji University; Military General Hospital of Beijing, PLA; General Hospital, Tianjin Medical University; Zhong-Shan Hospital, Xiamen University; Nan Fang Hospital, Nan Fang Medical University; and Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School) across 6 provinces in China (Shanghai, Beijing, Tianjin, Fujian, Guangzhou, Jiangsu).

Eligibility criteria
Inclusion criteria: (1) Individuals aged 18-75 years; (2) Individuals who had at least one and no more than 6 histologically confirmed CRAs removed within 6 months before recruitment; (3) Individuals who are able to swallow pills; (4) Individuals who voluntarily sign the consent form after being fully informed and understanding the purpose and procedure of this study, characters of the disease, effect of medication, methods of related examinations, and potential risk/benefits of the study.

Exclusion Criteria: (1) Individuals whose adenoma was not completely removed during previous colonoscopy; (2) Individuals with a history of familial adenomatous
polyposis (FAP) or hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome); (3) Individuals who are taking regularly aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), cyclo-oxygenase 2 (COX2) inhibitors, calcium or vitamin D; (4) Individuals with a history of subtotal/total gastrectomy or partial bowel resection; (5) Individuals who are intolerant to another colonoscopy examination; (6) Individuals who are hypersensitive or intolerant to the drugs; (7) Individuals with severe heart, liver or kidney disease, or any cancer history; (8) Individuals presenting severe constipation; (9) Pregnant women, women during breast-feeding period, or women with expect pregnancy; (10) Individuals with mental diseases who are not able to cooperate; (11) Individuals who are involved in designing, planning or performing this trial.

**Interventions**

Participants are randomized to receive either BBR hydrochloride (intervention group) or placebo (control group). BBR hydrochloride is a faint yellow pill with a slightly bitter taste, at a 0.1g dose and the placebo pill with an identical appearance is simply made of starch. The pills are packaged in a white pill bottle with a label ‘A’ or ‘B’ containing 100 pills, and both study pills are formally manufactured by a qualified company (Shanghai SINE Pharmaceutical Co., Ltd.). Participants are instructed to take continuously 3 study pills twice per day for 2 years after recruitment.

Participants are required to avoid taking study agents outside the trial, thus any use of nonstudy BBR hydrochloride during the study period will be regarded as a dropout. When severe constipation occurs to participants after taking study pills, they will be allowed to take one prokinetic drug within 2 weeks for help.

To ensure subsequent adherence, a run-in period of 15 days has been carried out after enrollment to identify and exclude participants who were considered unlikely to follow study procedures. Follow-up has been performed by telephone call and clinical visit. All participants have recorded a home phone and mobile phone number, a WeChat account (if any), as well as a complete home address to keep in touch. A regular monthly phone call has been made by our study staffs in charge to monitor curative effect and side effect/adverse event till the end of the trial. A newly-applied
phone number at each centre serves especially for our follow-up. Once participants have problems or something to consult, they are always welcome to contact our staffs. Records will be collected that included data on major medical events, antibiotic and probiotic uses, colorectal surgical procedures, and endoscopic examinations. A clinical visit every 3 months has been planned to return any unused pill and the bottle and change for another 3-month dosage of drugs with the same label. If participants have difficulties to reach the hospital, we will mail the bottles of study pills every 3 months. A net pot is delivered to each participant at the initial visit, the first-year visit and the second-year visit for stool collections. Participants are required to deliver the pot with fresh stool to hospital within 4 hours, and the samples will be collected and marked by our trained study staff and frozen immediately at -80°C.

Participants at each centre are informed to undergo a colonoscopic follow-up examination per year by a training endoscopist to investigate whether new adenoma will be taken place, using a standard colonoscope (Olympus Optical Co., Ltd.). Bowel preparation includes 1000ml polyethylene glycol electrolyte solution administered the previous evening and 2000ml in the early morning before an intraday examination. If the colonoscopy does not reach the cecum, it cannot be included for further analysis and the patient will be required to undergo colonoscopy by another skilled endoscopist on next occasion. The mean colonoscopy withdrawal time are required to be 6 minutes or more. During the examination, the location and size of all detected polypoid lesions should be recorded. The size of each polyp will be measured in vitro and all retrieved polypoid lesions will be sent to local pathology laboratories for histologic evaluation. Disagreements for histologic diagnosis will be resolved by re-checks to reach consensus.

To evaluate safety and effect of the drugs, participants will be required to run laboratory tests at the baseline, 1st year, and 2nd year under medication, including blood routine, hepatic and renal function, lipid and glucose level and carcinoembryonic antigen (CEA) level.

Outcomes
The primary endpoint is the recurrence of CRA at a follow-up colonoscopy in both groups. The number, size, location and histologic subtype of adenoma will be assessed. The secondary outcomes are incidence rates of all polypoid lesions (hyperplastic polyps, inflammatory polyps, serrated polyps, adenomas, etc.), as well as advanced adenoma or colorectal cancer at a follow-up colonoscopy in both groups. Other outcome parameters are changes in fecal microflora in both groups at the baseline, 1st year, and 2nd year under BBR medication.

**Participant timeline**

The time schedule of enrollment, interventions and visits for participants is shown in a schematic diagram (Table 1) following the instructions in the SPIRIT 2013 (Standard Protocol Items: Recommendations for Interventional Trials) statement [15].

**Sample size**

We estimated 918 individuals for recruitment after calculation on the basis of an expected CRA relapse rate of 21% (30% reduction) for the intervention group and 30% for the placebo group. This study sample size was decided to provide power of 80% at a statistical significance level of 0.05, allowing a 20% dropout rate.

**Recruitment**

Patients who met the inclusion criteria were identified by investigators during their routine clinical practices. Majority of potentially eligible participants were approached by our study staffs in charge through a face-to-face visit for the first time, so as to give a full explanation of the trial and record contact information, and the rests were contacted by telephone. After 2 weeks, our staffs would call the patient for a confirmation and make an appointment for the formal baseline visit. All participants provided written informed consent and baseline information, and they began to take blinded BBR or placebo after the 2-week run-in period.

**Allocation**

Computer-generated randomization is used to allocate participants in a 1:1 ratio to group ‘A’ or ‘B’. The person who generates the allocation sequence is an expert on biostatistics, who is not involved in this study. Investigators who enroll participants receive the excel table with serial numbers and the ‘A’ or ‘B’ grouping. Participant
enrollment has been carried out according strictly to the serial numbers. Those who enroll participants and assign interventions are unaware of the details of grouping.

**Blinding**

The participant, the investigator in charge of follow-up, the data analyst, the endoscopist and the pathologist are all blinded of which treatment the participant is receiving. The pharmaceutical company have provided blinded bottles of study pills with the label ‘A’ or ‘B’. Unblinding will be advanced for an appropriate clinical management only in case of emergency.

### Table 1: Participant timeline

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<th>Post-allocation</th>
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Unblinding will be advanced for an appropriate clinical management only in case of emergency.
**Data collection**

Investigators follow a schedule to record data, including: (1) informed consent, inclusion and exclusion criteria, and enrolment data; (2) baseline information (age, gender, birth date, height, weight, medical history, recent medication (antibiotics, probiotics, etc.), related family history, clinical manifestations before enrollment, diet, lifestyle, recent colonoscopy and biopsy); Participants are asked to complete a brief lifestyle and dietary questionnaire, which is subsequently transferred (via double entry) to an electronic database system. (3) follow-up information at every visits (changes of clinical symptoms, major medical events, antibiotic and probiotic uses, colorectal surgical procedures, laboratory tests results, etc.); (4) endoscopic examinations (date, endoscopist, hospital, number of new adenoma, site, size and type).

Data are collected using paper-based case report forms (CRFs) and a secure electronic database (Bo Shi, China) that conformed with Good Clinical Practice (GCP) requirements for quality control. A timely training of personnel has been conducted before electronic data input, and access to the corresponding system rights has been granted. The investigators of each centre can only reach their own data page by using a specific account and pass code, and the principal investigators in Ren-Ji Hospital can view and centralise all the baseline and follow-up information of participants, yet with no permission to modify the data of other centres. The principal investigators are planned to carry out on-site verifications of CRFs of each centre every 6 months and organize annual summary meetings for better communication and quality management.

**Statistical methods**

All statistical tests will be two tailed and will be analysed using SAS software. In general, quantitative variables will be analysed by calculating the mean, SD, median, minimum value, maximum value Categorical variables will be described using cases and percentages for each category. The significance of differences between two groups will be determined using the χ² test or Fisher’s exact test for categorical data.
The primary and secondary outcomes are planned to be analysed using the $\chi^2$ test with a significance level of 5%. The trial results will be evaluated by intention-to-treat and per-protocol analysis. Risk ratios (RR) and 95% confidence intervals (CI) will be also used comparing BBR supplementation and control groups by Cox regression methods. Exploratory subgroup analyses and safety analysis will be conducted.

**Monitoring**

The Data Monitoring Committee is composed of clinical experts on oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed to the principal investigators. Researchers should record adverse events and severe adverse events in the corresponding CRFs, including signs and symptoms, date, duration, severity, relationship with therapy, measurements and outcomes. All severe adverse events must be reported to the drug administration department and the ethics committee within 24 hours.

**Ethics and dissemination**

The Ethics Committee of Ren-Ji Hospital, Shanghai Jiao Tong University School of Medicine has granted ethics approval for this study on 13th August 2014. All amendments will be reviewed by Ethics Committee of Ren-Ji Hospital. The protocol has been registered at the ClinicalTrials.gov registry (No. NCT02226185). Written informed consents are obtained from all participants. In the event of additional studies from the database, all the investigators should keep the results confidential until these are publicly available, and they cannot publish any data related to the database without the approval of the principle investigators.