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Efficacy of quadruple regimen with polaprezinc for gastric *Helicobacter pylori* infection eradication: protocol for a single-centre, single-blind, non-inferiority, randomised clinical trial

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ABSTRACT

Introduction *Helicobacter pylori (H. pylori)* is the most well-known risk factor for gastric cancer. At present, *H. pylori* shows varying levels of resistance to different treatments, leading to a lower rate of *H. pylori* eradication. The aim of this study is to evaluate the efficacy of polaprezinc-containing quadruple therapy (PQT) for the eradication of *H. pylori* infection and, thus, to provide more evidence to inform the clinical treatment of *H. pylori* infection in China.

Methods and analysis This is a single-centre, single-blind, non-inferiority, randomised controlled trial, enrolling 158 patients with *H. pylori* infection. Patients are randomised (1:1) to the two groups for a 14-day therapy. Treatment group: PQT (esomeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg, polaprezinc 75 mg) two times per day; control group: bismuth-containing quadruple therapy (esomeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg, bismuth potassium citrate 220 mg) two times per day. The primary outcome is the rate of *H. pylori* eradication. Secondary outcomes are the incidence of adverse events and the gastrointestinal microbiota distribution. The 16S ribosomal RNA (16S rRNA) next-generation sequencing (NGS) is used to evaluate the effect of two different therapies on the distribution of the gastrointestinal microbiota.

Ethics and dissemination This study was approved by the Ethics Committee of Sichuan Cancer Center & Hospital (No. SCCHEC-02-2019-015). Any amendment to the research protocol will be submitted for ethical approval. All participants must provide informed consent. On completion, the results of the study will be published in the appropriate peer-reviewed journal.

Trial registration number ChiCTR1900025800; preresults.

Strengths and limitations of this study

- The study design (randomised controlled trial, RCT) is the gold standard of clinical evidence.
- This is the first RCT to compare the efficacy of polaprezinc-containing quadruple therapy and bismuth-containing quadruple therapy on *H. pylori* eradication.
- The results of this study could provide more evidence to inform the clinical treatment of *H. pylori* infection in China.
- This is the first prospective study with next-generation sequence detecting strategy in Chinese mainland to explore the possible drug-resistance mechanism of *H. pylori*.
- The single-centre clinical trial may not represent the *H. pylori* eradication rate of general population.

INTRODUCTION

Helicobacter pylori (H. Pylori) is a multiflagellum, microaerobic Gram-negative, bacillus, that is specifically colonised in the gastric epithelium.[1] It is estimated that about 50% of the world's population is infected with *H. pylori*,[2 3] but the infection rate for *H. pylori* varies widely by population, age, geographic region, race, socioeconomic status, health status, population density and eating habits.[2 3] *H. pylori* is one of the most important pathogenic factors of gastrointestinal diseases such as chronic gastritis, peptic ulcer, gastric adenocarcinoma and gastric mucosa - associated lymphoid tissue (MALT) lymphoma.[4 5] Moreover, *H. pylori* infection is correlated with a 1-fold to 10-fold increase in the risk of gastric or duodenal ulcer and with 0.1%–3% risk of developing gastric cancer.[6] The eradication of *H. pylori* is an important method for the treatment of digestive tract ulceration and chronic gastritis and for the prevention of gastric cancer.

At present, a combination of therapy is recommended for the treatment of *H. pylori* infection in clinical practice as well as for the

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treatment of gastritis and digestive ulcer caused by *H. pylori* infection. Among available treatments, the use of bismuth, in combination with antibiotics, such as metronidazole, amoxicillin, clarithromycin, azithromycin, levofloxacin, chloramphenicol and tetracycline, has been widely recommended. Polaprezinc, a chelating agent of L-carnosine and zinc, can block the colonisation of *H. pylori* by inhibiting its activity pharmacologically and the associated inflammatory chain reaction and removing urea, enzymes, monochloramines to achieve the *H. pylori* eradication. Polaprezinc combination with lansoprazole, amoxicillin and clarithromycin can increase the *H. pylori* eradication rate from 24/31 (77.4%, triple therapy alone) to 33/35 (94.3%).[7] However, the efficacy of *H. pylori* infection treatment in high-risk population in China is needed to be established.

With the frequent use of antibiotics, the rate of antibiotic resistance also showed an upward trend. Studies reported on the resistances of *H. pylori* to various antibiotics, which lead to lower *H. pylori* eradication rates. A large number of studies outside China have been conducted to detect the drug-resistance sites of *H. pylori*, with the plausible mechanisms leading to resistance for many antibiotics having been defined.[8–12] Moreover, the use of drugs to treat *H. pylori* infection has been associated with significantly different degrees of overgrowth of the gastric microbiota.[13] Compared with patients with gastritis, the diversity of gastric microbiota among patients with gastric cancer is significantly lower, with the abundance of Helicobacter species being specifically decreased, while the abundance of other species is increased.[14] The effect of different treatment options for treatment of *H. pylori* infection on the distribution of the other microbiota in the gastrointestinal is unknown.

The 16S ribosomal RNA (16S rRNA) next-generation sequencing(NGS) technology is a powerful tool for microbial detection and classification. The 16S rRNA sequence information is obtained by comparing the gene sequences of the 16S rRNA gene fragment of various organisms, with the sequence information obtained by cloning, sequencing or enzyme cutting or probe hybridisation. The 16S rRNA sequence information is then compared with the data in the 16S rRNA database for identification and is not affected by antibiotics. It can identify dead bacteria and microorganisms that currently cannot be cultivated artificially as well as identify new types of microorganisms that play an important role in intestinal microbiota detection.[15 16]

The aim of our study is to evaluate the efficacy of polaprezinc-containing quadruple therapy (PQT) compared with the traditionally bismuth-containing quadruple therapy (BQT) for *H. pylori* eradication and, thus, to provide evidence for clinical treatment of *H. pylori* infection in Chinese population.

METHOD AND ANALYSIS

Design

This protocol is a single-centre, single-blind, non-inferiority, randomised clinical trial, in which 158 patients with *H. pylori* infection will be enrolled. Patients will be randomly assigned into one of the study groups in a 1:1 allocation ratio (figure 1). Patients are required to come to hospital on day 7 after taking drugs for receiving next-cycle drugs and follow-up the adverse events (AEs). Phone follow-up is used to monitor AEs on day 14 after taking drugs. All patients will be called back within week 4–8 after the end of therapy. This study follows the Consolidated Standards of Reporting Trials guidelines.

Measured outcomes (and associated processes) are summarised in table 1. The 16S rRNA will be performed to sequence the gastrointestinal microbiota in the collected samples.

This study will be conducted at Yanting County Cancer Hospital. It began in December 2019 and is projected to take 1 year to complete.

Objectives

The three objectives of the study are as follows. The first is to evaluate the efficacy and safety of PQT for eradication of *H. pylori* infection. The second is to detect the antibiotic drug-resistance mutation sites of *H. pylori* and explore the association between the therapy and the drug-resistance sites. The third is to compare the distribution of gastrointestinal microbiota, before and after the therapy and between the different therapies.

Inclusion criteria

The enrolled patients should be 18–65 years of age, residents who participated in early screening for upper gastrointestinal cancer and the outpatients at Yanting County Cancer Hospital, with a positive primary ^{14}C urea breath test (^{14}C-UBT) for *H. pylori* infection, with no evidence of gastric cancer or other severe gastric disease identified on gastroscopy and should provide written informed consent.

Exclusion criteria

Potential participants will be screened on the following exclusion criteria: prior antibiotics or bismuth therapy within 4 weeks of enrolment; prior proton pump inhibitor (PPI) therapy within 2 weeks of enrolment; pregnancy or lactation in women; history of cardiovascular and other severe diseases; participation in other clinical studies within 3 months; unable to follow the study procedures (eg, due to mental illness or severe neurosis); history of allergy to medications used and any contraindication to gastroscopy.

Criteria for termination in the study

The following criteria will support termination of a patient's participation in the study: deterioration of health status or severe complications; severe adverse effect on the drug used that cannot be tolerated by the patient; other conditions that would affect the measurement of the outcomes; pregnancy during the treatment and lost to follow-up.

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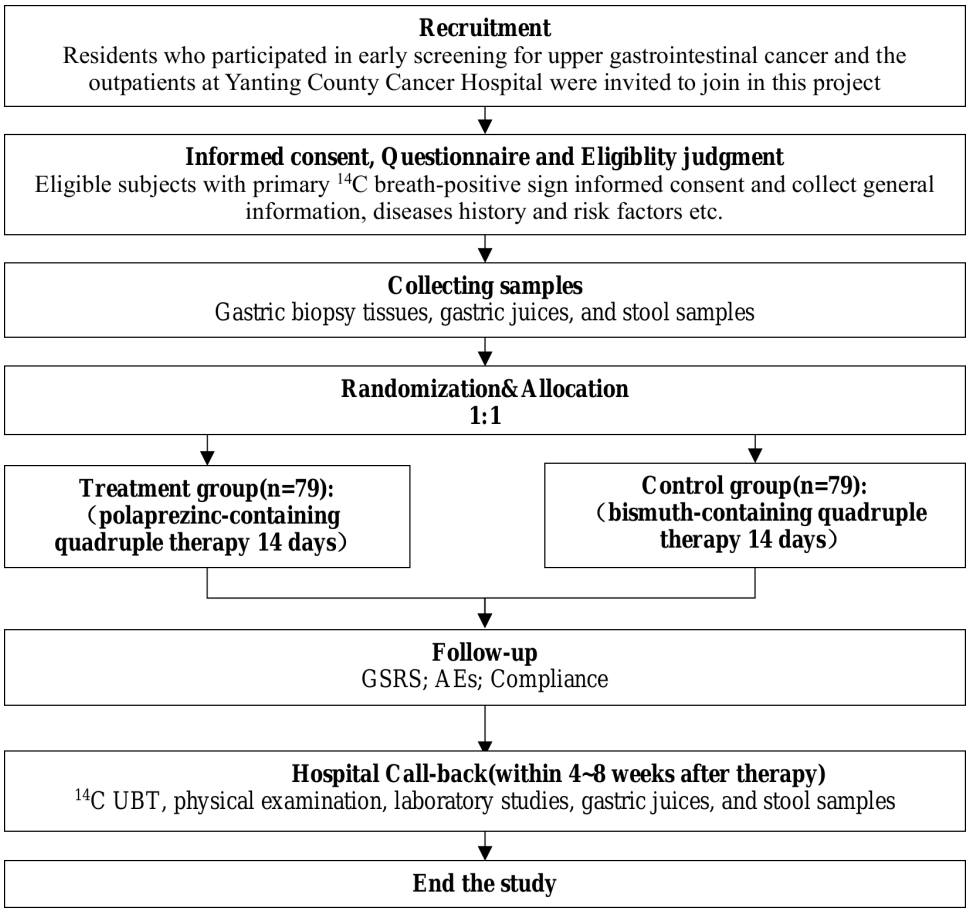

Figure 1 Flowchart of the clinical study. treatment group: esomeprazole, amoxicillin, clarithromycin and polaprezinc. Control group: esomeprazole, amoxicillin, clarithromycin and bismuth potassium citrate. ^{14}C UBT, ^{14}C-urea breath test; AEs, adverse eventsGSRS, gastrointestinal symptom rating scale.

Allocation and blinding

Patients will be randomly allocated to the two groups of the study, namely, the treatment group (PQT) and the control group (BQT), by an independent researcher using a computer-generated random sequence, with a 1:1 allocation ratio. The drugs are packaged in identical boxes, ensuring that the drugs cannot be identified on packaging appearance. The drugs are controlled by an independent drug administrator and the doctor assigned the drugs to the participants strictly according to the protocol. All patients in the study will be blinded to the assignment.

Interventions

All enrolled patients will complete physical examination and laboratory tests, including complete blood count, blood chemistry and urine tests, electrocardiogram (ECG) and gastroscope. Stools, gastric juices and biopsy tissues will be collected before therapy. Patients with a confirmed *H. pylori* infection, who meet the inclusion criteria, will be randomly assigned to the two groups of the study. As per the sample size calculation described below, 79 patients will be enrolled into each group. Patients in the treatment group will receive a 14-day PQT (esomeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg, polaprezinc 75 mg) two times per day. Patients in the control group will receive a 14-day BQT (esomeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg, bismuth potassium citrate 220 mg) two times per day.

Outcomes

The primary outcome of this study is the rate of *H. pylori* eradication, 4 weeks after the end of therapy, as measured by a ^{14}C-UBT. The secondary outcomes are the incidence of AEs and the gastrointestinal microbiota distribution before and after the therapy and between the different therapies.

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Table 1 Project flow and follow-up items.

	Before treatment/ baseline	Period of treatment/intervention and follow-up (14 days)		After the treatment/ call-back
Project	0 week (–5 to –1 days)	1 week/day 7	2 weeks/day 14	Within 4–8 weeks
Screening	×			
Informed consent	×			
Inclusion and exclusion criteria	×			
Medical history collection	×			
Vital signs	×	×	×	×
Randomisation	×			
Blood routine test	×			×
Urine routine test	×			×
Renal function test	×			×
liver function test	×			×
ECG	×			×
^{14}C UBT	×			×
Stool *H. pylori* antigen	×			×
Specimens	×			×
Treatment and follow-up		×	×	×
Concomitant medications	×	×	×	×
AEs		×	×	×
Surplus medicines recycling			×	

The '×' is the item or operation content that must be performed in the project.
^{14}C UBT, ^{14}C-urea breath test; AEs, adverse events.

Follow-up

Self-reported severity of gastrointestinal symptoms is assessed on day 7 and 14 after taking drugs. The Gastrointestinal Symptom Rating Scale (GSRS) has 15 items included in five symptom clusters depicting reflux, abdominal pain, indigestion, diarrhoea and constipation, its symptoms are graded on a 7-point Likert scale (1 means absence of symptoms, 7 means with much trouble symptoms). The GSRS was found to be good, with acceptable reliability and validity.[17] Self-reported symptom severity is obtained by patient interviews on day 7 and day 14 after taking drugs. Patients are required to come to hospital on day 7 after taking drugs for receiving next-cycle drugs and follow-up the AEs. Phone follow-up is used to monitor AEs on day 14 after taking drugs. All AEs and serious AEs are recorded and will be submitted to the Medical Ethics Committee of Sichuan Cancer Center & Hospital(SCC).

^{14}C-UBT is carried out for confirmation of *H. pylori* eradication.[18] Physical examination and laboratory studies (including complete blood count, blood chemistries, urine test, ECG and gastroscopy) will be repeated 4–8 weeks after the end of therapy. Meanwhile, stools, gastric juices will be collected.

Sample size estimation

As appropriate for a non-inferiority study, the calculation of the sample size was based on the primary outcome, the eradication rate of *H. pylori*. A previous single-centre, small sample size study conducted in Japan determined that polaprezinc combined with triple therapy increased the *H. pylori* eradication rate from 77.4% to 94.3%.[7] Another randomised, open-label, non-inferiority, phase 3 study reported an *H. pylori* eradication rate of 80% when treated using bismuth subcitrate potassium, metronidazole and tetracycline, in combination with omeprazole, as a quadruple therapy.[19] Based on these data, we calculated that 63 participants were required in each group for a one-sided α value of 0.025 and power of 80%. A high proportion is better, control group proportion of 80% and the non-inferiority margin of −20% are set to identify group difference in the rate of *H. pylori* eradication. Considering a drop out rate of 20%, a sample of 79 patients in each group is required. The sample size analysis was performed using PASS (V.15.0; NCSS, LLC, USA).

Patient and public involvement

No patients or members of the public participated in the conception of our study. On completion, however, the results of the study will be published in the appropriate journal.

Data collection and management

A project-specific data entry group will be established at SCC before the initiation of the study. Two individuals are responsible for the data entry and double check the accuracy of the data entered. Data will be entered in

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an encrypted ACCESS database, developed by the data administrator at SCC, who is responsible for monitoring the data.

Access to the original case report form (CRF) and database is restricted to the data entry personnel, investigators and other relevant researchers authorised by the principal investigator.

Data analysis

All statistical analyses are performed by the statistician at SCC using SPSS (V.17.0; SPSS, Chicago, Illinois, USA). Intention-to-treat (ITT) and per-protocol (PP) analyses are used to evaluate the primary outcome of *H. pylori* eradication. The ITT population included patients who meet the criteria, randomised, take at least one dose of drugs after enrolled. Missing observations are accounted for using the predictive mean matching (PMM) method. The PP population included patients who complete the designated therapy of this protocol. Categorical variables are compared using χ^2 test or Fisher exact test. Continuous data are compared using Student's t-test or Wilcoxon rank test. The primary analysis (evaluating non-inferiority of the PQT) is assessed through hypothesis testing and derivation of a one-sided 95% CIs. P values <0.05 were considered statistically significant.

The following processes are applied to the microbiota distribution of the secondary outcome data: quantitative insights into microbial ecology[20] (QIIME2, https://qiime2.org/) is used to clean and assemble the original offline data. The amplicon sequence variants (ASVs) are assigned to taxa (domain, kingdom, phylum, class, order, family, genus and species) by matching to SILVA database V138. The alpha diversity of gastrointestinal microbiota is based on Shannon's diversity index, observed ASVs, Faith's phylogenetic diversity and Pielou's evenness. Microbiota statistical analyses are performed using R (V.3.6.1 platform; https://www.r-project.org/). Principal coordinate analysis(PCoA) is performed to represent the differences of the beta diversity based on Bray-Curtis, Jaccard distances, weighted unifrac distance and unweighted unifrac distance. The composition of microbiota is compared between different groups, and the different taxa are identified based on linear discriminant analysis(LDA) effect size(LEfSe). Adonis function in R vegan is used to quantify the impact of host lifestyle on microbiome community.

Specimen preservation

Special specimen administrators are designated, for this study, at SCC and the study site to ensure the unified storage of specimens and the management of refrigerator keys. The specimen administrator sorts samples according to their ID number in a sequence. The specimens will be transferred to the SCC in drikold and stored at a temperature of −80°C.

Privacy measures for patient data

All specimens and forms collected as part of this study are coded using unique patient identifiers; electronic data are stored in an encrypted database on a password-protected platform, which can only be identified by authorised research personnel. As well, patient records will be accessed only as per the regulations of the Ethics Research Committee at the SCC, and only when necessary. To the extent permitted by applicable laws and/or regulations, any records relating to patient identification are confidential and will not be made public. Patients or their legal representatives are notified in a timely manner if new information arises that may affect a patient's continued participation in the study.

Ethics and dissemination

This study was approved by the Ethics Committee of Sichuan Cancer Center & Hospital (No. SCCHEC-02-2019-015). Any amendment to the research protocol will be submitted for ethical approval. All participants must provide written informed consent (online supplemental material 1). On completion, the results of the study will be published in the appropriate peer-reviewed journal.

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