Chinese Herbal Medicine Combined with Entecavir to Reduce the Off-therapy Recurrence Riks in HBeAg-positive Chronic Hepatitis B Patients: a Multi-center, Double-blind, Randomized Controlled Trial in China

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Study protocol

Keywords: chronic hepatitis B, Chinese herbal medicine formula, protocol, randomized-controlled trial

Posted Date: December 19th, 2019

DOI: https://doi.org/10.21203/rs.2.19291/v1

Version of Record: A version of this preprint was published on August 12th, 2020. See the published version at https://doi.org/10.1186/s13063-020-04417-9.

Abstract

Background: Nucleos(t)ide analogs (NAs) are the first-line options against chronic hepatitis B (CHB). NAs produce a potent suppression of viral replication but are associated with tiny opportunity of HBsAg seroclearance and high risks of virological relapse after discontinuation. The combined therapy of NAs plus traditional Chinese medicine (TCM) is widely accepted and has been recognized as a prospective alternative approach in China. Based on preliminary works, this study is designed to observe the therapeutic effect of TCM plus entecavir (ETV) against HBeAg-positive chronic hepatitis B with respect to reducing the recurrence risk after NAs withdrawal.

Methods/design: The study is a nationwide, multi-center, double-blind, randomized, placebo-controlled trial with a designed duration of 120 weeks. A total of 18 hospitals and 490 eligible Chinese HBeAg-positive CHB patients will be enrolled and randomly allocated into the Experimental group and Control group in a 1:1 ratio. Patients in the experimental group will be prescribed with TCM formulae (Tiao-Gan-Jian-Pi-Jie-Du granule) plus ETV 0.5mg per day for consolidation therapy of 96 weeks. Patients in the control group will be prescribed with TCM granule placebo plus ETV 0.5mg per day for the same course. After the consolidation therapy, all patients will discontinue their trial drugs and closely monitored in the next 24 weeks. Once clinical recurrence (CR) occurs, ETV treatment will be restarted. The primary outcome is the accumulative rate of CR at the end of this trial.

Conclusion: This study is the first of its kind to observe therapeutic effects in respect of reducing recurrence after drug withdrawals after a unified integrative consolidation therapy in CHB population.

Background

Chronic hepatitis B (CHB) has been one of the most concerning diseases worldwide. Every year, approximately 1 million people die of CHB-related cirrhosis and hepatocellular carcinoma[1]. Nucleos(t)ide analogues (NAs) therapy is widely applied in patients with chronic hepatitis B virus (HBV) infection. Currently, six NAs are approved for the treatment of CHB infection: lamivudine, telbivudine, entecavir (ETV), adefovir, and tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF), of which ETV and TDF are recommended as first-line therapy[2-4]. NAs inhibit the reverse transcriptase activity of the HBV polymerase and thus suppress viral replication. However, NAs exert little effect on the viral covalently closed circular DNA (cccDNA) in the nucleus[5]. Hence they can not permanently eradicate the virus. Currently, hepatitis B surface antigen (HBsAg) loss is the most widely accepted endpoint to guide cessation of NA treatment. However, as HBsAg loss is an infrequent event, this strategy entails an indefinite therapeutic duration that could be lifelong for the vast majority of NAs-treated patients[6].Therefore, the benefit of long-term treatment should be weighed against the burden of life-long medication, monitoring, and adherence. In addition, the long-term risk for the development of resistance and adverse events remains unclear.

In recent years, some scholars had attempted to find approaches for preventing relapses after NAs withdrawal. A previous study observed the outcomes after the cessation of ETV therapy among patients who fulfilled the stopping rules of the Asia-Pacific Association for the Study of the Liver (APASL). The 2-year cumulative rates of virological and clinical relapse were 41.3% and 33% in hepatitis B e antigen (HBeAg)-positive patients, and 3-year cumulative rates of virological and clinical relapse were 62.7% and 48.3% in HBeAg-negative patients, respectively. The risk is alarming after discontinuation of the therapy.

Traditional Chinese medicine (TCM) therapy has a long history and definite curative effect treating various chronic liver diseases, and abundant data and experience have been accumulated in long-term clinical practices and scientific research. Although TCM therapy alone has no explicit antiviral effect, it has some advantages in improving clinical symptoms, alleviating liver inflammation, anti-fibrosis, and regulating immune function. In China, supported by major national special research projects funded by the Ministry of Health, the treatment of chronic hepatitis B by the combination of TCM and NAs has achieved some research results, the advantages of TCM therapy in the treatment of chronic hepatitis B have been preliminarily clarified,[7] the integrated TCM and NAs therapeutic schedule has been developed,[8] and it has been proved that the combination of TCM and NAs can significantly enhance the negative conversion ratio of HBeAg, including that of refractory diseases.[9,10] Based on the preliminary work, this study carries out research of integrated TCM and NAs for HBeAg-positive chronic hepatitis B. Through national multi-center double-blind randomized controlled study, the effect of integrated TCM and NAs in reducing the recurrence rate after drug withdrawal is to be verified.

Methods And Results

Registration

This multi-center, double-blind, randomized placebo-controlled trial was registered before recruitment on the Chinese Clinical Trial Registry (No. ChiCTR1900021232) and conducted in accordance with the principles of the Declaration of Helsinki (2004 version). The study protocol was approved by Ethics Committee of Dongzhimen Hospital, affiliated to Beijing University of Chinese Medicine, before recruiting participants (Ethical approval No.DZMEC-KY-2018-61).

Recruitment

A total of 490 patients will be recruited by eighteen clinical trial centers in China listed as follows: Dongzhimen Hospital affiliated to Beijing University of Chinese Medicine, Shenzhen Traditional Chinese Medicine Hospital, Guangdong Hospital of Traditional Chinese Medicine, Liaoning Hospital of Traditional Chinese Medicine, the First Affiliated Hospital of Guangxi University of Chinese Medicine, Shaanxi Hospital of Traditional Chinese Medicine, Mengchao Hepatobiliary Hospital of Fujian Medical University, Beijing Chinese Medicine Hospital, Beijing Ditan Hospital, Chongqing Traditional Chinese Medicine Hospital, Affiliated traditional Chinese medicine hospital of Southwest Medical University, Shanghai Shuguang Hospital, the Sixth People's Hospital of Shenyang, 302 Military Hospital of China, Shandong

Hospital of Traditional Chinese Medicine, the Second People's Hospital of Tianjin, Public Health Clinical Center of Chengdu, the Third People's Hospital of Shenzhen. The case inclusion obeyed the principle of geographical balance nationwide. Volunteers were mainly recruited from outpatient clinics. The principal investigator will introduce the protocol as well as the benefits and risks of the study to the participants. An informed consent form is mandatory for enrollment.

The case inclusion obeyed the principle of geographical balance nationwide. Volunteers were mainly recruited from outpatient clinics. The inclusion and exclusion criteria were listed below. The criteria for premature withdrawal from the study included protocol deviation, pregnancy, or investigator discretion. Participants were also entitled to halt the study any time.

Inclusion Criteria

(1) Diagnosed CHB with positive HBeAg, who has been achieved HBeAg loss and/or seroconversion; (2) Aged between 18 and 65; (3) TBIL < 3×ULN; (4) HBsAg < 5000 IU/ml; (5) TCM symptoms are classified as Stagnation of liver and deficiency of Spleen Qi, Damp-Heat in the liver and gallbladder; (6) Voluntarily sign informed consent.

Exclusion Criteria

Any of the following cases will be excluded: (1) Diagnosed with chronic hepatitis caused by non-viral causes, overlapped virus infections, or cryptogenic hepatitis; (2) Accompanied by liver failure, hepatocirrhosis (including stage 4 fibrosis), hepatic encephalopathy, electrolyte disorders, gastrointestinal bleeding, fatal infections or any other severe complications; (3) Diagnosed with malignant tumors or with a progressive elevation of serum tumor markers; (4) Diagnosed with primary or secondary cardiovascular, cerebrovascular, pulmonary, renal, endocrine, nerve and hematology diseases; (5) Participating or participated in other clinical trials within one month; (6) Diagnosed mental disorders; (7) Confirmed Hepatitis B virus Pre C/C or P gene variants; (8) Pregnant or lactating women, individuals that are scheduled to conceive or fertilize; (9) With other unfavorable situations.

Sample Size Estimation

We calculated the samples size by the equation: N = (Uα + Uβ)2 × 2P × (1 - P) / (P1 - P0) 2. We referred to a study published in 2016, in which the recurrence rate was 32% CHB patients after withdrawal NAs at 24weeks. In this study, we assumed to reduce the recurrence rate by 10% to 22%. The certainty was set at 80%, considering 20% of sample lose. The sample size was set to 245 for each group, 490 in total.

Study Drugs

The trial ingested TCM granules named Tiaogan-Buxu-Jiedu Granule(□□□□□□□□ TGBXJD) and corresponding placebos. All patients were randomized to treatment with Tiaogan-Buxu-Jiedu Granule or placebos combined with entecavir (ETV). TGBXJD Granules (including placebos, 30g per dose) were provided by PuraPharm Co. Ltd, China (lot No. A190069710). The manufacturing procedure was as

follows: every herb, including Bupleurum, atractylodes rhizome, scutellaria root, was dynamic extracted of hanging basket method with 97±2°C water. The extract was then filtered through 200 mesh filter cloth, and the filtrate was concentrated under vacuum to relative density 1.10 ~ 1.20 (temperature 60 ± 5 ° C), a suitable amount of dextrin and water was added, the concentrate was mixed after stirring for 30 minutes, the dried extract powder was obtained after spray drying, the calculated amount of dextrin was added to mix uniformly, so the herbal granulates were obtained by dry granulation method. ETV tablet (0.5 mg per tablet) were provided by CHIATAI TIANQING company, China (lotNo.181214101,181203201,181024101). The major ingredients of TGBXJD are scutellaria, Bupleurum and atractylodes.

Therapeutic Regimen

Patients who meet the inclusion criteria will be allocated in a 1:1 ratio to either the experimental group or the control group. Patients in the experimental group will receive TGBXJD Granule along with ETV. Patients in the control group will receive placebo along with ETV. The treatment duration is 96 weeks. All patients will accept drug withdrawal and observe for 24 weeks after treatment course. Once clinical recurrence occurs, antiviral treatment is suggested.(Figure 1)

Randomization and Blind

Stratified block randomization is conducted by the third party (Chinese Academy of Chinese Medicine Sciences, CACMS) with their online central randomization system. Clinical physicians in sub-centers oversaw recruitment. They would apply for an ID through a web interface provided by CACMS for each patient, by which the patients would be randomly allocated to each group at a ratio of 1:1 via a central randomization system. Each trial drug was assigned to a patient with a unique number, which would be dispatched by the online system. All experimental drugs and corresponding placebo were consistent in appearance and taste. The grouping information of each participant was concealed to all research personnel and data analyzers until the trial ended.

Interviews

From 1st day to the observation point of week 96, patients would be interviewed once every 24 weeks (± 3 days). Corresponding drugs were dispatched each visit. From week 96 to week 120, interviews were conducted every 4 weeks (± 3 days). Medications will be reintroduced in patients who had a relapse during the period of observation.

Assessments

To ensure safety and reveal the curative effects of trail drugs, assessments were adopted regularly throughout the whole trial. (Table 1)

Primary Outcomes

The primary outcome measurement will be the recurrence rate after drug withdrawal.

Secondary Outcomes

Secondary outcome measurements include the virus serological indicator, HBV DNA, liver function, liver biopsy, cccDNA, HBcrAg, HBV-pgRNA, L-HBsAg, symptomatic score of TCM, SF-36 and the CLDQ, which will be measured at the intervention.

Safety Monitoring

Primary vital signs, physical examinations, and some laboratory tests will be performed for safety assessment daily. Primary vital signs include body temperature, blood pressure, heart rate, and respiratory rate. Laboratory tests include routine blood, urine, and stool tests, along with fecal occult blood tests, ECG, abdominal B ultrasound scan. In particular, the relapse after drug discontinuation was defined HBV DNA levels > 2,000 IU/mL as while serum ALT > 2-fold of the upper limit of the normal.

Adverse events

All unexpected AEs occurring during the intervention period will be reported, and the causality related to the TGBXJD will be analyzed. If any adverse event occurs, the study managers will ensure that the participant receives adequate treatment. The AEs will be immediately reported to the principal investigator and to the ethics committee to decide whether the participant should withdraw from the trial. If any symptoms aggravate, the patient will be withdrawn from the study and will be referred for further treatment.

Statistics Analysis

The statistical report is made by an independent statistical analysis team using the proposed blind method in the statistical analysis plan and then submitted to the personnel responsible for summarizing clinical research data for comprehensive analysis. The statistical analysis plan covers objectives, indicators, analysis types, hypothesis testing, significance level, statistical software, analysis, and result expression methods, curative effect analysis, safety evaluation, and analysts, etc. The statistical analysis team conducts intention-to-treat (ITT) analysis of the main effect indicators.

Data description: The enumeration data is described by the constituent ratio, the measurement data is represented by mean ± standard deviation, and non-normal distribution is described by median and interquartile range.

Comparison of baseline data: Pearson chi-square test or Fisher test is used for comparison of enumeration data, group variance test is used for the comparison of normal distribution measurement data, and non-parametric analysis and test is used for the comparison of non-normal distribution measurement data.

Comparison of curative effect: The total effect of the scale scores is tested by Wilcoxon rank-sum test. Variance test is adopted for the baseline comparison of symptom score and rate, the comparison after treatment and the difference comparison among groups before and after treatment, and paired t-test is used for the comparison before and after treatment within groups.

Safety evaluation: The comparison of quantitative data in the laboratory is made using the variance test. Meanwhile, all the laboratory data is classified according to whether they are normal or not. The number of normal and abnormal cases before and after treatment is respectively described according to different intervention groups. The adverse events are described using incidence, and the specific performance and degree of all the adverse events in each group and their relationship with drugs are described in detail.

Discussion

HBV infection remains a major global health concern, as the disease itself and its complications, mainly hepatocellular carcinoma (HCC) and cirrhosis, caused 887,000 deaths in 2015 alone[11]. Currently, therapeutic drugs for CHB are limited to two types of NAs and interferon. Nucleotide drugs can inhibit virus replication for a long time, but it cannot prevent the synthesis of cccDNA[12], so it is still challenging to achieve clinical cure. Functional cure refers to continuously negative HBV DNA and negative conversion or seroconversion of HBsAg, but it can only be realized in a few patients. A systematic evaluation included into 34 published studies and involving 42,588 patients showed that the annual clearance rate of HBsAg in CHB patients is about 1% (including treated and untreated populations), and that in HBeAg-negative patients and HBeAg-positive patients is 1.33% (95%CI, 0.76-2.05) and 0.40% (95%CI, 0.25-0.59), respectively[13]. Therefore, lifelong medication is required for most patients.However, long-term treatment by nucleotide drugs still has problems that cannot be ignored. Although the drug resistance rate of the currently recommended Entecavir and TDF is low, the forward incidence rate is unclear. Meanwhile, the hygienic economic burden brought by long-term oral drugs is a vital trade-off factor. The withdrawal of NAs can significantly reduce expenditures for medical care and public health. Also, the side effects of NAs can not be neglected[14], such as the renal toxicity and skeletal toxicity of the first-line therapeutic drug TDF. The new drug TAF can solve this problem, but its price is high and thus the drug has not been widely applied in many countries.

In recent years, a number of scholars have proposed the concept of immune control, namely, the host's immune system still exerts significant and explicit control over HBV after drug withdrawal, HBV DNA is in an immeasurable level, and no virology or clinical relapse will occur a long time after drug withdrawal (above 6 months). Therefore, the long-term virology inhibition after the withdrawal of nucleotide drugs can be regarded as the possible endpoint of therapy. According to the Asia Pacific Association for the Study of the Liver (APASL) Guidelines, HBeAg-positive patients can try to discontinue drugs under strict follow-up visits after serological conversion of e-antigen and consolidation therapy for 12 months[15].

Current studies show that the risk of relapse after NAs withdrawal is high. A prospective study included 178 CHB patients, and 59.5% of the patients suffered virological relapse within two years, and 48%

suffered clinical relapse. However, the safety of drug withdrawal for HBeAg-positive patients seems to be higher. Liu F et al. [16] conducted a long-course observational study on drug withdrawal. A total of 223 patients were included in the study. The results showed that the 10-year relapse rate of HBeAg-positive patients was significantly lower than that of HBeAg-negative patients (30.9% vs 62.3%). Surprisingly, some studies show that a small number of patients eventually achieved negative conversion of HBsAg after withdrawal of nucleotide drugs and temporary relapse. A large Taiwan study included a total of 1,075 cases of HBeAg-negative patients treated by NAs. Thereinto, 6 patients obtained HBsAg clearance during the treatment, and the annual HBsAg clearance rate was about 0.15%. 691 patients discontinued NAs according to the APASL Guidelines. After a median follow-up visit of 155 weeks, 42 patients achieved HBsAg clearance, the 6-year cumulative HBsAg clearance rate was 13%, and the annual HBsAg clearance rate was about 1.78%. Some patients achieved HBsAg clearance after drug withdrawal and virological relapse[17]. Although there is no unambiguous interpretation for this research result, drug withdrawal seems to be possible. More and more studies have shown that the level of HBsAg during withdrawal is an important influencing factor of the relapse rate. A systematic evaluation including 11 studies and 1,716 patients showed that, regardless of the state of HBeAg during drug withdrawal, the relapse rate in patients with HBsAg level greater than 100IU/ml was significantly higher than that in patients with HBsAg level less than 100IU/ml, the virology relapse rate of the above two populations was respectively 9.1%-19.6% and 31.4%-86.8% after drug withdrawal for more than 12 months, and the clinical relapse rate was 15.4%-29.4% and 48.1%-63.6%, respectively. Moreover, patients with HBsAg level less than 100IU/ml at drug withdrawal were more likely to achieve spontaneous immune clearance after drug withdrawal.[18]. Besides, some scholars have carried out relevant research on other critical markers in the HBV replication cycle. Large and medium surface proteins of HBV (LHBs, MHBs)[19], HBV RNA, and hepatitis B core-related antigen (HBcrAg)[20,21] may become serological indicator of cccDNA, and have predictive value in the efficacy monitoring of anti-virus and the outcome after drug withdrawal. The risk of virological recurrence after drug cessation in patients who had undergone long-term NAs therapy is to be observed in the current study. It is also our goal to further investigate the criteria for safely drug withdrawal and to explore the predictive factors of relapse. Previous studies have shown that the combination of TCM and NAs can improve HBeAg loss, especially in refractory population, the long-term effect is better than NAs monotherapy without compromising the safety profile. Interestingly, certain herbal combinations could act as ‘immune-incubation agent’ or ‘synergist of NAs’ against HBV infection, particularly in HBeAg positive CHB patients[8]. This is the first time that integrated Chinese and western medicine has been used to reduce the off-drug recurrence risks in NAs treated population. The results will provide patients with alternative options, especially in those who had undergone long-term NAs treatment and are unwilling to take NAs lifelong.

Trial Status

The first subject was included in the trial in Mar. 2019. A total of 490 patients will be subsequently recruited, and the recruitment is still open. The trial status has been updated in the Chinese clinical trial

registry database. Recruitment is expected to end late 2019. The version number and date of the protocol are v1.0, and December 11, 2018, respectively.

Declarations

Ethics approval and consent to participate

The study protocol was approved by Ethics Committee of Dongzhimen Hospital, affiliated to Beijing University of Chinese Medicine, before recruiting participants (Ethical approval No.DZMEC-KY-2018-61). and we will not begin recruiting at other centers in the trial until local ethical approval has been obtained. The purpose, procedures, and potential risks of the RCT will be explained clearly to the participants. All participants will give their written informed consent to the research assistant before joining the RCT.

Consent for publication

Not applicable.

Availability of data and materials

Data are all contained within the paper. And we will share the clinical research data in the system of Chinese Clinical Trials Registry (No. ChiCTR1900021232, 2019/02/02, http://www.chictr.org.cn/showproj.aspx?proj=35297)

Competing interests

The authors declare that they have no competing interests.

Funding

This study is funded by China National Science and Technology major projects 13th 5-year plan, No.2018ZX10725505.

Author Contributions

Xiaoke Li is the vice PI of the project and academic consultant, Ludan Zhang is the executive secretary. Mei Qiu, Yi Huang, Huanming Xiao, Bingjiu Lu, Yuyong Jiang, Fuli Long, Hui Lin, Jinyu He, Qikai Wu, Mingxiang Zhang, Li Wang, Xiaoning Zhu, Man Gong, Xuehua Sun, Jianguang Sun, Fengxia Sun, Wei Lu, are PIs of co-centers. Zhiguo Li, Danan Gan, Xianzhao Yang participated in program and helped with polishing the manuscript. Hongbo Du is the vice PI of the project, and Yong'an Ye is the PI of the project.

Acknowledgements

We thank the patients for participating in the trial dissemination. We also thank the authors of the primary studies and our colleagues supporting the study

Abbreviations

ALT: alanine aminotransferase; cccDNA: closed circular DNA; CHB: chronic hepatitis B; ECG: electrocardiogram; ETV: entecavir; HBeAg: hepatitis B envelop antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; IFN: interferon; NAs: Nucleot(s)ide analogue; RCT: randomized controlled trial; RGT: biejia ruangan; TBIL: total bilirubin; TCM: traditional Chinese medicine; WM: Western medicine TGBXJD: Tiaogan-Buxu-Jiedu Granule; ULN: upper limit of normal.

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Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Figures

Figure 1

Flowchart of the study design

Supplementary Files

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