Effect of Jianpi Qinghua Decoction on Gut Microbiota, Intestinal Permeability and Glycolipid Metabolism in Newly Diagnosed T2DM Patients: Study Protocol for a Randomized, Double-blind, Placebo-controlled Clinical Trial

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Abstract

Background

The gut microbiome is a key target for traditional Chinese medicines (TCM) to ameliorate type 2 diabetes mellitus (T2DM). Patients with T2DM have quite distinct gut microbiota in comparisons with normal individuals, and it directly influences intestinal permeability. This study aims to investigate the underlying effects of Jianpi Qinghua Decoction on gut microbiota and intestinal permeability in newly diagnosed T2DM patients.

Methods / design

This study is a single-center, randomized, double-blind, placebo-controlled clinical trial in 120 subjects with newly diagnosed T2DM. The patients will be randomized in a 1:1 ratio into placebo group (1/10 Jianpi Qinghua Decoction + maltodextrin twice daily, n = 60) and treatment group (Jianpi Qinghua Decoction twice daily, n = 60). The study drugs will be double blinded to both investigators and participants. The visits will be done at baseline (visit 0), 1 month (visit 1), 2 months (visit 2), 3 months (visit 3). Measures include: anthropometric measures, blood pressure, glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), 2-hour postprandial blood glucose (2hPG) and serum insulin, total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL), safety indexes (including blood routine examination, clinical urine tests, liver/renal profile), identifying intestinal permeability used ELISA testing serum LPS, serum intestinal fatty acid binding protein (IFABP), serum Zonulin concentrations and fecal sample collection for gut microbiota profiling through 16S rRNA gene sequencing. Data will be analyzed using SPSS version 26.

Discussion

We describe a clinical research protocol evaluating the impact of Jianpi Qinghua Decoction on gut microbiota and intestinal permeability in newly diagnosed T2DM patients. We assume that administration of Jianpi Qinghua Decoction will correct gut microbial dysbiosis and intestinal permeability, leading to improved glycemic control.

Trial registration

Chinese clinical trial registry, identifier: ChiCTR2000039423 <http://www.chictr.org.cn/edit.aspx?pid=62537&htm=4>. Registered on 27 October 2020.

Background

Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases in the world, moreover, brings a heavy economic burden to individuals and society. The ADA guidelines pointed out that after adjusting for inflation, economic costs of diabetes increased by 26% from 2012 to 2017 [1]. Insulin

resistance (IR) is the major predisposing factor to T2DM, whereas the pathological mechanism of IR is still unclear [2].

In recent decades, gut microbiota disorders are gradually recognized as risk factor of developing T2DM, abnormalities in gut microbiota composition and/or its functionality also contribute to IR [3]. Gut dysbiosis results in aberrant metabolite concentrations that disturb gut barrier integrity. Those bacterial deleterious metabolites such as lipopolysaccharide (LPS), trimethylamine (TMA) etc. translocate into the circulation through loose gut barrier and contribute to the development of systemic chronic low - grade inflammation [4]. Chronic low-grade inflammation is not only a central factor contributing to the development of T2DM, but also is an established factor in the etiopathogenesis of IR [5]. For instance, this gut - derived LPS could alter epithelial permeability by affecting the expression of tight junction proteins, subsequently launch an inflammatory cascade via activation of pattern recognition receptors (PRRs) such as TLR4 in adipose and liver tissues [6-7]. Increased proinflammatory markers in adipose and liver tissues will lead to insensitivity of insulin signaling pathway which decreases uptake of glucose [8]. The pivotal role of intestinal permeability in chronic low - grade inflammation therefore makes the microbiome a core player in the metabolic inflammation - related T2DM [9].

Jianpi Qinghua Decoction is the modified formulation of a classical Chinese prescription named Gegen Qinlian Decoction including *Pueraria lobata*, *Scutellaria*, *Coptis chinensis*, *Euonymus alatus*, *Codonopsis pilosula*, *Astragalus*, *Dioscorea polystachya*, *Polygonatum*. On the basis of our previous retrospective clinical research, individuals of T2DM with Jianpi Qinghua Decoction displayed improvement in weight and glucolipid metabolism parameters [10]. In vivo studies, Jianpi Qinghua Decoction could mitigate IR level of T2DM rats via regulating the transcription factors expression and inflammatory factors concentration of Th1 and Th17 cells in intestinal tissues [11]. Qing-Hua Granule, a simplified Jianpi Qinghua Decoction, can induce GLP-1 secretion in db/db mice partly through activation of intestinal bitter taste receptor pathway (Gagust/PLC β 2/TRPM5) [12]. Meanwhile, most of the herbs that make up the Jianpi Qinghua Decoction can exert anti-diabetic effects by regulating the gut microbiota and/or gut barrier. *Pueraria lobata* can increase the relate abundance of certain microbial communities that contribute to anti-obesity and anti-inflammatory activities in diet - induced obesity (DIO) mice [13]. *Scutellaria-Coptis* (SC) could upregulate the expression levels of the tight junction proteins, significantly improve intestinal epithelial damage; moreover SC had a strong inhibitory effect on some potential enteropathogenic bacteria and LPS-producing bacteria, such as *Proteobacteria*, *Enterobacteriaceae*, *Enterobacter*, *Escherichia-Shigella*, and *Enterococcus*, and could also promote the proliferation of butyrate-producing bacteria, such as *Lachnospiraceae* and *Prevotellaceae* [14]. administration of *Astragalus* significantly increased microbial richness and diversity in T2D mice and significantly altered the abundance of several bacterial taxa, inducing increased abundance of *Lactobacillus* and *Bifidobacterium* [15]. *Dioscorea polystachya* 'yam gruel' composed of *Dioscorea polystachya* improves cognitive function of diabetic rats partially through mediating gut microbiota and its metabolites [16]. Extract saponin from *Polygonatum sibiricum* could increase the number of probiotics, and reduce the number of harmful bacteria of diabetic mice, thus exerts hypoglycemic effects [17]. We also have identified the key active ingredients of Jianpi Qinghua Decoction by UPLC-MS analysis including

Dulcitol, Puerarin, Magnoflorine, Daidzoid, Berberamine Hydrochloride, Scutellarin, Ferulic acid, D-Tetrahydrojatrorrhizine, Lobetyolin, Baicalin, Epiberberine, Jatrorrhizine, Coptisine, Daidzein, Palmatine, Wogonoside, Berberine, Baicalein, Astragaloside IV, Wogonin (Supplementary Fig 1 and Supplementary Table 1). Most of these ingredients could improve glucose metabolism by adjusting gut microbiota.

Based on above research, we speculate that the target of Jianpi Qinghua Decoction which ameliorates clinical outcomes may be in the intestine. Therefore, we hypothesize that a shift in the gut microbiome induced by Jianpi Qinghua Decoction will alleviate intestinal epithelial permeability and result in improved glucose and lipid metabolism management.

Methods / Design

Objectives and hypothesis

Objectives

Main research objective is to explore the underlying effects of Jianpi Qinghua Decoction on gut microbiota and intestinal permeability in newly diagnosed T2DM patients.

Secondary objective of this trial are to compare the changes in glycemic and lipid control between the control and treatment groups.

Hypothesis

Jianpi Qinghua Decoction improves the metabolism of diabetes patients by regulating the structure and function of the microbiota as well as the intestinal permeability of newly diagnosed T2DM patients.

Overview

This study is a randomized, double-blind, placebo-controlled clinical trial designed following the CONSORT SPIRIT 2013 guidelines (see Additional file 1) [18], will be conducted over a period of 3 years from 2020, at Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine in Shanghai, China. 120 patients with newly diagnosed T2DM as determined by 1999 World Health Organization criteria (FBG 7 mmol/l or greater and / or 2-h OGTT 11.1 mmol/l or greater) [19] will be randomly allocated in a 1:1 ratio into a placebo or treatment group (n = 60 / group) and will receive either placebo (1/10 Jianpi Qinghua Decoction + maltodextrin) and granule (Jianpi Qinghua Decoction) twice daily, respectively, for 12 weeks. The eligibility criteria for the subjects are presented in Table 1. The participants will be recruited on a voluntary basis from the outpatient clinic of Shuguang Hospital and sign informed consent. The progression of trial enrollment, interventions and assessments is presented in Fig. 1.

Interventions and Lifestyle guidance

Interventions

The period of intervention will last for 3 months, the visits and the evaluations will be done as follows: screening (visit 0), 1 month (visit 1), 2 months (visit 2), 3 months (visit 3) (Fig. 1). The treatment drug is Jianpi Qinghua Decoction granule composed of *Pueraria lobata* 30g, *Scutellaria* 9g, *Coptis chinensis* 6g, *Euonymus alatus* 15g, *Codonopsis pilosula* 30g, *Astragalus* 30g, *Dioscorea polystachya* 30g, *Polygonatum* 30g, oral administration, twice daily, for 12 weeks. The placebo medication comprises 1/10 Jianpi Qinghua Decoction and dextrin, oral administration, twice daily, for 12 weeks. Patients with hyperlipidemia and / or hypertension should be treated with corresponding hypolipidemic and / or antihypertensive drugs according to specialist' s advice.

Jianpi Qinghua Decoction granule and placebo will be prepared by Lei Yun Shang Pharmaceutical Group Co., Ltd according to a standard production process. All of the drugs will be quality controlled throughout the trial. All herbs used in this study are from the same place and in the same batch.

Lifestyle guidance

Participants in both groups of the study will receive lifestyle advice from a specialist. This advice is according to Guideline for the prevention and treatment of type 2 diabetes mellitus in China for Chinese adults [20]. The lifestyle intervention of the guideline focus on introduction of T2DM – related knowledge, daily nutrition management and physical exercise.

Anthropometric parameters and blood pressure measurement

Anthropometric parameters will be measured at visit 0 (baseline visit) and visit 3 (at 12 weeks). Height (cm) will be measured using tape measure to the nearest 0.1 cm. Body weight (kg) will be measured in light, indoor clothing to the nearest 0.1 kg using a universal weight scale. Calculating Body Mass Index (BMI, kg / m²) according to the formula: BMI = Body weight / Height². Waist circumference (WC, cm) will be measured at midway between the iliac crest and the lower costal margin at the end of normal expiration using a tape measure to the nearest 0.1 cm. Similarly, the hip circumference (HC, cm) also will be measured at widest part of the buttocks at the intertrochanteric level to the nearest 0.1 cm using a tape measure. Calculating Waist-to-hip ratio according to the formula:Waist-to-hip ratio =WC / HC. All anthropometric parameters will be made by a same researcher.

Blood pressure for each participant will be measured by a same researcher at visit 0 (baseline visit) and visit 3 (at 12 weeks) using a mercury sphygmomanometer in both of the hands and the mean blood pressure readings. Participants will be required to rest for 10 minutes prior to the first blood pressure measurement with a minimum of 15 minutes between the two occasions.

Biochemical assays

Blood samples will be collected for fasting blood glucose (FBG, mmol/L), 2-h plasma glucose (2hPG, mmol/L), glycated hemoglobin (HbA1c, %), fasting insulin (FINS, mU/L), total cholesterol (TC, mmol/L), total triglycerides (TG, mmol/L), high-density lipoprotein cholesterol (HDL, mmol/L), and low-density

lipoprotein cholesterol (LDL, mmol/L). These parameters will be measured in a clinical laboratory of Shuguang Hospital. The homeostatic model assessment of insulin resistance (HOMA - IR) index will be used to determine insulin resistance using the following formula:

$$\text{HOMA - IR} = \text{FBG} \times \text{FINS} / 22.5.$$

Safety Assessment Index

Safety indices will be tested on visit 0 (baseline visit) and visit 3 (at 12 weeks). Renal function tests for serum creatinine (Scr, $\mu\text{mol/L}$), serum uric acid ($\mu\text{mol/L}$), serum carbamide (mmol/L). Hepatic function tests for aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), AST / ALT, γ -glutamyltransferase (U/L), total bilirubin ($\mu\text{mol/L}$). Blood routine and urine routine also will be tested. Adverse events will be recorded in the Case Report Forms (CRF), the specific treatment measures of adverse events see the *supplementary materials*. All of parameters will be measured in a clinical laboratory of Shuguang Hospital.

Intestinal permeability

Intestinal permeability will be assessed by using ELISA detecting serum Lipopolysaccharide (LPS)(Lipopolysaccharide ELISA Kit, Cloud – Clone Corp, China), serum intestinal fatty acid binding protein 2 (IFABP2) (intestinal fatty acid binding protein 2 ELISA Kit, Cloud – Clone Corp, China), serum Zonulin (Human Haptoglobin ELISA Kit, BOSTER, China) concentrations.

Gut microbial profiling

Stool samples will be collected at baseline and 3months. Microbial community genomic DNA will be extracted from stool samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's instructions and quantify using NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene will be amplified by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR product will be extracted from 2% agarose gel and purify using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantify using Quantus™ Fluorometer (Promega, USA). Purified amplicons will be pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Data analysis

Sample size

In this study, the sample size was estimated based on data from a previous study [21]. A mean difference in HbA1c level of 1.3% between the two groups is aimed to be detected for a specified α of 0.05 and a study power of 90%. Thus the calculated sample size was 45 cases in the Jianpi Qinghua Decoction

group and 45 cases in the control group. It is estimated that the drop-out rate in this study is 20%, so the final sample size of this study included 60 cases in the Jianpi Qinghua Decoction group and 60 cases in the placebo group, total 120 cases.

Randomization

An independent statistician will use IBM SPSS Statistics version 26.0 (SPSS Inc., Chicago, USA) to generate random sequences according to the central block randomization method. Eligible patients will be randomly assigned to the treatment group and Placebo group at a ratio of 1:1.

Blinding

Both of the investigators and patients are blind to the treatment allocations. The medication will be delivered in analogical packages and labels, each one with its own serial number. The allocation serial number will be generated by a independent statistician. Envelopes containing a monthly supply of either placebo or Jianpi Qinghua Decoction will be prepared according to the randomization sequence and supplied to the patients by the research assistants when they are randomized to the trial.

Data collection, management and monitoring

Questions in the CRF collect a series of data including basic information of the patient, outcome and adverse events. Without the consent of the investigator and the patient, the CRF data shall not be released to the public. The same research investigator throughout the study standardize the data collection procedure. CRF will be used to record data for all participants, and data will enter an electronic database to preservation.

Establishing a special data entry system for this study based on the CRF. The data entry clerk will perform entry of the data and check it. After checked, the data manager will inspect data and manufacture a database list. The data manager confirm that the contents on the list are completed, the researcher will approve the database lock and sign the name and date. The data manager should save the relevant documents of data management as required, and transfer the locked database and related materials to the statistician for statistical analysis. All samples will be kept in a constant temperature and humidity storage and saved by expert staff. The main researcher will have access to the final trial dataset.

Statistical analysis

All data were presented as the mean $\pm$ standard deviation. the measurement data which not conforming to the normal analysis are described by the median, and the count data are described by the composition ratio. Data will be processed and analyzed using SPSS 26.0 software, Parametric (e.g., ANOVA) and non-parametric (e.g., Kruskal-Wallis test) statistical tests will be used for comparison between groups. $P < 0.05$ will be considered to indicate statistical significance.

Discussion

In this article, we describe a protocol designed for a clinical trial to evaluate the efficacy and safety of Jianpi Qinghua Decoction in the newly diagnosed T2DM patients. This trial will provide a potential therapeutic drug for improving glycemic and lipid profiles among T2DM individuals by correcting gut dysbiosis and Intestinal permeability.

The innovations of this experiment include: (1) There are few rigid Randomized controlled trials (RCTs) to prove the effect of hypoglycemic effect of TCM, so we hope to prove the effect of Jianpi Qinghua Decoction on improving metabolism through normative RCT; (2) ameliorating metabolic disorders through gut microbiota is considered to be a promising approach, but there is still a lack of rigorous clinical evidence, especially for traditional Chinese medicine, our trial will get more evidence from the population; (3) the intestinal barrier prevents harmful substances and bacteria from entering the circulation, once barrier dysfunction occurs, it can cause serious metabolic problems, but drugs to reduce intestinal permeability are very scarce. Our experiment may provide a new choice of drugs to improve the intestinal barrier function.

The primary challenge of our study will be the short-term impact of the intervention. This challenge will be solved by further experiment.

If Jianpi Qinghua Decoction is shown to be effective in management of newly diagnosed T2DM patients, the formula would be a very tempting alternative for current medications.

Declarations

Trial status

Enrollment for the trial has not yet started (scheduled date – 31 December 2023).

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Not applicable

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Availability of data and materials

Not applicable

Authors' contributions

Shenyi Jin and Hao Lu substantially contributed to the general idea and design of the study. Shenyi Jin, Sheng Zhang, Qingguang Chen, Xu Han, Yahua Liu, Zihui Yan, Mengjie Cai, Hao Lu took part in designing

the protocol. Shenyi Jin and Sheng Zhang planned the data analysis. Shenyi Jin drafted the manuscript. All authors have read, consented and approved the manuscript.

Ethics approval and consent to participate

Study is approved by the Ethics Review Committee of the Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine in Shanghai, China.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Dissemination of study finding

The results of the above study will be published in local and international, peer-reviewed journals.

Ethical considerations

The study has been approved by the Ethics Review Committee of the Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine in Shanghai, China. The trial is also registered at the Chinese clinical trial registry (identifier: ChiCTR2000039423). The study will be conducted in compliance with the Declaration of Helsinki and the GCP guidelines. Any change in trial protocol will be informed to the relevant regulatory authorities and trial participants, with reconsent being taken from participants, if required.

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Tables

Table 2: Eligibility criteria for participants in the trial
Inclusion criteria:
(This study will enroll male and female patients who need to meet the following conditions)
☒ Newly diagnosed T2D patients according to the 1999 World Health Organization criteria (FBG 7 mmol/l or greater and / or 2-h OGTT 11.1 mmol/l or greater [20]) who had not previously received pharmacological treatment;
☒ $6.5\% \leq \text{HbA1c} \leq 10\%$; $7 \leq \text{FBG} \leq 13.0 \text{ mmol/L}$;
☒ Age 30 - 70 years old;
☒ Body mass index (BMI): $24.0 \leq \text{BMI} \leq 30.0 \text{ kg}\cdot\text{m}^{-2}$;
☒ Individuals who have not participated in any clinical trials within 3 months before being selected;
☒ No serious heart, liver, or kidney diseases;
☒ Able to keep taking granules for 3 months;
Exclusion criteria:
(Patients will be excluded if they meet the following criteria)
☒ Impaired liver function defined as AST / ALT exceeded the upper limit of the normal range by 2.5 times; Renal insufficiency defined as serum creatinine $> 132\mu\text{mol}\cdot\text{L}^{-1}$ or estimated glomerular filtration rate (eGFR) $< 60\text{mL} (1.73 \times \text{min} \times \text{m}^2)^{-1}$; mental illness; severe infection; severe anemia and neutral Neutropenia;
☒ Severe heart diseases, including but not limited to congenital heart disease, rheumatic heart disease, hypertrophic or dilated cardiomyopathy. New York Heart Association (NYHA) cardiac function grade $\geq$ III;
☒ Diabetes caused by type 1 diabetes, monogenic diabetes, pancreatic injury or other secondary diabetes (such as Cushing's syndrome, thyroid abnormalities and acromegaly);
☒ Treatment with anti-diabetic drugs including oral anti-diabetic drugs, GLP-1 agonists or insulin;
☒ A history of acute diabetic complications within 3 months, including diabetic ketoacidosis or hyperosmolar hyperglycemia nonketotic coma;
☒ Have used antibiotics, steroid hormones, cytokines that can stimulate the body's immune system, methotrexate and other immunosuppressants in the past three months; used topical antibiotics (such as mouthwash) in the past 7 days;
☒ Taking weight-loss drugs; corticosteroids administered by oral, intramuscular, intravenous, parenteral or intraarticular within the past 3 months;
☒ Gastrointestinal diseases which can cause accurate chronic diarrhea or severe constipation;
☒ Serious diseases such as tumor, immune system, blood system and other diseases in the past 5 years;
☒ The history of bowel resection or other gastrointestinal surgery (such as cholecystectomy) in the past 1 year, or other history of parenteral surgery in the past 6 months;

☒ History of substance addiction and alcohol abuse;
☒ Pregnancy (HCG-positive urine in women of reproductive period), breastfeeding;
☒ From the researcher's point of view, any factors that may interfere with the experiment;
Special standards for gut microbiome research: (The intestinal microbiota can be affected by many factors, hence in order to ensure the reliability of the results of 16S rRNA sequencing, the subjects are required to meet the following conditions during the trial)
☒ During the entire study period, a light diet should be maintained for 3 days, unless necessary, high-fat foods should be avoided;
☒ 7 days before the visiting, better not consume fermented dairy products (such as yogurt) and probiotics;
☒ Throughout the trial period, not take antibiotics (such as penicillin, cephalosporin, tetracycline, etc.) or other pharmaceuticals that may affect the gastrointestinal tract. If these drugs have to be taken, the use of this kind of medication must be recorded in detail in the accompanying medication form;
Rejection criteria:
☒ Participants that did not meet the inclusion criteria or were mistakenly included;
☒ Individuals who cannot complete the required observation items after attending the research;
☒ Participants who do not cooperate after inclusion;
☒ Patients with incomplete clinical data after inclusion;
Exit criteria: (Patients will withdraw from the trial if the following events occur)
☒ Adverse events;
☒ Other concurrent diseases or worsening conditions.

Figures

TIMEPOINT	STUDY PERIOD				
	Enrolment	Allocation	Post-allocation		
	-t ₁	Day 0	Visit 1 (1 month)	Visit 2 (2 month)	Visit 3 (3 month)
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Allocation		X			
INTERVENTIONS:					
JPQHF		X	X	X	X
placebo		X	X	X	X
ASSESSMENTS:					
Medical history taking	X				
Fasting Blood Glucose		X	X	X	X
2hPG		X			X
Fasting insulin		X			X
HOMA-IR		X			X
HbA1c		X			X
Blood routine examination		X			X
Clinical urine tests		X			X
High sensitivity C-reactive protein					
Lipid profile ¹		X			X
Liver/Renal profile ^{2,3}		X			X
Blood pressure		X			X
Physical examination ⁴		X			X

Figure 1

Summarized study schedule at each visit in the clinical trial

Supplementary Files

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