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Efficacy of calcium dobesilate in treating Chinese patients with mild to moderate non-proliferative diabetic retinopathy (CALM-DR): protocol for a single-blind, multicenter, cluster-randomized, controlled trial

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Abstract

Introduction

Calcium dobesilate (CaD) has been recommended to treat diabetic retinopathy (DR) due to its potential in protecting against retinal vascular damage. However, there is limited evidence exploring its efficacy in combating DR progression. This study is aimed at evaluating whether CaD could prevent DR progression into an advanced stage among Chinese patients with mild to moderate non-proliferative DR (NPDR).

Methods and analysis

This study is a single-blind, multicenter, cluster-randomized, controlled superiority trial. A total of 1,200 patients with mild to moderate NPDR will be enrolled and randomly assigned at a 1:1 ratio into the control group (conventional treatment group) and the intervention group (conventional treatment plus CaD [500 mg trice daily] for 12 months). Patients will be followed at 1, 3, 6, and 12 months after randomization and receiving treatments, with the severity of DR assessed by the Early Treatment Diabetic Retinopathy Study (ETDRS) scale. The primary endpoint is the progression of DR during follow-up, which is defined as an increase of 2 or more steps in the ETDRS scale. The secondary endpoints include the concomitant changes in visual acuity, presence, number, location and type of retinal lesions, and retinal blood vessel diameter as well as the arteriovenous ratio at different visits.

Ethics and dissemination

The Ethical Review Committees of Zhongda Hospital of Southeast University has approved the study (2019ZDSYLL132-P01). The results will be published in high

impact peer-reviewed scientific journals aimed at the general reader.

Trial registration numbers

NCT04283162.

Strengths and limitations of this study

(1) This will be the first cluster-randomized controlled trial with a large sample size assessing the efficacy of Calcium dobesilate (CaD) in treating diabetic retinopathy (DR) among individuals with mild to moderate non-proliferative DR.

(2) This study will collect longitudinal data on patients treated with CaD, and will report on the evolution of several important outcome measures over the first year.

(3) As with any longitudinal study, there is a risk of loss to follow-up throughout the study period, which may induce bias in the final results.

(4) A relatively short-term follow-up period of 12 months might also be a limitation for this study.

Introduction

Diabetic retinopathy (DR) is a common microvascular complication associated with diabetes, and encompasses a broad clinical spectrum from the mild non-proliferative DR (NPDR) to a more advanced stage of vision-threatening such as proliferative DR (PDR) and diabetic macular edema (DME).[1] The pathogenesis of DR involves a variety of abnormal pathways, which are triggered by a series of factors including chronic hyperglycemia, free radicals, advanced glycosylation end products, inflammatory cytokines, and vascular endothelial growth factors (VEGF).[2] These may eventually lead to blood-retinal barrier (BRB) breakdown, retinal neovascularization, and retinal neuronal apoptosis,[3] with BRB injury being considered the hallmark of DR.[4]

There is evidence that 30-50% of patients with diabetes may develop vision-threatening retinopathy during their lifetime,[5] and that timely intervention, such as intensive diabetes management, adjunctive anti-VEGF therapy, laser photocoagulation, and surgical management, can prevent up to 98% of the cases developing vision loss.[1] However, tight glycemic control and laser therapy could not reverse the existing ocular damage and may only slow its progression, while ocular surgeries have been questioned regarding its safety as well as its effectiveness in improving visual acuity.[6] Moreover, about three-quarters of the world's diabetic patients live in low-and middle-income countries, where healthcare resources are severely limited,[7] posing a serious challenge to the implementation of anti-VEGF therapy or surgical methods for DR.[2] Therefore, access to affordable medicines,

coupled with early detection of DR, may be a better way to prevent and delay diabetic blindness.[1]

CaD is an angioprotective agent that has been proposed as a treatment for DR by protecting against retinal vascular damage.[8 9] The earliest clinical study was a 2-year, double-blind, randomized controlled trial (RCT) involving 51 patients and 17 controls. The results showed that CaD was a potent angioprotector, which can prevent intra and extravascular hemorrhages of retina, reduce the incidence of exudate formation and improve visual acuity.[10] A recent meta-analysis of 8 RCTs consisting of 552 patients further demonstrated the angioprotective effects of CaD on the early stage of DR.[11] However, other studies have reported that CaD had no beneficial effect on DR.[12-15] One of them focused on the effect of CaD on the development of DME and was followed up for 5 years. This trial concluded that CaD did not reduce the risk of developing DME.[14] Therefore, whether CaD is beneficial to DR is unclear.

The reason for the discrepancy between clinical trials is unknown. It should be noted that most of these studies generally have small sample size (range from 18 to 194).[10 12 13 15-21] The sample size of such studies is too small to draw any definitive conclusions. Although a most recent study with negative results included a relatively large number of participants, however, the primary endpoint of that study was DME.[14] As we all know, the first proven effective treatment for DME is macular laser photocoagulation or intravitreal injection of anti-VEGF agent,[22] rather than CaD. Therefore, inconsistencies in the results of these studies may also be attributable to the inappropriate choice of primary endpoints. A review clearly identified the need for

well-designed, robust trials.[8] We aim to address these problems in the current study.

Methods and analysis

This study protocol was guided by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting guidelines.[23]

Trial setting

The trial is a multicenter study set in 24 tertiary hospitals in China. These centers are located in areas that spans the range of population densities, urbanization and socioeconomic development status in China.

Rationale for using CaD

Despite of the inconsistent results of its efficacy, CaD still remains the only angioprotective agent that reduces the progression of DR.[24] It was suggested in a review that some double-blind trials relating to permeability variables had better results with CaD than placebo, in particular a greater reduction in hemorrhage surface area and in fluorescein penetration into the vitreous body.[25] Others showed an improvement of visual acuity after CaD treatment.[25] Another review showed that CaD had significantly superior effects on the evolution of BRB permeability compared with placebo.[24] A recent meta-analysis indicated that CaD could effectively treat DR at the systematic and local ocular levels compared to control.[11]

Safety

Safety profiles of CaD have been well-established. Side effects are rare and uncommon with therapeutic doses of CaD. Uncommon side effect (0.1%-1%) is tachycardia. Rare side effects (0.01%-0.1%) include nausea, diarrhea, vomiting, rash,

fever, chills, arthralgia, and agranulocytosis.

Hypotheses

In relation to the overall objective, the following are the hypotheses: 1) patients with diabetes who take CaD can reduce the progression of mild to moderate DR; 2) this intervention can also improve visual acuity and retinal vascular function of these patients.

Objectives

The primary objective of this study is to assess the potential therapeutic effect of CaD on the progression of mild to moderate DR in adults with diabetes. The secondary objective is to investigate the effect of the intervention on visual acuity and retinal vascular changes in these patients.

Endpoints

Primary endpoint

The primary endpoint is the progression of DR during 12 months after randomization. After 12-month treatment, 2 digital fundus photographs will be taken for each eye according to Early Treatment Diabetic Retinopathy Study (ETDRS) scale standard of fundus photo description to evaluate the rate of improvement in the progression of patients with mild to moderate DR. DR progression is defined as an increase of 2 or more steps on the ETDRS scale during follow-up.[26] The progression of mild to moderate DR has been widely used in some well-known clinical studies of DR, for example, DCCT study, ACCORD study, and WESDR study.[1]

Secondary endpoints

(1) Progression of DR from baseline to 3, 6 and 12 months;

(2) Time from randomization to the occurrence of progression of DR;

(3) ETDRS scale at baseline, 3, 6, and 12 months;

(4) Changes in eyesight post-intervention from baseline to 1, 3, 6, and 12 months;

(5) Changes in the numbers, location, and types of the retinal lesions post-intervention from baseline to 3, 6, and 12 months;

(6) Changes in the retinal blood vessel diameter and arteriovenous ratio from baseline to 3, 6, and 12 months;

(7) Changes in metabolic biomarkers such as HbA1c post-intervention from baseline to 3, 6, 12 months.

Safety endpoints

(1) Changes in physical examination and vital signs before and after treatment;

(2) Changes in laboratory parameters (liver, renal function, blood routine, etc.);

(3) Adverse events/serious adverse events and their severities.

Selection of patients

Inclusion criteria

(1) Being diagnosed with mild to moderate diabetic retinopathy;

(2) Being older than 18 years;

(3) Being willing to attend this trial.

Exclusion criteria

(1) Being allergic hypersensitive to experimental drugs or comparator drugs;

(2) Having alanine aminotransferase or aspartate aminotransferase ≥ 2 times higher

than the upper limit of normal value, or total bilirubin ≥ 1.5 times higher than the upper limit of normal value upon the exclusion of mild fatty liver disease;

(3) Having severe renal insufficiency (defined as an eGFR ≤ 30 mL/min/1.73 m^2);

(4) Having malignant tumor and some other life-threatening diseases;

(5) Being in pregnancy, expecting pregnancy, or breast feeding;

(6) Being with unstable conditions, such as: 1) uncontrolled high blood pressure (e.g., BP > 180/100 mmHg); 2) glycosylated hemoglobin (HbA1c) > 8.0% or uncontrolled high blood glucose or hypoglycemia; 3) acute cardiovascular events like unstable angina, congestive heart failure, stroke, transient ischemic attack, or myocardial infarction within the previous 3 months; 4) uncontrolled infection; and 5) diabetic ketoacidosis or hyperosmolar state in the past 1 month;

(7) Being with glaucoma, cataracts, or other opacities that may interfere with retinal examination and fundus photography;

(8) Receiving laser treatment, cryocoagulation, or vitrectomy;

(9) Receiving drugs such as CaD or traditional Chinese medicine that may help to improve micro-vascular function in the past 2 weeks;

(10) Receiving VEGF therapy in the past 4 months or will be judged to take VEGF therapy because of disease progression;

(11) Having attended other clinical trials in the past 1 month, being attending some clinical trials, or some other conditions that are unfit for this trial judged by investigators.

Although the recruitment target is mainly middle-aged diabetic patients, the age

standard is set at ≥ 18 years to include young patients with type 1 diabetes, because of the prevalence of DR in type 1 diabetes after an average of 23 years is 77-95% despite adequate treatment for diabetes.[27] The majority of exclusion criteria are based on reducing the risk of adverse effects associated with the intervention, as well as the rate of loss of follow-up.

Because some drugs may have an effect on DR,[6] patients who have used these medicines within 1 month will also be excluded.

Number and source of subjects

It is expected that approximately 1200 patients will be enrolled into the study from the 24 centers (hospitals) in China.

Screening procedures and pre-randomization investigations

The scope of our study presents a variety of potential barriers to participant recruitment, for which we will use an assortment of recruitment methods. Our recruitment strategies will rely on community-level advertising, primary health service center referrals, and word-of-mouth recommendations of patients treated in center in order to inform prospective participants of our study. Given that the focus of this study is on the treatment of DR, it is important to provide potential participants with an assessment of their retinopathy. We also ensure that participation in treatment conditions and data collection are convenient for the participants. A member of each center research team will be in contact with people interested in participating in the study, screening for eligibility, and explaining the main requirements for participation in the study. Written information will be sent to the potential participant who are

eligible for participation.

No research-related procedures will be conducted until detailed written information about the study is provided to participants and informed consent is obtained. Those who are still interested in participating in this study after reading the materials will be invited to a health examination (visit 0): 1) recording of lifestyle characteristics, including smoking and alcohol; 2) recording of medical history, including glucose-lowering medications taken within the last 6 months, and other prescription-only medications taking or have taken in the last month; 3) physical examination, including measurement of height, body weight, waist and hip circumferences, and vital signs; 4) laboratory measurements of blood specimens; and 5) a comprehensive eye examination (see below). Lifestyle education will also be provided.

Recruitment of patients will operate for 12 months in total in each center.

Randomization and masking

Extensive consultation has been conducted among clinical stakeholders in each potential center to ensure a willingness to be randomized to one of two interventions and agreement to implement the allocated plan on a hospital wide level.

Randomization will be opened to participants and investigators for safety and practical considerations, but researchers related to participant's identification, informed consent acquisition, enrolment, and outcome evaluation will be blinded about the treatment allocation. 24 hospitals will be randomized to the intervention and control arms on a 1:1 allocation ratio using a permuted-block randomization method with block size of four. The allocation sequence will be generated using SAS PROC

PLAN independent of the investigators, by the Global Health Trials Unit at the Liverpool School of Tropical Medicine (UK). This is achieved by the study team sharing a list of centers, each of which has a corresponding number. Allocation status will be communicated to the research team coordinator, who will inform respective centers after the investigators obtain the consents of the participants to participate in the trial. To ensure that all baseline measurements of allocated interventions by the investigators are unbiased, the allocation of the intervention groups will be made at the end of the baseline examination (day -6).

Treatment of patients (Figure 1)

Control group

Participants allocated to the control group are requested not to alter their original lifestyle habits and treatment plan after randomization and at the end of the study, with reviews by a study doctor and nurse. Management of blood glucose, blood pressure, and lipid profiles is undertaken by the study team during the period of the trial. Any changes to the treatment of these features are made by a doctor who unaware of treatment allocation to minimize the risk of performance bias. During the period of this study, patients are treated as appropriate to maintain the following targets: HbA1c level < 7.5%, blood pressure < 140/80 mmHg, total cholesterol level < 4.0 mmol/L, triglyceride level < 2.0 mmol/L, high-density lipoprotein level > 1.0 mmol/L, and low-density lipoprotein level < 2.0 mmol/L.

Treatment group

Patients in the treatment group will receive the same intervention as those in the

control group. Additionally, participants will be asked to take a 1500 mg CaD per day in three divided doses of 500 mg for one year. The product will be delivered by the investigator at each center.

Follow-up of patients

The duration of follow-up after randomization is 12 months. Patients will be seen at baseline and subsequently at months 3, 6, and 12 by the study team. We have included extra visits at 1 month to the patients' routine schedule of visits. This extra visit is intended to re-enforce adherence to study drugs, to re-enforce their understanding of side effects and the actions that they need to take if these occur, and to examine the participant clinically.

Recording and monitoring of adverse events

Adverse events will be recorded by the attending physicians and recorded details checked by an independent clinically qualified monitor.

Measures of adherence

Participants in CaD treatment will be asked to bring their study medication to visit 2 (month 1), visit 3 (month 3), visit 4 (month 6), and visit 5 (month 12). The investigators will examine and register the amounts of tablets taken and thereby assess compliance to the drug. A compliance rate ≥ 80% is considered satisfactory.

Schedule for follow-up/Flow Chart

Eligible participants after screening will be included in the study (visit 1-visit 5) (Table 1 and Figure 1). Study visits are scheduled for the morning, after an overnight fast of ≥ 8 h and omission of any morning doses of medications. As mentioned above,

during the period of randomized treatments, visits are scheduled to occur at baseline and then after 1, 3, 6 and 12 months (± 7 days). For those who are no longer receiving randomized treatments, they are still scheduled to visit every 6 months, starting from the baseline visit.

At the baseline visit (visit 1), physical examination and blood sampling are repeated, and the following process are completed: 1) first-morning urine sample for measurement of urinary albumin/creatinine ratio; 2) visual acuity; 3) retinal photographs; 4) an examination of concomitant medications; and 5) a review of current dietary and physical exercise.

Routine assessments to be performed when patients receive randomized treatment include: 1) complete physical examination; 2) laboratory test of fasting blood samples; 3) urinary albumin/creatinine ratio; 4) visual acuity; 5) retinal photographs; 6) recording of concomitant medications; 7) recording of adverse events; and 8) a review of current dietary and physical exercise.

Public and patient involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Assessments

Anthropometric

Height and body weight are measured in light indoor clothing without shoes. Height and weight are measured to the nearest 0.1 cm and 0.1 kg, respectively. Waist circumference, the mid-way between lowest rib and iliac crest, hip circumference, and

the level of the great trochanters, will be measured to the nearest 0.1 cm. Blood pressure measurements are taken after at least 5 minutes of rest in duplicate separated by at least 5 minutes. The mean value of the two measurements will be used in the analysis. Subjects are required to refrain from smoking or ingesting caffeine for 30 minutes prior to the examination.

Sociodemographic

Sociodemographic information, including age, gender, ethnicity, civil status, education, occupation, income, health history, smoking status and alcohol consumption, will be collected at baseline.

Fasting blood and first-morning urine samples

Fasting blood and first-morning urine samples will be collected and analyzed for fasting plasma glucose, HbA1c, liver and renal functions, blood lipid profiles, and urinary albumin/creatinine ratio at baseline and follow-up visits (Table 1).

Visual acuity

Visual acuity will be measured in both eyes using ETDRS visual acuity charts at 4 m by optometrists at baseline and follow-up visits (Table 1).

Retinal photographs

All persons with diabetes attending this study will undergo routine digital retinal photography, which is conducted in a darkened room using a nonmydriatic digital camera capturing optic disc and macular centered images per eye without the use of mydriasis by trained and certified photographers.

All retinal images from the patients will be independently reviewed and graded by

one of three senior retinal graders according to a grading protocol used in the Multi-Ethnic Study of Atherosclerosis (MESA), which is modified from the Airlie House Classification system.[28] Levels of DR are classified as no DR (levels 10-13) if no lesions are detected, and any DR (levels 14-80) when at least one microaneurysm and/or a blot hemorrhage are detected. DR is further divided into minimal-moderate NPDR (levels 14-20), mild–moderate NPDR (levels 31-41), and severe NPDR to proliferative retinopathy (levels 51-80). DME is defined by hard exudates in the presence of microaneurysms and blot hemorrhage within 1-disc diameter from the foveal center or the presence of focal photocoagulation scars in the macular area. Clinically significant macular edema (CSME) is considered present when the macular edema is within 500 μm of the foveal center or if focal laser photocoagulation scars are present in the macular area. These are confirmed with central macular thickness measurements by optical coherence tomography using the macular thickness cube scan protocol. Vision-threatening retinopathy is defined as the presence of severe NPDR, proliferative retinopathy, or CSME. The level of retinopathy is graded based on the worse eye. If an eye is ungradable, the score for the other eye will be used to define these outcomes.

Any discrepancies between the two initial graders will be adjudicated by a senior grader using standardized edit rules.

Retinal vascular caliber

Retinal vascular caliber is measured using a computer-based program following a previously validated protocol.[29] Optic disc-centered photograph of the right eye of

each participant are measured. Left eye measurements are performed when photographs of the right eye are ungradable. For each photograph, all arterioles and venules coursing through an area 0.5 to 1-disc diameter from the optic disc margin are measured and summarized as the average central arteriolar and venular equivalents. These equivalents represent the average of projected calibers for the central retinal vessels, and have a high intergrader repeatability with intra- and inter-grader intraclass correlation coefficients ranging from 0.78 to 0.99.[30]

Procedures for assessing safety

Throughout the course of the study, a steering committee meets to review progress every 6 months, and an independent data safety monitoring board (DSMB) has been established to monitor safety and outcomes. The investigators are responsible for ensuring that all serious adverse events are reported timely to the sponsor, who will then notify the ethics committee of the corresponding centers and the Chinese Medicines Agency according to the current laws and ICH/GCP guidelines. In case of unexpected severe adverse reactions to medication during the study, the trial will be discontinued. In addition, the DSMB may also recommend termination of the study for other serious safety reasons.

Subjects who withdraw from the study for any reason at any time will not be replaced. Subjects who are excluded or who decide to stop participating will be referred to their ophthalmologist for advice on how to manage their DR. At the end of the study, data on withdrawn subjects will be collected and used for efficacy and safety analyses.

Loss to follow-up

All subjects will be followed up for 12 months duration of the trial. Participants will be sent text message reminders prior to their appointment. If they fail to show, then they will be contacted by phone or by home visit if phoning is not possible to understand the reasons and reschedule another appointment within a week.

Trial closure

Study follow up will be for 12 months following randomization. The trial will be considered closed after the last patient enrolled has completed 12 months of follow up.

Withdrawal from trial intervention

Participants can withdraw from the intervention at any time. Participants may be withdrawn from the trial at the discretion of the investigator due to a safety concern or a serious violation of the protocol. Participants will be withdrawn in occurrence of pregnancy or at the intention to become pregnant. Withdrawn participants will be invited for the following assessments every 6 months unless written consent is withdrawn: 1) complete physical examination; 2) fasting blood specimens; 3) visual acuity; and 4) retinal photographs.

Statistical methods

Sample size

Progression of DR assessed by an increase of 2 or more steps on the ETDRS severity scale has not previously been used as primary outcome in RCTs of patients with DR treated with CaD. A prospective observational cohort study showed that with conventional treatment, the proportion of patients with 2-step or greater progression

of DR at 1 year was 16.2%.[31] It is expected that combined medical therapy of DR in addition to conventional treatment will reduce the development or progression of DR by approximately 50%.[1] With a two-sided significance level of 5%, a power of 80%, and an intracluster correlation coefficient of 0.01,[32] a clinically meaningful difference in the progression of DR over the 12-month intervention of ≥ 8.1% between two groups can be detected with 432 participants in each group. To allow for dropouts as 20%, we plan to include 1200 subjects (600 in each of the two study groups), or 50 in each cluster.

Data management

Subjects will be identified by study ID. Study data will be collected and managed using the Smart CR secure web-based system[33] developed by Suzhou MetroHealth Medical Technology, where electronic case report forms (CRF) will be created. During the intervention phase, data will be entered directly into the Smart CR by study personnel and will be extracted by the investigators or sponsor. The fundus photograph data will be transmitted electronically from the person performing the operation to the same certified photographic grader and will be archived on a secure hard drive with backed up in Southeast University. All blood and urine samples obtained will be stored in a biobank for future use. Samples will be labelled with a unique study identifier.

Statistical analysis

All statistical analyses will be performed using SAS 9.4 and Stata 14 based on intention-to-treat principle and in accordance with the CONSORT guidelines for

reporting cluster randomized trials. Per-protocol analysis will also be performed as supplementary analysis.

A generalized linear mixed-effects model (GLMMIX) will be fitted to analyze the primary endpoint. The model will have a binomial distribution and logit link function and include treatment, time, and interaction between treatment and time as fixed factors; baseline ETDRS severity scale as covariate, and cluster and subject as random effects. Odds ratio with its 95% CI at each time point (3, 6 and 12 months) will be derived, which will also be converted into risk ratio using the mathematical relationship between odds ratio and risk ratio.[34] A covariate-adjusted analysis of the primary endpoint will be performed by adding pre-specified covariates at baseline into GLMMIX. Missing efficacy data will be treated as missing at random and no imputation will be made, because the GLMMIX model is a likelihood-based procedure and handles missing at random as ignorable.[35] To evaluate the sensitivity of the result of this assumption, the multiple imputation method will be used to impute missing primary endpoint during follow-up. Pre-specified subgroup analyses are performed to explore the influence of covariates on primary endpoint.

For secondary binary outcomes, the similar GLMMIX will be used. For secondary continuous outcomes, the change from baseline for each of the outcomes will be modelled using GLMMIX with normal distribution and identity link function and with treatment, time and interaction between treatment and time as fixed factors, baseline measurement as covariate, and cluster and subject as random effects. Mean difference and its 95% CI at each time point will be derived. Time-to-event outcome

will be analyzed using Cox proportional hazards regression model with frailty at the center (cluster) level and treatment as the study variable and intervention effects will be reported as hazard ratio with 95% CI. Kaplan-Meier plots will also be produced for the two study arms separately and log-rank test used to compare two time-to-event curves.

Detailed statistical analyses will be described in the statistical analysis plan, which will be developed and finalized before the database lockup.

Ethics and dissemination

The study is carried out in accordance with the Helsinki Declaration after approval by the Ethical Review Committees of Zhongda Hospital of Southeast University (2019ZDSYLL132-P01).

CaD, which has been discovered more than 40 years ago and is registered for the treatment of DR in more than 20 countries remains, to date, the only angioprotective agent that reduces the progression of this disease.[24] Although CaD is effective in animal and/or in vitro models, however, the results of clinical trials are inconsistent. A large, multicenter study is warranted to provide a definitive conclusion.[11] To our knowledge, this study is the largest multicenter RCT, and is unique in embedding a cluster-randomized trial design within an intervention framework to study the effectiveness of CaD treatment on the progression of mild to moderate NPDR compared to conventional treatment. The efficacy of the intervention on visual acuity, the presence, number, location and type of retinal lesions, and retinal blood vessel diameter as well as arteriovenous ratio will also be tested. Our study will give us an

opportunity to explore in depth whether CaD is beneficial for the treatment of mild to moderate DR. A limitation of this study is the single-blinded study. Patients' knowing of their treatment groups may have a psychological impact on the trial results. However, the primary endpoint will be assessed by an independent committee and results regarding the primary endpoint will not be biased.

Even in the case of negative results, this trial will produce a large amount of illuminating data. Investigators will be able to closely monitor progression of DR in both arms during the 12-month follow-up period. If the treatment of CaD is effective, it will provide an additional therapy option for comprehensive management of the diabetic patients with mild to moderate DR.

The results of the trial will be analyzed, presented and published as soon as possible at high-impact peer-reviewed journals and presented at the international scientific meetings and conferences. Manuscripts will be written in accordance with the CONSORT guidelines for reporting cluster randomized trials.

Contributors HH and JL drafted the manuscript. HH, JL, DW, SQ, YY, FW, YW, QS, and ZS participated in the design and preparation of the study. DW provided statistical analysis support. DW, SQ, YY, FW, YW, QS, and ZS critically revised the manuscript's drafts. All authors approved the final version of the manuscript.

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Table 1. Trial design, visits and end points.

Visit	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Time (days from the start of intervention)	-14	-6	30	90	180	360
Informed consent	×					
History	×					
Inclusion/exclusion criteria	×					
Height	×	×	×	×	×	×
Body weight	×	×	×	×	×	×
Waist circumference	×	×	×	×	×	×
Hip circumference	×	×	×	×	×	×
Blood pressure	×	×	×	×	×	×
Glycosylated hemoglobin	×	×		×	×	×
Fasting plasma glucose		×				×
Liver function	×	×		×	×	×
Renal function	×	×		×	×	×
Blood lipid profiles		×		×	×	×
Urinary albumin/creatinine ratio		×		×	×	×
Fasting blood samples		×				×
Urine samples		×				×
Visual acuity		×	×	×	×	×
Retinal photographs	×	×		×	×	×
Retinal vascular caliber		×		×	×	×
Adverse events			×	×	×	×
Drug accountability		×	×	×	×	×

The maximum allowed time interval between screening (visit 0) and baseline examination (visit 1) will be 2 weeks (= 14 days). Otherwise, a new screening will be conducted before the participants are included in the study.

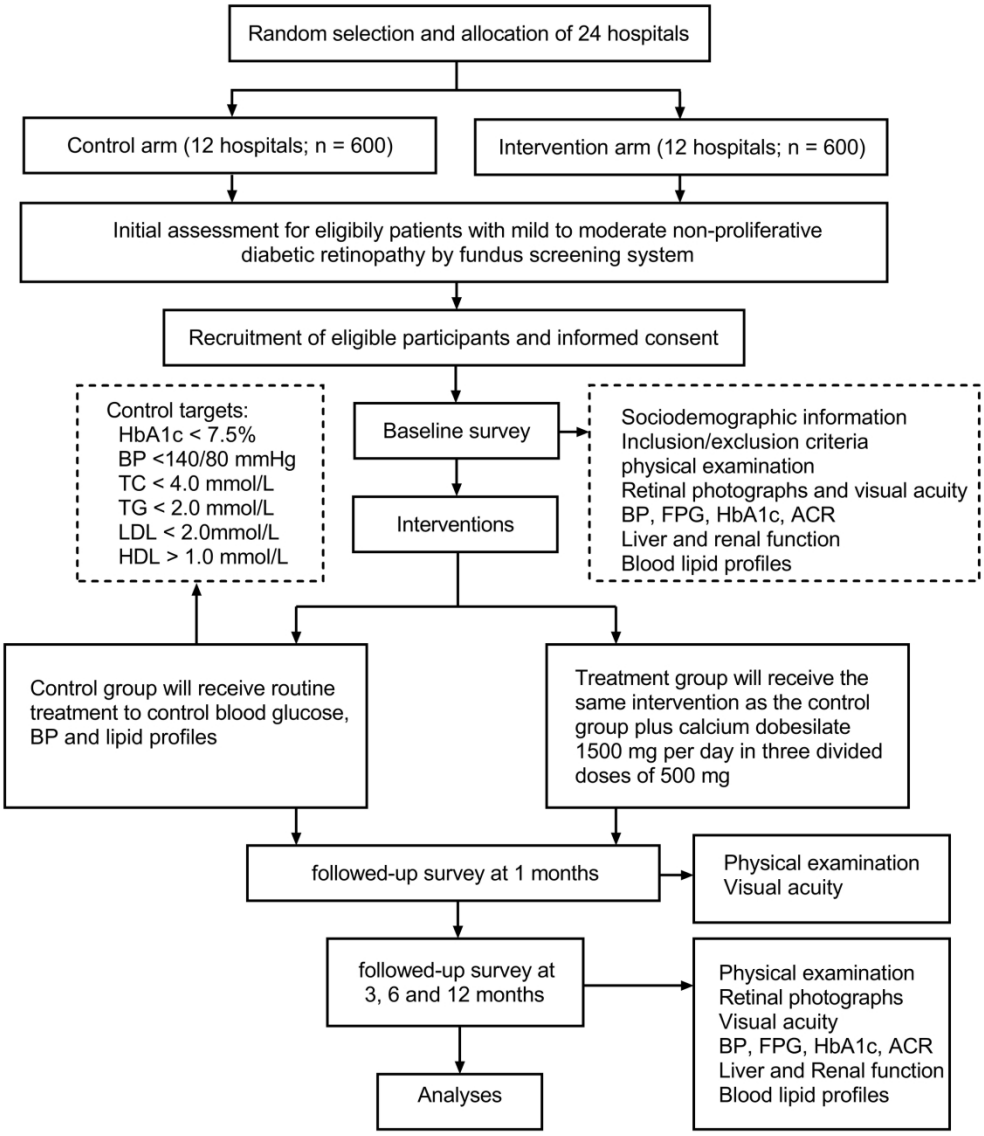

Figure 1. Treatment of patient flow chart. BP, blood pressure; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; ACR, urinary albumin/creatinine ratio; TC, total cholesterol, TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

197x227mm (300 x 300 DPI)

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Efficacy of calcium dobesilate in treating Chinese patients with mild to moderate non-proliferative diabetic retinopathy (CALM-DR): protocol for a single-blind, multicenter, 24-armed cluster-randomized, controlled trial

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Abstract

Introduction

Calcium dobesilate (CaD) has been used in the treatment of diabetic retinopathy (DR) due to its potential in protecting against retinal vascular damage. However, there is limited evidence exploring its efficacy in combating DR progression. This study is aimed at evaluating whether CaD could prevent DR progression into an advanced stage among Chinese patients with mild to moderate non-proliferative DR (NPDR).

Methods and analysis

This study is a single-blind, multicenter, cluster-randomized, controlled superiority trial. A total of 1,272 patients with mild to moderate NPDR will be enrolled and randomly assigned at a 1:1 ratio into the control group (conventional treatment group) and the intervention group (conventional treatment plus CaD [500 mg thrice daily] for 12 months). Patients will be followed at 1, 3, 6, and 12 months after randomization and receiving treatments, with the severity of DR assessed by the Early Treatment Diabetic Retinopathy Study (ETDRS) scale. The primary endpoint is the progression of DR during follow-up, which is defined as an increase of 2 or more steps in the ETDRS scale. The secondary endpoints include the concomitant changes in visual acuity, presence, number, location and type of retinal lesions, and retinal blood vessel diameter as well as the arteriovenous ratio at different visits.

Ethics and dissemination

Each local ethics committee (first Vote: Ethical Review Committees of Zhongda Hospital of Southeast University (2019ZDSYLL132-P01)) has approved the study.

The results will be published in high impact peer-reviewed scientific journals aimed at the general reader.

Trial registration numbers

NCT04283162.

Strengths and limitations of this study

(1) This will be the first cluster-randomized controlled trial with a large sample size assessing the efficacy of Calcium dobesilate (CaD) in treating diabetic retinopathy (DR) among individuals with mild to moderate non-proliferative DR.

(2) This study will collect longitudinal data on patients treated with CaD, and will report on the evolution of several important outcome measures over the first year.

(3) As with any longitudinal study, there is a risk of loss to follow-up throughout the study period, which may induce bias in the final results.

(4) A relatively short-term follow-up period of 12 months might also be a limitation for this study.

Introduction

Diabetic retinopathy (DR) is a common microvascular complication associated with diabetes, and encompasses a broad clinical spectrum from the mild non-proliferative DR (NPDR) to a more advanced stage of vision-threatening such as proliferative DR (PDR) and diabetic macular edema (DME).[1] The pathogenesis of DR involves a variety of abnormal pathways, which are triggered by a series of factors including chronic hyperglycemia, free radicals, advanced glycosylation end products, inflammatory cytokines, and vascular endothelial growth factors (VEGF).[2] These may eventually lead to blood-retinal barrier (BRB) breakdown, retinal neovascularization, and retinal neuronal apoptosis,[3] with BRB injury being considered the hallmark of DR.[4]

There is evidence that 30-50% of patients with diabetes may develop vision-threatening retinopathy during their lifetime.[5] Timely intervention, such as intensive diabetes management, adjunctive anti-VEGF therapy, laser photocoagulation, and surgical management, can prevent up to 98% of the cases developing vision loss.[1] However, tight glycemic control and laser therapy could not reverse the existing ocular damage and may only slow its progression. The safety of ocular surgery and its effectiveness in improving visual acuity have also been questioned.[6] Moreover, about three-quarters of the world's diabetic patients live in low-and middle-income countries with severely limited healthcare resources,[7] which poses a serious challenge to the implementation of anti-VEGF therapy or surgical methods for DR.[2] Therefore, access to affordable medicines, coupled with early detection of DR, may be a better way to

prevent and delay diabetic blindness.[1]

CaD is an angioprotective agent that has been proposed as a treatment for DR by protecting against retinal vascular damage.[8 9] The earliest clinical study was a 2-year, double-blind, randomized controlled trial (RCT) involving 51 patients and 17 controls. The results showed that CaD was a potent angioprotector, which can prevent intra and extravascular hemorrhages of retina, reduce the incidence of exudate formation and improve visual acuity.[10] A recent meta-analysis of 8 RCTs consisting of 552 patients further demonstrated the angioprotective effects of CaD on the early stage of DR.[11] However, other studies have reported that CaD had no beneficial effect on DR.[12-15] One of them focused on the effect of CaD on the development of DME and was followed up for 5 years. This trial concluded that CaD did not reduce the risk of developing DME.[14] Therefore, whether CaD is beneficial to DR is unclear.

The reason for the discrepancy between clinical trials is unknown. It should be noted that most of these studies generally have small sample size (range from 18 to 194).[10 12 13 15-21] The sample size of such studies is too small to draw any definitive conclusions. Although a most recent study with negative results included a relatively large number of participants, however, the primary endpoint of that study was DME.[14] As we all know, the first proven effective treatment for DME is macular laser photocoagulation or intravitreal injection of anti-VEGF agent,[22] rather than CaD. Therefore, inconsistencies in the results of these studies may also be attributable to the inappropriate choice of primary endpoints. A review clearly identified the need for well-designed, robust trials.[8] We aim to address these problems in the current study.

Methods and analysis

This study protocol was guided by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting guidelines.[23]

Trial setting

The Efficacy of **CAL**cium dobesilate in treating Chinese patients with **M**ild to moderate non-proliferative **D**iabetic **R**etinopathy (CALM-DR) is a multicenter randomized controlled study set in 24 tertiary hospitals in China. These centers are located in areas that spans the range of population densities, urbanization and socioeconomic development status in China. We will recruit patients between June, 2021, and December, 2021. Follow-up will complete in December, 2022.

Rationale for using CaD

Despite of the inconsistent results of its efficacy, CaD still remains the only angioprotective agent that reduces the progression of DR.[24] It was suggested in a review that some double-blind trials relating to permeability variables had better results with CaD than placebo, in particular a greater reduction in hemorrhage surface area and in fluorescein penetration into the vitreous body.[25] Others showed an improvement of visual acuity after CaD treatment.[25] Another review showed that CaD had significantly superior effects on the evolution of BRB permeability compared with placebo.[24] A recent meta-analysis indicated that CaD could effectively treat DR at the systematic and local ocular levels compared to control.[11]

Safety

Safety profiles of CaD have been well-established. Side effects are rare and

uncommon with therapeutic doses of CaD. Uncommon side effect (0.1%-1%) is tachycardia. Rare side effects (0.01%-0.1%) include nausea, diarrhea, vomiting, rash, fever, chills, arthralgia, and agranulocytosis.

Hypotheses

In relation to the overall objective, the following are the hypotheses: 1) patients with diabetes who take CaD can reduce the progression of mild to moderate DR; 2) this intervention can also improve visual acuity and retinal vascular function of these patients.

Objectives

The primary objective of this study is to assess the potential therapeutic effect of CaD on the progression of mild to moderate DR in adults with diabetes. The secondary objective is to investigate the effect of the intervention on visual acuity and retinal vascular changes in these patients.

Endpoints

Primary endpoint

The primary endpoint is the progression of DR during 12 months after randomization. After 12-month treatment, 2 digital fundus photographs will be taken for each eye according to Early Treatment Diabetic Retinopathy Study (ETDRS) scale standard of fundus photo description to evaluate the rate of improvement in the progression of patients with mild to moderate DR. DR progression is defined as an increase of 2 or more steps on the ETDRS scale during follow-up.[26] The progression of mild to moderate DR has been widely used in some well-known clinical studies of DR, for

example, DCCT study, ACCORD study, and WESDR study.[1]

Secondary endpoints

(1) Progression of DR from baseline to 3, 6 and 12 months;

(2) Time from randomization to the occurrence of progression of DR;

(3) ETDRS scale at baseline, 3, 6, and 12 months;

(4) Changes in eyesight post-intervention from baseline to 1, 3, 6, and 12 months;

(5) Changes in the numbers, location, and types of the retinal lesions post-intervention from baseline to 3, 6, and 12 months;

(6) Changes in the retinal blood vessel diameter and arteriovenous ratio from baseline to 3, 6, and 12 months;

(7) Changes in metabolic biomarkers such as HbA1c post-intervention from baseline to 3, 6, 12 months.

Additional repeat testing at 9 months is warranted for patient with type 1 diabetes.

Safety endpoints

(1) Changes in physical examination and vital signs before and after treatment;

(2) Changes in laboratory parameters (liver, renal function, blood routine, etc.);

(3) Adverse events/serious adverse events and their severities.

Selection of patients

Inclusion criteria

(1) Being diagnosed with mild to moderate diabetic retinopathy;

(2) Being older than 18 years;

(3) Being willing to participate in the trial.

Exclusion criteria

(1) Being allergic hypersensitive to experimental drugs or comparator drugs;

(2) Having alanine aminotransferase or aspartate aminotransferase ≥ 2 times higher than the upper limit of normal value, or total bilirubin ≥ 1.5 times higher than the upper limit of normal value upon the exclusion of mild fatty liver disease;

(3) Having severe renal insufficiency (defined as an eGFR ≤ 30 mL/min/1.73 m^2);

(4) Having malignant tumor and some other life-threatening diseases;

(5) Being pregnancy, expecting pregnancy, or breast feeding;

(6) Being with unstable conditions, such as: 1) uncontrolled high blood pressure (e.g., BP > 180/100 mmHg); 2) glycosylated hemoglobin (HbA1c) > 8.0% or uncontrolled high blood glucose or hypoglycemia; 3) acute cardiovascular events like unstable angina, congestive heart failure, stroke, transient ischemic attack, or myocardial infarction within the previous 3 months; 4) uncontrolled infection; and 5) diabetic ketoacidosis or hyperosmolar state in the past 1 month;

(7) Being with glaucoma, cataracts, or other opacities that may interfere with retinal examination and fundus photography;

(8) Receiving drugs such as CaD or traditional Chinese medicine that may help to improve micro-vascular function in the past 2 weeks;

(9) Receiving VEGF therapy in the past 4 months or will be judged to take VEGF therapy because of disease progression;

(10) Having attended other clinical trials in the past 1 month, being attending some clinical trials, or some other conditions that are unfit for this trial judged by

investigators.

Although the recruitment target is mainly middle-aged diabetic patients, the age standard is set at ≥ 18 years to include young patients with type 1 diabetes, because of the prevalence of DR in type 1 diabetes after an average of 23 years is 77-95% despite adequate treatment for diabetes.[27] The majority of exclusion criteria are based on reducing the risk of adverse effects associated with the intervention, as well as the rate of loss of follow-up.

Because some drugs (such as corticosteroids, non-steroidal anti-inflammatory drugs, antioxidants, inflammatory molecule inhibitors, renin-angiotensin system blockers, and fenofibrate) may have an effect on DR,[6] patients who have used these medicines within 1 month will also be excluded.

Number and source of subjects

It is expected that approximately 1272 patients will be enrolled into the study from the 24 centers (hospitals) in China.

Screening procedures and pre-randomization investigations

The scope of our study presents a variety of potential barriers to participant recruitment, for which we will use an assortment of recruitment methods. Our recruitment strategies will rely on community-level advertising, primary health service center referrals, and word-of-mouth recommendations of patients treated in center in order to inform prospective participants of our study. Given that the focus of this study is on the treatment of DR, it is important to provide potential participants with an assessment of their retinopathy. We also ensure that participation in treatment

conditions and data collection are convenient for the participants. A member of each center research team will be in contact with people interested in participating in the study, screening for eligibility, and explaining the main requirements for participation in the study. Written information will be sent to the potential participant who are eligible for participation. Researchers in each hospital will obtain informed consent signed by the participants.

No research-related procedures will be conducted until detailed written information about the study is provided to participants and informed consent is obtained. Those who are still interested in participating in this study after reading the materials will be invited to a health examination (visit 0): 1) recording of lifestyle characteristics, including smoking and alcohol; 2) recording of medical history, including glucose-lowering medications taken within the last 6 months, and other prescription-only medications taking or have taken in the last month; 3) physical examination, including measurement of height, body weight, waist and hip circumferences, and vital signs; 4) laboratory measurements of blood specimens; and 5) a comprehensive eye examination (see below). Lifestyle education will also be provided.

Recruitment of patients will operate for 12 months in total in each center.

Randomization and masking

Extensive consultation has been conducted among clinical stakeholders in each potential center to ensure a willingness to be randomized to one of two interventions and agreement to implement the allocated plan on a hospital wide level.

Randomization will be opened to participants and investigators for safety and

practical considerations, but researchers related to participant's identification, informed consent acquisition, enrolment, and outcome evaluation will be blinded about the treatment allocation. 24 hospitals will be randomized to the intervention and control arms on a 1:1 allocation ratio using a permuted-block randomization method with block size of four. The allocation sequence will be generated using SAS PROC PLAN independent of the investigators, by the Global Health Trials Unit at the Liverpool School of Tropical Medicine (UK). This is achieved by the study team sharing a list of centers, each of which has a corresponding number. Allocation status will be communicated to the research team coordinator, who will inform respective centers after the investigators obtain the consents of the participants to participate in the trial. To ensure that all baseline measurements of allocated interventions by the investigators are unbiased, the allocation of the intervention groups will be made at the end of the baseline examination (day -6).

Treatment of patients (Figure 1)

Control group

Participants allocated to the control group are requested not to alter their original lifestyle habits and treatment plan after randomization and at the end of the study, with reviews by a study doctor and nurse. Management of blood glucose, blood pressure, and lipid profiles is undertaken by the study team during the period of the trial. Any changes to the treatment of these features are made by a doctor who unaware of treatment allocation to minimize the risk of performance bias. During the period of this study, patients are treated as appropriate to maintain the following

targets: HbA1c level < 7.5%, blood pressure < 140/80 mmHg, total cholesterol level < 4.0 mmol/L, triglyceride level < 2.0 mmol/L, high-density lipoprotein level > 1.0 mmol/L, and low-density lipoprotein level < 2.0 mmol/L.

Treatment group

Patients in the treatment group will receive the same intervention as those in the control group. Additionally, participants will be asked to take a 1500 mg CaD per day in three divided doses of 500 mg for one year. The product will be delivered by the investigator at each center.

Follow-up of patients

The duration of follow-up after randomization is 12 months. Patients will be seen at baseline and subsequently at months 3, 6, and 12 by the study team. We have included extra visits at 1 month to the patients' routine schedule of visits. This extra visit is intended to re-enforce adherence to study drugs, to re-enforce their understanding of side effects and the actions that they need to take if these occur, and to examine the participant clinically.

Recording and monitoring of adverse events

Adverse events will be recorded by the attending physicians and recorded details checked by an independent clinically qualified monitor.

Measures of adherence

Participants in CaD treatment will be asked to bring their study medication to visit 2 (month 1), visit 3 (month 3), visit 4 (month 6), and visit 5 (month 12). The investigators will examine and register the amounts of tablets taken and thereby assess

compliance to the drug. A compliance rate ≥ 80% is considered satisfactory.

Schedule for follow-up/Flow Chart

Eligible participants after screening will be included in the study (visit 1-visit 5) (Table 1 and Figure 1). Study visits are scheduled for the morning, after an overnight fast of ≥ 8 h and omission of any morning doses of medications. As mentioned above, during the period of randomized treatments, visits are scheduled to occur at baseline and then after 1, 3, 6 and 12 months (± 7 days). For those who are no longer receiving randomized treatments, they are still scheduled to visit every 6 months, starting from the baseline visit.

At the baseline visit (visit 1), physical examination and blood sampling are repeated, and the following process are completed: 1) first-morning urine sample for measurement of urinary albumin/creatinine ratio; 2) visual acuity; 3) retinal photographs; 4) an examination of concomitant medications; and 5) a review of current dietary and physical exercise.

Routine assessments to be performed when patients receive randomized treatment include: 1) complete physical examination; 2) laboratory test of fasting blood samples; 3) urinary albumin/creatinine ratio; 4) visual acuity; 5) retinal photographs; 6) recording of concomitant medications; 7) recording of adverse events; and 8) a review of current dietary and physical exercise.

Public and patient involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Implementation quality and intervention fidelity

To ensure both the quality and motivation, a series of dialogue meetings between project partners were held before and after recruitment of hospitals. Meetings between the project and hospital leaderships, and the intervention implementers were held before and after recruitment, and regular meetings are planned throughout the study period. To ensure intervention fidelity, quarterly gatherings will be organised throughout the project period separately for the hospital leaderships and the study group members of each hospital to share experiences and motivate each other.

In each gathering, each hospital is required to develop an execution plan for their specific work the coming quarter. In addition, representatives from project leaderships will phone calls with each team every other week to discuss progress, challenges and solutions.

Assessments

Anthropometric

Height and body weight are measured in light indoor clothing without shoes. Height and weight are measured to the nearest 0.1 cm and 0.1 kg, respectively. Waist circumference, the mid-way between lowest rib and iliac crest, hip circumference, and the level of the great trochanters, will be measured to the nearest 0.1 cm. Blood pressure measurements are taken after at least 5 minutes of rest in duplicate separated by at least 5 minutes. The mean value of the two measurements will be used in the analysis. Subjects are required to refrain from smoking or ingesting caffeine for 30 minutes prior to the examination.

Sociodemographic

Sociodemographic information, including age, gender, ethnicity, civil status, education, occupation, income, health history, smoking status and alcohol consumption, will be collected at baseline.

Fasting blood and first-morning urine samples

Fasting blood and first-morning urine samples will be collected and analyzed for fasting plasma glucose, HbA1c, liver and renal functions, blood lipid profiles, and urinary albumin/creatinine ratio at baseline and follow-up visits (Table 1).

Visual acuity

Visual acuity will be measured in both eyes using ETDRS visual acuity charts at 4 m by optometrists at baseline and follow-up visits (Table 1).

Retinal photographs

All persons with diabetes attending this study will undergo routine digital retinal photography, which is conducted in a darkened room using a nonmydriatic digital camera capturing optic disc and macular centered images per eye without the use of mydriasis by trained and certified photographers.

According to a grading protocol modified by Airlie House Classification system used in the Multi-Ethnic Study of Atherosclerosis (MESA), all retinal images from the patients will be independently reviewed and graded by one of three senior retinal graders who do not know the clinical details.[28] Levels of DR are classified as no DR (levels 10-13) if no lesions are detected, and any DR (levels 14-80) when at least one microaneurysm and/or a blot hemorrhage are detected. DR is further divided into

minimal-moderate NPDR (levels 14-20), mild–moderate NPDR (levels 31-41), and severe NPDR to proliferative retinopathy (levels 51-80). DME is defined as retinal thickening or hard exudates at least one disc diameter to the center of the macular. Clinically significant macular edema (CSME), introduced by ETDRS, is defined as DME meeting at least one of three criteria: thickening at or within 500 μm of the foveal center, hard exudates within 500μm of foveal center with adjacent thickening, or at least one disk diameter of thickening with part of it located within one disc diameter of foveal center. These are confirmed with central macular thickness measurements by optical coherence tomography using the macular thickness cube scan protocol. Vision-threatening retinopathy is defined as the presence of severe NPDR, proliferative retinopathy, or CSME. The level of retinopathy is graded based on the worse eye. If an eye is ungradable, the score for the other eye will be used to define these outcomes.

Any discrepancies between the two initial graders will be adjudicated by a senior grader using standardized edit rules.

Retinal vascular caliber

Retinal vascular caliber is measured using a computer-based program following a previously validated protocol.[29] Optic disc-centered photograph of the right eye of each participant are measured. Left eye measurements are performed when photographs of the right eye are ungradable. For each photograph, all arterioles and venules coursing through an area 0.5 to 1-disc diameter from the optic disc margin are measured and summarized as the average central arteriolar and venular equivalents.

These equivalents represent the average of projected calibers for the central retinal vessels, and have a high intergrader repeatability with intra- and inter-grader intraclass correlation coefficients ranging from 0.78 to 0.99.[30]

Procedures for assessing safety

Throughout the course of the study, a steering committee meets to review progress every 6 months, and an independent data safety monitoring board (DSMB) has been established to monitor safety and outcomes. The investigators are responsible for ensuring that all serious adverse events are reported timely to the sponsor, who will then notify the ethics committee of the corresponding centers and the Chinese Medicines Agency according to the current laws and ICH/GCP guidelines. In case of unexpected severe adverse reactions to medication during the study, the trial will be discontinued. In addition, the DSMB may also recommend termination of the study for other serious safety reasons.

Subjects who withdraw from the study for any reason at any time will not be replaced. Subjects who are excluded or who decide to stop participating will be referred to their ophthalmologist for advice on how to manage their DR. At the end of the study, data on withdrawn subjects will be collected and used for efficacy and safety analyses.

Loss to follow-up

All subjects will be followed up for 12 months duration of the trial. Participants will be sent text message reminders prior to their appointment. If they fail to show, then they will be contacted by phone or by home visit if phoning is not possible to understand the reasons and reschedule another appointment within a week.

Trial closure

Study follow up will be for 12 months following randomization. The trial will be considered closed after the last patient enrolled has completed 12 months of follow up.

Withdrawal from trial intervention

Participants can withdraw from the intervention at any time. Participants may be withdrawn from the trial at the discretion of the investigator due to a safety concern or a serious violation of the protocol. Participants will be withdrawn in occurrence of pregnancy or at the intention to become pregnant. Withdrawn participants will be invited for the following assessments every 6 months unless written consent is withdrawn: 1) complete physical examination; 2) fasting blood specimens; 3) visual acuity; and 4) retinal photographs.

Statistical methods

Sample size

Progression of DR assessed by an increase of 2 or more steps on the ETDRS severity scale has not previously been used as primary outcome in RCTs of patients with DR treated with CaD. A prospective observational cohort study showed that with conventional treatment, the proportion of patients with 2-step or greater progression of DR at 1 year was 15.2% (we contacted the study investigators to clarify the unpublished estimate).[31] It is expected that combined medical therapy of DR in addition to conventional treatment will reduce the development or progression of DR by approximately 50%.[1] With a two-sided significance level of 5%, a power of 80%,

and an intracluster correlation coefficient of 0.01,[32] a clinically meaningful difference in the progression of DR over the 12-month intervention of ≥ 7.6% between two groups can be detected with 528 participants in each group. To allow for dropouts as 20%, we plan to include 1272 subjects (636 in each of the two study groups), or 53 in each cluster.

Data management

Subjects will be identified by study ID. Study data will be collected and managed using the Smart CR secure web-based system[33] developed by Suzhou MetroHealth Medical Technology, where electronic case report forms (CRF) will be created. During the intervention phase, data will be entered directly into the Smart CR by study personnel and will be extracted by the investigators or sponsor. The fundus photograph data will be transmitted electronically from the person performing the operation to the same certified photographic grader and will be archived on a secure hard drive with backed up in Southeast University. All blood and urine samples obtained will be stored in a biobank for future use. Samples will be labelled with a unique study identifier.

All collected data will only be used for the purposes of the present study. Participants' information will be confidential and anonymized and will only be treated at a collective level. Trial results will be shared with both the scientific community and health professionals, through publications in scientific peer-reviewed journals and presentations at national and international conferences.

Statistical analysis

All statistical analyses will be performed using SAS 9.4 and Stata 14 based on intention-to-treat principle and in accordance with the CONSORT guidelines for reporting cluster randomized trials. Per-protocol analysis will also be performed as supplementary analysis.

A generalized linear mixed-effects model (GLMMIX) will be fitted to analyze the primary endpoint. The model will have a binomial distribution and logit link function and include treatment, time, and interaction between treatment and time as fixed factors; baseline ETDRS severity scale as covariate, and cluster and subject as random effects. Odds ratio with its 95% CI at each time point (3, 6 and 12 months) will be derived, which will also be converted into risk ratio using the mathematical relationship between odds ratio and risk ratio.[34] A covariate-adjusted analysis of the primary endpoint will be performed by adding pre-specified covariates at baseline into GLMMIX. Missing efficacy data will be treated as missing at random and no imputation will be made, because the GLMMIX model is a likelihood-based procedure and handles missing at random as ignorable.[35] To evaluate the sensitivity of the result of this assumption, the multiple imputation method will be used to impute missing primary endpoint during follow-up. Pre-specified subgroup analyses are performed to explore the influence of covariates on primary endpoint.

For secondary binary outcomes, the similar GLMMIX will be used. For secondary continuous outcomes, the change from baseline for each of the outcomes will be modelled using GLMMIX with normal distribution and identity link function and with treatment, time and interaction between treatment and time as fixed factors,

baseline measurement as covariate, and cluster and subject as random effects. Mean difference and its 95% CI at each time point will be derived. Time-to-event outcome will be analyzed using Cox proportional hazards regression model with frailty at the center (cluster) level and treatment as the study variable and intervention effects will be reported as hazard ratio with 95% CI. Kaplan-Meier plots will also be produced for the two study arms separately and log-rank test used to compare two time-to-event curves.

Detailed statistical analyses will be described in the statistical analysis plan, which will be developed and finalized before the database lockup.

Ethics and dissemination

The study is carried out in accordance with the Helsinki Declaration after approval by each local ethics committee (first Vote: Ethical Review Committees of Zhongda Hospital of Southeast University (2019ZDSYLL132-P01)). Clinicaltrial.gov NCT04283162.

CaD, which has been discovered more than 40 years ago and is registered for the treatment of DR in more than 20 countries remains, to date, the only angioprotective agent that reduces the progression of this disease.[24] Although CaD is effective in animal and/or in vitro models, however, the results of clinical trials are inconsistent. A large, multicenter study is warranted to provide a definitive conclusion.[11] To our knowledge, this study is the largest multicenter RCT, and is unique in embedding a cluster-randomized trial design within an intervention framework to study the effectiveness of CaD treatment on the progression of mild to moderate NPDR

compared to conventional treatment. The efficacy of the intervention on visual acuity, the presence, number, location and type of retinal lesions, and retinal blood vessel diameter as well as arteriovenous ratio will also be tested. Our study will give us an opportunity to explore in depth whether CaD is beneficial for the treatment of mild to moderate DR. A limitation of this study is the single-blinded study. Patients' knowing of their treatment groups may have a psychological impact on the trial results. However, the primary endpoint will be assessed by an independent committee and results regarding the primary endpoint will not be biased.

Even in the case of negative results, this trial will produce a large amount of illuminating data. Investigators will be able to closely monitor progression of DR in both arms during the 12-month follow-up period. If the treatment of CaD is effective, it will provide an additional therapy option for comprehensive management of the diabetic patients with mild to moderate DR.

The results of the trial will be analyzed, presented and published as soon as possible at high-impact peer-reviewed journals and presented at the international scientific meetings and conferences. Manuscripts will be written in accordance with the CONSORT guidelines for reporting cluster randomized trials.

Contributors HH and JL drafted the manuscript. HH, JL, DW, SQ, YY, FW, YW, QS, and ZS participated in the design and preparation of the study. DW provided statistical analysis support. DW, SQ, YY, FW, YW, QS, and ZS critically revised the manuscript's drafts. All authors approved the final version of the manuscript.

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Competing interests None declared.

Figure 1. Treatment of patient flow chart. BP, blood pressure; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; ACR, urinary albumin/creatinine ratio; TC, total cholesterol, TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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Table 1. Trial design, visits and end points. Additional visit* at 9 months is warranted for patient with type 1 diabetes

Visit	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit*	Visit 5
Time (days from the start of intervention)	-14	-6	30	90	180	270	360
Informed consent	×						
History	×						
Inclusion/exclusion criteria	×						
Height	×	×	×	×	×	×	×
Body weight	×	×	×	×	×	×	×
Waist circumference	×	×	×	×	×	×	×
Hip circumference	×	×	×	×	×	×	×
Blood pressure	×	×	×	×	×	×	×
Glycosylated hemoglobin	×	×		×	×	×	×
Fasting plasma glucose		×					×
Liver function	×	×		×	×	×	×
Renal function	×	×		×	×	×	×
Blood lipid profiles		×		×	×	×	×
Urinary albumin/creatinine ratio		×		×	×	×	×
Fasting blood samples		×					×
Urine samples		×					×
Visual acuity		×	×	×	×	×	×
Retinal photographs	×	×		×	×	×	×
Retinal vascular caliber		×		×	×	×	×
Adverse events			×	×	×	×	×
Drug accountability		×	×	×	×	×	×

The maximum allowed time interval between screening (visit 0) and baseline examination (visit 1) will be 2 weeks (= 14 days). Otherwise, a

3 new screening will be conducted before the participants are included in the study.

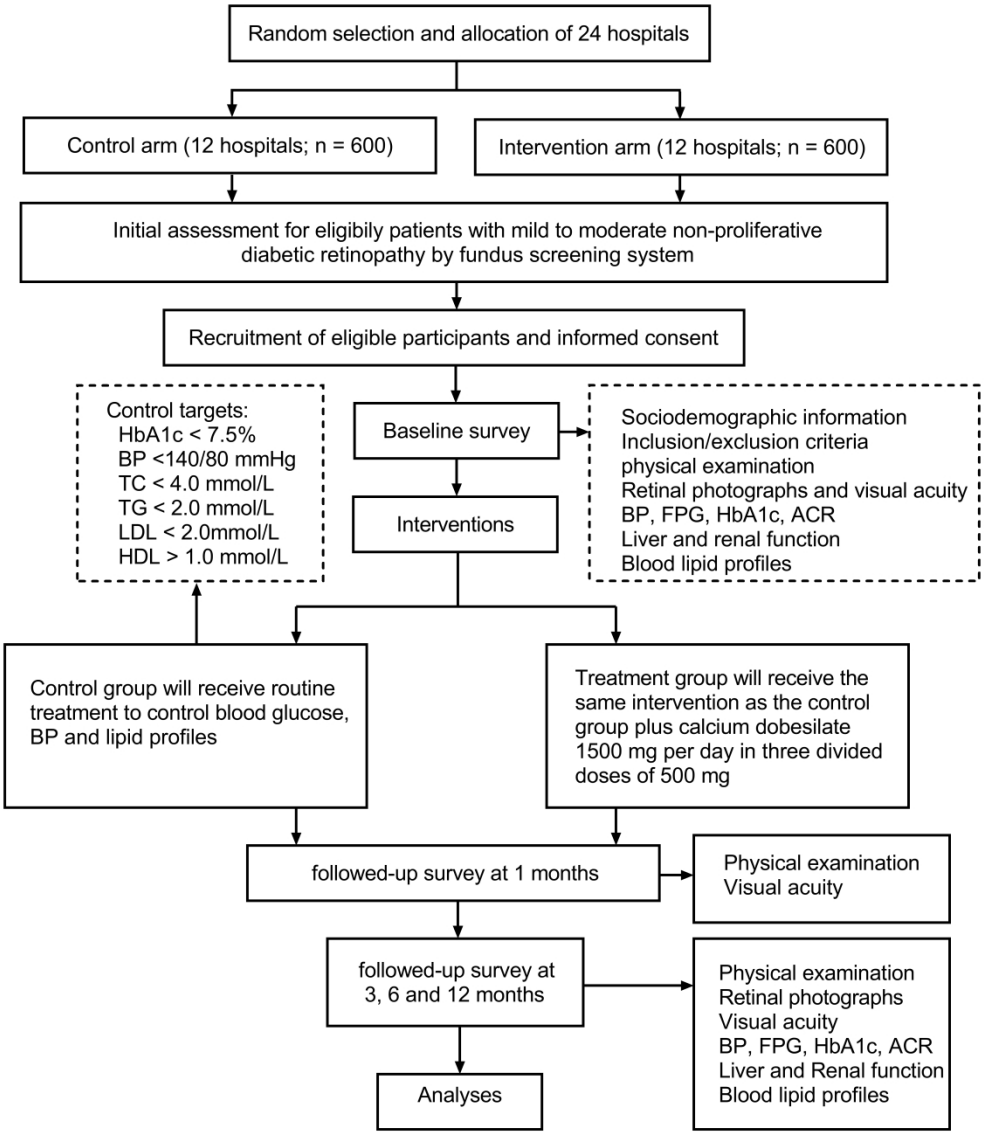

Figure 1. Treatment of patient flow chart. BP, blood pressure; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; ACR, urinary albumin/creatinine ratio; TC, total cholesterol, TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

197x227mm (600 x 600 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry P3 and P22
	2b	All items from the World Health Organization Trial Registration Data Set NA
Protocol version	3	Date and version identifier P22
Funding	4	Sources and types of financial, material, and other support P24
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors P1 and P24
	5b	Name and contact information for the trial sponsor P1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities P24
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) P18 and P20
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention P4-5
	6b	Explanation for choice of comparators P4-5
Objectives	7	Specific objectives or hypotheses P7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) P6 and P12

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained P6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) P8-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered P10-14
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) P18-19
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) P13-15
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial P12-13
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended P7-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) P10-14, P18, and Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations P19-20
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size P10

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions P11-12

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned P11-12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions P11-12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how P11-12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial NA
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol P15-18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols P18-19
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol P20
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol P20-22
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) P21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) P21
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed P18

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial P18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct P18-19
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor P18

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval P22
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) P18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) P11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial P20
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site P24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators P20
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions P23
	31b	Authorship eligibility guidelines and any intended use of professional writers NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code P20

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates P11 and P20
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable P20

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.