Clinical study protocol

Project title: Secreted heat shock protein 90 is involved in the pathogenesis of diabetic peripheral vascular disease

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Summary of Research Scheme

Package name	The role of secretory heat shock protein 90 in the pathogenesis of diabetic peripheral vascular disease
Bidding for the unit	Nanfang Hospital, Southern Medical University
Principal investigator	Zou Mengchen
Research center and responsible person	Zou Mengchen, Nanfang Hospital, Southern Medical University
The research object	Ⅱ with type 1 diabetes
Research purpose	Main purpose: Ⅱ with type 1 diabetes, vascular lesion severity, and the relationship between heat shock protein 90 in serum. Secondary purpose: serum heat shock protein 90 (HSP90) can predict the degree of vascular disease in diabetic patients.
The research group	The diabetic group had no PAD, had PAD, and had diabetic foot disease
Study design	According to the degree of peripheral artery disease, the patients were divided into three groups, and the content of heat shock protein 90 in serum of the patients was detected.To analyze the correlation between the degree of peripheral arterial disease and the content of heat shock protein 90 in serum.
The time limit	The 2020-9-1-2020-9-1
Sample size	150 cases, 50 cases in each group.
The inclusion criteria	1. Diagnostic criteria for diabetes: using WHO1999 diabetes diagnostic criteria, that is, (1) having diabetes symptoms (polyuria, polydipsia, and unexplained weight loss), and random (any time after meal) plasma glucose ≥11.1mmol/L(200mg/ dL);(2), or fasting (fasting for at least 8 hours) plasma glucose ≥7.0mmol/L(126mg/ dL);(3), or OGTT 2 hours plasma glucose ≥ 11.1mmol/L(200mg/ dL) 2. Inclusion criteria of the diabetes PAD group: patients diagnosed as T2DM according to the above-mentioned diagnostic criteria for diabetes, and with (1) symptoms and signs of atherosclerotic occlusion (intermittent claudication, resting pain, reduced or absent pulsation

	of dorsal foot artery, etc.);(2) Ankle-brachial arterial pressure index (ABI) ≤0.9;Toe brachial artery pressure index (TBI) ≤0.70; There was no three-phase pulse pattern of foot artery. Transcutaneous partial oxygen pressure (TcPO2) <30mmHg;(3) Evidence of atherosclerosis, atherosclerotic plaques, arterial stenosis or obstruction in carotid and/or lower extremity major arteries found by vascular color Doppler ultrasound. 3. Inclusion criteria for the diabetic foot group caused by PAD: the presence of infection, ulcer and/or deep tissue destruction of the foot was confirmed according to the above diagnostic criteria of diabetic PAD. 4. Agree to participate in the study and data collection, and sign the informed consent.
Exclusion criteria	1. Diabetic ketoacidosis or hyperosmolar state occurs within 30 days; Diabetic coma in 2 or 3 months, or severe hypoglycemia in nearly 1 month; 3. Diseases of tumor, immune system and hematopoietic system; 4. Other types of diabetes 5. Less than 40 years old
Validity analysis	The main observation indexes: Ⅱ with type 1 diabetes, serum content of heat shock protein 90.
Statistical analysis	Sample size determination: $n = \varphi^2 \left(\sum S_i^2 / g \right) / \left[\sum (\bar{X}_i - \bar{X})^2 / (g-1) \right]$ Statistical analysis: SAS 9.2 statistical analysis software will be used for statistical analysis.
follow-up	No follow-up
Statistical method	Analysis of variance
schedule	2020-9-1 -- 2020-10-10 Collect all samples 2020-10-10 -- 2020-10-15 Complete all sample tests 2020-10-15 -- 2020-10-20 Statistical analysis was completed

List of abbreviations and definitions of terms

Arterial lesions around PAD

OGTT oral glucose tolerance test

1. Research background

Diabetic foot disease is one of the most serious complications of diabetes, which brings great pain and economic burden to patients, with a global incidence of about 6%.In China, the incidence rate is 8.1%, and the amputation rate is 7.3%. Every year, more than 1 million diabetic patients have amputations, ranking first among non-traumatic amputations.According to the American Diabetes Association (ADA), the incidence of Peripheral Arterial Disease (PAD) in diabetic patients is twice that of non-diabetic patients, and the resulting lower limb ischemia is the main cause of the high mortality and disability rate of diabetic foot.According to the International Working Group on Diabetic Foot (IWGDF), about 50% patients with diabetic foot disease are complicated with PAD, and the degree of vascular stenosis is closely related to the prognosis.Severe limb ischemia is a higher cause of diabetic foot ulcer in China than in western countries. The main pathological change of diabetic PAD is atherosclerosis, and endothelial injury is the initial link of atherosclerosis.Oxidative stress is the central link in the mechanism of endothelial injury in diabetic vascular disease, and it is also the basis for us to model endothelial cells with high glucose and hydrogen peroxide.

Heat Shock Protein 90 (Hsp90) is an important type of Heat stress Protein. The molecular size of Hsp90 is 90-kDa. In eukaryotic cells, there are mainly two subtypes, α and β, and the content of Hsp90 accounts for 2-3% of the total Protein in cells.Involves in the correct folding and activation of intracellular proteins. Although Hsp90 is primarily involved in intracellular protective mechanisms, they can also be exposed to the plasma membrane and released in the extracellular space, resulting in detectable levels of Hsp90 in the blood.Extracellular heat shock proteins are involved in cell-cell

communication as well as immune and inflammatory processes.Hsp90 promotes cell survival, migration, inflammation and angiogenesis, and is therefore considered a promising target for cancer therapy.This led to the development of specific HSP90 inhibitors.More recently, these inhibitors have also been tested in diabetic animals. The use of the HSP90 inhibitor 17-DMAG significantly reduced atherosclerotic lesions and induced a more stable plaque phenotype in a mouse model with hyperglycemia and hyperlipidemia.Hsp90 is upregulated in human carotid atherosclerotic plaques (especially in unstable areas of plaques) and in patients' serums, triggering autoimmune antibodies against Hsp90 in patients.

2. Research Objectives

Main purpose: 2.1 Ⅱ with type 1 diabetes, vascular lesion severity, and the relationship between heat shock protein 90 in serum.

2.2 Secondary purpose: serum heat shock protein 90 was used to predict the degree of vascular disease in diabetic patients.

3. Study endpoint

3.1 Primary end point: Patients were screened for diabetic angiopathy during hospitalization.

4. Study design

This study is a prospective study. It is planned to enroll 150 subjects, with 50 subjects in each group.

Subjects will not be given or offered any randomization or any study regimen-driven treatment during the study.If it is clinically applicable, treatment decisions and treatment options are made at the discretion of the treating physician.

5. Subjects

The subjects of this study is Ⅱ crowd with type 1 diabetes.

Diagnostic criteria: 5.1 reference WHO1999 years diabetes diagnosis standard, specification and so on carries on the diagnosis of Ⅱ with type 1 diabetes.

5.2 Inclusion criteria:

●Voluntarily signed informed consent;

●Over 40 years of age, regardless of gender

WHO1999 year after the diagnosis of diabetes guidelines, specifications Ⅱ diagnosed with type 1 diabetes

5.3 Exclusion criteria:

- Diabetic ketoacidosis or hyperosmolar state occurs within 30 days;
- Diabetic coma in 3 months, or severe hypoglycemia in nearly 1 month;
- Diseases of tumor, immune system and hematopoietic system;
- Other types of diabetes
- The researcher judged that it was not suitable to participate in this study.

5.4 Case withdrawal and abscission

All enrolled subjects have the right to withdraw from the study at any time.Case withdrawal reasons:

–The subject requested to withdraw and did not want to continue in this study;

–For other reasons, the investigator determined that subjects were not appropriate to continue in the study;

Subjects who dropped out of the study were considered to have fallen out.When the patient falls off, try to contact the patient, complete the evaluation project, and fill in the study summary page.

6. Research methods and procedures

Because this is an observational study, no additional visits or laboratory analysis or evaluation beyond what is required by routine clinical practice are required. The doctor will decide the treatment plan according to the instructions and local regulations. The investigator will review the patient's medical history and laboratory reports to determine eligibility based on inclusion and exclusion criteria. Patients must sign the latest IRB/ IEC-approved informed consent form (ICF) prior to performing data collection.The number and duration of visits are at the discretion of the physician in accordance with local practice.

6.1 Informed consent and inclusion

Subjects who provide informed consent and meet all other inclusion/exclusion

criteria are considered to be enrolled in the study.

6.2 Subject identification number

Each subject was given a unique identification number, namely the initials of the patient and the serial number. The identification numbers of the diabetic group without PAD were: DM001, DM002……And so on;The identification numbers of PAD groups are DMP001 and DMP002……And so on;The identification numbers of diabetic foot disease group were: DF001, DF002……. All study documents (such as case report forms, clinical records, etc.) will use this identification number.In addition, under data privacy regulations, a unique identification number is allowed, as long as it does not contain combined information that identifies the subject.

6.3 Data source/data collection process

Data from this study were obtained by extracting routine clinical records of enrolled subjects. The investigator was required to complete the study's electronic case report form (eCRF) and/or record form based on the information entered in the patient's medical record throughout the monitoring period.

6.4 Data collection steps:

6.4.1 screening period

（1） Signed Informed Consent

（2） Complete inclusion and exclusion criteria verification

（3） Medical history and demographic data were obtained

（4） Laboratory tests: screening for diabetes-related complications, blood routine, CRP, coagulation function, renal function, etc

6.4.2 trial

(1) Serum collection: the nurses collected the patients' fasting blood at 6:00 am in accordance with the routine blood collection procedures: 1 tube of procoagulant blood and 1 tube of anticoagulant blood were collected, each tube was no less than 3ml of blood;Store in a 4-degree

refrigerator for disposal.

(2) Collection of serum and plasma: the blood samples collected by the nurse were placed in a centrifuge, centrifuged at 3000 RPM for 15 minutes, and then the supernatant was collected.

(3) Laboratory test: the collected serum or plasma were quantitatively tested by HSP90 ELISA, and the results were analyzed.The remaining serum or plasma was stored in a negative 80 refrigerator.

6.4.3 follow-up

A telephone interview will be conducted every 3 months to assess the patient's prognosis (blood glucose and medication status, complication progression, PAD or diabetic foot disease).

7. Safety monitoring, reporting and medical treatment

7.1 Definition of adverse event (AE)

An adverse event is any adverse medical event that occurs after a patient or subject receives a drug and is not necessarily cause-and-effect related to the treatment. Thus, an adverse event can be any adverse physical sign (including abnormal laboratory results), symptom, or disease that is time related to the use of the study drug, regardless of whether a causal relationship with the study drug is considered. Adverse events include Serious Adverse events (SAE) and non-serious Adverse events.

7.2 the SAE definition

A SAE is a medical event that occurs during a clinical study that requires or prolongs hospitalization, is disabled, affects work ability, endangers life or death, or results in birth defects.Includes the following medical events:

 1) death events;

 2) life-threatening events (defined as the subjects of the attack, there is immediate danger of death);

 3) requiring hospitalization or extend the length of time of events;

□ 4) can lead to permanent or severe disability/function/event affects the ability to work;

□ 5) anomaly or birth defects;

□ other important medical event (defined as events harm to subjects, or any need to intervene to prevent the above situation).

7.3. Recording, collecting, reporting and handling of adverse events

7.3.1 Collection, reporting and processing of AE

After signing the Informed Consent, all AEs that occur in relation to the prescribed operating procedures of the study protocol will be recorded in the eCRF.

A record of AE should include a description of AE and all related symptoms, time of occurrence, severity, duration, action taken, and final outcome and outcome. The AE should be recorded in medical terms, and if the symptoms and signs of a subject can be summarized by a common cause, the diagnosis should be recorded whenever possible. With the exception of progression-related indicators, all clinical events and clinically significant laboratory adverse events were treated according to the Common Event Evaluation Criteria (CTCAE) version 5.0.

7.3.2 SAE collection and reporting

All SAE incidents, regardless of cause, from the date the subject signed the informed consent will be reported using the SAE Report Form.In the event of SAE, the investigator should immediately take appropriate treatment measures to ensure the safety of the subjects.

7.3.4 Determining criteria for severity of AE

Severity will be assessed against the five-level criteria developed by the NCI CTCAE5.0:

■ Grade 1, mild;No symptoms or mild signs; For clinical or diagnostic observation only, no medical intervention is required;

■ Level 2, medium;Age-appropriate limitations in daily living functions (such as cooking, shopping, making phone calls, etc.);

■ Level 3, serious or medically important but not immediately life-threatening;Resulting in or prolonging hospitalization; Disabled; Restricted daily self-

care activities (daily self-care activities refer to bathing, dressing, undressing, eating, going to the toilet, taking medicine, etc., but not being bedridden);

■ Level 4, life-threatening and requiring emergency treatment;

■ Level 5, AE-related deaths

7.3.5 Other responsibilities of the investigator during follow-up of serious adverse events

Serious adverse events should be examined and treated according to clinical judgment, including necessary clinical laboratory tests, physical examinations, etc. The results of any examination or other updated SAE information must be followed up for the same time frame and procedure as the initial report.

8. Study termination/suspension criteria

8.1 Sponsor reserves the right to terminate/suspend the study.Before terminating/suspending a clinical study, the sponsor must notify the investigator, the ethics committee and the relevant regulatory authorities and state the reasons.After early termination/suspension of the study, study resumption must be reviewed and approved by the ERB.

8.2 Termination/Suspension as required by the IRB.

9. Provision for the termination of clinical studies

The study ended when all subjects met the following criteria:

1）All subjects completed at least a routine diabetes test

2）2) or death, loss of follow-up or withdrawal of informed consent of all subjects.

Data management

10.1 Data management

1) The researcher must ensure the authenticity, integrity and accuracy of the data;

2) When making any corrections to the research records, only the underlined and the modified data should be annotated with reasons, signed and dated by the researcher, and the original records should not be erased or overwritten;

3) Complete laboratory inspection items.

10.2. Data recording and file preservation

Subject data on the Case Report Form shall be recorded as Subject Numbers and

Subject can only be identified by Subject Numbers or their initials.

OC-RDC was used for data management. From the data entry to the verification requirements of the source data to the query and answer of the quality control data, finally to the operation of the data locking and export,

After confirming that the data is in doubt, the parties sign the database lock application form and the database is locked by the data administrator. After the database is locked, the analysis database is exported by the data administrator and sent to the statisticians for statistical analysis. The locked data cannot be edited again, and problems found after database locking can be corrected in the statistical analysis program after confirmation.

11. Statistical analysis

11.1 Sample size determination

This study was a non-interventional prospective study, and the scientific formula of sample size was estimated around the content of serum heat shock protein 90 in patients with diabetes.According to the statistical principle, the number of cases needed to achieve the desired purpose of the study was calculated as 50 cases in each group.

11.2 Statistical methods

The analysis included baseline analysis and analysis of the relationship between the degree of vascular disease and serum HSP90.

11.3 Statistical Software and General Requirements:

●All statistical analyses were performed using SAS9.2 (or higher).

●The measurement data were described as mean, standard deviation and group number.

12. Research management

12.1 Comply with the requirements of relevant laws and regulations

1) Investigators shall implement quality control and quality assurance systems for clinical studies using standard operating procedures;

2) The original data must comply with the requirements of relevant laws and regulations;

3) Laboratory test results must be accurate and reliable;

4) The observations and findings used should be verified to ensure the reliability of the data;

5) Establish a complete research organization and clarify the responsibilities of personnel at all levels;

6) The main researcher shall be responsible for overall quality control and carry out the responsibilities of personnel at all levels;

7) The principal investigator shall be responsible for designing the study plan and informed consent, and the principal investigator shall write the study summary report after the study is over;

8) The designated researcher shall be responsible for formulating the research implementation rules and SOP for use in the research;

9) Before the study, the research team shall organize the study program for all participants, and all participants shall receive GCP training;

10) Doctors and nurses participating in the study should strictly abide by the program provisions and follow the procedures without any arbitrary changes;

11) The designated statistician shall be responsible for overall statistical processing of data.

12.2 Subject's privacy shall be protected

Subjects during the study period of completely confidential data entry computer storage and analysis, the necessary customs agency may to audit records, in order to verify that the true, accurate and integrity of the data, the research data may also be published in the academic journal, but participants name will not be published, the privacy of the subjects to be confidential.

12.3 Problems occurred in the study and their treatment measures

1) Revision of the scheme: After the scheme has been approved by the ethics committee, if the plan is to be revised, the "Revision Description of the Scheme" shall be formulated and signed by the principal investigator. Only after the researcher and the drug registration applicant have negotiated and agreed to revise the scheme;

2) The revised scheme shall be submitted to the Ethics Committee for review and

approval before it can be implemented;

3) No participant in the study shall violate the protocol.

12.4 Quality control and quality assurance

12.4.1 Quality assurance:

The sponsor, entrusted by the sponsor is responsible for all or part of this study related responsibilities and tasks of the cooperation unit (including CRO, SMO, statistics institutions and clinical center, etc.) shall establish its own quality assurance system, to perform their respective duties, and strictly follow the clinical study scheme, adopt the corresponding standard operating procedures, to ensure the clinical research on the implementation of the quality control and quality assurance system.

12.4.2 Quality assurance of clinical study process

Before the start of clinical study, researchers should receive training on the study protocol, so that researchers can fully understand and recognize the specific connotations of the clinical study protocol and its indicators.The quality control personnel shall check the basic conditions of the clinical study to ensure that the clinical study conditions can meet the requirements of the protocol.During the study, researchers should conscientiously carry out clinical operations according to the requirements of the institution's SOP and the study protocol, and make true, timely, complete and standardized records.The quality control personnel will check the quality of the research process and the corresponding original records.After the end of the study, the research unit will sort out the corresponding project documents, which will be archived and stored after being checked by the quality control personnel.The quality assurance department of the clinical research unit checks the feasibility of the research carried out.When the non-conformance is found, the researcher and the person in charge of the unit shall be informed to make corrections in time, and the correction shall be tracked.

12.4 Anticipated progress and completion date of the clinical study

2020-9-1 -- 2020-10-10 Collect all samples

2020-10-10 -- 2020-10-15 Complete all sample tests

2020-10-15 -- 2020-10-20 Statistical analysis was completed

12.5 Responsibilities and other relevant work undertaken by the Parties

1) Sponsor Responsibilities

The sponsor is responsible for initiating, applying for, organizing, and funding the clinical study.

2) Investigator responsibilities

The investigator will conduct the clinical study in accordance with the ethical, ethical and scientific principles as well as protocol design and regulations laid down in the Declaration of Helsinki and relevant regulations. The investigator should be aware of the procedures and requirements for reporting SAE and should document and report these events as required.The investigator shall accurately, completely, timely and legally record the data into the eCRF and accept the inspection or inspection by the inspector or inspector dispatched by the sponsor or CRO company and the inspection and inspection by the pharmaceutical regulatory department to ensure the quality of the clinical study.

3) Sponsor research methods for data publication

Sponsor has exclusive rights to the research data. The final report should not be published on an individual's behalf until it is completed, unless it has been agreed in writing by the Sponsor or is reflected in the Co-operation Agreement.The sponsor has the final say regarding the manuscript and publication.

12.6 The collection, sale, export and exit of human genetic resources shall comply with the Interim Measures for the Management of Human Genetic Resources (Guo Ban Fa [1998] No. 36).

13. Research the relevant ethics

13.1 Ethics Committee

Prior to the commencement of the study, the investigator will submit the investigator's manual, study protocol, informed consent, CRF (based on actual submission), and any other information given to the subject to the IRB for approval.Any amendments to the study protocol must be approved by the IRB.

13.2 Informed Consent

The qualified investigator must explain in detail to each subject in informed

consent the nature, purpose, procedures, expected time, potential risks and benefits, and any discomforts that may arise of the study.Each subject must be aware that participation in the study is voluntary and that he/she may withdraw from the study and withdraw informed consent at any time without affecting his/her subsequent treatment or relationship with the treating physician.

Informed consents should be given in standard written form and in non-professional language as far as possible. Each informed consent must include all of the above and include a voluntary statement. The informed consent must be submitted to the ethics committee for approval.

After the basic content of the study has been explained and the Investigator has satisfied that each prospective subject understands the purpose of the study, each prospective subject shall be required to sign and date the informed consent form.The subject should read and consider the statement before signing and dating it, and should obtain an informed consent to keep after signing. Without Informed Consent Subjects will not be admitted to the study without signing informed consent.

13.3 other

When a subject is unable to participate in informed consent independently, a reliable fair witness/legal representative must be present throughout the informed consent process.The selection of fair witness/legal representative shall not violate the subject's right to confidentiality.Upon the subject's oral consent, the fairness witness/legal representative should sign and date the informed consent to prove that the information is accurate.

14. References

Clinical study protocol confirmation signature page

The role of secretory heat shock protein 90 in the pathogenesis of diabetic peripheral vascular disease

Principal Investigator's Consent on the Scheme:

I have read this plan carefully, and I agree that it contains all the information necessary to carry out the research, and I agree to carry out the plan as described. I understand that the research shall not be initiated without the approval of the ethics committee, and shall fully comply with the relevant regulations of the unit.

Informed consent forms and corresponding documentation of all participants should be obtained. Following the signing of the Informed Consent, clinical studies will be conducted in accordance with the requirements of the Declaration of Helsinki and the laws and regulations governing the clinical application of the research techniques.

Signature of Principal Investigator: Date: