Study's initial protocol

This trial was registered in ClinicalTrial.gov (NCT01108120).

The inclusion and exclusion criteria

The inclusion criteria：(1) Type 2 diabetes with Wagner grade 1～ 3 foot ulcers, (2) Age 30-80 years old, (3) Chinese Han from Hubei province. The exclusion criteria: (1) Patients with Wagner grade4 or 5 diabetic foot ulcers, (2) severe coronary, cerebral, renal vascular as well as severe liver diseases, malignant neoplasms, (3) bleeding individuals(4) > 80 years old, (5)heart failure (NYHA 3,4), (6) Cancer

Study objective

The primary endpoint was Healing rate of diabetic foot ulcers [Time Frame: < half a year] and during hospitalization, the healing rate of foot ulcers is observed. The recurrence rate of diabetic foot ulcers [Time Frame: 8 years]. During the 8 years of follow up period, recurrence rate of diabetic foot ulcers are observed. The secondary endpoint was cardiovascular events and death .During the follow up period of 8 years, the cardiovascular events, death from all causes are observed.

Study procedures

We select 200 diabetic patients with Wagner grade 1 ~ 3 foot ulcers. They are divided into two groups randomly: thrombolysis group and control group, 100 cases in each group between May 2010 and December 2015.

After diabetic dietary advice, all patients receive insulin therapy to control blood glucose within a range of 5 - 10 mmol/L. Then the patients receive conventional care for their ulcers. To remove extensive callus and necrotic tissue, wound debridement was performed. Broad spectrum antibiotics are prescribed if ulcers show clinical signs of infection. Adjustments to the treatment are performed when indicated on the basis of microbiologic cultures and sensitivity testing.

The conventional group patients receive an intravenous injection of prostaglandin E1 (20 ug per day) until the healing of ulcers or discharged from hospital. In the continuous intra-femoral thrombolysis group, first of all, a ultrasound Doppler examination of vessels including artery and venous of lower limbs were performed. To avoid pulmonary infarction, a filtrator is placed in the inferior vena cava before the thrombolysis process if ultrasound results show venous thrombosis. Then insert a percutaneous artery canal from femoral artery in another lower limb into the distal of popliteal artery as far as possible. After finishing this process, the outside part of this artery canal is fixed at thigh, and the patients must keep in supine position in the bed.Firstly,20 0000 ~ 40 0000 units urokinase is injected via the catheter to diseased foot. Then, continuous infusion urokinases via femoral artery by an artery pump (100 ml 0.9% sodium chloride + 100 0000 unit urokinase at a rate of 4 ml per one hour) for 7 - 10 days. Finally, patients receive an intravenous injection of prostaglandin E1 (20 ug per day) until the healing of ulcers or discharged from hospital. The healing rate of foot ulcers, the time of ulcers, neuropathy symptoms, the period of

hospitalization are compared between the two groups during hospitalization. The recurrence rate of foot ulcers, cardiovascular events, death from all causes are compared between two groups during follow up study.

The final protocol

The inclusion and exclusion criteria

The study protocol was approved by the ethics commission of General Hospital of Central Theater Command and all the patients gated informed consent before they entered the study. **The inclusion criteria：** (1) Type 2 diabetes with Wagner grade 1～ 3 foot ulcers, (2) Age 30-80 years old, (3) Chinese Han from Hubei province. **The exclusion criteria:** (1) Patients with Wagner grade4 or 5 diabetic foot ulcers, (2) Bleeding diatheses, (3) Malignant hypertension, (4) Hepatic or renal failure, (5) Mental illness, (6) Cancer, (7) Current pregnancy, (8) Diabetic ketoacidosis or diabetic lactic acidosis. **Diagnosis criteria:** (1) Diabetes mellitus was defined in 1999 WHO standard, (2) The DFU was classified by Wagner's scale, (3) Cardiovascular events included coronary heart disease events (non-fatal myocardial infarction, angina), total stroke, cardiovascular death, coronary and carotid revascularization. (4) Cardiovascular death was deemed when a fatal event occurred after myocardial infarction, ventricular fibrillation, sudden death, or heart failure. All the other deaths were classified as noncardiovascular, (5) Cigarette smoker was defined as subjects who had smoked at least one cigarette daily for 1 year, (6) Malignant hypertension was defined in the ACC/AHA Guideline, (7) Albuminuria was defined as ADA criteria,-(8) Major Amputation was defined as any above-the-ankle amputation.

Study objective

The primary endpoint was ulcer closure rate at 1 month and terminal of the follow-up study. The secondary endpoint was the time of ulcer healing, incidence of ulcer recurrence, incidence of amputation, the cardiovascular event and the cardiovascular death during the follow up period.

Study procedures

A total of 195 diabetic foot ulcer patients were randomly divided into either continuous intra-femoral thrombolysis group (98 patients) or conventional therapy group (97 patients) between May 2010 and December 2015. The continuous intra-femoral thrombolysis group received continuous intra-femoral urokinase injection for 10 days, and conventional therapy group just received wound debridement and dressing change. Then, a follow-up of average 6.5 years was performed.

Eligible patients were randomly assigned to two groups for therapies. After diabetic dietary advices, all patients received insulin therapy to control blood glucose within a range of 5～10

mmol/L during first month intervention period as much as possible. Then the patients received conventional care for their ulcers such as wound debridement including remove extensive callus and necrotic tissue, and broad spectrum antibiotics when ulcers showed clinical signs of infection. Adjustments to the treatment are performed when indicated on the basis of microbiologic cultures and sensitivity testing. All participants received a regular dressing change with sterile gauze 2～3 times per week to the end of the study if needed.

Patients were examined weekly after the terminal of the intra-femoral urokinase injection assay. Then the patients were performed a follow-up study monthly for the first year, and followed trimonthly to December 2020. At each study visit, ulcers were assessed for area via wound tracing, ulcer closure, or adequate granulation tissue formation. The ulcer area was determined in square millimeters by multiplying the largest width and length of the ulcer. The largest ulcer was considered as the study ulcer when more than one ulcer was present. The measurement was performed after revision of the ulcer. During the follow-up period, the treated target of haemoglobinA1c (HbA1c) was < 7.0%, and the therapy medicines and strategy were determined by the responsible physicians based on CDS guidelines 2010.

The continuous intra-femoral urokinase injection approach

A continuous intra-femoral urokinase injection approach was performed for patients in continuous intra-femoral thrombolysis group. In brief, an ultrasound Doppler examination of vessels including artery and venous of lower limbs was performed. To avoid pulmonary infarction, a filtrator was placed in the inferior vena cava before the thrombolysis process if ultrasound results showed venous thrombosis. Next, a percutaneous artery canal was inserted into the distal of popliteal artery in affected lower limb as far as possible from femoral artery. After finishing this process, the outside part of this artery catheter is fixed at thigh, and the patients must keep in supine position in the bed. When the above operation was completed, 20 0000 ～ 40 0000 units urokinase was injected via the catheter to diseased foot. Afterwards, the continuous infusion Urokinase (100 ml 0.9% sodium chloride + 100 0000 unit urokinase at a rate of 4 ml /h) was administered with an artery pump via femoral artery for 10 days. In addition, the safety outcomes such as infections and bleeding or oozing blood for continuous intra-femoral urokinase injection approach were observed.

Summary of all amendments：

Because prostaglandin E1 is not available in our clinic, thus we modified the protocol that the control group was just treated by dressing change and wound debridement.