

Assessment of maximum tolerated dose of a new herbal drug, Semelil (ANGIPARS™) in patients with diabetic foot ulcer: A Phase I clinical trial

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ABSTRACT

Background and the purpose of the study: In many cases of diabetic foot ulcer (DFU) management, wound healing is incomplete, and wound closure and epithelial junctional integrity are rarely achieved. Our aim was to evaluate the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of Semelil (ANGIPARS™), a new herbal compound for wound treatment in a Phase I clinical trial.

Methods: In this open label study, six male diabetic patients with a mean age of 57±7.6 years were treated with escalating intravenous doses of Semelil, which started at 2 cc/day to 13.5 cc/day for 28 days. Patients were assessed with a full physical exam; variables which analyzed included age, past history of diabetes and its duration, blood pressure, body temperature, weight, characteristics of DFU, Na, K, liver function test, Complete Blood Count and Differential (CBC & diff), serum amylase, HbA1c, PT, PTT, proteinuria, hematuria, and side effects were recorded. All the measurements were taken at the beginning of treatment, the end of week 2 and week 4. We also evaluated Semelil's side effects at the end of weeks 4 and 8 after ending therapy.

Results and major conclusions: Up to the drug dose of 10 cc/day foot ulcer dramatically improved. We did not observe any clinical or laboratory side effects at this or lower dose levels in diabetic patients. With daily dose of 13.5 cc of Semelil we observed phlebitis at the infusion site, which was the only side effect. Therefore, in this study we determined the MTD of Semelil at 10 cc/day, and the only DLT was phlebitis in injection vein. The recommended dose of Semelil I.V. administration for Phase II studies was 4 cc/day.

Keywords: Semelil, ANGIPARS™, Diabetic foot ulcer, wound healing, *Melilotus officinalis*, Clinical Trial Phase I

INTRODUCTION

Diabetes mellitus has become one of the major health problems with diabetic foot ulcer (DFU) as one of the most important complications (1). The prevalence of DFU in different populations has been estimated 2-10% (2) and the lifetime risk of developing a foot ulcer for diabetic patients could be 15% (3). Currently, every 30 seconds one person around the world undergoes DFU related amputation (4).

The most frequent risk factors for ulceration are neuropathy, feet deformity, high plantar pressure, and uncontrolled hyperglycemia (5). Treatment of some DFUs is complicated, and patients need hospitalization due to existence of ischemia or infection (6,7). The most reason of hospital admission of diabetic patients is foot ulcer and amputation (8). In 1994, nearly 67000 diabetic patients were discharged after lower limb

amputation in the US (9) which have been accounted for 984000 hospital days with a mean length of stay about 15 days. In Iran, 34.7% of total reasons for lower limb amputation are related to diabetes (10), and the mean length of pre- and post-operative stay in hospital has been 3.8 weeks. Overall, the diabetic foot patients are hospitalized 59% more than other diabetic patients (11).

Although diabetes is among the reasons for more than half of nontraumatic lower limb amputations, diabetes foot complications are preventable by changing modifiable risk factors, patient education, correct care of foot, effective local treatments, and revascularization (12,13). There are different treatments for foot ulceration: debridement (enzymatic, autolytic, and surgical), off-loading (total contact cast), dressings, antibiotic therapy for resolution of infection, accompanied in all of them control of

hyperglycemia (14-18). There are also novel treatments for DFUs such as local therapy with growth factors (platelet-derived or epidermal growth factors), skin replacement with human skin equivalents (Dermagraft, Apligraf), hyperbaric oxygen therapy, vacuum assisted closure, heat therapy, and laser therapy (19-27). Although these methods are useful for prevention and treatment of DFU, all of them have partial, albeit significant, efficacy in the prophylaxis of amputation and more effective therapies are required. Semelil (ANGIPARS™) is a new herbal formulation with wound healing activity without toxic effects in pre-clinical and toxicology and genotoxicity studies (28-31). The main goal of this study was the assessment of the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of Semelil in a Phase I clinical trial.

MATERIALS AND METHODS

Drug solution preparation

Semelil (ANGIPARS™) was prepared by ParsRoos Co. (Tehran, Iran) according to the following procedure. The ethanol extract of leaves and small stems of *Melilotus officinalis* was mixed with various amounts of selenium, urea, fructose and sodium phosphoglycerol. The compound was then obtained by filtration using a cloth filter followed by successive filtrations through 5 and 0.22 µm filters. At the end of this procedure, Semelil was ready for the preclinical toxicity evaluation in laboratory animals and volunteers. The Semelil was freshly diluted in sterile solution of sodium chloride 0.9% and used in the study.

Patients and eligibility criteria

Six male diabetic foot patients (Type 1 or 2 of diabetes mellitus) in range of 18-75 years were enrolled to the study based on the following inclusion and exclusion criteria. Diabetic foot patients with peripheral sensory neuropathy or other neuropathies, foot deformities, trauma or inappropriate shoes, past history of foot ulcer or amputation, limitations in articular movements, uncontrolled hyperglycemia, long term diabetes or similar conditions could be enrolled. Lesions could be single or multiple and occurred within the last 2 weeks. Patients' inclusion was completely informative and voluntary. After inclusion of each volunteer, complete explanation was given about the goal of study, probable side effects of the drug and patients' rights during the research process. Then, written informed consent according to institutional guidelines was obtained before treatment.

Exclusion criteria were refusal to sign the informed consent form, existing liver or kidney disorders, malignancy, serious cardiovascular

conditions such as congestive heart failure, infectious ulcer, osteomyelitis, vasculitis and progressed diabetic retinopathy.

Study Design

The open label Phase I clinical trial was carried out on human subjects. The trial was designed based on the Storer two-stage dose escalating method as depicted in the overall scheme (flowchart: Fig. 1).

Dose Escalation

Patients were treated with intravenous administration of Semelil diluted in 100 cc normal saline. Baseline dose was determined based on LD₁₀ (lethal dose for 10% of the treated animals within 30 days). The initial dose was 2.0 cc daily administered as an i.v. infusion over 30 minutes at a rate of 200 cc/h., and it was escalated in next patients. Dose escalation was performed according to a modified Fibonacci method. In other groups, patients received 4, 6.7, 10 and 13.5 cc of intravenous Semelil, respectively. In the absence of toxic effects, treatment was continued for 28 days. Treatment ceased in patients experiencing progressive ulceration or drug adverse effects (Fig. 1). If dose limiting toxicity occurred in a patient at a given dose level, then the dose escalation ceased and the next lower dose was declared the MTD. Dose reduction by one level was considered for patients who developed DLT. Patients were followed for additional 4 weeks.

Assessments

For assessment of probable DLTs, a complete set of evaluations was performed by professional team (a nurse, a researcher physician, and an endocrinologist). The assessment included:

- medical history and physical examination taken at the baseline, and at the end of the second and forth weeks of treatment;
- measurements of body temperature, headache evaluation, allergic responses, neuropathy, heart and vascular parameters, blood pressure, ECG, gastrointestinal symptoms (nausea, vomiting, diarrhea), local reactions (rash or dermatitis) after injection, dyspnea, ocular, muscular and arthritis symptoms, neurologic and psychologic signs and symptoms, paresthesia;
- measurements of Na, K, Cr, PT, PTT, WBC, ALKP, SGOT, SGPT, HCT, Hb, HbA_{1c}, serum amylase, bilirubin, proteinuria and hematuria;
- weekly documentation of patient's compliance to therapy and his acceptance of side effects;
- recording any probable side effects and necessary managements;
- follow up, visit and evaluation of patients in weeks 4 and 8 after ending the therapy.

Laboratory Methods

All laboratory evaluations were done in the Hormone laboratory of EMRC, Shariati Hospital, Medical Sciences/University of Tehran (Tehran, Iran). HbA1C was measured by Drew-DS5. Evaluations of biochemical tests were performed by auto analyzer (Parsazmoon Co, Iran, kit), and enzymatic methods. Measurement of Na, K were performed by flame photometry; PT & PTT - by Stago (Germany); CBC & diff - by Sysmex (Japan) for cell counting, proteinuria - by kit of Parsazmoon Co. (Iran), and hematuria - by Kimia Co, urine strip-enzymatic method, respectively.

Ethical Considerations

To observe ethical aspects of this study, probable side effects of the drug were completely presented to the patients. All patients were also participated after signing the informed consent form and were excluded in occasion of any side effects and dissatisfaction at any time point of the study. The study protocol was approved by the Medical Ethics Committee of Medical Sciences/University of Tehran.

RESULTS AND DISCUSSION

Six diabetic patients with DFU complications were examined prospectively for DLT effects. The range of their ages was 40-70 years; with 57 ± 12 years. The mean duration of diabetes was 7.6 years. They were treated with escalating intravenous doses which started at 2.0 cc/day to 13.5 cc/day for 28 days, plus standard treatment of hyperglycemia or infection, if present. All Semelil treatment options are described in Table 1.

Mr. M.B. was 48 years old with 8 years history of diabetes. He received daily 2.0 cc of Semelil for 28 days and was evaluated eight times. Following all the examinations, we did not observe any clinical or laboratory side effects neither during the treatment no at the end of the week 8 after ending therapy.

Mr. Z.M. was 67 years old with duration of diabetes for 7 years. He received daily 4.0 cc of the drug for 28 days and was evaluated eight times. No side effects were observed according to clinical and laboratory evaluations in the period of follow up.

Mr. M.H. was 70 years old who had diabetes for 3 years. He received daily 6.7 cc for 28 days and was evaluated seven times. In this patient no side effects were recorded according to clinical and laboratory data.

Mr. Gh.A was 40 years old with 10 years duration of diabetes. He received daily 10.0 cc for 28 days and was evaluated eight times. Up to this dose level foot ulcer was dramatically improved. The

patient did not have any side effects according to clinical and laboratory assessments in all the period of study.

Mr. M.H. was 55 years old and had diabetes for 10 years. He received daily 13.5 cc for 8 days and was evaluated three times. We observed phlebitis in injection place, on the 8th day of treatment. After changing the place of injection, the phlebitis was continued. So, the administered dose was declined up to the half of it (6.7 cc/day). Phlebitis has vanished consequently. No other side effects were observed at 13.5 and then 6.7 cc/day dose levels during the treatment. The patient did not attend the clinic after treatment follow-ups possibly because of his dissatisfaction.

Mr. D.N. was 64 years old and started drug with 13.5 cc/day. Because phlebitis was observed, on the day of 15, the drug dose was decreased to 6.7 cc/day and continued for remaining 13 days at this (6.7 cc/day) level. By declining the dose to its half, phlebitis disappeared. No other side effects were observed at 13.5 and then 6.7 cc/day dose levels.

In this Phase I clinical trial (without control group), all patients were treated with escalated doses of the drug which started out at 2 cc/day. The doses were easily tolerated up to level of 10 cc/day. Two cases with 13.5 cc/day showed phlebitis which gradually disappeared after decreasing the drug dose level to 6.7 cc/day. Other clinical and laboratory evaluations demonstrated no side effects up to the dosage of 10 cc/day.

The main objective in a Phase I clinical trial is to find MTD of the drug (32) with a higher probability of response without or acceptable toxicity (33). In fact, the underlying assumption of all Phase I clinical trials is that the dosage of a drug is related to probable toxic response (34). Since the dose established as the MTD will be passed for further testing in phase II clinical trials, accurate determination of the MTD is of great importance (35). Perhaps, because phase I clinical trials are generally non-randomized, do not involve large sample sizes, and are not hypothesis-driven, statistical considerations are largely ignored (34).

Like in other phase I clinical trials, our main goal was determination of the MTD and DLT. We used the common toxicity criteria scale that was published by National Cancer Institute (36) for assessment of DLT. By considering DFU as not life-threatening (not as severe as uncontrolled cancer), we chose the grade 2 of adverse events that was published by WHO (37), for definition of DLT in this study. For each adverse event, grades were assigned and defined using a scale from 0 to 5 with 0 representing no adverse events within

Table 1. Escalated dose groups and observed dose limiting toxicities of Semelil (ANGIPARS™) at phase I clinical trial

Patient's Name	Description	Initial dose (Daily)	Continued dose (Daily)	DLT
M.B.	Based on LD10	2.0 cc	2.0 cc	No
Z.M.	100% upper than baseline	4.0 cc	4.0 cc	No
M.H.	67% upper than the second level	6.7 cc	6.7 cc	No
Gh.A.	55% upper than the third level	10.0 cc	10.0 cc	No
M.H.	40% upper than the fourth level	13.5 cc	6.7 cc	Phlebitis
D.N.	40% upper than the fourth level	13.5 cc	6.7 cc	Phlebitis

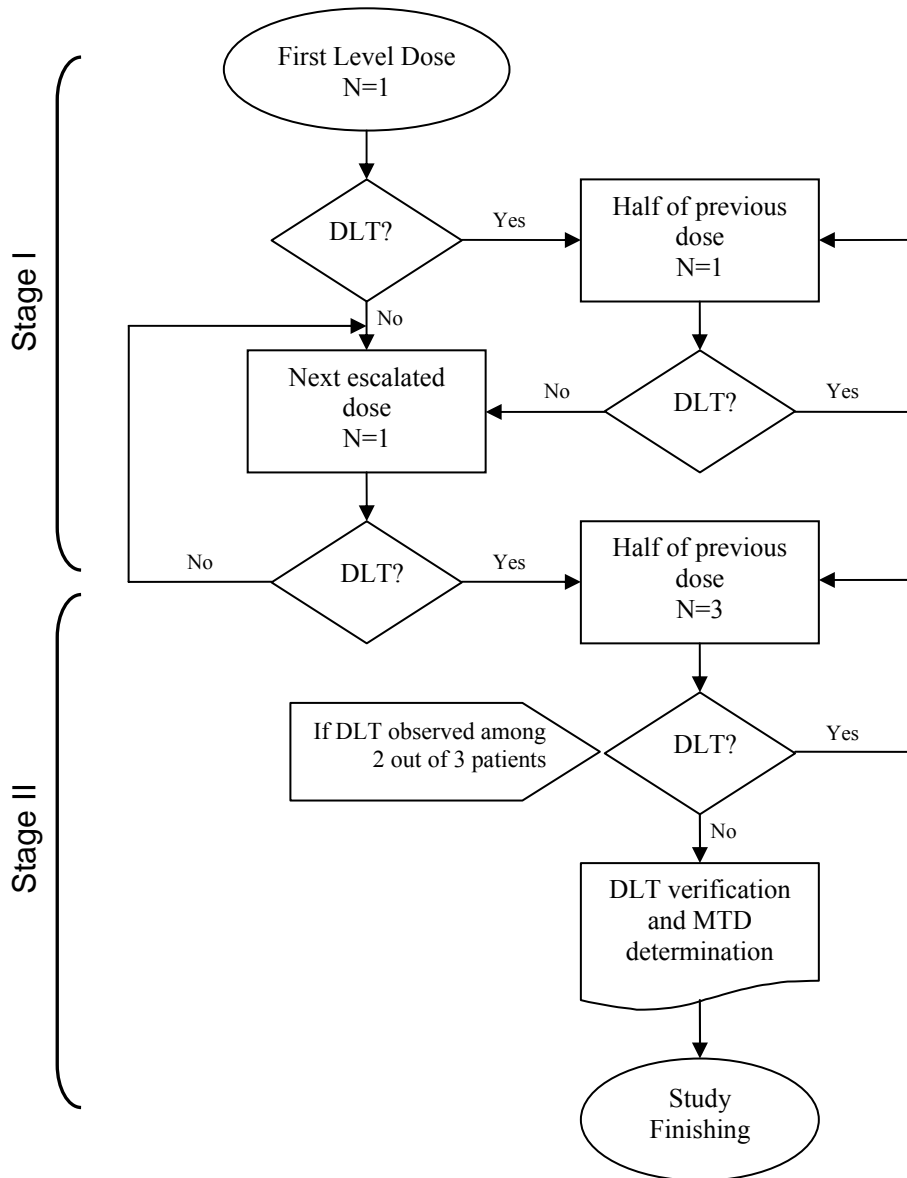


Figure 1. Study flowchart for MTD determination of ANGIPARS™
 MTD: Maximum Tolerated Dose; DLT: Dose Limiting Toxicity

normal limits and 5 representing a death related to an adverse event. Grade 2 meant moderate adverse event(s).

Our study was performed in two sections and we reported the results of the first section in this paper. At the baseline, for the first patient we used a fraction of LD₁₀ which was estimated from animal studies. When we observed DLT, we considered a half of the maximally used dose and repeated at the same level for one or two additional patients. In our study, we increased the dosage up to 10 cc/day without clinical or laboratory side effects. When we increased the daily dosage to 13.5 cc (Table 1), we observed phlebitis in our patient. Although we performed suitable practical consideration (changing the injection place) occurrence of the phlebitis continued to occur. Thus, another (the sixth) patient were treated at the same dose. After detecting phlebitis at the dose level of 13.5 cc/day in this patient, too, we were forced to use 6.7 cc as a daily dose. At this level, we did not observe

clinical or laboratory side effects. Thus, phlebitis was the only detectable side effect in our patients.

CONCLUSIONS

It is concluded that the dose of 10.0 cc/day was as Maximal Tolerated Dose (MTD) and local phlebitis was the only DLT. The i.v. dose of 4.0 cc/day of Semelil was recommended for Phase II clinical studies. Finally, ANGIPARS™ is a safe drug with without any side effects other than phlebitis and it can be recommended with high confidence for further trials in diabetic patients.

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