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Clinical Research on Diabetic Foot Ulcers: Demonstration of a Comprehensive Methodology

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Abstract

The best way to approach research on Chinese medicine would be one that takes full reference to the methodology being used in modern medicine. This would enable traditional medicine to be elevated to a level of universal acceptance. However, problems lie in difficulties to achieve uniform herb supply; randomization and placebo arrangement in clinical trials; and uncertain chemical structures and pharmacological effects. A detailed account is given on the clinical research on diabetic foot ulcers, in which a strictly evidence-based, placebo-controlled, randomized clinical trial was conducted, while parallel mechanistic studies were done in the laboratory and the herbs used were adequately authenticated at the same time. The encouraging clinical efficacy supports further studies encouraged by the revealed mechanisms of action of the herbs. Reformulation of the herbal composition could also be planned with the knowledge of their quality and mode of action.

KEYWORDS: Chinese medicine, methodology, evidence-based medicine, diabetic ulcer

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Introduction

While modern medicine has offered a lot and is still advancing at fast speed which is beyond our imagination many years ago, alternative methods of treatment are still gaining much support and popularity (Hannallah et al, 2002; Lieberman et al, 2002; Eisenberg et al, 1998). In the recent decade, clinicians and patients both adopted a realistic attitude towards alternative medicine. They wanted to know more about the true efficacy while they are using the alternative means, either as a treatment option or as supplement. Users of Traditional Chinese Medicine (TCM) in Chinese communities might have taken a much more committed attitude as they were obviously culturally influenced (Klemman, 1975, WHO 2000). However the quest for more evidence on the effectiveness of the traditional treatment, inevitably, is also mounting (Goldbeck et al, 1996; Kaptchuk, et al, 1998).

We should all critically look at TCM with the aim of revealing its efficacy. The exploration starts with the records of usefulness, then it looks at the current publications and current practices.

Targets of Concern

Modern medicine, as a matter of fact, has made tremendous achievements in the past decades, so that, only in those areas where, in spite of the advances, modern medicine is still finding difficulties, that alternative medicine (including TCM) should be considered. (Cheng, 2001)

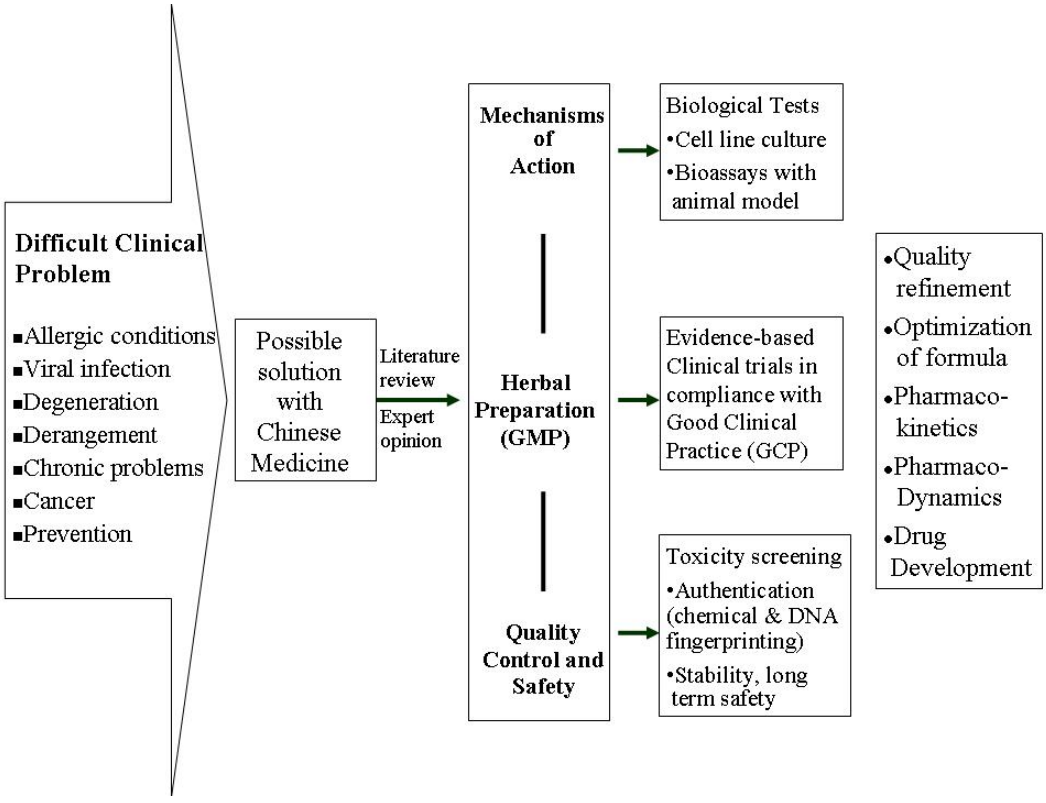
Where are the areas of difficulties in modern medicine? Modern Medicine works on a deductive, target orientated principle. Wherever, the cause of disease is clear, a variety of solution would have been worked out to specifically remove the pathology. However, when the pathology was unclear, modern medicine, did not work so well. Examples where the causative pathology is yet unclear, uncertain or too complicated include: allergy, chronic degenerative diseases, chronic pain, endocrine and muscles-skeletal derangements, and cancers. (Lai, 2002)

The research on TCM could concentrate on these areas, aiming at the reveal of alternative effective means of treatment that could supplement conventional means that were currently available.

Methodology

Research on Herbal Medicine in the past century has been focused on many aspects: from pharmacognosy, to quality control, biological tests in the laboratory, authentication and clinical efficacies. Of these approaches many resource have been spent on the identification of the active components and subsequently working out the chemical formula responsible for the efficacy, with the obvious intension of developing an effective drug. There are a few successful examples like taxol and vincristine in France and artemisinin in China. But these were limited number of successes in spite of the many attempts. It is believed that the production of chemicals of botanical origin are too complicated and extraction and identification of active principles require excessive innovative and financial input. A compromise, aiming at proving the efficacy of the raw herbal material or raw formula of herbs, before considering further sophisticated analysis is proposed.

The Efficacy Driven Approach



The Centre of research is the clinical trial which is targeting on an important clinical problem which fails to find perfect solution in modern medicine. Choosing a methodology of universal acceptance for the clinical trial would enable the results of the trial to be widely accepted, thus convincing the clinical experts on the efficacy of the treatment modality.

The clinical trial utilizes the modern principles of evidence-based medicine which include proper randomization, blinding technique and placebo control. With this fulfillment of the fundamental requirements of clinical trials, the results would gain international support.

Parallel to the clinical trial, laboratory in-vitro and in-vivo explorations are carried out to explore the biological mode of action of the herbal formula being used for the clinical trial. Using the results of the laboratory tests, the scientific basis of the clinical efficacy could be worked out.

Since herbs are well known to have varying chemical compositions with different species and different environments of cultivation, extra procedures need to be taken to identify the chemical profiles (or finger-prints) of the combined formula as well as the single herbs. This extra work is essential to allow future repetition of laboratory experiments and extensions of clinical trials.

The methodology of clinical research utilizing herbs therefore, will be centered on a trial designed on evidence based medicine, while biological tests are done in parallel to work out the mechanisms of action and further supporting work must include sophisticated tests of authentication, which establish the chemical and if necessary, the molecular DNA profiles of the individual herbs being used.

An Example – Treating Chronic Unhealing Diabetic Foot Ulcers with a Herbal Formula

As the incidence of diabetes mellitus was obviously increasing all over the world in the recent decades, those suffering from diabetes were under the constant threat of ulcer formation. Ulcer formation in the diabetic patient happened as a result of inadequate arterial flow, peripheral neuropathy and high susceptibility to infection. (Reiber, 1996; Reiber et al, 1999; US Department of Health and Human Services, 1998) For the same reasons, once an ulcer developed in the diabetic patient, healing was difficult and chronicity commonly resulted. The concern with regard to chronic, unhealing foot ulcers among the diabetics was justified because they formed a high risk factor for amputation. (Mayfiled et at, 1996; Reiber et al, 1992; Chen et al 2002)

Treatment for chronic diabetic foot ulcers included the standard local care, different types of wound dressing, debridement of necrotic tissues while the state of diabetes was effectively controlled with hypoglycaemic agents, and infection was treated with antibiotics. (Steed et al, 1996; Kantor et al, 2000) Such routine treatment, however, was not always successful. Some randomized clinical studies indicated that not more than 30% of those receiving standard treatment succeeded to heal in 20 weeks. (Margolis et al, 2000) The unhealing ulcers were further threatened by unusual infections, notably necrotizing fasciitis which was really life-threatening.(Kovaco, 2001) The unhealing nature of these chronic ulcers and the genuine threat of spreading fatal infection encouraged the orthopaedic surgeon to be more radical with the decision of amputation. Even with the introduction of new wound healing agents, like the local use of growth factors and artificial skins, (Bennett et al, 2003; Gentzkow, 1996) no real change in the prospect of ulcer healing was observed. In the United States of America, the overall rate of leg amputation among diabetic patients was 4.1 per 1000 persons. (US Department of Health and Human Services 2000)

In the orthopaedic specialty, patients with chronic diabetic foot ulcers were refered from other specialties because of uncontrolled infections, gangrene of some toes, or, when the attending physician was quite certain that the chronic ulcer would not heal. The orthopaedic surgeon should have the option of doing only limited surgery like amputating the toe, fore-foot or mid-foot. However, the frequently occurring poor vascular and neuropathic states usually led to poor stump healing which required repeated surgical operations.(Taylor et al, 1987) Such unpleasant experience prompted surgeons to choose amputation below the knee level as a standard practice, so that secondary surgical procedures could be avoided. However, we have to be aware that the diabetic patients suffering from chronic ulcers were most of the time, old and flail, so that healing after amputation could still be a problem and, moreover, the artificial limb would not serve them well.

Attempts trying to rescue the affecting limbs from amputation, should therefore be justified. Indeed, Chinese physicians in Shanghai used traditional herbal formulae to treat chronic diabetic foot ulcers and reported good results of ulcer healing. (Xi, 1996)

The Study

The study consisted of three major sections: a clinical trial, biological tests and authentication studies.

I. Clinical Trial

The study was conducted in two orthopaedic units of two different hospitals. The patients selected from the orthopaedic wards were all suffering from extensive ulcerations, complicated with infection, or gangrenous parts. Amputation was already the recommended treatment from the orthopaedic teams. The study protocol was properly screened and approved by the Clinical Research Ethics Committees of the Chinese University of Hong Kong. All patients were required to give written informed consent before registration.

Objective of Study

The objective of the study was to see whether simple wound care together with standard diabetic and infection control, supplemented by a herbal drink, could promote ulcer healing in the diabetic patients, in an attempt to avoid major amputation.

Design of Study

The study was a double-blinded, randomized placebo-controlled trial. Basing on the reported result of 85% successful limb salvage, a total of 80 patients formed the target of study. Safety of the herbal formula being used was ascertained through a thorough literature search to exclude poisonous items in the formula, and a practical laboratory screening of the preparation to rule out toxic contaminants including heavy metals, insecticides, and biological agents, as were required by the guidelines for the production of herbal health products set by the Department of Health in Hong Kong.

Outcome Measures

The primary outcome measure was ulcer healing with no major amputation. Secondary outcomes included skin temperature, surface oxygen consumption, quality of life measures and liver/renal functions. (to rule out toxicity)

Methodology

Recruitment started with hospital admission. With the exception of life-threatening sepsis, which would require emergency amputation, consent was obtained from the patients on recruitment, subject to standard randomization.

Treatment consisted of standard diabetic control measures and standard courses of antibiotic treatment as required. Blood tests for renal and liver functions were routinely done. Only those with normal function qualified for recruitment. Either the herbal drink or placebo would be given as treatment options. Treatment continued for three to four weeks while close monitoring on the ulcer size and ulcer bed went on. If three to four weeks' blind treatment, either using the herbal formula or placebo, did not bring obvious improvement, but instead, deterioration, the patient would be given the herbal option irrespective of whether he/she belonged to the placebo group originally. No unblinding was necessary at this stage of conversion to open treatment.

Other routine treatment consisted of: daily local wound care, i.e. antiseptic bathing, cleaning and dressing; weekly or biweekly debridement of necrotic tissues; limited removal of gangrenous toes and soft tissues; and daily administration of the herbal drink or placebo. Wound assessments were done carefully until, in the worst situation, uncontrolled wound deterioration led to major amputation, or, either spontaneous wound healing occurred, or, in most circumstances the ulcer bed became so healthy that split thickness skin graft could be applied under local anesthesia to facilitate healing. Treatment continued for a maximum of 24 weeks.

Other assessment parameters included the following:- surface oxygen tension[*], skin temperature[†], and Neuropathic status using the 10gm Weinsteim Monofilament test.

[*] Surface Oxygen tension was measured with a Transcutaneous Blood Gas Monitor (Microgas 7650-500) Linde, Switzerland

[†] Skin temperature was measured with a digital thermometer with probe (model no. 15-077-8B) Fisher Scientific, USA.

The Herbal Formula

The herbal formula was modified from a popular classic preparation consisting of 12 herbs used traditionally for the maintenance of health among patients under the threat of chronic infection or debilitating diseases. The herbs were well documented to be non-toxic and the use of individual ones had been thoroughly studied for their safety. A qualified laboratory[‡] made the herbal preparation into user-friendly granules ready for consumption by mixing with warm water. The different herbs used were listed in the Table 1. Before the recruitment of patients, the preparation went through standard laboratory procedures to rule out toxicity for human consumption, according to the strict regulations on medicinal herbal preparations set by the Department of Health of Hong Kong. (Department of Health Hong Kong, 2004)

Statistic Analysis

1. Demographic data
   Baseline characteristics of the two groups were all included. For continuous variables, means were compared using analysis of variance. Categorical variables were compared using the X^2 test.
2. Maturation of granulation tissues (duration before end stage skin grafting)
   A Kaplan-Meier plot was used to examine the duration in each group. A log-rank test was used to compare different groups.
3. Oxygen tension and skin temperature
   Changes from baseline were assessed within the treatment groups by using the pared t-test. Comparison with the placebo group was conducted by using ANCOVA wit baseline as a covariate.

[‡] Hong Kong Institute of Biotechnology Ltd. Biotechnoogy Avenue, 12 Miles, Tai Po Road. Shatin N.T. Hong Kong

Table 1. List of Herbs contained in the herbal formula:-

	Name in Chinese Medicine	Description of used parts and botanical origins	Raw herb proportions
1	*Radix Astragali*	Dried root of Astragalus membranaceus (Fisch.) Bge. In the family Leguminosae	40g
2	*Radix Rehmanniae*	Dried rhizome dervied from Rehmannia glutinosa Libosch. In the family Scrophulariaceae	21g
3	*Rhizoma Atractylodis Macrocephalae*	Dried rhizome of Atractylodes macrocephala Koidz. In the amiliy Compositae	9g
4	*Radix Stephaniae Tetrandrae*	Dried root tuber of Stephania tetrandra S. Moore in the family Menispermaceae	9g
5	*Radix Polygoni Multiflori Preparata*	Dried root tuber of Polygonum multiflorum Thunb. In the family Polygonaceae	9g
6	*Rhizoma Smilacis Chinensis*	Dried rhizome of Smilax china L. in the family Smilacaceae	9g
7	*Poria*	Dried sclerotium of the fungus, Poria cocos (Schw.) Wolf in the family Polyporaceae	6g
8	*Rhizoma Dioscoreae*	Dried fruit of Dioscorea opposita Thunb. In the family Dioscoreaceae	9g
9	*Fructus Schisandrae Chinensis*	Dried fruit of Schisandra chinensis (Turcz.) Barll. In the family Dioscoreaceae	6g
10	*Cortex Moutan*	Dried root bark of Paeonia suffruticosa Andr. In the faimly Ranunculaceae	6g
11	*Fructus Corni*	Dried ripe sarcocarp of Cornus officinalis Sieb. Et Zucc. In family Cornaceae	9g
12	*Rhizoma Alismatis*	Dried tuber of Alisma orientalis (Sam.) Juzep. In the family Alismataceae	6g

Results

Patients

From January 2002 to April 2006, eighty diabetic patients admitted into the orthopaedic units because of unhealing foot ulcers satisfied the selection criteria and were recruited into the study. Expert orthopaedic judgement advised major amputation at below-knee level because of the chronic nature of the ulcers, together with obvious infection in most of these diabetic patients. 86% of the recruited patients were above 65 years of age.

The patients gave full consent to the clinical trial of ulcer healing using a herbal formula in an attempt to limb salvage, and were randomized into the herbal treatment group and placebo group. The demographic data of the two groups and their clinical conditions were listed under (Table 2).

The chronic ulcers were mainly located in the toes, dorsum, the heel and the sole of the foot (Table 3). 80% of the feet were having gangrene of either the ulcerated part or adjacent toe(s).

Table 2. Baseline Demographic characteristics of 80 patients treated with Herbal Treatment or Placebo.

	Herbal Treatment	Placebo	Herbal vs. Placebo
No. of patients	40	40	
Sex			
Male	25/40	22/40	0.496
Female	15/40	18/40	
Age (years)	66.3±12.6	68.5±11.1	0.408
Range	40 - 85	49 - 86	
Diabetes, duration (years)	8.4±7.6	12.4±8.8	0.034
Ulcer duration (week)	7.8±8.2	12.9±24.6	0.296
Ulceration size (cm^2)	28.7±31.3	26.7±27.3	0.789
Diabetes types			
Type I	5/40(13%)	9/39(23%)	0.218
Type II	35/40(88%)	30/39(77%)	
Diabetes current medication			
Oral hypoglycemic	28/40(70%)	26/40(65%)	0.891
Insulin injection	7/40(18%)	8/40(20%)	

Table 2, continued.

Diet control	5/40(13%)	6/40(15%)	
Diabetes control			
Good (Blood check steady)	19/37(51%)	17/35(49%)	
Fair (Blood check occasional fluctuate)	14/37(38%)	17/35(49%)	0.321
Poor (Blood check fluctuate)	3/37(8%)	1/35(3%)	
Smoking			
Smoking experience (3 cigarettes per week)	13/40(33%)	16/40(40%)	0.485
Non-smoker	27/40(68%)	24/40(60%)	
Ulcer bed			
Infected with slough	28/35(80.0%)	30/36(83.3%)	
Edematous with patchy	6/35(17.1%)	0	0.171
Relatively clean	1/35(2.9%)	2/36(5.6%)	
Gangrenous tissue			
Dry	12/38(32%)	8/31(26%)	
Wet	19/38(50%)	12/31(39%)	
None	7/38(18%)	11/31(35%)	
Nutrition state			
Body weight (kg)	59.1±12.3	61.2±12.3	0.601
Serum albumin(g/L)	317±45	322±42	0.647

Table 3. Ulceration sites

Ulceration site	Herbal Treatment	Placebo	P value
Toes	23/37 (62%)	24/35 (69%)	
Foot dorsum	8/37 (22%)	4/35 (11%)	
Heel	2/37 (5%)	1/35 (3%)	0.730
Sole	2/37 (5%)	3/35 (9%)	
other	2/37 (5%)	3/35 (9%)	

There were no differences in the distribution of ulceration site between the two groups (p=0.730).

Clinical Outcome

Early Deterioration and Amputation

All patients were eager to retain their ulcerated limb but the outcome was hinged on the clinical behaviour of the ulcers. If rapid deterioration to the extent of life-threatening level was observed, amputation had to be done. When there was no progress in the first 4 weeks, and yet the deterioration was not bad enough to justify amputation, the treatment was shifted over to herbal supplement without unblinding, so as to offer the best opportunity to limb salvage until either improvement or sacrifice could be properly determined.

There were 6 patients amputated (3 herbal, 3 placebo) because of rapid deterioration. Another 18 patients (6 herbal, 12 placebo) were found no progress in first 4 weeks and thus they were put directly under herbal treatment. The difference between the two groups was reaching statistical significance (p=0.048). When continual open-treatment failed to give obvious clinical indications of ulcer improvement, or, if necrosis/gangrene further developed, or, if infection spread out of control, limb salvage management had to be stopped and amputation performed. 12 of the 18 open-treatment patients were rescued (herbal 6/6, placebo 6/12). Summarized in Table 4, altogether 12 amputations (3 herbal, 9 herbal) were done for the two groups within the 24 weeks treatment period.

Table 4 Outcome of Cases with Poor Responses

	Amputation because of rapid deterioration in First 4 weeks	No improvement in First 4 weeks	Shift to open Herbal Treatment	Limb salvaged after Herbal Treatment	Amputation in spite of Herbal Treatment	Total no. of Amputation
Herbal Treatment	3	6		6	0	3
Placebo	3	12		6	6	9
Total	6	18		12	6	12

Healing Parameters

When there was no threat to limb survival, local management and herbal/placebo treatment continued. Local management consisted of repeated removal of necrotic tissues and toe amputations when gangrene occurred. Raw areas were not sutured after limited amputations. When the granulation tissues appeared clean and healthy, split skin grafting was taken from the patients' own leg under local anaesthesia, and applied to the ulcer. Perfect skin graft taking was the rule. The duration of herbal/placebo treatment, therefore, represented the time taken for the granulation to mature before the final stage of skin grafting. (Figure 1)

Figure 1 Diabetic foot ulceration before and after herbal treatment

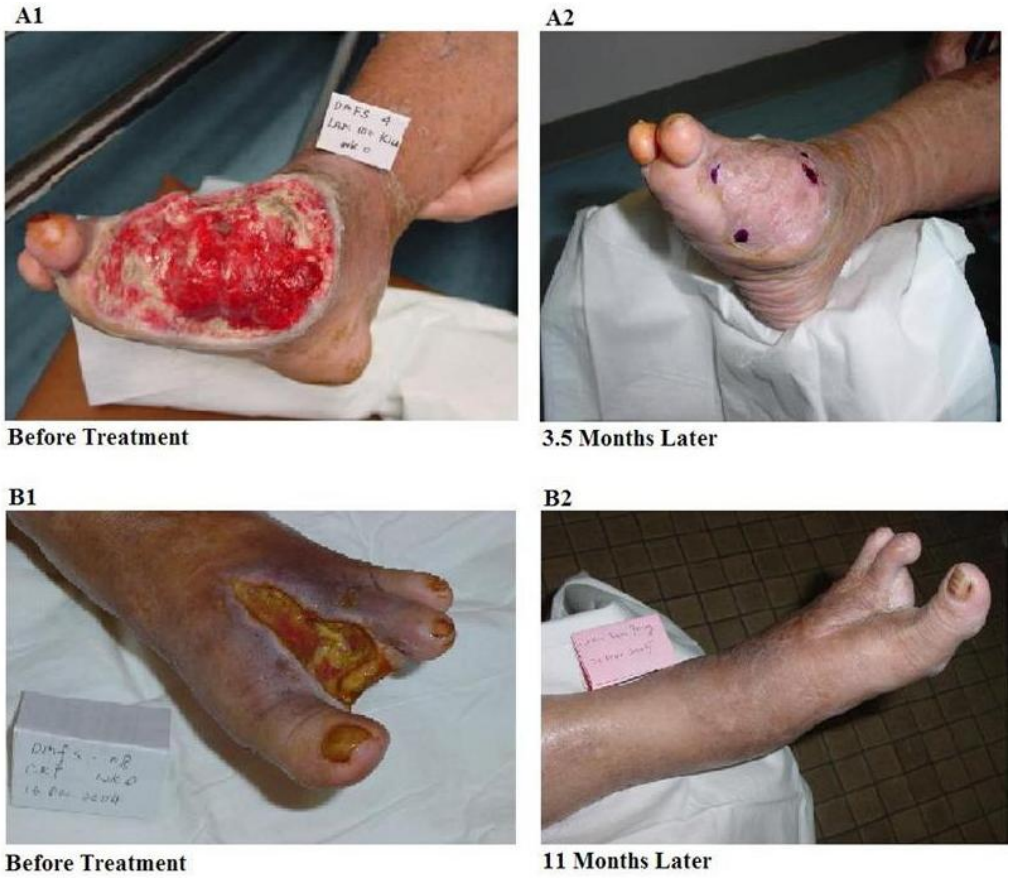


Table 5 gives the duration taken by the two different groups for granulation maturation. Among patients healing within the 24-week period, the mean time to healing was shorter in patients treated with herbal treatment compared with those treated with placebo. (5.91±1.36 vs. 9.15±1.90)

Table 5 Time taken for granulation maturation (before skin grafting)

Group	Healing time (week)
Herbal Treatment(n=32)	
Mean	5.91±1.36
Median	3.43
Placebo(n=32)	
Mean	9.15±1.90 (↑76%)
Median	6.86 (↑100%)
P value	0.147

Local Circulation State

Diabetic patients with foot ulcers admitted into hospital for treatment gradually showed improved circulatory state in the affected limb, due to the control of infection, good ulcer care and bed rest. It was important to critically look at the possible facilitating effects of the herbal treatment by the use of objective measurements.

A. Clinical State of Ischaemia

The state of ischaemia was classified into 4 grades (grade A: no ischaemia, B: clinical ischemic, C: toe gangrene and D: forefoot gangrene) and the results after 2 and 4 weeks of treatment are put under Table 6.

Table 6 State of Ischaemic

	Herbal Treatment	Placebo	P value
Ischaemca			
Before treatment			
A	6/37 (16%)	4/35 (8%)	0.142
B	4/37 (11%)	10/35 (31%)	
C	27/37 (73%)	20/35 (58%)	
D	0/37	1/35 (4%)	
Week 2			
A	6/19 (32%)	6/22 (27%)	0.290
B	9/19 (47%)	6/22 (27%)	
C	4/19 (21%)	9/22 (41%)	
D	0/19	1/22 (5%)	
Week 4			
A	10/15 (67%)	5/12 (42%)	0.084
B	1/15 (7%)	5/12 (42%)	
C	4/15 (27%)	2/12 (17%)	
D	0/15	0/12	
P value (baseline vs. week 2)	0.000	0.440	
P value (baseline vs. week 4)	0.002	0.036	

A=no ischaemic; B=ischaemic without gangrene; C: toe gangrene D: forefoot gangrene.

B. Other Parameters of Local Circulatory State

The surface O_2 tension changes around the foot ulcer before and 4 weeks after treatment were entered in Table 7. Skin temperature changes were represented in Table 8.

Table 7. TcPO2 2cm proximal to ulcer

Group	Week 0	Week 4	P value (week0 vs. week4)
Herbal Treatment			
Mean	7.02	8.28	0.005
N	38	20	
SD	5.42	3.55	
Placebo			
Mean	5.86	6.93	0.760
N	37	18	
SD	3.70	3.48	
P value	0.280	0.051	

Table 8. Skin Temperature 10cm above ulcer

Group	Week 0	Week 24	P value (week0 vs. week24)
Herbal Treatment			
Mean	30.1	30.6	0.030
N	37	18	
SD	2.2	2.3	
Placebo			
Mean	29.9	30.4	0.362
N	36	16	
SD	2.5	2.6	
P value	0.748	0.677	

C. Cytokine Study

Serum cytokine could be studied to realize the state of inflammatory responses. Tumor necrotizing factor TNF α was taken as an effective marker indicating the active state of inflammation. A decline of TNF α level indicated smoothening down of inflammatory processes which was an indicator of good healing. Patients treated with herbal treatment showed a significant gradual decline of TNF α when compared with placebo (Table 9).

Table 9. Change of TNF-α after at each visit of 80 patients

Visit	Week 0 (Baseline)	Week 2	Week 4	% change at Week 4
Herbal Treatment	48.42± 116.34	35.60± 75.95	28.25± 61.83	-41.7
Placebo	43.86± 83.35	40.59± 68.46	39.46± 63.82	-10.0
p value	0.841	0.433	0.703	

Comparing the two groups in percentage change from baseline to week 4 by paired-t test, p-value is 0.037.

Discussion

As the type II diabetic patients are increasing and they are getting more and more prone to develop foot ulcers, which as a rule, turn chronic and fail to heal in spite of conventional treatment, patients commonly turn to alternative, unconventional methods of treatment. New, innovative agents are already available, e.g. the use of growth factors, stem cells, and synthetic material for re-epithelialisation. (Huang et al, 2005, Margolis, 2005) However, such measures are still under early trials. Moreover, diabetic chronic foot ulcers have very complicated background which might not be redeemable with means of epithelialisation alone. Other alternative methods including traditional remedies like local applications or systemic consumption of various herbal preparations might deserve special consideration.

In the first 4 weeks of blinded management, some ulcers deteriorated rapidly in spite of treatment, while others gradually cleaned up and started to show granulations. 18 patients were thus detected as not responding to treatment

and were transferred to the herbal treatment group (now with open label) to make sure that the best offer was given. 12 of the 18 patients thus transferred for open-label treatment were from the placebo group. Once changed to herbal treatment, 6 patients showed gradual improvement and limb salvage was subsequently successful. 6 others did not improve and later received below-knee amputation. In the large group of patients who apparently responded satisfactorily, 6 did not do well and eventually required a major amputation.

The observations indicated that :-

i) Some chronic foot ulcers in the Diabetic patients were resistant to both conventional treatment and special measures to stimulate granulation growth and eventually required major amputations.
ii) Some chronic foot ulcers in the Diabetic patients, on the other hand, responded well to the herbal consumption which stimulated granulation growth and later, ulcer healing.
iii) When placebo cast no effects on granulation growth, shifting to herbal treatment reversed the position in 50% of cases.

When the overall data were analysed and the two groups of patients receiving different forms of treatment compared, with regard to the secondary endpoints, the following observations were made:-

1) 12 failures ending in amputations were found: all of these patients whether initially belonging to the herbal group or placebo group, were observed in the first 3-4 weeks of treatment to be poor respondents. The initial treatment, therefore did not bring much improvement among the poor respondents of either group.
2) The secondary endpoints, might be more useful to illustrate the efficacy of the herbal treatment. The herbal group was found to be more superior to the placebo group ($p=<0.05$) in the following parameters:-
   a) Treatment time taken before granulation was ready for skin grafting,
   b) Speed of maturation of granulation tissues,
   c) Clinical improvement of local vascular state (ischaemia),
   d) O_2 tension around the ulcer, and
   e) Skin temperature around the ulcer.
3) A more significant progressive decline of TNF α was observed in the herbal group compared with the placebo group.

4) As was illustrated by the clinical well-being, liver and renal function tests, adverse effects related to the herbal treatment were not significant.

II. Biological Test

In-vitro Study

CRL-7522 fibroblast cell line was used to model the granulation enhancing activities of the herbal formula. Results showed that the formula and four of its component herbs, viz Radix astragali, Radix Rehmanniae, Rhizoma Alismatis and Rhizoma Atractylodis significantly enhanced the cell viability. (Lau et al, 2007)

In-vivo Study

A foot ulcer model in a diabetic rat was developed with the aim of using it for the evaluation of the efficacy of the herbal formula. Digital photographs of the ulcers, under treatment with the herbal formula or plain water were serially taken. A specially designed image analytical program was utilized to objectively read out the ulcer area changes. Results showed that the average wound area after 7 days of treatment with the herbal formula was 11.45mm^2, which was significantly smaller than the control, which was 15.12mm^2 ($p=0.04$).(Lau et al, forthcoming)

III. Authentication

Chinese Medicine is monitored by using a few characteristic chemical markers which could be active ingredients. These chemical markers are usually taken as reference standards in chromatographic analysis such as HPLC (high performance liquid chromatograph) and GC-MS (gas chromatography-mass spectrometry) which are instruments widely employed for quality control of Chinese Medicine. Thin layer chromatography (TLC) with less chemical information obtained is another technique utilized in most Chinese Medicine pharmacopoeias and monographs. However, more and more evidence shows that the therapeutic effects of Chinese Medicine can rarely by attributed to one or two active components alone and more markers or chemical information are desirable.

The authentication part of the herbal formula used for the treatment of chronic diabetic ulcer started with the identification of the Standard HPLC profile of the whole formula and its components, followed by a more sophisticated search for other active components within the formula using special chemometric techniques.(Gong et al, 2001)

Twelve compounds with known pharmacological activities are identified through this method. They included benzamide, butylated hydroxy toluene, apocynine, azabicylo, benzenedicarbonylic acid, tetradecanoic acid, dimethoxy naphthalene, naphthaquinone, octadecadienol, octadecynoic acid, schizandrin and gomisin A.

One other essential step towards the comprehensive approach on Chinese Medicine is the provision of voucher specimens of the individual herbs for archive keeping after full morphological authentication. The voucher specimens provide a good basis for both further laboratory research and possible drug development. Both future research and drug development would demand an acquisition of the identical herbs.

Conclusion

The example given on the clinical trial of chronic diabetic ulcers succeeded to confirm the efficacy of a herbal formula on wound healing. Although other causative factors were obviously preventing perfect efficacy, our clinical observations showed clearly the facilitating effects on granulation formation and improvement of local circulation. The clinical observations were supported by in-vitro experiments of fibroblasts culture, and in-vivo tests on and diabetic rats with induced foot ulcers. Further studies on the use of the herbal formula for other types of chronic ulcers are indicated. Further extractions to identify the active components responsible for the promotion of healing would be equally justified. The parallel study on authentication of the herbal formula has laid down useful ground work towards the optimization of the herbal formula.

The comprehensive methodology adopted for the study of herbal medicine might be criticized for being over-ambitious and expensive, as much as not sufficiently focused. Our early experience, as exemplified by the diabetic foot ulcer study, however, has demonstrated that this approach was practical and provided an environment of mutual support between the three groups of scientists. The clinicians, while sticking to the strict requirements of evidence based medicine to test the efficacy of the herbal preparation, were particularly comfortable because they would be able to give explanations to the mechanisms of action. The authentication group on the other hand, facilitated the optimization of the herbal formula. The non-clinical scientists, as a whole, were made aware of the important targets to be addressed and the particular emphasis that would facilitate their future pursue.

While remaining optimistic about our comprehensive endeavor, we are aware of the need to subject the methodology for further tests in other relevant areas of concern.

The Recommendations on the line of approach for Research on Chinese Medicine are therefore summarised as follows:-

i) Identify specific clinical problems in spite of past efforts, which have not found satisfactory solutions.
ii) Work out a practical option for the resolution of the clinical problem by creating a herbal formula for the treatment trial.
iii) Maintain a Quality supply of herbal preparation for the clinical trial,
iv) Work out a clinical protocol built on Evidence based Medicine (EBM) and Good Clinical Practice (GCP).
v) Design a series of laboratory experiments to go parallel with the clinical trial, to understand the mode of action of the herbal preparation through Cell Cultures, Bioassays and to authenticate the herbs being used, so that proper finger prints and quality control of the herbal product could be maintained.

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