Vascular Response of Raynaud's Phenomenon to Nifedipine or Herbal Medication (Duhuo-Tisheng Tang with Danggui-Sini Tang): A Preliminary Study

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Background: Raynaud's phenomenon (RP) is a common manifestation in connective tissue diseases. Calcium channel antagonists are most effective and frequently used for treating RP. This study compared the efficacy, digital vascular response, and tolerability between nifedipine and a combination of 2 Chinese herbal medications (Duhuo-Tisheng Tang and Danggui- Sini Tang) for treating RP.

Methods: This open-label non-randomized clinical trial included 47 connective tissue disease patients with RP. The herbal group and the nifedipine group included 26 and 21 patients, respectively. The duration of therapy was 4 weeks. Baseline and posttreatment laser Doppler blood flow imaging of both hands were performed at room temperature and after cold challenge. Nailfold capillary microscopy was performed at the baseline and after 4 weeks of therapy. Serum levels of soluble intercellular adhesion molecule-1 (sICAM-1), prostaglandin E_2 (PGE_2), nitrite (NO_2), and nitrate (NO_3), and plasma levels of endothelin-1 (ET-1) were also measured. Self-reported symptoms, using a visual analog scale (VAS) and a physician global assessment (PGA), were recorded at the baseline and after treatment.

Results: After 4 weeks of treatment, VAS scores improved ($p = 0.0035$) and the physician's global assessment of RP severity decreased in the nifedipine group ($p = 0.0078$) but not in the herbal group. Episodes of RP attacks decreased in the nifedipine group after treatment ($p = 0.008$). The nifedipine group had increased laser Doppler flow (116.3 ± 70.7 AU) compared to the baseline (72.4 ± 49.0 AU, $p = 0.0008$). Laser Doppler images improved at various time points after cold challenge in the nifedipine group after therapy. Laser Doppler flow in the herbal group did not significantly change with therapy. Capillary microscopy demonstrated no significant difference in enlargements, avascularity, or hemorrhagic spots between groups. Serum NO_2 concentrations were higher in the nifedipine group than in the herbal group. Levels of sICAM-1, PGE_2, NO_3, and ET-1 after therapy were similar to those at the baseline in both groups.

Conclusions: The digital vascular response in RP improved with nifedipine but was unchanged with a combination of the Chinese medicines Duhuo-Tisheng Tang and Danggui-Sini Tang.

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Key words: Raynaud's phenomenon, laser Doppler flow, NO_2, herbal medicine, nifedipine

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Raynaud's phenomenon (RP) was first described by Maurice Raynaud in 1862. This microcirculatory disorder is characterized by triphasic color changes in pallor, cyanosis, and hyperemia, often involving the extremities. The condition is generally mild but may cause pitting scars, painful ulcers, gangrene, loss of digits, and refractory ischemia.

The prevalence of RP is approximately 3%~5%.[1] Raynaud's phenomenon can be classified as either primary or secondary. Primary RP is defined as at least 2 years of symptoms with no apparent associated disease. Secondary RP is associated with connective tissue diseases such as progressive systemic sclerosis (PSS), systemic lupus erythematosus (SLE), polymyositis, dermatomyositis (DM), mixed connective tissue disease (MCTD), rheumatoid arthritis (RA), or overlapping syndrome. Secondary RP reportedly occurs in more than 90% of scleroderma, 10%~57% of SLE, 33% of primary Sjogren syndrome, 20% of dermatomyositis or polymyositis, and 10%~20% of rheumatoid arthritis.[2-4]

Symptoms of RP are frequently exacerbated by emotional stress and/or cold temperature. The pathogenesis of this microcirculatory disorder is not fully understood. Possible mechanisms include dysregulated neuroendothelial control of vascular tone and derangement of the endothelium.[5] Abnormalities in the vascular wall (including the endothelium and smooth muscle) and circulating mediators may impair blood flow and/or cause endothelial injury.[6] Evaluation of blood flow is a useful tool for the early diagnosis and follow-up of RP. Lau *et al*. found that steady blood flow during warming-cooling-rewarming cycles were significantly lower in RP secondary to PSS than in normal subjects, and the vasodilatation response during rewarming was slower.[7] In recent years, laser Doppler perfusion imaging of the digits has been employed to objectively measure RP and may be useful in evaluating therapeutic effects of medical treatment.[8,9] Nailfold capillary microscopy, another vascular evaluation tool, may aid the diagnosis and follow-up of RP patients.

Currently, the most effective and widely used therapies for RP in outpatient settings are calcium channel antagonists such as nifedipine.[10,11] However, some patients may be intolerant to such therapy due to possible side effects including palpitations, headaches, dizziness, facial flushing, or peripheral edema.[12] In Taiwan, many patients turn to traditional Chinese medicine believing that herbal medicines have fewer or no side effects compared with Western medicines. Duhuo-Tisheng Tang[13-15] and Danggui-Sini Tang[16,17] are known to increase cardiovascular and peripheral circulation and are often prescribed to treat RP. However, studies of Chinese herbal medicines in the English medical literature are limited. This study investigated the therapeutic efficacy and tolerability of Duhuo-Tisheng Tang and Danggui-Sini Tang for RP in comparison with nifedipine. Laser Doppler flow imaging with cold challenge, nailfold capillary microscopy, and serum or plasma levels of vascular markers of soluble intercellular adhesion molecule 1 (sICAM-1), prostaglandin E_2 (PGE_2), nitrite (NO_2), nitrate (NO_3), and endothelin-1 (ET-1) were evaluated to elucidate the vascular responses to these traditional therapies.

METHODS

Fifty-three RP patients were recruited from the Rheumatology Clinic at Chang Gung Memorial Hospital (CGMH), Linko medical center, Taiwan. All patients were diagnosed with secondary RP. Six patients withdrew from the study due to drug side effects (3 in the nifedipine group and 3 in the herbal group). Of 47 patients completing the study, 19 had SLE, 11 had PSS, 10 had undifferentiated connective tissue disease (UCTD), 3 had MCTD, 2 had Sjogren's syndrome (SS), 1 had DM, and 1 had RA. Table 1 summarizes the clinical characteristics of the 47 study patients. A diagnosis of RP was based on a history of episodic digital vasospasms with triphasic color changes. To be included in the study, patients had to have an average of 6 or more episodes per week. Exclusion criteria were as follows: digital gangrene, severe finger contractures, pregnant or using inadequate contraception methods, currently taking angiotensin-converting enzyme (ACE) inhibitors, and a history of significant cardiorespiratory disease, liver or renal impairment, or noncompliance.

This study received approval from the Institutional Review Board (IRB) of CGMH, and informed consent was obtained from all subjects. The open-label design of this nonrandomized clinical trial attempted to compare the effects of nifedipine with those of a combination of 2 herbal compound mixtures (Duhuo-Tisheng Tang and Danggui-Sini

Table 1. Comparisons of Demographic Characteristics and Baseline Laboratory Parameters between the Nifedipine and Herbal Groups

	Nifedipine (n = 21)	Herbal (n = 26)	*p*
Gender, Female	19 (90.5%)	25 (96.2%)	0.5791‡
Age (yr)	42.2 ± 12.0	38.6 ± 11.7	0.3038§
Smoking, Yes	2 (9.5%)	3 (11.5%)	1.000‡
RP duration (yr)	7.3 ± 6.8	6.7 ± 6.0	0.7186II
Disease type			0.3359‡
UCTD	5 (23.8%)	5 (19.2%)	
SLE	6 (28.6%)	13 (50.0%)	
Scleroderma	5 (23.8%)	6 (23.1%)	
Others	5 (23.8%)*	2 (7.7%)†	
Creatinine (mg/dl)	0.7 ± 0.12	0.7 ± 0.2	0.8690II
ALT (mg/dl)	26.9 ± 38.4	18.0 ± 6.4	0.8063II
Triglyceride (mg/dl)	71.3 ± 28.2	96.6 ± 56.2	0.2169II
Cholesterol (mg/dl)	175.1 ± 34.8	162.8 ± 31.0	0.1327II
sICAM-1 (ng/ml)	365.9 ± 185.4	423.6 ± 189.9	0.3097II
ET-1 (pg/ml)	1.2 ± 0.7	1.7 ± 0.9	0.1130II
PGE_2 (pg/ml)	371.4 ± 211.5	438.4 ± 335.8	0.3513II
NO_3 (μM/ml)	62.6 ± 49.2	66.6 ± 40.9	0.2328II
NO_2 (μM/ml)	0.5 ± 0.3	0.5 ± 0.2	0.2411II

Abbreviations: UCTD: undifferentiated connective tissue disease; SLE: systemic lupus erythematosus; RP: Raynaud's phenomenon; ALT: alanine transferase; sICAM-1: soluble intercellular adhesion molecule-1; ET-1: endothelin-1; PGE_2: prostaglandin E_2; NO_3: nitrate; NO_2: nitrite; *: One with mixed connective tissue disease, 1 with dermatomyositis, 1 with rheumatoid arthritis, 2 with Sjogren's Syndrome; †: Two with mixed connective tissue disease; ‡: By Fisher's exact test; §: By unpaired t-test; II: By the Wilcoxon rank-sum test.

Tang) on the vascular response in RP after 4 weeks of therapy. As the herbal medications are powders with distinct odors, performing a double-blind study was difficult. Each patient was given the choice of being in the herbal or nifedipine group and entered the study group of his or her preference. The study was conducted between the months of November and March 2001. Patients were required to refrain from use of vasodilators, anticoagulants, or antiplatelet agents for at least 2 weeks before the start of the study. After a baseline assessment, study subjects were enrolled in either the herbal or nifedipine group for a 4-week treatment. Fasting samples for creatinine, alanine aminotransferase (ALT), sICAM-1, PGE_2, NO_2, NO_3, and ET-1 were drawn before and after 4 weeks of therapy. Triglyceride and cholesterol levels were measured at the baseline. Nifedipine was administered at 10 mg t.i.d. (Hexal Pharma, Germany). The herbal group was administered a combination of Duhuo-Tisheng Tang and Danggui- Sini Tang each at 2.5 g q.i.d. The herbal compound mixtures were obtained from Suenn Tian Tarng Chinese Herbal Company, a Good Medical Practice (GMP) pharmaceutical company (Taiwan). Duhuo-Tisheng Tang extracts in powdered form were prepared from the following raw herbs: pubescent angelica root (Radix Angelica Pubescentis), Loranthus mulberry mistletoe (Ramulus Loranthi), Eucommia bark (Cortex Eucommia), Achyranthes root (Radix Achyranthis Bidentatae), wild ginger (Herba Asari), large-leaf gentian root (Radix Gentianae Macrophyllae), tuckahoe (Poria), cinnamon bark (Cortex Cinnamoni), Ledebouriella root (Radix Ledebouriellae), Chuanxiong rhizome (Rhizoma Ligustici Chuanxiong), ginseng (Radix Ginseng), licorice root (Radix Glycyrrhizae), Chinese angelica root (Radix angelicae Sinensis), white peony root (Radix Paeniae Alba), and dried Rehmannia root (Radix Rehmanniae). The extract powder of Danggu-Sini Tang was prepared from the following raw herbs: Chinese angelica root (Radix Angelicae Sinensis), cinnamon twig (Ramulus Cinnamomi), peony root (Radix Paeoniae), Asarum herb (Herba Asari), prepared licorice root (Radix Glycyrrhizae Praeparata), five-leaf Akebia stem (Caulis Akebiae), and Chinese dates (Fructus Ziziphi Fujubae). Both compounds in the herbal mixture remained unchanged over the 4-week study period. Patients were examined at 2 and 4 weeks after treatment began. Full compliance with therapy was ensured by oral confirmation from subjects at each visit.

Assessment of RP symptoms

Patients were provided symptom diary cards and were instructed to record their symptoms and number of attacks at their convenience but at least on 2 days per week.[18] An RP attack was defined as a blanching episode followed by cyanosis. Patients recorded the number of RP attacks and graded the severity at the end of the day of recording on a visual

analog scale (VAS) of 0~10 where 0 was defined as no episode and a VAS score of 10 as the worst episode ever experienced. All numbers and scales were averaged over the number of days of recording. The severity of RP in each patient was also assessed by a physician on the day the patient attended the clinic. Assessment was based on the day of visit and was consistently performed at the same clinic section time for the individual study subject. The temperature of the examination room was maintained at 21± 1°C. Physicians scored RP for all subjects on a global assessment scale, the physician global assessment (PGA), where 0 was no attack, 1 was a mild attack, 2 a moderate attack, and 3 a severe attack. After drug therapy, the physician's assessment of the overall treatment effect was graded as 1 for greatly improved, 2 for improved, 3 for no change, or 4 for worsened.

Vascular studies

Skin microvascular blood flow was measured by a laser Doppler flow study at the baseline and after therapy. These measurements were performed via a scanning laser Doppler flow meter using a helium-neon laser at 633 nm (Moor LDI Laser Doppler Imager, Moor Instruments, Axminster, Devon, U.K.). Patients were required to avoid consuming alcoholic or caffeinated beverages and to fast for at least 8 h. Measurements were taken after patients had rested for 20 min in a temperature-controlled room at 21± 1°C. Bilateral second to fourth digits on each hand were scanned. With both hands gloved, patients then underwent a cold water challenge involving the complete immersion of the hands in a 15°C cold water bath for 1 min.[19] Laser Doppler flow (LDF) measurements were taken immediately and at 5, 10, 15, 20, and 30 min after the cold challenge.

Nailfold capillary microscopy with a stereoscopic microscope (SMZ-10, Nikon Fx 35A, Japan) was performed at room temperature maintained at 21 ±1°C at the baseline and after 4 weeks of drug therapy. The stereomicroscope was set with a magnification of 15x. Nailfold capillary microscopy was performed on the bilateral fourth digits, and images were recorded. The same author (YJJW) aided by 1 research assistant assessed all nailfold capillaries for vascular loop enlargement, avascularity, hemorrhagic spots, and vascular density. A vascular loop was defined as "definitely enlarged" with a width 4~10 times that of normal.[20] An "extremely enlarged loop" was defined as ≥ 10 times normal. "Avascularity" was classified as follows: grade 0 was no avascularity; grade 1 was mild, with 1 or 2 discrete areas of vascular deletion; grade 2 was moderate, with > 2 discrete areas of vascular deletion; and grade 3 was severe, with large or confluent avascular areas. Nailfold hemorrhagic spots per finger were classified as follows: grade 1 was fewer than 2 punctate hemorrhages per finger; grade 2 was more than 2 punctate hemorrhages per finger; and grade 3 was confluent areas of hemorrhage.[21] The density of the vascular loops was calculated as the number of capillaries per 3 mm.

Serum and plasma markers

Blood samples were collected immediately before and after 4 weeks of therapy. After centrifugation of whole blood at 3000 xg for 10 min, plasma and serum samples were stored in aliquots at –20°C. Creatinine, ALT, triglyceride, and cholesterol levels were measured on the day of the baseline visit. Plasma ET-1 levels and other parameters were determined at later dates by enzyme-linked immunosorbent assay (ELISA; QuantiGlo, Human Endothelin-1 Immunoassay, R&D Systems, Minneapolis, MN, U.S.A.) according to the manufacturer's instructions. Serum sICAM-1 and PGE_2 were also measured by an ELISA method (Parameter, Minneapolis, MN, U.S.A.), (High Sensitivity, Minneapolis, MN, U.S.A.) respectively. Serum nitric oxide (NO) concentrations, measured as NO_2 and NO_3, were determined by chemiluminescence with an NO analyzer (NO ATM 280, Sievers Instruments, Boulder, Co., U.S.A.).[22]

Statistical analysis

Categorical data such as gender, tobacco use, and disease type between the nifedipine and herbal groups were compared by Fisher's exact test. The Shapiro-Wilk test was used to check the normality of continuous data including age, RP duration, creatinine, ALT, triglyceride, cholesterol, sICAM-1, ET-1, PGE_2, NO_2, NO_3, and capillary density. Only age was normally distributed in our sample. Hence, ages were compared between the nifedipine and herbal groups by unpaired t-test. Continuous data which did not follow a normal distribution or ranking data including VAS, PGA, capillary microscopy on enlarge-

ment, avascularity, and hemorrhagic spots were analyzed between the 2 study groups using the Wilcoxon rank-sum test. Continuous data which did not follow a normal distribution or ranking data between the baseline and posttreatment were compared using the Wilcoxon signed-rank test. Repeated-measures analysis of variance was performed to compare how LDF measurements, of a continuous nature, differed over time and between the 2 groups.

RESULTS

Table 1 presents demographic and baseline clinical characteristics of both study groups. The nifedipine and herbal groups did not significantly differ in gender distribution, tobacco use, age, duration of RP, creatinine, ALT, triglyceride, cholesterol, sICAM-1, ET-1, PGE_2, NO_3, or NO_2. As Table 2 shows, baseline RP episodes per day, VAS scores, and physician global assessments were similar in the two groups. The herbal group had more-enlarged capillary loops than the nifedipine group (0.7 ± 0.7 vs. 1.2 ± 0.6, $p = 0.0259$) on nailfold capillomicroscopy; however, avascularity, hemorrhagic spots, and capillary density were similar in the 2 groups.

Following treatment, the VAS score improved in the nifedipine group, decreasing from the baseline at 4.3 ± 2.1 to 2.5 ± 1.9, $p = 0.0035$. The VAS score in the herbal group did not significantly improve (baseline 4.2 ± 2.6 to 3.5 ± 2.7, $p = 0.3854$). The RP episodes in the nifedipine group improved (2.8 ± 1.5 at the baseline to 2.1 ± 1.8 at 4 weeks, difference between 2 visits (V3 - V1) = -0.7 ± 1.1) compared with the herbal group (baseline 3.1 ± 2.3 to 3.3 ± 2.9 at 4 weeks, V3 - V1 = 0.2 ± 2.0, $p = 0.0186$). The number of RP episodes in the nifedipine group decreased from 2.8 ± 1.5 to 2.1 ± 1.8 ($p = 0.0080$); however, no change in the number of RP episodes was observed in the herbal group (3.1 ± 2.3 to 3.3 ± 2.9, $p = 0.5046$). The PGA of RP severity decreased in the nifedipine group (from 1.4 ± 0.5 to 1.0 ± 0.5, $p = 0.0078$), but remained unchanged in the herbal group (1.8 ± 0.8 to 1.6 ± 0.8, $p = 0.3438$). Capillary microscopic studies revealed no difference in the number of enlarged loops, avascularity, hemorrhagic spots, or capillary density after treatment when compared to baseline measurements in each therapy group (Table 3). In the herbal group with normal capillaries, 66.7% of patients showed improvements in the VAS, episodes of RP, and overall physician assessment. In the nifedipine group with normal capillaries, 88.8% showed improvements in the VAS and overall physician assessment, and 77.8% showed an improvement in RP episodes. In the herbal group with definite enlargement, 40% showed improvements in the VAS scores and episodes, and 53.3% showed an improvement in physician assessment; in the nifedipine group with definite enlargement, 66.7% showed an improvement in the VAS scores, 77.8% in episodes, and 88.8% in the physician assessment. In the herbal group with extreme enlargement, 50% patients showed improvements in the VAS scores and number of episodes, and 37.5% in physician assessment; in the nifedipine group, all

Table 2. Baseline Clinical Characteristics of the Nifedipine and Herbal Groups

	Nifedipine (n = 21)	Herbal (n = 26)	*p*
RP episodes/day	2.8 ± 1.5	3.1 ± 2.3	0.8564†
Patient VAS (1~10)	4.3 ± 2.1	4.2 ± 2.6	0.6630†
PGA of RP			0.1203†
1 = mild	13 (61.9%)	12 (46.2%)	
2 = moderate	8 (38.1%)	8 (30.7%)	
3 = severe	0	6 (23.1%)	
Mean ± SD	1.4 ± 0.5	1.8 ± 0.8	
Capillary microscopy			
Enlargement			0.0259*†
0 = none	9 (42.9%)	3 (11.5%)	
1 = definite	9 (42.9%)	15 (57.7%)	
2 = extreme	3 (14.3%)	8 (30.8%)	
Mean ± SD	0.7 ± 0.7	1.2 ± 0.6	
Avascularity			0.0803†
0 = none	8 (38.1%)	1 (3.9%)	
1 = mild	5 (23.8%)	10 (38.5%)	
2 = moderate	4 (19.1%)	10 (38.5%)	
3 = severe	4 (19.1%)	5 (19.2%)	
Mean ± SD	1.2 ± 1.2	1.7 ± 0.8	
Hemorrhagic spots			0.7063†
0 = none	9 (42.9%)	8 (30.8%)	
1 = < 2/finger	1 (4.8%)	10 (38.5%)	
2 = > 2/finger	7 (33.3%)	5 (19.2%)	
3 = confluent	4 (19.1%)	3 (11.5%)	
Mean ± SD	1.3 ± 1.2	1.1 ± 1.0	
Density (no./3 mm), right	18.1 ± 5.8	16.3 ± 4.1	0.1260†
Density (no./3 mm), left	18.3 ± 4.2	16.7 ± 4.2	0.2035†

Abbreviations: PGA: physician global assessment; RP: Raynaud's phenomenon; VAS: visual analogue scale; *: $p < 0.05$; †: By the Wilcoxon rank-sum test.

Table 3. Effects of Nifedipine or Herbal Therapy on Clinical Parameters

	Nifedipine (n = 21)	Herbal (n = 26)	$p^{\ddagger}$
VAS (1~10)	2.5 ± 1.9	3.5 ± 2.7	0.1100
V3 - V1	-1.8 ± 2.7	-0.7 ± 2.3	0.0738
$p^{\ddagger}$	0.0035*	0.3854	
RP episodes	2.1 ± 1.8	3.3 ± 2.9	0.0672
V3 - V1	-0.7 ± 1.1	0.2 ± 2.0	0.0186*
$p^{\ddagger}$	0.0080*	0.5046	
PGA of RP	1.0 ± 0.5	1.6 ± 0.8	0.0031*
V3 - V1	-0.4 ± 0.6	-0.2 ± 0.6	0.1935
$p^{\ddagger}$	0.0078*	0.3438	
Capillary microscopy			
Enlargement	0.7 ± 0.7	1.1 ± 0.7	0.0306*
V3 - V1	-0.05 ± 0.4	-0.08 ± 0.4	0.8097
$p^{\ddagger}$	1.000	0.6250	
Avascularity	1.2 ± 1.1	1.8 ± 0.9	0.0875
V3 - V1	0.05 ± 0.5	0.04 ± 0.6	0.9789
$p^{\ddagger}$	1.000	1.000	
Hemorrhagic spots	1.4 ± 1.2	1.2 ± 1.1	0.6782
V3 - V1	0.1 ± 0.9	0.1 ± 0.7	0.5365
$p^{\ddagger}$	0.8281	0.5625	
Density (no./3 mm), right	18.2 ± 5.6	16.0 ± 3.8	0.0928
V3 - V1	0.1 ± 0.9	-0.2 ± 2.0	0.5121
$p^{\ddagger}$	0.5625	0.8130	
Density (no./3 mm), left	18.5 ± 4.1	16.8 ± 4.3	0.1925
V3 - V1	0.2 ± 0.8	-0.1 ± 1.4	0.6641
$p^{\ddagger}$	0.2734	0.7285	

Abbreviations: V1: baseline; V3: after 4 weeks of treatment; VAS: visual analogue scale; PGA: physician global assessment; RP: Raynaud's phenomenon; *: $p < 0.05$; †: By the Wilcoxon rank-sum test; ‡: By the Wilcoxon signed-rank test.

patients with extreme enlargement demonstrated improvements in all measures.

LDF measurements before treatment were similar in the herbal and nifedipine groups. After treatment, the nifedipine group (116.3± 70.7 AU) had greater LDF compared to the baseline (72.4 ± 49.0 AU, $p=0.0008$) and better perfusion than the herbal group (73.9 ± 62.1 AU, $p=0.0215$). The nifedipine group after therapy showed improved blood flow as measured by LDF at various times after the cold challenge. Fig. 1 shows LDF measurements at room temperature for the baseline, and 1, 5, 10, 15, 20, and 30 min after the cold challenge in both groups before and then after 4 weeks of therapy. Among the serological markers, serum NO_2 in the herbal group decreased from 0.5 ± 0.2 μM/ml to 0.4 ± 0.2 μM/ml, while it increased in the nifedipine group from 0.5 ± 0.3 μM/ml to 0.7 ± 0.9 μM/ml after treatment, $p=0.0469$. Other parameters, including creatinine, ALT, sICAM-1, ET-1, PGE_2, and NO_3, did not significantly differ after therapy in either group (Table 4).

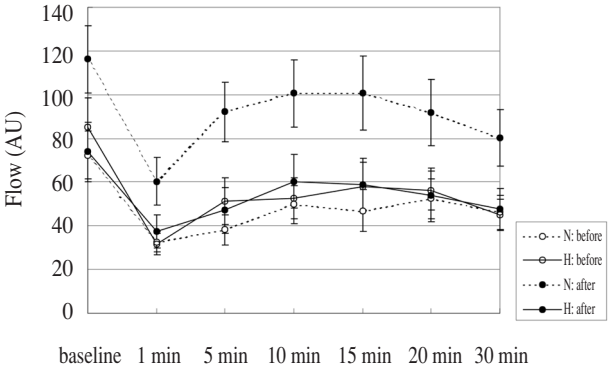

Fig. 1 Comparison of laser Doppler flow (LDF) at room temperature and 1~30 minutes after cold challenge before and after 4 weeks of therapy. N: nifedipine group; H: herbal group.

Of 53 patients enrolled in the study, 6 patients withdrew due to adverse effects. In the nifedipine group, 1 patient complained of headaches, 1 developed flushing, and 1 developed palpitations. In the herbal group, 1 patient withdrew due to pruritus, 1 for flushing, and 1 for palpitations and pruritus. Of the 47 patients completing the study, 7 (33%) patients in the nifedipine group experienced adverse effects: 2 patients experienced dizziness, 2 chest tightness, 1 developed palpitations, 1 experienced headaches, and 1 experienced a burning sensation in the throat. In the herbal group, 8 (31%) patients experienced 1 or more side effects: 2 patients reported menorrhagia, 1 complained of pruritus during the first 3 days of therapy, 1 experienced increased appetite along with constipation, 1 had vomiting, 1 had diarrhea, 1 had hand cramps, 1 had a burning sensation in the chest, 1 experienced neck tightness, and 1 experienced tongue numbness.

DISCUSSION

Reportedly effective pharmacological therapies for RP include calcium channel antagonists such as nifedipine, amlodipne, and felodipine;[10-12]

Table 4. Effects of Nifedipine or Herbal Therapy on Laboratory Parameters

	Nifedipine (n = 21)	Herbal (n = 26)	$p^{\dagger}$
Creatinine (mg/ml)	0.7 ± 0.1	0.7 ± 0.2	0.8898
V3 - V1	-0.01 ± 0.10	-0.02 ± 0.13	0.7991
$p^{\ddagger}$	0.8828	0.4812	
ALT (mg/ml)	21.5 ± 23.9	17.3 ± 6.6	0.5434
V3 - V1	-5.4 ± 16.9	-0.7 ± 5.9	0.7325
$p^{\ddagger}$	0.4059	0.5681	
sICAM-1 (ng/ml)	339.5 ± 209.2	415.3 ± 211.1	0.5158
V3 - V1	-26.4 ± 145.3	-8.3 ± 139.7	0.6630
$p^{\ddagger}$	0.3606	0.7389	
ET-1 (pg/ml)	1.4 ± 0.6	1.8 ± 1.1	0.8778
V3 - V1	0.1 ± 0.4	0.1 ± 0.5	0.3353
$p^{\ddagger}$	0.1245	0.7389	
PGE2 (pg/ml)	372.1 ± 181.0	400.9 ± 217.7	0.7443
V3 - V1	0.7 ± 196.3	-37.4 ± 386.7	0.7903
$p^{\ddagger}$	0.9333	0.5916	
NO_3 (μM/ml)	62.2 ± 45.5	66.1 ± 72.2	0.9292
V3 - V1	-0.4 ± 50.7	-0.5 ± 63.9	0.9068
$p^{\ddagger}$	0.8015	0.6123	
NO_2 (μM/ml)	0.7 ± 0.9	0.4 ± 0.2	0.0469*
V3 - V1	0.2 ± 0.9	-0.1 ± 0.3	0.4641
$p^{\ddagger}$	0.9599	0.1080	

Abbreviations: V1: baseline; V3: after 4 weeks of treatment; ALT: alanine transferase; sICAM-1: soluble intercellular adhesion molecule; ET-1: endothelin-1; PgE_2: prostaglandin E_2; NO_3: nitrate; NO_2: nitrite; * $p < 0.05$; †: By the Wilcoxon rank-sum test; ‡: By the Wilcoxon signed-rank test.

angiotensin-II type 1 receptor antagonists such as losartan;[18] topical nitrates;[9,23] prostacyclin; iloprost;[24] serotonin reuptake inhibitors;[25,26] sildenafil (a phosphodiesterase type 5 inhibitor);[27,28] and bosentan (an endothelin receptor antagonist).[29] Nifedipine remains the gold standard and is mediated through peripheral vasodilatory, antiplatelet, and anti-white cell effects.[30] This study also demonstrated a reduction in severity of Raynaud's attack in the nifedipine group after 4 weeks of therapy with 10 mg 3 times daily.

The following traditional Chinese medications have been shown to increase cardiovascular and peripheral circulation: Panax Notoginseng (1 of its main components is trilinolein),[31-34] Salvia miltiorrhiza Bunge,[35-37] Duhuo-Tisheng Tang,[13-15] Bu Yang Huan Wu Tang,[38] and Danggui-Sini Tang.[16,17] This study evaluated a combined prescription of 2 traditional Chinese medicines, Duhuo-Tisheng Tang and Danggu-Sini Tang, in treating RP. Duhuo-Tisheng Tang is traditionally prescribed for rheumatism, arthralgia, and poor circulation. An animal study of Duhuo-Tisheng Tang revealed increased capillary flow and reduced vessel spasms in mice measured by vessel caliber and time to spasms after administration of adrenalin.[14] Danggui-Sini Tang has been shown to increase blood circulation. Clinical studies have also revealed anti-anxiety, analgesic, anti-inflammatory, and anti-spasm properties.[17] A decoction of this combined prescription was found to be effective in treating RP.[16] However, in this study, Duhuo-Tisheng Tang with Danggui-Sini Tang group revealed no reductions in RP symptoms.

Several studies have demonstrated the value of a blood flow evaluation for the diagnosis and follow-up of RP.[8,9] Laser Doppler imaging can measure flux over an area rather than at a single point and does not require direct contact between a probe and the skin surface, which can influence blood flow.[39] The experimental results of this study verified that patients taking nifedipine had a good vascular response as measured by the laser Doppler imaging.

Nailfold capillary microscopy is a vascular imaging tool also used for evaluating vascularity and severity of disease and aids in differentiating primary and secondary RP.[39,40] Semiquantitative assessment of nailfold capillary abnormalities can measure microvascular disease progression over time. The severity of capillary dilation and avascularity has been shown to be correlated with clinical severity such as the extent of organ involvement and disease duration.[41,42] In this study, nailfold capillary microscopy was employed to evaluate capillary density and avascularity, and these showed no significant difference between values at the baseline and after 4 weeks of therapy in either group. The length of time before vascular differences can be observed by nailfold capillary microscopy is unclear. ter Borg *et al*., in a 6-year study of serial nailfold capillary microscopy in scleroderma reported an ongoing decrease in the total number of capillary loops over 3 years.[43] Grassi *et al*. suggested that scleroderma nailfold capillary morphology can vary over the course of a few days.[44] In the present study, patients evaluated after 4 weeks of therapy revealed similar nailfold morphologies before and after treatment, suggesting that visible vascular differences may take longer than 4 weeks to manifest.

At the baseline, both groups of patients had comparable demographic and baseline clinical characteristics except that the herbal group had more-enlarged capillary loops. This discrepancy raises the possibility that patients in the herbal group may have had a poorer response. Thus the effect of treatment was analyzed using the changes (V3 - V1) rather than the measurement at week 4 (V3). Patients were compared to their own values before and after 4 weeks of treatment, and the changes within groups (V3 - V1) were compared between the 2 study groups to avoid the bias due to baseline differences. Furthermore, comparisons of subgroups by normal, definite, or extreme enlargement of the capillaries by microscopy were made and revealed that patients in the nifedipine group enjoyed greater improvements in VAS scores, RP episodes, and overall physician assessment than did the herbal group in all 3 subgroups.

In this study, the nifedipine group had increased levels of NO_2 after therapy compared with the herbal group, suggesting that the effect of nifedipine on vasorelaxation may involve NO. This finding is consistent with those of previous studies. Berkels *et al*. demonstrated that nifedipine stimulated NO liberation of porcine coronary endothelium[45] and enhanced platelet NO production in porcine platelet-rich plasma.[46] More recently, Ding and Vazire, in an investigation of cultured human coronary artery endothelial cells, found that nifedipine upregulated endothelial nitric oxide synthase expression.[47]

In both groups, ET-1 concentrations revealed no change after treatment. This finding is consistent with that obtained by Dziadzio *et al*. in a study comparing losartan and nifedipine therapy for RP and scleroderma, who found that ET-1 did not significantly decrease after losartan or nifedipine therapy.[18] The potent vasoconstrictor, ET-1, has been found to significantly increase in RP[48] and PSS.[49] However, a recent study by Smyth *et al*. showed no difference in ET-1 concentrations between primary versus secondary RP and between RP and a control group. Smyth *et al*. suggested that circulating ET-1 might not be of direct importance but might be an indicator of more functionally significant local release.[50] None of the other circulating parameters, sICAM-1, PGE_2, and NO_3, significantly differed after treatment in either group in this study. These findings also agree with those obtained by Dziadzio *et al*. in their 12-week study which showed no reduction in sICAM-1 levels after nifedipine therapy.[18]

Similar side effects of palpitations and flushing were noted in evaluating the tolerability and side effects of nifedipine and the herbal mixture of Duhuo-Tisheng Tang and Danggu-Sini Tang. This finding suggests that these herbal mixtures have vasodilatory effects on the circulation. Further, in contrast to commonly held beliefs in Chinese culture, Duhuo-Tisheng Tang and Danggui-Sini Tang did exert other side effects: in addition to palpitations and flushing, some patients experienced pruritus, numbness, and menorrhagia.

However, the lack of measurable improvements after herbal medicine therapy may have been due to the more-gradual effects of Chinese herbal medicine. Furthermore, in traditional Chinese medicine, the basic formula is often modified for individual patients after examination by a traditional Chinese medicine practitioner. In this study, all patients received identical herbal mixtures throughout the therapeutic course. Another possibility accounting for the lack of significant therapeutic results may have been that the herbal medication in powdered form is less effective than the decoction form, which is the traditional method of preparing these prescriptions. A longer duration of treatment and a larger patient population are required to further study the effects of this mixed herbal prescription in comparison with Western medications.

The limitation of this preliminary study is the open label administration of the Chinese medicine treatments. Blinding and randomizing patients was difficult due the distinct odor of the herbal powders, and patients may have harbored personal preferences for herbal or Western medication. To minimize bias, the present study investigated various objective and laboratory measures of therapeutic effects in addition to RP symptoms and severity. Potential biases may still have existed because of discrepant baseline characteristics of the 2 groups in terms of the vascular enlargement on capillary microscopy.

In conclusion, the digital vascular response improved in secondary RP after 4 weeks of nifedipine therapy but not after therapy with a herbal mixture of Duhuo-Tisheng Tang and Danggui-Sini Tang as measured by the number and severity of RP attacks and by LDF imaging. After treatment, the nifedipine group showed increased levels of NO_2

compared to the herbal group, suggesting that NO may play a role in the therapeutic effect of nifedipine. Both groups experienced some side effects. A double-blind randomized clinical trial is required to clarify the observations of this preliminary study.

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獨活寄生湯合當歸四逆湯或 nifedipine 在雷諾氏症候群之循環反應：初步研究

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背 景： 雷諾氏症候群常見於結締組織疾病，其最常使用及有效治療爲鈣離子阻斷劑。本研究探討中藥獨活寄生湯合當歸四逆湯在雷諾氏症候群之效果及耐受性和 nifedipine 作比較。

方 法： 本研究收案 47 位雷諾氏症候群病人，其中 26 位參與中藥組， 21 位參與 nifedipine 組。測量治療前及治療四週後手指雷射杜卜勒影像掃描 (室溫下及接受冷刺激後)，微血管甲摺鏡檢查，及血液中的可溶性細胞間粘連分子-1 (sICAM-1)，前列腺素 E2 (PGE2)，二氧化氮 (NO_2)，三氧化氮 (NO_3)，內皮素-1 (endothelin-1)，加以分析比較。另外收集病人在治療前及治療後自我症狀評分，發作次數紀錄，及醫師對雷諾氏症嚴重度評估。

結 果： 經過治療四週以後，nifedipine 組病人雷諾氏症發作次數、自我症狀評分及醫師對雷諾氏症嚴重度評估改善，手指雷射杜卜勒流速增加 (治療前 72.4 ± 49.0 AU，治療後 116.3 ± 70.7 AU)，中藥組則沒有明顯差異。用微血管甲摺鏡檢查評估血管擴大，血管減少及出血點在兩組治療前後相似，血清 NO_2 在 nifedipine 治療後比中藥組增加。

結 論： 雷諾氏症候群手指循環反應 nifedipine 較獨活寄生湯合當歸四逆湯達到改善。
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關鍵詞： 雷諾氏症候群，雷射杜卜勒流速，二氧化氮，獨活寄生湯合當歸四逆湯，nifedipine

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