Danshen Gegen capsule for intermittent claudication in patients with peripheral arterial disease: abridged secondary publication

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KEY MESSAGES

1. We demonstrated in vitro dose-dependent vasodilation and angiogenesis responses to Danshen and Gegen via endothelium-independent mechanism and involvement of inwardly rectifying K^+ channels and Ca^{2+} channels.
2. We demonstrated an in vivo effect in functional limb recovery and in vitro improvement in blood micro-vessel density and blood perfusion responses to Danshen and Gegen in a limb ischaemic animal model.
3. Danshen and Gegen may be an effective treatment for patients with intermittent claudication, especially for those with severe symptoms compared with placebo. However, longer-term research in a larger population is needed to deteremine its safety and efficacy.

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Introduction

The prevalence of peripheral arterial disease (PAD) secondary to lower limb arterial blockages is increasing.[1] Intermittent claudication is the most common manifestation of PAD and induces dysfunction and muscle pain (claudication pain). Claudication is associated with impairment in walking capacity and physical activity as well as quality of life including social functioning and emotional and mental health.[2] Effective pharmacotherapy for intermittent claudication is limited. Two traditional Chinese medicines—Danshen and Gegen (DG)—have shown positive therapeutic effect on vascular function and structure in patients with coronary artery disease.[3] We hypothesised that a herbal formula consisting DG is an effective treatment for patients with PAD and symptomatic intermittent claudication.

Methods

We evaluated vasodilatory and angiogenic response to DG in an animal chronic limb ischaemic model and assessed efficacy and safety of DG in patients with PAD in a randomised placebo-controlled trial.

For in vitro vasodilation studies, rat femoral artery rings were used. Dose-dependent vasodilatory response to DG was tested with various ion-channel inhibitors. For in vivo studies, the hindlimb ischaemia rat model (right femoral artery ligation) was used to evaluate functional limb recovery and blood perfusion increase in response to low dose (300 mg/mL) and high dose (600 mg/mL) DG for 28 days. Immunofluorescent and immunohistochemical assessments were performed to evaluate changes in muscle structure and angiogenesis.

A 24-week, prospective, randomised, double-blind, placebo-controlled study was approved by the Clinical Research Ethics Committee of Joint Chinese University of Hong Kong – New Territories East Cluster (reference: 2012.561-T) [ClinicalTrials.org. ID NCT 02380784]. Informed consent was obtained before the study. People aged ≥40 years with stable intermittent claudication (Rutherford class 1-3) secondary to PAD (resting ankle-brachial index <0.90 and a ≥10 mmHg decrease in ankle artery blood pressure after exercise) and without critical limb ischaemia, major lower limb amputation, or surgical or endovascular revascularisation for PAD within 3 months before enrolment were recruited. Patients were allocated at random to the treatment group (n=48) with daily oral DG capsules (1.5 g twice daily) or the control group with placebo (n=47). Primary outcome was the change in maximal walking distance (MWD) in a standardised graded treadmill test (Gardener Protocol), defined as total distance walked from beginning treadmill walking until the subjects can walk no further. Secondary outcomes included changes in pain-free walking distance (PFWD), defined as the distance walked at the onset of claudication. Functional status, quality-of-life, and pro-inflammatory biomarkers including

C-reactive protein, interleukin-6, tumour necrosis factor alpha (TNF-α) were assessed.

Results

In in vitro vasodilation studies, endothelium-independent mechanism was shown to involve in the response to DG in a dose-dependent manner. Involvement of both inwardly rectifying K^+ channel and Ca^{2+} channels in the mechanism of vasodilatory response to DG was confirmed. In the in vivo hindlimb ischaemia rat model, significant improvement in functional limb recovery including positive change in maximum contact area, stance phase duration, and print area was observed in DG groups at high and low doses at week 28. Blood perfusion ratio of both DG treatment groups was significantly higher at the end of study. In addition, low dose DG was associated with a significant increase in capillary density, whereas high dose DG achieved a two-fold increase compared with the control group.

Of 95 patients randomly allocated to the treatment group (n=48) or the control group (n=47), 17 (17.9%) dropped out (Fig). Baseline characteristics of patients in both groups were comparable. Overall compliance of patients reached 94.8%. After the 24-week intervention, a significant proportion of participants in the treatment group showed improvement in MWD and PFWD. Considering >50% improvement in walking distance as a clinically meaningful benefit, the proportion of patients who achieved ≥50% improvement in either PFWD or MWD was significantly higher in the treatment group (43.2% vs 22.0%, P=0.044). Significant increase in PFWD was observed in the treatment group after 24-week (P=0.033) and after log transformation, with a 6.8% increase (P=0.075) [Table]. However, the two groups were comparable in terms of the absolute (P=0.287) and the log transformed increases in PFWD (P=0.299).

Subgroup analysis was performed based on patients' baseline walking capacity on an exercise treadmill. Patients with moderate to severe intermittent claudication (baseline MWD <200 m) improved in terms of % change in log-transformed PFWD, compared with placebo (9.9% vs -0.4%, P=0.072), whereas patients with baseline MWD ≥200m did not improve significantly, compared with placebo (P=0.736, Table).

Although ankle-brachial index improved by 23.6% in the treatment group (P=0.029), the two groups were not significantly different in improvement (P=0.213). There were no significant changes in pulse wave velocity within or between groups. There was no significant change in self-perceived quality of life or functional status. The two groups were comparable in terms of results of haematology tests and serum chemistry tests, including lipid profiles. Both plasma interleukin-6 (P=0.011) and plasma TNF-α (P=0.011) levels changed significantly in the treatment group, but the two groups were marginally significantly different in terms of change in plasma TNF-α level (P=0.072).

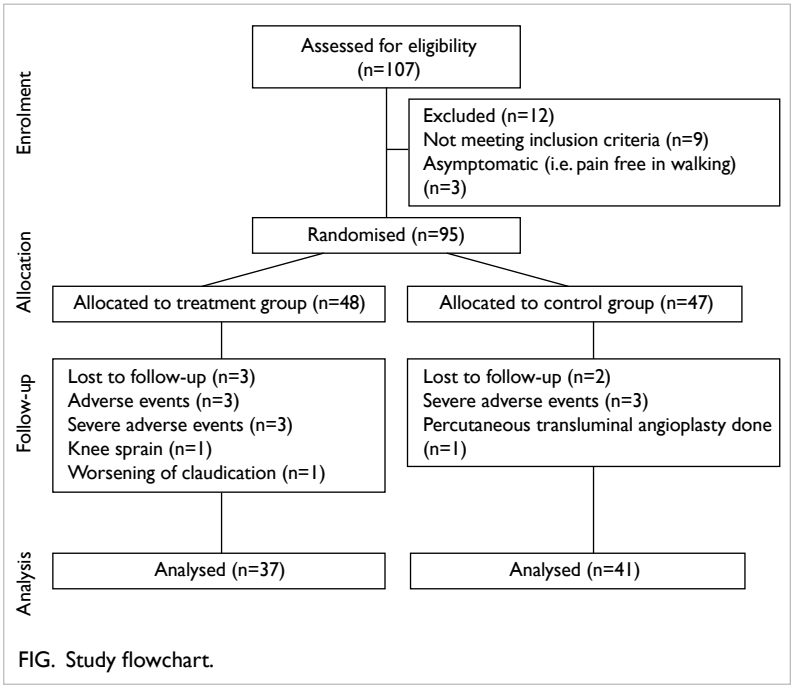

FIG. Study flowchart.

Discussion

In in vitro studies, vasodilation effect of DG was demonstrated in the rat femoral arteries in a dose-dependent manner. Therefore, we speculated that DG could be beneficial to PAD patients to improve the tissue perfusion by dilating the femoral artery and microvasculature. Identifying the involvement of the inwardly rectifying K^+ channels and Ca^{2+} channels in the vasodilatory response of DG may help subsequent modification on the herbal formula of the capsule for optimising its efficacy. In in vivo studies, DG were found to be effective for functional limb recovery in rats with hindlimb femoral artery ligation. The significant improvement was associated with positive changes on blood perfusion and microvessel density after DG treatment.

In patients with PAD, a significant proportion of patients in the treatment group showed a positive response in MWD and PFWD, but the degree of improvement was not significant. The beneficial impact of DG may have been diluted owing to wide variation in baseline walking distances (ie, type II error). Moreover, the study duration of 24 weeks may be insufficient owing to the highly variable onset of clinical responses reported by the patients. Using walking distances as examples, the ranges observed in treatment group (MWD: -55.7% to 276.2%; PFWD: -74.4% to 421.6%) were wider than that in control group (MWD: -56.1% to 197.8%;

TABLE. Subgroup analysis on maximal walking distance and pain-free walking distance with patients stratified by severity of claudication.

Claudication	Treatment (n=37)						Control (n=41)						Between-group comparison
	Baseline	Week 24	Absolute change	% Change	P value	P value (moderate vs mild)	Baseline	Week 24	Absolute change	% Change	P value	P value (moderate vs mild)	P value
Maximal walking distance													
Distance, m													
Moderate	119.6±45.2	173.7±111.8	54.1±97.1	43.1±92.8	0.030	0.362	137.0±37.4	162.9±111.6	25.9±99.7	17.0±62.8	0.984	0.514	0.248
Mild	388.9±180.3	389.9±184.4	1.1±157.1	6.2±44.7	0.977		395.2±147.8	439.0±220.4	43.8±144.2	10.8±40.0	0.217		0.731
Natural log transformation													
Moderate	4.7±0.4	4.9±0.8	0.2±0.6	3.6±13.0	0.306	0.288	4.9±0.3	4.9±0.6	0.1±0.4	1.0±9.0	0.608	0.549	0.520
Mild	5.9±0.4	5.9±0.5	0.0±0.4	-0.2±7.0	0.852		5.9±0.3	6.0±0.5	0.0±0.4	0.6±6.6	0.647		0.653
Pain-free walking distance													
Distance, m													
Moderate	65.4±25.2	110.3±74.4	45.6±72.3	87.2±143.8	0.023	0.249	73.0±36.7	61.1±25.1	-8.0±35.1	7.6±60.3	0.335	0.044	0.064
Mild	148.3±126.1	154.6±162.2	8.9±88.5	40.6±109.3	0.381		107.9±58.1	154.8±115.8	46.9±98.1	47.7±90.9	0.025		0.758
Natural log transformation													
Moderate	4.1±0.4	4.5±0.8	0.4±0.7	9.9±18.5	0.051	0.339	4.2±0.6	4.0±0.4	-0.1±0.6	-0.4±14.5	0.569	0.060	0.072
Mild	4.7±0.8	4.8±0.7	0.1±0.7	3.8±17.8	0.603		4.6±0.5	4.8±0.6	0.3±0.5	6.1±10.1	0.015		0.736

PFWD: -59.9% to 355.0%). In subgroup analysis, patients with more severe intermittent claudication symptoms at baseline showed significant positive responses to DG, compared with those with mild claudication. Therefore, further longer-term study in patients with severe walking impairment and more restricted distance (eg, <200 m) is warranted.

Conclusion

We demonstrated positive dose-dependent vasodilation and angiogenesis responses to DG via endothelium-independent mechanism. We also demonstrated beneficial effects of DG in functional limb recovery in an in vivo hindlimb ischaemia animal model and significant in vitro improvement in blood microvessel density and blood perfusion rate. DG may be an effective treatment for patients with intermittent claudication, especially for those with severe symptoms. However, longer-term research in a larger population is needed to determine its safety and efficacy.

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