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Long-term low molecular weight heparin therapy for severe Raynaud's phenomenon: A pilot study

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ABSTRACT

Objective

To investigate tolerability and efficacy of low molecular weight (LMW) heparin therapy in patients with severe Raynaud's phenomenon.

Methods

A prospective parallel group study comparing patients receiving regular subcutaneous LMW heparin (n=16) with a matched control group (n = 14). Endpoints were change in Raynaud's attack severity, non-invasive vascular studies or serum levels of circulating soluble adhesion molecules.

Results

There was overall improvement in Raynaud's attack severity during heparin therapy (p = 0.0002). This was observed after 4 weeks, and was maximal by 20 weeks. Mean finger blood flow recovery time improved, and serum levels of circulating ICAM-1, VCAM-1 and E-selectin were lower at completion of heparin therapy, but changes did not reach statistical significance.

Conclusion

This study suggests that LMW heparin therapy is well tolerated, and potentially beneficial, in patients with severe Raynaud's phenomenon, and justifies further evaluation.

Introduction

Although calcium channel blockers and other vasodilators have been shown to be beneficial treatments for Raynaud's phenomenon, responses are often variable (1). Intermittent infusions of vasodilator prostanoids are also effective (2), but are necessarily invasive. New therapies are therefore needed, especially agents offering sustained benefit over several months, for example during the winter, when patients experience more severe symptoms (3).

Heparin is a naturally occurring proteogycan, structurally related to heparan sulphate, a ubiquitous constituent of plasma membranes and extracellular matrix. In addition to being a powerful anticoagulant, heparin has a broad range of biological properties, including effects on the vasculature, immune system and connective tissue [reviewed in (4)]. This led us to undertake a pilot study to assess the feasibility of long-term LMW heparin (5) therapy for Raynaud's phenomenon in a group of patients with severe and refractory symptoms.

Methods

Study design and patients

This was a prospective parallel group study, comparing heparin with conventional treatment, over a 24-week period. The aims were to evaluate tolerability and safety and to provide some preliminary information regarding efficacy. Eligible patients had Raynaud's phenomenon of at least Grade 3 severity (9), with attacks on most days throughout the year. Thirty-five patients were screened for possible inclusion. Thirty were eligible and consented to enter the study. Of these, 19 patients had Raynaud's associated with scleroderma. Fourteen were classified as limited cutaneous systemic sclerosis and 5 had diffuse disease. Other patients (n = 11) were designated primary Raynaud's phenomenon. These had negative antinuclear antibody reactivity, normal nailfold capillaroscopy, and no clinical evidence of an associated connective tissue disorder (such as rash, arthralgia or oesophagitis) at recruitment, although one case developed anticentromere antibodies during the study. Exclusion criteria included any risk of pregnancy, history of easy bruising, menorrhagia, or severe epistaxis. Patients with evidence of reduced bone density and those on maintenance corticosteroid therapy were also excluded because of the association between heparin treatment and osteoporosis (6). Iloprost administration within 3 months of the study was not permitted. All patients were taking at least one oral anti-Raynaud's agent, such as a calcium channel blocker (mainly nifedipine or diltiazem, n = 14), angiotensin converting enzyme inhibitor (n = 8) a 5-hydroxytryptamine blocker (ketanserin, n = 3) or fish oil capsules (maxepa, n = 10), and heparin was added onto this treatment. The study was approved by the Institutional Ethical Practices Sub-committee.

Patients were randomly allocated, using previously coded unmarked envelopes, to heparin or control arms. Mean clinical and demographic features were similar for each arm of the study; mean age 44.2 ± 3.1 years for heparin and 48.5 ±

4.2 for control patients; mean duration (years) of Raynaud's symptoms was 14.9 ± 2.5 (heparin) and 9.6 ± 2.8 (control); and time since diagnosis of scleroderma was 7.7 ± 1.8 and 11.1 ± 3.4 for heparin and control arms respectively. None of these differences reached statistical significance.

Assessment of Raynaud's symptom severity

Raynaud's attack severity was recorded by visual analogue scale. Patients scored their symptoms on a pre-selected day every 2 weeks, ranking them between 10 (worst ever) and 0 (no attacks). Objective assessments were made during bimonthly assessments - digital ulcers were counted, routine biochemical and haematological tests performed, including coagulation profiles, and patients were examined.

Non-invasive vascular studies

Nailfold capillary videomicroscopy was performed and graded between I and III (7). Finger blood flow was also estimated using Laser Doppler flowmetry (8). At each visit the recording probe was attached to the pulp surface of the left ring finger. Basal flow was measured over 5 minutes, in a temperature controlled room (23±1°C). Recovery times following cold challenge (hand immersed in a waterproof glove in water at 15°C for 60 seconds) were compared at baseline and at the end of the study. Data were displayed and analysed using Thermosoft image analysis software (EIC Inc., USA).

Circulating adhesion molecule levels

Circulating levels of the soluble isoforms of ICAM-1, VCAM-1 and E-selectin may provide indirect information about activity and severity of underlying disease processes and their organ-based complications (9). Measurements were made by solid-phase ELISA of replicate samples using a commercial assay kit (R and D Systems Inc., USA) according to the manufacturer's instructions.

Results

Clinical end-points

At completion of the study there was significant improvement in Raynaud's attack severity in the patients receiving heparin, but not in the control group. In view of the relatively small number of patients in each treatment arm, overall analysis was used as a starting point for determining a treatment effect. Mean change-from-baseline was statistically significantly different between heparin-treated and control subjects from 4 weeks ($p < 0.05$), and was maximal at 20 weeks ($p < 0.001$), representing a mean improvement of 25 ± 4% of the baseline severity for Raynaud's patients compared with 6 ± 3% change in the control group (Fig. 1). Similar trends of improvement from baseline were observed after heparin treatment for primary (20 ± 5% n = 4) and secondary (26 ± 4%, n = 12) Raynaud's phenomenon. Subset analysis for the patients with scleroderma-associated Raynaud's suggested greater response for limited (27 ± 6%, n = 9) than diffuse (14 ± 9%, n = 3) cutaneous subsets, although formal statistical comparison is precluded by this small number of cases.

Vascular studies

There was no improvement in the nailfold capillaroscopic score or the digital ulcer count, but a trend of improvement in recovery time following cold challenge, determined by laser Doppler flowmetry. This did not reach statistical significance and there was a wide variation in individual measurements. Data for these end-points are summarised in Table I.

Circulating adhesion molecules

Overall mean levels of circulating ICAM-1, VCAM-1 and E-selectin were elevated compared with healthy donor control levels reported previously (9). Subgroup analysis demonstrated substantially higher levels for all three adhesion molecules in patients with Raynaud's secondary to scleroderma. Levels were lower at completion of heparin therapy, but differences were not significantly different (Table II).

Side-effects

Four patients failed to complete the study, including 2 who had started heparin. One patient developed metastatic adenocarcinoma of unknown primary. Heparin was stopped upon diagnosis of the malignancy. Another developed widespread bruising two weeks after starting heparin injections and heparin was discontinued. Two of the control group withdrew from the study. Most patients experienced temporary discom-

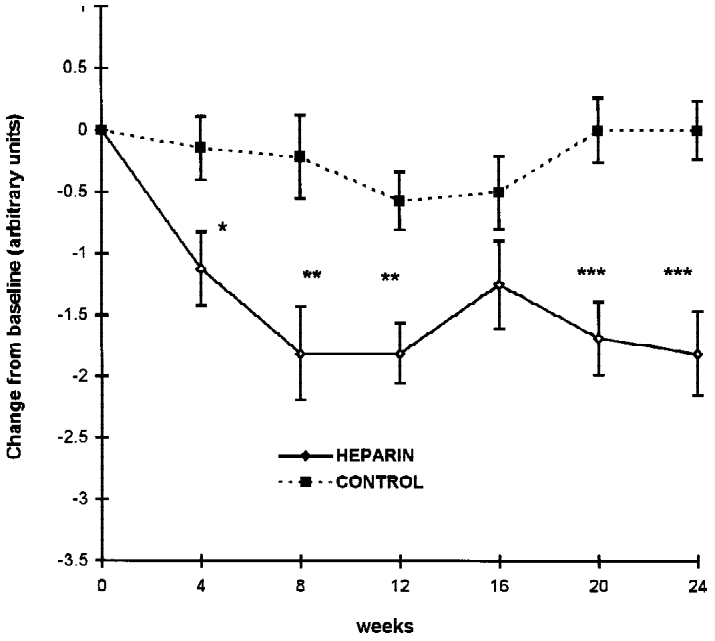

Fig. 1. Improvement in severity of Raynaud's symptoms in patients treated with heparin. Attack severity was assessed by self-reported symptom diary on a visual analogue scale (0 to 10). Mean changes from baseline are shown. Significance levels for each time point (Student's unpaired t-test): *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$.

fort and some bruising at the site of heparin injections. No significant changes in biochemical or haematological indices was observed during heparin treatment.

Discussion

The results of this pilot study suggest that subcutaneous LMW heparin administration is well-tolerated, and may be beneficial in Raynaud's phenomenon. Overall response data are presented, in view of the relatively small number of patients in each treatment arm; however, subgroup analysis (primary Raynaud's, diffuse cutaneous systemic sclerosis or limited cutaneous systemic sclerosis) did not suggest differential response between these groups, although this should be addressed in future studies. The absence of a injectable placebo in the control group necessitates cautious interpretation of treatment effect since there is often a significant placebo response to novel therapies for Raynaud's phenomenon (10). However, the time course of improvement would be unusual for a placebo, and improvement at 20 weeks was significantly greater than at 4 weeks. Another consideration is that differences in baseline characteristics between the heparin and control groups, arising by chance, might have been important. There were differences in the case-mix of the two groups, including a greater proportion of patients with systemic sclerosis in the active treatment arm. It is possible that a study focusing on only one subset, or on either primary or secondary Raynaud's patients would provide more conclusive results.

Heparin therapy was generally well tolerated, and although most patients reported episodic local discomfort or bruising at injection sites. this was inconvenient rather than intolerable. There was no evidence of a clinically significant reduction in bone mineral density after heparin treatment, although formal densitometry was not undertaken, and patients at high risk of osteoporosis had been excluded. This should be assessed in any future larger study. It is disappointing that objective assessments of vasculopathy did not demonstrate statistically significant benefit. For nailfold capillaroscopic assessment this is likely to reflect the difficulty of demonstrating a change based upon our current scoring system with only three grades of severity (7).

Table I. Changes in clinical and laboratory variables in the heparin-treated and control groups. There was a marked improvement in mean Raynaud's attack severity after treatment. This effect was absent in the control cohort. Statistical analysis was by Student's paired t-test.

		Baseline		Post-treatment		Pre- minus post		t-test
		Mean	SEM	Mean	SEM	Mean	SEM	p
Raynaud's severity score								
0 - 10	Heparin	7.5	0.3	5.6	0.4	1.9 (25%)	0.3	0.0002
	Control	7.0	0.3	6.6	0.4	0.4 (6%)	0.2	0.16
LDF recovery time (sec.)								
	Heparin	335	64	256	50	79 (24%)	79	0.50
	Control	201	59	290	61	-89	87	0.20
Digital ulcer count								
	Heparin	2.0	0.7	1.4	1.0	0.9 (45%)	0.7	0.10
	Control	1.0	0.4	1.3	0.8	-0.3	0.9	0.40
Capillaroscopy grade (1-3)								
	Heparin	2.3	0.1	2.5	0.1	-0.2	0.1	0.10
	Control	2.4	0.1	2.3	0.1	0.1 (4%)	0.1	0.30

LDF: laser Doppler flowmetry
% change from baseline indicated for variables showing any improvement during the study period.

Table II. Levels of circulating soluble adhesion molecules at baseline and after heparin treatment. Changes in levels of circulating soluble ICAM-1, VCAM-1 and E-selectin which occurred during heparin therapy did not reach statistical significance. Levels of these adhesins were consistently higher in scleroderma-associated Raynaud's than in primary Raynaud's phenomenon. Comparison within groups or between groups was by Student's paired or unpaired t-test respectively.

A. Change in levels (ng/ml) during the study period for heparin-treated and control groups.

	Baseline			Post-treatment			Paired t-test (p)		
	ICAM-1	VCAM-1	E-selectin	ICAM-1	VCAM-1	E-selectin	ICAM-1	VCAM-1	E-selectin
Heparin									
Mean	363	714	61	313	665	58	0.40	0.98	0.93
SEM	34	84	9	44	75	12			
Control									
Mean	309	540	48	323	583	56	0.31	0.28	0.20
SEM	46	40	12	68	79	12			

B. Differences in base levels (ng/ml) for primary Raynauds and SSc patients.

	Primary Raynaud's			SSc			Unpaired t-test (p)		
	ICAM-1	VCAM-1	E-selectin	ICAM-1	VCAM-1	E-selectin	ICAM-1	VCAM-1	E-selectin
Mean	208	565	42	370	693	62	0.07	0.09	0.1
SEM	39	44	14	31	74	8			

There are several possible mechanisms by which an improvement in Raynaud's symptoms might occur in patients receiving low molecular weight heparin. The relevance of intravascular microthrombi or emboli to Raynaud's phenomenon is uncertain, but these could be influenced heparin therapy. Other mechanisms include interaction with a variety of cytokines and growth factors including the heparin binding group of fibroblast growth factors (11), and chemokines such as monocyte chemoattractant protein-1 (12). Heparin also has a less well understood immunosuppressive effect in animal experiments, perhaps through alterations of lymphocyte trafficking (13). Modulation of endothelial cell properties, for example reduction of endothelin-1 (ET1) release and promotion of prostacyclin synthesis has also been reported (14). An effect on the vasoconstrictor peptide ET1 may be responsible for the antihypertensive properties of heparin (15). Heparin may also up-regulate endothelial cell expression of superoxide dismutase, providing protection from oxidant stress occurring in Raynaud's phenomenon and scleroderma.

In conclusion, our results provide preliminary evidence that LMW heparin may be a worthwhile therapeutic option for severe Raynaud's phenomenon. If confirmed in future studies, then a clinically significant beneficial effect on symptoms may justify its use, and allow its disease-modifying properties in scleroderma to be more fully explored.

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