A Randomised, Double Blind, Placebo-controlled Study to Determine the Efficacy of Immune Modulation Therapy in the Treatment of Patients Suffering from Peripheral Arterial Occlusive Disease with Intermittent Claudication

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Objectives: this study examined the effect of immune modulation therapy (IMT) on claudication distances.

Materials and methods: a double-blind placebo controlled trial was performed on patients with disabling intermittent claudication with randomisation stratified for short and long distance IC. For IMT, following exposure to UV light, oxidation and 42.5°C, 10 ml of citrated autologous blood was administered by intra-muscular injection. One course consisted of 6 injections in 3-weeks followed by 3-weeks rest. Patients received 2, 3 or 4 courses depending on response. The primary end-point was the number of responders (>50% increase in initial claudication distance (ICD)) in each group. Secondary end-points included percentage changes in ICD and change in quality of life.

Results: at week 24, there were more responders in the IMT group (20/31, 65%) compared to placebo (16/39, 41%) (p = 0.06). In the subgroup of short distance claudicants this difference reached significance (IMT 17/26, 65%) (Placebo 12/33, 36%) (p = 0.04). The median increase in ICD was significantly greater in the IMT group (81%) compared to placebo (44%, p = 0.04). These results were supported by quality of life measurements.

Conclusions: IMT is a safe and apparently effective treatment for patients with short distance claudication.

Key Words: Peripheral arterial occlusive disease; Intermittent claudication; Immune modulation therapy.

Introduction

Intermittent claudication (IC) is the most common symptom of peripheral arterial occlusive disease (PAOD), affecting between 1–9% of the population, increasing with age.1,2 Patients with IC can be severely impaired in their ability to perform daily activities3 and have a lower quality of life.4 Current treatments include risk factor modification, exercise therapy, balloon angioplasty, medication and surgery.

Vascular physicians have long advised patients with IC to “stop smoking and keep walking”.5 There is longstanding evidence that a well structured supervised exercise programme can be of benefit,6-8 however, patient compliance can be poor.9 Balloon angioplasty is a popular choice of treatment, but not all patients are suitable and even with good patient selection, there is technical failure to recanalise and/or dilate a lesion in 10–20% of cases.10 Up to 2% will require surgery, thrombolysis, embolectomy or repair of false aneurysm and there are risks of amputation (0.3%) and death (0.17%).10 Surgical reconstruction is more durable than angioplasty but again there are significant risks of early and late complications. In consequence, operations are reserved for patients with more severe symptoms.9 Many pharmacological treatments have been tried for the treatment of IC, though without major benefit.11

Immune modulation therapy (IMT) is a new therapeutic approach for treating IC and involves the administration of autologous blood components following their ex vivo processing by exposure to thermal and oxidative stress.12 Both open-label and controlled clinical studies have indicated that IMT may be helpful in relieving symptoms of claudication.13 The exact mechanism of therapy is under investigation. However, pre-clinical and clinical data support a role for IMT in improving endothelial function13 and in

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reducing levels of inflammation in pre-clinical models of atherosclerosis\textsuperscript{14} and Th1-driven models of inflammation.\textsuperscript{15,16}

Patients with PAOD have a high prevalence of coronary artery atherosclerosis,\textsuperscript{18} suggesting widespread vascular disease.\textsuperscript{19} Endothelial dysfunction has been demonstrated in the brachial artery of patients with known coronary artery disease and diabetes.\textsuperscript{17} Endothelial dysfunction may be, in part, due to inflammation caused by chronic ischaemia and reperfusion.\textsuperscript{20} IMT reduces inflammatory responses in animal models\textsuperscript{13} and protects against injury following ischaemia and reperfusion.\textsuperscript{21}

Improvements in endothelial function following IMT have been demonstrated by an increased vasodilatory response to acetylcholine in rabbit arterial ring preparations.\textsuperscript{13} This improvement in vasodilatory function is further supported by the detection of an increase in endothelium dependent skin blood flow in patients with severe Raynaud’s syndrome following a course of IMT.\textsuperscript{22} IMT also increases the rate of recovery of skin blood flow following occlusion in patients with PAOD.\textsuperscript{13}

Pre-clinical work in LDL receptor deficient mice showed that aortic lipid deposition, was reduced following treatment with IMT, compared to untreated mice.\textsuperscript{23} The authors proposed that as this study demonstrated reduction in the development of atherosclerosis in its early stages, in a model of human familial hypercholesterolaemia, IMT may induce regression of established atherosclerotic lesions.

Thus, in patients suffering from IC as a result of PAOD, IMT could stem the progression of atherosclerosis, reduce inflammatory responses and improve endothelial function, leading to increased microvascular blood flow in the claudicating muscle and improved walking distance.

\section*{Patients and Methods}

This randomised double-blind placebo-controlled parallel group study was performed in two centres in the U.K.; Department of Vascular Surgery, Bristol Royal Infirmary and Department of Medicine, Ninewells Hospital and Medical School, Dundee. Approval to conduct the study was obtained from the Local Research Ethical Committees and the study was conducted in accordance with the Declaration of Helsinki and International Committee for Harmonisation Good Clinical Practice guidelines. All patients gave written informed consent prior to participation.

Between May 1998 and September 2000, 92 patients were consented to participate. Each centre recruited 46 patients. All entrants to the trial had stable IC of at least three months duration. A clinical diagnosis of atheromatous occlusive disease was supported by a resting ankle brachial pressure index of less than 0.8 in either or both legs. The distances walked before onset of pain, initial claudication distance (ICD) and before stopping due to pain, absolute claudication distance (ACD) were assessed using a standardised constant load treadmill test (2 km/h, 10\% incline). At entry, a minimum of 2 and maximum of 4 treadmill tests were performed and the ICD of the most recent 2 tests had to have a variance of less than 25\%. Exclusions at this stage included: rest pain, ulcers and/or gangrene; diminished walking performance for any reason other than arterial disease (e.g. shortness of breath, angina); vascular surgery or angioplasty within 3 months; anticipated vascular intervention during course of study; myocardial infarction within 6 months; severe concomitant diseases including haemorrhagic and coagulation disorders; concomitant use of vasoactive therapies such as prostacyclin analogues, niconaril, cinnarazine, naftidrofuryl, nicotine acid derivatives, oxpentifylline and thyroxamine; use of immunosuppressants, cytotoxic agents, including systemic corticosteroids, photosensitising agents and standard unfractionated heparin. ACE inhibitors, nitrates and calcium channel blockers were permitted provided that drug and dosage had remained stable in the 3 months prior to the study. Aspirin was permitted. Concomitant drug therapy remained unchanged during the study where feasible. Patients had previously received advice on risk factors such as smoking cessation, diet and exercise as part of their normal management. No further advice was given during the study period.

Randomisation to active agent or placebo was computer generated and stratified for diabetes and walking ability to ensure homogeneity between treatment groups. Patients were classified as being either short distance claudicants, ACD less than 100 m, or long distance claudicants, ACD between 100 m and 300 m. Forty-three were randomised to receive IMT and 49 placebo.

For patients receiving IMT, a 10 ml aliquot of venous blood was drawn from the antecubital fossa, into 2 ml of anti-coagulant sodium citrate solution, 3.8\% and placed in a disposable vessel (VC7002, Vasogen Inc. Toronto, Canada). It was then inserted into the Vasogen VC7001 medical device. Within the device the blood was exposed to controlled levels of UV light (wavelength: 253.7 nm), oxidative stress (a gas mixture of medical oxygen containing 14.5 \pm 1.0 \mu g/ml of ozone, at a flow rate of 240 \pm 24 ml/min) and thermal stress (42.5 \pm 1.0°C), for a period of 3 min.
Following an injection of local anaesthetic 10 ml of the treated blood was administered to the patient by intramuscular injection into the upper gluteal region. Similarly a 10 ml blood sample was collected from patients allocated to receive placebo. However, this sample was discarded and a 10 ml intra-muscular injection of warm sterile saline administered, also following local anaesthetic. In order to maintain the blindness of the process, there were two investigators. One was responsible for handling the blood samples and administering injections, whilst the other performed the venepunctures and study assessments. The patients were also blinded during treatments. Patients received between 12 and 24 injections of either IMT treated autologous blood or placebo. After receiving 12 injections, non-responders (as judged by a less than 30% improvement in ICD during treadmill testing) received an additional 6 injections, which could be repeated again if no response was achieved.

Treadmill tests were performed at baseline and at 3 and 9 weeks following each course of treatment. Quality of life was assessed using the Short Form 36 (SF-36) at the start and finish of the study. The primary end-point was whether a cladribic responded, as defined by a greater than 50% increase in ICD over baseline. The number of responders in each treatment group was compared using the Fisher’s exact test. Assuming a 10% placebo response rate, 40 evaluable patients in each treatment group were needed to detect a 30% difference in response rate between treatment groups with a 90% power and 5% level of significance ($p < 0.05$).

The secondary end-points were changes in ICD and ACD. These data were expressed as percentage increase from baseline. The SF36 quality of life questionnaires were scored, coded, summed and transformed. The results for each health domain as well as the standardised physical and mental components were compared for treatment effect. Analysis for safety was performed on adverse events using the Coding System Thesaurus for Adverse Reaction Terms (COSTART). Each event was coded into one of the following categories: body as a whole, cardiovascular system, digestive system, haemic and lymphatic systems, metabolic and nutritional disorders, musculoskeletal system, nervous system, respiratory system, skin and appendages, special senses, urogenital tract. The occurrence rate and severity for each category was then compared between treatment groups.

Following examination for prevalence of normal distributions, parametric data (age, weight, height, blood pressure, ankle brachial pressure index) were summarised as means and compared using the independent $t$-test, whilst non-parametric data (duration of symptoms, ICD and ACD, quality of life and safety) were summarised as medians and analysed using the Mann–Whitney $U$-test. Results were considered significant at the 5% level ($p < 0.05$).

The design of the trial was such that if a patient “responded” then subsequently deteriorated they were considered to have reached an end-point and terminated the study. For this reason, along with other patients lost to follow-up, the number of patients in the study decreased over time (Fig. 1).

Results

Of the 92 patients enrolled in the study, 7 patients were excluded from analysis prior to unblinding due to major protocol violations (Fig. 1) during the first 12-week period. This left 85 patients in the intention-to-treat (ITT) population. The randomised distribution of these patients is given in Table 1. At baseline there were no significant differences between groups in baseline demographic and efficacy characteristics (Table 2).

At week 24, after all treatments had been administered, intention to treat analysis of the primary end-point showed a greater number of responders in the IMT group (20/31, 65%) compared to placebo (16/39, 41%) which approached statistical significance ($p = 0.06$, Fisher’s exact test). In the subgroup of patients with short distance claudication there was a significantly greater number of responders in the IMT group (17/26, 65%) compared to placebo (12/33, 36%), ($p = 0.04$, Fisher’s exact test).

The median ICD and ACD results for the ITT population are given in Table 3. At week 24, median ICD had increased by 81% in the IMT group compared to 44% in the placebo group ($p = 0.04$, Mann–Whitney $U$-test). At the same time-point, median ACD also increased in favour of IMT, however, this did not reach statistical significant (IMT 50%, placebo 30%, $p = 0.07$ Mann–Whitney $U$-test).

Quality of life at the end of the study, as measured by the standardised physical component (Table 4) was significantly better in the IMT treated group compared to the placebo group ($p = 0.041$, Mann–Whitney $U$-test). This was predominantly due to a significant difference in favour of IMT in the physical role domain ($p = 0.03$, Mann–Whitney $U$-test). The pain index and vitality domains also improved significantly over time for the IMT group ($p = 0.03$, Wilcoxon $t$-test) however the difference between groups did not reach statistical significance ($p = 0.06$, Mann–Whitney $U$-test). The IMT treated group scored significantly higher in the mental health index domain compared to placebo.
Fig. 1. Participant flow.

* Responders at week 12 who became non-responders at week 18 exited the study at this point and thus had no week 24 assessment, as per protocol.

** Breathlessness n = 1, Angina n = 1, Back pain n = 1.

*** Decreased n = 1, withdrew consent n = 1, loss to follow-up n = 1.

' Withdrawed consent n = 1.

LOCF = Last observation carried forward.
group \( p = 0.02 \), Mann–Whitney \( U \)-test) although this was not reflected in the standardised mental component \( p = 0.2 \), Mann–Whitney \( U \)-test). No significant differences were noted in the general health perception, social functioning, emotional role and health transition domains.

### Adverse Events

In total 518 adverse events were reported during the study. All except a small minority were insignificant. A preponderance of events (IMT 43%, placebo 46%) occurred in the “body as a whole category” with symptoms of influenza being the main complaint. The remaining 288 events were evenly split between the other 10 COSTART categories and were also evenly distributed between treatment groups. The most severe cardiac event reported was angina pectoris, but again there was no difference in the occurrence rate or severity between IMT and placebo. No significant irregularities were noted in laboratory parameters. Two patients died during the study, one of peritonitis and pneumonia (placebo) and a second of a perforated duodenum and acute liver failure, secondary to past alcoholism (IMT). Neither death was related to treatment.

### Discussion

The Transatlantic Inter-Society Consensus (TASC) recommends that to be valid, a trial assessing a new treatment for IC must have parallel groups, be randomised and double blind. As the symptom under scrutiny is claudication, change in walking performance should be a primary end-point and assessed objectively. Constant-load or graded treadmill testing is an acceptable means to do this. Quality of life should be assessed using a questionnaire validated for this group of patients.\(^{11}\) Although the TASC report was not available at the time this study was designed it complies well with the recommendations.

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**Table 1. Randomisation distribution, ITT data set.**

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Baseline ACD</th>
<th>Diabetes Yes/No</th>
<th>Placebo (%)</th>
<th>IMT (%)</th>
<th>Total (%)</th>
</tr>
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<tbody>
<tr>
<td>&lt; 100 m</td>
<td>Yes</td>
<td>5 (11)</td>
<td>5 (13)</td>
<td>10 (12)</td>
<td></td>
</tr>
<tr>
<td>&gt; 100 m</td>
<td>Yes</td>
<td>1 (2)</td>
<td>0 (0.0)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>&lt; 100 m</td>
<td>No</td>
<td>34 (74)</td>
<td>29 (74)</td>
<td>63 (74)</td>
<td></td>
</tr>
<tr>
<td>&gt; 100 m</td>
<td>No</td>
<td>6 (13)</td>
<td>5 (13)</td>
<td>11 (13)</td>
<td></td>
</tr>
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</table>

**Table 2. Baseline demographic and efficacy characteristics, ITT data set.**

<table>
<thead>
<tr>
<th>Randomisation</th>
<th>Placebo (n = 46)</th>
<th>IMT (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male:female)</td>
<td>24 : 22</td>
<td>25 : 14</td>
</tr>
<tr>
<td>Race – Caucasian (n)</td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Weight (kg) (mean)</td>
<td>74</td>
<td>76</td>
</tr>
<tr>
<td>Height (cm) (mean)</td>
<td>165</td>
<td>168</td>
</tr>
<tr>
<td>Current or past smoker (n)</td>
<td>36 (78%)</td>
<td>31 (80%)</td>
</tr>
<tr>
<td>Diabetes (n)</td>
<td>6 (13%)</td>
<td>5 (13%)</td>
</tr>
<tr>
<td>Systolic BP (mean)</td>
<td>148</td>
<td>159</td>
</tr>
<tr>
<td>Diastolic BP (mean)</td>
<td>76</td>
<td>77</td>
</tr>
<tr>
<td>Duration of Fontaine stage II (months)</td>
<td>37 (16–62)</td>
<td>42 (24–99)</td>
</tr>
</tbody>
</table>

**Table 3. Median ICD and ACD, ITT data set.**

<table>
<thead>
<tr>
<th>Placebo (n = 46)</th>
<th>IMT (n = 39)</th>
<th>Mann–Whitney ( U )-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (Inter-quartile range)</td>
<td>Median (Inter-quartile range)</td>
<td>( p ) value</td>
</tr>
<tr>
<td>ICD (m)</td>
<td>33 (23–46)</td>
<td>33 (22–53)</td>
</tr>
<tr>
<td>Week 6</td>
<td>38 (21–62)</td>
<td>46 (29–67)</td>
</tr>
<tr>
<td>Week 12</td>
<td>41 (23–60)</td>
<td>54 (27–73)</td>
</tr>
<tr>
<td>Week 18</td>
<td>51 (22–72)</td>
<td>56 (36–83)</td>
</tr>
<tr>
<td>Week 24</td>
<td>47 (30–76)</td>
<td>69 (38–93)</td>
</tr>
<tr>
<td>Week 30</td>
<td>48 (27–74)</td>
<td>76 (37–97)</td>
</tr>
</tbody>
</table>

**Table 4. Quality of life, ITT data set.**

<table>
<thead>
<tr>
<th>End of study</th>
<th>Placebo (n = 46)</th>
<th>IMT (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQ range)</td>
<td>Median (IQ range)</td>
<td>( p ) value</td>
</tr>
<tr>
<td>Role ± physical</td>
<td>25 (0–75)</td>
<td>75 (25–100)*</td>
</tr>
<tr>
<td>Mental health index</td>
<td>76 (66–84)</td>
<td>84 (72–92)*</td>
</tr>
<tr>
<td>Standardised physical component</td>
<td>36 (27–43)</td>
<td>40 (33–48)*</td>
</tr>
</tbody>
</table>

* Significant at 0.05 level.
In this study the baseline demographic data is typical for a group of patients suffering with IC, with more males than females, increasing age, a proportion of diabetics and a high occurrence of current or past smoking. The results consistently demonstrate greater symptomatic improvement in the IMT group compared to placebo. Potential mechanisms for these improvements were not directly studied here but support the hypothesis that treatment with IMT may result in increased microvascular blood flow through improved endothelial function and reduced inflammatory responses. It is likely that ability of IMT to reduce atherosclerotic progression had little effect on the outcomes studied here. However, the benefits of this effect on long term mortality and morbidity could be great.

Endothelium dysfunction occurs in patients with diabetes and arterial disease. It is possible that this damage may become too severe for IMT to be effective. Thus a poorer outcome may be expected from patients with severe arterial disease, with diabetes or with both. When there is a possibility that the treatment may be effective in only a subgroup randomisation stratification is recommended to ensure comparability between the active and control groups. The relatively small sample size of this study could have lead to an unequal number of patients with severe arterial disease, diabetes or both in the treatment groups; however, this was successfully overcome by stratifying the randomisation. Due to the small number if diabetic patients recruited, possibly a reflection of the small sample size, it was not appropriate to analyse this group separately thus examining the response to IMT in these patients alone is beyond the scope of this study. However, in contrast to exercise and Cilostazol trials the majority of patients recruited here were short distance claudicants. The increased severity of IC in the study population can be attributed to patients being recruited from two specialist units. Although subgroup analysis of this population was not pre-planned and must therefore be considered exploratory, the improvements seen in the IMT treated short distance claudicants do not support the hypothesis that IMT may be less effective in patients more severe disease. Alternatively when endothelium damage is severe and widespread the potential for systemic improvement and thus symptomatic improvement may be the same or greater.

In this study IMT was associated with an increase in ICD that exceeded the typical increase of 32% seen in similar pharmacological studies. Trials of supervised exercise however, report greater improvements, 170% though compliance is often a problem. Given the potential systemic and symptomatic improvements resulting from treatment with IMT a combination of this therapy and exercise may be advised.

The overall physical improvement in quality of life reflects the increases seen in walking distance. IMT had no significant effect on the standardised mental component although the placebo group deteriorated. This may be a reflection of placebo patients’ having a higher level of dissatisfaction due to less of an improvement in walking ability.

In accordance with the International Committee for Harmonisation Good Clinical Practise Guidelines, any and every change in a patient’s condition was prospectively recorded as an adverse event, whether or not it was considered to be related to the study. The number of adverse events recorded demonstrates rigorous and meticulous data collection. Subsequent analysis using the COSTART system raised no concerns about the safety of IMT.

Conclusion

In this study IMT produced significant increases in walking distances in comparison to the placebo group and these increases were reflected in improvements in the patients’ quality of life. IMT is a safe and apparently effective treatment for patients with disabling IC.

Acknowledgements

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