Assessment of endothelial function in complex regional pain syndrome type I using iontophoresis and laser Doppler imaging

R. Gorodkin¹, T. Moore¹ and A. Herrick¹,²

Objectives. To assess microvascular endothelial function in patients with complex regional pain syndrome type I (CRPS) compared with healthy controls, as measured by iontophoresis of vasoactive chemicals and laser Doppler imaging.

Methods. Microvascular blood flow was stimulated locally in affected and contralateral limbs of patients with CRPS (n = 17) and in control subjects (n = 16) using iontophoresis of the endothelial-dependent vasodilator acetylcholine (ACh) and the endothelial-independent vasodilator sodium nitroprusside (NaNP). Changes in blood flow were measured using laser Doppler imaging. Comparisons were made between right and left limbs and between patients and controls.

Results. No significant differences in blood flow [expressed as a median percentage increase from baseline (interquartile range)] were detected between affected and contralateral limbs in patients with CRPS for ACh [affected 237 (95–344); unaffected 251 (152–273)] or for NaNP [affected 102 (49–300); unaffected 190 (53–218)]. In addition, there were no significant differences between patients and healthy controls [controls, ACh 216 (119–316); controls, NaNP: 122 (48–249)].

Conclusions. In this pilot study, CRPS was not associated with impairment of microvascular endothelial function. This may be a true result or may reflect the diversity of the CRPS disease process.

Key words: Complex regional pain syndrome, Reflex sympathetic dystrophy, Endothelium, Laser Doppler imaging, Iontophoresis.

Complex regional pain syndrome (CRPS) is a condition of the peripheries, typically occurring after trauma (sometimes minimal) and characterized by severe pain, allodynia, oedema, motor dysfunction and marked autonomic changes. These abnormalities spread beyond the site of the initial injury and are not confined by anatomical boundaries such as nerve innervation. The formal International Association for the Study of Pain (IASP) criteria include the presence of an initiating noxious event, continuing pain disproportionate to the inciting event, evidence at some time of oedema, changes in skin blood flow, or abnormal sudomotor activity in the affected area, and the absence of any coexisting condition that might otherwise account for the symptoms [1]. CRPS has been split into type I (reflex sympathetic dystrophy), in which there is no overt nerve damage, and type II (causalgia) in which there is definite evidence of a nerve lesion [1].

Numerous hypotheses have been proposed to explain the features of CRPS [2–5]. Whatever the pathogenesis of the condition, there is no doubt that vasomotor changes are an integral part of the disease process. Patients often but not always progress from a warm red limb to a cool, atrophic one, and it is well known that control of microvascular blood flow is abnormal in CRPS [4, 6].

Some of the blood flow abnormalities of CRPS are due to sympathetic hypersensitivity following an initial period of reduced sympathetic input [4, 6]. An increase in sympathetic tone causes increased vasoconstriction and hence a reduction in blood flow, and it has recently been suggested that enhanced vasoconstrictor activity in CRPS contributes to hyperalgesia [7]. However, other mechanisms may be involved in the pathophysiology of the vascular abnormalities. It has been demonstrated that free radical infusion produces CRPS-like symptoms [8], and that treatment with free radical scavengers can both reduce the risk of developing CRPS [9] and help in its treatment [10]. Because free radicals damage the endothelium, and the endothelium plays a key role in the regulation of vascular tone, it therefore seems likely that endothelial dysfunction might occur in CRPS. This suggests (regardless of the initial pathogenesis) the possibility of a vicious circle involving altered blood flow, leading to hypoxia, production of free radicals, endothelial damage and further reductions in blood flow. If so, then endothelial-dependent vasodilation of the microvasculature might be impaired.

We tested this hypothesis using iontophoresis (a procedure whereby small quantities of chemicals are driven into the skin using a small electrical current) of acetylcholine (ACh; an endothelial-dependent vasodilator) and sodium nitroprusside (NaNP; an endothelial-independent vasodilator). We have previously applied this technique on patients with Raynaud’s phenomenon and systemic sclerosis [11]. Changes in blood flow were assessed using laser Doppler imaging, a non-invasive method of monitoring blood flow over an area of skin [12].

Patients and methods

Subjects

Seventeen patients with CRPS fulfilling the IASP criteria [1] were recruited, and 16 control subjects were chosen on the basis that...
they had suffered a previous fracture but had not developed CRPS. Clinical characteristics are shown in Table 1, including (in the case of patients with CRPS) whether the affected limb was principally hot or cold. No subjects were known to suffer from cardiovascular disease. All patients and controls were asked to abstain from any vasoactive medication for 1 week prior to the study and from smoking and any caffeine-containing products on the day of the study. In addition, one patient with a spinal cord stimulator was asked not to use it for the 24 h preceding the study. All patients tolerated the procedure well. The study was approved by the Salford and Trafford Local Research Ethics Committee.

<table>
<thead>
<tr>
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<th>CRPS (n = 17)</th>
<th>Controls (n = 16)</th>
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</thead>
<tbody>
<tr>
<td>Median age (yr)</td>
<td>48 (22–69)</td>
<td>37 (19–66)</td>
</tr>
<tr>
<td>Male:female</td>
<td>6:11</td>
<td>5:11</td>
</tr>
<tr>
<td>Median duration of symptoms/time since fracture (range)</td>
<td>5 yr (3 months–20 yr)</td>
<td>7.5 yr (3 months–38 yr)</td>
</tr>
<tr>
<td>Upper/lower limb</td>
<td>8 upper, 9 lower</td>
<td>9 upper, 7 lower</td>
</tr>
<tr>
<td>Mean limb temperature (°C) (s.d.)</td>
<td>Affected 29.2 (3.2), unaffected 29.6 (3.6)</td>
<td>N/A</td>
</tr>
<tr>
<td>Hot/cold limb subgroups (mean thermographic temperature difference between limbs)</td>
<td>4 hot (+0.9°C), 13 cold (–0.8°C)</td>
<td>N/A</td>
</tr>
<tr>
<td>Oedema</td>
<td>8 (2 hot, 6 cold)</td>
<td>0</td>
</tr>
<tr>
<td>Smoking</td>
<td>7 smokers, 10 non-smokers</td>
<td>2 smokers, 14 non-smokers</td>
</tr>
<tr>
<td>Median VAS (interquartile range)</td>
<td>60 (20–90.5)</td>
<td>N/A</td>
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</table>

**Methods**

Subjects were acclimatized for 20 min at 23°C. Once acclimatized, limb temperature in the CRPS subjects was assessed using thermographic imaging, and the temperature difference between the affected and unaffected limb calculated (i.e. affected limb temperature minus unaffected limb temperature). The degree of discomfort was measured using a visual analogue scale (VAS) of 1–100 where 100 was the worst imaginable pain (Table 1) [13]. In patients with CRPS the most severely affected area where contact with the skin could be tolerated was studied, together with a similar area on the unaffected limb. In the control subjects the area to be scanned was chosen to lie close to the previous injury, with the same location on the opposite limb being used as an internal control.

The skin was cleaned using an alcohol wipe and the two 9.5 mm (internal) diameter iontophoresis chambers (connected to an iontophoresis controller) (Moor Instruments Ltd, Axminster, Devon, UK) were attached immediately adjacent to each other. The chamber attached to the positive terminal was filled with 1% ACh solution (Sigma-Aldrich, Dorset, UK) made up with distilled water. The chamber attached to the negative terminal was filled with 1% NaNP solution (David Bull Laboratories Pty Ltd, Victoria, Australia) also made up with distilled water. A coverslip was placed over both chambers to minimize reflection.

**Iontophoresis protocol**

The injured limb was always studied first, immediately followed by the control limb. When the iontophoresis chambers were in place, the limb was placed under a scanning laser Doppler imager (Moor Instruments Ltd, Axminster). The area to be scanned was delineated to include both chambers. Scan time was 43 s with the scan repeating every 45 s. After the baseline scan a 90-s period of iontophoresis at 30 μA was commenced. At the same time a series of 14 further scans was started.

**Analysis**

From the 15 scans the following were calculated for each iontophoresis protocol, i.e. for ACh and NaNP, in both affected and contralateral limbs, for each subject:

- the maximum flux expressed as a percentage increase from baseline (%max)
- the area under the curve (AUC) expressed as a percentage of the baseline AUC (%AUC).

Statistical analysis was carried out using the Wilcoxon signed ranks test for paired samples to compare between left and right limbs in individual subjects. The Mann–Whitney U-test was used to look for differences between groups. Sub-analysis of the ‘hot’ CRPS and ‘cold’ CRPS groups was also carried out. All analysis was carried out using SPSS for Windows (version 10.1 USA).

**Results**

**Intragroup comparisons**

- Control subjects: There were no significant differences in microvascular blood flow responses to ACh and NaNP between right and left limbs within the control group (data not shown).
- CRPS patients: Comparison of microvascular responses in the CRPS group demonstrated no significant differences between the affected and unaffected limbs (Table 2).

**Intergroup comparisons**

- The affected and unaffected limb results from the CRPS group were compared with the ‘combined’ control group. Again, no significant differences were detected (Table 2).
- Subgroup comparisons: When ‘hot’ and ‘cold’ CRPS subgroups were considered separately, again there were no statistically significant differences between affected, unaffected and healthy control limbs (data not shown).

**Discussion**

To our knowledge, microvascular responses to vasoactive agents have not previously been examined in patients with CRPS. This study confirms that the technique is safe and well tolerated in this group of patients, even in those with marked allodynia. Therefore iontophoresis affords a non-invasive method of measuring microvascular responses in patients with CRPS. Most studies with
Iontophoresis have measured blood flow response with single-probe laser Doppler, but this study uses laser Doppler imaging (LDI), i.e., measuring blood flow over an area rather than at a single point. The advantage of this is that measurements are less likely to be influenced by the inherent inhomogeneity of skin microvascular flow.

The study did not demonstrate any abnormality of endothelial function in patients with CRPS compared with the control group. However, there are some methodological issues, which may be relevant.

1. This was intended as a pilot study, so the numbers involved were relatively small. CRPS is in itself a very heterogeneous condition, affecting differing regions of the body and presenting with variable features [14], and so larger studies would be needed to give a more conclusive result. In particular, a larger group would allow better analysis of hot versus cold differences, although it should be borne in mind that many patients describe very variable limb temperatures.

2. It would have been of interest to compare baseline perfusion values between the two groups as it is possible that there is an altered baseline perfusion in patients with CRPS (in keeping with the thermography results), but a technical problem with the laser Doppler scanner calibration prevented this. This problem did not, however, influence the percentage increases in results reported here.

3. In addition to this variation in the clinical presentation, there is a considerable degree of physiological variation, even amongst healthy controls. Blood flow is not the same in all areas of the skin. It has been demonstrated to be higher in the face compared with the trunk and limbs, and lowest over the buttocks, and both dorsal and plantar aspects of the feet [15]. Not only is baseline perfusion as measured by laser Doppler imaging variable in different parts of the body, but the effect of iontophoresis is also diverse. Gardner-Medwin et al. [16] demonstrated site-dependent vasodilation to ACh iontophoresis. Palmar sites had higher baseline flux but no response to ACh, while dorsal hand and volar forearm sites had lower baseline flux but were able to vasodilate in response to ACh. Therefore, regional blood flow variability in both baseline and degree of response to ACh is a further potential confounding factor in interpreting the results between groups of patients.

4. Iontophoresis is known to produce axon reflex vasodilatation. Preliminary studies with distilled and deionized water suggested that this was not an issue for healthy controls using the experimental protocol above. In theory, patients with CRPS might have more pronounced axon reflex vasodilatation as this is a component of neurogenic inflammation, which has been implicated in the pathogenesis of CRPS [17]. Birklein et al. [18] looked at the effects of cutaneous histamine application but found no differences in the degree of response in patients with CRPS compared with control subjects. A second study by the same group, however, did demonstrate significantly greater axon reflex vasodilatation in the CRPS group [19]. It is therefore impossible to rule out axon reflex vasodilatation as a cause of the increased blood flow in the CRPS group, but in practice its effect is likely to be minimal at the low current used. It is also worth noting that at no stage did the vasodilation associated with each iontophoresis chamber extend to ‘contaminate’ results in the adjacent chamber.

5. Another methodological issue is the reproducibility of iontophoresis, which is known to be poor using the single-point technique [11]. Although we have not carried out reproducibility studies, Morris and Shore [20] reported that iontophoresis studies using LDI gave a coefficient of variation of 12.1% compared with 34–42% with single-point measurement. This suggests improved reproducibility when LDI is used as an alternative to single-point measurement, although further reproducibility studies with LDI are required.

In summary, the lack of significant results implies that there is no underlying endothelial dysfunction. As most of the published data supporting our hypothesis relate to free-radical mediated injury, it is possible that the free radicals exert their effect via a non-endothelial pathway. However, bearing in mind the difficulties implicit in the measurement of physiological parameters in this highly variable condition, it is also possible that experimental limitations have obscured a true difference. Larger studies will need to be carried out to decide this.

<table>
<thead>
<tr>
<th>CRPS affected limb (n = 17)</th>
<th>CRPS unaffected limb (n = 17)</th>
<th>All controls (n = 16)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>% ACh max.</td>
<td>237 (95–344)</td>
<td>251 (152–273)</td>
<td>216 (119–316)</td>
</tr>
<tr>
<td>% NaNP max.</td>
<td>102 (49–300)</td>
<td>190 (53–218)</td>
<td>122 (48–249)</td>
</tr>
<tr>
<td>% ACh AUC</td>
<td>87 (24–158)</td>
<td>77 (40–111)</td>
<td>61 (41–118)</td>
</tr>
<tr>
<td>% NaNP AUC</td>
<td>47 (26–179)</td>
<td>73 (29–124)</td>
<td>73 (25–141)</td>
</tr>
<tr>
<td>Affected vs unaffected</td>
<td>0.831</td>
<td>0.971</td>
<td>0.829</td>
</tr>
<tr>
<td>Affected vs all controls</td>
<td>0.309</td>
<td>0.943</td>
<td>0.640</td>
</tr>
<tr>
<td>Unaffected vs all controls</td>
<td>0.687</td>
<td>0.885</td>
<td>0.971</td>
</tr>
</tbody>
</table>

**Key messages**

- Endothelial function in CRPS type I appears to be normal.
- Larger studies are needed in this heterogeneous patient group.

**Acknowledgements**

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The authors have declared no conflicts of interest.

**References**