Postural vasoconstriction and leg ulceration in homozygous sickle cell disease

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1. Chronic leg ulceration is a major cause of morbidity in patients with homozygous sickle cell disease; the ulcers commonly resolve on bed rest. We have therefore compared the cutaneous vascular response to dependency in three groups of eight patients with sickle cell disease (those with an active ulcer, with an ulcer scar and with no history of ulceration) and in eight subjects with normal haemoglobin and no history of leg ulceration.

2. We monitored, with a laser Doppler flowmeter, the change in red cell (erythrocyte) flux induced in the skin of the leg, at two sites proximal to the malleoli, with the leg horizontal and 5 and 10 min after moving the leg to the dependent position.

3. With the leg horizontal, mean cutaneous red cell flux was substantially higher in normal skin of patients with sickle cell disease than in normal subjects and was higher still at the site of the ulcer or scar. On dependency, red cell flux fell not only in normal subjects but also in the patients with sickle cell disease, both in the normal skin and at the site of the ulcer or scar; there was no difference in any group between the 5- and 10-min values. The fall in red cell flux in normal skin of patients with sickle cell disease was smaller than in normal subjects when considered as a percentage of the control values (32%, 36%, 30% and 61% respectively in sickle cell patients with an active ulcer, with an ulcer scar and with no history of ulceration and in normal subjects), but in absolute terms the falls in red cell flux were similar in sickle cell patients and normal subjects. By contrast, the fall in red cell flux at the ulcer or scar site was greater than in normal skin from sickle cell patients whether considered as a percentage of the control value (48% and 49% respectively in those with an active ulcer or ulcer scar) or in absolute terms.

4. We propose that high resting perfusion is important in patients with sickle cell disease to maintain normal integrity of cutaneous tissue and that pronounced vasoconstriction on dependency hinders the healing and encourages recurrence of leg ulcers.

INTRODUCTION

Chronic leg ulceration is a major cause of morbidity in homozygous sickle cell (SS) disease. In Jamaica, the cumulative incidence of leg ulceration reaches a plateau of 75% of all patients with SS disease at the age of 30 years [1]. Leg ulcers typically occur around the malleoli. They may result from minor trauma, but about 50% apparently occur spontaneously. They often deteriorate on prolonged standing and generally heal with prolonged bed rest [1]. These observations suggest that an impaired cutaneous circulation and, particularly, the cutaneous vascular response to dependency may be important in the genesis and maintenance of these lesions.

In normal subjects, lowering the leg to the dependent position produces cutaneous vasoconstriction in that leg. This is, in part, a myogenic constrictor response to the rise in intravascular pressure, but is mainly attributable to a local, neurally mediated vasoconstrictor response that is thought to occur via an 'axon reflex' (venoarteriolar reflex) initiated by the rise in venous pressure [2]. The increase in precapillary resistance limits the rise in capillary pressure resulting from the height of the column of blood between the heart and the dependent extremity and is thought to protect against oedema formation.

In chronic venous insufficiency and in diabetes, which are also associated with ulceration in the lower extremities, this venoarteriolar reflex is reduced [3, 4]. It has also been reported to be absent in SS disease [5]. However, this report was based on experiments on only two female SS patients. One had a history of leg ulceration while the other did not, and there was no mention of when the experiments were performed relative to the menstrual cycle: it is known that in normal subjects the venoarteriolar reflex is greatly attenuated in the luteal phase [6].

We have therefore carried out a larger study on male SS patients so as to avoid any influence of the menstrual cycle. We monitored resting cutaneous red cell (erythrocyte) flux (RCF) in the malleolar...
region and its response in the normal skin and affected skin of SS patients who had an ulcer or scar and in the normal skin of SS patients and of control (AA) subjects with normal haemoglobin who had no history of ulceration. Our aim was to test the hypothesis that abnormal regulation of cutaneous circulation contributes to leg ulceration in SS disease. All experiments were performed in the morning to avoid the different patterns of within-day variability in cardiovascular baselines that occurs in SS and AA subjects [7]. The results have been reported in brief to the British Microcirculation Society [8].

MATERIALS AND METHODS

Subjects

The SS patients were recruited from those attending the Sickle Cell Clinic of the University Hospital of the West Indies for routine appointments. Control subjects with a normal haemoglobin (AA) genotype were recruited from the Cohort Study [9] and from volunteers in the university community. All were male and predominantly of West African origin, the AA subjects being matched for age and racial origin with the SS patients. The subjects were divided into four study groups, each comprising eight subjects. These were: SS\textsubscript{a}, those with active leg ulcers <5 cm diameter; SS\textsubscript{n}, those with scars of previously healed ulcers; SS\textsubscript{m}, those with no history of leg ulcers; and AA, subjects with no history of leg ulcers. There were no age differences between the groups, mean (±SD) ages in groups SS\textsubscript{a}, SS\textsubscript{n}, SS\textsubscript{m} and AA being 25.1±5.9, 25.4±6.1, 25.2±5.1 and 22.6±3.2 years respectively. All subjects were normotensive; none had evidence of cardiac or renal failure. The patients were in the 'steady state' of the disease and had not had a painful crisis within the preceding 4 weeks or a blood transfusion within 3 months before this study. These experiments were carried out in accordance with the Declaration of Helsinki (1989) and approved by the local ethics committee. Each subject gave informed consent to participate in the study.

Methods

We monitored cutaneous RCF simultaneously from two sites by using a dual-channel laser Doppler flowmeter (MBF3D; Moor Instruments, Axminster, Devon, U.K.) and standard probes (P1). RCF is the product of the velocity and concentration of moving erythrocytes in a hemisphere of approximately 1 mm diameter in the tissue sample volume, and is considered to be directly related to blood flow [10]. This particular Doppler flowmeter uses two 3-mW semiconductor diodes operating at a wavelength of 780–810 nm. This near-infrared wavelength is not as strongly absorbed by melanin as the red wavelength of other Doppler flowmeters and the higher level of backscattered light results in an increased sensitivity for darkly pigmented skins [11]. It was therefore a suitable instrument for the present study.

One probe (RCF1) was placed 1 cm from the periphery of the active ulcer in group SS\textsubscript{a} or on the middle of the scar in group SS\textsubscript{m}. In both groups the second probe (RCF2) was placed 6–10 cm from RCF1, on apparently normal skin and in a plane that was the same proximal distance from the malleolus RCF1. In groups SS\textsubscript{n} and AA, the probes were placed on the medial and lateral sides of the leg approximately 4 cm proximal to the malleoli. Arterial blood pressure was monitored continuously and non-invasively with a Finapres instrument (Ohmeda 2300; Biomedical International, Miami, FL, U.S.A.) and standard probes (P1). RCF is the product of the velocity and concentration of moving erythrocytes in a hemisphere of approximately 1 mm diameter in the tissue sample volume, and is considered to be directly related to blood flow [10]. This particular Doppler flowmeter uses two 3-mW semiconductor diodes operating at a wavelength of 780–810 nm. This near-infrared wavelength is not as strongly absorbed by melanin as the red wavelength of other Doppler flowmeters and the higher level of backscattered light results in an increased sensitivity for darkly pigmented skins [11]. It was therefore a suitable instrument for the present study.

At the end of the experimental session on all AA subjects, a sample of venous blood was taken for analysis. Routine haematological indices were measured in a Coulter S plus 4 (Coulter Electronics, Hialeah, FL, U.S.A.) and packed cell volume was determined by centrifugation. For the SS patients, comparable data were obtained on the day of the study or were obtained from the Sickle Cell Clinic database using analyses done in the steady state before the study. In the SS patients, fetal haemoglobin (HbF) levels, measured by alkali desaturation [13], was also obtained from the Sickle Cell Clinic database. The database was used for many of the SS patients, so as to avoid taking another blood sample on the day of the study. All of the SS patients are closely monitored by the Sickle Cell Clinic: direct comparisons made for 15 patients between haematological values taken from the database and presented in this study were fully comparable (P<0.05, paired t-test) with those measured in new samples taken in the steady state after the study. It is also unlikely that HbF changed between the time of measurement and the time of the study given there is no age-related trend in HbF in male SS patients over 14 years old [1].

Protocol

All studies were conducted in the morning (see [7]) at a room temperature of 24–25°C and relative humidity of 60%. Each experiment began with a 30 min period during which the subject rested in a supine position with the legs held horizontal on a hinged couch. This was followed by a 20-min experimental period. The legs were then passively lowered
until the toes were approximately 50 cm below horizontal and remained dependent for 10 min. The legs were then passively returned to the horizontal position for a further 6 min period. Data were collected on to the computer for analyses off-line for 30 s periods at 2 min intervals when the legs were horizontal and at 1 min intervals when the legs were dependent.

Analyses

For each subject, resting RCF, MAP and HR recorded over the last 10 min of the initial rest period (see above) were averaged to give mean resting values and were then pooled to give a mean (±SD) for each subject group. During dependency, mean values of each variable for each subject group were calculated for the fifth and tenth minute of dependency to represent the early and late phases of the response. RCF1 and RCF2 did not differ between the two normal skin sites in groups SSn and AA, so values at these two sites were averaged and the average value used for further analyses.

Mean measurements with the legs horizontal and at the fifth and tenth minute after lowering the legs were compared within subjects and between the four groups by using analysis of variance with one between-subjects factor. MAP, HR and RCF all appeared to be normally distributed. Variances of MAP and HR were approximately constant between groups, but the variance of RCF in AA subjects was significantly lower than in the three SS groups (P<0.001, using Levene's test, see Fig. 1). The between-subjects effect for RCF was therefore tested by the Brown-Forsythe test, which does not assume homogeneity of variance; pairwise contrasts were performed using separate variance estimates, the P-value being adjusted using Dunnett's T3 procedure. The within-subject main effects and interaction effects were tested using the Wilks' lambda test [14].

In the SSn and SSa groups, differences between normal and abnormal (scarred or ulcerated) skin and the interactions with group and dependency were assessed by using the multivariate approach to two-way within-subject analysis of variance with one between-subjects factor. Interactions were further investigated by looking at simple effects (i.e. looking at the effects of one variable within each of the levels of the other), the P-value being adjusted using Bonferroni's technique.

Mean haematological data for the four groups were compared using one-way analysis of variance. Kramer's modification of Tukey's HSD procedure was used to adjust P-values for the pairwise comparisons.

Throughout, 5% was taken as the level of statistical significance.

RESULTS

RCF in normal skin of AA and SS subjects

There was no interaction between the four groups and dependency for RCF (P = 0.68), indicating that the effects of dependency were the same for each group, and the group effects were the same, regardless of leg position. Main effects only are described below.

There was a significant difference in mean RCF between the four groups (P<0.001): RCF in the normal skin of SSn and SSa patients was higher than in AA subjects (P<0.05 for both comparisons, see Fig. 1). The difference between RCF in the normal skin of SSn and AA subjects was of borderline significance (0.05<P>0.1).

On dependency, mean RCF in normal skin fell significantly (P<0.001) from resting horizontal values, with no significant difference between values recorded at the fifth and tenth minute (P = 1.00). The absolute fall in mean RCF was similar in SS patients and AA subjects (i.e. no interaction) so that, even during dependency, SSn and SSa patients continued to have higher RCF than AA subjects (Fig. 2). The fall in RCF expressed as percentage change (±SD) from baseline was 32±15% in SSa patients, 36±22% in SSn patients, 30±23% in SSn patients and 61±17% in AA subjects.

RCF in abnormal skin (ulcer/scar)

The difference in mean RCF between normal and abnormal skin was similar in the SSn and SSa groups (P = 0.92). There was a significant interaction (P<0.001) between values of RCF in normal and abnormal skin and dependency, meaning that the relationship between RCF values in normal and
abnormal skin changed with leg position. Indeed, RCF at the ulcer/scar site in the SS and SS* groups tended to be higher than in adjacent normal skin when the leg was horizontal, but not at 5 or 10 min dependency (Fig. 2).

As described above for normal skin, mean RCF at the ulcer/scar site in SS and SS* patients fell significantly on dependency (P<0.001), with no difference between the 5- and 10-min values (P = 1.00), but the mean absolute fall at the ulcer/scar site was significantly greater (P<0.001) than at the normal skin site in the same subjects (Fig. 2).

In group SS*, the percentage (±SD) fall in RCF at the ulcer site was 48±23%, compared with only 32±15% at the normal skin site. Similarly, for group SS*, the fall in RCF at the scar site was 49±26%, while that at the normal skin site was 36±22%.

MAP and HR

There was no interaction between the four groups and dependency for MAP (P = 0.59) and HR (P = 0.23). There were no significant differences in mean arterial pressure (MAP) or heart rate (HR) between the groups with the legs horizontal: MAP was 71±10, 72±9, 77±13 and 81±9 mmHg in groups SS*, SS, SS* and AA respectively, whereas HR was 63±13, 65±11, 73±8 and 58±7 beats/min respectively. There were no significant changes in MAP or HR during dependency in any group.

Haematological analyses

Mean erythrocyte count, haemoglobin and packed cell volume were significantly lower in SS patients than in AA subjects (Table 1). There were no significant differences between the different SS groups.

DISCUSSION

This study has described four main features of the leg microcirculation in SS disease. First, cutaneous RCF in the normal skin of the legs in SS patients was significantly higher than in AA subjects. Second, within SS patients, resting cutaneous RCF at the site of an ulcer or scar with the leg horizontal tended to be higher than in normal skin. Third, on dependency, there was a significant fall in RCF in the normal skin site in SS patients, regardless of ulcer history. Fourth, within SS patients with ulcers or scars, the fall in RCF at the site of an ulcer or scar on dependency was greater than in normal skin.

These findings apparently differ in several respects from those of Gniadecka et al. [5]. They reported that RCF proximal to the malleolus was similar in SS and AA subjects when the legs were horizontal. However, as this was based on only two female patients (see Introduction), and given the higher intersubject variability of the RCF values we recorded in SS patients, their observation is not necessarily inconsistent with ours. Gniadecka et al. [5] also reported that the postural vasoarteriolar reflex was absent in SS patients. This disparity is more difficult to explain, although their studies may have been conducted during the luteal phase of the menstrual cycle when the vasoarteriolar reflex is attenuated (see [6]). We can only state that, in a much larger group of male SS patients, a pronounced postural vasoconstriction on dependency was seen in every patient irrespective of their

Table 1. Haematological indices in SS patients and AA subjects. Values are given as means ± SD; n = 8 in each group. NM, not measured; P-values are for comparisons between SS patients and AA subjects by using ANOVA (see text), except for HbF, which is for comparison between groups of SS patients. Abbreviations: SS*, SS patients with active ulcers; SS, SS patients with scars; SS*, SS patients with no history of ulcers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SS*</th>
<th>SS*</th>
<th>SS*</th>
<th>AA patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte count (10¹² cells/l)</td>
<td>2.6±0.7</td>
<td>2.9±0.5</td>
<td>3.1±0.4</td>
<td>5.3±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total haemoglobin (g/dl)</td>
<td>7.4±1.6</td>
<td>8.3±0.8</td>
<td>8.4±1.2</td>
<td>14.4±0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>22.0±4.5</td>
<td>24.5±2.3</td>
<td>25.9±3.8</td>
<td>43.7±2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fetal haemoglobin (%)</td>
<td>3.1±2.4</td>
<td>2.8±1.4</td>
<td>5.0±3.5</td>
<td>NM</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Fig 2. RCF at abnormal and normal skin sites in SS patients and in AA subjects with legs in the horizontal position and at 5 and 10 min dependency. Abbreviations for experiment groups as in Fig 1. Each column shows mean ± SD. Open columns, legs horizontal; shaded columns, 5- and 10-min values.
history of ulceration and of whether measurements were made on normal, ulcerated or scarred skin.

In the present study the raised cutaneous RCF in the normal skin of the leg in SS patients compared with AA subjects is consistent with previous observations of higher forearm cutaneous RCF and higher total forearm blood flow in SS patients than in AA subjects (see [15]). As RCF is a product of erythrocyte concentration and velocity of erythrocytes, a raised RCF may reflect increased volume flow, greater capillary recruitment and/or increased vascularity. Such changes might be expected as a compensatory adjustment to the reduced oxygen delivery caused by chronic anaemia in SS patients. The finding that RCF at the ulcer or scar site was even higher than at normal skin may simply reflect hyperaemia or neovascularization associated with a chronic inflammatory process; neovascularization has been demonstrated in histological studies of the ulcer base in SS disease [16].

If the fall in RCF on dependency is considered in absolute terms, then the fall in the normal skin of SS patients was similar to that in AA subjects. By contrast, if the fall in RCF is considered as a percentage of the baseline RCF with the legs horizontal, the change was substantially less in the SS patients than in AA subjects, simply because the SS patients had a higher baseline RCF. But, regardless of the way the response is expressed, the consequence of this postural vasoconstriction was that RCF in the normal skin of SS patients remained markedly higher than in AA subjects: 2–3 times greater with the legs horizontal and 3–6 times greater with the legs dependent. All of these observations are similar to those of Rayman et al. [4] in their study on diabetic patients. They also noted that RCF in the plantar region of the great toe with the limb dependent was even higher in patients with neuropathy than in those with no recognized neuropathy, the former being the very group that is more susceptible to leg ulceration [4]. Rayman et al. [4] argued that impairment of the neurally mediated component of the postural vasoconstriction in diabetes leads to a raised capillary pressure on dependency, excess fluid filtration into the tissue spaces and eventually to increased capillary–cell diffusion distances for oxygen, so creating the conditions for tissue necrosis and ulceration.

In SS disease, there is no evidence for an associated neuropathy [1]. Thus, the relative hyperperfusion on dependency may simply reflect the inability of neurally mediated vasoconstriction to override the local factors that contribute to the high resting perfusion. This hyperperfusion on dependency may, as in diabetes, increase the likelihood of ulcer formation [4].

If this is the case then the fact that the fall in RCF on dependency was substantially greater at the ulcer and scar sites of the SS patients than in their normal skin is of particular interest. If the higher resting RCF at the ulcer and scar sites with the leg horizontal is a response to local factors associated with tissue ischaemia and is required to promote healing or maintain tissue integrity, then it may be that the pronounced reduction in perfusion on dependency hinders healing and encourages recurrence of ulceration.

An important question that remains is ‘why are some SS patients, such as the SSΔ group of the present study, apparently resistant to leg ulceration?’ Although any differences between the HbF values in the different groups of SS patients did not reach statistical significance, the mean HbF value tended to be higher in SSΔ patients (5.5%) than in those with active ulcers (SSn, 2.8%), or scars (SSn, 3.1%). In a Jamaican study on SS patients with ulcers, those who had ulcers of ‘spontaneous onset’ had a significantly lower HbF than those in whom the ulcers could be attributed to external trauma [17]. Moreover, in a 7 year epidemiological study of leg ulcers in 2075 patients with SS disease in the U.S.A., the incidence of leg ulcers decreased significantly with increase in HbF levels [18]. Thus, resistance to ulcer formation seems to be positively correlated with HbF. This may be because a higher HbF within the erythrocyte inhibits polymerization of haemoglobin S [1] and therefore helps to reduce the increase in cell rigidity and consequent blockage of vessels that can occur in microcirculation when tissue blood flow and oxygenation fall [1].

In summary, we have demonstrated that, irrespective of ulcer history, SS patients show substantial vasoconstriction in the skin of the legs upon dependency, but that in absolute terms the fall in perfusion is greater in ulcerated and scarred skin than in normal skin. Our results also indicate that the hyperperfusion relative to that of AA subjects that is present in the skin when the leg is horizontal persists when the leg is dependent. We propose that the relative hyperperfusion upon dependency helps to protect against ulcer formation in SS disease by limiting the poor tissue oxygenation and erythrocyte sickling that may lead to skin infarction, whereas the exaggerated fall in perfusion in those with ulcers and scars may hinder healing and encourage new ulcer formation.

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REFERENCES


