Dynamic Cerebral Autoregulation in Homozygous Sickle Cell Disease

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Background and Purpose—Sickle cell disease (SCD) is associated with cerebral hyperperfusion and an increased risk of stroke. Also, both recurrent microvascular obstruction and chronic hemolysis affect endothelial function, potentially interfering with systemic and cerebral blood flow control. We addressed the question whether cerebrovascular control in patients with SCD is affected and related to hemolysis.

Methods—Systemic and cerebrovascular control were studied in 18 patients with SCD and 10 healthy subjects. Dynamic cerebral autoregulation was evaluated by transfer function analysis assessing the relationship between mean cerebral blood flow velocity and mean arterial pressure.

Results—Normal baroreflex sensitivity and postural cardiovascular reflex responses indicated integrity of systemic cardiovascular control. In the low- (0.07 to 0.15 Hz) frequency region, mean arterial pressure variability was comparable for both groups, but a larger mean cerebral blood flow velocity variability in SCD (6.1 [4.6 to 7.0] versus 4.2 [2.6 to 5.2] [cm·s⁻¹]²·Hz⁻¹; *P<0.05) indicated a reduced capacity to buffer the transfer of blood pressure surges to the cerebral tissue. Impairment of dynamic cerebrovascular control was confirmed by a reduced mean arterial pressure-to-mean cerebral blood flow velocity transfer function phase lead in SCD versus healthy subjects (32±17° versus 50±19°, *P<0.05) that was unrelated to the severity of hemolysis.

Conclusions—In patients with SCD, dynamic cerebral autoregulation is impaired but appears unrelated to hemolysis.

Key Words: baroreflex sensitivity • brain circulation • cerebral blood flow • hemodynamics • transcranial Doppler
Table 1. Group Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CTRL (n=10)</th>
<th>SCD (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>3/7</td>
<td>5/13</td>
</tr>
<tr>
<td>Male, years</td>
<td>35 (9)</td>
<td>33 (12)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6 (4.6)</td>
<td>22.1 (3.0)</td>
</tr>
<tr>
<td>History of hypertension*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>120 (9)</td>
<td>120 (11)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76 (6)</td>
<td>72 (7)†</td>
</tr>
<tr>
<td>Mean</td>
<td>92 (6)</td>
<td>88 (8)</td>
</tr>
<tr>
<td>SpO₂, %</td>
<td>97.4 (0.8)</td>
<td>96.3 (1.3)†</td>
</tr>
<tr>
<td>Hb, mmol/L</td>
<td>8.0 (7.6–8.3)</td>
<td>5.7 (5.3–6.1)‡</td>
</tr>
<tr>
<td>Hematocrit, L/L</td>
<td>0.39 (0.03)</td>
<td>0.26 (0.04)‡</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>154 (24)</td>
<td>392 (328–454)‡</td>
</tr>
<tr>
<td>Reticulocyte, %</td>
<td>1.4 (0.8–1.7)</td>
<td>7.1 (6.4–9.5)‡</td>
</tr>
<tr>
<td>Organ damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

*Defined as BP >140/90 mm Hg.
†P<0.05 and ‡P<0.01 versus CTRL. Data are presented as means (SD) or medians (interquartile range).
SpO₂ indicates O₂ saturation.

Subjects and Methods

Subjects
Afro-Caribbean black subjects with SCD and age- and ethnicity-matched healthy subjects (CTRL) were consecutively recruited from the outpatient clinic at the Academic Medical Center. Group characteristics are presented in Table 1. All subjects gave their written informed consent as approved by the Academic Medical Center Medical Ethical Committee and experiments were performed in accordance with the Declaration of Helsinki. Only patients with SCD with genotype HbSS or HbS-thalassemia, confirmed by high-performance liquid chromatography, were included in the study. Exclusion criteria consisted of: a sickle cell crisis in the preceding 4 weeks, history of symptomatic cerebrovascular disease, clinical manifestation of heart failure or other cardiovascular diseases, uncontrolled hypertension (BP >160/100 mm Hg), orthostatic hypotension, use of medication with potential influence on autonomic cardiovascular function, or a blood transfusion in the preceding 4 months. The studies were performed in morning sessions in a room at 22°C. Subjects were requested to abstain from caffeinated beverages for at least 12 hours before measurements.

Blood Samples
Degree of hemolysis was represented by Hb, reticulocyte count, lactate dehydrogenase (LDH), and haptoglobin plasma levels. Venous blood samples were drawn from all subjects and centrifuged immediately at 3000 g for 15 minutes and then stored at −80°C. Complete blood counts (Hb, hematocrit, leukocyte, reticulocyte percentage) were determined (Cell-Dyn 4000; Abbott) and LDH was analyzed using spectrophotometry (Roche Hitachi Modular P800, Basel, Switzerland).

Hemodynamic Parameters
Continuous BP was measured noninvasively by a servo-controlled finger photoplethysmograph (Portapres; FMS, Amsterdam, The Netherlands) with the cuff placed on the middle phalanx of the left middle finger kept at heart level. Changes in BP measured by photoplethysmography are not different from intra-arterial BP measurements both at rest and during orthostatic stress. An automated noninvasive BP measuring device (HEM-705CP; Omron, Kyoto, Japan) was used to calibrate the finger BP measurements. Stroke volume was determined by pulse wave analysis using the ModelFlow method (BeatScope 1.0 software; BMEye, Amsterdam, The Netherlands). Heart rate (HR) was the inverse of the interbeat interval. Cardiac output was the product of HR and stroke volume, and systemic vascular resistance was mean arterial pressure (MAP) divided by cardiac output. The transcranial Doppler- (DWL MultiDop X4, Sipplingen, Germany) derived CBFV was measured in the proximal segment of the right middle cerebral artery (MCA) and the MCA was imaged using the posterior temporal window. Once the optimal signal-to-noise ratio was obtained, the probe was secured with a headband (Marc 600; Spencer Technologies, Seattle, Wash). Arterial CO₂ tension influences CBF independently of cerebral autoregulation. To account for the cerebrovascular effects of CO₂, end-tidal CO₂ tension was measured by a sampling infrared capnograph (Tonocap; Datex-Ohmeda, Madison, Wisc). Transcutaneous O₂ saturation was measured using a pulse oximeter (Novametrics 515A; Wallingford, Conn). The signals of BP, spectral envelope of CBF, and end-tidal CO₂ tension were analog/digital converted at 100 Hz and stored on a hard disk for offline analysis.

Systemic Cardiovascular Control
The MAP, HR, and systemic vascular resistance responses to orthostatic stress assessed efferent sympathetic vasomotor function. Afferent, central, and vagal efferent baroreceptor reflex pathways were evaluated by quantifying baroreflex sensitivity using the sequential method. Beat-to-beat values of systolic BP and interbeat interval were interpolated and resampled at 1 second. Cross-correlations were calculated using a 10-second window containing systolic BP for delays in the interbeat interval window of 0 to 5 seconds. The highest coefficient of correlation was selected and accepted if P<0.01. Baroreflex sensitivity was the slope of the regression line between changes in interbeat interval versus systolic BP, expressed as ms · mm Hg⁻¹.

Cerebral Blood Flow Control
The steady-state response of mean MCA velocity (MCA V̅mean) to postural change was assessed from steady-state arterial pressure and CBFV sampled from 1 minute before standing up to 5 minutes upright. With standing, the positioning of the head approximately 30 cm above heart level within a few seconds results in an abrupt reduction in cerebral perfusion pressure of approximately 20 mm Hg, with a decrease in cerebral tissue oxygenation,16,17 and CBF,18 reflected by the transcranial Doppler determined MCA V̅mean.13 Such steady-state reductions in cerebral perfusion take place even though the cerebral perfusion pressure remains within, what is considered to be, its autoregulatory range. Static cerebral autoregulation limits the steady-state postural reduction in MCA V̅mean to approximately 15%.16,17 Beat-to-beat values for MCA V̅mean and MAP were derived as the integral over one beat divided by the corresponding beat interval. MAP at brain level (MAPbrain) was calculated from MAP measured at heart level and the vertical finger-to-transcranial Doppler probe distance.15 Cerebrovascular resistance was the ratio of MAPbrain and MCA V̅mean. The Gosling pulsatility index of the MCA was taken as an index of cerebral microangiopathy expressed as the amplitude of CBFV divided by time-averaged CBFV.

Frequency domain analysis quantified the counterregulatory capacity of dynamic cerebral autoregulation from spontaneous BP oscillations in the upright position.20,21 A 4-minute tracing of beat-to-beat data of MAP and MCA V̅mean was spline interpolated and resampled at 4 Hz. To quantify the variability of BP and CBFV, the power spectra of the 2 variables were estimated by transforming the time series of BP and CBFV with discrete Fourier transformation to the frequency domain. From the cross-spectrum, transfer function phase shift and gain were derived. According to the high-pass filter...
model of cerebral autoregulation, autoregulatory capacity is reflected by the positive phase relation between oscillations of BP (input function) and CBFV (output function). At high frequencies, less resistance; PETCO2, end-tidal CO2 tension; BRS, baroreflex sensitivity.

Table 2. Cerebrovascular and Cardiovascular Response to Postural Change

<table>
<thead>
<tr>
<th>Groups</th>
<th>Supine</th>
<th>Standing t=5 Minutes</th>
<th>Δ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPbrain mm Hg</td>
<td>CTRL 83 (10)</td>
<td>93 (10)</td>
<td>+13%*</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>SCD 80 (7)</td>
<td>91 (11)</td>
<td>+14%*</td>
<td></td>
</tr>
<tr>
<td>MCA Vmean cm·s⁻¹</td>
<td>CTRL 64 (13)</td>
<td>56 (11)</td>
<td>−14%*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SCD 87 (16)</td>
<td>76 (14)</td>
<td>−13%*</td>
<td></td>
</tr>
<tr>
<td>CVR, mm Hg·cm⁻¹·s⁻¹</td>
<td>CTRL 1.35 (0.23)</td>
<td>1.30 (0.25)</td>
<td>−4%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SCD 0.94 (0.16)</td>
<td>0.90 (0.16)</td>
<td>−5%</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>CTRL 72 (13)</td>
<td>81 (4)</td>
<td>+13%*</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>SCD 75 (8)</td>
<td>86 (12)</td>
<td>+14%*</td>
<td></td>
</tr>
<tr>
<td>SV, mL</td>
<td>CTRL 96 (14)</td>
<td>68 (9)</td>
<td>−29%*</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>SCD 96 (16)</td>
<td>73 (12)</td>
<td>−25%*</td>
<td></td>
</tr>
<tr>
<td>CO, L·min⁻¹</td>
<td>CTRL 6.8 (1.2)</td>
<td>5.5 (0.8)</td>
<td>−19%*</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>SCD 7.1 (1.1)</td>
<td>6.0 (0.9)</td>
<td>−15%*</td>
<td></td>
</tr>
<tr>
<td>SVR, dyn·s·m⁻¹</td>
<td>CTRL 731 (182)</td>
<td>1022 (229)</td>
<td>+40%*</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>SCD 638 (78)</td>
<td>871 (135)</td>
<td>+37%*</td>
<td></td>
</tr>
<tr>
<td>PtpCO2, mm Hg</td>
<td>CTRL 39.8 (2.7)</td>
<td>37.3 (2.3)</td>
<td>−6%*</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>SCD 39.3 (2.6)</td>
<td>37.5 (2.4)</td>
<td>−4%*</td>
<td></td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>CTRL 0.78 (0.15)</td>
<td>0.75 (0.14)</td>
<td>−4%</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>SCD 0.78 (0.10)</td>
<td>0.78 (0.10)</td>
<td>−1%</td>
<td></td>
</tr>
<tr>
<td>BRS, ms·mm Hg⁻¹</td>
<td>CTRL 19 (8)</td>
<td>10 (5)</td>
<td>−49%*</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>SCD 19 (12)</td>
<td>10 (4)</td>
<td>−47%*</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.01 versus supine. P, probability value represents overall difference between CTRL and SCD groups. Data are given in means (SD) for n=10 (CTRL) versus n=18 (SCD).

CVR indicates cerebrovascular resistance; SV, stroke volume; CO, cardiac output; SVR, systemic vascular resistance; PtpCO2, end-tidal CO2 tension; BRS, baroreflex sensitivity.

Statistical Analysis

Data are presented as means (SD) or medians (interquartile range). Differences between groups were identified by unpaired Student t test when data fit a normal distribution, and a Mann-Whitney rank sum test was applied when data were not normally distributed. Two-way analysis of variance was used to identify differences across condition (SCD versus CTRL) and body position. P<0.05 was considered to indicate a statistically significant difference. To investigate the relationship between hemolysis and dynamic cerebral autoregulatory capacity, a multivariate stepwise regression model was constructed with MAP-to-MCA Vmean transfer function phase as the dependent variable and Hb and LDH as the independent variables as well as age, systolic and diastolic BP, hematocrit, reticulocyte count, presence of chronic organ failure (pulmonary hypertension, retinopathy, microalbuminuria), use of hydroxyurea or folic acid, and body mass index with forward entry and removal.

Results

Group Characteristics and Baseline Measurements

Eighteen Afro-Caribbean black subjects (5 male and 13 female) with SCD (17 patients with HbSS and one with HbSβ⁰ thalassemia) and 10 age- and ethnicity-matched healthy subjects (3 male and 7 female, CTRL) were included. Among the SCD versus CTRL groups, there were no differences with regard to gender ratio and age, whereas body mass index, O₂ saturation, diastolic BP (P<0.05; Table 1), and systemic vascular resistance (P=0.012) were lower in SCD. In the SCD group, plasma LDH and reticulocyte count were higher and Hb lower (P<0.01), whereas haptoglobin was undetectably low. A limited (<15%) variation of these values in the preceding 2 years conformed to a relatively stable level of hemolysis. Baseline MAP, HR, stroke volume, cardiac output, end-tidal CO2 tension, and pulsatility index were comparable between groups (Table 2). MCA Vmean was higher (87±16 versus 64±13 cm·s⁻¹, P<0.01) and cerebrovascular resistance lower (0.94±0.16 versus 1.35±0.23 mm Hg·cm⁻¹·s⁻¹, P<0.01) in SCD.

...
Systemic Cardiovascular Control

A normal orthostatic increase in systemic vascular resistance confirmed intact efferent sympathetic vasomotor function in SCD (Figure 1). The postural reduction in stroke volume and cardiac output did not differ between groups. A normal orthostatic HR response together with normal baroreflex sensitivity indicated intact parasympathetic HR control in SCD.

Cerebral Blood Flow Control

The postural decline in MAP\textsubscript{brain} (−15%), MCA \textit{V}_{\text{mean}} (−13% versus −14%), and end-tidal CO\textsubscript{2} tension (−4% versus −6%) was comparable. In one subject from the CTRL group and in 2 subjects from the SCD group, coherence was <0.5 and these data were excluded from further analysis.

MAP low-frequency power was comparable for both groups (Table 3), whereas MCA \textit{V}_{\text{mean}} low-frequency power was higher in the SCD group (6.1 [4.6 to 7.0] versus 4.2 [2.6 to 5.2] \textit{cm·s}^{-1}\textit{Hz}^{-1}; \textit{P}=0.043). Representative examples of individual recordings are given in Figure 2. In the SCD group, the transfer function phase lead between MCA \textit{V}_{\text{mean}} and MAP was lower (32° [23° to 38°] versus 50° [44° to 60°]; \textit{P}<0.05; Figure 3) with phase lead lower than 40° in 83% of the patients. MAP-to-MCA \textit{V}_{\text{mean}} transfer function phase did not relate to Hb, LDH, hematocrit, reticulocyte count, age, systolic and diastolic BP, body mass index, use of hydroxyurea or folic acid, and presence of chronic organ damage. Also, no relationship was found between Hb levels and MCA \textit{V}_{\text{mean}}.

Discussion

The present study provides novel information regarding the control of CBF in patients with SCD. We found evidence for impairment of dynamic cerebral autoregulation in SCD that appeared unrelated to the degree of hemolysis, whereas systemic cardiovascular control was unaffected.

Impaired cerebral autoregulation has been linked to an increased risk for stroke, particularly in severe obstructive...
carotid artery disease.23 Silent cerebral infarctions are present in approximately one third of homozygous patients with SCD.5 The observation in the present study that cerebrovascular autoregulatory capacity was affected in the majority of consecutively recruited patients with SCD with asymptomatic cerebrovascular disease supports that impairment of dynamic CBF control precedes cerebral ischemic events. Our data represent the findings in adult patients and may therefore not be applicable in young children with homozygous SCD. However, although the clinical presentation of stroke appears to differ between children and adult patients with SCD, it seems likely that these clinical forms represent the same pathophysiological process. Both homozygous SCD and low Hb are major risk factors for both ischemic and hemorrhagic stroke in SCD. In addition, many patients with SCD present with secondary hemorrhagic stroke after a previous ischemic stroke.4 Future long-term follow-up studies in both young and adult patients with SCD are needed to strengthen these assumptions.

Impairment of dynamic cerebral autoregulation in patients with SCD puts forward a potential linkage to cerebral small vessel disease. This notion is supported by the finding that cerebral autoregulation is equally impaired in patients with Type 2 diabetes.24,25 The higher variability in CBFV in the SCD group indicated a reduced capacity to buffer the transfer of BP surges to the cerebral tissue. Impairment of dynamic cerebrovascular control was confirmed by a reduced phase lead of the BP-to-CBFV transfer function in SCD. In a previous study, the cerebral arterial pulsatility index has been proposed as an indicator of cerebral microangiopathy in patients with diabetes.19 However, this was not substantiated in the present study, questioning its applicability in patients with SCD.

SCD is characterized by an ongoing state of vascular inflammation with endothelial activation26 resulting in microvascular damage. This process is enhanced by the elevated oxidative stress associated with chronic hemolysis added to ischemia–reperfusion injury due to transient vaso-occlusive events.27 Both result in reduced NO bioavailability due to NO scavenging by cell-free hemoglobin and reduced NO formation due to increased arginase levels.28 We did not measure NO concentrations, but consider that the relationship between hemolysis and NO bioavailability is well established.29 Controversies exist regarding the role of NO in dynamic cerebral autoregulation in humans.30,31 We acknowledge that in the present study, indirect markers of hemolysis were used based on a small sample size; however, no relationship was found between dynamic cerebral autoregulatory capacity and severity of hemolysis. Larger studies are needed to establish the functional significance of this observation.

An inverse relationship between CBF and hematocrit level has been reported under physiological conditions both in animal models,32–34 and in humans.35,36 In the present study, no relationship was found between either CBFV or dynamic cerebral autoregulatory capacity and Hb, rendering a role for anemia as an independent factor in the impairment of dynamic cerebral autoregulation unlikely. The finding of an elevated CBFV in the SCD group does not in itself imply affected dynamic cerebral autoregulation. For instance, in healthy subjects, CBFV changes in response to postural stress, exercise, and hyperglycemia independently of cerebral autoregulatory capacity.37

Hydroxyurea has been demonstrated to reduce CBFV in children with SCD.38 Although hydroxyurea may reduce cellular adhesion of leukocytes and erythrocytes to endothelial cells,39 there are no data indicating an effect of folic acid or hydroxyurea on dynamic cerebral autoregulation in humans.50,51 We acknowledge that in the present study, indirect markers of hemolysis were used based on a small sample size; however, no relationship was found between dynamic cerebral autoregulatory capacity and severity of hemolysis. Larger studies are needed to establish the functional significance of this observation.

The finding of comparable cardiac output but lower systemic vascular resistance and diastolic blood pressure in SCD versus CTRL is consistent with earlier observations.5,40,41 In contrast to an earlier report,42 we found intact baroreflex cardiovascular control and no signs of cardiovascular autonomic dysfunction in the SCD group. Thus, integrity of cardiovascular control contrasts to impairment of dynamic cerebrovascular control in patients with SCD.
Sickle cell disease is associated with an increased CBFV assessed by transcranial Doppler ultrasonography. Blood flow velocity rather than blood flow was monitored and it could be considered that changes in the diameter of the insonated vessel by enhanced sympathetic activity modulate blood flow velocity independently of flow. However, the large cerebral arteries are conductance rather than resistance vessels and moderate sympathetic activation does not modify the luminal diameter of a systemic conduit artery. Thus, CBF increases in proportion to changes in MCA mean blood flow velocity, or in internal carotid flow, and constancy of the diameter links changes in CBFV to changes in flow. An increased blood flow velocity may reflect intracranial stenosis in the MCA at the background of an unchanged global CBF, but it also may indicate cerebral hyperperfusion as a compensatory response to a SCD-associated reduced O2-binding capacity. Recently, an increased CBFV in patients with SCD has been attributed to hyperperfusion rather than intracranial stenosis, which conforms to earlier studies reporting an increased CBF as determined by 133Xe and MRI.

In conclusion, the capacity to buffer the transfer of blood pressure surges to the cerebral tissue is reduced in patients with SCD but appears unrelated to hemolysis. Whether a hampered dynamic cerebral autoregulation plays a role in the high incidence of cerebrovascular complications in SCD remains to be elucidated.

Appendix

The CURAMA study group is a collaborative effort studying sickle cell disease in The Netherlands Antilles and The Netherlands. Participating centers are: the Red Cross Blood Bank Foundation, Curacao, The Netherlands Antilles; the Antillean Institute for Health Research, Curacao, Netherlands Antilles; the Department of Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands; the Department of Vascular Medicine and the Department of Hematology, Academic Medical Center, Amsterdam, The Netherlands; the Department of Hematology, Erasmus Medical Center, Rotterdam, The Netherlands; the Department of Pathology, Groningen University Hospital, The Netherlands; the Department of Internal Medicine, the Laboratory of Clinical Thrombosis and Hemostasis, and the Cardiovascular Research Institute, Academic Hospital Maastricht, The Netherlands.

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Disclosures

None.

References


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