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Running title: Sickle cell disease and cerebral oxygenation

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Abstract

Sickle cell anemia (SS) is characterized by a reduced cerebral microvascular oxygen saturation (cerebral TOI), which is not associated with hemoglobin concentration. Cerebral TOI has never been studied in sickle cell-hemoglobin C disease (SC). We focused on the relationships between hemorheological alterations and cerebral TOI in sickle cell patients with no cerebral vasculopathy and on the usefulness of TOI variability to assess the cerebral vasomotion activity. The blood rheological profile, the level of cerebral TOI (spatial resolved spectroscopy) and the cerebral TOI variability, which reflects vasomotion activity, were compared between 20 healthy subjects (AA), 21 SC patients and 21 SS patients. Cerebral TOI exhibited the following order: AA > SC > SS. The low cerebral TOI in SS patients was related to red blood cell aggregation and deformability properties. The cerebral TOI variability of SS and SC patients was increased above healthy values and vasomotion activity was negatively associated with the reduced cerebral TOI in SS patients. We demonstrated that 1) blood rheology could be involved in the reduced cerebral TOI in SS patients but not in SC patients; 2) vasomotion activity is increased in SS and SC patients to compensate for the reduced cerebral TOI.

Introduction

Cerebral microvascular oxygen saturation (cerebral TOI) measured by Near-Infrared Spectroscopy (NIRS) was demonstrated to be 20-30% lower in asymptomatic patients with sickle cell anemia (SS) than in healthy individuals, which has been interpreted as representing a certain degree of cerebral hypoxia in SS patients [1, 2]. The resulting rise of cerebral blood flow [3], if too large, may be a concern for these patients by increasing the risks for cerebral complications such as impaired neurocognitive functions [4] or enhanced risk for stroke [5]. Although the cerebral complications are less frequent in patients with sickle cell-hemoglobin C disease (SC) [6], it is unknown whether this population also suffers from a reduced cerebral TOI.

The causes of cerebral TOI reduction in asymptomatic SS patients have not been elucidated yet. It seems not to be related to the level of anemia [1, 2]. Several studies performed in non-sickle cell disease patients have demonstrated that hemorheological properties may be involved in cerebrovascular accident, and might determine blood flow in brain microcirculation and adequate brain perfusion [7-9]. Sickle cell disease (SCD) patients are characterized by wide hemorheological abnormalities, with SS and SC patients having a very different hemorheological profile [10]. But the impact of altered blood rheology on the cerebral TOI of SCD patients is unknown. Studies demonstrated that hydroxyurea therapy in SS patient was able to improve red blood cell (RBC) deformability [11] and also cerebral TOI [12]. Taken together these findings suggest that the hemorheological alterations could be involved in the reduction of the cerebral TOI in SCD.

Despite the fact that cerebral oxygen consumption has been reported to be slightly decreased in few SS patients [13], few previous studies on small SS groups reported that the mean value of cerebral oxygen consumption was close to the normal values [14]. This finding contrasts

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with the idea that a reduction of the cerebral TOI could be a sign of cerebral hypoxia in SS patients [2]. Although the fact that cerebral oxygen consumption in asymptomatic SS patients appears normal seems surprising, it could be explained by an enhanced vasomotion. Vasomotion is a form of spontaneous localized oscillations induced by spontaneous contraction and relaxation of the muscular components in the small blood vessel walls which generate rhythmic changes in their diameter. Although vasomotion mechanisms are still under debate [15], several studies demonstrated that vasomotion may have beneficial effects on tissue oxygenation [16, 17] and can be appreciated by the spectral analysis of the cerebral TOI variability over time [18, 19].

The present study compared the blood rheological profile, the level of prefrontal cortical microcirculatory oxygen saturation and its variability between healthy subjects (control group, AA) and SS and SC patients without cerebral vasculopathy history. We focused on the relationships between hemorheological parameters and cerebral microvascular oxygen saturation in sickle cell patients. Moreover, because SC patients are usually less prone than SS patients to cerebrovascular accident [6, 20, 21], we hypothesized that 1) cerebral microvascular oxygen saturation should be more preserved in SC than in SS patients, in comparison with AA subjects and 2) cerebral microvascular oxygen saturation variability, which reflects vasomotion, should be higher in SS than in SC patients.

Material and methods

Patients

Sixty-two age- and ethnicity-matched volunteers participated in the study: 20 AA subjects (10 males and 10 females), 21 SC patients (10 males and 11 females) and 21 SS patients (11 males and 10 females).

The SS and SC patients recruited are regularly followed by the Sickle Cell Unit of the Academic Hospital of Pointe-à-Pitre (Pointe-à-Pitre, Guadeloupe) and had a magnetic resonance imaging less than 3 months before enrollment to exclude the presence of cerebral vasculopathy or silent cerebral infarcts. All participants were aged ≥ 18 yrs old and were Afro-Caribbean native from Guadeloupe. SCD patients were in clinical steady state at the time of the study (*i.e.,* without vaso-occlusive crisis, acute medical complication or blood transfusion/phlebotomies within the last 3 months). Exclusion criteria for all subjects were recent infectious episode (in the last month), stroke or cerebral vasculopathy history, β-thalassemia, pregnancy or breast-feeding. Patients taking medication that could affect the hemorheological parameters studied, such as hydroxyurea, were excluded. All participants received verbal and written explanation of the objectives and procedures of the study and subsequently provided written informed consent. The study was approved by the Regional Ethics Committee (CPP Sud-Ouest Outre-Mer III, Bordeaux, France). The experiments were performed in accordance with the guidelines set by the Declaration of Helsinki.

Protocol

For each participant, a physician from the Sickle Cell Unit (Guadeloupe) performed a clinical exam with anthropometric, transcutaneus oxygen saturation (SpO_2) and blood pressure measurements. Mean arterial pressure was calculated: 1/3 systolic + 2/3 diastolic pressures. Venous blood was sampled in EDTA tubes from the antecubital vein to perform hematological and hemorheological (blood viscosity, RBC deformability, RBC aggregation and disaggregation properties) measurements. Then, near-infrared spectroscopy (NIRS, NIRO-200, Hamamatsu Photonics, Hamamatsu City, Japan) measurements were performed for the determination of a tissue oxygenation index (TOI) at the prefrontal cortex and flexor

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digitorum superficialis muscle levels. The TOI value reflects the microvascular oxygen saturation. The muscular oxygen consumption (mVO_2) was measured by NIRS using the venous occlusions (50 mmHg) method [22].

A Fast Fourier Transform was applied on the cerebral TOI signal for the evaluation of the total power spectrum in the frequency interval 0.004-2 Hz (*i.e.,* total cerebral microvascular oxygen saturation variability) and calculation of the power across 5 band frequencies: interval I (0.004-0.02 Hz) reflects nitric oxide metabolism and/or endothelial function, interval II (0.02-0.06 Hz) depends on neurogenic activity of the vessel wall, and interval III (0.06-0.15 Hz) corresponds to the myogenic activity, interval IV (0.15-0.4 Hz) reflects the breathing frequency and interval V (0.4-2 Hz) is under the influence of heart rate and cardiac output [18]. The whole oscillations recorded (*i.e.,* cerebral TOI variability or total power of the spectrum) reflect the global flowmotion. The low frequency domain (intervals I, II and III) corresponds to the vasomotion activity [18].

For additional information on the experimental methods and data analysis techniques, see the online data supplement.

Results

Subjects' characteristics and hematological parameters

Subjects' characteristics and hematological parameters are summarized in the Table I. Age and mean arterial pressure were not significantly different between the three groups. Transcutaneus oxygen saturation (SpO_2) was significantly lower in SS group than in AA and SC groups.

Fetal hemoglobin level was significantly higher in SS group than in both SC and AA groups. Hemoglobin and hematocrit were different between the three groups: AA > SC > SS. The percentage of reticulocytes was different between the three groups with SS > SC >AA.

Hemorheological parameters

Hemorheological values are shown in the Table II. Blood viscosity was higher in SC group than in AA and SS groups. The SS and AA groups were not significantly different regarding blood viscosity. The RBC elongation index at 3 Pa was higher in AA group compared to SC and SS groups and the 16% higher RBC elongation index at 3 Pa in SC patients in comparison with SS patients did not reach statistical significance (Table II). At 30 Pa, the RBC elongation index was significantly different between the three groups: SS < SC < AA. The RBC aggregation index was different between the three groups such as SC < SS < AA. In contrast, the RBC disaggregation threshold was higher in the two SCD groups in comparison with AA subjects.

Muscular TOI and oxygen consumption (mVO_2)

As shown in the Table II, the muscular TOI was higher in both AA and SC groups compared to SS group. In contrast, no difference was observed between the three groups for mVO_2 (Table II).

Cerebral TOI

The cerebral TOI (Fig 1) was higher in AA than in SS group. SC patients exhibited an intermediate level. The presence of α-thalassemia in SS or SC patients had no effect on the cerebral TOI (data not shown).

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In AA group, cerebral TOI was positively correlated with hemoglobin concentration (r = 0.62; P < 0.01) and negatively correlated with the RBC aggregation index (r = -0.63; p < 0.01). No correlation was observed between cerebral TOI and SpO_2, RBC elongation index, blood viscosity, RBC disaggregation index, percentage of reticulocytes or vasomotion activity in AA group. In the SC group, no correlation was found between cerebral TOI and hemoglobin concentration, percentage of reticulocytes, SpO_2, blood rheology or vasomotion activity. In the SS group, we observed positive correlations between cerebral TOI and RBC elongation index at 3 Pa (r = 0.54; p < 0.05), and cerebral TOI and RBC aggregation index (r = 0.54; p < 0.05). At 30 Pa, the positive association between RBC elongation index and cerebral TOI tended to be significant (r = 0.43; p = 0.055). Moreover, a negative correlation was observed between cerebral TOI and vasomotion activity (r = -0.61; p = 0.004) in SS patients. No correlation was observed between cerebral TOI and SpO_2, hemoglobin concentration, percentage of reticulocytes, blood viscosity or the RBC disaggregation threshold in SS patients.

Cerebral TOI variability

Figure 1 supplemental online material shows typical examples of cerebral TOI recordings in one AA, SC and SS subject. The total power of the signal (*i.e.,* cerebral TOI variability) was significantly different between the three groups: SS > SC > AA (Fig 2). Figures 2a and 2b supplemental online materials show the power spectral density in the five frequency intervals with SS > SC > AA.

Discussion

This study demonstrates that: 1) cerebral microvascular oxygen saturation (cerebral TOI) is lower in asymptomatic SS adults in comparison with the control group, and SC patients have an intermediate level; 2) the whole cerebral TOI variability and the power spectral density in each frequency interval are higher in sickle cell patients (intermediate level for SC group) than in the control group reflecting higher flowmotion and vasomotion; 3) the lower cerebral TOI in SS or SC patients is not related to the degree of anemia; 4) the cerebral TOI of SS patients is correlated with vasomotion activity and RBC rheological properties; 5) no relationship is observed between the hemorheological parameters and cerebral TOI in SC group.

We showed that cerebral TOI was lower in asymptomatic SS patients (*i.e.,* without cerebral vasculopathy history) than in AA group. SC patients exhibited an intermediate cerebral TOI level (Fig 1). This is the first study giving information about cerebral TOI in SC patients compared to a group of healthy subjects. Cerebral TOI is of primary importance since a reduction of cerebrovascular oxygen saturation may result in a rise of the cerebral blood flow [3] and could precede the appearance of cerebral complications [4, 23].

We did not find any correlation between the cerebral TOI and hemoglobin concentration in the sickle cell groups indicating that anemia is probably not the only factor involved in the decreased cerebral microvascular oxygen saturation of SS or SC patients, as previously reported [1, 2]. This finding contrast with a very recent study performed in SCD children [24]. The lack of correlation between cerebral TOI and SpO_2 in SS or SC patients supports

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previous findings [2] and suggests that systemic hypoxia could be not the main cause of the reduced cerebral TOI in SCD.

Our results demonstrated that the cerebral TOI of AA group was not related to RBC deformability. The RBC deformability in the healthy AA subjects is probably optimal and does not disturb the microvascular blood flow and, hence, cerebral TOI. For SCD patients, only the SS group demonstrated a positive correlation between RBC deformability and cerebral TOI suggesting that a greater RBC deformability in this group positively affects cerebral TOI. In this way, Parthasarathi and Lipowsky [25] demonstrated that reduced RBC deformability in rat cremaster muscle severely affected tissue oxygenation. In contrast, our findings do not support a key role of RBC deformability on the cerebral TOI modulation of SC patients.

The cerebral TOI of AA subjects was negatively related to RBC aggregation which supports previous findings showing that RBC aggregation properties may strongly affect hemodynamics and vascular resistance in both the macro- and microcirculation [26, 27]. Although we did not observe any correlation between the low RBC aggregation level and the reduced cerebral TOI in SC patients, we reported a surprising positive correlation between these parameters in SS patients suggesting that a rise of RBC aggregation in this population could be associated with cerebral TOI improvement. This result contrasts drastically with the finding reported in the AA group and with the traditional view that increased RBC aggregation may impair microcirculation. However, the SS group had a 21% RBC aggregation reduction in comparison with controls. Yalcin et al [28] studied the effects of a graded increase of RBC aggregation on the blood flow resistance in guinea pig hind limb and

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found a tri-phasic relation suggesting that there is an optimal level of RBC aggregation to reduce blood flow resistance and provide adequate tissue perfusion.

The larger total spectral power variability of cerebral TOI and the greater absolute power of each frequency interval of the cerebral TOI signal suggest higher cerebral flowmotion and vasomotion [29, 30] in SCD patients than in controls which can be interpreted as a mechanism to compensate for the reduced cerebral TOI [17, 30]. The low deformable RBCs in SCD patients could trigger an oscillatory vasomotion pattern that helps to maintain the microvascular blood flow and tissue oxygenation by rheological abnormal SCD blood [20, 21]. This is supported by a significant negative correlation found between vasomotion activity and RBC deformability at 3 Pa in SS patients (r = -0.54; p < 0.05).

Although the decreased cerebral TOI and the increased TOI variability could support the presence of a certain degree of cerebral hypoxia in SS patients, and to a lesser extent in some SC patients, we did not measure the cerebral oxygen consumption in the present study. Few previous studies on small asymptomatic SS groups reported that the mean value of cerebral oxygen consumption was close to the normal values [14] suggesting that, even if the cerebral TOI is reduced in this population, enough oxygen could be provided for brain functioning. The measurements of muscular TOI and mVO_2 in our patients support this hypothesis with mVO_2 being normal in SS patients despite a reduction of muscular TOI (*i.e.,* -8.9 %). This result demonstrates that a reduction of TOI is not always a synonym of tissue hypoxia. Although the RBCs rheological alterations could impair the cerebrovascular flow and participate to the decrease of the cerebral oxygen reserve in SCD patients, the reduced hemoglobin S affinity for oxygen [31] could result in a higher oxygen release to the tissues in

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SS and SC patients, hence participating in the decrease of cerebral TOI but limiting to some extent tissue hypoxia. In addition, we suspect that the increased vasomotion activity in SS and SC patients limits brain hypoxia.

The clinical meanings of reduced cerebral TOI and enhanced cerebral TOI variability in SCD have never been studied and this was not the aim of the present study. Nevertheless, we had the case of a SS patient (not included in the present study) who presented not excessively low cerebral TOI (i.e., 62.4%) but a very surprising TOI variability with values ranging intermittently between 0 and 99% (data not shown). Based on this very large TOI variability, it was asked to the patient to perform magnetic resonance imaging which demonstrated the presence of an intermittent trickle flow at the level of the anterior cerebral and anterior communicating arteries. This observation suggests that the presence of an excessive cerebral periodic microcirculatory flow could reflect severe cerebrovascular complications in SCD, as it can be the case at the peripheral level [32].

In conclusion, we demonstrated that asymptomatic SCD patients without a history of vasculopathy had lower cerebral microvascular oxygen saturation than normal subjects. This lower cerebral microvascular oxygen saturation was not related to the degree of anemia or to the systemic arterial oxygen saturation level but could be related to the reduced deformability and aggregation properties of RBCs. Moreover, although the cerebral microvascular oxygen saturation was reduced in SCD patients, the cerebral flowmotion and vasomotion activities were higher than in the control group, with the higher activity found in SS patients. This higher cerebral vasomotion activity could be a way to compensate for the reduced cerebral microvascular oxygen saturation. Future longitudinal studies will have to assess if a reduced

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cerebral microvascular oxygen saturation and an enhanced vasomotion activity could be predictor of cerebrovascular complications in SCD.

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Figure 1. Cerebral microvascular oxygen saturation (cerebral TOI) in AA, SC and SS groups. Different from AA group (***$p < 0.001$); different from SC group ($^{†††}p < 0.001$). AA and SC were not significantly different ($p = 0.08$).

Figure 2. Fast Fourier Transform analysis of TOI signal for total power spectral density (flowmotion activity).* Different from AA group ($p < 0.05$); † different from SC group ($p < 0.05$).

Table I. Subjects characteristics and hematological parameters

	AA	SC	SS
Age (yrs)	34.7 ± 11.9	35.9 ± 12.2	33.6 ± 11.9
MAP (mmHg)	91 ± 11	89 ± 12	85 ± 8
SpO_2 (%)	99.6 ± 0.8	99 ± 1.2	96.0 ± 3.1***†††
Fetal hemoglobin (%)	0.6 ± 0.7	1.3 ± 0.9	7.6 ± 5.4***†††
Hemoglobin S (%)	-	47.4 ± 0.9	$83.6 \pm 5.7^{†††}$
Hemoglobin C (%)	-	43.4 ± 1.3	-
Hematocrit (%)	41.9 ± 3.2	32.3 ± 2.8***	25.2 ± 4.2***†††
Hemoglobin (g/dL)	13.4 ± 1.3	11.1 ± 1.2***	8.5 ± 1.0***†††
Reticulocytes (%)	1.1 ± 0.5	2.6 ± 1.6*	8.4 ± 3.9***†††

Values represent mean ± S.D. MAP = mean arterial pressure, SpO_2 = transcutaneus oxygen saturation. Different from control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$); different from SC group ($^{†}p < 0.05$; $^{††}p < 0.01$; $^{†††}p < 0.001$).

Table II. Hemorheological parameters, muscular microvascular oxygen saturation and muscular oxygen consumption

	AA	SC	SS
ηb at $225s^{-1}$ (mPa/s)	6.4 ± 1.1	7.8 ± 1.2***	$6.3 \pm 1.5^{\dagger\dagger\dagger}$
EI at 3 Pa	0.32 ± 0.05	0.17 ± 0.04***	0.14 ± 0.07***
EI at 30 Pa	0.59 ± 0.02	0.43 ± 0.05***	0.34 ± 0.13***†
AI (%)	65.0 ± 6.5	43.5 ± 9.2***	51.3 ± 9.7***††
γthr (s^{-1})	149 ± 40	299 ± 126***	361 ± 169***
mTOI (%)	62.4 ± 6.4	60.5 ± 7.9	53.5 ± 6.4**††
muscVO$_2$ ($\mu molO_2 \cdot cm \cdot min^{-1}$)	145 ± 50	143 ± 65	160 ± 63

Values represent mean ± S.D. ηb = blood viscosity, EI = elongation index (*i.e.,* RBC deformability), AI = aggregation index, γthr = disaggregation threshold - *i.e.,* the minimal shear rate needed to prevent RBC aggregation or to break down existing RBC aggregates, muscTOI = flexor digitorum superficialis muscle microvascular oxygen saturation, muscVO$_2$ = flexor digitorum superficialis muscle oxygen consumption, Different from control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$); different from SC group ($^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$).