Hematologic and hemorheological determinants of resting and exercise-induced hemoglobin oxygen desaturation in children with sickle cell disease

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Design and Methods

Patients

The study took place between January 2010 and January 2011, and included 107 children with SCD (50=SC; 57=SS) between the ages of 8 and 16 years old at steady state, which was defined as no blood transfusions in the previous three months and absence of acute episodes (infection, vaso-occlusive crises (VOC), acute chest syndrome (ACS), stroke, priapism, splenic sequestration) at least one month before inclusion into the study. Mean age (SS: 11.5 ± 2.3 vs. SC: 12.0 ± 2.3 yr) was comparable between the two groups. This is an expanded cohort that represents 92% of the SCD pediatric population in this age range who were enrolled in the SAPOTILLE project,1 and have been followed since birth by the Sickle Cell Center at the Academic Hospital of Pointe-à-Pitre (Guadeloupe, French West Indies). All children were identified by neonatal screening, and diagnosis was made as previously described.2 Polymerase Chain Reaction (Gap-PCR) was used to detect 6 common α-thalassemia deletions, including -α- and -α- alleles and triplication defects of the α-globin genes.3,4

Charts were retrospectively reviewed by 3 physicians to recognize all ACS and VOC episodes from birth to the time of blood sampling based on previously described criteria.1 The rates of ACS and VOC were calculated for each child by dividing the number of ACS or VOC event episodes by the number of patient-years.5,6

The associations between asthma or lung dysfunction (data not shown) and resting or exercise-induced hemoglobin oxygen desaturation were also addressed. Patients were considered as asthmatic when using medication for asthma and/or when the patients had experienced several repeated asthmatic-like events in the past. However, the pulmonary function tests performed were not designed to detect asthma or airway hyper-responsiveness specifically, and thus asthma frequency could have been underestimated.

Pulmonary function tests were performed on the same day as the blood sampling/6MWT, and included forced expiratory volume in 1 second (FEV), forced vital capacity (FVC) and FEV/FVC. The results of these pulmonary function tests were then used to define common phenotypes: normal lung function, obstructive lung syndrome, restrictive lung disease or mixed syndrome.6

The study was conducted in accordance with the guidelines set by the Declaration of Helsinki and was approved by the Regional Ethics Committee (CPP Sud/Ouest Outre Mer III, Bordeaux, France, registration number: 2009-A00211-56). All children and their parents were informed of the purpose and procedures of the study, and gave written consent.

Hematologic and hemorheological measurements

Blood was drawn by venipuncture into EDTA tubes between 8:00 a.m. and 10:00 a.m. and immediately used for measurements of hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, reticulocytes, RBC, platelets and leukocytes count (Max M-Retic, Coulter, USA). Hematocrit was measured after blood microcentrifugation at 9500 g (JOUAN-HEMA-C, Saint Herblain, France). Serum lactate dehydrogenase and total bilirubin concentrations were determined by standard biochemical methods.

Hemorheological parameters were measured immediately after sampling and after full re-oxygenation of the blood for 10-15 min as recommended.7 We followed the guidelines for international standardization in blood rheology techniques/measurements.7 Blood viscosity was measured at native hematocrit and at room temperature (~25°C) using a cone-plate viscometer (Brookfield DVII+ with CPE40 spindle) at 90 s⁻¹. RBC deformability was determined at 37°C at two shear stresses (3 and 30 Pa) by laser diffraction analysis (eck-
tacometry) using the Laser assisted Optical Rotational Cell Analyzer (LORCA, RR Mechatronics, Hoorn, The Netherlands). The system calculates the average RBC elongation index. The higher this index, the more deformable the RBC are. RBC aggregation was determined at 37°C via syllectometry (i.e. laser backscatter vs. time), using the LORCA (RR Mechatronics, Hoorn, The Netherlands), after adjustment of the hematocrit to 40%. The system calculates the aggregation index. The RBC disaggregation threshold, i.e., the minimal shear rate needed to prevent RBC aggregation or to breakdown existing RBC aggregates, was determined using a re-iteration procedure. Note that the RBC disaggregation threshold mainly reflects the RBC aggregate strength while the RBC aggregation index is a measure of the extent of aggregation integrated during a time period of 2 minutes.

Six-minute walk test

Immediately after blood sampling, a self-paced 6MWT was conducted according to the guidelines of the American Thoracic Society. The 6MWT reflects the functional exercise level for daily physical activities. It is a submaximal exercise test often used in SCD population to determine functional status or changes in status as a result of an intervention. The total distance walked was recorded for all patients. The percentage of predicted distance was calculated according to the models of Geiger et al. obtained in healthy children. Hemoglobin oxygen saturation (SpO₂) by finger pulse oximetry (SureSigns VS3 No. 3000, Philips Medical System, Andover, MA, USA) was obtained before and immediately after the 6MWT. The criteria outlined by Campbell et al. were used to define hemoglobin oxygen desaturation at rest and after exercise in SCD children. At rest, SCD children were classified into three groups as a function of SpO₂: no desaturation (SpO₂ > 98%), mild desaturation (95 ≤ SpO₂ ≤ 98%) and moderate desaturation (SpO₂ < 95%). EIHOD was defined as a drop in SpO₂ of 5% or more during exercise compared to the resting level.

Statistical analysis

All values were expressed as means ± SD. The data were tested for the normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene test). ANOVA (and Tukey post hoc test) or unpaired Student’s t-test were used to compare the different parameters. When the rules for parametric test application were not fulfilled, a Kruskall-Wallis test (and Dunn’s multiple comparison test) or a Mann-Whitney test was used. A χ² test was used to test the influence of sex, α-thalassemia or hydroxyurea treatment on the hemoglobin oxygen desaturation at rest or after exercise.

To identify the independent predictors of oxygen desaturation at rest (Table 2, main text), we used an ordinal multivariate logistical model with the baseline SpO₂ defining three ordered categories, as mentioned above.

To identify factors independently associated with exercise-induced oxygen desaturation in SC and SS children, we used a binary (i.e. no desaturation = decrease of SpO₂ < 3 %, or exercise-induced hemoglobin desaturation = decrease of SpO₂ ≥ 3 %) multivariate logistical model. All variables at P < 0.2 by univariate analyses were included as covariates in the multivariate model. Significance level was defined as P < 0.05. Analyses were conducted using SPSS (v. 20, IBM SPSS Statistics, Chicago, IL, USA).

References