Brain injury pathophysiology study by a multimodal approach in children with sickle cell anemia with no intra or extra cranial arteriopathy

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Online Supplement methods

Patients

Consecutive patients from two French referral centers for sickle cell disease (University Hospital Necker-Enfants malades and Centre Hospitalier Intercommunal de Creteil) were screened during routine visits according to the following inclusion criteria: 1) SS or S-β°thalassemia genotype; 2) no past history of abnormal or conditional transcranial and extracranial TCD; 3) at steady state (> 3 months from any vaso-occlusive event, transfusion or infection); 4) Age between 5 and 17 years. The study was offered to all children meeting the inclusion criteria and regularly followed up since neonatal screening in the participating centers, between March 2015 and July 2016, with a target of 60 children (based on the expected number of SCA patients meeting the inclusion criteria in these centers and the pilot design of the study). Treatment by hydroxyurea was not an exclusion criterion, but chronic transfusion was. After written informed consent was obtained, a visit during which all investigations were performed was scheduled.

The protocol was approved by the ethics committee "Comité pour la Protection des Personnes Ile de France III" (014-A01575-42) and registered in ClinicalTrials.gov: NCT 02909283.

Clinical parameters

Clinical parameters were collected: Systolic, diastolic and mean arterial pressures as well as pulse oximetry (Mindray iPM10, Shenzhen, China) were recorded at steady state. Relevant past medical events were collected: history of acute splenic sequestration, vaso-occlusive complications (VOC) requiring hospitalization (including either painful episodes and/or acute chest syndrome (ACS)), history of blood transfusion, ongoing treatments including hydroxyurea.

Biological parameters:

Full blood count as well as hemolysis markers were determined. Fetal Hb (HbF) percentage was quantified by high performance ion-exchange liquid chromatography (HPLC) VARIANTTMnbs system (BioRad Laboratories, Marnes La Coquette, France). Soluble E-Selectin, and soluble P-Selectin were quantified using ELISA kits from R&D Systems

(Minneapolis, MN, USA). Blood viscosity was determined at native hematocrit (Hct) and a shear rate of 225 s^{-1} at 37°C, using a cone/plate viscometer (Brookfield DVII+ with CPE40 spindle, Brookfield Engineering Labs, Natick, MA). The ratio Hct/blood viscosity was calculated for each subject and represent an hemorheological index of oxygenation, as it reflects a balance between the capacity of blood to transport oxygen (i.e., the number of red blood cells) and the ability of blood to circulate (i.e., blood viscosity).[1,2] Red blood cell (RBC) deformability was measured at 37°C and 30 Pa by laser diffraction analysis (ektacytometry), using the Laser-assisted Optical Rotational Cell Analyzer (LORRCA Maxsis, RR Mechatronics, Hoorn, The Netherlands). RBC aggregation was determined at 37°C via syllectometry, (i.e., laser backscatter versus time), using the LORRCA, after adjustment of the Hct to 40%. RBC aggregates strength, i.e. the minimal shear rate needed to prevent RBC aggregation or to breakdown pre-existing RBC aggregates, was determined using a re-iteration procedure using the LORRCA. [3] The guidelines for international standardization in blood rheology techniques/measurements [4], as well as for hemorheological measurements in sickle cell disease were strictly followed. [5]

Transcranial Doppler (TCD) procedure:

TCD imaging (TCDI) was performed using a LOGIQ E9 XDclear 2.0 ultrasound system (GE Healthcare, Milwaukee, WI, USA) at Necker – Enfants malades and an Acuson S 2000 ultrasound system (Siemens Healthineers, Erlangen, Germany) at Centre Hospitalier Intercommunal de Creteil. All procedures were performed by TCD-experienced radiologists in both sites (DG and SV) and followed the same protocol. [6] Morphology of arteries and time-averaged mean of the maximum velocities (TAMMV) were recorded respectively by color and pulsed Doppler sonography without angle correction. TCDI velocities data were classified according to the Stroke Prevention Trial in Sickle Cell Anemia (STOP) study. [7] Additionally, sharp angled loops (>120°) of extra cranial ICA were noted if present.

Brain MRI

MR imaging was performed with a GE Signa HDxt 1.5-T system (GE Medical Systems, Milwaukee, WI, USA) and a 12-channel head-neck-spine coil in non-sedated children. The MR investigation included standard pulse sequences for brain (three-dimensional T1-weighted [3DT1], fluid-attenuated inversion recovery [FLAIR], diffusion-weighted [DWI] sequences)

three-dimensional time-of-flight sequence [3D TOF]) for intracranial arteries and an additional 3-D TOF multislab unenhanced MR angiography (MRA) sequence for extracranial internal carotid arteries and carotid bifurcations.

Silent cerebral infarcts (SCIs) were defined on MRI as hypersignals of cerebral parenchyma of at least 3 mm on T2 FLAIR sequence. Given their ovoid shape, hypersignal volume was obtained as follows: X*Y*Z*0.5 (X = antero-posterior diameter, Y = right-left diameter, Z = cranio-caudal diameter). X and Y were measured on the FLAIR-weighted 2D sequence in which the slices had a thickness of 4 mm spaced 0.4 mm from each other. Thus, the measurement of diameter Z could only be performed with an accuracy of 4.4 mm. In order to overcome this limitation, Z diameter was measured on the T1-weighted 3D sequence whose resolution was infra-millimetric.

Cerebral blood flow was measured using a three-dimensional pseudo-continuous arterial spin labeling (pCASL) sequence (repetition time msec/echo time msec, 4428/10.5; postlabeling delay= 1025 msec; 80 axial partitions; field of view, 240 x 240 x 4 mm; acquisition matrix, eight spiral arms in each three-dimensional partition with 512 points per arm; flip angle, 155°; acquisition time, 4 minutes 17 seconds).

Image analysis

All MR images were interpreted by two MRI-experienced neuro-radiologists blinded to the patient's history (SV, DG). Only procedures including complete and good quality FLAIR, 3DTOF and ASL sequences were analyzed using a medical image viewer (Vue PACS, version 11.4.0.1253; Carestream Health, Toronto, Ontario, Canada) and the **Advantage Windows Imaging Workstation**, GE Healthcare.

Regions of interest were manually delimited on T1-weighted images based on anatomical landmarks and reported on ASL images to obtain average cerebral blood flow (CBF) values for each arterial territory, as given by the device software. Five cerebral cortical territories (ACA, MCA, PCA, anterior and posterior border zone [AW, PW]), two basal ganglia regions (putamen and caudate nucleus), and cerebellar cortical territories were thus delineated on both hemispheres to obtain sixteen vascular regions of interest (Figure 1). CBF expressed in mL/min/100 g brain tissue was analyzed as raw data.

Near Infra-Red Spectrometry (NIRS):

Transcranial Near Infra-Red Spectroscopy (NIRS) was performed using a dual-channel absolute cerebral oximeter FORE-SIGHT® (Branford, CT, USA). Bilateral oximeter sensors were applied to the subject forehead, symmetrically along the midline to avoid the sagittal sinus and as far above the orbital ridge as possible to maximize brain analysis. Cerebral tissue hemoglobin oxygen saturation (ctSO2) on the right and the left sides was obtained in patients at rest in supine position during room air breathing. The physical principles upon which NIRS is based on have been described [8]. NIRS can be used in SCA patients since the near-infrared spectra absorbance of HbS is similar to normal Hb [9].

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