SUPPLEMENTARY APPENDIX

Hemodynamic provocation with acetazolamide shows impaired cerebrovascular reserve in adults with sickle cell disease

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Supplementary materials

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MR imaging protocol

All images were acquired on a 3T clinical MR system (Philips Ingenia, Philips Healthcare, Best, The Netherlands), with a 32-channel receive head-coil, and body-coil transmission. Sequences were planned on sagittal, coronal and axial scouts and angiograms. For CBF, a pseudo-continuous arterial spin labelling (pCASL) sequence was used with a 2D gradient echo FFE single shot echo-planar imaging (EPI) readout. The pCASL labelling plane was placed 90 mm below the center of the imaging volume and angulated perpendicular to the brain feeding arteries as seen on initial angiograms. Labelling was implemented with flip angle 27.81°, radio frequency interval of 1.21 ms and duration of 0.48 ms, and gradient average of 0.36 mT/m and maximum of 5.0 mT/m. The sequence had a TR/TE of 4400/14 ms, FOV 240 x 240 mm, voxel size 3 x 3 x 7 mm, post-label delay 1800 ms, label duration 1800 ms, 19 axial slices, flip angle 90°, SPIR fat suppression with a frequency offset of 130 Hz (in 16 cases this was erroneously set to 220 Hz which leading to an artefact in 13 cases), 140 label-control pairs, two suppression pulses at 1830 Hz and 3155 Hz to reduce background tissue signal, and a total scan duration of 20 min. After 5 min of continuous scanning, participants received acetazolamide (Diamox®, Mercury Pharmaceuticals Ltd., London, UK) at a dose of 16 mg/kg bodyweight and a maximum of 1400 mg. Acetazolamide dissolved in 20 mL saline (NaCl 0.9%) was injected intravenously using an MR-compatible contrast injection pump at a flow rate of 0.1 mL/sec (<500 mg/min acetazolamide), and flushed with 10 mL saline also at a flow rate of 0.1mL/sec. Scanning was continued throughout the injection time to observe the dynamic response. A scaling image (M0), was acquired with the same settings as pCASL. For quantification purposes, the longitudinal relaxation time of blood (T1b) was measured using an inversion recovery (T2-TRIR) sequence in a single slice perpendicular to the venous compartment of the sagittal sinus before and after acetazolamide with a 2D single shot FFE EPI Look-Locker read-out (TR/TE/TI1/ΔTI 150/24/10/130 ms, FOV 202 x 243, voxel size 2 x 2 mm, slice thickness 4 mm, flip angle 95°, 4 dynamics, scan duration 0:50 min[1]. For labelling efficiency estimation and velocity measurement, we acquired a 2D phase-contrast (PC-) MRI sequence at the same level and angulation as the pCASL labeling plane (TR/TE 15/5 ms, FOV 230 x 230 mm, voxel size 0.45 x 0.45 mm, flip angle 15°, maximum velocity-encoding sensitivity 80 cm/s, slice thickness 4 mm, scan duration 1 min). For arterial transit time, we acquired a multiple time-point pulsed ASL sequence utilizing quantitative STAR labelling of arterial regions (Turbo-QUASAR) using single-shot FFE EPI Look-Locker readouts between each labelling pulse. The parameters for Turbo-QUASAR were TR/TE/TI/TI1 6800/16/600/30 ms, FOV 240 x 240 mm, voxel size 3.75 x 3.75 x 7 mm, flip angle 35°, scan duration 3:24 min [2]. 3D time-of-flight magnetic resonance angiography (TOF MRA) images and maximum intensity projection reconstructions of right-left and feet-head projections were acquired using a multiple-overlapping-thin-slab-acquisition (MOTSA) sequence, using 3D T1 FFE RF-spoiled gradient echo inflow angiography (TR/TE 21/4 ms, FOV 200 x 200 x 90 mm, voxel size 0.39 x 0.39 x 0.5 mm, flip angle 20°, 180 axial slices, scan duration 5:45 min). 3D fluid-attenuated inversion recovery (FLAIR) images were acquired using a 3D TSE multi-shot inversion recovery sequence (TR/TE 4800/356 ms, FOV 250 x 250 x 180 mm, voxel size 0.98 x 0.98 x 1.12 mm, flip angle 90°, inversion recovery delay of 1650 ms, scan duration 5:11 min). None of the scans required contrast.

Cerebral blood flow quantification

CBF was quantified by scaling the images using a dual compartment flow model [3]. Recommended parameters for the quantification of CBF from this model have been calibrated for healthy or elderly subjects [4], and require additional consideration in patients with SCD to avoid potential errors in CBF. We measured the three most dominant parameters to enhance the accuracy of the quantification. The first was T1 of blood (T1b), measured directly in each subject in the sagittal sinus using T2-TRIR MRI according to de Vis et al. [5]. The second was labelling efficiency, simulated from the pCASL sequence parameters according to the method proposed by Maccotta et al.[6,7] and corrected in the quantification based on a match to the measured velocity from PC-MRI. The third was arterial transit time, quantified using a model-based approach from the vascular crushed signal of the Turbo-QUASAR experiment [8], plus 700 ms to account for the more distant labelling location (90 mm) of the labelling plane in pCASL compared to Turbo-QUASAR (20 mm) [9].

Image analysis

We used the ExploreASL toolbox [10], implemented in MATLAB (Version 2014b, MathWorks, Natick, MA, USA) for automated processing of ASL images to obtain quantitative parametric maps. Image quality was assessed by standard deviation of CBF and visually evaluated for artefacts. Image inspection revealed that an artefact resulting from incomplete fat suppression appeared in the WM in thirteen images resulting in unreliable CBF and CVR values in the WM. We excluded participants with the artefact (12/36 patients and 1/11 controls) from the final WM CBF and CVR analysis. The remainder (24 patients and 10 controls) had high quality data. Registration steps included affine and subsequent non-linear registration of anatomical images bringing them to common atlas space, hence allowing voxel-based comparisons of parameters, including lesions, CBF and CVR. Anatomical images were segmented into gray matter (GM) and white matter (WM) tissue probability maps. GM was defined as >25% of the GM tissue probability map, and WM as >90% of the WM tissue probability map excluding lesion voxels (lesion definition is described below). Before calculating CVR, a 3D Gaussian filter (FWHM 9.2 mm) was first applied to the WM-CBF images to reduce noise. CBF time-series analysis was done by first applying a 3D Gaussian filter (FWHM 3.5 mm) for initial de-noising. The time-series curve represents average GM CBF in increments of 8.8 sec. Each subject's time-series was de-noised with a Butterworth low-pass filter using a frequency cut-off of 0.002 Hz, in order to observe the expected smooth baseline and ramp to plateau [11–13], without ripple. These filtered time-series were averaged per group and plotted over time with the standard deviation. Voxel-wise CVR was calculated using the formula:

$$CVR\ (\%) = (\Delta CBF) / CBF_{PRE} \times 100\%,$$

where ΔCBF represents the average of the first 5 min (CBF_{PRE}) of the CBF time-series subtracted from the average of the final 5 min.

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Supplementary tables

Table 1. Incidence of side-effects of acetazolamide

Side effects in total cohort (n=47)	Incidence (n)	Incidence (% of total)	Total dose mg *
None	20	43	768 – 1400 mg
Dizziness	12	26	489 – 1376 mg
Headache	6	13	896 – 1400 mg
Paresthesia, tingling in lips and/or extremities	6	13	880 – 1376 mg
Heaviness, pressure	5	11	864 – 1376 mg
Fatigue, drowsiness	5	11	848 – 1200 mg
Light headedness	2	4	976 – 1200 mg
Strange taste	1	2	928 mg

*Range of total doses at which the side effects occurred.
Side-effects were de-briefed 30 minutes after the intravenous acetazolamide administration in all participants (n=47). There was no difference in incidence of side-effects between patients and healthy controls. Some participants had more than one type of side-effect so appear in the table more than once.

Table 2. Cerebral hemodynamics in transfused patients

Participant receiving regular transfusions (CTT)	Days since last transfusion	Hemoglobin (g/dL)	GM CBF (mL/100g/min)	GM CVR (%)
Pt A (CTT + Hydroxyurea)	35	8.9	108.8	32.6
Pt B (CTT + Hydroxyurea)	14	10.6	64.8	47.0
Pt C (CTT only)	4	10.3	78.8	57.6
Average of Pts A,B, and C	-	9.9	84.1	45.8
Average of other SCD Pts	-	8.8	87.3	38.4*
Average of controls	-	13.7	53.8	81.8

*SCD patients on CTT versus SCD patients not on CTT: $P = 0.62$