Effects of Hydroxyurea on Brain Function in Children with Sickle Cell Anemia

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Word count: abstract 241; main text 3296

Tables: 3; Figures: 1

Running title: Hydroxyurea and brain function in children with SCA

Key words: sickle cell, hydroxyurea, brain function, neurocognition, children

Abbreviations

ASL	arterial spin labeling
BOLD	blood oxygen level dependent (signal)
CBF	cerebral blood flow
fMRI	functional MRI
FSIQ	full scale IQ
GM-CBF	gray matter cerebral blood flow
HbF	hemoglobin F
IRB	Institutional Review Board
MDI	Mental Developmental Index
MRA	magnetic resonance angiography
OEF	oxygen extraction fraction
p^{FDR}	false discovery rate adjusted p-value
PRI	Perceptual Reasoning Index

PSI	Processing Speed Index
SCA	sickle cell anemia
SCD	sickle cell disease
SCI	silent cerebral infarct
SD	standard deviation
TAMV	time-averaged mean maximum velocity
TCD	transcranial Doppler ultrasound
TWiTCH	TCD with Transfusions Changing to Hydroxyurea trial
VCI	Verbal Comprehension Index
WAIS	Wechsler Adult Intelligence Scale
WISC	Wechsler Intelligence Scale for Children
WJ	Woodcock Johnson Tests of Academic Achievement
WM	white matter
WM-CBF	white matter cerebral blood flow
WMI	Working Memory Index

ABSTRACT

Introduction: Sickle cell anemia (SCA) results in numerous adverse effects on the brain, including ischemic lesions and neurocognitive dysfunction. Hydroxyurea has been utilized extensively for management of SCA, but its effects on brain function have not been established.

Methods: We examined prospectively the effects of one year of treatment with hydroxyurea on brain function in a cohort of children with SCA (HbSS/HbSβ^0-thalassemia) by baseline and exit evaluations, including comprehensive neurocognitive testing, transcranial Doppler ultrasound (TCD), and brain MRI [silent cerebral infarcts (SCI), gray matter cerebral blood flow (GM-CBF), and blood oxygen level dependent (BOLD) signal from visual stimulation].

Results: Nineteen patients with SCA, mean age 12.4 years (range 7.2-17.8), were evaluated. At baseline, subjects had these mean values: full scale IQ (FSIQ) 81.9, TCD velocity 133 cm/sec, GM-CBF 64.4 ml/100g/min, BOLD signal 2.34% increase, and frequency of SCI 47%. After one year of hydroxyurea, there were significant increases in FSIQ (+2.8, p=0.036) and reading comprehension (+4.8, p=0.016), a significant decrease in TCD velocity (-11.4 cm/sec, p=0.007), and no significant changes in GM-CBF, BOLD, or SCI frequency. Furthermore, FSIQ was associated with higher hemoglobin F (HbF) and lower GM-CBF, but not with hemoglobin level.

Discussion: Significant improvement of neurocognition and decreased TCD velocity following one year of treatment support the use of hydroxyurea for improving neurocognitive outcomes in SCA. Understanding the mechanisms of benefit, as indicated by relationships of neurocognitive function with HbF, hemoglobin, and CBF, requires further evaluation.

INTRODUCTION

Sickle cell anemia (SCA) is a devastating hematological disease marked by acute and chronic cerebrovascular changes leading to cerebrovasculopathy, brain injury, stroke, and neurocognitive deficits.[1] Without intervention, about 11% of children with SCA will develop clinically overt stroke before age 20 years.[2] Furthermore, 39% of children develop "silent cerebral infarcts" (SCI), indicated by focal ischemic damage on MRI without clinical signs or symptoms of overt stroke.[3] Hydroxyurea, an oral ribonucleotide reductase inhibitor, has proven efficacy in the prevention of vasoocclusive events of SCA in adults[4] and in children[5] and is approved by the U.S. Food and Drug Administration.

Neurocognitive deficits have been noted from an early age in children with sickle cell disease (SCD) and are exacerbated by environmental factors such as low socioeconomic status.[6] In a meta-analysis of 110 studies involving 3600 participants with SCD, deficits in full scale IQ (FSIQ), verbal reasoning, perceptual reasoning, and executive function increased from preschool to school-age samples.[7] However, the effects of hydroxyurea on neurocognitive performance in children with SCD have not been clearly established despite several studies which have addressed that question.[5,8,9]

Children with SCA, including those with transcranial Doppler ultrasound (TCD) velocities in the abnormal range, have had markedly decreased velocities following treatment with hydroxyurea, indicating a reduced risk for stroke.[10,11] In the randomized TCD with Transfusions Changing to Hydroxyurea trial (TWiTCH), in which children with an abnormal TCD velocity were randomized to continue chronic transfusion or convert to hydroxyurea treatment, non-inferiority of the drug indicated that it may be substituted for transfusion.[12]

The role of hydroxyurea in the prevention or management of SCI is unclear. In a single institution prospective trial, children with SCA had a 38% prevalence of SCI at baseline, and a similar prevalence after 3 and 6 years of hydroxyurea at maximum tolerated dose.[13]

To improve understanding of neuropathology in SCD, arterial spin labeling (ASL) MRI neuroimaging has been utilized to quantify cerebral blood flow (CBF) at the capillary level and identify small vessel disease.[14,15] Increased gray matter cerebral blood flow (GM-CBF) has been found in children with SCA compared to normal controls, and has been inversely correlated with neurocognitive function.[16] Blood oxygenation level dependent signal (BOLD) functional MRI (fMRI) non-invasively measures brain hemodynamic responses to neural activities.[17,18] Individuals with SCA were shown to have a compromised or diminished BOLD response to visual stimuli.[19] SCI occur in cerebral artery border zone regions and are associated with low CBF.[20] Elevated oxygen extraction fraction (OEF) in border zones, indicating cerebral metabolic stress, has been mitigated by hydroxyurea.[21] The MRI techniques that we utilized have not been studied together in a prospective cohort of children with SCA to evaluate the role of hydroxyurea on brain function globally.

The primary objective of this study was to examine prospectively the effect of one year of hydroxyurea treatment on measures of CBF, particularly GM-CBF. It was hypothesized that hydroxyurea would significantly lower CBF velocity and might have secondary benefits on brain function in SCA. Therefore, additional objectives included exploration of the effects of hydroxyurea on neurocognitive performance, TCD velocity, and BOLD response to visual stimulus.

METHODS

Subjects

Subjects were eligible for the study if they were 7-18 years of age, had a diagnosis of SCA (HbSS, HbSß^{0}thalassemia), and had not been previously exposed to hydroxyurea, but met clinical criteria for initiation of this therapy.[22] Indications for hydroxyurea treatment were primarily recurrent vasoocclusive pain events (≥3/year requiring medical visits) and acute chest syndrome. Subjects were excluded from participation if they had a history of clinical stroke, were receiving chronic transfusion therapy, had received a hematopoietic stem cell transplant, or were unable to tolerate MRI examination without sedation or anesthesia. Subjects were recruited from the Sickle Cell Outpatient Clinic at St. Jude Children's Research Hospital (St. Jude) between June 2011 and January 2015, at the time that they were initiating hydroxyurea therapy.

Study Design

This was a prospective clinical pilot study. The protocol was approved by the St. Jude Institutional Review Board (IRB). The legal guardian of each participant gave informed consent and subjects gave assent according to the requirements of the IRB. Participants were evaluated prior to hydroxyurea initiation with baseline MRI imaging, TCD examination, neurocognitive testing, and blood work, usually over a two-day period. Hydroxyurea dosing was initiated at 20 mg/kg/d and gradually escalated as per standard local protocol.[22] All the evaluations were repeated after 12 ± 1 months of hydroxyurea treatment.

Anatomical and functional MRI Imaging

Siemens MRI 3T scanners were used in the study. MR images were acquired to identify morphologic abnormalities, facilitate spatial processing of functional images, and visualize functional imaging results. Sagittal 3D T1-weighted (MPRAGE), axial 2D T1-weighted (FLASH), sagittal 3D T2-weighted (SPACE), sagittal 3D T2 FLAIR (SPACE), axial fat-

saturated 2D T2 FLAIR, axial diffusion-weighted, and axial 3D time-of-flight MRA images were acquired and were evaluated by pediatric neuroradiologists (KH, SH). Participants were not sedated. CBF was measured with Q2TIPS ASL sequence and perfusion weighted images were used to quantify CBF based on the method of Luh.[23] CBF was calculated in whole brain, gray matter, and white matter (WM), but GM-CBF is primarily reported here because it was highly correlated with WM and whole brain CBF values and is what is usually reported in the literature. The BOLD signal percentage changes in the primary visual cortex were measured during visual stimulation using a T2* weighted echo-planar MRI sequence.[19] Functional images were processed and analyzed using SPM (htttps://www.fil.ionucl.ac.uk/spm/) and in-house software.

TCD Examination

Standard non-imaging TCD examinations were performed in the Sickle Cell Clinic by certified TCD examiners.[24] Time averaged mean maximum velocities (TAMV) of the middle cerebral and internal carotid arteries were utilized to classify patients into normal (<170 cm/sec), conditional (170-199 cm/sec) and abnormal (≥200 cm/sec) risk groups.

Neurocognitive Testing

Measures were selected that could assess the widest study age range possible using a battery that could be administered in approximately 2-3 hours. All measures had age-specific norms from large, representative standardization samples and had demonstrated reliability and validity. Measures were chosen with appropriate test-retest reliability and negligible practice effects for the one-year interval between testing time points.

Intellectual Function: Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV).[25] The WISC was used to assess intelligence in children 7-17 years of age and the Wechsler Adult Intelligence Scale- Fourth Edition (WAIS-IV)[26] for subjects older than 17.0 years. A

shortened administration of 8 subtests allowed the derivation of the index scores of Full-Scale IQ (FSIQ), Verbal Comprehension (VCI), Perceptual Reasoning (PRI), Working Memory (WMI), and Processing Speed (PSI).

Academic Achievement: Woodcock Johnson Tests of Academic Achievement-Third Edition (WJ-III).[27] The WJ-III Achievement Tests allowed interpretation of broad and specific academic factors. Selected subtests included Letter-Word Identification, Reading Fluency, Passage Comprehension, Calculation, Math Fluency and Applied Problems.

Statistical Analysis

The descriptive statistics of the study were summarized using frequencies for categorical variables and mean, median, standard deviation (SD) and range for continuous variables. The Shapiro-Wilk test was performed to test for normality of the data. One-sample t-tests or Wilcoxon signed rank tests, depending on the normality, were used to compare continuous variables between two time points. Mixed effect linear modeling was used to estimate effects of HbF level, hemoglobin concentration, age, and sex on CBF, BOLD, and neurocognitive performance and relationships among CBF, BOLD, TCD and neurocognitive results. Scatter plots by time points were used to visually examine relationships with FSIQ. For all analyses, p values were two sided and $p<0.05$ was considered statistically significant. False discovery rate adjusted p-values (p^{FDR}) <0.05 were indicated for consideration of significance after adjustment for multiple comparisons. A sample size of 19 evaluable patients was needed to obtain power 86%, based on a correlation between two paired CBF $\rho = 0.4$, an assumed decrease of CBF of 15% (i.e., 13.5 mL/100g/min) based on previous data,[19] and a common standard deviation of 19.2 at a significance level of 0.05 using a one-sided t-test.

RESULTS

Subjects

Twenty-nine subjects with SCA were approached and consented to the study. Ten were later excluded because they never initiated hydroxyurea treatment, discontinued the drug shortly after starting it, and/or failed to complete baseline evaluations. Therefore, 19 subjects were evaluated. These subjects had a mean age of 12.4 years (SD 3.3 years, range 7.2-17.8 years) at enrollment; 11 (58%) were female.

Hydroxyurea Treatment

All subjects began treatment with a dose of hydroxyurea of 20 mg/kg/day and doses were gradually escalated reaching an average of 23.8 (range 13.3 - 33.4) mg/kg/day after 12 months of treatment. Hematologic responses to hydroxyurea at 12 months were excellent, suggesting good adherence with treatment (Table 1). Overall, the mean hemoglobin level increased from 8.7 g/dL to 10.1 g/dL, mean HbF increased from 8.2% to 23.1%, and mean absolute reticulocyte count decreased from 260,000/μL to 140,000/μL. The dose of hydroxyurea at study exit was not associated with results for GM-CBF, TCD velocity, or neurocognitive performance.

Anatomical and Functional MRI (Table 1)

Silent Cerebral Infarcts

Nine of 19 subjects (47%) had SCI on their initial brain MRI examination. After one year of hydroxyurea, 8 remained positive for SCI, none of these had new cerebral infarcts, 8

were negative, one previously negative had new cerebellar infarcts, and two exams were not performed. Therefore, after one year of treatment, SCI were present in 9 out of 17 (53%).

Cerebral Blood Flow

GM-CBF decreased from a mean of 64.4 ml/100g/min at baseline to 62.2 ml/100g/min at exit, but this was not statistically significant (p=0.18). In addition, WM-CBF declined, but not significantly, from a mean of 37.9 to 37.2 ml/100g/min (p=0.67).

BOLD Signal

BOLD signal response to visual stimulation was evaluated in 13 participants at baseline, and 8 had a repeat evaluation at 12 months. At baseline, mean increase in BOLD signal with stimulation was 2.34%. In the 8 participants with paired BOLD comparisons, BOLD signal response increased from a mean of 2.17% to a mean of 2.52%, but this change was not statistically significant (p=0.32).

TCD Examinations

At baseline, the mean TCD velocity (time averaged mean maximum velocity, TAMV) was 133 (±26) cm/sec; two results were in the conditional range (170-199 cm/sec) and none were abnormal (≥200 cm/sec). At exit, the mean TAMV was significantly lower at 120 (±25) cm/sec, p=0.007. One result was in the conditional range and none were abnormal.

Neurocognitive Evaluations (Table 1)

Within the neurocognitive battery, two significant improvements were found in comparing performances at baseline and exit. Mean FSIQ increased from 81.9 (±12.9) to 83.4 (±13.2) (p=0.036) and Woodcock-Johnson Passage Comprehension improved from 79.5 (±16.7)

to 82.6 (±17.1) (p=0.016). No significant changes were seen on the VCI, PRI, WMI, or PSI, or with other WJ achievement subscales.

Linear Mixed Effect Models (Tables 2 and 3)

Linear mixed effect models incorporating both baseline and exit data were used to assess the effects of age, sex and laboratory results on GM- and WM-CBF, BOLD signal, TCD velocity, and neurocognitive performance. Increased age was associated with decreased TCD velocity (p=0.005). After adjusting for age, increased hemoglobin was associated with decreased GM-CBF (p=0.029) and TCD velocity (p=0.015), as well as increased working memory (p=0.02), but not increased FSIQ (p=0.085, Figure 1A), (Table 2). However, after adjusting for age, increased HbF was associated with increased FSIQ (p=0.045, Figure 1B), as well as increased VCI (p=0.042), WJ Calculation (p=0.044) and WJ Passage Comprehension (p=0.016) (Table 2). After adjusting for age, female sex was associated with increased GM-CBF and WM-CBF. Also, after adjusting for age, the presence of SCI was not associated with any measures of neurocognitive performance.

In exploring the effects of GM-CBF and BOLD signal on neurocognitive performance (after adjusting for age), decreased GM-CBF was found to be associated with increased FSIQ (p=0.048, Figure 1C) (Table 3), but not with other neurocognitive measures. GM-CBF was not associated with TCD velocity. BOLD signal increase was associated with faster processing speed (p=0.031). After adjusting for age, decreased TCD velocity also was associated with faster processing speed (p=0.049) and with improved performance on the WJ Math Fluency subtest (p=0.017), but not with other neurocognitive measures or with BOLD signal.

DISCUSSION

Hydroxyurea treatment is a standard of care for children with SCA, and yet its effect on brain function has not been carefully examined in prospective longitudinal studies. In our cohort of children with SCA who initiated hydroxyurea, most notable were improvements in FSIQ and reading comprehension observed after 12 months of treatment. The gain in FSIQ was associated with higher HbF and decreased GM-CBF but not with reduction in TCD velocity or increased hemoglobin level.

Only a limited number of studies, mostly cross-sectional and retrospective, have addressed the effect of hydroxyurea on neurocognitive performance. In the earliest of these, 15 children with SCD (mean age 14.9 years) being treated with hydroxyurea had significantly higher scores on tests of verbal comprehension, fluid reasoning, and general cognitive ability compared to 50 children (mean age 11.6 years) who were not receiving the drug.[8] More recently, a cross-sectional evaluation of neurocognition in adolescents (ages 16-17 years) with sickle cell disease (SCD) followed at our institution compared patients with a history of hydroxyurea exposure (n=64) and those never treated with hydroxyurea (n=39).[9] Thirty-one percent of these patients had repeated a grade and 81% received educational support at school. Among those with HbSS/ HbSβ^0thalessemia (SCA), those who had received hydroxyurea had higher scores on reaction speed and sustained attention than never-treated patients. In those with HbSC/HbSβ^+thalassemia, the hydroxyurea-exposed group had higher scores on nonverbal IQ, working memory and verbal memory. It was concluded that cognitive impairment may be mitigated by exposure to hydroxyurea in adolescents with SCD.

Another recent cross-sectional study examined laboratory biomarkers, cerebral blood flow velocity, and intellectual function in 38 children with HbSS, aged 4-11 years.[28] LDH level, less

maternal education, and older age were negative predictors of cognitive function, but TCD velocity did not have a significant effect. The 26% who were taking hydroxyurea showed a significantly greater estimated verbal IQ compared with those not taking hydroxyurea (p=0.005). Finally, in a randomized prospective trial of hydroxyurea (BABY HUG), very young children with SCA (mean age 13 months) received hydroxyurea or placebo for two years.[5] Mean scores on the Bayley Scales of Infant Development Mental Developmental Index (MDI) in the hydroxyurea group were 97 at baseline and 97 at exit from the trial, which were not significantly different from mean scores in the placebo group (97 at baseline, 94 at exit). Interestingly, 5 of 80 subjects in the placebo group had an MDI <70 (impaired range) at exit, whereas none of the 85 in the hydroxyurea group scored below 70, suggesting that hydroxyurea may provide a level of neurodevelopmental advantage in infants with SCA.

A mechanism for improved cognition from hydroxyurea treatment might be increased hemoglobin level, which could impact brain oxygenation and function. In a study of 37 children with SCA, aged 6-18 years, low hemoglobin was associated with a decrease in verbal short-term memory.[29] In another report, older age and low hemoglobin were associated with increased neurocognitive impairment.[30] HbF has been associated with significantly better verbal fluency in a study of adolescents[9] and better scores on particular cognitive measures in a study of adults.[31]

Our findings, using linear mixed modeling of the overall cohort, showed that higher hemoglobin levels were associated with decreased GM-CBF and TCD velocity and increased working memory (although not increased FSIQ). However, higher HbF was associated with increased FSIQ, verbal comprehension, and WJ Calculation and Passage Comprehension. Taken together, our data indicate that hydroxyurea may provide specific neurocognitive benefits, possibly by increasing both HbF and hemoglobin levels. Our data also suggest that increased

BOLD signal and decreased CBF may be contributing to the process, because changes with hydroxyurea were in the expected direction although not statistically significant.

Neurocognitive dysfunction has been associated with elevated TCD velocities in previous reports. Children with SCA and abnormal TCD values performed more poorly than children with conditional TCDs on measures of verbal intelligence and executive function.[32] In a subsequent study based on teacher reports, children who had abnormal TCD velocities had clinically significant executive dysfunction with difficulties in working memory, planning and organization.[33] In a meta-analysis, TCD velocity was negatively associated with visual-spatial and perceptual reasoning.[7]

Numerous studies have shown that hydroxyurea treatment results in diminished TCD velocities.[34] In our patients, as expected, hydroxyurea significantly reduced TCD velocities and only one patient had a conditional velocity after one year of treatment. TCD velocities were not associated with GM- or WM-CBF measurements, but increased velocities were associated with low performance on two neurocognitive measures--the PSI and WJ Math Fluency (Table 3).

An inverse correlation between CBF and neurocognitive function (performance IQ) has been noted in children with SCA.[16] In our study, CBF decreased, but not significantly, after one year of hydroxyurea treatment in both gray matter and white matter. FSIQ was inversely related to GM-CBF.

SCI occur in cerebral artery border zone regions and are associated with low CBF.[20] Elevated OEF, particularly in border zones, indicating cerebral metabolic stress, has been mitigated by hydroxyurea.[21] In a comparison of three groups of SCA patients, those with no treatment had the highest OEF (median 42.9%), whereas those receiving chronic transfusion

therapy had a much lower OEF (35.3%) and those on hydroxyurea were intermediate (40.7%). The authors concluded that hydroxyurea may provide neuroprotection by mitigating cerebral metabolic stress in patients with SCA.

Our study evaluated BOLD signal responses to visual stimulation, which are driven primarily by changes in blood flow and oxygen extraction. BOLD signal reflects the interplay among dynamic changes of CBF, cerebral metabolic rate of oxygen use, cerebral blood volume, and oxygen extraction fraction (OEF) in response to changing neural activity. Previously, we had used BOLD- and CBF-based fMRI to measure primary visual cortex responses to photic stimulation in 23 children with SCA (almost all receiving hydroxyurea) and 21 clinical controls.[19] The BOLD responses were diminished in children with SCA, although blood hemoglobin level and CBF were not predictive of BOLD signal amplitude. However, BOLD amplitude was associated with higher IQ scores ($p \leq 0.05$), possibly reflecting less oxygen extraction from the arterial circulation and consequently a higher level of oxygenation in the cerebral venous circulation. In our current study, we found that BOLD signal increase was associated with faster processing speed. Average BOLD signal activation levels went from 2.34% (baseline) to 2.75% (after one year). This was not a statistically significant change, possibly because of the limited number of comparisons that were available (n=8), but the changes were in a positive direction. These data suggest that hydroxyurea results in better oxygen delivery to brain tissue, and possibly improved cognitive function.

The major strength of our study was that the effects of hydroxyurea on brain function were evaluated prospectively and comprehensively both before and after a monitored one-year treatment course. To our knowledge, this approach using fMRI, ASL MRI, anatomical MRI, TCD, neuropsychologic evaluation, and hematologic evaluation has not been previously

performed to evaluate the effects of hydroxyurea on brain function. There were some significant limitations to the study, most notably the relatively small number of subjects and the lack of a randomized control group. Some patients who began hydroxyurea during the enrollment period declined to participate and not all the planned evaluations were completed, especially the BOLD signal examinations. However, our study provides novel evidence for the benefit of hydroxyurea therapy on brain function, including both neurocognitive and vascular function.

In summary, in this prospective longitudinal trial of one year of treatment, hydroxyurea had positive effects on measures of neurocognition, including FSIQ and reading comprehension. A significant decrease in TCD velocity was found, but not significant MRI changes in CBF, BOLD signal and SCI. Using linear effects modeling of the overall data, FSIQ was associated with higher HbF and lower GM-CBF, and several subtests of neurocognition were associated with increased HbF or Hb or with decreased TCD velocity. These results support the use of hydroxyurea for improving neurocognitive outcomes in SCA and suggest possible mechanisms for improvement based on functional changes in the brain. Larger numbers of subjects should be tested to obtain better understanding of the mechanisms of benefit from hydroxyurea and other potential interventions.

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FIGURE LEGEND

Figure 1. *Factors Influencing FSIQ.* Scatterplot of FSIQ versus hemoglobin (Panel A), HbF (B) and GM-CBF (C) by baseline and exit time points. *Abbreviations:* FSIQ=Full Scale Intelligence Quotient, HbF=hemoglobin F, GM-CBF=gray matter cerebral blood flow. *P values* were calculated to test the main effect on FSIQ of hemoglobin, HbF or GM-CBF from linear mixed effects models with adjustment for age. All false discovery rate adjusted p-values were >0.05.

Author contributions: JSH, KH, JS and WCW designed the study. WCW, PZ, SH, AMH, JS, KH, and JSH performed the research. PZ processed and analyzed the functional images. GK and JD analyzed the data. All authors contributed to the drafting and editing of the manuscript and have approved the manuscript.

Conflicts of Interest: WCW had consultative fees from Agios and Novartis. JSH had research funding from Global Blood Therapeutics (GBT) and consultative fees from GBT and MJH Lifesciences. The other authors had no conflicts of interest.

Acknowledgments: The authors thank Evelyn Harris and Patsy Burnside for excellent administrative assistance.