HYDROXYUREA THERAPY LOWERS
TRANSCRANIAL DOPPLER FLOW VELOCITIES
IN CHILDREN WITH SICKLE CELL ANEMIA

Short Title: Hydroxyurea lowers TCD velocities

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ABSTRACT

Hydroxyurea has hematological and clinical efficacy in SCA, but its effects on transcranial doppler (TCD) flow velocities remain undefined. Fifty-nine children initiating hydroxyurea therapy for clinical severity had pre-treatment baseline TCD measurements; 37 with increased flow velocities (≥140 cm/sec) were then enrolled in an IRB-approved prospective Phase II trial with TCD velocities measured at maximum tolerated dose (MTD) and one year later. At hydroxyurea MTD (mean ± 1SD = 27.9 ± 2.7 mg/kg/day), significant decreases were observed in the right MCA (166 ± 27 cm/sec to 135 ± 27 cm/sec, p<.001) and left MCA velocities (168 ± 26 cm/sec to 142 ± 27 cm/sec, p<.001). The magnitude of TCD velocity decline was significantly correlated with the maximal baseline TCD value. At hydroxyurea MTD, 14 of 15 children with conditional baseline TCD values improved, while 5 of 6 with abnormal TCD velocities whose families refused transfusions became <200 cm/sec. TCD changes were sustained at follow-up. These prospective data indicate that hydroxyurea can significantly decrease elevated TCD flow velocities, often into the normal range. A multicenter trial is warranted to determine the efficacy of hydroxyurea for the management of increased TCD values, and ultimately for primary stroke prevention in children with SCA.
INTRODUCTION

Transcranial doppler (TCD) ultrasonography is now recommended as a routine screening test for children with sickle cell anemia (SCA) from 2 to 16 years of age, to identify patients at highest risk for primary stroke [1,2]. TCD studies measure flow velocity within the large intracranial arteries, which are the vessels most often involved in sickle cerebral vasculopathy and stroke [3,4]. TCD ultrasonography is particularly useful in children because it is painless, non-invasive, relatively inexpensive, easy to perform, and requires no sedation.

Landmark studies performed almost two decades ago by Adams and colleagues [5] found that children with SCA have higher baseline TCD flow velocities than age-matched children without SCA. These increased TCD velocities were related in part to the anemia, since the TCD values were inversely correlated with the hematocrit [6]. Among children with SCA, however, increased TCD flow velocities have also been associated with younger age and pathological arterial stenosis [7]. In the cohort of patients from the Medical College of Georgia, 36.8% had TCD velocities ≥140 cm/sec, 17.5% had velocities ≥170 cm/sec termed “conditional”, and 7.9% had velocities ≥200 cm/sec termed “abnormal” [5,8,9].

Abnormal TCD velocities are identified in 5-10% of children with SCA and confer a 10% annual risk for developing primary stroke [8,9]. The NHLBI-sponsored multicenter randomized Stroke Prevention (STOP) trial demonstrated that monthly blood transfusions can reduce this stroke risk by 90% compared to observation alone [10], and a Clinical Alert recommended chronic transfusions for children with SCA and abnormal TCD velocities [11]. However, the follow-up STOP 2 study concluded that transfusions must
be continued indefinitely in this setting, due to an increased risk of conversion to abnormal TCD velocities and the development of new neurological events after halting transfusions [12]. Children with conditional TCD velocities also have an elevated (1-3% annual) risk of developing primary stroke, most commonly after conversion to the abnormal category [13]. Accordingly, frequent monitoring of children with conditional TCD velocities is recommended, although no specific therapy is currently recommended for this potentially vulnerable patient population.

An alternative effective therapy would be beneficial for children with SCA and abnormal TCD velocities to reduce the long-term risks associated with chronic blood transfusions, including erythrocyte alloimmunization, infectious risks, and iron overload [14]. In addition, a simple treatment option might be beneficial for children with conditional TCD values, who currently receive increased monitoring but no specific treatment. The increasing availability of hydroxyurea therapy offers a reasonable therapeutic option in these settings, but to date no prospective studies have focused on the effects of hydroxyurea therapy on TCD velocities in children with SCA. The Belgian registry recently reported a beneficial effect of hydroxyurea in 11 children who had abnormal TCD and serial measurements, with a significant decrease observed in TCD velocities [15]. A recent small retrospective analysis suggested that hydroxyurea significantly decreased the TCD velocities compared to age-matched controls, but only 8 children had TCD velocities ≥140 cm/sec and the effects were not related to hematological changes [16]. In the current study, we describe a prospective single-institution IRB-approved Phase II trial of hydroxyurea for children with SCA and increased TCD flow velocities.
METHODS

TCD Screening. Beginning in 1995, the Duke Pediatric Sickle Cell Program enrolled subjects in the Phase I/II pediatric hydroxyurea safety trial (HUG-KIDS), and thereafter began using hydroxyurea for the routine clinical management of selected children with severe vaso-occlusive complications such as recurrent painful events and acute chest syndrome. Routine TCD screening began at Duke in 1999, and analysis of the initial TCD screening results provided the pilot data and rationale for a prospective Phase II trial to determine the effects of hydroxyurea on TCD flow velocities.

Prospective Phase II Study. Between late 2000 and 2004, all pediatric patients with severe forms of sickle cell anemia (HbSS, HbS/β\(^0\)-thalassemia, HbS/O\(_{Arab}\)) underwent TCD screening before initiating hydroxyurea treatment. In most cases, these children were prescribed hydroxyurea therapy for severe clinical complications (e.g., recurrent pain or acute chest syndrome), including children who received hydroxyurea as part of an open-label trial to determine the effects of hydroxyurea on organ function and quality of life for young children (age 1-3 years) with SCA. Children initiating hydroxyurea therapy who had increased baseline TCD flow velocities identified were eligible for a prospective study to determine the effects of hydroxyurea on their TCD values.

All TCD screening studies were performed in the Neurodiagnostic Laboratory at Duke University Medical Center using a non-duplex TCD instrument, according to the published techniques for children with SCA [5]. Briefly, the left and right middle cerebral, distal internal carotid, anterior cerebral, and posterior cerebral arteries were insonated at 2mm increments using a special pediatric transducer and a transtemporal approach. Maximal systolic and diastolic peak velocities were recorded, but the time-averaged
maximum velocity (TAMV) was used to determine the TCD value for the major intracranial arteries on each side, as previously described [5].

After TCD screening, those children with at least one increased TCD value (defined as a right- or left-sided TAMV ≥ 140 cm/sec) were offered enrollment in an IRB-approved trial to determine prospectively the effects of hydroxyurea therapy on TCD velocities. Approval for this prospective study was obtained from the Duke University Medical Center Institutional Review Board and all subjects and families gave written informed consent. Hydroxyurea dosing and monitoring were performed according to routine clinical practice, including escalation to the maximum tolerated dose (MTD) with monthly blood counts as previously described [17]. After a stable hydroxyurea MTD was reached, typically after 6-12 months of therapy, a follow-up TCD study was performed. A third long-term follow-up TCD study was then performed at least one year after reaching MTD, to determine if there were sustained treatment effects. Baseline brain MRI/MRA imaging was performed in children as part of this protocol, using standard techniques.

**Statistical analysis.** TCD flow velocities were recorded for the right and left middle cerebral artery (MCA), anterior cerebral artery (ACA), and posterior cerebral artery (PCA). Patient and treatment characteristics such as age, hydroxyurea dose, and routine blood counts including % HbF values, were also recorded. Standard statistical calculations such as mean ± standard deviation, median, and range were performed using Excel software. Comparisons of TCD studies for a given patient, e.g. pre- and post-treatment with hydroxyurea, were performed using the nonparametric Wilcoxon rank sum test while between-group comparisons used a two-tailed t test. Regression plots to determine
correlations between parameters such as baseline TCD velocity and change in TCD values were performed using SAS software (Cary, NC).

RESULTS

Initial TCD Screening. Analysis of the first 102 children who received TCD screening [18] revealed fewer than expected children with conditional and abnormal flow velocities (12.7% and 3.9% respectively), compared to Adams’s published prevalence values of 17.5% and 7.9% [8,9]. Subsequent analysis of these initial screening results according to concurrent hydroxyurea therapy revealed a significant difference: 28 children on hydroxyurea therapy had an average TCD flow velocity of 116 ± 25 cm/sec, while 74 children not on hydroxyurea therapy had an average TCD flow velocity of 141 ± 32 cm/sec (p<.001, data not shown). These pilot data suggested that hydroxyurea therapy might lead to decreased TCD flow velocities, but no baseline pre-treatment measurements were available, and there were substantial differences between these two patient cohorts (e.g., the hydroxyurea-treated children were older and had more clinical severity). To test this hypothesis, we designed a prospective Phase II study to determine the effects of hydroxyurea therapy on TCD flow velocities in children with SCA.

TCD screening for Phase II study eligibility. Over a 45-month period, 59 children with SCA underwent TCD screening before initiating hydroxyurea therapy for clinical indications (e.g., pain, ACS). A total of 37 children (62.7%) were identified with at least one increased TCD velocity value ≥ 140 cm/sec (right MCA in 31, left MCA in 32, right ACA in 12, and left ACA in 14 patients), all of whom were enrolled in the prospective study. Only one child had an elevated PCA flow velocity of 156 cm/sec on the left side.
Fifteen children (25.4%) had at least one conditional TCD value between 170 and 199 cm/sec (right MCA in 11, left MCA in 10, right ACA in 3, and left ACA in 1 patient). Six other children (10.2%) had at least one abnormal TCD value ≥ 200 cm/sec (right MCA in 3 and left MCA in 5 patients); their families declined transfusions and elected to proceed with hydroxyurea therapy as already planned for clinical severity.

**Baseline MRI/MRA results.** A total of 33 children completed baseline brain MRI and MRA studies, typically within 2 months of initiating hydroxyurea therapy; 4 children were unable to complete the study. Eleven children had abnormal MRI results, 10 had abnormal MRA findings, and 2 had both abnormal MRI and MRA results. Thirteen children with abnormal MRI findings included 11 with small T2-weighted bright foci consistent with small vessel ischemia, and 3 with an unsuspected Arnold Chiari Type I malformation. Twelve children with abnormal MRA findings included 9 with mild vessel narrowing, 2 with moderate vessel stenosis, 2 with prominent lenticulostriate collateral vessels, and 1 with a hypoplastic arterial segment.

**Hydroxyurea effects.** The age, baseline laboratory characteristics, and pre-treatment TCD values for the 37 children with increased flow velocities who enrolled in the prospective study are included in Table 1. Their pre-treatment laboratory values were very similar to those reported in the HUG-KIDS trial [19]. The average pre-treatment TCD velocities were highest in the MCA, followed by ACA and then PCA vessels, but no significant differences were observed between the right- and left-sided vessels.

All 37 children were treated with hydroxyurea and eventually reached a stable MTD of 27.9 ± 2.7 mg/kg/day (median 28.6, range 18.8 to 32.6 mg/kg/day). Compliance
with hydroxyurea therapy was estimated to be excellent for most of the patients, although six children (16%) had presumed poor medication adherence based on hematological parameters and failure to attend clinic appointments regularly. Laboratory values at hydroxyurea MTD for the entire cohort of 37 patients illustrate the expected significant increases in hemoglobin concentration, hematocrit, MCV, and %HbF (Table 1).

**TCD Changes.** Follow-up TCD screening was performed on 36 children after reaching hydroxyurea MTD, with an average interval of 10 ± 5 months (median 8 months) of therapy. The results of these follow-up TCD examinations demonstrated a significant decrease in the maximal TCD flow velocities, for either the MCA or ACA vessels (Table 1). Follow-up TCD examination was not obtained in one non-compliant patient, who was lost to follow-up 14 months after initiating hydroxyurea treatment.

The magnitude of the decrease in TCD flow velocity was significantly associated with the baseline TCD velocity. Figure 1 illustrates that children with the highest baseline TCD velocity measurements had the greatest decrease in response to hydroxyurea therapy ($r^2 = .12, p = .04$). The decline in TCD flow velocities observed in association with hydroxyurea therapy at MTD was then compared to the corresponding increases in hematocrit for each patient. There was no correlation between the magnitude of the TCD decrease and the hematocrit increase ($r^2 = .06, p = .17$), but on average the median TCD velocity decreased 4.8 cm/sec for every percent increase in hematocrit (data not shown).

An additional TCD examination was then attempted at least 1 year after reaching MTD, to determine if the effects of hydroxyurea treatment were sustained. A total of 28 long-term follow-up TCD studies were performed at an average of 25 ± 7 months (median 24 months) after starting hydroxyurea therapy. In almost every case, the TCD velocity
was similar to the initial follow-up study (Figure 2), with an average further decrease of 12 ± 15 cm/sec (median 10 cm/sec decline, p = NS).

**Outcome of initial conditional and abnormal TCD velocities.** Fifteen children had conditional baseline TCD flow velocities, including 8 with “low conditional” values (170-184 cm/sec), and 7 with “high conditional” values (185-199 cm/sec). Upon reaching hydroxyurea MTD, 14 of 15 children had decreased TCD velocities with only 3 low conditional studies on follow-up examination, and none at long-term follow-up. One child with an initial left MCA flow velocity of 166 cm/sec (baseline HbF = 10.0%) had an initial decline after 11 months of hydroxyurea therapy to 147 cm/sec (HbF= 14.9%), but after 20 months of hydroxyurea therapy with suspected non-compliance (HbF= 11.4%) the flow velocity had increased again to 171 cm/sec.

The six children with initial abnormal TCD flow velocities had relatively large decreases in TCD flow velocities in association with hydroxyurea therapy. The TCD values for the 8 vessels with initial abnormal values decreased from 216 ± 14 cm/sec to 173 ± 31 cm/sec (p<.001). One 5-year-old male with a conditional TCD but admitted hydroxyurea non-compliance had conversion to an abnormal TCD; the right MCA remaining unchanged at 171 cm/sec, but the left MCA increased from 167 to 234 cm/sec and the right ACA increased from 100 to 221 cm/sec. Hydroxyurea was discontinued for this child and he was changed to monthly blood transfusions.

One new neurological event occurred in a 14-year-old female with abnormal TCD after 7 months of hydroxyurea therapy. The overall incidence of new neurological events for the entire patient cohort during treatment was 1 in 193.2 patient-years of follow-up, or 0.52 events per 100 patient-years of observation.
DISCUSSION

Despite the definitive results of the STOP and STOP 2 trials, there are several unresolved issues regarding TCD flow velocities and stroke risk in children with SCA. First, the positive predictive value of an abnormal TCD result for stroke is relatively low, since only one-third of children with abnormal TCD velocities and even fewer with conditional TCD velocities will develop an overt clinical stroke [8,9]. Long-term follow-up from the original STOP trial has also identified a subset of patients who remain stroke-free off transfusions despite persistent abnormal TCD flow velocities, and also some transfused patients who continue to have abnormal TCD velocities [20]. Discordance between TCD and brain MRI/MRA results is also recognized, including the observation that most children with abnormal TCD velocities will have no abnormalities on brain MRI or MRA [21,22]. In addition, there are well recognized risks of blood transfusions, including iron overload and alloimmunization, for children with SCA receiving indefinite monthly transfusions in accordance with current NHLBI recommendations for abnormal TCD velocities. In the STOP trial, 10 of 63 subjects randomized to the transfusion arm developed new RBC alloantibodies, despite extended phenotypic matching of blood, 5 required central line placement to facilitate transfusions, and almost all of the patients had increases in serum ferritin to the range requiring initiation of iron chelation therapy [23,24]. Finally, the projected costs of chronic blood transfusions (including chelation therapy) approaches $400,000 per patient decade [25]. Taken together, these observations suggest that an alternative and effective treatment for primary stroke prevention would be a useful option for patients with SCA and an abnormal TCD velocity.
Although monthly transfusions are currently recommended for children with abnormal TCD velocities, the mechanisms by which transfusions reduce stroke risk have not been fully elucidated. Acutely, transfused erythrocytes quickly raise the circulating erythrocyte mass and increase the hematocrit with concomitant increased blood viscosity, and have been demonstrated to lower the TCD velocity within 30 minutes of starting the transfusion [26]. Chronic transfusion therapy also directly inhibits erythrocyte sickling, improves blood rheology, reduces red cell adhesion, and lowers the plasma-free hemoglobin and other laboratory evidence of hemolysis [27-29]. Similar effects are observed in association with hydroxyurea therapy, which also increases the hematocrit, inhibits erythrocyte sickling by increased fetal hemoglobin, improves red cell rheology, reduces red cell adhesion, and lowers LDH and total bilirubin concentrations [19,30-32]. Both transfusions and hydroxyurea therapy might, therefore, be expected to lower TCD flow velocities in children with SCA. In the setting of secondary stroke prevention, hydroxyurea therapy has been shown to provide similar protection as transfusion prophylaxis [33,34].

Gulbis and colleagues [15] recently reported updated results from the Belgian registry, which included 34 children with SCA at risk for primary stroke on the basis of an abnormal TCD. Although these patients were treated with hydroxyurea below MTD (median dose <20 mg/kg/day), 11 children with serial TCD measurements had a significant decrease in TCD velocities from 235 ± 25 cm/sec to 204 ± 27 cm/sec (p<.01). Only 1 of the 34 children developed a new neurological event over 96 patient-years of follow-up. In contrast, our Phase II trial provides the first prospective data focused specifically on the ability of hydroxyurea therapy to lower TCD velocities in children with SCA. In 37 pediatric patients with increased TCD flow velocities ≥140 cm/sec,
hydroxyurea therapy significantly lowered the TCD values and this effect was sustained for at least one year after reaching MTD. Children with conditional TCD velocities had improvement, with resolution of the conditional velocities at long-term follow-up in 14 of 15 children. Compliance with hydroxyurea remains a concern, however, since those children with poor medication adherence had minimal TCD responses, and one admittedly non-compliant child had conversion from conditional to abnormal flow velocities, leading to a change in therapy with chronic transfusions. The long-term risks of hydroxyurea also remain undefined for young patients with SCA.

These data do not demonstrate definitively that hydroxyurea is an efficacious or effective treatment for the prevention of primary stroke in children with SCA. Although most of the children with increased TCD flow velocities in this study had a significant decrease in the TCD values while on hydroxyurea therapy, there was one new neurological event in a child with an initial and follow-up abnormal result. A controlled multicenter trial is needed to determine the efficacy of hydroxyurea therapy to lower TCD velocities and ultimately, to prevent primary stroke in children with abnormal TCD flow velocities.

ACKNOWLEDGMENTS

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REFERENCES


Figure 1. The decrease in TCD flow velocity on hydroxyurea at MTD is correlated with the maximal baseline TCD flow velocity. The magnitude of the decline in TCD flow velocity (shown on the y-axis in absolute value) was significantly correlated to the maximal baseline TCD flow velocity ($r^2 = 0.12$, $p = .04$).
Figure 2. Long-term effect of hydroxyurea therapy on TCD flow velocities. Children with SCA and increased baseline TCD velocities received hydroxyurea therapy at MTD. The maximum TCD velocity is shown after reaching MTD and then in long-term follow-up for 28 children. There was a significant decrease from baseline TCD flow velocities to the MTD values (p<.001), and this decrease was sustained at long-term follow-up.
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Table 1. Laboratory parameters and TCD flow velocities for 37 children with SCA, before hydroxyurea therapy and after reaching MTD.
Hydroxyurea therapy lowers transcranial doppler flow velocities in children with sickle cell anemia

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