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Urine Concentrating Ability in Infants with Sickle Cell Disease: Baseline Data from the Phase III Trial of Hydroxyurea (BABY HUG)

Scott T. Miller, MD,
SUNY-Downstate Medical Center/Kings County Hospital Center

Winfred C. Wang, MD,
St. Jude Children's Research Hospital

Rathi Iyer, MD,
U Mississippi Medical Center

Sohail Rana, MD,
Howard University

Peter Lane, MD,
Children's Healthcare of Atlanta

Russell E. Ware, MD PhD,
St. Jude Children's Research Hospital

Daner Li, MS, and
Clinical Trials & Surveys Corp

Renée C. Rees, PhD
Clinical Trials & Surveys Corp

for the BABY HUG Investigators

Abstract

Background—A urine concentrating defect is quite common in sickle cell anemia, has its onset in early childhood, and may be reversible with transfusion. The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) is a double-blind, placebo-controlled trial to assess efficacy of hydroxyurea in preventing organ damage in young children with sickle cell anemia.

Procedures—Enrolled infants were subjected to parent-supervised fluid deprivation and urine and serum osmolality were determined.

Results—Of 185 infants age 7.5 – 17.9 months (mean 13.0+/-2.7) and fluid-deprived 7.4+/-2.4 hours (range 4-13), 178 had concurrent determinations of urine and serum osmolality. Mean serum osmolality was 286+/-6 mOsm/kg H_2O (range 275-312) and independent of age, height, weight, or duration of fluid deprivation. Urine osmolality (mean 407+/-151, range 58-794 mOsm/ kg H_2O) was greater than serum ($p<0.0001$) and correlated with duration of fluid deprivation ($p=0.001$). Of 142 (77.2%) who concentrated urine, 54 (29.4%) had urine osmolality > 500 mOsm/kg H_2O. Urine osmolality correlated with ^{99m}Tc-DTPA clearance ($p=0.02$) and serum urea nitrogen

Corresponding Author: Scott T. Miller MD, Professor of Clinical Pediatrics, Division of Hematology/Oncology, SUNY-Downstate Medical Center, 450 Clarkson Ave., Box 49, Brooklyn, NY 11203. Phone 718-270-2843, Fax 718-270-1692, Pager 917-760-1077, stmseelig@aol.com.

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($p<0.0001$), but not with serum osmolality, gender, age, height, weight or serum creatinine. Infants able to produce urine with osmolality > 500 mOsm/kg H_2O had higher mean fetal hemoglobin concentrations than did those who could not ($p=0.014$).

Conclusions—Even with often limited fluid deprivation, 77.2 percent of young infants with sickle cell anemia were able to concentrate urine. Preservation of concentrating ability was associated with higher fetal hemoglobin concentration. Assessment will be repeated after two years of hydroxyurea or placebo treatment. (ClinicalTrials.gov number, NCT00006400.)

Keywords

nephropathy; urine osmolality; isosthenuria; hyposthenuria

Introduction

Dr. Hugh Josephs first noted that individuals with sickle cell anemia have “a tendency to fixation of specific gravity of the urine” in 1928 [1]. Loss of concentrating capacity may occur quite early in life [2,3] and is transiently reversible in young children by transfusion of normal blood [2,4]. Clinical implications include nocturia, enuresis [5] and a tendency toward dehydration, the latter perhaps predisposing to vaso-occlusive episodes [6].

The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) enrolled 193 infants with sickle cell anemia (188 with homozygous disease and only five heterozygous for sickle hemoglobin and β^0-thalassemia) in a double-blind placebo-controlled trial to determine whether early (age nine months to 17 months at entry) initiation of hydroxyurea therapy may reduce or delay chronic organ damage. While hydroxyurea reduces pain and acute chest syndrome frequency in adults with sickle cell anemia [7] and has shown similar results in uncontrolled trials in children [8-10], its impact on organ damage and function is not clear.

The primary endpoints of the BABY HUG trial are spleen and renal function. As a secondary endpoint, pretreatment data were collected during a pre-randomization screening phase of BABY HUG with the goal of measuring baseline urine concentrating ability after a modified period of fluid restriction. Eligible infants were then randomized to receive two years of therapy with either hydroxyurea or placebo. The randomized portion of the trial is ongoing with completion anticipated in September 2009.

Methods

Subjects were recruited from 14 centers. Each center was expected to identify all infants with sickle cell anemia shortly after birth and ultimately introduce them to the BABY HUG trial; there was no attempt to select or exclude infants based on clinical severity. Randomization was restricted to eligible infants age nine through 17 months. Screening could begin a maximum of eight weeks prior to randomization and required baseline blood and urine data.

Over one night during the screening phase, parents were asked to withhold food and fluids from their infants for a period approaching but not exceeding 10 hours. They were instructed to record on a worksheet the time of last oral intake the evening prior to a clinic visit; capture the first void at least four hours after the last fluid intake and the subsequent void (urine bags were provided); record times of all voids on the worksheet; and bring urine samples to the clinic in the morning. While parents were encouraged to submit first and second urine specimens collected at home, generally the first urine specimen was obtained at the clinic and this was the specimen used. Blood to measure serum osmolality was obtained as close in time as feasible to the urine specimen. Parents were permitted to provide a small

amount of fluid (enough to appease the infant) orally if no urine had been obtained after 10 hours of restriction to avoid overly dehydrating the infants and thereby risk inducing vaso-occlusive problems; however, specimens obtained after 10 hours were included in the analysis. Serum and urine specimens were sent to a central laboratory for determination of osmolality by the technique of freezing point depression using the Model 2430 Automatic Osmometer Precision System. Urine specific gravity was measured locally at each center.

Infants with less than four hours of fluid deprivation were excluded from analysis. Isosthenuria was defined as urine osmolality within 1 standard deviation (SD) of the mean serum osmolality; hyposthenuria and hypersthenuria were defined as less or greater concentration than isosthenuria. Urine osmolality was analyzed to determine potential relationships to gender, age, height and weight; duration of fluid restriction reported; urine specific gravity; serum urea nitrogen, osmolality and creatinine; and glomerular filtration rate as estimated by ^{99m}Tc-DTPA clearance and the Schwartz formula [18].

Descriptive statistics and statistical analyses were performed using SAS version 8.2 (SAS Institute, Cary NC). Univariate linear regression was used to determine significant associations with the various measures of kidney function, as well as estimates of the relationships. The Student t test was used to identify significant associations between the continuous clinical and laboratory variables and urine concentrating ability. The Chi-square test was used to assess the association between gender and urine concentrating ability. A multivariate logistic regression model was constructed to explore the effects of fetal hemoglobin, duration of fluid deprivation, and serum urea nitrogen on urine concentrating ability by controlling the other demographic and clinical factors. Analyses were repeated for a subgroup of infants able to concentrate to a urine osmolality > 500 mOsm/kg H_2O [2]. The paired T test was used to compare urine and serum osmolality. ANOVA was done to determine whether subgroups of infants defined by duration of fluid deprivation differed significantly in glomerular filtration rate and urine osmolality achieved. P values ≤ 0.05 were considered statistically significant.

Results

Of 233 infants enrolled in the screening phase of the BABY HUG trial, 185, age 7.5 – 17.9 months (mean 13.0 +/- 2.7), had a reported fluid deprivation of at least four hours. Urine and serum osmolality were determined for 184 and 179 of these infants, respectively; 178 infants had both tests done concurrently. The mean duration of fluid deprivation was 7.4 +/- 2.4 hours (range 4 – 13). Mean urine osmolality was 407 +/- 151 mOsm/kg H_2O (range 58 - 794) and was significantly greater than the mean serum osmolality of 286 +/- 6 mOsm/kg H_2O (range 275 - 312) ($p<0.0001$). One hundred forty-two (77.2%) infants were hypersthenuric. There was an average increase of 13.2 mOsm/kg H_2O (95% CI: 4.5 – 22.0, $p=0.003$) in urine osmolality for every hour of fluid deprivation (Figure 1) and 23.0 mOsm/kg H_2O (95% CI: 17.0 – 29.0, $p<0.001$) for every 1.0 mg/dL increase in serum urea nitrogen. Urine osmolality was correlated with glomerular filtration rate as determined by ^{99}Tc-DTPA clearance ($p=0.02$). There was no relationship between urine osmolality and genotype (SS versus Sβ^0), gender, age, height, weight, serum osmolality or creatinine level. The number of subjects with various degrees of urine concentrating capacity is shown in Table I.

Serum osmolality did not correlate with age, height, weight, serum creatinine, duration of fluid deprivation, or creatinine clearance as estimated by either ^{99}Tc-DTPA clearance or the Schwartz formula. However, serum osmolality was associated with an average increase of 0.31 mOsm/kg H_2O (95% CI: 0.06 – 0.56, $p=0.017$) for every 1.0 mg/dL increase in serum urea nitrogen. Mean urine specific gravity was 1.011 +/- 0.005 gm/ml (range 1.000 - 1.025)

with 54 (29%) infants having urine specific gravity > 1.015 gm/ml. Urine osmolality correlated with urine specific gravity, increasing on average 18.9 mOsm/kg H_2O (95% CI: 15.7 – 22.0, p<0.001) for every 0.001 increase in urine specific gravity. Urine specific gravity was also associated with length of fluid deprivation, increasing on average 0.00034 gm/ml (95% CI: 0.00004 – 0.00065, p=0.029) for every one-hour increase in fluid deprivation.

Additional analyses were conducted looking at subgroups of infants. Infants with urine osmolality greater than 500 mOsm/kg H_2O had higher fetal hemoglobin concentrations (28.4+/-8.6 gm/dl vs.25.0+/-8.3 gm/dl, p=0.014) and serum urea nitrogen (11.5+/-3.3 vs. 9.0+/-2.9 mg/dl, p<0.001) than those with lower osmolality but did not have significant associations with other clinical and laboratory parameters. Multivariate logistic regression showed fetal hemoglobin (odds ratio 1.051, 95% CI 1.007-1.097, p=0.022), serum urea nitrogen (odds ratio 1.308, 95% CI 1.160-1.474, p<0.0001), and duration of fluid deprivation (odds ratio 1.162, 95% CI 1.008-1.339, p=0.039) to be the significant clinical factors that predict urine osmolality > or < 500 mOsm/kg H_2O. Among infants with at least seven hours of reported fluid deprivation, urine osmolality correlated with creatinine clearance, as measured by the Schwartz formula (p=0.05) and ^{99}Tc-DTPA clearance (p=0.03), and serum urea nitrogen (p<0.001). Serum osmolality also correlated with urea nitrogen (p=0.007) and duration of deprivation (p=0.01). Infants with deprivation of greater than six hours had mean urine osmolality greater than those with only four to six hours (p<0.03).

Discussion

In normal adults serum osmolality is maintained at 285 mOsm/kg H_2O (normal range 282-295). To achieve this stability urine may be diluted to as little as 50 mOsm/kg H_2O or concentrated to as much as 1400 mOsm//kg H_2O. These changes are accomplished in the loops of Henle and the countercurrent multiplier of the renal medulla. Newborns have relatively reduced capacity but attain adult concentrating ability by four to six months of age [11].

The renal concentrating defect in sickle cell anemia is apparently related to polymerization of sickle hemoglobin in the hyperosmolar renal medulla, which increases the concentration of hemoglobin within red cells, thus potentiating the polymerization of sickle hemoglobin. The eventual result is almost complete destruction of the vasa recta and particularly disruption of the juxtamedullary nephrons with long loops of Henle extending into the intra-medullary zone. The latter results in an inability to super-concentrate urine in the presence of water deprivation [12].

Studies examining the prevalence and severity of the renal concentrating defect in sickle cell disease were largely done in the 1950s. Kunz et al. fluid-deprived 15 children age one to 14 years (median nine years) for 18 hours, then administered 10 units of pitressin prior to collecting urine. Urine specific gravity ranged from 1.008 to 1.014 (median 1.011) in 14 (93%) and one child (7%) had a specific gravity of 1.036 [3]. In our younger, less vigorously fluid-restricted infants, 54 (29%) had urine specific gravity > 1.015. Keitel et. al. fluid-deprived 29 persons with sickle cell disease (27 Hb SS, two transfused recently, median age six years, range 9 months – 41 years) for 14-17 hours, followed by two doses of pitressin. While 24 controls (age two to 30 years) had a mean urine osmolality of 1055+/-118 mOsm/kg H_2O (range 900-1250), urine of sickle cell patients had a mean osmolality of 493 mOsm/kg H_2O (range 369-767). No untransfused sickle cell patient age seven years or greater had an osmolality of greater than 500 mOsm/kg H_2O. The four infants under age two, in the

same range as our population, had a mean osmolality of 550 mOsm/kg H_2O (range 465-682) [2].

In our group of infants 77 percent% were able to concentrate urine above serum osmolality; 29 percent had an osmolality greater than 500 mOsm/kg H_2O and 7 percent greater than 600 mOsm/kg H_2O. We were surprised at the correlation between concentrating capacity and increasing glomerular filtration rate, speculating that a concentrating defect might rather be associated with hyperfiltration, though these processes have previously been reported as independent from one another [15].

Our fluid deprivation was less stringent than that utilized in the 1950s; we were limited by our concern both for precipitation of a sickle cell crisis by dehydration and by an unwillingness to insert urinary catheters and have strictly enforced withholding of fluid. In a study of the effect of indomethacin on renal function, fluid deprivation of 10 hours was adequate to demonstrate significant differences in urine concentrating capacity between adults with sickle cell disease (mean urine osmolality 414+/-10 mOsm/kg H_2O) and controls (911+/-39 mOsm/kg H_2O) [13]. That 20 percent of our infants had dilute urines in spite of our attempt to have parents withhold fluid indicates the limitation of our methodology to fully assess concentrating function. Although even older sickle cell patients with a concentrating defect maintain free-water clearance and thus the ability to dilute urine if fluid-loaded [13,14], it was not anticipated that any fluid restricted subjects would have osmolalities below that of serum. We speculate that some parents may have aggressively hydrated their children just prior to commencing the period of deprivation, resulting in dilute urine.

Patients with variants of sickle cell disease, notably hemoglobin SC or sickle β-thalassemia, also have concentrating defects, often with later onset than those with hemoglobin SS; individuals with sickle trait also may have concentrating defects [15]. Concurrent alpha thalassemia attenuates the defect; increasing loss of alpha globin genes correlates with diminished percentage of hemoglobin S and better ability to concentrate [16]. In young children the renal concentrating defect may at least temporarily be restored by blood transfusion [2,4], suggesting not only reversibility but also potential for prevention.

Hydroxyurea therapy results in an increase in fetal hemoglobin production with dramatic impact on the frequency of pain and acute chest episodes in individuals with sickle cell anemia [7], but there are few data regarding hydroxyurea's impact on urine concentrating ability. Hydroxyurea could reduce hyperfiltration and the abnormal increase of glomerular filtration that occurs over the early years of life [18]. However, none of eight children with hemoglobin SC disease age 10-17 years showed improved concentrating capacity during 15 months of hydroxyurea therapy at a dose of 15 mg/kg per day [17].

In conclusion, although fluid deprivation was suboptimal, a majority of the infants with sickle cell disease screened for the BABY HUG trial did show an ability to concentrate urine. Correlation between urine osmolality and duration of fluid deprivation was improved in infants with deprivation exceeding six hours. When urine concentrating capacity is repeated at exit from the BABY HUG trial, with infants two years older, parents will likely be more successful in achieving the recommended fluid deprivation and urine collection. It is noteworthy that fetal hemoglobin concentration was higher in infants with demonstrable concentrating ability. The fetal hemoglobin-promoting action of hydroxyurea may indeed prove to preserve concentrating ability in infants assigned to treatment in BABY HUG.

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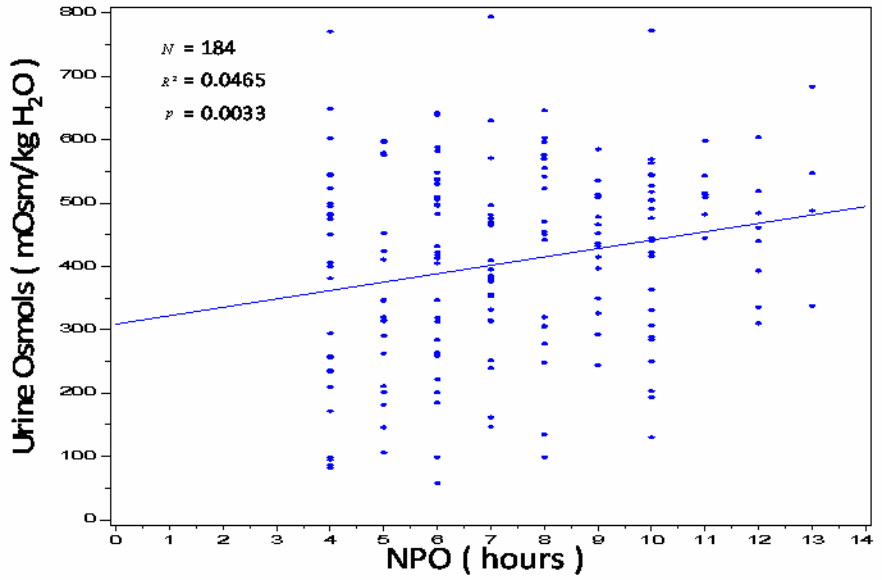

Figure 1.
Urine osmolality increased significantly with duration of fluid deprivation.

Table I

Number of Infants Demonstrating Various Measures of Urine Concentrating Capacity

Measure of Urine Concentrating Capacity	Number of Infants
Urine osmolality < mean serum osmolality – 1 SD*	37 (20.1%)
Urine osmolality within +/- 1 SD of mean serum osmolality**	5 (2.7%)
Urine osmolality > mean serum osmolality + 1 SD***	142 (77.2%)
Urine osmolality > 2 X (serum osmolality)	23 (12.9%)
Urine osmolality > 500 mOsm/kg H_2O	54 (29.4%)
Urine osmolality > 600 mOsm/kg H_2O	12 (6.5%)

* Hyposthenuria-in our cohort, osmolality < 280 mOsm/kg H_2O

** Isosthenuria-osmolality 280-292 mOsm/kg H_2O

*** Hypersthenuria-osmolality > 292 mOsm/kg H_2O