



Pharmacokinetics of hydroxyurea 1,000 mg coated breakable tablets and 500 mg capsules in pediatric and adult patients with sickle cell disease

Mariane de Montalembert
Dora Bachir
Anne Hulin
Linda Gimeno
Agnès Mogenet
Jean Louis Bresson
Isabelle Macquin-Mavier
Françoise Roudot-Thoraval
Alain Astier
Frédéric Galactéros

Little is known about the pharmacokinetics of hydroxyurea in patients with sickle cell disease (SCD). Our aims were to evaluate bioequivalence between standard hydroxyurea capsules and a new formulation of 1,000 mg coated breakable tablets in adults and to compare pharmacokinetic parameters in adults and children with SCD. Fifteen adults received hydroxyurea capsules and tablets in a randomized cross-over study. Eleven children received hydroxyurea tablets. The results showed bioequivalence between capsules and tablets in adults. Pharmacokinetic parameters were not significantly different between adults and children. Considerable inter-individual variability was noted.

Key words: sickle cell disease, pharmacokinetics, hydroxyurea.

Haematologica 2006; 91:1685-1688

©2006 Ferrata Storti Foundation

From the Service de Pédiatrie Générale, Hôpital Necker, Paris (MdM); Maladies Génétiques du Globule Rouge (DB, LG, FG); Service Pharmacie, Hôpital Henri Mondor, Créteil (AH, AA); Centre d'Investigation Clinique, Hôpital Necker (AM, JLB); Service de Pharmacologie clinique (IM-M); Département de Santé Publique, Hôpital Henri Mondor, Créteil (FR-T).

Correspondence:

Mariane de Montalembert, MD,
Service de Pédiatrie Générale,
Hôpital Necker, Paris. E-mail: mariane.demontal@nck.aphp.fr

Hydroxyurea stimulates the production of fetal hemoglobin in patients with sickle cell disease (SCD), which inhibits sickling.¹ In a randomized placebo-controlled study of adults with SCD, hydroxyurea decreased the rates of vaso-occlusive crisis, acute chest syndrome, and blood transfusion,² leading the Food and Drug Administration to approve hydroxyurea for the treatment of SCD in adults. In children with SCD, hydroxyurea treatment significantly reduced the number of days spent in hospital.³ Subsequent studies confirmed this beneficial effect.⁴⁻⁸ In addition to increased production of fetal hemoglobin, decreased endothelial adhesiveness and increased bioavailability of nitric oxide may mediate the therapeutic effects of hydroxyurea.⁹ The widespread use of hydroxyurea to treat SCD contrasts with the dearth of pharmacological data in this condition. Most pharmacokinetic studies of hydroxyurea were done in patients with cancer given hydroxyurea intravenously¹⁰⁻¹³ or orally.^{12,14,15} Only two studies assessed the pharmacokinetics of hydroxyurea capsules in patients with SCD,^{1,16} and both were confined to adults. A single study assessed the pharmacokinetics of a liquid hydroxyurea formulation in SCD infants aged 12 to 18 months.¹⁷ The paucity of data is all the more troublesome since clinical responses to hydroxyurea treatment vary widely. Dosage adjustments for body weight are difficult with current hydroxyurea capsules, especially in children. Coated breakable 1,000 mg tablets have been developed and are under review by European regulatory authorities. The aims of

our study were to compare the bioequivalence of hydroxyurea tablets and capsules in adults with SCD and to compare pharmacokinetic parameters of hydroxyurea tablets in adults and in children with SCD.

Design and Methods

Patients

Patients with a definite diagnosis of homozygous SCD or S/β^0 thalassemia were enrolled if they had been taking hydroxyurea capsules for at least 3 months, with a stable daily dosage for the last month or longer. Women of childbearing potential had to use effective contraception and to have a negative urine pregnancy test. Exclusion criteria were total white blood cell count $<2.5 \times 10^9/L$ or platelet count $<100 \times 10^9/L$, serum alanine aminotransferase more than twice the upper limit of normal, serum creatinine level $>120 \mu\text{mol}/L$, and infection or blood transfusion within the last month. We included 15 adults and 11 children aged 4 to 18 years. Written informed consent was obtained from legal representatives for children, from children able to understand, and from adults. The study was approved by the ethics committee of the Kremlin-Bicêtre Hospital.

Study design

A two-way cross-over design was used in adults, who were allocated at random to capsules (500 mg, Hydrea®, Bristol Myers Squibb Laboratories) or tablets (1,000 mg coated, breakable into parts of 250 mg each;

Siklos®, OTL-Pharma Laboratories) taken at home from day 1 to day 7. Pharmacokinetic variables were measured on day 8 after administration of the last dose at the study center. After collection of the last blood sample on the morning of day 9, patients returned to their usual hydroxyurea capsule regimen for 14 to 28 days, when they switched to the other study drug, using the same procedures. Children took hydroxyurea tablets at home from day 1 to day 13. Pharmacokinetic variables were measured on day 14 after administration of the last dose at the study center.

Pharmacokinetic studies

Patients arrived at the study center between 7.30 and 8.30 a.m after overnight fasting. The investigator evaluated compliance with the hydroxyurea regimen, recorded concomitant medication and vital signs, and checked that no criteria for study withdrawal had developed. Each patient underwent baseline blood sampling then took a hydroxyurea dose in the presence of a member of the study staff. Breakfast was served 30 minutes later.

Further blood samples were collected 45, 90, 120, 150, 180, 240, 360, and 480 minutes after hydroxyurea administration. The patient then spent the night at home and returned to the center for collection of the last blood sample on next morning, when adverse events and changes in concomitant medications were recorded. Each blood sample consisted of venous blood drawn into a heparin-coated tube, centrifuged immediately, and frozen at -80°C at the study center. Urine was collected from 0 to 4 h, 4 to 8 h, and 8 to 24 h after the hydroxyurea dose. For each urine collection, total volume was recorded, the urine was stirred, and four aliquots were frozen at -80°C .

Hydroxyurea in plasma and urine was assayed by high-performance liquid chromatography using the modified method of Manouilov *et al.*¹⁸ The equipment consisted of a Surveyor® chromatographic system (Thermoelectron, Les Ulis, France) coupled with Chromquest acquisition software®. The wavelength for detection was set at 449 nm. Compounds were separated by reverse-phase chromatography on a Nucleosil C18, 250-4.6 column (5- μm particle size, Macherey-Nagel, Hoerdt, France) filled to a pre-column Nucleosil 8-4.6. The mobile phase was 13% water (Millipore, St Quentin en Yvelines, France) and 87% acetonitrile at a flow rate of 1.2 mL/min. Detection was linear between 7 to 1,000 μM . Intraday and interday coefficients of variation were both $<10\%$. Accuracy ranged from 97.7 to 103.9%. The limit of detection was 5 μM and the limit of quantification was 7 μM .

Data analysis

Pharmacokinetic parameters were computed by non-compartmental analysis using the WinNonLin® software version 3.0. The terminal-phase rate constant

(β [reciprocal hours]) was determined as the slope of the terminal monoexponential decline in concentration in plasma using the least-squares method. The terminal half-life ($t_{1/2\beta}$) was calculated as $t_{1/2\beta}=0.693/\beta$. The area under the concentration-time curve (AUC) from time zero to time t of the last sample was calculated as $\text{AUC}_{0-t}=C_t/\beta$ where C_t was the drug plasma concentration in the last blood sample taken at time t . Total clearance (CLT) from plasma was calculated as $\text{CLT}=\text{dose}/\text{AUC}_{0-t}$ where AUC_{0-t} is the AUC between two doses at steady state. The apparent volume of distribution (V) was determined as $V=\text{CLT}/\beta$. The apparent volume of distribution at steady state (V_{ss}) was $V_{ss}=\text{MRT}(\text{dose}/\text{AUC})$. Maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were experimental values. In adults, pharmacokinetic variables for hydroxyurea capsules and tablets (C_{max} , AUC_{0-t} , $\text{AUC}_{0-\text{inf}}$) were analyzed by two-way analysis of variance (ANOVA). Wilcoxon's paired test was used to evaluate T_{max} . As recommended by Health Canada and the United States Food and Drug Administration, criteria for bioequivalence were p values smaller than 0.05 for the comparisons of AUC_{0-t} and C_{max} with a 90% confidence interval of the mean ratio of these parameters between tested and reference products between 0.8 and 1.25 for log-transformed data (Schuirmann's bilateral t -test). Pharmacokinetic parameters in adults and children were compared using the non-parametric Mann-Whitney test.

Results and Discussion

The 15 adults had a mean age of 30.1 ± 8.9 years (range, 21-49) and a mean body weight of 61.0 ± 7.6 kg (range, 42-71). The diagnosis was homozygous HbSS in 13 patients and S/ β^0 thalassemia in two patients. The mean daily hydroxyurea dosage was 20.9 ± 5.6 mg/kg (range, 15-35). The mean age of the 11 children was 10.2 ± 5.5 years (range, 4-19) and their mean body weight was 35.5 ± 15.6 kg (range, 16-55); ten children had homozygous HbSS and one had S/ β^0 thalassemia. Their mean daily hydroxyurea dosage was 21.9 ± 7.2 mg/kg (range, 14-36.5). No significant differences were found between adults and children regarding hydroxyurea dosage normalized for body weight. Concomitant medications in children were folic acid and phenoxymethylpenicillin ($n=11$), deferoxamine ($n=1$), insulin ($n=1$) and fluticasone with salmeterol ($n=1$). The adults were taking folic acid ($n=15$), oral contraceptives ($n=3$), enalapril ($n=1$), molsidomine ($n=1$), ketoprofen ($n=1$) and allopurinol ($n=1$).

In adults, C_{max} and AUC did not differ significantly between patients taking hydroxyurea tablets and capsules (26.5 and 26.1 mg/L for C_{max} and 121.1 and 127.3 mg/h/L for AUC, respectively) (Figure 1). Pharmacokinetics parameters with the tablet in adults and in chil-

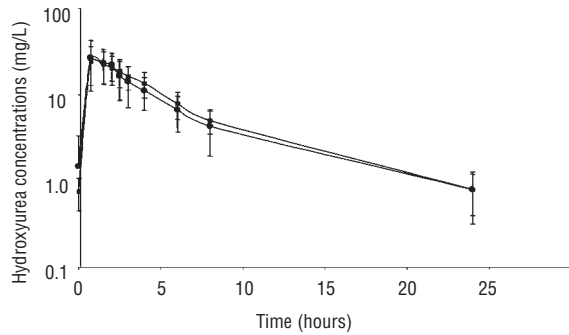


Figure 1. Bioequivalence. Mean concentrations (SD) of hydroxyurea in 15 adults receiving capsules (squares) or tablets (circles).

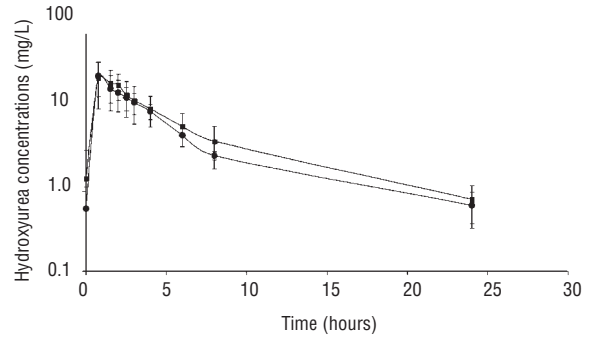


Figure 2. Hydroxyurea tablets in adults and children. Mean concentrations (SD) of hydroxyurea in 15 adults (squares) and 11 children (circles) receiving tablets.

Table 1. Pharmacokinetic parameters of hydroxyurea tablets.

Parameters	Children Median (1 st and 3 rd quartiles)	Adults Median (1 st and 3 rd quartiles)
C _{max} (mg/L)	24.5 (22.6-38.5)	26.5 (18.3-37.0)
C _{min} (mg/L)	0.63 (0.42-0.76)	0.72 (0.63-1.24)
T _{max} (h)	0.75 (0.75-0.75)*	0.86 (0.75-2.0)
AUC ₀₋₂₄ (mg/h/L)	111.2 (91.8-153.4)	121.1 (102.9-152.9)
AUC _{0-∞} (mg/h/L)	115.8 (95.2-156.3)	128.4 (105.6-157.9)
T _{1/2} (h)	6.3 (5.6-8.8)	6.3 (5.2-7.5)
V (L/kg)	1.98 (1.54-2.39)	1.58 (1.05-1.86)
Total clearance (L/h)	4.5 (3.6-10.8)	9.4 (8.1-11.8)

C_{max}: maximum concentration; C_{min}: minimum concentration; T_{max}: time to maximum concentration in plasma; AUC₀₋₂₄: area under the concentration-time curve from time zero to time 24 h; AUC_{0-∞}: area under the concentration-time curve from time zero to infinity; T_{1/2}: terminal half-life; V: apparent volume of distribution; *p<0.05.

dren are shown in Figure 2 and Table 1; no significant differences were noted except for an earlier T_{max} in children, possibly related to the different blood sample times in the two populations. Coefficients of variation were 45% and 39% for C_{max}, 44% and 136% for C_{min} and 40 and 45% for AUC in adults and children, respectively. Responses to hydroxyurea treatment vary widely across patients, for reasons that remain unclear. We found large coefficients of variation for hydroxyurea pharmacokinetic parameters, suggesting that differences in pharmacokinetics might contribute to the differences in clinical responses. Polymorphisms in genes involved in hydroxyurea metabolism are being investigated.¹⁹

We used a new 1,000 mg coated breakable tablet formulation currently under European regulatory review. Our first aim was to evaluate bioequivalence between this formulation and the standard capsules that were

used in efficacy studies in adults and children.^{2,3} To minimize blood-sample volume in children, we confined the bioequivalence study to adults. Our results indicate bioequivalence of the tablet and capsule form in adults with SCD.

We then compared tablet pharmacokinetics in adults and children. The only previous study of hydroxyurea pharmacokinetics in pediatric patients investigated a liquid formulation in infants aged 12 to 18 months.¹⁷ We studied children and adolescents aged 4 to 19 years. C_{max} and T_{max} were similar in the two populations. The median AUC and half-life were greater in children (112.2 mg/h/L and 6.3 h) than in infants (67.2 mg/h/L and 1.7 h), suggesting age-related differences in drug metabolism. The pharmacokinetics of hydroxyurea tablets was not different between adults and children with SCD in our study. Thus, pharmacokinetic factors cannot explain the greater reported efficacy of hydroxyurea treatment in children (all patients in the Duke pediatric cohort responded)⁷ compared to adults (half the adults in the Multicenter Study of Hydroxyurea showed nearly unchanged fetal hemoglobin levels after 2 years, despite identical treatment protocols).²⁰

We are planning population pharmacokinetic studies with the goal of modeling hydroxyurea pharmacokinetics in patients with SCD. We hope to identify clinical and biological parameters that correlate with treatment efficacy.

MdM collected the data in children, analyzed the data and wrote the paper; DB, LG collected the data in adults; AH, IM-M, AA performed the pharmacokinetics studies and analyzed the data; AM performed the pharmacokinetic studies in children; FR-T performed the statistical analysis; J-LB, FG analyzed the data.

The authors are indebted to Sam Charache for his helpful comments.

Manuscript received April 26, 2006. Accepted September 25, 2006.

References

- Charache S, Dover GJ, Moore RD, Eckert S, Ballas SK, Koshy M, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. *Blood* 1992;79:2555-65.
- Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med* 1995;332:1317-22.
- Ferster A, Vermylen C, Cornu G, Buyse M, Corazza F, Devalck C, et al. Hydroxyurea for treatment of severe sickle cell anemia: a pediatric clinical trial. *Blood* 1996;88:1960-4.
- Kinney TR, Helms RW, O'Branski EE, Ohene-Frempong K, Wang W, Daeschner C, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. *Blood* 1999;94:1550-4.
- Hankins JS, Ware RE, Rogers ZR, Wynn LW, Lane PA, Scott JP, et al. Long-term hydroxyurea therapy for infants with sickle cell anemia—the HUSOFT extension study. *Blood* 2005; 106:2269-75.
- Gulbis B, Haberman D, Dufour D, Christophe C, Vermylen C, Kagambega F, et al. Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events. The Belgian experience. *Blood* 2005; 105: 2685-90.
- Zimmerman S, Schultz W, Davis J, Pockens CV, Mortier NA, Howard TA, et al. Sustained long-term hematological efficacy of hydroxyurea at maximum tolerated dose in children with sickle cell disease. *Blood* 2004;103: 2039-45.
- De Montalembert M, Bégue P, Bernaudin F, Thuret I, D, Mischeau M. On behalf of the French Study Group on Sickle Cell Disease. Preliminary report of a toxicity study of hydroxy-urea in sickle cell disease. *Arch Dis Child* 1999; 81:437-9.
- Halsey C, Roberts IA. The role of hydroxyurea in sickle cell disease. *Br J Haematol* 2003;120:177-86.
- Newman EM, Carroll M, Akman SA, Chow W, Coluzzi P, Hamasaki V, et al. Pharmacokinetics and toxicity of 120-hour continuous-infusion of hydroxyurea in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 1997;39:254-8.
- Gandhi V, Plunkett W, Kantarjian H, Talpaz M, Robertson LE, O'Brien S. Cellular pharmacodynamics and plasma pharmacokinetics of parenterally infused hydroxyurea during a phase I clinical trial in chronic myelogenous leukemia. *J Clin Oncol* 1998;16:2321-31.
- Rodriguez GI, Kuhn JG, Weiss GR, Hilsenbeck SG, Eckardt JR, Thurman A, et al. A bioavailability and pharmacokinetics study of oral and intravenous hydroxyurea. *Blood* 1998;91: 1533-41.
- Garcia AA, Muggia FM, Spears CP, Jeffers S, Silberman H, Pujari M, et al. Phase I and pharmacologic study of I.V. hydroxyurea infusion given with i.p. 5- fluoro-2'-deoxyuridine and leucovorin. *Anticancer Drugs* 2001;12: 505-11.
- Veale D, Cantwell BMJ, Kerr N, Upfold A, Harris AL. Phase I study of hydroxyurea in lung cancer. *Cancer Chemother Pharmacol* 1988;21:53-6.
- Raymond E, Faivre S, Weiss G, McGill J, Davidson K, Izbicka E, et al. Effects of hydroxyurea on extrachromosomal DNA in patients with advanced ovarian carcinomas. *Clin Cancer Res* 2001; 7:1171-80.
- Yan JH, Ataga K, Kaul S, Olson JS, Grasela DM, Gothelf S, et al. The influence of renal function on hydroxyurea pharmacokinetics in adults with sickle cell disease. *J Clin Pharmacol* 2005;45:434-45.
- Rogers ZR, Thompson B, Ware RE, Wang WC, Iyer RV, Millet ST, et al. Pharmacokinetics of hydroxyurea in young children with sickle cell anemia: a report from the Baby HUG trial. *Blood* 2005;106:890a[abstract].
- Manouilov KK, McGuire TR, Gwilt PR. Colorimetric determination of hydroxyurea in serum using high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1998; 708:321-4.
- Wyszynski DF, Baldwin CT, Cleves MA, Farrell JJ, Bisbee A, Kutlar A, et al. Genetic polymorphisms associated with fetal hemoglobin response to hydroxyurea in patients with sickle cell anemia. *Blood* 2004; 104:34a [abstract].
- Steinberg MH, Lu ZH, Barton FB, Terrin ML, Charache S, Dover GH. Fetal hemoglobin in sickle cell anemia: determinants of response to hydroxyurea. *Blood* 1997;89:1078-88.