Impact of renal function on hydroxyurea exposure in sickle-cell disease patients

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Abstract

Aims:

This prospective study aimed to develop a population pharmacokinetics (PK) model of hydroxyurea (HU) in patients with sickle cell disease (SCD). This model can be used to determine the impact of glomerular filtration rate (GFR) on HU kinetics.

Methods:

We included 30 patients. They underwent HU pharmacokinetics analyses of plasma and urine. Six underwent PK analyses in two periods with and without angiotensin-converting enzyme (ACE) inhibitor. HU was assayed with a validated HPLC-UV method. Non-compartmental PK analysis was conducted and a population PK model built with Monolix®. This model was validated externally on another 56 patients. HU PK was simulated as a function of GFR.

Results:

The HU PK model was constructed as a two-compartment model with first-order absorption and elimination. The quality criteria were good, including for external validation. We found that estimated GFR (eGFR) and body weight affected HU PK, with lower eGFR or body weight associated with a higher HU AUC. We recommend the monitoring of HU through eGFR and body weight, which together account for 47% of its variability. Urinary HU fractions and renal clearance were higher in the glomerular hyperfiltration group and lower in the moderate CKD group, respectively. No differences in non-renal HU clearance were observed.

Conclusion: eGFR has an impact on the kinetics of hydroxyurea, and HU dose should be adapted accordingly. ACE inhibitor seems to have minor effect on HU PK in adults with SCD.

KEYWORDS

Sickle cell disease, hydroxyurea, glomerular hyperfiltration, moderate chronic kidney disease

What is already known about this subject?

- Hydroxyurea is administered at a daily dose of 15-35 mg/kg.
- The considerable variability of the response to HU is partly due to the pharmacokinetics and pharmacodynamics of HbF at steady state
- Sickle cell disease alters renal function, inducing a glomerular hyperfiltration, and a decrease in glomerular filtration rate.

What this study adds

- eGFR and body weight have an impact on the kinetics of hydroxyurea in sickle cell disease patients, accounting for 47% of HU variability.
- Some recommendations are made concerning HU dose with respect to eGFR.

1. INTRODUCTION

Hydroxyurea (hydroxycarbamide, HU) is the first approved pharmacological treatment for sickle cell disease (SCD) in children and adults[1]. There is compelling evidence to suggest that its beneficial effects are mediated by an increase in HbF synthesis by erythroid regeneration, nitric oxide (NO)-related increases in soluble guanylate cyclase activity and cyclic guanidine monophosphate leading to a stimulation of γ-globin expression, myelosuppression, decreases in red blood cell and leukocyte adhesion, in the percentage of dense red blood cells and hemorrheological improvement.[2-7] Clinically, HU decreases the incidence of vaso-occlusive crisis, transfusion requirement, hospital stays and it has been suggested that it also decreases mortality.[7-9] This treatment is usually administered orally, at a daily dose of 15-35 mg/kg, or less in cases of renal insufficiency.[5]

We have shown that the high variability of the response to HU is partly related to its pharmacokinetics (PK) and pharmacodynamics (PD).[10] Fetal hemoglobin (HbF) expression, currently assessed as a global percentage, may be a more relevant biomarker than mean corpuscular volume (MCV) for monitoring HU treatment.

However, SCD substantially alters renal structure and function, frequently causing chronic kidney disease in these patients.[11] A broad spectrum of renal manifestations (hyperfiltration, impaired urinary concentration ability, albuminuria, decrease in glomerular filtration rate (GFR), leading to-end stage renal disease) has been described in SCD patients. The optimal dosing schedule, the best strategy for monitoring and adjusting treatment, and the impact of the prior determination of renal function on HU dose have yet to be investigated in adults with SCD. Dong et al. identified body weight and cystatin C level as predictors of HU clearance in children.[12] Our objective were 1) to determine the impact of glomerular hyperfiltration or renal failure on the HU PK and 2) to develop and validate a model for adjusting the dosage of HU according to renal function.

2. METHODS

2.1. Patients and study design

The study was a phase IV, single-center, prospective, open-label, non-randomized pharmacokinetic study in patients treated with HU (Siklos®) at an optimal dose. It aimed to compare PK profiles as a function of renal function. The inclusion criteria were: SS or S−β^0 thalassemia patients, over the age of 18 years, with normal renal function, glomerular hyperfiltration or moderate chronic kidney disease, on HU treatment. The initial dose of HU was defined at 15 mg/kg, and adjusted by the clinicians on the clinical and hematological response.

Estimated glomerular filtration rate (eGFR) was determined with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation, without adjustment for ethnicity.[13] Moderate kidney disease was defined as a permanent (lasting at least three months) decreased eGFR between 30 and 59 mL/min/1.73 m^2, according to the CKD-EPI formula.[14] Hyperfiltration was defined as an eGFR > 130 mL/min/1.73 m^2 for women and an eGFR > 140 mL/min/1.73 m^2 for men.

The exclusion criteria were severe kidney disease, defined as an eGFR of less than 29 mL/min/1.73 m^2, treatment with erythropoiesis-stimulating agents (ESA), chronic blood transfusion and pregnancy. The patients included in the study were at steady state, defined as more than one month after a vaso-occlusive crisis episode or acute event and more than three months after the last blood transfusion.

This study, conducted at the Adult Sickle-Cell Referral Center of Henri Mondor Hospital, was approved by the Ile-de-France IV Ethics Committee (2014/77) and declared on the clinicaltrials.gov site (NCT02522104). All participants gave signed informed consent.

A second patient population was included for the validation of our model. This population included HU-treated patients treated for SS or S−β^0 thalassemia SCD

who were undergoing routine pharmacological treatment monitoring in our laboratory (56 patients).

2.2 Pharmacokinetics protocol

Blood samples for PK analysis were taken before HU (T0) and after (T0.75, T1.5, T3, T4, T6, T7.5, T24 hours) HU administration. Urine samples were collected from T0 to T4 hours, T4 to T7.5 hours and from T7.5 to T24 hours.

Patients treated with ARBs (angiotensin receptor blockers) or ACE (angiotensin-converting enzyme) inhibitors underwent two periods of blood sampling for PK analysis. The first blood samples for PK analysis (Period 1) were collected while the patients were taking these treatments, and the second set was collected (Period 2) after a 30-day washout period. Patients not treated with ARBs or ACE inhibitors underwent blood sampling for PK analysis directly.

Blood samples were collected in heparinized tubes and centrifuged at 2000 x *g* for 10 minutes at 4°C. Urine samples were collected in dry tubes. Plasma and urine samples were then stored at –80°C until analysis; they were assayed after derivatization with xanthydrol, by high-performance liquid chromatography (HPLC) with UV-detection at 240 nm, as previously described.[15] The analytical method was linear between 0.38 and 76 mg/L, precise (coefficients of variation ranging from 6.8 to 13.6%), and accurate (95.4 to 104.8%). The lower limit of quantification (LLOQ) was 0.38 mg/L.

2.3 PK study design (rich sampling)

Noncompartmental PK analysis was conducted to evaluate 1) the impact of coadministered treatments (ARB or ACE) and 2) urinary data. HU PK parameters in blood and urine were calculated with Phoenix WinNonLin® software v6.4 (Certara).

The experimental parameters measured were the maximal (Cmax, mg/L) and trough (Cmin, mg/L, measured 24 h after dose administration) concentrations.

The PK parameters calculated were the area under the curve of HU concentrations over time $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}inf}$ (mg.h/L, by linear up – log down method), total clearance (CLT/F, L/h, dose/$AUC_{0\text{-}inf}$), distribution volume (Vd/F, L or L/kg Vd = CLT/F/λz), and apparent plasma half-life (T1/2, h, T1/2 = Ln2/ λz).

The urinary pharmacokinetics parameters calculated were:

- Total quantity excreted in mg per 24 h
- Urinary fraction (%) = Total quantity excreted in mg per 24 h (mg)/ 24 h-dose in mg x 100
- Renal clearance CLR/F (L/h): amount excreted in 24 h (mg/24 h)/$AUC_{0\text{-}24h}$ in mg.h/L
- Non-renal clearance/F (L/h): Total clearance CLT/F - renal clearance CLR/F

2.4 Statistical analyses

The continuous variables are summarized with descriptive statistics. Statistical plasma PK analyses for Periods 1 and 2 were performed with paired *t*-tests for a log-normal distribution for Cmax/dose, $AUC_{0\text{-}24}$/dose and $AUC_{0\text{-}inf}$/dose. Point estimates and 95% confidence intervals (CIs) for least-squares mean paired differences between Periods 1 and 2 are estimated. ~~Results are presented with back-transformed data corresponding to the original scale.~~ A global test was performed on the three subgroups for urinary PK parameters with the non-parametric Kruskal-Wallis test.

2.5 Population PK models

The model was developed using a non-linear mixed-effect modeling approach (Monolix® version 2019.R2 (available at www.lixoft.eu)).[16] Parameters were

estimated using the stochastic approximation expectation maximization (SAEM) algorithm. One-, two and three compartment structural models with first-order elimination were tested to define the basic structural model. Between-subject variabilities were ascribed to an exponential model. Residual variability was described by a proportional model.

Categorial covariates were tested as follows:

$$\theta_i = \theta_{pop} \times \theta^{COV}$$

where θ_i is the individual parameter (elimination clearance: CL, volume of distribution of the central compartment: V_c, inter-compartment clearance Q, and apparent volume of distribution of the peripheral compartment Vp) for the *i*th patient, θ_{pop} is the typical value of the parameter, θ^{COV} is the covariate parameter, and COV is the category 0 or 1 for the covariate.

Continuous covariates were associated with PK parameters by a power function:

$$\theta_i = \theta_{pop} \times \left(\frac{Cov_i}{(Median(Cov))} \right)^{PWR},$$

where Cov_i is the covariate value for the *i*th patient and PWR is the exponent. For body weight (BW), the adult value of 70 kg was taken for the reference value, and the exponents (PWR) were 0.75 for clearance (CL) and inter-compartment clearance (Q) and 1 for the volumes of distribution, according to the allometric rule. Other covariates were tested: age, sex, cotreatment, HbF (%), hemoglobin, reticulocytes, leukocytes, microalbumin and lactate deshydrogenase.

The effect of a covariate on a structural parameter was retained if it caused a decrease in the Bayesian information criterion (BIC) and/or reduced the corresponding between subject variability (BSV) with $P < 0.05$. The objective function value reduction was tested for significance via a likehood ratio test. Diagnostic graphics were used for evaluation of the goodness-of-fit. Concentration profiles were simulated and compared with the observed data with the aid of the visual predictive check in order to validate the model. Empirical percentiles

(percentiles of the observed data (5th, 50th and 95th), calculated either for each unique value of time, or pooled by adjacent time intervals) and theorical percentiles (percentiles of simulated data) were assessed graphically.

2.6 External validation

Additional ill patients with the same inclusion and exclusion criteria were used to validate the model. Blood samples were collected and analysed using the same methods as for the building model. The individual predicted concentrations (Cpred) were obtained by fixing the parameters of the structural and variance models to the final estimates, and were compared with observed concentrations (Cobs), using R software. The mean prediction error (MPE, or bias), the mean absolute error (MAE) and root mean square error (RMSE) were defined as follows:

$$\text{MPE} = \frac{1}{n}\sum_{i=1}^{i=n}\left(C_{obs_i} - C_{pred_i}\right)$$

$$\text{MAE} = \frac{1}{n}\sum_{i=1}^{i=n}\left|C_{obs_i} - C_{pred_i}\right|$$

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{i=n}\left(C_{obs_i} - C_{pred_i}\right)^2}$$

Moreover, median prediction error (MrPE) and median absolute prediction errors (MArPE) were calculating and expressing as percentages. PrE, MDPrE and MDAPrE were defined as follows:

$$\text{PrE}_i = \frac{C_{obs_i} - C_{pred_i}}{C_{pred_i}} \times 100$$

$$\text{MrPE} = median\left\{\frac{C_{obs_i} - C_{pred_i}}{C_{pred_i}} \times 100, i = 1,...,N\right\}$$

$$\text{MArPE} = median\left\{\frac{\left|C_{obs_i} - C_{pred_i}\right|}{C_{pred_i}} \times 100, i = 1,...,N\right\}$$

2.7 Simulation of HU pharmacokinetics for GFR

Using our final model, we simulated the recommended dosing schemes for the solid oral form of HU (1,000 Monte Carlo simulations) for patients for renal function: normal eGFR, moderate CKD, hyperfiltration; 10000 patients were simulated in each case. The results were compared graphically by representing the HU AUC estimated with different GFR values and the covariates retained in the final model. Estimated personalized treatment dose regimens (500 mg/day, 750 mg/day, 1000 mg/day, 1250 mg/day, 1500 mg/day) were simulated regarding to the dosage adjustment by the clinicians.

RESULTS

3.1 Patient characteristics

A summary of the characteristics of the population evaluated in the model-building process (rich dataset) is provided in Table 1A. This population included 30 patients, with a mean age of 34.4 (19-61) years, and a mean weight of 61.0 (45-83) kg, receiving 946 (±277) mg of HU once a day. Ten of these patients had normal renal function, 12 had glomerular hyperfiltration and 8 had moderate CKD, with mean eGFR values of 112, 138 and 47 mL/min, respectively. Six patients underwent two rounds of PK analysis with and without the co-administration of an ACE inhibitor (ramipril). The characteristics of the population used to validate our PK model (sparse dataset) are shown in Table 1B: 56 patients with a mean age of 39.5 (19-79) years, and a mean weight of 66 (42-108) kg, receiving 975 (±410) mg of HU. In

total, 41 of these patients had normal renal function, 7 had glomerular hyperfiltration and 8 had moderate CKD, with mean eGFR values of 105, 140 and 35 mL/min, respectively. The characteristics of these two populations were not significantly different.

3.2 Pharmacokinetic analysis

The PK parameters are summarized in Table (A) for the population used to build the model (rich dataset) and (B) for the population used to validate the model (sparse dataset). In the rich dataset, 53% of the patients received 1000 mg HU (60, 67 and 25% of the patients with normal renal function, hyperfiltration and moderate CKD, respectively), 33% received doses below 1000 mg (750 or 500 mg; 30, 8 and 75% of the patients with normal renal function, hyperfiltration and moderate CKD, respectively) and 14% received doses above 1000 mg (1500 or 1250 mg, 10 and 25% of the patients with normal renal function, and in the two groups of patients with hyperfiltration and with moderate CKD, respectively). In the sparse dataset, 39% of the last doses before the determination of concentrations were 1000 mg, 43% were below 1000 mg, and 18% were above 1000 mg. Median numbers of samples per patient in the sparse dataset and in the rich dataset were 1 and 10 respectively (Figure 1).

3.3 PK analysis with ACE inhibitor treatment

Only six patients underwent HU pharmacokinetic analysis during two periods: four had moderate CKD and two, hyperfiltration. Mean PK plasma parameters and summary statistics for these patients are presented in Table 2 for Period 1 and Period 2, with and without concomitant ramipril treatment, respectively. With ramipril associated, a higher renal clearance of HU was observed.

3.4 Urinary data for the population used for model estimation

The urinary PK data are presented in Table 3. Median urinary fraction in the hyperfiltration group was 25.3% higher than that in the normal renal group, whereas it was 36.5% lower in the moderate CKD group. Median eGFR in the hyperfiltration group was 26.2% higher than that in the normal renal group, whereas it was 73.8% lower in the moderate CKD group. A statistical analysis comparing the three groups revealed significant differences for urinary fraction (p=0.0039) and renal clearance (p=0.0025). A pairwise analysis of the groups showed that both urinary fraction and renal clearance differed significantly between the normal renal function and moderate CKD groups (p=0.049 and p=0.003 respectively). No significant differences were observed between the normal renal function and hyperfiltration groups.

The median non-renal clearance of HU was slightly higher (+7.2%) in the hyperfiltration group than in the normal renal function group, and lower (-14.5%) in the moderate CKD group, although these differences were not statistically significant.

The only urinary parameter for which a significant difference was detected between Periods 1 and 2 was renal clearance (median 2.6 *vs.* 1.5 L/h, respectively; p=0.03; Table 4).

3.5 PK population model

Data were best fitted using a two-compartment model with first-order elimination. PK parameters were rate constant of absorption (Ka), apparent elimination clearance (CL/F), apparent volume of distribution of the central compartment (V_c/F), apparent elimination clearance of the peripheral compartment (Q/F), and apparent volume of

distribution of the peripheral compartment (Vp/F). The Ka could not be well estimated, but the stabililty of the model was improved when the value was fixed to 3.29 h-1, a value reported by Paule et al (10). BSV was estimated for CL/F, and residual variability was estimated using a proportional error model.

Weight-based allometry reduced the BIC by 77 points. Implementation of GFR for clearance decreased the BSV by 14% and the BIC by 22 units. The others covariates had no significant effect. The final PK parameters are summarized in Table 5. For patient *i*, the final equations were:

$$CL_i = 8.14 \times \left(\frac{BW_i}{70}\right)^{0.75} \times \left(\frac{GFR_i}{112}\right)^{0.56}$$

$$Vc_i = 42.6 \times \left(\frac{BW_i}{70}\right)^{1}$$

$$Q_i = 8.3 \times \left(\frac{BW_i}{70}\right)^{0.75}$$

$$Vp_i = 40.2 \times \left(\frac{BW_i}{70}\right)^{1}$$

3.6 Model evaluation

The goodness-of-fit plots and normalized prediction distribution error (NPDE) are depicted in Figure 2, while the prediction-corrected visual predictive check is shown in Figure 3. Plots of observations *versus* population and individual predictions were generated (Figure 2A). Individual weighted residuals (IWRES), predicted weighted residuals (PWRES) and NPDE *versus* time and predicted concentrations (Cc) were presented in Figure 2B. The PC-VPC (Figure 3A) performed on the final model showed that mean predictions matched the observed concentration-time courses and that variability was reasonably well estimated. Results of PC-VPC stratified on GFR were presented in Figure 3B.

3.7 Model validation

291 samples of 56 additional patients were used to externally validate the final model. Graphical evidence that the model describes the validation data adequately, were provided in Figure 4, with the use of VPC. MPE, MAE and RMSE were -0.11 mg/L, 1.57 mg/L and 2.18 mg/L, respectively. MdPE were 2% and MdAPE were 14%.

3.8 Simulation of HU PK for eGFR

The simulated HU pharmacokinetic profiles for eGFR are shown in Figure 5A. For a dose of 1000 mg HU, we obtained a simulated AUC of 136 mg.h/L for patients with a normal eGFR, increasing to 228 mg.h/L in patients with moderate CKD, and decreasing to 107 mg.h/L in patients with hyperfiltration (Figure 5B). Simulations of the HU AUC obtained for patients weighing 50, 70 and 90 kg, with different eGFR values, are presented in Figure 5C. As an illustration, to obtain the same AUC in a 50 kg patient with an eGFR of 30 mL/min/1.73 m^2 as obtained in a patient of the same weight with an eGFR of 90 mL/min/1.73 m^2, the dose should be adjusted to 500 mg. Conversely, to obtain the same AUC in a 50 kg patient with an eGFR of 150 mL/min/1.73 m^2 as in a patient of the same weight with an eGFR of 90 mL/min/1.73 m^2, the dose should be adjusted to 1250 mg.

3. Discussion

In this study, the population PK model was developed to characterize the impact of renal function on HU pharmacokinetics in adults with SCD.

HU was found to display linear pharmacokinetics.[18] Other studies in rats and humans, with doses of 10 to 800 mg/kg in cancer patients, demonstrated parallel linear renal and saturable non-renal elimination.[18] This non-renal elimination was not detectable here, probably because the doses administered were not high enough to achieve the saturation of non-renal elimination pathways (10 to 35 mg/kg *per os* in SCD). The model developed here is consistent with published data, with Vc/F of 45.3 L, CL/F of 11.6 L/h in adults weighing 70 kg, and Vc/F of 49.6 and CL/F of 6.9 in children.[10,12,18]

We show here, for the first time, that eGFR has an impact on the HU pharmacokinetics in adult SCD patients. Patients with a high eGFR linked to glomerular hyperfiltration display higher levels of renal HU clearance, with little effect on total clearance, and therefore require a higher-dose regimen. Conversely, patients with a low eGFR due to CKD have lower total and renal HU clearances and require lower-dose regimens. Renal elimination is a mean of 39% of total clearance in patients with normal function, 46% in patients with glomerular hyperfiltration and 20% in patients with moderate CKD.

From a pathophysiological standpoint, sickle cell nephropathy largely reflects an underlying functional vasculopathy. This vasculopathy leads to a perfusion paradox, with medullary hypoperfusion occurring in conjunction with kidney and/or cortical hyperperfusion, and aberrant renal vascular responses to stress occurring

systemically or in distant organs and tissues. This response is characterized by an enhancement of renal vasoconstriction and the resulting vasoocclusion.[11,19] These processes culminate in the initiation and progression of sickle cell nephropathy.
A relationship between PK HU variations and kidney function, characterized by cystatin C determinations, has already been described in children.[12] Kidney failure develops with age in patients with sickle cell anemia.[11]

Both for previous studies in children and ours in adults, two types of significant predictors of HU PK have been identified: markers of renal function, such as cystatin C in children and eGFR in adults, and body weight, in both groups. Our model shows that 47% of the variability in HU CL/F and hence AUC can be explained by eGFR and body weight. Most of this variability is explained by weight (32%). The remaining variability may be related to HU metabolism in cells, galenic formulation, intestinal absorption via urea transporters B and organic cation transporter (OCTN1) and/or urea renal transporters.

Based on our population model, we propose an adaptation of HU dose schedules in adult patients with SCD, according patient's covariates (eGFR, body weight) and concentrations determined in the patient for the simulation of HU clearance, making it possible to determine the optimal dose regimen. However, our study was carried out in patients at steady state, in whom HU treatment was initiated based on weight (15-35 mg/kg) and then adapted for clinical and biological efficacy. Indeed, our model can estimate hydroxyurea AUC in a given patient and compare it with that for the study population, making it possible to determine the dose adjustment required. If its utility is confirmed, this model will be useful for estimating the most appropriate HU dose schedule, and could be used for Bayes feed-back driven therapeutic drug monitoring. We will now validate this approach to HU therapeutic monitoring in a prospective multicenter population.

The interindividual variability of the urinary data was so great that it was not possible to include these data in our model. This variability is related to 1) the method of collecting urine in our protocol, as some urine was collected at home, 2) the daily variability of PK, which can be high in patients with sickle cell disease. However, we found that the renal function of adult patients with SCD influences not only the renal excretion fraction of HU, but also its cellular metabolism. In patients with glomerular hyperfiltration, the renal clearance of HU is about 26% higher than that in individuals with normal renal function, but the non-renal clearance of HU is unaffected (7%) due to the very low level of hyperfiltration. In patients with moderate CKD, the renal and non-renal clearances of HU are lower than those in individuals with normal renal function, by 74% and 15%, respectively. In all cases, including our patients with glomerular hyperfiltration, the excreted fractions of urinary HU are smaller than those reported for adolescents and children (about 50%).[20-22]

In the six patients presenting an increase in HU renal clearance on associated ramipril treatment, there was no difference of plasma PK between the two periods. Ramipril inhibits dipeptidylcarboxypeptidase I, an angiotensin-converting enzyme, leading to a decrease in angiotensin II formation and an inhibition of bradykinin degradation, resulting in vasodilation. This mechanism of action can explain our observation. This drug is frequently associated with HU in SCD patients. However, plasma HU exposure did not differ between the periods, and the interindividual variability of renal clearance was high due to the small number of patients and the large range of GFR values considered. To our knowledge, this is the first time that this drug interaction, between ramipril and HU, has been studied. Based on our findings, we think that an adjustment of HU dose is probably not required in patients also treated with ramipril, but it will be necessary to validate this lack of drug-drug interaction in a larger number of SCD patients. None of the patients included in this

study were on any other angiotensin-converting enzyme inhibitor or angiotensin II inhibitor.

A recent observational cohort study suggested modifying the eGFR criteria for CKD in SCD.[23] Patients with SCD display a rapid decrease in eGFR over time, associated with markers of disease severity and comorbid conditions. These authors proposed defining CKD as an eGFR<90 mL/min/1.73 m^2 in SCD patients. This proposal has yet to be validated and was not, therefore, retained in our model; it will be the subject of subsequent analyses.

In conclusion, our study showed that eGFR affected the CL/F and AUC of HU in adult patients with SCD. Patients with a high eGFR linked to glomerular hyperfiltration present a higher renal clearance of HU, with little effect on total clearance, resulting in a need for higher dose regimens. Conversely, patients with a low eGFR due to CKD have lower total and renal clearances of HU total and therefore require lower-dose regimens. We validated a model including eGFR and body weight as covariable. Too few patients have received Ramipril to be able to conclude on its possible effects on HU kinetics. In a future study, we will consider the PK/PD relationship after adjusting HU therapy for renal function and body weight.

Competing interests

Pablo Bartolucci is a consultant for ADDMEDICA. Vincent Audard and Anoosha Habibi report receiving personal fees from ADDMEDICA outside of the submitted work. The others authors have no competing interests to declare.

Authors' contributions

Study design: P Bartolucci, A Hulin and V Audard; inclusion and follow-up of patients: P Bartolucci, and MG Rakotoson; Production and management of samples: R Arrouasse and C Barau; HU determination and monitoring of biological data: MG Rakotoson and A Hulin; statistical analysis, modeling and simulations: C Pressiat, C Barau and A Hulin; writing of the manuscript: A Hulin, V Audard, C Pressiat, P Bartolucci, T Stehle.

All the authors have read and approved the final manuscript.

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FIGURE 1 Mean concentration *vs.* time profiles in patients with normal renal function (NR), glomerular hyperfiltration (HF) and moderate renal failure (IR) – Log scale

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FIGURE 3: Predicted corrected visual predictive check (PC-VPC) of model building
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FIGURE 4: Predicted corrected visual predictive check (PC-VPC) of model validation: comparison between the 5th, 50th, and 95th percentiles obtained from 1000 simulations and the observed data (points) for HU concentrations.

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Data Availability statement

Data available on request from the authors

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