Hydroxyurea treatment does not increase blood viscosity and improves red blood cell rheology in sickle cell anemia

The presence of sickle hemoglobin (HbS) in red blood cells (RBC) of patients with sickle cell anemia (SCA) is at the origin of their rheological abnormalities. Abnormal blood rheology has been shown to modulate the clinical severity and to also be involved in several complications of SCA.[1-3] Hydroxyurea (HU) therapy improves the clinical course of both children and adults with SCA.[4,5] However, no study has of yet investigated the effects of HU therapy on blood viscosity and RBC rheology. The aim of the present study was to test the effects of one year of HU therapy on blood rheology (RBC deformability and aggregation, blood viscosity), hematology and clinical profile in SCA adults. We demonstrated that the improvement in RBC deformability and the decrease of RBC aggregates robustness in HU-treated patients limit the consequences of the increase of hemoglobin on blood viscosity.

24 adult patients with SCA (38.3 ± 11.8 yrs, 61.9 ± 12.7 kg, 172.3 ± 10.9 cm; 14 males/10 females) regularly followed by the Sickle Cell Center of Pointe-à-Pitre (Academic Hospital, Guadeloupe) and at steady-state,[2] were recruited consecutively. Frequent hospitalizations for vaso-occlusive crises (VOC; n=13) and/or at least one acute chest syndrome (ACS; n=8) event in the preceding

Table 1. Effects of Hydroxyurea treatment on haematological, biochemical and routine clinical parameters.

	Before	1 month	3 months	6 months	12 months
Dose of HU mg/kg/j		9.9±3.6	14.2±6.1	16.2±6.4	15.7±5.2
VOC and/or ACS events[a]	2.0±1.6				0.1±0.2 ***
Leg ulcers frequency (%)	25				17
Priapism frequency (%)	38				15
Proteinuria (%)	62				62
Microalbuminuria (%)	13				8
HbF (%)	5.6±3.3	6.7±3.5 ***	10.8±5.4 ***###	13.3±6.6 ***#	15.3±7.7 ***# #
WBC (10^9/dL)	9.7±3.0	8.2±2.7 ***	6.6±1.8 ***# #	6.4±1.8 ***	6.6±2.3 ***
Neutrophils (10^9/dL)	5.6±2.5	4.0±2.1 ***	3.1±1.3 ***# #	2.9±1.1 ***	3.2±1.7 ***
Platelets (10^9/dL)	399±144	339±147 ***	310±144 ***	279±88 ***	283±105 ***
Hb (g/dL)	8.0±1.1	8.1±0.9	8.7±0.9 ***	8.9±1.1 ***	9.0±1.1 ***
MCV (fl)	81.5±7.1	88.0±8.4 ***	94.8±11.9 ***# # #	97.6±12.6 ***#	98.4 ± 12.5 ***
MCHC (pg)	35.6±1.3	35.6±1.0	35.2±1.4	35.1±1.1 *	34.7±1.3 **
Reticulocytes (10^9/dL)	278±73	219±76 ***	162±87 *** # #	151±66 ***	158±95 ***
LDH (IU/L)	572±174	–	–	–	406±146 ***
Total BIL (M/L)	65±38	–	–	–	39±22 **
AST (IU/L)	41±12	–	–	–	32±10 **
ALT (IU/L)	21±10				22±9
Creatinine (M/L)	60±21				59±20
Mean haemoglobin saturation (%)	95±3	–	–	–	97±3 *
Heart rate (bpm)	77±12	–	–	–	74±9
Systolic BP (mmHg)	120±10	–	–	–	120±10
Diastolic BP (mmHg)	66±8	–	–	–	67±9

*[a]VOC and/or SCA events were calculated on the 1 year-period preceding HU treatment and after 1 year HU medication in the subgroup of SCA patients for which HU was given for frequent VOC and/or ACS (n=16); HbF: fetal hemoglobin; WBC: white blood cell; Hb: hemoglobin; MCV: mean cell volume; MCHC: mean corspuscular hemoglobin concentration; LDH: lactate dehydrogenase; BIL: bilirubin; AST: aspartate aminotransferase; ALT: alanine aminostransferase; BP: blood pressure. Different from before treatment (*P<0.05; **P<0.01; ***P<0.001); different from the preceding value (#P<0.05; ##P<0.01; ###P<0.001).*

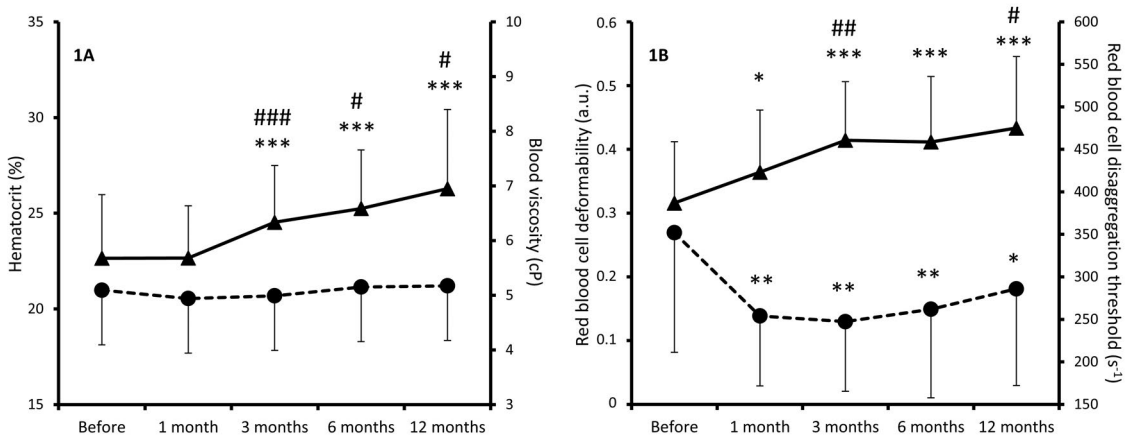

Figure 1. Effects of Hydroxyurea treatment on hematocrit (solid line, Figure 1A), blood viscosity measured at 225 s^{-1} (dotted line, Figure 1A), red blood cell deformability measured at 30 Pa (solid line, Figure 1B) and red blood cell disaggregation threshold (i.e. RBC aggregates strength; dotted line, Figure 1B). Different from before treatment (*P<0.05; **P<0.01; ***P<0.001); different from the preceding value ($^{\#}P$<0.05; $^{\#\#}P$<0.01; $^{\#\#\#}P$<0.001).

year were the main criteria used to propose HU therapy to these patients: 5 patients met the two criteria. ACS and VOC were defined as previously described.[1,2] For the other patients (n=8), HU therapy was proposed due to their need for frequent ambulatory care for subclinical VOC and a persistent low value of hemoglobin in comparison to usual steady-state values. Other complications within the preceding year were listed by an experienced physician. Routine examination before starting HU therapy included the measurements of anthropometric characteristics, blood pressure, mean hemoglobin saturation (by pulse oximetry) and heart rate. The same parameters, as well as the occurrence of the above complications, were prospectively recorded for one year after the initiation of HU therapy. Blood was sampled before HU therapy and at 1, 3, 6 and 12 months of treatment for biological measurements. The study was performed in accordance with the Declaration of Helsinki, was approved by the IRB of the Academic Hospital of Pointe-à-Pitre (DRCI-CHUPAP 230514, Guadeloupe), and written consent was obtained from all patients.

A diagnosis of SCA and α-thalassemia was made as previously described.[2] Hematological parameters were determined using a hematology analyzer (Max M-retic, Coulter, USA). Biochemical parameters in blood and urine (24 hours urine sample) were performed using standard methods.[2] All hemorheological measurements were carried out within 4 hours of the venipuncture to avoid blood rheological alterations, and after complete oxygenation of the blood.[6] Blood viscosity was determined at native Hct, at 25°C and at shear rates of 45, 90 and 225 s^{-1} using a cone/plate viscometer (Brookfield DVII+, Brookfield Engineering Labs, Natick, MA, USA). The hematocrit/blood viscosity ratio was calculated at 225 s^{-1} and is considered an index of RBC oxygen transport effectiveness.[7] RBC deformability was measured at 37°C at 3 and 30 Pa by ektacytometry (LORCA, RR Mechatronics, Hoorn, The Netherlands).[6] RBC aggregation was determined at 37°C via syllectometry using the LORCA (hematocrit adjusted to 40%). The RBC disaggregation threshold (i.e., RBC aggregates strength) was determined using a re-iteration procedure.[8]

A one-way analysis of variance (ANOVA) with repeated measurements and post-hoc Tukey tests was used to study the biological changes occurring with HU therapy. Biochemical and clinical routine parameters were compared prior to and at 12 months of HU therapy by using a paired student's t-test. The effects of HU therapy on the different complications (VOC, ACS and other complications) were assessed by using the McNemar test. Significance level was defined as $P < 0.05$ (SPSS, v. 20, IBM-SPSS Statistics, Chicago, IL, USA).

HU treatment was effective in patients for whom HU was given for frequent VOC and/or ACS (Table 1). The frequency of leg ulcers did not decrease after HU. The frequency of male patients having recurrent priapism events tended to decrease after one year of HU treatment ($P<0.1$). No change was observed in the frequency of patients having microalbuminuria or proteinuria.

Doses of HU were progressively escalated (Table 1) and adjusted for each patient, based on the values obtained for the hematological parameters.[5] Fetal hemoglobin (HbF) increased immediately after the beginning of HU therapy (i.e., at 1 month) and continued to rise until the end of the follow-up. White blood cells, neutrophils and reticulocytes count decreased to below pre-treatment values at 1 month, continued to decrease at 3 months and then stabilized at these lowest values until the end. Platelet count decreased at 1 month of therapy and remained stabilized until the end. Hemoglobin and hematocrit did not significantly change at 1 month but increased at the 3rd month of therapy: values measured at 3, 6 and 12 months were not significantly different. Mean cell volume increased immediately after starting HU medication and continued to rise before stabilizing at 6 months. Mean corpuscular hemoglobin concentration decreased to below pre-HU values at 6 and 12 months. Lactate dehydrogenase, bilirubin and aspartate aminotransferase values had decreased at 12 months of therapy. No change was observed for alanine aminotransferase, creatinine, heart rate and blood pressure. Mean hemoglobin saturation had significantly increased with regard to

Table 2. Effects of hydroxyurea treatment on blood rheology.

	Before	1 month	3 months	6 months	12 months
Blood viscosity at 45 s^{-1} (cP)	8.4±3.1	8.2±2.7	8.1±3.1	8.2±3.1	7.7±2.9
Blood viscosity at 90 s^{-1} (cP)	6.5±2.0	6.3±1.6	6.2±1.9	6.4±1.9	6.3±1.9
Hematocrit/blood viscosity (225 s^{-1})	4.6±1.1	4.7±0.9	5.0±0.8 *	5.0±0.9 *	5.2±0.9 *
RBC deformability at 3 Pa	0.14±0.05	0.16±0.04 *	0.18±0.05 *** #	0.19±0.06 ***	0.22±0.07 *** # # #
Red blood cell aggregation	52±10	51±8	52±8	52±9	55±10

*Different from before treatment (*P<0.05; ***P<0.001); different from the preceding value (#P<0.05; ###P<0.001).*

pre-HU values at the end of the follow-up.

Blood viscosity measured at 3 shear rates did not change during the entire follow-up (Table 2 and Figure 1A). In contrast, the hematocrit/blood viscosity ratio calculated at 225 s^{-1} (Table 2) and at other shear rates (data not shown) increased with regard to pre-HU values after 3 months of therapy, and remained as such until the end. RBC deformability measured at 3 and 30 Pa (Figure 1B) exhibited an early increase (at 1 month) and continued to rise until the end of the follow-up. RBC aggregation remained unaffected by HU (Table 2) but the strength of RBC aggregates decreased at 1 month until the end of the study (Figure 1B).

50% of patients had 1 α-gene deleted (-$\alpha^{3.7}$ deletion). In agreement with previous studies,[1,9] they exhibited lower mean cell volume, higher hematocrit and hemoglobin, increased RBC deformability, decreased RBC aggregates strength (P<0.05 for all parameters) and similar blood viscosity compared to patients without α-thalassemia (*data not shown*). The magnitude of the hematological/hemorheological responses under HU treatment was similar in the two groups, except for hemoglobin (+16 *vs.* +8%, P<0.05), hematocrit (+23 vs. +11%, P<0.05), the hematocrit/blood viscosity ratio (+34 *vs.* +8%, P<0.05) and RBC deformability (at 30 Pa: +65 *vs.* +26%, P<0.05), which increased more in patients without α-thalassemia than in patients with it. The effect of HU on the decrease of the number of VOC/STA events was similar in the two subgroups.

In agreement with previous studies, HU significantly decreased the rates of hospitalization for VOC and ACS in SCA patients who frequently exhibited these complications.[4,5] HU therapy decreased reticulocyte, white blood cell, neutrophil and platelet counts and the severity of anemia, and increased HbF levels, which participates in the improvement of SCA clinical status through the inhibition of HbS polymerization by HbF and the decrease in the number of highly adherent circulating cells.[5] Of the 6 men with frequent priapism, 4 reported having no episodes after one year of HU therapy, but more studies are needed to accurately demonstrate the long-term efficacy of HU on this complication.[10] Of importance, mean hemoglobin saturation increased with HU, hence improving the quality of life of patients.

Despite the increase in hemoglobin and hematocrit levels, blood viscosity measured at various shear rates reflecting the hemodynamic conditions of different vascular compartments of the body[6] did not rise, and remained unchanged throughout the study. This is of particular importance since increased steady-state blood viscosity has been shown to increase the risk of frequent VOC in SCA.[2,3] The hematocrit/blood viscosity ratio increased after 3 months of HU treatment until the end of the follow-up, suggesting improved RBC oxygen transport effectiveness.[7] Our data strongly suggest that concerns about increased blood viscosity related to HU treatment[5] may not be justified and may legitimate its use in other sickle cell syndromes, such as SC disease, where blood viscosity may be highly increased at steady-state compared to SCA.[11] The lack of change in blood viscosity in SCA patients receiving HU is caused by the large improvement of RBC deformability (+45% at 12 months of HU compared to steady-state) and the decrease of the RBC aggregates strength (-15%), which compensate for the increase of hemoglobin and hematocrit. Our findings also suggest that the magnitude of the hemoglobin and RBC deformability responses under HU treatment depends on their initial levels, which partly depend on the α-thalassemic status of the patients. Nevertheless, the improvement in RBC deformability compensated for the increase in hemoglobin in both α-thalassemic and non α-thalassemic patients, resulting in a lack of change in blood viscosity during the follow-up in the two subgroups.

Even when oxygenated, the deformability of sickle RBCs is lower than that of normal RBCs.[12] This loss of deformability is involved in the pathophysiology of SCA and the occurrence of VOC-like and hemolytic-like events.[1,13] Cross-sectional studies, including a limited number of SCA patients under HU treatment, previously demonstrated that those patients had higher RBC deformability than patients not receiving HU.[14,15] The progressive rise in RBC deformability observed in patients receiving HU for one year, in association with the decrease of RBC adhesiveness,[5] may enhance blood flow through the microcirculation and tissue perfusion, hence limiting the occurrence of VOC-like episodes.

Increased RBC aggregates strength in SCA was first reported by Tripette *et al.*[11] and is highly suspected to increase the risk for ACS.[2] Our finding demonstrated a positive effect of HU on the robustness of the RBC aggregates in SCA. The mechanisms at the origin of this observation are unknown and could simply be the consequence of the improved RBC mechanical properties, since it has been demonstrated that rigid RBCs are prone to form robust aggregates.[1] Further studies are needed to better understand how HU may change RBC aggregation properties in SCA.

In conclusion, we demonstrated that HU therapy does not increase blood viscosity in SCA patients and positively impacts the rheology properties of sickle RBC (i.e. deformability and strength of RBC aggregates). All these

hemorheological changes may limit the occurrence of acute vaso-occlusive like complications in SCA. Our findings apply mainly to patients having a hemoglobin increase of around 1 g/dL under HU, which is the usual finding, but cannot be extrapolated to the minority of those with higher hemoglobin increase under treatment.

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