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Hydroxurea treatment in sickle cell children

Marie-Hélène Odièvre[1,4], Malika Benkerrou[2], Marie Belloy[3], Jacques Elion[1]

[1]*Inserm, UMR 763, Paris, F-75019 France ; Université Paris Diderot, Paris, France*

[2]*AP-HP, Hop Robert-Debré, Centre de la Drépanocytose, Paris, F-75019 France*

[3]*Hop de Gonesse, Service de Pédiatrie, Gonesse, F-95500 France*

[4]*AP-HP, Hop Louis-Mourier, Service de Pédiatrie, Colombes, F-92700 France*

Corresponding author

Jacques Elion, Inserm U763, Hôpital Robert Debré, 48 Bd Sérurier, 75019 Paris, France. Email : jacques.elion@inserm.fr

Sickle cell anaemia, the most prevalent genetic disease in the black population, is characterised by the polymerisation of the abnormal haemoglobin S, which leads to vascular occlusion and severe painful crisis. Hydroxyurea is the first drug that has been shown to reduce the clinical severity of sickle cell anaemia in adults. Several trials have been carried out in more than 400 children in the last decade, using different therapeutic schedules. Enrolment was based on clinical vasoocclusive severity and few studies included children with cerebrovascular events. Hydroxyurea enhanced fetal haemoglobin expression in almost all the children, despite a large inter-individual variability. Hydroxyurea significantly reduces the number of vasoocclusive crises, hospitalisations, the frequency of the acute chest syndrome and the rate of transfusion. Long-term tolerance to hydroxyurea is good. However, clinical response does not correlate consistently with the degree and with the time of increment in fetal haemoglobin, suggesting that hydroxyurea may mediate some of its clinical benefits through other mechanisms such as the reduction of sickle erythrocytes – endothelial cells adherence and of the vasoconstrictive stimulus.

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Introduction

Sickle cell anaemia (SCA) is the most prevalent hereditary disorder in black individuals. It was first described in 1910, when the abnormal red cell shape named "sickle cell" was recognised[1]. Identification of the abnormal haemoglobin (HbS) in 1949 made it the first described molecular disease[2] leading to the definition of a precise pathophysiological scheme in the early 1960s. Despite these biological progresses, advances in therapy remain modest contrasting with the large prevalence of the disease particularly among black people. The basic pathophysiological scheme is the abnormal tendency of HbS to polymerise under hypoxic conditions with resulting sickling. This scheme explains chronic haemolytic anaemia, episodic occurrence of painful crises believed to result from vascular occlusion by undeformable rigid sickle cells inside the microvasculature leading to tissue ischaemia, and infectious complications. However, this mechanism is proving insufficient as laboratory data point out that the time necessary for developing cell sickling is longer than the microvascular transit time[3].

Recent evidence supports the hypothesis that abnormal adhesiveness of erythrocytes from SCA patients to vascular endothelial cells increases the transit times through small vessels and precipitates the occurrence of vasoocclusive crises (VOC)[4]. This observation makes red cell-endothelial cell interactions a central point of the pathophysiological scheme[5].

When present in the erythrocyte, fetal haemoglobin (HbF) inhibits deoxy-HbS polymerisation and hydroxyurea (HU, also referred to as hydroxycarbamide) treatment was initially given to reinduce HbF expression. In 1995, a large clinical trial in adults (*n*=299) with a history of at least three VOC per year showed that HU therapy caused a significant reduction in the frequency of painful crises, the frequency of acute chest syndrome (ACS) and the number of transfusions[6]. Others confirmed that adult patients taking HU for severe VOC had reduced mortality after nine years follow up[7]. Since 1996, studies have not involved randomisation because of the proven efficacy of HU therapy. Marketing authorisation of HU for use in adult SCA patients was awarded in the United States of America (USA) in 1998 (Droxia®) and authorisation for use in adult and children with SCA was awarded in the European Union in 2007 (Siklos®).

Paediatric trials

Between 1996 and 2006, data from more than 400 paediatric patients treated with HU for a severe clinical course of SCA have been published[8-31] (Table 1). The majority were conducted in the USA[8,10,13,15,17-19,22-24,26-28,31]. There have also been studies in Belgium[9,14,21,29], France[11,12,20,30], Israel[16] and Brazil[25]. Most children were SS homozygotes but 46 were S/β thal compound heterozygotes[8,11-14,16,20,23,25,27-31], five were S/O Arab compound heterozygotes[13,17,27,28], three had S/D Punjab compound heterozygosity[21,29,30]; one study included only severely affected S/C children[22].

Most trials involved < 40 children, but four studies included 84–225 children[18,20,21,29,30]. The French study has been both the largest and had the longest follow up[11,12,20,30]. The mean age of patients ranged from 9 to 15.3 years in the majority of the studies; however, several studies have involved young children under the age of 5 years[19,21,23,25,27-31]. One study has 14 children younger than two years with a minimum follow up of two years of HU therapy[29].

Clinical indication

In the majority of studies, enrolment (Table 1) was made on the basis of clinical evidence of disease severity[8,11-13,15,16,18-20,22,24-27,29,30]; usually three or more severe VOC during the last year and/or two or more ACS requiring hospitalisation during the last two years, and/or priapism. Two studies[17,28] included only patients with cerebrovascular events while five other studies[9,21,27,29,30] added aseptic hip necrosis and/or history of splenic sequestration and/or cerebrovascular event or abnormal transcranial doppler (TCD) and/or cardiac ischaemia to the VOC. Severe anaemia with haemoglobin (Hb) levels less than 7.0 g/dl[10,30] and/or requiring more than one transfusion per month to maintain haemoglobin level over 8.0 g/dl[14] was also an indication for treatment. The 28 very young patients treated in two studies[23,31] were not selected on the basis of disease severity but to test the potential for HU to preserve organ function, principally splenic function.

Dosage

The treatment schedule varied largely from one study to the other (Table 1). The initial daily dose (7 to 20 mg/kg) was usually systematically increased by 5 mg/kg every eight weeks until the maximum tolerated dose (MTD) avoiding bone marrow toxicity[8,10,13-15,17-19,22,24-28] was reached. The HU dose to reach MTD ranged from 15 to 35 mg/kg. In the "HUG-KIDS Study"[18], HU was discontinued for a least one week when the patient experienced a haematological toxicity; once the toxicity resolved, HU was restarted at a dose 2.5 mg/kg lower than the dose at which the toxicity occurred and was subsequently increased by 2.5 mg/kg every eight weeks, provided toxicity did not occur. In this study, MTD was defined as the dose 2.5 mg/kg below which two successive haematological toxicities occurred or when the daily dose reached 30 mg/kg and was sustained without toxicity for eight weeks.

For their very young children, Wang et al.[23] used a fixed dose of 20 mg/kg/day, adjusted for weight every eight weeks. In other studies, the daily maintenance dose was planned between 25 and 35 mg/kg[9,16,20,21,23,29,31] or 40 mg/kg four days a week (i.e. 20-23 mg/kg/day) in the French studies[11,12,30]. In their pilot study, Ferster et al.[9] used an initial daily dose of 20 mg/kg increased to 25 mg/kg if no change in HbF level had occurred after two months (increase < 2%). Their patients were randomly allocated to the sequence HU for six months followed by placebo for six months, or placebo followed by HU for an additional six months. It should be stressed that this is the only randomised paediatric study even though, in retrospect, the six month crossover period can be questioned as it is now established that the HbF level can increase up to 12 months after the onset of HU therapy. Another schedule was used by

Table 1 Different paediatric trials

Ref.	State	Number of patients	Ages of patients (years)	Phenotype	Enrolment	Maximum tolerated dose (mg)	Usual maintenance dose (mg/kg/day)
8	United States	15	Mean 14 Range 10–17	SS: *n*=11 Sβ^0: *n*=2 SS + α thal: *n*=2	VOC, ACS, priapism	Yes	Mean 21.4 ± 5.2 Range 14–34.7
9	Belgium	25	Mean 9 Median 8 Range 9–22	SS SS + α thal	VOC, ACS, stroke Splenic sequestration	No Max 25	Range 20–25
10	United States	15	Median 15.3 Range 4.4–18.8	SS	VOC, ACS, low Hb	Yes Max 35	Median 25.9
11 12	France	35	Mean 10.9 ± 4.1 Median 11.4 Range 4–19	SS: *n*=33 Sβ^0: *n*=1 Sβ$^+$: *n*=1	VOC	No Max 40	Mean 34.2 ± 4.6
13	United States	16	Median 14.2 Range 5.3–18.4	SS: *n*=13 Sβ^0: *n*=1 Sβ$^+$: *n*=1 SOArab: *n*=1	VOC, ACS, priapism	Yes	35
14	Belgium	8	Range 5–16	SS: *n*=6 Sβ: *n*=2	VOC, low Hb	Yes	Range 14–27
15	Canada + United States	17	Mean 12.4 ± 1.2 Range 5.2–18		VOC, ACS	Yes	Mean 21.8 ± 2.8 Range 6.7–32
16	Israel	19	Mean 15.1 ± 4 Range 7–23	SS: *n*=9 Sβ^0: *n*=9 Sβ$^+$: *n*=1	VOC, ACS Stroke (*n*=1) Hypersplenism (*n*=1)	No	Mean 21.3 ± 4.9 Range 16.4–31.2
17	United States	16	Mean 7.1 ± 4.4 Median 6.4	SS: *n*=15 SOArab: *n*=1	Stroke	Yes or Max 30 overlap with transfusion: none	Mean 24.9 ± 4.2 Range 19.1–32.7
18	United States "HUG-KIDS Study"	84	Mean 9.8 ± 3.2 Median 9.1 Range 5–15	SS: *n*=83 ?: *n*=1	VOC, ACS	Yes or Max 30	Mean 25.6 ± 6.2 Range 7.5–30
19	United States	8	Mean 3.7 Median 3.7 Range 2–5	SS	VOC, ACS	Yes or Max 30	Mean 27 ± 4
20	France	101	Mean 9.8 ± 0.4 Range 2–20	SS: *n*=99 Sβ^0: *n*=1 Sβ$^+$: *n*=1	VOC	No	Mean 21.4 ± 0.5 Range 9–30
21	Belgium	93	Median 7 Range 0.67–45	SS: *n*=92 SDPunjab: *n*=1	VOC, ACS, priapism Stroke Ischaemic bone necrosis Splenic sequestration (*n*=1)	No	Range 20–30
22	United States	6	Range 6.7–17.5	SC	VOC, ACS	Yes or Max 30	Median 1000 mg/day Range 750–1600 mg/day
23	United States	28	Median 15 month Range 6–28 m	SS: *n*=27 Sβ^0: *n*=1	Not on the basis of the disease severity	No Fixed dose 20	
24	United States	5	Range 11–14	SS	VOC, ACS	Yes Max 30	Mean 27 Range 20–30
25	Brazil	21	Mean 11.7 Range 3–22	SS: *n*=14 Sβ^0: *n*=7	VOC, ACS, priapism	Yes Max 30	
26	United States "HUG-KIDS Study"	68 achieved MTD 84 enrolled	Mean 9.5	SS	VOC, ACS	Yes or Max 30	
27	United States "HUG-KIDS Study" and "HUSOFT trial" and other studies	122	Median 11.1 Range 0.5–19.7	SS: *n*=106 SC: *n*=7 Sβ^0: *n*=6 Sβ$^+$: *n*=1 SOArab: *n*=2	VOC, ACS Stroke	Yes or Max 30–35	Mean 25.4 ± 5.4
28	United States	35	Mean 11.9 ± 4 Range 3.0–19.9	SS: *n*=33 Sβ^0: *n*=1 SOArab: *n*=1	Stroke	Yes overlap with transfusion: 6 ± 3 months	Mean 26.7 ± 4.8 Range 17–34.8
29	Belgium	127	Median 6 Range 0.67–19	SS: *n*=119 Sβ^0: *n*=4 SC: *n*=3 SDPunjab: *n*=1	VOC, ACS, priapism Stroke Abnormal transcranial doppler Ischaemic bone necrosis	No	After 1 year of treatment 49% patients: mean <20 46% patients: mean 20–25 1.9% patients: mean 25–30 0.9% patients: mean >30
30	France	225	Mean 9.2 ± 4.4 Range 1.42–19	SS: *n*=212 SC: *n*=3 Sβ: *n*=8 SDPunjab: *n*=2	VOC, ACS, low Hb Stroke Abnormal transcranial doppler Cardiac ischaemia	No Max 40	Mean 21.2 ± 6.1 Median 20 Range 6.25–42
31	United States "HUSOFT extension"	21	Median 3.4 Range 2.6–4.4	SS: *n*=20 Sβ^0: *n*=1	Not on the basis of the disease severity	30	Mean 30 ± 1.2

Altura et al.[24] with escalating doses of HU every six months over a period of 18 months (maximum dose: 30 mg/kg/day). Finally, the doses used in the French and Belgian studies are close to the MTD.

The mean duration of treatment ranged between 10[14] and 47 months[29] (range: 3 to 101 months). Several studies involved longer periods of treatment[29-31]. In Ferster's study, 39 of the 127 patients have been treated for five years or more[29]. In the French study 75 of the 225 children have been treated for more than five years and 10 for more than 10 years[30]. In the "HUSOFT extension study", 11 out of 21 patients were treated for a total of six years[31].

Biological effects

Despite the difference in the study designs, results were rather concordant. HU induced a rise in HbF levels in almost all children (Figure 1A). Only seven children were considered as non responders[8,11,12,24,25]. However, a large inter-individual variability was observed, both in the rate of HbF increase and in the maximal HbF (HbF max) level achieved. The relative increase over the initial values varied from 1.5 to 16 fold[8-13,15,16,18,19,21,22,24-29] with a HbF max greater than 35% in a few children[9,11,12,25].

Significant HbF max is *usually* reached after six months treatment. HbF max was reached at 24 months in one patient[12]; i.e. HbF value at six months is *not* predictive of HbF max. After reaching its maximum, HbF generally remained sustained at this level or at a level just slightly lower. In one study, the HbF max was reached after nine months[13]. Most of the young patients treated for two years by Wang et al.[23] had a significant upward trend in Hb and HbF (20.3 ± 4.9 %) compared with the pre-treatment values and the expected age-related levels (10.9 ± 7.9 %) documented in untreated SCA infants. Ware et al.[26] found that baseline HbF values and Hb concentration, MTD dose and compliance to treatment were significantly associated with a higher HbF level at MTD.

The treatment-associated changes in laboratory variables were also highly predictive of the HbF response: greater positive changes in Hb concentration and mean corpuscular volume (MCV) as well as greater negative changes in reticulocyte and white blood cell count were associated with a higher HbF level at MTD suggesting that HU dose escalation to mild myelosuppression is desirable for Hb max response[26]. Steinberg et al.[7] showed that adult patients with low reticulocyte counts and low Hb levels also had lower HbF levels and received lower doses of HU when treated in the Multi-Center Study of Hydroxyurea (MSH) randomised trial[6], suggesting that these patients had more severe disease and perhaps reduced marrow reserve unable to tolerate sufficient HU treatment to increase HbF levels.

Among the other laboratory values, the MCV and total Hb (Figures 1B and 1C) were consistently increased when compared with the pre-treatment values in almost all studies[8-13,15-19,21,24,27-29]. These values were also significantly increased in very young children when compared to the expected age-related levels[23,31]. In two studies[22,27], concerning S/C children, the MCV increased significantly without change in Hb concentration but the pre-treatment Hb values were already relatively high.

A significant decrease in the reticulocyte count was reported in six studies[9-12,15,18,27] while this decrease was not significant in two other studies[8,22] (Figure 1D). Reticulocyte count was also significantly lower when compared to the expected age-related levels[31].

The number of white blood cells and neutrophils (Figure 1E) decreased consistently in 12 studies[9-12,15,16,18,19,21,22,27,29], a decrease also observed when compared to the expected age-related values in the infants[23,31]. A significant decrease in platelets was reported in five studies[10,15,16,18,27] and a non significant decrease in five others[9,11,12,19,22,23].

Serum bilirubin concentration decreased significantly in six studies[8,10,15,18,27,32]. HU decreases in serum bilirubin level were significantly different among the three repetition polymorphism genotypes in the uridine diphosphoglucuronate glucuronosyl-transferase 1A (UGT1A) promoter[32]. Children with the homozygous 6/6 genotype have a significant reduction in total bilirubin to within the normal range after 12 to 18 months of therapy, whereas heterozygous 6/7 and homozygous 7/7 children (representing approximately two thirds of African children) have a significant reduction in total bilirubin, but not to the normal range.

Clinical effects

In all the studies, the beneficial effects of HU treatment were clinically evident by a 39–68% decrease in the VOC frequency[10,13-16,21,22,25] and in the number of admissions (about 55%)[8,9,19,21,22,29] and admission days (37–74%)[8-16,19,21,22,29]. A significant decrease in the ACS frequency (68-85%)[13,15,16,21] and need for transfusion (75–90%)[10,13-16,19,25] was also reported. In one study[9], 16 of the 22 patients studied did not require any hospitalisation during the six-month

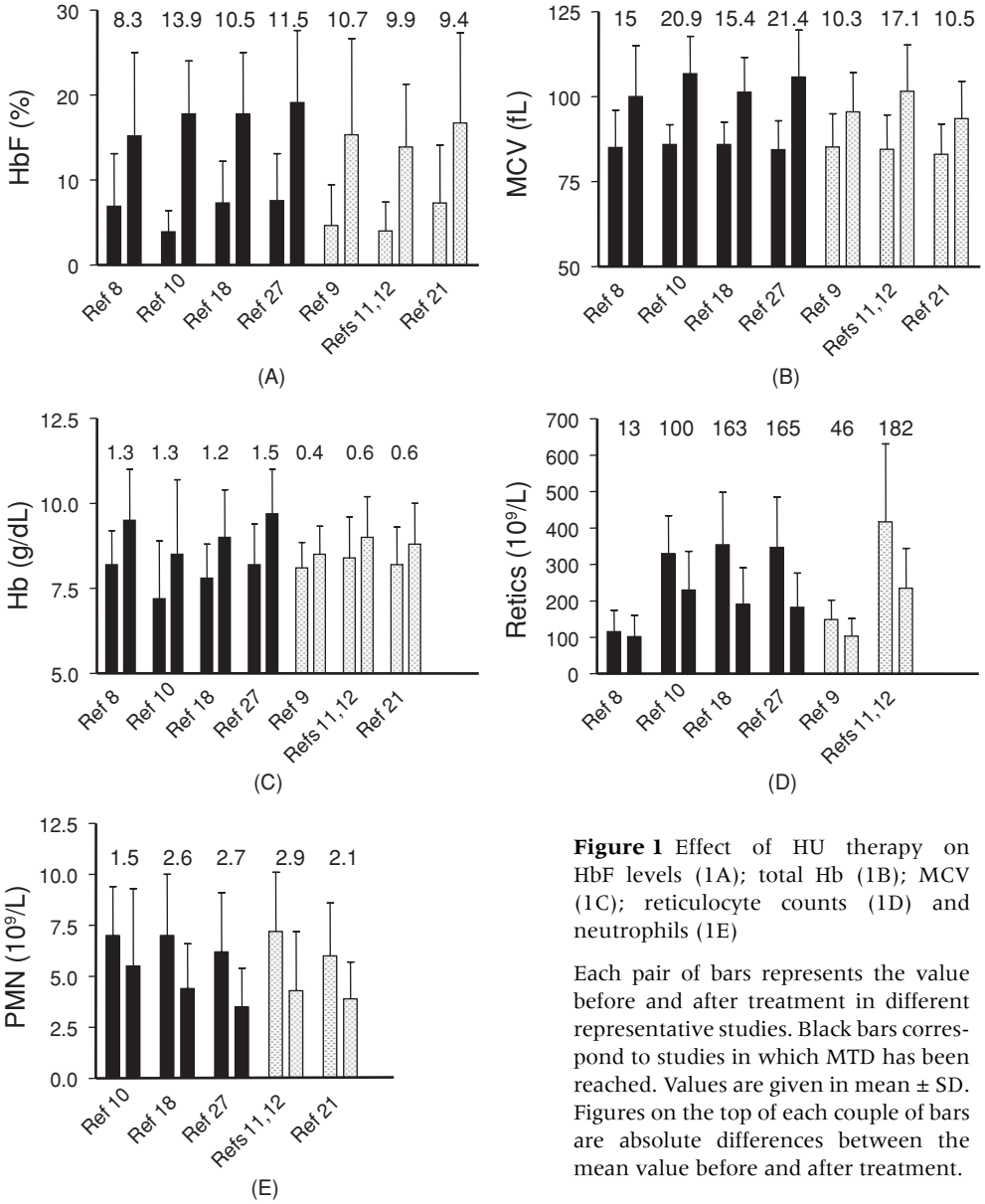

Figure 1 Effect of HU therapy on HbF levels (1A); total Hb (1B); MCV (1C); reticulocyte counts (1D) and neutrophils (1E)

Each pair of bars represents the value before and after treatment in different representative studies. Black bars correspond to studies in which MTD has been reached. Values are given in mean ± SD. Figures on the top of each couple of bars are absolute differences between the mean value before and after treatment.

trial period, even when previously seriously disabled. Most investigators noted that there was no correlation between the delayed biological effect appreciated on the HbF, total Hb or even MCV values and the rapid clinical improvement with the exception of one study in which the clinical improvement was greater after one year of treatment[8].

Cerebrovascular

Nine patients with cerebrovascular events were studied[21] and remained free of new clinical neurological complications after a follow up of four years; some of them had received a blood transfusion during the first months after starting HU treatment. Unfortunately, it is not mentioned if a cerebral vasculopathy in these patients was documented by magnetic resonance angiography (MRA). In another study[29], one recurrent stroke was observed after six years of follow up among the eight patients included for stroke. Among the other 34 patients considered at risk of primary stroke on the basis of abnormal TCD, only one presented with a cerebrovascular event (seizures). Eleven of them have been prospectively followed by TCD; the mean TCD velocities significantly decreased and two patients had TCD velocities that returned to normal value on HU. Among these 34 patients, 26 underwent magnetic resonance imaging (MRI)/MRA studies, which were interpreted as abnormal (arterial stenosis) in seven patients[29].

Among the 35 patients with infarctive stroke in whom HU treatment was used as an alternative to blood transfusion[17,28], seven (20 % or 5.7 events per 100 patient years) had neurological events considered as recurrent stroke (all infarctive events, no transient ischaemic attacks or seizures),

i.e. just slightly higher to the secondary stroke rate on chronic transfusions. Among these 35 patients, the 20 patients who overlapped HU therapy with transfusions had a lower stroke recurrence rate of 3.6 events per 100 patient years (two patients of the 20, i.e. 10 %). No data regarding TCD studies were reported by the authors. Here again, in absence of data concerning MRA studies, we do not know if the patients who experienced a recurrence of stroke had a cerebral vasculopathy. Recurrent stroke occurred in several patients who had a history of stroke[27].

Young children

In Wang's study[23], 21 young children (not enrolled on the basis of clinical history) completed two years of follow up at a median age of 39 months (range 30–52 months). They experienced a number of clinical events. One child died, two developed splenic sequestration, two had clinical central nervous system events and three had a total of seven episodes of ACS. Two children had invasive pneumococcal infections without neutropenia, two had a total of eight mild VOC and two had priapism. 47% had functional asplenia (scintigraphic measurement) at an age when about 80% would be expected to have it. No patient had decreased growth velocity compared with untreated age-matched control SCA patients. Therefore, for a drug exposure of two years at a median dose of 20 mg/kg/day, no prevention of severe clinical events occurred. Even though haematological responses occurred and neutropenia was observed in some children, the authors argue that their study cannot be compared to others because of its scope, the limited period of drug exposure and the use of an HU dose lower than predicted MTD. Similar results were observed in the "HUSOFT extension study"[31].

Splenic function

In another study[25] aimed to evaluate the effects of HU treatment on long-term splenic function evaluated by spleen scintigraphy, this function improved in 10 patients, remained unchanged in eight and worsened in three.

Deaths

In all these studies, six children and one adult died. One aged 15 years died after three months of treatment because of an acute intracranial haemorrhage[8]. One who had a history of previous stroke died 2.5 years after inclusion from suspected intracranial haemorrhage[9]. One infant, aged 16 months, died from an acute episode of splenic sequestration after two months of treatment[23]. One four-year old SS child, splenectomised at the age of two years, died of pneumococcal sepsis after 3.5 years of treatment[27,31]. One 15-year old SS child died of acute transfusion reaction after 5.4 years of treatment[27]. One patient with previous history of splenic sequestration, 13-months old at inclusion, died during the second year of HU therapy from an episode of splenic sequestration[29]. Additionally, one 18-year old patient, who had received HU for 11 months, died suddenly from asystole while he was hospitalised for VOC[30].

Short-term side effects

Haematological toxicity has been reported in the majority of studies, including cytopenia[8,11,12,16,17,19,21,23,27,29,30], and/or significant decrease in total white cell[10,13,15,16,18,20,22,23,27,30,31], platelet[9,15,18,20,22,23,27,29-31], and absolute reticulocyte counts[8,11,12,18,20,27,30]. This toxicity was usually transitory, only requiring temporary dose reduction or temporary discontinuation of HU treatment[8,11,13,15-18,20-23,27,29-31]. In one case, HU therapy was definitively interrupted after idiopathic thrombocytopenic purpura occurred[14]. A few cases with transient increase in transaminase activity have been reported[11,18,19,23], also requiring a decrease or cessation, both transitory, of HU administration. A significant increase in serum creatinine from 43 ± 11 μmol/l to 50 ± 14 μmol/l ($P = 0.05$) was noted in children receiving MTD of HU[15]. One 13-year old girl without any history of renal disease developed acute renal failure when a systemic lupus erythematous syndrome occurred requiring haemodialysis and definitive cessation of HU treatment[11,12,30].

Other reported side effects included skin rash[8,15,18,20,30], hair loss[10,11,15,20], melanonychia or hyperpigmented ridges on fingernails[11,13,20,27,33] and conjunctivitis[15]. It is noticeable that seven out of 50 African–American children developed hyperpigmentation on nails, palms and other skin surfaces after short term HU therapy (mean 8–12 weeks) at relatively low doses (17–25 mg/kg/day)[34]. Nausea[10,15,18,27] and diarrhoea[18] have been occasionally reported, as have headache[8,18,20,30] and drowsiness[20] at the beginning of HU treatment. Two patients had an accidental overdose of HU[23], followed by a mild transient neutropenia, HU having been withheld for 21 and 11 days respectively.

The reasons for interrupting HU therapy can be its failure[8,10,11,13,20,21,29,30], including the appearance of stroke[18,19,23,30] or the necessity for a bone marrow transplant[21,29], bad compliance to treatment[8,10,11,13,18,20,21,23,27,30], pregnancy[8,20,29,30], side effects such as skin rash[8,20,30], leg ulcer[20,30], nausea[10], neutropenia[18], thrombopenia[18],

idiopathic thrombocytopenic purpura[14], serum alanine aminotransferase increase[18], renal failure[11,20], leukaemia[20,29,30], systemic lupus syndrome diagnosed one year after the beginning of treatment[29], Hb level higher than 13 g/dl in a S/C patient[29], hypersplenism[30], osteonecrosis of femoral head[30], headache[30] and dizziness[30].

Long-term side effects

Long-term tolerance to HU therapy in terms of growth is good. There was no growth retardation in studies where growth was monitored[8,11-13,18-20,22,23,27,31,35]. One study reported normal pubertal development[8]; however, two cases of secondary amenorrhea have been reported[11,20].

The risk of potential malignancy is poorly documented in paediatric studies. To date, three cases of malignant disease have been published[20,29,30,36,37] without apparent relationship between HU treatment and malignancy. The first case concerned a 10-year old child with the SS genotype who developed acute lymphoblastic leukaemia with evidence of Philadelphia chromosome, seven weeks after the onset of HU therapy; in that case, the painful bone crises for which HU was begun may have been due to leukaemia[20,30,36]. The second case was an acute promyelocytic leukaemia diagnosed after eight years of HU therapy in a 21-year old patient[29]. The third case concerned an 8-year old $S\beta^0$ thal child who developed Hodgkin's disease six months after initiation of HU treatment for painful crises[37]. There was no increase in the number of acquired somatic DNA mutations after HU treatment for either eight years[27] or 30 months[38] in the two studies which looked for this. However, DNA damage in blood leukocytes of 28 SCA individuals treated with HU was significantly higher than in 28 controls[39]; DNA damage was positively correlated with the mean HU dose (> 20 mg/kg/day) and with the duration of treatment (≥ 42 months).

A reversible azoospermia has been reported in a SS patient in whom HU therapy was initiated at the age of 17 years[40]. While spermatic fluid analysis was normal before therapy, azoospermia was discovered six months later leading to interrupt HU. A reduced number of spermatozoa with reduced motility were present 10 months after cessation of HU therapy, a complication otherwise well known in untreated patients with SCA. A retrospective study[41] of four adult men showed that HU generally reduces sperm counts and mobility; cessation of HU in three cases resulted in recovery of spermatogenesis in one case, however sperm morphology and mobility remained impaired in the two other cases.

Specific problems to childhood and unsolved problems

Studies concerning HU therapy in children show that this drug seems to prevent VOC and their consequences. However, several points specific to childhood remain to be discussed.

Compliance

HU compliance has not been evaluated in all studies; it was precisely assessed in the "HUG–KIDS study" concerning 84 children[18] among them 74 % were compliant. Three other studies found that between 7.5 and 14% of children discontinued HU therapy because of poor compliance[27,28,30].

Age of initiation of therapy

One question concerns the age at which treatment should be initiated to prevent tissue damage while cytotoxicity of HU could affect the development of brain cells. Only a few studies have involved children aged less than five years[19,20,29] or less than two years[23,29,31]. With a mean follow up of three years, no side effect has been observed, particularly concerning growth. However, administration of high doses of HU to young mice has been shown to affect brain development and behaviour[42]. For this reason and also to avoid adverse effect of HU upon growth, to date not proven, it seems reasonable not to initiate HU therapy before the age of two years.

Splenic function

Therapy in children aged less than 5 years may delay functional asplenia and, despite the risk of severe infections, might prolong the period of risk for acute splenic sequestration[23,31]. A retrospective study[43] suggests that HU possibly preserves spleen function in SCA children, and can even result in recovery of splenic function.

Cerebrovascular

It has been postulated that HU could be effective as stroke prophylaxis in reducing adhesion molecule expression on sickle erythrocytes and in increasing steady-state Hb[44]. HU was used in 35 children with SCA and stroke in whom transfusions given to prevent stroke occurrence were discontinued because of antibodies, recurrent stroke despite transfusions, iron overload, non compliance and/or desferroxamine allergy[28]. Seven patients (20% or 5.7 events per 100 patient years) had neurological events considered to be recurrent strokes[28]. When HU therapy overlapped with transfusions, the recurrence rate

was lower (3.6 events per 100 patient year) and comparable with the published stroke recurrence rates for children with SCA receiving transfusion prophylaxis against secondary stroke (2.2 events per 100 patient years)[45,46]. The secondary stroke rates from these three articles[28,45,46] are not, however, directly comparable[47].

These results suggest that HU could be effective for preventing secondary stroke in children with SCA, but when performed with an overlap period allowing escalation of HU to MTD while still receiving transfusions. Other studies, particularly large clinical trials, are necessary in order to answer the question whether HU is effective for prevention of stroke in comparison to the effect of transfusion prophylaxis well documented by large studies. The Stroke with Transfusions Changing to Hydroxyurea (SWiTCH) Trial is a randomised phase 3 multicentre study which began enrolment in 2006 and is ongoing[48]. Furthermore, no controlled study has been made in those children considered at risk because of abnormal results on TCD. In a French study[49], 10 patients with abnormal TCD, whose velocities normalised on transfusion, were switched to treatment with HU. Four patients reverted to abnormal TCD and were placed back on transfusion, but six continued to receive HU after a median of 4.4 years of follow up. A recent study[50] showed that 24 HU-treated children experienced statistically significant decreases in TCD velocities compared to age-matched controls, which was not a consequence of changes in haematological parameters. A prospective phase 2 trial[51] included 37 children with pre-treatment increased TCD velocities. TCD velocities were then measured at MTD and one year later. At MTD, significant decreases were observed and the magnitude of TCD velocity decline was significantly correlated with the maximal baseline TCD value. TCD changes were sustained at follow up. A recent retrospective study summarising the Belgian experience[52] found that the significant increase with age of the TCD velocities did not appear in the patients treated with HU, and that in the 21 patients with an abnormal first TCD, the velocities decreased to the normal range in eight of them. Furthermore, children with highest baseline TCD velocities had the greatest treatment-related decreases.

To date, chronic transfusion to maintain HbS below 30% in high-risk group remains the only proven prevention strategy for stroke in SCA[53], but the data indicate that HU might be an alternative to chronic transfusion for primary stroke prevention in children at risk for stroke on the basis of abnormal TCD[54].

Limitations of clinical trials

After publication of the MSH[6] and the Stroke Prevention (STOP)[55] trials, use of HU and TCD assessment has drifted towards a standard practice without evidence of therapeutic efficacy among groups that were excluded from the trials[56]. Therefore, despite the potential differences in dosing, safety, and efficacy between drug trials in adults when compared with children, pediatricians are often forced to rely on data from studies performed in adults to make treatment decisions in children[56]. It is also difficult to extend the results of the MSH and the STOP trials to patients with haemoglobin SC[56,57], since both trials have limited the inclusions to individuals with haemoglobin SS (and $S\beta^0$-thalassaemia in the STOP trial).

Predictive response to HU therapy

Children seem to be better responders to HU therapy than adults, although the clinical and haematological response is variable[58]. As already quoted, efficacy of treatment is not predicted from the initial HbF level[12]. In fact, clinical response to HU in children is rapid and constant while resultant HbF increase is late and variable from one patient to another in level (a few to 30%) and in time (a few weeks to more than one year)[12,59]. Furthermore, the beneficial efficacy of HU upon clinical manifestations quickly decreases when discontinued while HbF levels remain high. A recent study[60] showed that some genetic determinants were associated with the HbF response to HU. Thus, it appears clear that induction of HbF is unlikely to explain all the clinical effects of HU. Adhesion and/or other mechanisms seems to be implicated in the individual response[61].

Mode of action of HU

HU, also named hydroxycarbamide, is a hydroxylated derivative of urea, $H_2N–\overset{\overset{O}{\|}}{C}–NH–OH$. It is a cytotoxic agent used since the sixties for treating malignant diseases (polycythemia vera, head and neck cancers, chronic granulocytic leukaemia), cyanotic congenital heart diseases[62,63] and even more recently psoriasis and HIV infection in association with nucleoside analogues[64]. HU is a freely water-soluble molecule with a molecular weight of 76. Given orally, it crosses the intestinal wall by passive diffusion and its maximal plasma concentration is rapidly reached (T_{max} = 1.22 hours); its oral bioavailability is nearly complete[64]. It is rapidly metabolised and converted into urea in the liver. Elimination of HU and its metabolites is mainly renal.

HU crosses the meningeal barrier, placental tissue and is present in maternal milk. Urinary HU was present for at least 12 hours following HU ingestion, but marked differences in urinary HU concentrations were noted[65]. There was a positive relationship between plasma HU concentrations and time elapsed between oral HU intake and sampling in 37 adults[66]. Little is known about the pharmacokinetics of HU in children with SCA. A recent study[67] showed that pharmacokinetic parameters were not significantly different between adults and children, but considerable inter-individual variability was noted suggesting that differences in pharmacokinetics might contribute to the differences in clinical responses.

HU inhibits the ribonucleotide reductase that catalyses conversion of ribonucleotides into deoxyribonucleotides. Consequently, HU inhibits DNA synthesis and arrests cells in S-phase. As already mentioned, the efficacy of HU in SCA was initially attributed to pharmacological stimulation of HbF[68]. The cytoreductive effect of HU was thought to result in differentiation of erythroid precursors with a relative increase of cells with high level of HbF. In fact, absence of complications during the first months of life seemed to be correlated with elevated HbF levels while secondary occurrence of painful crisis appeared to be coincidental with physiological decrease in HbF. Moreover, patients with low HbF level frequently develop a more severe disease[69] and have a reduced life expectancy[70], and it was even suggested that HbF level should be considered an important factor in selecting children for high-risk interventions such as bone marrow transplantation and long term treatment with agents that stimulate the production of HbF[70]. HbF within the cell interferes with HbS polymerisation and sickling. However, HU therapy was often associated with clinical improvement before any observable rise in HbF and the achieved level of HbF did not regularly predict response[6,12,71]. In addition, improvement vanishes rapidly if HU is discontinued, even if the patient still maintains a high level of HbF.

These data indicate that HbS polymerisation and sickling are not the only phenomenon influenced by HU. Indeed, new data suggest that HU may act by additional mechanisms affecting other aspects of the pathophysiology of SCA. Reduction by HU of adhesion molecule expression on sickle red cells and reticulocytes to endothelial cells[72], and to the subendothelial matrix proteins thrombospondin and laminin[73] can play a role as does a decrease in the percentage of reticulocytes expressing VLA-4 and CD36[74] and soluble VCAM-1 molecules[75]. HU significantly reduced sickle red cell adhesion to fibronectin, and reticulocytes from patients on HU had significantly lower CD36 and CD49d (α-subunit of VLA-4) surface expressions, as well as significantly lower CD36, CD49d and CD29 (β-subunit of the VLA-4) gene expressions[76]. It has been recently shown that HU decreases the VCAM-1 expression and the ET-1 peptide release from endothelial cells in culture[77,78] and the plasma ET-1 level in children with SCA[79]. Finally, our group[80] recently showed that on reticulocytes from SCA children treated with HU, expression of CD36, VLA-4 and ICAM-4 (to a lower extent) was decreased, but surprisingly Lu/BCAM expression (also CD47 and CD147 to a lower extent) was significantly increased. Alterations of adhesion receptor levels could be recapitulated in two-phase liquid cultures of erythroid progenitors from controls and the symptomatic patients grown in the absence or presence of HU.

These results suggest that in addition to red cells, endothelial cells are other cellular targets for HU[81]. A variety of alterations in sickle erythrocytes could result from HU administration[71,82]. This drug could also exert its impact through myelosuppression[71,82] with reduction in neutrophil counts and through reduction in neutrophil activation[83]. Finally, it has recently been suggested that the action of HU on gene expression, at the molecular level, might result from its capacity to act as a nitric oxide (NO) donor and thereby to activate the NO-mediated soluble guanylate cyclase (sGC) and cGMP signalling pathway[84-86]. Therefore, NO release[71,82] might also lead to vasodilatation.

Thus, clearly HU has pleiotropic effects on various aspects of SCA. It is a very effective drug, but potential long-term adverse effects are still uncertain. A better understanding of its mode of action is likely to provide important new data crucial for the identification of new therapeutic targets and an in-depth understanding of SCA pathophysiology. This is crucial if HU is to be used early on in SCA patients and for a life-long treatment.

Conclusion

HU has become an essential drug for the management of severe cases of SCA in children. According to the data reported above and in accordance with market authorisations by the EMEA and FDA (Table 2), its seems reasonable; (1) not to initiate HU therapy before the age of two years; (2) to start the treatment at the initial dose of 15 mg/kg/day, then to increase by 5 mg/kg until the optimal dose which is the dose leading to a clinical effect and avoiding bone marrow toxicity (remaining below 35 mg/kg/day); (3) to start HU in cases of severe vaso-occlusive disease (usually three or more severe VOCs during the last year

Table 2 Recommendations for use of hydroxyurea (HU) = hydroxycarbamide
Note that FDA recommendation apply to adult patients only

	EMEA (European Medicines Agency)	**FDA (Food and Drug Administration)**
Name of the medicinal product	SIKLOS®	DROXIA®
Date of issue of marketing authorisation	29 June 2007	25 February 1998
Orphan medicinal product designation date	9 July 2003	
Therapeutic indication	Prevention of recurrent painful vaso-occlusive crises including acute chest syndrome in paediatric and adult patients suffering from symptomatic sickle cell syndrome	To reduce the frequency of painful crises and to reduce the need for blood transfusion in adult patients with sickle cell anaemia with recurrent moderate to severe painful crises (generally at least 3 during the preceding 12 months)
Oral use as	1000 mg breakable tablets (into four equal parts)	200, 300, 400 mg capsules
Recommendation for administration	Initiation by a physician experienced in the management of sickle cell syndrome	
Starting dose	15 mg/kg/day as single dose	
Usual dose	15–30 mg/kg/day If necessary, maximum dose of 35 mg/kg/day under close haematologic monitoring Stop treatment if no response (35 mg/kg/day over 3–6 months) Reduced dose if mild renal impairment	If blood counts are in an acceptable range : – neutrophil levels ≥ 2500/mm^3 – platelets ≥ 95000/mm^3 – hemoglobin (Hb) > 5.3 g/dl – reticulocytes ≥ 95000/mm^3 when Hb < 9 g/dl the dose may be increased by 5 mg/kg/day every 12 weeks until a maximum tolerated dose (the highest dose that does not produce toxic blood counts over 24 consecutive weeks), or 35 mg/kg/day Initial dose reduced if renal impairment
	Discontinued until haematological recovery (then be reinstituted at a reduced dose) if : – neutrophil levels < 2000 /mm^3 – platelets < 80000 /mm^3 – haemoglobin < 4.5 g/dL – reticulocytes < 80000 /mm^3 when haemoglobin < 9 g/dL	
Checking before and during treatment	– Blood cell counts every 2 weeks for the first 2 months (and if > 35 mg/kg/day), then every 2 months – Renal and liver functions	– Blood counts prior to, and every 2 weeks during the duration of therapy – Renal and liver functions
Cautions	– Not recommended for children aged below 2 years – Continuous follow-up of the growth of treated children is recommended – Use of effective contraception is strongly recommended for women; if becomes pregnant while taking HU should be advised of the potential risk to the fetus – Patients with leg ulcer	– Safety and effectiveness in paediatric patients have not been established – Women of childbearing potential should be advised to avoid becoming pregnant; if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential harm to the fetus – Discontinue nursing, or discontinue Droxia if breast-feeding
Contraindications	– Hypersensitive to hydroxycarbamide or to any of the excipients – Severe renal or liver impairment – Toxic ranges of myelosuppression – Breast–feeding must be discontinuated while taking Siklos	– Previous hypersensitivity to hydroxyurea or any other component of its formulation
Risk management plan	– Cohort follow–up : 2000 patients up to 8 years recruitment, total duration 10 years – Information kit for physicians and patients	– Information kit for patients
Reference	http://emea.europea.eu/humandocs/Humans/EPAR/siklos/siklos.htm	http://www.fda.gov/cder/foi/nda/2003/16-295S036_Droxia_Prntlbl.pdf

and/or two or more ACSs requiring hospitalisation during the last or the two last years, and/or priapism); and (4) that the initial prescription is made under the guidance of the sickle cell centre, and needs to take into account, and to carefully monitor the potential haematological, liver and renal toxicities.

In addition, we can suggest that: (5) HU could be used also in cases of severe anaemia with Hb level less than 7.0 g/dl, and/or as an alternative to chronic transfusion for primary stroke prevention in children at risk for stroke on the basis of abnormal TCD; (6) we must also be careful of the possibility of prolonging the period of risk for acute splenic sequestration; and finally (7) we must inform the parents about uncertainties concerning long-term effect on fertility in young boys, and/or offer storage of frozen sperm to adults.

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