bjh short report

The presence of α-thalassaemia trait blunts the response to hydroxycarbamide in patients with sickle cell disease

Nisha Vasavda,[1] Sheela Badiger,[2] David Rees,[1,3] Sue Height,[3] Jo Howard[2] and Swee Lay Thein[1,3]

[1]Molecular Haematology, Division of Gene and Cell Based Therapy, King's College London School of Medicine, [2]Department of Haematology, St Thomas' Hospital, and [3]Department of Haematological Medicine, King's College Hospital, Denmark Hill, London, UK

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Correspondence: Swee Lay Thein, Department of Haematological Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK. E-mail: sl.thein@kcl.ac.uk

Summary

Hydroxycarbamide (HC), although a key drug therapy in sickle cell disease (SCD), does not result in a clinical response in all patients. Increases in fetal haemoglobin (HbF) and mean corpuscular volume of erythrocytes are standard clinical measures of HC efficacy in SCD. Genetic studies have determined that the majority of HbF regulation occurs outside the β-globin locus. Approximately 30% of SCD patients have co-inherited α-thalassaemia resulting in hypochromic and microcytic erythrocytes. We provide data from 30 SCD patients (10 with α-thalassaemia) demonstrating that co-existing α-thalassaemia significantly affects several standard measures of HC efficacy in SCD.

Keywords: sickle cell disease, hydroxycarbamide, alpha thalassaemia.

Hydroxycarbamide (HC) has been a major advance in sickle cell disease (SCD) in that it has reduced the frequency of sickle cell complications and transfusion requirements, and improved overall survival (Charache *et al*, 1995, 1996). Although the efficacy of HC therapy in SCD has been established there are still a significant number of patients who do not respond clinically; one study found this proportion to be as high as 40%, despite positive serum assays to confirm adequate adherence (Steinberg *et al*, 1997). Changes in fetal haemoglobin (HbF, $\alpha_2\gamma_2$) levels have been used as a clinical measure of HC efficacy in SCD, and the response has repeatedly been shown to be highly variable (Bakanay *et al*, 2005; Hankins *et al*, 2005). Studies investigating the reasons for the variable HbF response concluded that a primary determinant is the baseline HbF level and 'bone marrow reserve' (Steinberg *et al*, 1997). The *Xmn*1-Gγ SNP found on certain β^S haplotypes accounts for part of the variable HbF response, but the majority of the differences in HbF among patients are due to factors outside the β-globin locus (Steinberg *et al*, 1997; Ware *et al*, 2002).

Loci on chromosomes 6q and 8q associated with HbF regulation, and loci associated with HC metabolism and erythroid progenitor proliferation have been implicated in genetic association studies (Ma *et al*, 2007). More recently, another study suggested that genetic variants within the promoter of the *SAR1A* gene (on chromosome 10) may contribute to individual differences in HbF levels and patient response to HC in SCD (Kumkhaek *et al*, 2008). *SAR1A* encodes a guanosine-triphosphate (GTP)-binding protein that has been shown to have a key role in induction of the human γ-globin gene (Tang *et al*, 2005).

Another clinical measure of response to HC is the change in the red blood cell (RBC) mean corpuscular volume (MCV) (Charache *et al*, 1996; Ware *et al*, 2002). About one-third of patients with SCD have co-inherited α-thalassaemia and these individuals have hypochromic and microcytic RBCs (Embury *et al*, 1982; Steinberg & Embury, 1986). We have analysed laboratory data from patients taking HC to determine if, and how, the presence of α-thalassaemia affects these laboratory measures of HC efficacy.

Subjects and methods

The study was based on routine care laboratory results in patients with SCD attending specialist haematology clinics (adult and paediatric) in King's College Hospital (KCH) and St Thomas' Hospital (STH), London.

Patients were included if they were on HC treatment, had been genotyped for α-thalassaemia, and had data on steady state blood tests prior to, and while on HC therapy. α-globin genotyping was carried out using polymerase chain reaction-based methods on specifically amplified DNA in the routine diagnostic haematology laboratories at KCH or STH. Patients

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with poor adherence and who had been on HC treatment for <6 months were excluded.

Ethical approval was granted by the King's College London Research Ethics Committee (CREC/07/08-146).

Hydroxycarbamide therapy

Criteria for commencing HC therapy were more than two admissions for acute sickle pain in the previous 12 months or previous episodes of acute chest syndrome. Therapy in adults was started at 500 mg/d (or 15 mg/kg/d, whichever was higher) and increased in 500 mg steps after 4–6 weeks to a maximum tolerated dose in the adult patients. The children commenced at a dose of 15 mg/kg/d (minimum therapeutic dose), and this was only increased by 5 mg/kg/d increments after several weeks if there was apparent failure to respond to the starting dose. HC was reduced to the starting dose if toxicity was observed, as defined by standard blood count data. The mean dose was 1 g/d, and doses ranged from 300 mg to 2 g, with only one patient at either extreme, both of which were paediatric patients.

Data collection

Data were collected from the written and electronic patient records (EPR). 'Pre-HC' results referred to the period prior to the onset of HC therapy (over a 2–3 year span). 'During-HC' results were collected while the patient was on HC treatment and at least 6 months after the patient had been on their stable dose. For both periods, the extreme values were taken (minimum and maximum) for each parameter so as to capture the maximum effect possible from the HC therapy. The dates of any blood

Table I. Descriptive information for patients included in the study, including male: female ratio, age ranges, alpha genotypes and the average min (pre HC), max (on HC) and magnitude change for each parameter.

	Patient group															
	KCH adult				ST adult				KCH paed				Overall			
N	12				12				6				30			
Age [median, (range)]	38 (25–54)				39 (20–50)				10 (7–18)				36 (7–54)			
Male:female	7:5				6:6				5:1				18:12			
	αα/αα		αα/−α		αα/αα		αα/−α		αα/αα		αα/−α or −α/−α		αα/αα		αα/−α	
	n = 8		*n* = 4		*n* = 10		*n* = 2		*n* = 2		*n* = 4		*n* = 20		*n* = 10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Hb (g/l)																
Pre HC-Min	69	13	80	3	80	18	97	10	55	10	69	6	74	17	79	12
During HC-Max	105	11	91	6	108	13	104	4	76	6	97	12	104	14	96	9
Magnitude change	35	12	10	6	23	14	7	13	22	5	28	5	28	13	17	15
Total HbF (g/l)																
Pre HC-Min	7	7	3	1	3	3	7	–	1	–	5	1	5	6	4	2
During HC-Max	21	13	5	3	19	6	17	–	2	–	10	15	19	11	9	8
Magnitude change	15	9	3	2	16	5	10	–	1	0	3	13	13	8	5	8
Red blood cells ($\times 10^{12}$/l)																
Pre HC-Min	2·1	0·4	2·8	0·3	2·5	0·7	3·7	0·5	2·0	0·1	2·9	0·3	2·3	0·6	3·0	0·4
During HC-Max	2·9	0·4	2·9	0·3	2·9	0·4	3·6	0·4	2·6	0·1	3·2	0·3	2·8	0·4	3·2	0·4
Magnitude change	0·7	0·6	0·1	0·1	0·2	0·5	0·0	0·1	0·6	0·1	0·3	0·2	0·4	0·6	0·2	0·2
MCV (fL)																
Pre HC-Min	85·2	5·6	77·4	2·0	90·5	6·5	78·5	3·5	77·9	4·4	70·0	6·0	86·9	7·0	74·6	5·6
During HC-Max	117·3	15·3	98·1	7·4	122·8	11·2	93·0	14·1	89·0	10·0	88·8	7·7	117·3	15·2	93·4	8·9
Magnitude change	31·4	15·8	20·7	7·4	34·2	11·6	14·5	17·7	13·6	9·1	17·6	6·9	30·8	14·3	18·7	8·6
MCH (pg)																
Pre HC-Min	29·8	2·3	27·1	1·4	31·6	2·7	26·5	0·6	27·0	2·7	21·9	2·4	30·3	2·8	24·9	3·1
During HC-Max	41·4	6·0	34·9	1·3	42·2	4·1	30·6	4·7	32·1	5·6	30·9	3·5	40·8	5·4	32·4	3·4
Magnitude change	11·2	5·5	7·8	2·7	11·4	3·0	4·2	4·0	5·2	3·1	9·0	3·2	10·7	4·5	7·6	3·3
White blood cells ($\times 10^{9}$/l)																
Pre HC-Min	9·8	3·5	9	1·4	8·6	2·5	8·7	1·9	11	1·2	6·9	3·3	9·8	3	8·1	1·8
During HC-Max	11·3	2·9	11·1	1·7	12·9	7·2	12·2	1·3	13·2	5·6	9·7	3·4	11·9	3·7	10·8	1·7
Magnitude change	1·5	3·3	2·1	3	4·3	4·7	3·6	0·6	2·3	4·4	2·9	2·5	2·1	3·5	2·7	2·1

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transfusions were noted, and these dates avoided when recording the data. Laboratory data retrieved included: haemoglobin concentration (Hb g/l), HbF (g/l), RBC ($\times 10^{12}$/l), WBC ($\times 10^{9}$/l), MCV (fL), MCH (pg), total bilirubin (μmol/l), C-reactive protein (CRP) (mg/l), lactate dehydrogenase (LDH) (IU/l) and reticulocyte count (10^{9}/l).

Data analysis

Data analysis was performed using Microsoft Excel and GraphPad Prism software (La Jolla, CA, USA). The magnitude change in each parameter was compared for individuals with co-existing α-thalassaemia and those without. The magnitude change for each parameter was defined as the difference between the maximum value while on HC therapy, and the minimum value prior to HC therapy.

Results

A total of 30 patients homozygous for haemoglobin S mutation i.e. SCD-HbSS (confirmed by haemoglobin and/or DNA studies) on HC therapy with α globin genotypes were studied (Table I). Ten of the 30 patients had co-inherited α-thalassaemia. The single patient with homozygous α-thalassaemia (α−/α−) was included with the group of nine heterozygous (αα/−α) patients for the purposes of the analysis.

We observed varying magnitudes of increases in HbF levels, MCV and MCH in patients on HC therapy. As adherence to HC treatment is likely to be variable, to capture the impact of HC therapy, we recorded the minimum and maximum values of the laboratory parameters, both prior to, and during HC therapy. The pre-HC minimum and during-HC maximum values were used to calculate the magnitude change in each parameter because of HC therapy, and this change was compared among SCD patients with co-existing α-thalassaemia ($n = 10$) and those without ($n = 20$). We found the magnitude change differed significantly for the following parameters, as shown in Fig 1. Total Hb ($P = 0{\cdot}033$), HbF ($P = 0{\cdot}024$), MCV ($P = 0{\cdot}002$), MCH ($P = 0{\cdot}043$) and RBC ($P = 0{\cdot}035$). Magnitude change was not significantly different between the α-thalassaemia and no α-thalassaemia groups for WBC, total serum bilirubin, reticulocyte count, LDH or CRP (data not shown).

Discussion

α-Thalassaemia, which is present in one-third of SCD-SS patients, is an important modulator of the disease (Embury *et al*, 1982; Steinberg *et al*, 1984). Co-inheritance of α-thalassaemia in SCD is associated with reduced haemolysis, higher haematocrit, lower MCV and lower reticulocyte counts. This observational study showed that the co-inheritance of

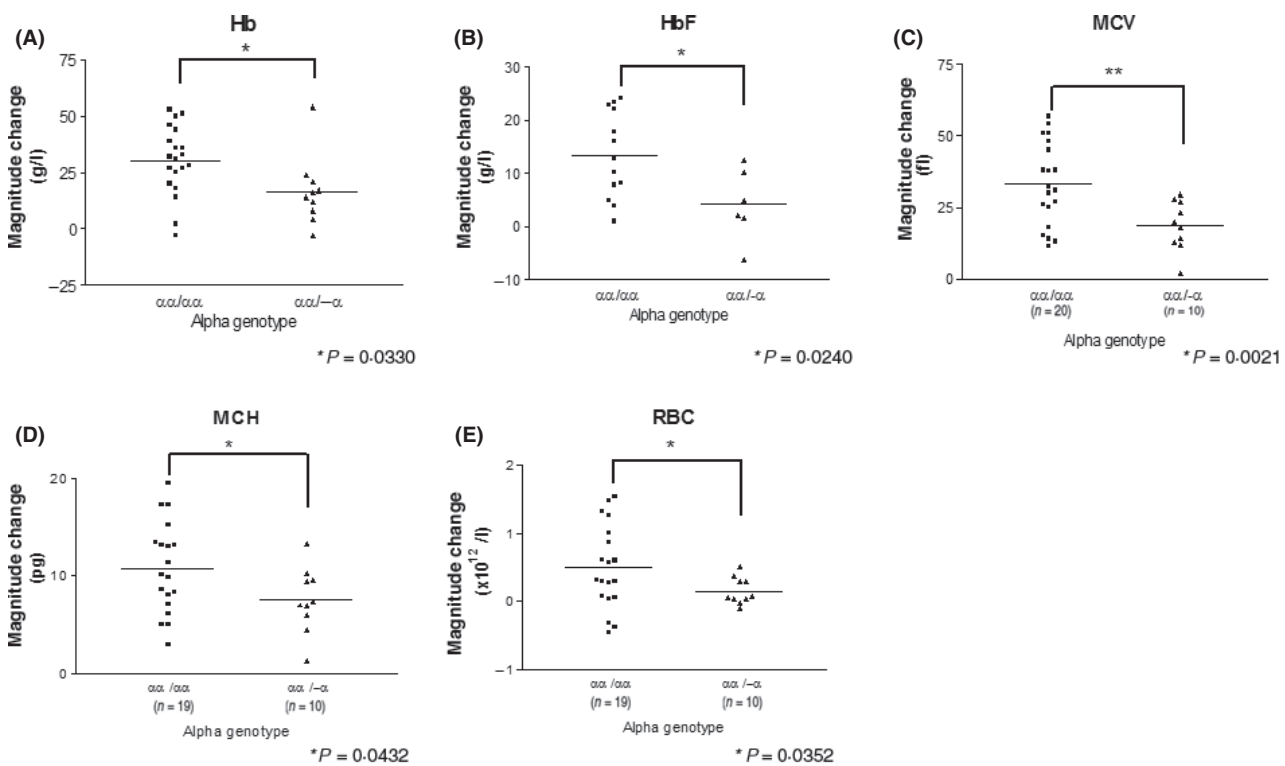

Fig 1. Graphs depicting magnitude change, in response to HC therapy, in a range of red cell parameters: (A) total haemoglobin (Hb, g/l); (B) HbF (g/l) (C) mean corpuscular volume (MCV, fL); (D) mean corpuscular haemoglobin (MCH, pg) and (E) red blood cell count (RBC, 10^{12}/l). Magnitude change is defined as the maximum value during HC therapy, minus the minimum value prior to hydroxycarbamide therapy. As a full data set was not available for all patients, exact numbers for each analysis are shown. The horizontal line represents the mean for each data set.

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α-thalassaemia in SCD patients significantly attenuated the response to HC treatment, as measured by changes in RBC count, total haemoglobin, HbF, MCV and MCH.

Hydroxycarbamide is increasingly used in the treatment of SCD for both adult and paediatric patients but the clinical response is relatively variable and unpredictable. Several mechanisms are thought to contribute to its therapeutic efficacy including increased HbF and increased water content of the erythrocytes (as measured by MCV). Indeed, the Multicentre Study of Hydroxyurea in sickle cell anaemia (MSH), has shown that HC patients with lower crisis rates have higher F cell counts, higher HbF levels and higher MCV and vice versa (Charache *et al*, 1996; Steinberg *et al*, 1997). Thus, in addition to clinical measures, changes in HbF levels and MCV are frequently used to assess HC response, and indeed, adherence. However, the MSH study also showed that plasma HC assays were not consistent with changes in either HbF levels or MCV values, and concluded (rightly or wrongly) that irregular compliance may account for some of the inconsistencies. The results of our study suggest that some cases of 'poor compliance' may be related to the modifying effect of coincidental α-thalassaemia.

Coincidental α-thalassaemia may not affect baseline HbF or F cell levels in SCD (Steinberg *et al*, 1997) but our data showed that the presence of α-thalassaemia certainly impacts the amplitude of HbF response to HC treatment. We propose that the blunted HbF response in patients with co-incidental α-thalassaemia is related to the limiting α-globin chains, while the attenuated amplitude in MCV values in these patients, when compared with SCD patients without α-thalassaemia, is probably due to the lower baseline MCV and MCH. Although our data are preliminary and based on small numbers, we believe that α-thalassaemia is an important factor to consider when making decisions on HC response and adherence based on changes in HbF levels and MCV values. This is of note when it is considered that α-thalassaemia genotyping is not carried out as a routine assessment in all sickle clinics; a factor evident from our own data that led to the exclusion of several patients for this study. The clear difference in magnitude change in red cell parameters among those sickle patients with and without co-existing α-thalassaemia in our patient group suggests that the area warrants further investigation in a larger prospective study with documentation of clinical response. Furthermore, studies seeking genetic determinants of variable HbF response to HC therapy in SCD should consider co-existing α-thalassaemia in the analysis. Not doing so could result in quite distinct data sets being grouped together, and important associations being missed.

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