Effects of co-existing α-thalassaemia in sickle cell disease on hydroxycarbamide therapy and circulating nucleic acids

Hydroxycarbamide (HC) is a key treatment option for sickle cell disease (SCD) (Charache *et al*, 1995; Ferster *et al*, 2001; Ware & Aygun, 2009; Voskaridou *et al*, 2010), but not all patients respond clinically, despite compliance (Steinberg *et al*, 1997). HC response is monitored by increases in fetal haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin. We have previously shown that such increases were muted in SCD patients with co-inherited α thalassaemia (α-SCD) compared to those without (SCD) (Vasavda *et al*, 2008). Cell-free DNA (cfDNA), a marker of tissue damage, is elevated during sickle acute pain (Vasavda *et al*, 2007) and reduced with HC therapy (Ulug *et al*, 2008). It is generally accepted that co-inherited α thalassaemia reduces haemolysis in SCD, subsequent to which the haematocrit is relatively increased with predisposition to vaso-occlusive complications (Ballas, 2001). We hypothesized that an increase in vaso-occlusive events would, in turn, have an effect on cfDNA levels in SCD.

Here we describe the effect of co-inherited α thalassaemia on clinical response to HC, and on cfDNA levels.

Fifty-two participants were recruited from the specialist haematology clinics at King's College ($n = 38$) and Guy's and St Thomas' ($n = 14$) Hospitals, London. All participants from Guy's and St Thomas' hospital were treated with HC. The study was approved by the South London REC Office (08/H0803/185); all participants gave informed, written consent. Inclusion criteria were regular attendance at the sickle cell outpatient clinics, and homozygous sickle cell disease (HbSS) or compound heterozygotes with β^0 thalassaemia (HbSβ^0). Exclusion criteria were: lack of data on α globin genotype, regular blood transfusion and poor compliance (in the treated group). Commencing or terminating HC therapy was a purely clinical decision, and dosing criteria were as previously described (Ulug *et al*, 2008).

For patients treated with HC, clinical data were collected retrospectively for the year prior to starting HC therapy ('Pre-HC') and prospectively throughout the study ('On-HC'). For patients commencing HC during the study, 'On-HC' data were collected from 6 months after starting HC therapy. Clinical response was assessed in terms of attendance at accident and emergency, hospital admissions per year, and days spent as an inpatient per year due to sickle-related pain ('inpatient days'). Blood samples were collected at each 'steady state' clinic visit for cfDNA measurement, and plasma cfDNA extracted and quantified as previously described (Vasavda *et al*, 2007). Clinical data or blood samples were not collected for 3 months after any *pro rata* blood transfusion. Data was analysed using an unpaired Student's *t*-test to compare data between the α-SCD and SCD groups (significance threshold $P = 0{\cdot}05$). To avoid skewing of data in favour of patients with a greater number of samples and clinical data points, a 'patient mean' was calculated and used in further analyses.

Clinical response was assessed for those patients treated with HC. Mean 'on HC' and 'pre HC' laboratory values were obtained, and response calculated as the magnitude change in each parameter; this change represents an intra-patient change in response to HC. An overall mean was then calculated for each group (SCD and α-SCD). For the cfDNA analysis data was not analysed intra-individually: results were stratified according to HC therapy, then further by α thalassaemia status. Laboratory (haematological) data were available for 'pre-HC' and 'on-HC', as these are routine care tests, whereas cfDNA is a research test, requiring a separate sample. Only a very small number of patients started HC during the study period, so sufficient pre and post intra-individual cfDNA values were not available for analysis. However, we did show in a previous study that HC therapy resulted in a significant change in cfDNA levels (Ulug *et al*, 2008). Patients with heterozygous (αα/-α) and homozygous (-α/-α) α thalassaemia were analysed as one group. Mean cfDNA levels in the α-SCD and SCD groups were compared separately for HC-treated and untreated patients.

33 patients were on HC therapy with a mean HC dose of 1151 mg (18·3 mg/kg/d); 19 were untreated during the study duration. Participants were followed for a mean of 10·4 months (range 3·6–19·2 months). Three participants required blood transfusions during the study (all in the 'No-HC' group). Further patient characterization is shown in Table I. Pre-HC data was not available for all participants; numbers used are shown with each result.

HC therapy dramatically reduced the total number of hospital admissions and accident and emergency department (A&E) attendances per year in both groups (SCD and α-SCD) (Table I) but the difference between the groups was not significant. However, α-SCD patients had a significantly ($P = 0{\cdot}02$) smaller reduction in the number of inpatient days (3·833 d ± 3·146 $N = 7$) compared to the SCD group (19·08 ± 3·932. $N = 12$) (Fig 1A); changes in haematological indices reflected our previous findings (Table I).

Table I. Patient characteristics. Breakdown of patients recruited to the study and summary of data. α-SCD refers to patients with SCD and alpha-thalassaemia and SCD alone to patients without co-existing alpha-thalassaemia; HC, hydroxycarbamide; A&E, accident and emergency department.

Category			Total
N recruited			52
On HC			33
Not on HC			19
Male : Female			24:28
Age, years : Median (range)			28·5 (17–56)
Alpha genotype			
αα/αα			32
αα/ααα			2
αα/-α			15
-α/-α			3
Clinical Information – *Patients on HC therapy (N = 33)*			
		α-SCD αα/-α & -α/-α) *N = 10*	SCD Alone (αα/αα) *N = 23*
Mean HC Dose : mg/d (mg/kg/d)		428 mg (15 mg/kg/d)	503 mg (16·6 mg/kg/d)
A&E attendance/year : mean (range)			
Pre HC		4·81 (0–11)	3·71 (0–6)
On HC		0	1·45 (0–5)
Admissions/year : mean (range)			
Pre HC		4·52 (0–11)	2·78 (0–6)
On HC		1·21 (0–5·3)	0·87 (0–5)
Inpatient days/year : mean (range)			
Pre HC		11·50 (0–32)	18·31 (0–48)
On HC		2·61 (0–12)	0·53 (0–6)
Haematological Indices – Magnitude Increases *when on HC therapy*			
	α-SCD (N = 20) Mean ± SD	SCD Alone (N = 20) Mean ± SD	*P*-value
Mean corpuscular volume (MCV, fL)	11·4 ± 5·02 (7)	22·97 ± 10·71 (19)	$P = 0·02$
Mean corpuscular haemoglobin (MCH, pg)	3·95 ± 0·62 (7)	7·01 ± 4·09 (19)	$P = 0·01$
Total haemoglobin (g/l)	0·058 ± 0·82 (8)	2·03 ± 2·92 (20)	$P = 0·01$
Fetal haemoglobin (HbF%)	4·35 ± 2·88 (7)	8·24 ± 6·03 (17)	$P = 0·04$

CfDNA analysis was performed for 40 patients (15 were α-SCD). CfDNA levels were significantly higher in the α-SCD group whether on HC (α-SCD 31960 genome equivalents/ml (GE/ml) ± 6569 $N = 7$; SCD 12680 GE/ml ± 1513 $N = 13$, $P = 0·04$) or off HC (α-SCD 20470 GE/ml ± 4047 $N = 8$; SCD 10180 GE/ml ± 996·0 $N = 12$, $P = 0·04$) (Fig 1B and C, respectively).

In summary, we found that α-SCD patients demonstrate a reduced response to HC, in terms of haematological indices and (crucially) symptomatic response. Both SCD and α-SCD patients showed a reduction in the mean duration of inpatient stay in response to HC therapy, but the reduction was significantly smaller in α-SCD patients. The decrease in A&E attendance or number of admissions was not statistically different between the groups.

α-SCD patients had higher cfDNA levels than SCD patients, whether on, or not on HC therapy. As cfDNA is a marker of tissue damage, we suggest that the increased cfDNA levels may result from tissue damage related to an increased predisposition to vaso-occlusive events (Steinberg & Embury, 1986; Ballas, 2001) in α-SCD patients. α-SCD patients also had a relatively lower reduction in duration of inpatient stay; we suggest that the vaso-occlusive events in such patients may be more severe, requiring a longer resolution period.

Thus, although α-SCD patients had a comparable response to HC in the reduction in number of sickle-pain events per year, the length of hospital stay required to resolve episodes was not reduced as much in response to HC as for the SCD group. Nonetheless, although HC therapy response is attenuated by α thalassaemia, it remains highly efficacious in SCD patients with or without α thalassaemia.

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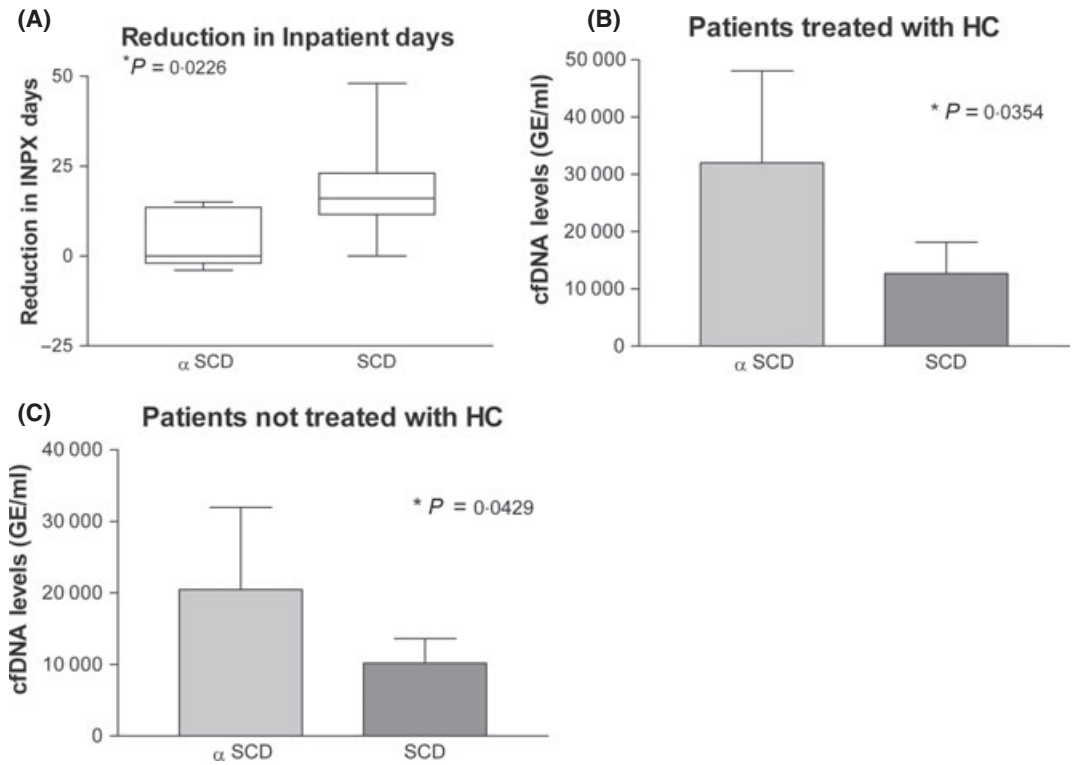

Fig 1. (A) Comparison of response to hydroxycarbamide (HC) therapy in sickle cell disease (SCD) patients with and without co-existing α-thalassaemia. Mean reduction in inpatient days (due to sickle pain). (B) and (C) Comparison of circulating DNA levels in SCD patients with and without co-existing α-thalassaemia; (B) patients treated with HC (C) separate patient group, not treated with HC. GE, genome equivalents.

Authorship contributions

NV performed the cfDNA experiments, collected and analysed the data and co-wrote the manuscript; CW recruited participants and collected clinical data; M Allman helped with recruitment and collected blood samples; ED recruited participants, collected clinical data and gave feedback on the final manuscript; M Awogbade reviewed the final manuscript; JH commented on the protocol, recruited participants, collected clinical data and gave feedback on the final manuscript; SLT conceived the project, recruited participants, collected clinical data and co-wrote the manuscript.

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Treatment of primary intraocular lymphoma with rituximab, high dose methotrexate, procarbazine, and vincristine chemotherapy, reduced whole-brain radiotherapy, and local ocular therapy

Primary intraocular lymphoma (PIOL) is a rare type of non-Hodgkin lymphoma that appears in the retina, vitreous, subretinal pigment epithelial space, or optic nerve head (Levy-Clarke *et al*, 2005). Sixty to eighty percent of PIOL patients develop central nervous system (CNS) lymphoma (Peterson *et al*, 1993; Akpek *et al*, 1999). Despite high rates of initial response, most patients usually succumb to recurrent disease and die with a median survival of 12–20 months (Coupland *et al*, 2004). Several treatment protocols for PIOL have been reported. In these reports, however, the relapse rate is relatively high and the side effects of therapy cannot be ignored; an appropriate treatment for PIOL has not been established.

In this study, five patients (three women and two men; mean age, 65 years; range, 43–72 years) with untreated PIOL diagnosed in our hospital from November 2007 to December 2009 were treated with high-dose methotrexate (MTX)-containing chemotherapy and reduced whole-brain radiotherapy (WBRT) combined with intravitreous MTX injections or ocular radiotherapy. PIOL was diagnosed following cytological analyses of vitrectomy samples, intraocular cytokines IL10 and IL6, flow cytometry, and immunoglobulin heavy chain gene (*IGH@*) rearrangements. All patients underwent systemic computed tomography, brain magnetic resonance imaging, bone marrow biopsy, and lumbar puncture to confirm that the lymphoma was localized to the eyes. This study was approved by the institutional ethical committee and written informed consent was obtained from all participants.

Initially, patients underwent intravitreous MTX injections or ocular radiotherapy after receiving the PIOL diagnosis. An intravitreal injection of 400 μg MTX was administered once a week for 2 months, followed by monthly injection for 2 months. Concurrent with intravitreous MTX, patients were administered five cycles of induction chemotherapy (R-MPV) as follows: day 1, rituximab 375 mg/m^2; day 2, MTX 3·5 g/m^2 and vincristine 1·4 mg/m^2 (maximum 2 mg). Procarbazine 100 mg/m^2/d was administered for 7 d with odd-numbered cycles. Patients received dose-reduced WBRT to a total dose of 23·4 Gy (1·8 Gy/fraction × 13 daily) for prophylactic irradiation following R-MPV chemotherapy. The treatment schedule is summarized in Fig 1. After chemo-radiotherapy, clinical and ophthalmological examinations were performed, intraocular IL10 and IL6 were measured, and computerized tomography was performed. Complete response (CR) was defined as complete disappearance of lymphomatous infiltrates and undetectable IL10 in the vitreous on post-treatment ophthalmological examination.

Patient characteristics, clinical findings, treatment, disease-free survival (DFS), and complications are summarized in Table I. The median follow-up time was 32 months, with a range of 21–42 months. At the time of writing, all five patients were alive without relapse.

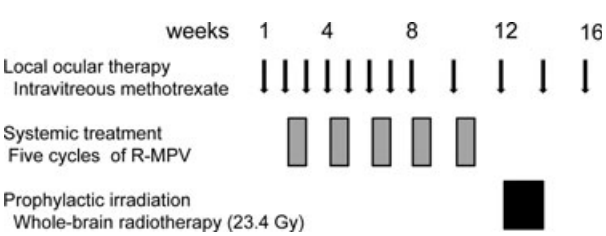

Fig 1. Treatment protocol for primary intraocular lymphoma. Intravitreous methotrexate (MTX): 400 mg of MTX, once a week for 2 months, followed by monthly injection for 2 months; R-MPV: day 1, rituximab 375 mg/m^2; day 2, MTX 3·5 g/m^2 and vincristine 1·4 mg/m^2, and procarbazine for 7 d. Whole-brain radiotherapy: 1·8 Gy/fraction × 13 daily (total dose 23·4 Gy).