bjh short report

The effects of hydroxycarbamide and magnesium on haemoglobin SC disease: results of the multi-centre CHAMPS trial

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Summary

In a phase-II multi-centre double-blinded trial, we evaluated haematological effects of oral hydroxycarbamide (HC) and magnesium (Mg) in patients with HbSC, aged 5–53 years old. Subjects were randomized to HC + placebo, Mg + placebo, HC + Mg, or placebo + placebo. The primary endpoint was the proportion of hyperdense red blood cells after 8 weeks. Thirty-six subjects were evaluable, but the study was terminated early because of slow enrollment. In the combined HC groups, mean cell volume and HbF were increased, but differences were not seen in hyperdense red cells or vaso-occlusive events. Mg had no effects. Further investigation of hydroxycarbamide as monotherapy in HbSC disease is warranted.

Keywords: sickle cell, Hb SC disease, hydroxycarbamide, hydroxyurea, magnesium.

The pathophysiology of HbSC disease involves erythrocyte dehydration, partly due to increased K-Cl co-transport, which allows the intracellular sickle haemoglobin (HbS) to reach concentrations that induce HbS polymerization and cell sickling. Magnesium (Mg) interferes with cation transport and previously published literature suggested that Mg might

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prevent red cell dehydration (De Franceschi *et al*, 1996, 1997, 2000; Nagel *et al*, 2003).

Hydroxycarbamide (HC), previously termed hydroxyurea, is an antineoplastic agent that inhibits ribonucleotide reductase and has been extensively evaluated in patients with HbSS, but data regarding the effects of HC in patients with HbSC disease are limited (Steinberg *et al*, 1997; Iyer *et al*, 2000; Miller *et al*, 2001).

Our primary goal in the CHAMPS Trial was to compare the efficacy of HC alone, Mg pidolate alone, and HC + Mg in combination to placebo in reducing the density of HbSC erythrocytes. Secondarily, interventions were examined for their effects on haematological parameters, biological measures of erythrocyte activity and red cell-endothelial interactions, and the prevention of vaso-occlusive episodes.

Methods

Study design

This phase-II factorial trial was a double-blinded, multicentre study of subjects with HbSC who were ≥5 years of age. The protocol (ClinicalTrials.gov, NCT00532883) was approved by a National Heart, Lung, and Blood Institute Protocol Review Committee and by local Institutional Review Boards. Eligible subjects had HbSC disease in steady state with at least one vaso-occlusive event (pain or acute chest syndrome) in the previous 12 months, but none within the previous 4 weeks. Eligibility criteria included a haemoglobin of 80–125 g/l and >3% RBC with density >410 g/l. Exclusion criteria included recent hydroxycarbamide or Mg treatment or transfusion. Two pre-randomization visits were performed to evaluate eligibility and obtain baseline laboratory measurements. Subjects were randomized to one of four arms: HC + Mg, HC + Mg placebo (Pbo), HC Pbo + Mg, and HC Pbo + Mg Pbo, stratified within site and age group (5–15 vs. >15 years). Due to small numbers within strata, a sequential allocation algorithm was used (Pocock & Simon, 1975). Subjects were evaluated at biweekly intervals for the first 8 weeks after beginning study drugs and every 4 weeks thereafter for a total of 44 weeks. At each visit, an interim history was obtained and standard blood counts were performed. Evaluations at baseline and weeks 8, 16, 24, and 44 included measures of erythrocyte density, cation content, K-Cl co-transport, Gardos channel activity, Na/Mg exchange, endothelial adhesion, HbF levels, and plasma Mg.

Study drugs

HC capsules were 'over-encapsulated' by UPM Pharmaceuticals (Baltimore, MD, USA) to disguise their appearance; identical appearing Pbo capsules were used. HC (and HC Pbo) was administered at a dose of 20 mg/kg/d PO. Oral Mg pidolate (1 mmol/ml) was formulated by Xcelience (Tampa, FL, USA) along with an indistinguishable Pbo liquid, and administered at a dose of 0·3 mmol/kg/d, BID.

Toxicity adjustments

HC/HC Pbo was discontinued for 1 week if a subject experienced haematological toxicity (absolute neutrophil count $<1 \times 10^9$/l, platelet count $<75 \times 10^9$/l, ≥20% decrease in Hb concentration). Mg/Mg Pbo was discontinued for 1 week if a subject had grade 3 or 4 diarrhoea (CTCAE version 3.0).

Haematological evaluations

The primary endpoint was the proportion of hyperdense red blood cells (cell density >410 g/l), measured centrally with the Advia 120 System (Siemens, Tarrytown, NY), along with reticulocyte count, haemoglobin, cell haemoglobin (CH), MCH, MCHC, MCV, RBC, red cell distribution width (RDW), and WBC count. HbF was measured with high performance liquid chromatography. Plasma total and ionized Mg (iMg) levels were quantitated using the Nova CCX Analyzer. Red cell surface phosphatidylserine was measured by flow cytometry using fluorescent annexin V binding (Sigma-Aldrich, St Louis, MO, USA). Erythrocyte Na, K and Mg content was determined by atomic absorption spectrophotometry and K-Cl co-transport activity by chloride-dependent net K efflux (De Franceschi *et al*, 1997, 2000). Na/Mg exchange was studied by determining the Na-dependent component of Mg efflux (Rivera *et al*, 2005). Gardos channel activity was determined from ^{86}Rb influx using the calcium ionophore A23187.

Adhesion of HbSC cells to the endothelial cell line ECRF-24 was assessed using both epinephrine-stimulated and unstimulated RBCs (Zennadi *et al*, 2004). Adhesion to laminin on coated glass slides was measured after a continuous-flow wash in a graduated-height flow chamber. Selected adhesion receptors were measured by quantitative Western blotting with anti-ICAM4 (Abnova, Jhongli City, Taiwan) and anti-BCAM/Lu, anti-CD47, and anti-stomatin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (Zennadi *et al*, 2004).

Statistical considerations

The primary endpoint and all laboratory secondary endpoints were compared across all four treatment groups using an *F*-test from a longitudinal mixed model controlling for baseline measurement and using a random subject effect. Adverse events were classified using the MedDRA coding system (version 10.0) and proportions of subjects with adverse events and acute pain crises were compared across treatment groups using Fisher's exact test. Although $P = 0{\cdot}05$ was used to determine statistical significance in this report, all *P*-values should be considered exploratory.

Results

Patients

Although the original sample size calculations called for the randomization of 188 subjects across the four treatment

groups, the study was closed early due to slow enrollment, with 64 subjects enrolled and 44 subjects randomized. Thirty-six completed 8 weeks of study drug, thereby reaching the primary endpoint evaluation, and 22 completed the full 44 weeks. Randomized subjects were evenly distributed across the four treatment groups with regard to baseline characteristics including age, gender, HbF, and hyperdense cells ($P = 0{\cdot}13$–$0{\cdot}68$). Mean age was 13·6 years (range 5–53), 57% were male and 73% <18 years old.

Haematological changes

No differences among the four treatment arms were observed for the primary outcome of hyperdense cells (Fig 1A). The two treatment arms that included HC showed significant increases in MCV (weeks 8, 16 and 24; all $P < 0{\cdot}001$), HbF (week 8: $P < 0{\cdot}05$ and week 24: $P < 0{\cdot}001$) and CH (weeks 8, 16 and 24; all $P < 0{\cdot}001$) (Fig 1B–D) compared to the non-HC arms (Table I). Children treated with HC showed greater increases in MCV and HbF at week 24 than adults ($P = 0{\cdot}03$, $0{\cdot}003$, respectively; data not shown). No treatment effect was seen for haemoglobin, absolute reticulocyte count, and MCHC. WBC counts declined significantly at weeks 8, 16, and 24 ($P \leq 0{\cdot}005$) in the HC-treated group, as did platelet counts at week 8 ($P < 0{\cdot}01$).

Cation transport

No differences across treatment groups were observed for erythrocyte Na and K content, K-Cl co-transport, or Na/Mg exchanger activity (Table I). The HC group showed modestly decreased Gardos channel activity at week 24 ($P = 0{\cdot}04$). Mean Mg levels at week 8 were slightly greater in the two groups that included Mg compared with those receiving Mg placebo [iMg 0·62 vs. 0·59 mmol/l ($P = 0{\cdot}02$); total Mg 0·90 vs. 0·86 mmol/l ($P = 0{\cdot}04$)].

Cell adhesion

Erythrocyte BCAM-Lu (laminin receptor) expression was significantly greater in the HC group at week 24 (Table I). However, expression of other adhesion receptors, RBC adhesion to endothelial cells and laminin, and red cell exposure of phosphatidylserine did not differ significantly among the four treatment arms (data not shown).

Toxicity

Both HC and Mg were well tolerated. Eight subjects experienced diarrhoea and 7 had abdominal pain, but these possible gastrointestinal toxicities were evenly distributed across the four groups. Only two subjects (one receiving HC) experienced mild neutropenia.

Clinical events

Thirty-eight subjects reported 293 adverse events (AE) while receiving study drugs; of these, 22 events in 10 subjects were serious adverse events (SAE). No differences were observed across the four treatment groups in the distribution of these events. Fifteen of the SAEs (in 9 subjects) and 111 of the AEs (in 27) were vaso-occlusive pain crises (VOC), but there were no significant differences among groups. Other common

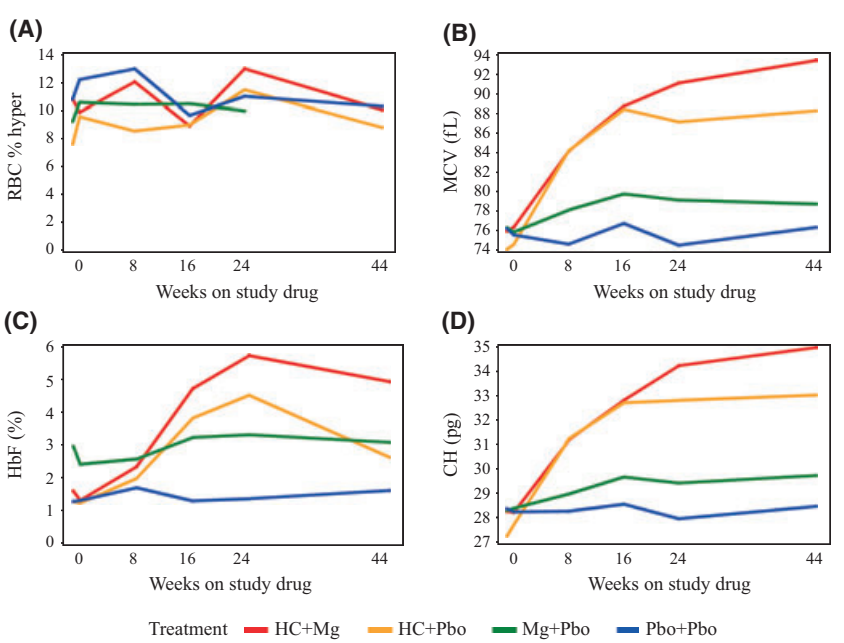

Fig 1. Red blood cell parameters by study visit and treatment group. Mean levels of haematological parameters by treatment arm *versus* weeks on study drug. Panel (A) percentage of red blood cells that are hyperdense (RBC % Hyper); Panel (B) mean cell volume (MCV); Panel (C) percent fetal haemoglobin (HbF); and Panel (D) cell haemoglobin content (CH).

Table I. Mean levels of biomarkers by visit and treatment group.

	Hydroxycarbamide†			No Hydroxycarbamide†		
Parameter – mean	Baseline	Week 8	Week 24	Baseline	Week 8	Week 24
N (min, max)	20, 23	13, 17	12, 13	19, 21	17, 19	13, 14
Red cell parameters						
MCV (fl)	75·6	84·9**	89·3**	76·1	76·4	76·6
HbF (%)	1·3	2·3*	5·2**	2·0	1·9	2·2
Cell Haemglobin (pg)	28·0	31·6**	33·6**	28·6	28·6	28·6
MCHC (g/l)	360	366	369	363	367	360
RBC ($\times 10^{12}$/l)	4·1	3·6**	3·4**	4·0	4·0	4·0
Reticulocyte count ($\times 10^{9}$/l)	0·2	0·2	0·2	0·2	0·2	0·2
RBC hyper (%)‡	9·6	11·7	12·3	11·1	11·8	10·6
Other blood cell parameters§						
WBC ($\times 10^{9}$/l)	8·4	6·8**	6·7**	7·6	7·8	7·6
ANC ($\times 10^{9}$/l)	4620	4410	3710	3870	3660	3640
Platelet count ($\times 10^{9}$/l)	320	294**	274	319	344	343
Adhesion factors						
BCAM/Lu		1·2	1·9**		0·9	0·9
Ion content/transport						
K-Cl co-transport (mmol/10^{13} cells × h)	13·1	14·2	13·8	13·3	12·5	13·2
Cell Na (mmol/kg Hb)	42·4	49·5	43·8	40·5	40·9	40·1
Cell K (mmol/kg Hb)	231	239	228	230	231	232
Gardos channel activity (mmol/l cells × h)	1·3	1·1	1·0*	1·4	1·3	1·3
	Magnesium¶			No Magnesium¶		
Ion content/transport						
Plasma ionized Mg (mmol/l)	0·57	0·62*	0·58	0·59	0·59	0·57
Plasma total Mg (mmol/l)	0·85	0·90*	0·87	0·87	0·86	0·84
K-Cl co-transport (mmol/10^{13} cells × h)	13·3	13·6	13·9	13·1	14·9	13·1
Cell Na (mmol/kg Hb)	43·5	48·6	43·7	39·6	41·2	40·2
Cell K (mmol/kg Hb)	229	233	223	232	236	236
Gardos channel activity (mmol/l cells × h)	1·3	1·2	1·2	1·4	1·3	1·2

†HC includes groups HC + Mg and HC + Pbo; No HC includes groups Pbo + Mg and Pbo + Pbo.
‡Proportion of red blood cells with cell haemoglobin concentration >410 g/dL (Advia).
§Other blood cell parameters derived from local laboratory results.
¶Mg includes groups HC + Mg and Pbo + Mg; No Mg includes groups HC + Pbo and Pbo + Pbo.
*$P < 0{\cdot}05$, **$P < 0{\cdot}01$; *P*-values are based on an *F*-test from a longitudinal mixed model controlling for baseline measurement testing the hypothesis of no difference between subjects receiving HC (HC/Mg and HC/Pbo) and subjects not receiving HC (Pbo/Mg and Pbo/Pbo) at week 8 and 24.

adverse events included gastrointestinal disorders ($n = 42$), headache/migraine ($n = 22$), upper respiratory infection ($n = 18$), and rash ($n = 9$). There were no deaths during the 26·3 patient-years of follow-up.

Discussion

Conclusions from the CHAMPS trial are limited because of the study's early termination with <25% of the targeted number randomized. Nevertheless, we were able to observe significant haematological effects from HC and a lack of response to Mg. Subjects who received HC had significant increases in MCV at week 8 and week 24 with parallel changes in CH and MCH. However, the proportion of dense red cells was not significantly changed by HC, suggesting that the drug's potential benefit would need to result from other mechanisms, such as increased HbF or effects on endothelial adhesion or nitric oxide metabolism. Interestingly, HC was associated with increased expression of the red cell laminin receptor at 24 weeks, without an increase in adhesion to laminin, similar to findings with HbSS red cells (Hillery *et al*, 2000; Odièvre *et al*, 2008).

In contrast with previously published literature in Hb SS disease (De Franceschi *et al*, 1997, 2000), we did not find significant effects of Mg pidolate on HbSC red cell properties, nor did we see an increase in the Mg content of erythrocytes, suggesting that permeability to Mg may differ in HbSS and HbSC cells. In our study Mg was given at a lower than maximally tolerated dose (Hankins *et al*, 2007), perhaps limiting our ability to find expected biological effects on RBC density.

In summary, HC had significant effects on HbSC red cells, including increased HbF and MCV. Disappointingly, Mg

pidolate had no measurable effect on red cell properties. Importantly, no significant toxicity was associated with HC or Mg either alone or in combination in this study. Our data provide a basis for performing clinical efficacy trials using hydroxycarbamide, perhaps at higher doses and for longer duration, in subjects with HbSC disease.

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Conflict of interest

Dr Brugnara and Children's Hospital Boston are named inventor and owner respectively of US Patent 6,331,557, issued December 18, 2001 on the 'Use of divalent cations for inhibiting erythrocyte dehydration *in vivo*'. The authors have no other conflicts of interest to report.

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