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Phase I study of magnesium pidolate in combination with hydroxycarbamide for children with sickle cell anaemia

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Summary

In sickle cell anaemia, red cell dehydration increases intracellular HbS concentration and promotes sickling. Higher erythrocyte magnesium reduces water loss through negative regulation of membrane transporters. Hydroxycarbamide (also known as hydroxyurea) reduces sickling partly by increasing intracellular HbF. Combining drugs with distinct mechanisms could offer additive effects. A phase I trial combining oral magnesium pidolate and hydroxycarbamide was performed to estimate the maximum tolerated dose (MTD) and toxicity of magnesium. Cohorts of three children with HbSS, who were on a stable dose of hydroxycarbamide (median 28·5 mg/kg/d), received magnesium pidolate for 6 months beginning at 83 mg/kg/d. The dose was escalated by 50% for subsequent cohorts. Laboratory evaluations were performed at 0, 3, 6 and 9 months. Sixteen children (aged 4–12 years) participated. All four dose-limiting toxicities (grade III diarrhoea and abdominal pain) occurred within the first month of starting magnesium. Additionally, diarrhoea grades I ($n = 1$) and II ($n = 3$), and abdominal pain grade II ($n = 3$) occurred. Hydroxycarbamide dose reduction or interruption was not required. The MTD for magnesium pidolate used in combination with hydroxycarbamide was 125 mg/kg/d. KCl co-transporter activity declined after 3 months of magnesium pidolate ($P = 0{\cdot}02$). A phase II study is needed to investigate the efficacy of this drug combination.

Keywords: magnesium pidolate, hydroxycarbamide, sickle cell anaemia, red cell dehydration.

Red blood cell (RBC) sickling is a complex phenomenon that involves the aggregation of sickle haemoglobin (HbS) under hypoxic conditions with intracellular polymer formation, resulting in altered erythrocyte morphology and rheology. The propensity of the RBC to undergo sickling is uniquely dependent on the intracellular concentration of deoxy HbS; a higher HbS concentration markedly reduces the lag time to polymer formation, thereby accelerating sickling (Ferrone *et al*, 1985). HbS concentration, in turn, is directly dependent upon cellular hydration status; dehydrated RBCs have higher intracellular HbS concentrations. Three main pathways are involved in red cell dehydration: the Gardos channel (Vandorpe *et al*, 1998), the KCl co-transporter (Brugnara *et al*, 1986) and the Na^+ pump (Joiner *et al*, 1986). Inhibition of any of these pathways will help prevent RBC dehydration. Magnesium (Mg^{++}) is an important regulator of cellular cation transporters such as the KCl co-transporter, Ca^{++} and K^+ channels (De Franceschi *et al*, 1996). Increased intracellular Mg^{++} inhibits K^+ efflux from the sickle erythrocyte and consequently prevents RBC dehydration (Brugnara & Tosteson, 1987). Patients with sickle cell anaemia (SCA) may have decreased magnesium levels in plasma and erythrocytes (Olukoga *et al*, 1990). Their urinary excretion of magnesium is elevated, however, suggesting a high rate of clearance and net movement of magnesium from the erythrocyte to the plasma (Olukoga *et al*, 1993).

Preliminary studies in transgenic sickle mice have shown that magnesium supplementation can significantly reduce KCl co-transport activity (measured by cell K^+ efflux), and thereby decrease mean corpuscular haemoglobin concentration

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(MCHC), red cell density, and reticulocyte count, when compared with mice receiving a low magnesium diet (De Franceschi *et al*, 1996). Administration of magnesium supplementation to adults patients with SCA has been associated with minimal toxicity (mostly diarrhoea) and possibly a reduced frequency of vaso-occlusion (De Franceschi *et al*, 1997, 2000).

Hydroxycarbamide (also known as hydroxyurea) stimulates fetal haemoglobin production in erythrocytes and inhibits formation of intracellular HbS polymers (Platt *et al*, 1984), consequently reducing RBC sickling and vaso-occlusion. Hydroxycarbamide may exert its beneficial effects in SCA via a number of additional mechanisms, including decreased RBC adhesion to endothelium (Hillery *et al*, 2000), a reduction in absolute white blood cell count (Zimmerman *et al*, 2004), and increased levels of nitric oxide, a potent vasodilator (Gladwin *et al*, 2002). Hydroxycarbamide offers clinical benefit to both children and adults with SCA by reducing the frequency of vaso-occlusive events (Charache *et al*, 1995; Jayabose *et al*, 1996; Scott *et al*, 1996).

For many years, the principle of drug combination has been successfully used in other settings, such as the treatment of infections and malignancy. Combining drugs with different mechanisms of action, but with non-cumulative toxicities, may offer the benefit of additive or synergistic effects without enhanced toxicity. Combining hydroxycarbamide and magnesium for the treatment of SCA is conceptually logical because both drugs act by different mechanisms but result in a common goal: reduction of intracellular sickling. In addition, these two compounds have different side effects, minimizing the likelihood of enhanced or overlapping toxicity. Furthermore, the principle of drug combination has not been investigated in SCA and could potentially represent a significant advance in the treatment of this disease.

We conducted a single institution, phase I clinical trial of the combination of hydroxycarbamide and magnesium pidolate (HUMG1 – clinicaltrials.gov identifier: NCT00143572) to investigate the maximum tolerated dose (MTD) and toxicities of magnesium pidolate when used in combination with steady-state hydroxycarbamide therapy in children with SCA. Haematological parameters, intracellular cation content, RBC adhesion to endothelium, and membrane transporter activity were measured during the study, to identify possible biological effects and potential phase II endpoints.

Methods

Patient selection

Patients were eligible to enroll in this Phase I study if they had a diagnosis of HbSS or HbSβ^0-thalassemia, were aged between 3 and 15 years, and had been receiving treatment with hydroxycarbamide for at least 6 months at a stable dose of 15–30 mg/kg/d. In addition, only subjects whose documented adherence to hydroxycarbamide (measured by comparing dispensed and returned drug) was ≥70% in the preceding 6 months were eligible for the trial. Exclusion criteria included RBC transfusion in the last 3 months, pregnancy, serum creatinine and/or alanine transaminase >1·5 times the normal for age, concomitant use of an antisickling agent other than hydroxycarbamide, current use of magnesium containing drugs, and serum ferritin <10 μg/l. The study was approved by a National Heart, Lung, and Blood Institute (NHLBI)-appointed Protocol Review Committee and the St Jude Children's Research Hospital Institutional Review Board. Study progress was reviewed by an NHLBI-appointed Data and Safety Monitoring Board. All study subjects' parents or guardians provided signed informed consent prior to any study-related activity and children older than 7 years signed an informed assent form.

Study design and treatment

This study was a single institution, phase I clinical trial, designed to estimate the MTD and toxicities of magnesium pidolate in children with SCA, when used in combination with steady-state hydroxycarbamide therapy. As a pilot study of 10 patients with SCA reported minimal toxicity (mostly diarrhoea) associated with magnesium pidolate at 83 mg/kg/d (0·6 mEq/kg/d; De Franceschi *et al*, 1997), our first cohort of three children received magnesium pidolate at this dose, given orally divided in two daily doses. Magnesium pidolate doses were escalated by 50% for subsequent cohorts of children [i.e. doses of 83, 125, 181 and 264 mg/kg/d (0·9, 1·3, and 1·9 mEq/kg/d)], following the principles of classical phase I study design (Friedman *et al*, 1998). Magnesium pidolate was given concurrently with hydroxycarbamide for a total of 6 months, unless dose-limiting toxicity (DLT) prompted early discontinuation of the magnesium. After 6 months, magnesium pidolate was discontinued and hydroxycarbamide was continued alone. Adherence to hydroxycarbamide and magnesium pidolate was monitored during the study by clinical pharmacists through cross-checks that compared the amount of dispensed and returned treatment drugs.

Patient and laboratory monitoring

Study participants were monitored for toxicities from both magnesium pidolate and hydroxycarbamide at each clinic visit. Patients had a thorough history, physical examination, complete blood count (CBC), and chemistry panel performed every 2 weeks for the first 2 months, and then monthly until the end of the study. In addition to routine laboratory studies, the following tests were performed at baseline, at 12 and 24 weeks of combination treatment, and finally 12 weeks after discontinuation of magnesium pidolate: CBC and reticulocyte count using the ADVIA 120 Hematology analyser (Siemens Medical Solutions Diagnostic, Tarrytown, NY, USA), haemoglobin F (HbF), intracellular cations (K^+, Na^+ and Mg^{++}), activity of erythrocyte membrane channels [K–Cl co-transporter, Na/Mg

exchange, and calcium-activated K channel (Gardos pathway)] and adhesion of RBCs to purified immobilized thrombospondin. The ADVIA, intracellular cation, membrane channel and RBC adhesion measurements were performed as previously described (De Franceschi *et al*, 1997, 2000; Hillery *et al*, 2000).

Statistical analysis

Because most of the toxicity from magnesium was expected to occur in the first month of treatment, DLT was assessed at week 4 of magnesium pidolate therapy. Toxicities were documented using the National Cancer Institute's Common Terminology Criteria (NCI-CTC) for Adverse Events, v3·0 (Cancer Therapy Evaluation Program, NCI, 2006). Grade III diarrhoea lasting longer than 48 h was considered DLT. All other NCI-CTC grade III toxicities were considered DLT, with the following modifications: (i) haemolysis only if ≥ grade III, (ii) haemoglobin decline only if ≥20% from baseline and (iii) exclusion of splenic dysfunction. These changes were made to more accurately distinguish toxicities from prestudy values in subjects with SCA.

To determine any effect of the combination of hydroxycarbamide and magnesium pidolate on haematological parameters, baseline studies were compared with those after 12 and 24 weeks of magnesium pidolate therapy. Baseline studies also were compared with those at 36 weeks (12 weeks after discontinuation of magnesium pidolate). All statistical comparisons were made using a two-sided paired *t*-test and *P*-values <0·05 were considered significant. If the normality assumption was violated, the Wilcoxon signed-rank test was used instead. Statistical analysis was conducted using StatXact-5 software (Cytel Software Corporation, Cambridge, MA, USA, Copyright 2001).

Results

Patient characteristics and study retention

Sixteen paediatric subjects with HbSS participated in this prospective study; nine were boys. Their median age at study enrolment was 7·3 years (range, 4·2–12·2), and the median duration of hydroxycarbamide therapy prior to study initiation was 1·9 years (range, 0·7–8·4). The median dose of hydroxycarbamide at enrolment was 28·5 mg/kg/d (range, 25·0–30·0). No study participant missed any study visits or exited the study early. All patients had excellent adherence to both hydroxycarbamide and magnesium pidolate as measured by the amount of dispensed *versus* returned treatment drugs. The mean ± SD adherence to hydroxycarbamide and magnesium pidolate were 98 ± 6% and 96 ± 12% respectively.

Maximum tolerated dose and toxicities of magnesium pidolate

Cohorts of three children were evaluated at each successive dose level of magnesium pidolate. There were a total of four DLT events reported and the MTD of magnesium pidolate when used in combination with hydroxycarbamide in children with SCA was determined to be 125 mg/kg/d (Table I).

Table I. Magnesium pidolate dose escalation.

					Total
Dose (mg/kg/d)	83	125	181	264	
Subjects enrolled (*n*)	3	6	5	2	16
Subjects discontinued secondary to DLT (*n*)	0	0	2	2	4

The magnesium pidolate dose was progressively increased from 83 to 264 mg/kg/d. There were two DLTs at 264 mg/kg/d, and two at 181 mg/kg/d. All four DLTs resulted in subject discontinuation from the study. Six patients received magnesium pidolate at the dose of 125 mg/kg/d without DLTs. The maximum tolerated dose of magnesium pidolate used in combination with hydroxycarbamide is, therefore, 125 mg/kg/d.

Four study subjects experienced DLTs that led to early magnesium pidolate discontinuation. All DLTs occurred within 4 weeks of beginning treatment; the specific events were expected and were all felt to be related to the study treatment. Of the DLTs, 4 were grade III diarrhoea, which was accompanied by grade III abdominal pain in two subjects. Only one subject required hospitalization; he was treated with IV fluids and analgesics for abdominal pain and discharged after 24 h of observation. Other cases of diarrhoea included 3 episodes of grade II and 1 episode of grade I toxicity. All episodes of diarrhoea (including all 4 cases of grade III diarrhoea) resolved within 24 hours of discontinuing magnesium pidolate. There were 27 other adverse events not felt to be associated with study medication: pain in the extremities ($n = 9$), bronchospasm ($n = 5$), acute chest syndrome ($n = 2$), fever ($n = 2$), otitis media ($n = 2$), vomiting ($n = 1$), a scalp nodule ($n = 1$), umbilical hernia repair ($n = 1$), constipation ($n = 1$), vulvitis ($n = 1$), cellulitis ($n = 1$), and excessive tearing ($n = 1$). There were no cases of haematological or other laboratory toxicity, and no patients required hydroxycarbamide dose reduction or interruption.

Haematological results and RBC properties

Twelve subjects completed 6 months of combination therapy: three at a magnesium pidolate dose of 83 mg/kg/d, six at 125 mg/kg/d, and three at 181 mg/kg/d. The activity of the KCl co-transport channel significantly decreased 3 months after the introduction of magnesium pidolate (Table II). There were no other significant changes in haematological parameters or red blood cell properties during the study, and measurement of other haematological parameters performed 3 months after discontinuation of magnesium pidolate were not significantly different from those at baseline. Overall, the proportion of hyperdense mature erythrocytes, mean corpuscular haemoglobin concentration, and level of adhesion to immobilized thrombospondin under conditions of flow were

Table II. Red blood cell parameters in children with SCA on steady state hydroxycarbamide who received phase I dose escalation of magnesium pidolate.

	Week (N = 12)			
	0	12	24	36
Hb (g/l)	98 ± 8	95 ± 8	95 ± 11	94 ± 12
Mean CV (fl)	102 ± 8	100 ± 7	100 ± 10	102 ± 10
Absolute reticulocyte count ($\times10^9$/l)	105 ± 28	102 ± 40	126 ± 54	132 ± 67
Hb F (%)	24·8 ± 5·6	25·7 ± 6·5	22·7 ± 6·5	22·2 ± 6·1
WBC ($\times10^9$/l)	6·9 ± 2·5	7·8 ± 4·2	7·2 ± 1·3	7·4 ± 2·6
ANC ($\times10^9$ cells/l)	3220 ± 1365	4397 ± 3999	3683 ± 1161	3750 ± 2482
Platelet count ($\times10^9$/l)	359 ± 88	406 ± 231	367 ± 95	410 ± 137
Hyperdense RBCs (%)	3·5 ± 3·0	3·5 ± 2	3·9 ± 2·7	3·1 ± 2·1
RDW (%)	16·0 ± 1·0	15·9 ± 1·2	16·7 ± 1·4	16·3 ± 1·3
MCHC (g/l)	330 ± 13	332 ± 10	331 ± 14	328 ± 15
HDW (g/l)	38 ± 7	38 ± 6	39 ± 7	37 ± 1
KCl Co-transport channel (mmol/l cell/min)	6·6 ± 4·0	3·8 ± 3·9*	5·5 ± 3	4·6 ± 2
Calcium activated K^+ channel (mmol/l cell/min)	71·6 ± 23·6	69·0 ± 22·6	78·0 ± 23·1	84·2 ± 19·8
Na^+/Mg^{++} exchange activity (mmol/l cell/min)	2·2 ± 1·0	2·8 ± 2	2·5 ± 1·1	2·5 ± 1·5
Intracellular K^+ (mmol/kg Hb)	328 ± 147	275 ± 39·6	282 ± 37·8	294 ± 41·1
Intracellular Mg^{++} (mmol/kg Hb)	6·6 ± 0·8	6·3 ± 0·9	5·6 ± 2·1	6·3 ± 1·7
Intracellular Na^+ (mmol/kg Hb)	61·9 ± 19·9	65·8 ± 21·7	63·8 ± 22·5	54·3 ± 17·2
Thrombospondin adhesion (RBCs/mm^2)	389 ± 298	278 ± 180	294 ± 268	271 ± 183

Results presented as means ± 1 standard deviation.
Hb, haemoglobin; MCV, mean corpuscular volume; WBC, white blood cell count; ANC, absolute neutrophil count; RBC, red blood cell; RDW, red cell distribution width; MCHC, mean corpuscular haemoglobin concentration; HDW, haemoglobin distribution width.
Comparisons were made between weeks 0 and 12, 0 and 24, and 0 and 36.
*P = 0·02.

low at baseline and remained stable throughout the study among patients treated at MTD. Interestingly, patients treated at MTD whose red cell properties were more aberrant at baseline (hyperdense RBCs >10%, adhesion to thrombospondin >200 RBCs/mm^2) showed a trend toward more normal levels when magnesium pidolate was introduced; however these changes were not statistically significant.

Discussion

Because multiple different mechanisms are involved in the pathophysiology of vaso-occlusion, SCA is an ideal disease for the investigation of combination drug therapy by offering drugs with different targets. Magnesium pidolate and hydroxycarbamide work through different mechanisms and have different toxicities, and both treatments have the potential to prevent or improve vaso-occlusive pathologies. Hydroxycarbamide treatment increases HbF and has proven laboratory and clinical efficacy for adults and children with SCA (Charache *et al*, 1995; Jayabose *et al*, 1996; Scott *et al*, 1996). Clinical and laboratory data regarding the effect of magnesium therapy in SCA are more limited, although patients treated with Mg pidolate for 28 d exhibited a significant decrease in RBC density when compared with baseline values (De Franceschi *et al*, 1997). The choice for combining magnesium pidolate and hydroxycarbamide seems logical and appropriate because both drugs are easily administered, work by different principles, and do not appear to have additive toxicities.

This phase I clinical trial determined that the MTD of oral magnesium pidolate in children with SCA, when used in combination with hydroxycarbamide therapy, was 125 mg/kg/d divided into two daily doses. Toxicities from magnesium pidolate were mostly gastrointestinal (diarrhoea and abdominal pain secondary to diarrhoea), and promptly resolved upon discontinuation of magnesium pidolate. There was no enhancement of hydroxycarbamide toxicity from the addition of magnesium pidolate over this 24-week treatment period.

A significant reduction of KCl co-transport activity occurred after introduction of oral magnesium pidolate. A decrease in this co-transport activity is known to reduce cellular efflux of potassium, therefore preventing RBC dehydration. Our finding supports the reported effect of magnesium pidolate use in adults with SCA (De Franceschi *et al*, 2000). No other significant changes in RBC properties were observed during combination therapy, but our study was neither designed nor statistically powered to identify significant differences in RBC parameters caused by drug effect. Our enrolled subjects had been receiving hydroxycarbamide therapy for a median of almost 2 years with both clinical benefit and laboratory response [mean MCV (mean corpuscular volume) 102 fl, HbF 25%], and they had documented excellent adherence with this treatment prior to recruitment. Their baseline RBC

properties were, therefore, less abnormal than those of untreated patients with SCA, and this observation may have blunted or obscured effects of adding magnesium pidolate. However, within our small sample, those subjects with greater baseline RBC abnormalities (e.g. higher proportion of hyperdense cells) tended to have a greater improvement in laboratory parameters with magnesium pidolate treatment. It is possible that magnesium would most benefit patients who are not very responsive to hydroxycarbamide. A phase II clinical trial of magnesium pidolate at MTD (125mg/kg/d) in combination with hydroxycarbamide is warranted to definitively determine effects on RBC properties. Appropriate endpoints would be % hyperdense erythrocytes, KCl cotransport channel activity, and adhesion to thrombospondin. A similar study is currently underway for patients with the Hb SC genotype (CHAMPS study, Clinical Trials.gov identifier NCT00143572) to examine the role of both hydroxycarbamide and magnesium pidolate in individuals with Hb SC disease. If combination drug treatment is proven successful, this approach may provide a major advance in the management of patients with SCA.

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Author contributions

Jane Hankins, MD, MS: designed and conducted study, interpreted data, and wrote manuscript. Lynn Wynn, MSN, PNP: co-ordinated and performed clinical activities. Carlo Brugnara, MD: performed studies of cellular metabolism. Cheryl Hillery, MD: performed red cell adhesion studies. Chin-Shang Li, PhD: performed statistical analysis. Winfred Wang, MD: designed study, interpreted data and wrote manuscript.

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