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The effect of dietary magnesium supplementation on the cellular abnormalities of erythrocytes in patients with β thalassemia intermedia

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Abstract

Background and Objective. **Reduced serum or erythrocyte Mg have been reported in human β thalassemia. These deficiencies may play a role in the cellular abnormalities characteristic of this disorder. We have therefore studied the effect of dietary Mg supplementation in patients with β thalassemia intermedia in order to establish whether it improves the abnormalities of thalassemic erythrocytes.**

Design and Methods. **Plasma and erythrocyte Mg were determined in 11 patients with b thalassemia intermedia, not requiring chronic transfusion therapy, and in 17 normal controls. Inclusion criteria included normal renal and liver function and performance status of 70% or greater. Seven patients were enrolled for the Mg supplementation study, after the appropriate informed consent was obtained. They were given a starting dose of 0.6 mEq/kg/day of magnesium pidolate, divided into two oral daily doses, for four weeks. In a 70-kg subject, a daily Mg dose of 42 mEq corresponds to 504 mg of Mg, with the daily Mg intake of normal subjects being 418±120 mg for males and 343±94 mg for females. After 28 days of treatment, five of the patients continued the protocol with a daily dosage increased to 1.2 mEq magnesium pidolate/kg/day, divided into two oral administrations, for an additional four weeks.**

Results. **In patients with untransfused β thalassemia intermedia we found reduced erythrocyte Mg (in mmol/kg Hb, 6.12±1.5, n=11 vs. 8.69±0.89, n=17, respectively, p < 0.0001) and normal serum Mg. In the seven patients given oral Mg supplements, at Mg dosages of 0.6 mEq/kg/day we observed significant increases in erythrocyte Mg, and significant improvement in some of the characteristic abnormalities of β thal erythrocytes (increased Na-K pump, K-Cl cotransport, cell dehydration, increased osmotic resistance). These changes were maintained in the 5 patients who were treated with 1.2 mEq of Mg/kg/day. Follow-up studies showed a return to baseline conditions. There were no signs of Mg toxicity, with the only side effect being diarrhea, which was generally mild, but led to discontinuation for one patient after the first four weeks.**

Interpretation and Conclusions. **These data indicate that dietary Mg supplementation improves some of the characteristic cellular function abnormalities of b thalassemia intermedia. The possible therapeutic value of this strategy should be further tested in these patients.**

Key words: magnesium, erythrocyte, thalassemia, transport

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Human β thalassemia (β thal) is characterized by ineffective erythropoiesis and reduced survival of circulating erythrocytes.[1] Several different molecular defects responsible for decreased globin β chain synthesis have been described. The hypochromic microcytic anemia of β thal is characterized by erythrocyte membrane damage imposed by the presence of free α globin chains, which involves both whole membrane and cytoskeletal proteins.[1-4] Several alterations in the ion content or transport have been reported in β thalassemia. They include an increased cell Ca content with presence of endocytic inside-out vesicles,[5,6] an increased permeability of the cell membrane to Na and K ions[7-9] and an increased activity of K-Cl cotransport.[10,11] The increased K-Cl cotransport activity is associated with K loss and relative erythrocyte dehydration. The abnormal activity of K-Cl cotransport in β thalassemias is likely due to oxidative membrane damage, since it can be reproduced *in vitro* when normal human red cells are exposed to oxidative agents[11] and it can be diminished by the use *in vitro* of dithiothreitol (DTT)[12] and *in vivo* by the iron chelator deferiprone (L1).[13]

In a mouse model of β thal, cell deformability and dehydration improved upon reduction of the excess of free α globin chains.[14] Thus, the pathogenesis for the increased K-Cl cotransport in β thal erythrocytes differs from that of cells homozygous for Hb C

(CC)[15] or Hb S (SS)[16,17] or double heterozygous for these two Hb variants (SC).[18] In these abnormally positively charged hemoglobin variants, K-Cl cotransport activation probably results from an interaction of Hb with the transporter and/or its regulators,[10,19] although the relative youth of the cells[17,20] and oxidative damage[21] may play a role in the activation observed in SS cells.

While there are no pharmacological inhibitors of K-Cl co-transport that can be used *in vivo*, the erythrocyte Mg concentration is a potent modulator of K-Cl cotransport and a modest increase in cell Mg induces marked inhibition of the transport system.[22,23] In addition, Mg is an important regulator of other ion transport pathways, and of various cellular and membrane functions.[24,28] A reduced cell Mg content has been shown to lead to increased susceptibility to oxidative damage.[29] Dietary Mg supplementation in a transgenic mouse model for sickle cell disease[30] led to significant changes in red cell Mg, Hb concentration, and improvement of the anemia. A study in patients with sickle cell disease has shown that oral Mg pidolate supplements reduce erythrocyte dehydration.[31]

Abnormalities of erythrocyte Mg metabolism have been described in human β thal and low serum Mg has been reported in children affected by the homozygous form of the disease.[32,33] An abnormally low erythrocyte Mg content has been reported in patients with heterozygous β thal.[33]

We found normal serum Mg and decreased red cell Mg content in a mouse model of β thal.[34] In these β thalassemic mice, we have shown that dietary Mg supplementation resulted in increased serum and erythrocyte Mg, reduced K-Cl cotransport activity and dehydration, and improved anemia.

We present here studies on the effect of dietary Mg supplementation in patients with β thal intermedia, and demonstrate that dietary Mg supplementation induces significant changes and improvements of the cellular abnormalities of β thal intermedia erythrocytes.

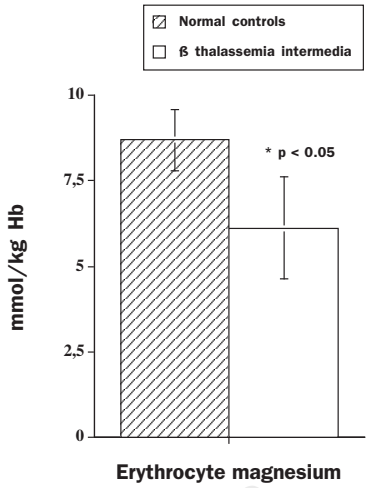

Figure 1. Erythrocyte Mg content (mean±SD) in normal controls (n=17) and in untransfused patients with b thalassemia intermedia (n=11).

Materials and Methods

Drugs and chemicals

NaCl, KCl, ouabain, bumetanide, Tris(hydroxymethyl) aminomethane (Tris), 3 (N-morpholino) propanesulfonic acid (MOPS), choline chloride and Acationox® were purchased from Sigma Chemical Co. (St. Louis, MO, USA). $MgCl_2$, dimethyl-sulfoxide (DMSO), n-butyl phthalate and all other chemicals were purchased from Fisher Scientific Co. (Bromall, PA, USA). All solutions were prepared using double distilled water.

Experimental design

Study protocol. Informed consent for collection of a blood sample was obtained from control subjects

Table 1. Effects of Mg dietary supplementation on plasma Mg, and red cell cation composition in β thalassemia intermedia.

		Plasma Mg (mM)	*Cell Mg*	*Cell Na (mmol/kg Hb)*	*Cell K*	*Cell Na+ K*
Time days	*Mg (mEq/kg/day)*					
0	–	0.81±0.06 (7)	6.8±1.7 (7)	36.1±1.2 (7)	217.1±11.9 (7)	256.3±9.3 (7)
28	0.6	0.89±0.06 (7)	8.6±1.3 (7)°	41.6±5.1 (7)°	271.8±18.1 (7)*	316.3±15.2 (7)*
56	1.2	0.96±0.08 (5)°	10.5±2.9 (5)°	42.0±2.6 (5)*	281.2±14.2 (5)*	323.0±14.4 (5)*
Wash-out	–	0.90±0.1 (7)	5.7±1.08 (7)	38.3±2.1 (7)	225.4±8.8 (7)	262.1±10.5 (7)

*Seven patients were treated with Mg supplements for 28 days at 0.6 mEq Mg/kg/day. Five of these patients received 1.2 mEq Mg/kg/day for an additional 28 days. Data are presented as means±SD (n of measurements). °p<0.05 and *p<0.005 compared to baseline.*

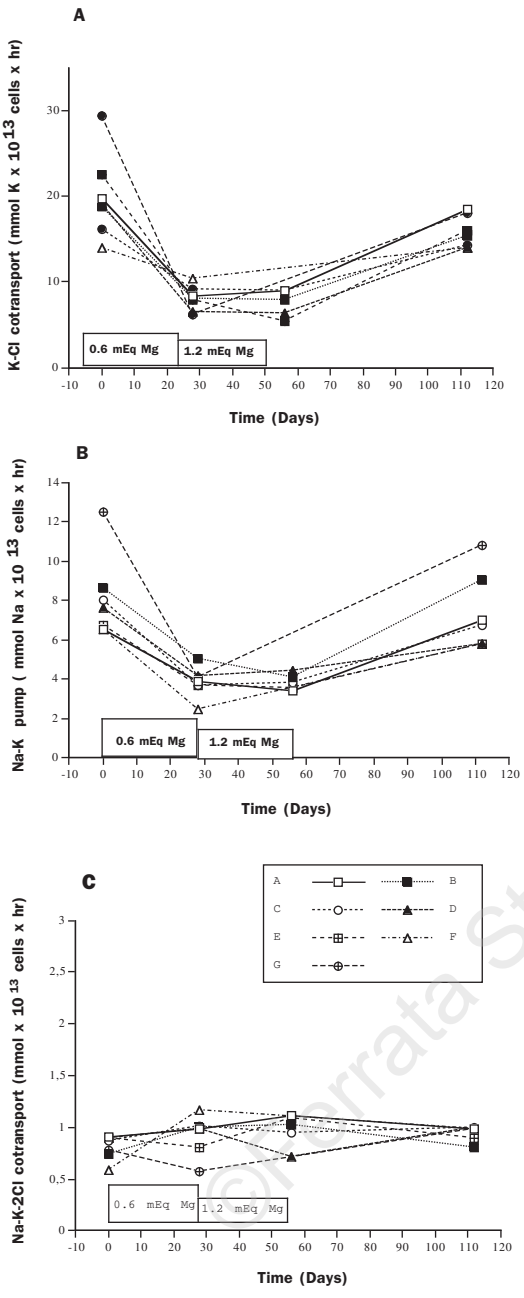

Figure 2. Activities of erythrocyte K-Cl cotransport (A), Na-K pump (B), Na-K-Cl cotransport (C), at baseline, during Mg supplementation, and after wash-out in patients with β thalassemia intermedia.

and patients. Blood was drawn after overnight fasting into heparinized tubes and processed within 24 hours. Plasma and erythrocyte Mg were determined in 11 patients with β thal intermedia, not requiring chronic transfusion therapy, and in 17 normal controls. Inclusion criteria included normal renal and liver function and performance status of 70% or greater. None of patients had received blood transfusions during the preceding 2 months.

Seven of these patients (identified with the letters A through G) were enrolled for the Mg supplementation study, after the appropriate informed consent was obtained.

Subjects were given a starting dose of 0.6 mEq/kg/day of magnesium pidolate (MAG-2, Synthelabo SpA, Limito, MI, Italy; each bag containing 184 mg of Mg, corresponding to 15.3 mEq), divided into two oral daily doses, for four weeks. In a 70-kg subject, a daily Mg dose of 42 mEq corresponds to 504 mg of Mg, with the daily Mg intake of normal subjects being 418±120 mg for males and 343±94 mg for females.[35] A slightly lower dosage had been shown to increase erythrocyte magnesium in diabetic patients.[36,37] After 28 days of treatment, five of the patients continued the protocol with a daily dosage increased to 1.2 mEq magnesium pidolate/kg/day, divided into two oral administrations, for an additional four weeks.

The following studies were performed at the time of entry, after 4 and 8 weeks of Mg therapy, and 2 months after the end of the trial: complete blood and reticulocyte counts, electrolytes, blood urea nitrogen (BUN), creatinine, ALT, AST, total and direct bilirubin, erythrocyte phthalate density profiles and membrane transport studies. Female subjects (n = 4) had negative pregnancy test results at the beginning the study. All the patients were treated as outpatients and none required hospitalization during the study.

Hematological and chemical assays. Erythrocyte cation content and phthalate density distribution curves were determined as previously described.[30,38,39] We calculated for each phthalate density curve the values of D_{20}, which represents the density value separating the 20% of the cells with the highest density. This value is often increased in β thal intermedia patients as a results of erythrocyte dehydration.

The osmotic fragility was determined by adding 40 µL of whole blood to 1 mL of media with different NaCl concentration.[40] After 20 minutes at room temperature, hemolysis was stopped by centrifugation at 1,200 g at 4°C. The absorption of the supernatant was measured at 546 nm, and the percentage hemolysis was calculated in relation to the 100% value obtained in distilled water. The T50%L is the tonicity (in mosM) yielding 50% cell lysis.

Complete blood count (CBC), mean corpuscular volume (MCV) and percent reticulocyte were measured with a Coulter STK instrument (Coulter Electronics, Hialeah, FL, USA). Plasma levels of BUN, creatinine, ALT, AST and other blood chemistry was measured using standard assays on a Boehringer/Hitachi 911 chemistry analyzer. Erythrocyte ATP and 2,3-DPG contents were measured after extraction with percloroacetate with standard biochemical

assays.

Cation transport measurements. Plasma and buffy coat were removed after centrifugation at 1,200 g for 10 minutes and the cells were washed four times with choline washing solution containing 152 mM choline chloride, 1 mM $MgCl_2$, 10 mM Tris-MOPS, pH 7.4 at 4°C.

K-Cl cotransport from fresh cells was measured as chloride-dependent K efflux. Flux media for chloride-dependent K efflux contained 100 mM Na and 1 mM Mg (the anion being either Cl^- or NO_3^-), 10 mM glucose and 10 mM Tris-MOPS (pH 7.4 at 37°C). Chloride-dependent K efflux was calculated as the difference between the K efflux in chloride and nitrate. Incubation times at 37°C for flux measurements were 5 and 15 minutes.

The maximal rates of Na-K pump and Na-K-2Cl cotransport activity were measured in cells containing equal amounts of Na and K (50 mmol/L of cells, obtained with nystatin technique), to achieve saturation of the internal sites for both transport system.[41,42] The nystatin-loading solution contained 70 mM NaCl, 70 mM KCl, and 55 mM sucrose. The Na-K pump was estimated as the ouabain-sensitive fraction on Na efflux into a media containing 130 mM choline chloride and 10 mM KCl. Triplicate samples were incubated for 5 minutes and 25 min at 37°C. The ouabain concentration was 0.1 mmol/L.

Na-K-2Cl co-transport was estimated as the bumetanide-sensitive fraction of the Na efflux into a media containing 140 mM choline chloride, 1 mM $MgCl_2$, and 0.1 mM ouabain. The efflux times were 5 and 25 minutes at 37°C with triplicate samples. The bumetanide concentration was 10 μM. All media contained 1 mM $MgCl_2$, 10 mM glucose, and 10 mM Tris-MOPS (pH 7.4 at 37°C).

Statistical analysis

All values are expressed as means±SD. Comparison of more than two groups was performed by one-way analysis of variance (ANOVA) with Tukey's test for *post hoc* comparison of the means.[43]

Results

Effects of dietary magnesium supplementation on plasma and erythrocyte Mg

In a preliminary phase of this study, plasma and erythrocyte Mg were determined in 11 patients with β thal intermedia and in 17 normal controls. Plasma Mg was similar in the two groups (0.81±0.06 mM for β thal vs. 0.82±0.03 mM in control). Erythrocyte Mg content was significantly lower in β thal intermedia (6.12±1.5 mmol/Kg Hb versus 8.69±0.89, $p<0.001$; see Figure 1). Seven of the β thal intermedia patients were entered into the dietary Mg supplementation trial.

After 28 days of treatment with oral magnesium pidolate (0.6 mEq/kg/day), a significant increase in erythrocyte Mg was observed ($p<0.05$, Table 1), with no changes in plasma Mg (Table 1). Five of the seven patients continued the trial for 28 more days, with an increased dosage to 1.2 mEq magnesium pidolate/kg/day. This regimen resulted in higher erythrocyte Mg level ($p<0.005$ vs. baseline; $p<0.05$ vs. 28 days of therapy; Table 1), and in significant increase in plasma Mg ($p<0.05$, compared to baseline, Table 1). Wash-out studies (56 days after Mg therapy was discontinued) showed plasma and red cell Mg returning to baseline values.

Effects of dietary magnesium supplementation on β thal erythrocytes

Figure 2 presents data on the activities of erythrocyte K-Cl cotransport, Na-K pump, and Na-K-2Cl cotransport at baseline, following dietary magnesium supplementation, and 56 days after therapy was discontinued. Baseline studies showed increased Na-K pump and K-Cl co-transport activities, with normal Na-K-Cl cotransport in β thal intermedia ery-

Table 2. Effects of Mg dietary supplementation on β thalassemia intermedia erythrocyte ATP, 2,3-DPG, density and osmotic fragility.

		ATP *(mmol/kg Hb)*	*2,3-DPG* *(mmol/kg Hb)*	*D_{20}* *–*	*T50%L* *mosM*
Time *days*	*Mg* *(mEq/kg/day)*				
0	–	5.8±0.2 (7)	16.1±2.4 (7)	1.115±0.003 (7)	93.4±20.8 (7)
28	0.6	4.6±0.3 (7)	17.4±5.2 (7)	1.106±0.003 (7)*	116.6±18.0 (7)°
56	1.2	6.0±0.1 (5)	16.8±1.5 (5)	1.105±0.002 (5)*	125.2±15.0 (5)*
Wash-out	–	5.7±0.4 (7)	18.1±3.3 (7)	1.117±0.002 (7)	94.7±12.3 (7)

*Seven patients were treated with Mg supplements for 28 days at 0.6 mEq Mg/kg/day. Five of these patients received 1.2 mEq Mg/kg/day for an additional 28 days. Data are presented as means±SD (n of measurements). °$p<0.05$ and *$p<0.005$ compared to baseline. T50%L, osmolarity yielding lysis of 50% of the cell in an osmotic fragility assay. D_{20}, density value which separates the 20% denser cells.*

throcytes, as it had been demonstrated in previous studies.[11] After 28 days of magnesium supplementation, a significant decrease in the activity of K-Cl cotransport activity was observed ($p<0.005$, Figure 2A). Twenty eight additional days of treatment with higher Mg supplements (1.2 mEq/kg/day) did not result in further reductions in the activity of the K-Cl co-transport system (Figure 2A).

After 28 days of treatment with 0.6 mEq magnesium pidolate/kg/day, a marked decrease in the Na-K pump activity was observed (Figure 2B). The additional 28 days of therapy with higher magnesium supplements (1.2 mEq/kg/ day) did not result in further reduction of the Na-K pump activity (Figure 2B). No significant changes in Na-K-2Cl cotransport activity were present in this trial (Figure 2C).

These changes in Na and K transport were accompanied by significant modifications of the erythrocyte Na and K contents. Erythrocyte Na increased after 28 day of treatment ($p < 0.05$, Table 1) and this increase persisted after 28 additional days at higher Mg supplements ($p <0.005$, Table 1). This result suggests that the observed reduction in Na-K pump activity (Figure 2B) was not accompanied by a similar reduction in the Na permeability, thus resulting in increased cell Na levels.

In all patients, erythrocyte K content increased after 28 days of Mg administration ($p <0.005$, Table 1). No further increase in the cell K content was observed after 28 additional days with higher Mg supplements (Table 1). This increase in erythrocyte K content is most likely due to the observed reduction in K-Cl co-transport activity (Figure 2A).

After 56 days of wash-out, the activities of Na-K pump and K-Cl co-transport increased, and erythrocyte Na and K contents returned to values which were similar to those observed at baseline (Figure 2, Tables 1 and 2).

Erythrocyte density studies, carried out with phthalate density profiles, indicate a significant reduction of dense cells (D_{20}) at 28 days of treatment with 0.6 mEq Mg pidolate/kg/day (Table 2). There were no further significant changes in red cell density for the patients who took 1.2 mEq/kg/day of Mg pidolate for 28 additional days (Table 2). No significant changes were observed in erythrocyte ATP and 2,3-DPG contents (Table 2).

Erythrocyte osmotic fragility (expressed as tonicity yielding 50% lysis, T50%L) increased from 93.4±20.8 mosM (n=7) to 116.6±18 mosM (n=7, $p<0.05$) with 28 days of Mg therapy. This change can be explained by the increased erythrocyte K content. Higher Mg dosages did not result in further changes of this parameter (Table 2).

Dietary Mg supplementation determined a significant decrease in the absolute reticulocyte count after 56 days of therapy ($174\pm42\times10^9$/L at day 56 vs. $286\pm78\times10^9$/L at baseline, $p < 0.024$; see Table 3 for individual values). A significant increase in the absolute reticulocyte count was observed at the wash-out point, with levels significantly higher than baseline ($1{,}331\pm832\times10^9$/L at wash out vs. $286\pm78\times10^9$/L at baseline, $p <0.05$) and 28 and 56 days ($p <0.02$ and <0.05, respectively). No significant changes were observed in Hb levels or MCV (Table 3).

Table 3. Effects of Mg dietary supplementation on hematological parameters in β thalassemia intermedia.

Time days	*Mg (mEq/kg/d)*	*Hb g/dL*	*MCV fL*	*Reticulocytes 10^9/L*
A Baseline		6.51	87.8	424
28	0.6	6.25	88.4	183
56	1.2	6.35	87.2	154
wash-out	–	6.20	87.1	708
B Baseline		8.1	62.3	229
28	0.6	8.2	62.5	380
56	1.2	8.1	62.3	190
wash-out	–	7.5	63.9	274
C Baseline		10.1	76.7	276
28	0.6	9.7	76.6	281
56	1.2	9.8	75.8	222
wash-out	–	9.1	77.4	1495
D Baseline		9.4	75.7	245
28	0.6	9.5	78.0	257
56	1.2	9.4	78.7	110
wash-out	–	9.1	77.2	2304
E Baseline		9.2	80.7	257
28	0.6	9.9	80.9	470
56	1.2	9.8	81.0	195
wash-out	–	9.6	81.0	1875
F Baseline		8.7	87.0	858
28	0.6	8.7	87.1	900
wash-out	–	8.0	86.4	1240
G Baseline		10.5	67.8	298
28	0.6	10.9	67.1	250
wash-out	–	10.7	67.4	293

All patients were treated with Mg for 28 days at 0.6 mEq Mg/kg/day. Patients A-E were treated for an additional 28d with 1.2 mEq Mg/kg/day.

During the first 28 days of treatment at 0.6 mEq/kg/day of Mg pidolate, one patient (subject F) experienced mild diarrhea; there was no evidence of volume depletion on physical examination. Two out of seven patients did not participate to the second phase of the study, one because of mild diarrhea (subject F) and the other for reasons unrelated to this trial (subject G).

During the additional 28 days of treatment with 1.2 mEq/kg/day of Mg pidolate, all five patients experienced mild diarrhea, which was well tolerated and did not lead to study discontinuation. There were no significant changes in plasma ALT, AST, creatinine and BUN levels at any time during the study (data not shown).

Discussion

Based on the findings of abnormally low erythrocyte Mg content in patients with β thalassemia intermedia, and the described cellular effects of Mg, we hypothesized that dietary Mg supplementation could increase erythrocyte Mg and affect some of the abnormal characteristics of β thal erythrocytes. This hypothesis was tested in a mouse model of β thalassemia, which showed significant changes in erythrocyte characteristics and improvement of anemia following dietary Mg supplementation.[34]

We have shown in the present study, which involved seven patients who had not received transfusion, that the reduced erythrocyte Mg of β thal intermedia can be significantly increased with dietary Mg supplements (Table 1). The changes in erythrocyte Mg content are associated with changes in, and/or normalization of, several of the characteristic abnormalities of β thal erythrocytes.

Erythrocyte Mg was significantly reduced in untransfused patients with thalassemia intermedia (Figure 1 and Table 1). In normal erythrocytes, there is a progressive decrease in the cell Mg content in the older and presumably denser cells. Most of Mg is bound to cellular proteins, ATP, and 2,3-DPG, with the major route for Mg exit from the erythrocyte being provided by the Na/Mg exchanger.[44,45] An increased activity of this system has been observed in sickle erythrocytes[46] and in β thal erythrocytes (Beuzard, unpublished observations). However, it has not been yet established whether β thal erythrocytes are also less Mg-endowed at birth, or just lose Mg more rapidly than normal cells. The normal levels of plasma Mg in these patients cannot be taken as an indicator of total body Mg balance or cell Mg state. Since it has not been clearly established that erythrocyte Mg is an indicator of the Mg state of other cells in the body, we do not know if the cellular Mg deficit of β thal intermedia is limited to erythrocytes or may affect other cells. Urinary Mg excretion studies may not identify an abnormality of Mg handling of β thal patients if this is restricted to the erythrocyte lineage.

Notwithstanding the mechanisms responsible for the reduced Mg content of β thal intermedia erythrocytes, dietary Mg supplements can increase erythrocyte Mg to levels which are similar to, or higher than those of normal controls (Table 1). This effect takes place in the absence of significant changes in plasma Mg, although transient increases in plasma Mg immediately following the oral administration of Mg pidolate cannot be ruled out. It is also possible that the increase in erythrocyte Mg may occur via transport of the Mg-pidolate complex inside the erythrocytes.[47] The possibility that this Mg preparation may offer some advantages compared to Mg lactate or Mg oxide will need to be investigated in future studies.

The β thal erythrocyte is characterized by the presence of several membrane transport abnormalities: the Na-K pump, Ca-pump and K-Cl co-transport are significantly increased. These abnormalities reflect the younger age of the circulating erythrocytes and the membrane damage imposed by the excess of free α globin chains. Accumulation of membrane free iron also leads to significant oxidative damage, which can be diminished with iron-chelation therapy.[48] Recent reports in hamsters and rats exposed to a Mg-deficient suggested that erythrocyte Mg deficiency enhances sensitivity to free radical injury, possibly via reduced glutathione levels.[28,29]

The increase of K-Cl co-transport in β thal intermedia erythrocytes probably has multiple origins: the younger age of the erythrocytes, membrane oxidative damage, which is sensitive to DTT[12] and L1 treatment,[13] and reduced erythrocyte Mg content. The reduction of K-Cl co-transport after dietary Mg supplementation can be attributed to the increased erythrocyte Mg (Figure 2A, Table 1), as suggested by the increase in activity of the K-Cl co-transport when Mg pidolate was withdrawn. There was, however, no additional inhibition of K-Cl co-transport when the erythrocyte Mg was further increased by greater Mg supplements (Table 1 and Figure 2A), suggesting the existence of a saturable mechanism(s). It is not clear whether the Mg-sensitive site resides on the K-Cl co-transporter itself or on one of its regulators. It has been speculated that Mg may affect the activity of the volume-dependent kinase or other key regulators of the functional state of the transporter.[49,50]

Dietary Mg supplementation induced a significant reduction in the activity of the Na-K pump, which was abnormally increased at baseline and reached at day 28 and 56 levels similar to those of normal controls (Figure 2B). Several lines of evidence indicate that Mg is an important regulator of the Na-K ATPase and an essential cofactor for the sodium-stimulated phosphorylation of the pump by ATP.[27,51-54] High red cell magnesium inhibits ATPase activity, perhaps partly stabilizing the E_2 forms of the pump and consequently inhibiting the activity of the transport system. However, it is the [Mg]/[ATP] ratio which is crucial in determining the activity of the Na-K pump. A [Mg]/[ATP] ratio near one is optimal for Na-K pump activity, with inhibition at higher and lower ratios.[54] In this study, erythrocyte ATP levels remained relatively constant while the erythrocyte Mg content increased, thus leading to an increase in the [Mg]/[ATP] ratio. In thalassemic cells, the increased Na-K pump activity partially compensates for the increased passive Na permeability of the erythrocytes, maintaining erythrocyte Na at normal levels.[11] Erythrocyte Na increased concurrently with the Mg-induced decrease in Na-K pump activity (Table 1). Thus, dietary Mg supplementation may not affect the increased Na permeability of β thal erythrocytes.

The erythrocyte K content was also significantly increased after 28 days of Mg supplementation and

remained unchanged when Mg was increased to 1.2 mEq of Mg/kg/day (Table 1). Although the most likely explanation of this effect lies in the observed reduction in K-Cl co-transport activity, additional effects on the increased passive permeability for K of the thalassemic erythrocyte cannot be ruled out.

We did not see significant changes in Hb levels with Mg pidolate therapy (Table 3). There was a significant reduction in the absolute reticulocyte count after 56 days of treatment, possibly a suggestion that the red cell survival might have improved (Table 3).[55,56] Reticulocyte counts returned to baseline or higher than baseline values after Mg was discontinued.

Our data indicate that dietary Mg supplementation at 0.6 mEq/kg/day is safe and free of severe side effects in this groups of patients with β thal intermedia. Higher Mg dosages (1.2 mEq/kg/ day) are associated with mild diarrhea. Diarrhea led to discontinuation of the study in one patient. We have also shown normalization of several abnormalities of the β thal erythrocyte following dietary Mg supplementation. These results suggest that the possible therapeutic value of this strategy should be further tested in patients with β thalassemia intermedia.

Contributions and Acknowledgments

LDF contributed to all aspects of this study and had main responsibility for writing the article. MDC, GG and FM were responsible for the clinical care of cases, analyzed clinical data, and commented on the draft. OO, RC, GF and YB discussed core ideas and commented on the draft. CB led the project, discussed core ideas, co-drafted and re-drafted the paper.

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Disclosures

Conflict of interest: none.

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