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Hydroxocobalamin dose escalation improves metabolic control in cblC

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Summary

Cobalamin C (cblC), a combined form of methylmalonic acidemia and hyperhomocysteinemia, is recognized as the most frequent inborn error of intracellular cobalamin metabolism. This condition can be detected by expanded newborn screening and can have an acute neonatal presentation that is life-threatening if not suspected and promptly treated. Intramuscular (IM) hydroxocobalamin (OHCbl) is the main treatment for patients with cblC, but formal dosing guidelines do not exist. A clinical improvement and a decrease of plasma methylmalonic acid (MMA) and total homocysteine (tHcy) levels, and an increase in methionine are typically observed after its initiation. It is well recognized that despite treatment, long-term complications such as developmental delay and progressive visual loss, may still develop. We describe the biochemical response of a 13-year-old boy with worsening metabolic parameters despite strict adherence to a conventional treatment regimen. We progressively increased the OHCbl dose from 1 to 20 mg IM per day and observed a dose-dependent response with an 80% reduction of plasma MMA (25 to 5.14 $\mu\text{mol/L}$; normal range $<0.27 \mu\text{mol/L}$), a 55% reduction of tHcy (112 to 50 $\mu\text{mol/L}$; normal range: 0–13 $\mu\text{mol/L}$) and a greater than twofold increase in methionine (17 to 36 $\mu\text{mol/L}$; normal range: 7–47 $\mu\text{mol/L}$). This suggests that higher OHCbl doses might be required to

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achieve an optimal biochemical response in cblC patients, but it is unknown whether it may slow or eliminate other complications. Future clinical trials to determine the benefits of higher-dose OHCbl therapy in patients with cblC and other disorders of intracellular cobalamin metabolism should be planned.

Introduction

Cobalamin C (cblC; OMIM#277400), a combined form of methylmalonic acidemia and hyperhomocysteinaemia, is recognized as the most frequent inborn error of intracellular cobalamin metabolism. Caused by mutations in the *MMACHC* gene OMIM *609831 (Lerner-Ellis et al. 2006), cblC results in impaired formation of 5'-deoxyadenosylcobalamin (AdoCbl) and methylcobalamin (Mellman et al. 1979), the coenzymes for methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthase (EC 1.16.1.8), respectively. Although the phenotype is variable (Lerner-Ellis et al. 2009; Mitchell et al. 1986; Rosenblatt et al. 1997), many patients become acutely ill in the newborn period and exhibit feeding difficulties, bone marrow involvement, and neurological symptoms (Rosenblatt et al. 1997). Death can ensue if the diagnosis is not suspected and promptly treated.

The mainstay of therapy for cblC consists of intramuscular (IM) hydroxocobalamin (OHCbl) injections (Ogier de Baulny et al. 1998; Rosenblatt et al. 1997; Smith and Bodamer 2002). Clinical improvement and a decrease of plasma methylmalonic acid (MMA) and total homocysteine (tHcy) levels, accompanied by an increase in methionine, is typically observed after OHCbl therapy is initiated (Bartholomew et al. 1988). Protein restriction and other cofactors and supplements, such as folate, carnitine and betaine, are variably used. Only a few studies have carefully documented the biochemical response to serial therapeutic interventions in cblC (Bartholomew et al. 1988; Van Hove et al. 2002). It is well recognized that despite treatment and improved metabolic parameters, severe complications such as developmental delay and progressive visual loss, may still develop (Andersson et al. 1999; Enns et al. 1999; Patton et al. 2000). The inclusion of cblC in most newborn screening panels highlights the importance of determining the most effective treatment regimen for this disorder (Fearing and Levy 2003).

Here, we report the case of a 13-year-old boy with worsening biochemical parameters despite strict adherence to a conventional treatment regimen. He improved considerably after the dose of OHCbl was increased above the range historically used to treat cblC patients.

Case report and methods

The patient is a 13-year-old boy who initially presented at 3 weeks of life with difficulty in feeding, lethargy and metabolic acidosis. MMA and tHcy levels were elevated and he was started on a low-protein diet, IM OHCbl 1 mg/day, betaine 250 mg/kg per day, oral folic acid 0.5 mg twice daily and oral levocarnitine 100 mg/kg per day. From complementation studies a diagnosis of cblC was made. Genotypic analysis revealed homozygosity for the c. 271dupA mutation (p.Arg91LysfsX14), associated with early severe presentation and the most common disease allele (Lerner-Ellis et al. 2006, 2009; Morel et al. 2006; Nogueira et al. 2008). Metabolic management remained unchanged until he was 6 years of age when the OHCbl injections were reduced to 1 mg twice weekly. On this regimen, his tHcy ranged between 60 and 80 $\mu\text{mol/L}$. At age 11 years, the tHcy levels increased above 95 $\mu\text{mol/L}$ (95–110 $\mu\text{mol/L}$; normal range 0–13 $\mu\text{mol/L}$). They remained elevated despite modest protein intake (1.2 g/kg per day), weight-appropriate adjustments in betaine and carnitine, and an increase of OHCbl to 1 mg three times weekly. The plasma MMA levels were consistently in the 20 $\mu\text{mol/L}$ range (normal range <0.27 $\mu\text{mol/L}$).

Patient studies were conducted as part of NIH study 04-HG-0127 'Clinical and Basic Investigations of Methylmalonic Acidemia and Related Disorders' after informed consent was obtained. At the time of assessment at 13 years of age, he had normal growth, a pigmentary retinopathy with severely constricted visual fields, and mild developmental delay. Metabolic measurements obtained after an overnight fast showed tHcy 112 $\mu\text{mol/L}$, plasma MMA 25 $\mu\text{mol/L}$, propionylcarnitine 3.92 $\mu\text{mol/L}$ (normal range $<0.8 \mu\text{mol/L}$), methionine 17 $\mu\text{mol/L}$ (normal range 7–47 $\mu\text{mol/L}$) and a plasma vitamin B₁₂ level of 58 680 pg/ml (normal range 211–946 pg/ml).

To determine the dose of OHCbl required for an optimal response, step-wise dose escalation was performed at 1-month intervals using the following dosing regimen: 10 mg IM three times weekly, 10 mg IM daily and 20 mg IM daily. OHCbl was formulated at either 10 mg/ml or 20 mg/ml (College Pharmacy, Colorado Springs, CO, USA). There were no other modifications in the regimen, which included betaine 8 g (140 mg/kg), carnitine 500 mg (9 mg/kg), folic acid 1 mg daily and modest whole-protein restriction (1.2 g/kg per day).

Monthly clinical and laboratory evaluations were performed in the fasting state and included measurements of the plasma tHcy, MMA, amino acids, acylcarnitines, cystathionine, *N,N*-dimethylglycine, *N*-methylglycine and 2-methylcitrate (Allen et al. 1993; Stabler et al. 1993). Trough vitamin B₁₂ levels were performed on serially diluted serum.

Results and discussion

We observed a considerable dose-dependent reduction of plasma MMA and tHcy and elevation of methionine as the dose of IM OHCbl was escalated (Fig. 1a); this resulted in an 80% reduction of plasma MMA (25 to 5.14 $\mu\text{mol/L}$; normal range $<0.27 \mu\text{mol/L}$), a 55% reduction of tHcy (112 to 50 $\mu\text{mol/L}$; normal range 0–13 $\mu\text{mol/L}$) and a greater than twofold increase in the concentration of methionine (17 to 36 $\mu\text{mol/L}$; normal range 7–47 $\mu\text{mol/L}$) (Fig. 1b). Each increase in the OHCbl dose led to a decrease in plasma MMA and tHcy levels and an increase in methionine. This was accompanied by a reduction in the levels of propionylcarnitine, 2-methylcitrate and cystathionine. The concentrations of *N*-methylglycine and *N,N*-dimethylglycine, products of methyl-donation from betaine to homocysteine, were similar throughout the study, indicating that the observed metabolic improvement was not due to an increased utilization of betaine (Allen et al. 1993). In addition to the biochemical changes, teachers subjectively reported an improvement in the patient's long-term memory. No adverse effects or complications at the site of injection were noted.

The serum cobalamin concentration showed a linear increase with the dose administered. This indicates that the trough serum cobalamin concentration measurements are an important and informative parameter in patients. In this boy, a peak response occurred when the serum cobalamin concentration was 4×10^6 pg/ml (normal <920 pg/ml) and required a dose of 20 mg/day (350 $\mu\text{g/kg}$ per day). Concentrated hydroxocobalamin preparations required to achieve these doses are not commercially available, but can be obtained at compounding pharmacies with a detailed physician's prescription.

The response of renal manifestations to very high doses of OHCbl in two cblC siblings with chronic thrombotic microangiopathy has been detailed (Van Hove et al. 2002). These siblings required a serum cobalamin concentration of approximately 1×10^6 pg/ml (dose 5 mg OHCbl IM or SQ) to control disease manifestations. This concentration approximates the levels we describe here, and suggests that serum cobalamin concentrations of this magnitude will be required to titrate the metabolic response to OHCbl therapy in some patients with cblC. The patient described here is a presumed homozygote for the most

common *MMACHC* mutation, which is associated with an early severe phenotype, suggesting that many others will respond in a similar fashion.

The treatment goals in patients with cblC should include normalizing the biochemical parameters to the greatest extent possible. Our observations indicate that the dose of OHCbl delivered to patients with cblC may need to be increased significantly to achieve an optimal biochemical response. Whether this intervention may slow or eliminate other complications in cblC is unknown, but worthy of study, particularly because numerous reports have demonstrated that disease progression can occur under treatment (Andersson et al. 1999; Enns et al. 1999; Huemer et al. 2005; Smith et al. 2006), even when therapy is initiated prenatally (Huemer et al. 2005; Patton et al. 2000). The safety of acute parenteral administration of much higher doses of OHCbl has previously been established (Uhl et al. 2006) and they are used in the treatment of cyanide poisoning (Shepherd and Velez 2008), but the long-term safety has still to be investigated. Future clinical trials to determine the benefits of higher-dose OHCbl therapy in patients with cblC and other disorders of intracellular cobalamin metabolism should be planned.

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Abbreviations

cblC	cobalamin C
IM	intramuscular
MMA	methylmalonic acid
OHCbl	hydroxocobalamin
SQ	subcutaneous
tHcy	total plasma homocysteine

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Fig. 1. Metabolite levels obtained during OHCbl dose escalation. Baseline levels correspond to the OHCbl dose of 1 mg 3×/week. **(a)** Dose-dependent reduction of plasma MMA and tHcy and increase in methionine levels. Normal levels: MMA<0.27 μmol/L; tHcy 0–13 μmol/L and methionine 7–47 μmol/L. **(b)** Table of relevant metabolite concentrations in plasma