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CASE REPORT

Glycine and L-Arginine Treatment Causes Hyperhomocysteinemia in Cerebral Creatine Transporter Deficiency Patients

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Abstract Our aim was to monitor folate status in five creatine transporter deficient (CRTR) patients undergoing glycine/L-arginine (Gly/Arg) therapy after the finding of severe hyperhomocysteinemia in one of these cases.

Five male patients (age range: 12–20; median = 13 years) genetically confirmed of CRTR deficiency, who were treated with oral glycine (200 mg/kg/day) and L-arginine (400 mg/kg/day) twice a day for 9 months. Clinical follow-up was done at baseline and every 3 months after the start of the therapy. Serum folate was assayed by automated procedures, and plasma total homocysteine (tHcys) by HPLC with fluorescence detection. The 677C→T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene was analyzed by PCR.

Case 1 presented severe hyperhomocysteinemia (81 μmol/L; control values < 10.8) 3 months after Gly/Arg therapy. Three out of the other four cases disclosed mildly increased plasma tHcys values. Serum folate was normal in all cases before therapy, but 3 months after, a deficient status was detected in two cases and a clear decrement in the others when compared with baseline conditions. Two cases were homozygous for the 677C→T polymorphism of the MTHFR, presenting the highest plasma tHcys values. In all cases, after 3 months of folate supplementation (5 mg/day), both serum folate and tHcys concentrations returned to normal values.

In conclusion, prior to the start of long-term Gly/Arg therapy, the monitoring of folate and plasma tHcys values, together with study of the 677C→T polymorphism of the MTHFR gene, seems necessary in order to correct hyperhomocysteinemia by means of folate supplementation.

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Introduction

Cerebral creatine deficiency (CCD) syndromes are inherited metabolic disorders causing a severe neurological handicap, with epilepsy, developmental language delay, intellectual disability, and autistic features as the main clinical signs. Three deficiencies have been recognized as causing CCD: guanidinoacetate methyltransferase deficiency (GAMT; OMIM 601240) (Stöckler et al. 1996), arginine–glycine amidinitransferase deficiency (AGAT; OMIM 602360) (Item et al. 2001), and X-linked creatine transporter

deficiency (CRTR; OMIM 300036) (Salomons et al. 2001). In contrast to GAMT and AGAT deficiencies, CRTR deficiency is not treatable by means of creatinesupplementation. In GAMT deficiency, this treatment may be combined with ornithine supplementation and arginine restriction. Different therapeutic approaches have been tried (Fons et al. 2008; Battini et al. 2010), but with no significant improvement in the neurological outcome. Recently, the association between glycine and L-arginine (Gly/Arg), with the rationale of increasing creatine syntesis from guanidinoacetate in the brain, has been assayed in several studies of patients (Leuzzi et al. 2008; Mercimek-Mahmutoglu et al. 2010), although no definitive results concerning neurological outcome have been published yet.

The association of Gly/Arg might increase the methylation demands, since creatine synthesis through guanidinoacetate methylation consumes an important amount of the folate pool. In fact, it has been suggested that L-arginine treatment alone might also increase these demands (Jahangir et al. 2009), elevating plasma total homocysteine (tHcys)/methionine ratio. In our own experience, during the period of 2007–2008, we treated our CRTR-deficient patients with L-arginine alone (Fons et al. 2008), but we did not detect low plasma methionine or decreased serum folate values (data not shown). In this report, our aim was to monitor folate status in five CRTR patients undergoing Gly/Arg therapy after the serendipitous finding of severe hyperhomocysteinemia in one of these cases.

Materials and Methods

Patients

Five male patients (age range: 12–20; median = 13 years) genetically confirmed of CRTR deficiency (mutations at *SLC6A8* gene). Clinical, biochemical, and molecular data of the patients have been published elsewhere (Fons et al. 2008). Written informed consent was obtained from parents of the patients before treatment. The clinical trial was approved by the ethical committee of Sant Joan de Déu Hospital.

Methods

Patients were treated with oral glycine (200 mg/kg/day) and L-arginine (400 mg/kg/day), twice a day for 6 months, according to previously reported experiences (Fons et al. 2008; Battini et al. 2010). No other changes in patient treatment or diet were made during this time. However, after the identification of hyperhomocysteinemia in several cases, folic acid oral supplementation was started in all patients (5 mg/day) during the following 3 months (total, 9 months of trial). Clinical and biochemical follow-up was done at baseline and at 6 and 9 months after the start of the therapy (except for case 1, whose severe hyperhomocysteinemia was detected at 3 months after the start of Gly/arg therapy).

Serum folate was assayed by automated chemimmunoluminescent procedures (Immulite 2500, Diamond Diagnostics, USA), plasma tHcys and urine guanidinoacetate by HPLC with fluorescence detection (Perkin-Elmer, Norwalk CT, USA) (Arias et al. 2006; Vilaseca et al. 1997), and plasma amino acids by ion-exchange chromatography with nynhydrin detection (Biochrom 30 analyzer, Biochrom, UK). The 677C→T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene was analyzed by PCR and the analysis of the digestion pattern of HinfI restriction enzyme.

Results

Treatment doses, and biochemical and genetic results are summarized in Table 1. After the first 3 months of monitoring (June of 2010), case 1 presented decreased methionine concentrations and severe hyperhomocysteinemia, firstly detected by ion-exchange chromatography with nynhydrin detection, and then confirmed by specific HPLC with fluorescence detection procedure of the SBDF derivatives. This led us to analyze plasma tHcys by HPLC with fluorescence detection in all patients, disclosing mildly increased values in all but one of them. Serum folate was normal in all cases before therapy, but after 3 months from the start of therapy, a deficient status was detected in two cases and a clear decrement in the others when compared with baseline conditions. Two cases were homozygous for the 677C→T polymorphism of the MTHFR (T/T genotype); they presented the highest plasma tHcys values when compared with age-matched reference values (Table 1). Other markers of hyperhomocysteinemia were normal, including plasma cobalamine (Table 1) and urine methylmalonic acid concentrations, which were below our reference values in all cases at the moment of hyperhomocysteinemia (reference values < 20 mmol/mol creatinine). Urine GAA concentrations increased in all patients after Gly/Arg supplementation, lending support to the effectiveness of the therapy in increasing GAA formation.

In all cases, after 3 months of oral supplementation with folic acid, both serum folate and plasma tHcys concentrations returned to normal values (Table 1).

Discussion

This is the first time that hyperhomocysteinemia is reported in association with Gly/Arg treatment in patients with CCD caused by genetic defects in the creatine transporter. There

Table 1 Treatment doses, and biochemical and genetic results of five male patients with CRTR deficiency at baseline and 3 months after starting glycine (200 mg/kg) and L-arginine (400 mg/kg) treatment

	Case 1[a]	Case 2	Case 3	Case 4	Case 5
Age (Years)	17	13	20	12	13
Weight (Kg)	74	45	61	34	32
Dosage (g/day)	Arginine: 30 Glycine: 15	Arginine: 18 Glycine: 9	Arginine: 24 Glycine: 12	Arginine: 13 Glycine: 7	Arginine: 12 Glycine: 6
Serum folate (nmol/L)					
Baseline (0 months)	10.0	19.9	n.a.	15.2	27.2
After Gy/Arg (6 months)	4.8	12.9	5.9	12.1	12.2
After folate (9 months)	>54.4	>54.4	>54.4	>54.4	>54.4
Normal values	9.0–48	10.1–51	9.0–48	10.1–51	10.1–51
Serum cobalamine (nmol/L)					
After Gy/Arg (6 months)	264	472	226	319	482
Normal values	142–530	142–530	142–530	142–530	142–530
Plasma tHcys (μmol/L)					
After Gy/Arg (6 months)	81	10.4	17.4	16.7	6.1
After folate (9 months)	8.4	3.7	10.3	8.2	5.1
Normal values	<10.8	<9.2	<12.8	<9.2	<9.2
Plasma methionine (μmol/L)					
Baseline	17	29	30	21	23
After Gy/Arg (6 months)	10	19	18	21	19
After folate (9 months)	32	34	28	23	25
Normal values	11–20	11–20	11–20	11–20	11–20
tHcys/methioine					
After Gy/Arg (6 months)	8.1	0.55	0.97	0.79	0.32
After folate (9 months)	0.26	0.11	0.37	0.36	0.20
Urine GAA (mmol/mol Cr)					
Baseline	59	162	46	60	93
After treatment	135	252	80	113	187
Normal values	8–94	8–94	8–94	8–94	8–94
MTHFR polymorphisms	T/T	C/T	C/T	T/T	C/C

tHcys total homocysteine; *GAA* guanidinoacetate; *MTHFR* methylentetrahydrofolate reductase; *T* thermolabile polymorphisms; *C/C* Wild-type

[a] Case 1. Biochemical controls were done 3 months after the star of Gly/Arg therapy

are at present several international therapeutic trials with these two amino acids in this kind of patient (Battini et al. 2010; Mercimek-Mahmutoglu et al. 2010) and it might be important to report such side effects, especially in light of the fact that one of our cases presented severe hyperhomocysteinemia.

Considering that the reduction of serum folate concentrations occurred just 3 months after the start of this supplementation, long-term therapy might cause severe folate depletion and hyperhomocysteinemia in most CRTR patients. In fact, case 1, who received the highest Gly/Arg total doses, showed severe hyperhomocysteinemia, and the lowest methionine and folate values. This patient is homozygous for the 677C→T polymorphism of the MTHFR gene. This polymorphism causes a thermolabile variant of the MTHFR enzyme, which leads to a decreased capacity for recycling 5-methyltetrahydofolate (the main one-carbon unit donor) from the folate pool (Guttormsen et al. 1996). Case 4, also homozygous for the T/T polymorphism, presented mildly increased tHcys values, but he was treated with low Gly/Arg total doses. The other patients were both heterozygous and wild type for this polymorphism and were treated with lower Gly/Arg total doses when compared with case 1. However, we cannot rule out the possibility that other underlying metabolic and genetic factors might have caused the severe hyperhomocysteinemia in case 1, which will deserve further investigation. In any case, both plasma cobalamin values and urine methylmalonate excretion were normal in all cases, suggesting that increased plasma tHcys values are likely caused by folate status disturbances triggered by high Gly/Arg doses.

Taken together, these data suggest that prior to the start of long-term Gly/Arg therapy, the monitoring of folate and plasma tHcys values, together with study of the 677 C→T polymorphism of the MTHFR gene, seems necessary in order to correct hyperhomocysteinemia by means of folate supplementation. Furthermore, folate supplementation seems advisable not only to prevent hyperhomocysteinemia but also to improve guanidinoacetate methylation so as to enhance creatine biosynthesis. Additionally, glycine in association with arginine (and other amino acids) may be administered to patients with other diseases (in the form of parenteral nutrition or as dietary supplements) and the side effect we have observed might be present in other diseases such as in patients with argininosuccinic acid lyase, argininosuccinic acid synthetase deficiencies, and isovaleric aciduria (high dose arginine intake in the first two disorders and high glycine intake in the latter). Furthermore, it might be hypothesized that in the general population harboring the 677C→T polymorphism of the MTHFR (T/T genotype), an excessive dietary intake of glycine and arginine would also trigger mild hyperhomocysteinemia.

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Synopsis

Hyperhomocysteinemia was observed in patients with creatine transporter deficiency under glycine and L-arginine treatment.

Details of the Contributions

C. Villar; Clinical assessment, data collection, and drafting the manuscript. J. Campistol; study design and critical revision of the manuscript. C. Fons; data collection, treatment protocol design, and critical revision of the manuscript. J. Armstrong; molecular genetic analysis and interpretation of results. A. Mas: clinical trial organization and critical revision of the manuscript. A. Ormazabal; Biochemical data collection and interpretation, and critical revision of the manuscript. R. Artuch; study design and drafting the manuscript.

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Ethics Approval

All children or their guardians signed an informed consent agreement in accord with the Helsinki Declaration of 1964, revised in Edinburgh in 2000. Our hospital ethics committee approved the study.

References

Arias A, Ormazabal A, Moreno J et al (2006) Methods for the diagnosis of creatine deficiency syndromes: a comparative study. J Neurosci Methods 156:305–309

Battini R, Casarano M, Tosetti M et al (2010) Clinical and MR spectroscopy follow-up of CT1 deficient Italian patients treated by oral arginine. J Inherit Metab Dis 33(suppl 1):S100

Fons C, Sempere A, Arias A et al (2008) Arginine supplementation in four patients with X-linked creatine transporter defect. J Inherit Metab Dis 31:724–728

Guttormsen AB, Ueland PM, Nesthus I et al (1996) Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (> or = 40 micromol/liter). The Hordaland homocysteine study. J Clin Invest 98:2174–2183

Item CB, Stöckler-Ipsiroglu S, Stromberger C et al (2001) Arginine: glycine amidinotransferase deficiency: the third inborn error of creatine metabolism in humans. Am J Hum Genet 69:1127–1133

Jahangir E, Vita JA, Handy D et al (2009) The effect of L-arginine and creatine on vascular function and homocysteine metabolism. Vasc Med 14:239–248

Leuzzi V, Alessandrì MG, Casarano M, Battini R, Cioni G (2008) Arginine and glycine stimulate creatine synthesis in creatine transporter 1-deficient lymphoblasts. Anal Biochem 375:153–155

Mercimek-Mahmutoglu S, Roland E, Huh L et al (2010) Six new patients with creatine deficiency syndromes identified by selective screening in British Columbia. J Inherit Metab Dis 33(Supp 1):S101

Salomons GS, van Dooren SJ, Verhoeven NM et al (2001) X-linked creatine-transporter gene (SLC6A8) defect: a new creatine-deficiency syndrome. Am J Hum Genet 68:1497–1500

Stöckler S, Isbrandt D, Hanefeld F, Schmidt B, von Figura K (1996) Guanidinoacetate methyltransferase deficiency: the first inborn error of creatine metabolism in man. Am J Hum Genet 58:914–922

Vilaseca MA, Moyano D, Ferrer I, Aruch R (1997) Total homocysteine in pediatric patients. Clin Chem 43:690–692