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Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) triggered by valproate therapy

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Abstract We report in this study a patient who developed repeated convulsions as a result of valproate therapy. MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) was subsequently diagnosed and a nucleotide 3243 A→G mutation was detected in the mitochondrial DNA. This mutation predisposes the patient to the detrimental effects of valproate on oxidative phosphorylation.

Conclusion We support the suggestion of Ponchaut et al. [14] that valproate should not be given to patients suspected of having mitochondrial diseases. In addition, for patients whose seizures worsen with valproate therapy, an inborn error of mitochondrial metabolism should be suspected. The underlying mitochondrial DNA defects should be sought for family screening and genetic counselling.

Key words Valproate · Mitochondrial encephalomyopathy · MELAS syndrome · Mitochondrial mutation

Introduction

Recent studies indicated that valproate (2-propylpentanoate) interferes with intermediary metabolism in both healthy subjects and in patients with latent inborn errors of metabolism [7, 10]. In rats, Rumbach et al. [16] showed that valproate at therapeutic concentrations modified the structural organization of the mitochondrial membrane by changing the conformation of the membrane proteins. It also affects the function of the mitochondria by depleting cytochrome aa₃, resulting in a decrease in mitochondrial respiration [13]. In addition, Chabrol et al. [1] observed in a patient that valproate decreased cytochrome c oxidase activity. However, whether there were underlying genetic defects in these patients is unknown. Thus it is difficult to determine whether valproate per se causes mitochondrial disease or if it merely augments a pre-existing condition [17]. The difference between acquired and genetic causes is important for prognostic assessment and for genetic counselling. In this report, we describe a patient who had mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), conditions which became aggravated by valproate therapy. Genetic studies revealed a nucleotide 3243 A→G mutation in the mitochondrial tRNA^{Leu(UUR)} gene of this patient, which is familial in origin.

Case report

A 12-year-old boy with mild mental retardation presented with headache, drowsiness, and right-sided focal convulsion. About 1 year later, he experienced a similar seizure and was then treated with sodium valproate 200 mg thrice daily. Eight days after initiation of valproate, the patient developed right hemiparesis, hypotonia and had two seizures. The serum valproate concentration measured at that time was 396 µmol/l (therapeutic range: 347–693 µmol/l). Plasma ammonium and serum transaminases were normal. Sodium valproate was increased to 300 mg thrice daily and carbamazepine was also started. However, there was no clinical improvement and the patient developed two more episodes of

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convulsions. Because of the idiosyncratic drug reactions, valproate was quickly tapered. Vitamins B₁ and B₂ were also started. The convulsions stopped and the patient's condition became stable.

Metabolic stroke and MELAS syndrome were suspected and blood for lactate and pyruvate drawn. Deproteinized blood lactate (L) was 2.8 mmol/L (reference range 0.5–1.3 mmol/L) and pyruvate (P) was 207 nmol/L (reference range 57–114 nmol/L) with a L/P ratio of 14 (reference range: 6–14). Brain CT scan showed the presence of a left parieto-occipital infarct and bilateral basal ganglia calcification. Muscle biopsy showed presence of ragged-red fibres on modified Gomori-trichrome stain. Electron microscopy showed increased numbers of mitochondria and atypical mitochondria in the subsarcolemmal region, some with inclusion bodies. Since the clinical features and the muscle biopsy findings strongly suggested MELAS syndrome, molecular mutation analysis was performed to confirm the diagnosis.

Material and methods

Mitochondrial DNA analysis

Genomic DNA was extracted from peripheral blood samples and formalin-fixed paraffin-embedded sections of the muscle biopsy tissue were performed as described [9]. The primers used for PCR were 5' CCTCCCTGTACGAAAGGAC3' and 3' CGCTAATCT-TACCCATGTTAC5', which were homologous to heavy chain nucleotide position 3116–3134 and light chain nucleotide position 3353–3333 of mitochondrial DNA sequence, respectively. The size of the PCR product was 238 bp. Digestion of the PCR product with 10 units of restriction enzyme *Hae*III at 37°C for 2 h produced DNA fragments of 169, 37, and 32 bp. The presence of the nt 3243 A→G mutation generates an additional *Hae*III restriction site splitting the 169 bp fragment into a 97 and a 72 bp fragment. The DNA fragments were separated by 12% polyacrylamide gel electrophoresis and the nt3243 A→G heteroplasmy was quantitated according to the procedure of Ciafaloni et al. [2].

Results

A point mutation at nucleotide position 3243 of mitochondrial DNA in the patient was present in 27% of peripheral blood leucocytes and in 84% muscle tissue. Family members of the patient were also examined for this mutation. The results are summarized in Fig. 1.

Discussion

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is a maternally inherited disease. The clinical features of MELAS syndrome were first described by Pavlakis et al. [11]. It was found to be associated with respiratory chain disorders, particularly complex I (NADH coenzyme Q reductase) [8]. The genetic defect was subsequently found to be a nt3243 A→G mutation in the mitochondrial tRNA^{Leu(UUR)} gene [5]. This mutation accounts for 80% of MELAS patients [4].

Our patient presented with a focal convulsion and was treated with sodium valproate. This treatment, contrary to expectation, enhanced the frequency of convulsions. In addition, valproate administration apparently triggered the development of the right hemi-

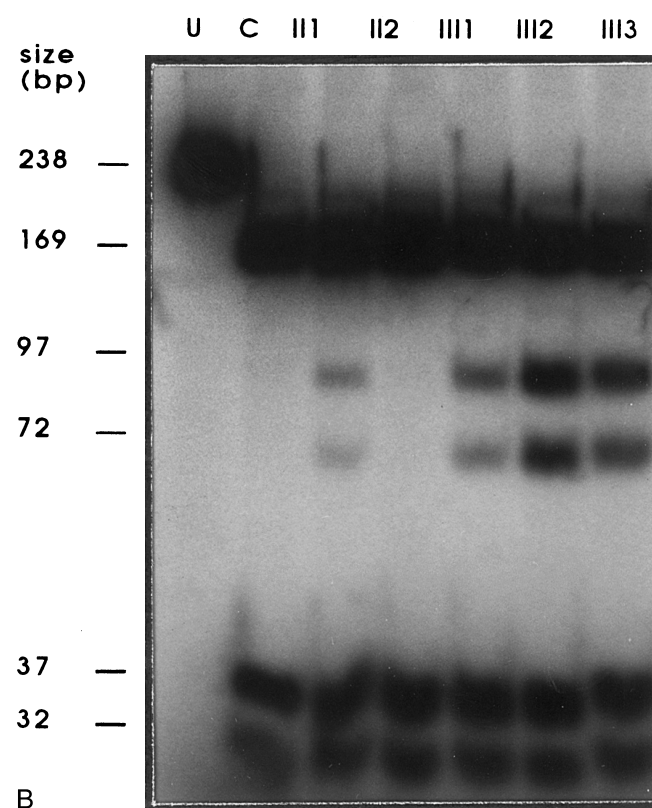
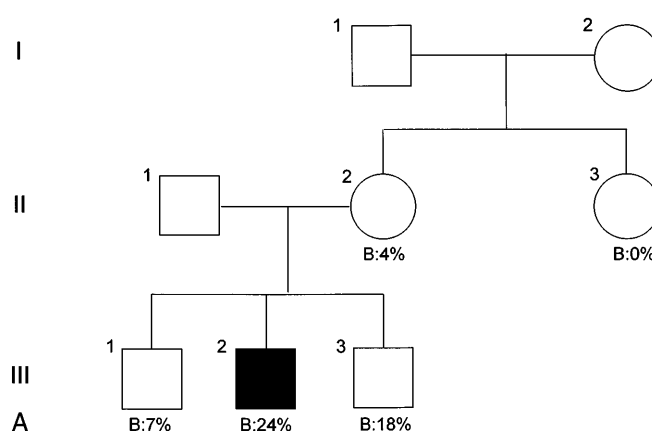


Fig. 1. **A** Pedigree of the family with MELAS. The *solid symbol* denotes the patient with MELAS. The *numbers* below the symbol represent the amount of mutant mitochondrial DNA in blood. **B** Restriction enzyme analysis of the PCR products from the MELAS patient and his family members. Digestion of normal mitochondrial DNA with restriction enzyme *Hae*III generates three fragments of 169, 37, and 32 bp, as shown in *lane C* (control); the presence of the nt3243 A→G mutation creates an additional *Hae*III site that causes the 169 bp fragment to be cut into 97 and 72 bp fragments. *U* uncut PCR products; *C* normal control

paralysis. Indeed, when valproate was tapered, the convulsions stopped and his clinical condition became stable. Although the dramatic improvement in our patient's condition may be attributed to the vitamins given in the treatment regime [12], no controlled study has clearly proven the benefit of such treatment [18]. Dichloroacetate which was proved to be effective in im-

proving mitochondrial function was not used in our patient [3]. Thus, it is evident that valproate directly triggered the mitochondrial myopathy in our patient.

While Rumbach et al. [15] showed that valproate inhibits oxidative phosphorylation in hepatic and cerebral mitochondria, Ponchaut et al. [13] demonstrated that valproate impaired the proton pumping activity of complex IV (cytochrome c oxidase) in the respiratory chain. This was observed in a patient who subsequently developed hepatic failure [1]. In this patient, while cytochrome oxidase activity was markedly decreased in the circulating lymphocytes, liver, and cultured skin fibroblasts, no gross alteration of mitochondrial DNA was detected by Southern blot. However, point mutations in the mitochondrial DNA were not investigated. Given the crucial role of complex IV in the respiratory chain, it is not surprising that valproate can precipitate severe symptomatology of MELAS by further suppressing the constitutively deficient complex IV activity, which occurs in 50% of MELAS patients [4]. In addition, this valproate-induced enzyme suppression can exceed the metabolic threshold for phenotypic expression in tissues having a lower amount of heteroplasmy. Since valproate normally inhibits oxidative phosphorylation [6], decreased mitochondrial enzymic activity determined during valproate therapy is not sufficient for a definitive diagnosis of mitochondrial diseases. In this situation, molecular genetic techniques should be used to confirm the diagnosis.

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References

- Chabrol B, Mancini J, Chretien D, Rustin P, Munnich A, Pinsard N (1994) Valproate-induced hepatic failure in a case of cytochrome c oxidase deficiency. *Eur J Pediatr* 153:133–135
- Ciafaloni E, Ricci E, Shanske S et al. (1992) MELAS: clinical features, biochemistry, and molecular genetics. *Ann Neurol* 31:391–398
- De Stefano N, Matthews PM, Ford B et al. (1995) Short-term dichloroacetate treatment improves indices of cerebral metabolism in patients with mitochondrial disorders. *Neurology* 45:1193–1198
- Goto Y, Horai S, Matsuoka T et al. (1992) Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. *Neurology* 42:545–550
- Goto Y, Nonaka I, Horai S (1990) A mutation in the tRNA^{Leu(UUR)} gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 384:651–653
- Haas R, Stumpf DA, Parks JK, Eguren L (1981) Inhibitory effects of sodium valproate on oxidative phosphorylation. *Neurology* 31:1473–1476
- Honeycutt D, Callahan K, Rutledge L, Evans B (1992) Heterozygote ornithine transcarbamylase deficiency presenting as symptomatic hyperammonemia during initiation of valproate therapy. *Neurology* 42:666–667
- Kobayashi Y, Morishita H, Sugiyama N et al. (1987) Two cases of NADH-coenzyme Q reductase deficiency: relationship to MELAS syndrome. *J Pediatr* 110:223–227
- Lam CW, Jain K, Chan KY, Silva DK, Chan YW, Wong LJC (1995) Diagnosis of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes in a Chinese family by PCR/restriction enzyme analysis. *J Clin Pathol Mol Pathol* 48:M 285–288
- Leao M (1995) Valproate as a cause of hyperammonemia in heterozygotes with ornithine-transcarbamylase deficiency. *Neurology* 45:593–594
- Pavlakakis SG, Phillips PC, DiMauro S, DeVivo DC, Rowland LP (1984) Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes: a distinctive clinical syndrome. *Ann Neurol* 16:484–488
- Penn AM, Lee JW, Thuillier P et al. (1992) MELAS syndrome with mitochondrial tRNA^{Leu(UUR)} mutation: correlation of clinical state, nerve conduction, and muscle ³¹P magnetic resonance spectroscopy during treatment with nicotinamide and riboflavin. *Neurology* 42:2147–2152
- Ponchaut S, Van Hoof F, Veitch K (1992) Cytochrome aa₃ depletion is the cause of the deficient mitochondrial respiration induced by chronic valproate administration. *Biochem Pharmacol* 43:644–647
- Ponchaut S, Van Hoof F, Veitch K (1995) Valproate and cytochrome c oxidase deficiency. *Eur J Pediatr* 154:79
- Rumbach L, Warter JM, Rendon A, Marescaux C, Micheletti G, Waksman A (1983) Inhibition of oxidative phosphorylation in hepatic and cerebral mitochondria of sodium valproate-treated rats. *J Neurol Sci* 61:471–523
- Rumbach L, Mutet C, Cremel G et al. (1986) Effects of sodium valproate on mitochondrial membranes: electron paramagnetic resonance and transmembrane protein movement studies. *Mol Pharmacol* 30:270–273
- Schapira AH, Cooper JN (1994) Inborn and induced defects of the mitochondrial respiratory chain. *Biochem Soc Trans* 22:996–1001
- Walker UA, Byrne E (1995) The therapy of respiratory chain encephalomyopathy: a critical review of the past and current perspective. *Acta Neurol Scand* 92:273–280