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Antiepileptic treatment and blood lactate level alteration in patients with myoclonic epilepsy with ragged-red fibers (MERRF) syndrome in a Chinese family

Fei Xiao *MD*, Jia Li *B*, Xiaogang Zhang *MD*, Xuefeng Wang *PhD*

Neurology Department, The First Affiliated Hospital of Chongqing Medical University, Chongqing; Key Laboratory of Neurology, Chongqing, China

Abstract

Background: Myoclonic epilepsy with ragged-red fibers (MERRF) is a type of mitochondrial encephalomyopathy, clinical experience with the antiepileptic treatment for myoclonus in MERRF is still limited. Myoclonus appears to be intractable, and some antiepileptic drugs may change the blood lactate level. *Objective:* In this study, we report on two patients, a girl and her mother, both with MERRF in a Chinese family. We aimed to study their myoclonus attack, response to AEDs and blood lactate level. *Methods:* The diagnosis was based on muscle biopsies and a genetic test. We recorded their myoclonus and detected alterations of blood lactate when the patients received antiepileptic drugs. *Results:* The patients displayed substantial differences in their responses to antiepileptic drugs. The mother exhibited a good response to valproic acid, although valproic acid is not recommended for mitochondrial disease; however, her daughter was refractory to many antiepileptic drugs until she received a combination treatment of levetiracetam and topiramate. We did not find valproic acid, levetiracetam or topiramate affected the blood lactate levels.
Conclusion: These findings imply that not all MERRF patients are resistant to antiepileptic drugs, and for those who are intractable, combination treatment involving levetiracetam and topiramate may be effective for treating myoclonus in MERRF and does not worsen lactic acidosis.

INTRODUCTION

Myoclonic epilepsy with ragged-red fibers (MERRF) is a type of mitochondrial encephalomyopathy; its clinical features include myoclonic epilepsy, ataxia, weakness, hearing loss, lactic acidosis and ragged-red fibers discovered in muscle. Short stature, optic atrophy and cardiomyopathy are also found in MERRF, as in other mitochondrial encephalomyopathies.

Approximately 80% of patients with MERRF have a family history of the disease, and the mode of inheritance is compatible with maternal inheritance, although not all maternal relatives were affected, and not all those who were affected exhibit the same symptoms.[1] The use of antiepileptic drugs (AEDs) for the treatment for myoclonus and seizures is an important symptomatic therapy for the management of MERRF. However, AED treatment of MERRF is challenging because it has not been investigated in randomized controlled study. Therefore, its underlying biochemical mechanisms are not well understood, and the treatment of myoclonus in MERRF is largely based on anecdotal evidence.[2,3] Previous studies have demonstrated the poor efficacy of valproic acid (VPA), phenytoin (PHT), phenobarbital (PB) and clonazepam in myoclonus in MERRF. Thus, myoclonus appears to be intractable to conventional treatments.[4] In addition, many drugs, including PB, VPA and PHT, have negative effects on mitochondrial function.[3] VPA can inhibit carnitine uptake and induce fulminant liver failure.[5] Studies have also shown that VPA can worsen epilepsy due to mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS).[6,7] Recently, levetiracetam (LEV) was reported to be effective against seizures that resulted from MERRF.[4,8] However, small samples of patients were recruited in those studies, and not all of the patients exhibited satisfactory response to LEV.

Lactic acidosis is one of the key features of MERRF and is toxic to all types of cells[3]; therefore, any medication that causes or increases lactic acidosis may be detrimental to the body. However, *in vivo* and *in vitro* studies have shown that many AEDs, including carbamazepine (CBZ), PB, and VPA, could lead to changes in the blood

Address correspondence to: Dr. Xuefeng Wang, Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Neurology, 1 You Yi Road, Chongqing 400016, China (tel.: 86 23 89012878; fax: 86 23 68708697; e-mail: xfyp@163.com).

lactate level.[9-12] An overdose of lamotrigine or VPA can also increase the lactate level.[13,14] Hence, in this study, we aimed to investigate the lactate levels in a MERRF pedigree in response to VPA, topiramate (TPM) and LEV.

Our study subjects consisted of 2 patients with MERRF in a Chinese family, a girl and her mother. The diagnosis was based on muscle biopsies and genetic test. We report here the response of the myoclonus to the different AEDs, and the effect of the AEDs on blood lactate level.

METHODS

Patients and family history

This particular Chinese family has two members (a mother and her daughter) with MERRF syndrome, which was confirmed with the mitochondrial DNA point mutation test and muscle biopsies. Myoclonus and lactic acidosis were observed in both patients; muscle weakness, ataxia and mental retardation were observed only in the daughter. Myoclonus was multifocal and was particularly evident in the proximal muscles of the limbs, causing sudden arm abduction and leg jerks. A detailed simplified myoclonus rating scale was administered.[4]

The daughter failed to respond to several AEDs, including VPA, PB and PHT. LEV monotherapy was also ineffective, at doses of up to 2000 mg per day. TPM was then added, 25 mg in the first week, 50 mg in the second week, 75 mg in the third week and 100 mg in the fourth week. The combination of LEV and TPM was given for 3 months. After that, we discontinued the LEV. However, the myoclonus recurred, so we resumed the previous combination treatment. The mother was administered VPA 200 mg tid, and this treatment resulted in successful control of the myoclonus. Although we strongly advised her to change from VPA to other AEDs because of the possible side effects, she refused because of its good efficacy and low cost. Informed consent was obtained from the patients or their relatives for the muscle biopsy, genetic test, and the use of any data and tissues. The study was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University.

Muscle biopsy

Muscle samples were obtained from the left biceps brachii. The samples were processed, and slices were stained with hematoxylin-eosin (HE), succinate dehydrogenase (SDH), nicotinamide-adenine dinucleotide-tetrazolium reductase (NADH-TR), ATPase, oil red O (ORO), and periodic acid-Schiff (PAS).[15,16]

Genetic test

A blood sample from the father of the affected daughter was obtained as a control. The analysis of the mitochondrial DNA was conducted according to the Choi *et al*.[17] procedure.

Blood lactate detection

Because blood lactate level may be affected by various factors and particularly under certain physiological conditions such as anoxia, shock and vigorous exercise, we obtained blood samples from the daughter on multiple days (before administering LEV, before administering TPM, when TPM was increased to 25 mg, 50 mg, 75 mg, 100 mg per day and after 3 months at 100 mg per day) and we ensured that no frequent jerks were observed during the 2 days prior to each scheduled blood taking to avoid interference. We obtained blood samples from the mother twice over one year.

Analysis of the activity of mitochondrial respiratory chain complex IV (cytochrome C oxidase, COX)

Muscle slices were stained according to the Greaves *et al*.[15] procedure to determine the activity of COX. The control consisted of sample from a patient with no neuromuscular disorders.

Statistical analysis

The average myoclonus score was evaluated when TPM was increased to 100 mg per day, after 3 months at 100 mg per day and TPM withdrawal. A Pearson linear correlation analysis was conducted to compare TPM dosage and blood lactate levels.

RESULTS

Myoclonus profile

The study subjects were two members of a Chinese family, a daughter and her mother. The daughter has no siblings. The mother has 3 siblings, but none of the other relatives were affected. The daughter had myoclonus for 12 years since she was 2 years old, and her mother had suffered the same symptom for 25 years since age 15 years. Cranial MRI revealed no abnormalities for both patients.

Multiple spike discharge was recorded in EEG when the daughter exhibited myoclonus. During follow-up, there were no obvious changes in the functional status other than the myoclonus.

The daughter's myoclonus was refractory to 2000 mg of LEV, with significant impairment of her activities of daily living. She reported a decrease in myoclonus with a dramatic improvement in the rating scale and quality of life after receiving TPM add-on therapy at a dosage of 100 mg per day (Table 1). However, 3 months later, when the LEV was withdrawn and TPM was continued as a monotherapy, the myoclonus became frequent again. No other side effects were observed.

The mother exhibited good response to VPA. There was only rare myoclonus, and there was no side effects from VPA over 8 years.

Muscle biopsy

Abundant typical raged red fibres were observed in both the daughter's muscle sample (Figure 1a) and her mother's.

Genetic test

After PCR-RFLP, sample 1 (daughter) and sample 2 (her mother) were partially digested with *Bgl* into two fragments (21 bp and 187 bp) because the A8344G point mutation generates a new *Bgl* restriction enzyme digestion site point. However, the product was not digested in sample 3 (control).

Gene sequencing analysis of the mitochondrial DNA confirmed a point mutation from A to G at the 8344th nucleotide position located in the tRNA (Lys) gene.

Blood lactate level

For the daughter, the blood lactate concentration was 12.5 mmol/L before LEV and 12.3 mmol/L before TPM. When TPM was increased to 25 mg, 50 mg, 75 mg, 100 mg per day and 3 months later, the concentrations of blood lactate were 12 mmol/L, 11.5 mmol/L, 12 mmol/L, 7 mmol/L and 10 mmol/L, respectively. TPM did not result in an increase in lactate. In addition, there was no linear relationship between the blood lactate level and the TPM dosage. For her mother, even after 8 years of VPA, her blood lactate level remained unchanged over the one-year period (3.9 mmol/L versus 3.8 mmol/L).

Mitochondrial respiratory chain complex IV (COX) activity

COX positive fibers were stained brown, and the color intensity was positively correlated with the level of enzyme activity. Compared with the control, the mother's COX activity decreased. The daughter's COX activity was more markedly decreased. (Figure 1b)

DISCUSSION

The most frequent mutation in MERRF is an A-to-G transition at nucleotide 8344 (m.8344A>G), which occurs in more than 80% of affected individuals. The mutations are usually detected in mtDNA from blood leukocytes.[3] Only some of the patients with this mitochondrial disorder have a positive genetic diagnosis. However, the absence of the mtDNA mutation in one tissue (e.g., blood) does not mean that the mutation is absent in other tissues. Thus, its precise diagnosis depends on clinical, metabolic, histological, enzymological, and molecular features.[18-20]

Table 1: The status of myoclonus of the daughter under various AEDs using simplified myoclonus rating scale

LEV		LEV & TPM 100mg/day		LEV & TPM for 3 months		TPM 100mg/day (LTV withdrawn)	
Territory	Score	Territory	Score	Territory	Score	Territory	Score
Face	1	Face	0	Face	0	Face	1
Arms	2	Arms	1	Arms	1	Arms	2
Legs	3	Legs	2	Legs	1	Legs	3

Score 0 = no myoclonus and no effets on daily living; score 1 = rare myoclonus with minor effets on daily living; score 2 = frequent myoclonus and moderate effets on daily living; score 3 = very frequent myoclonus with serious effets on daily living activities.
LEV = levetiracetam; TPM = topiramate

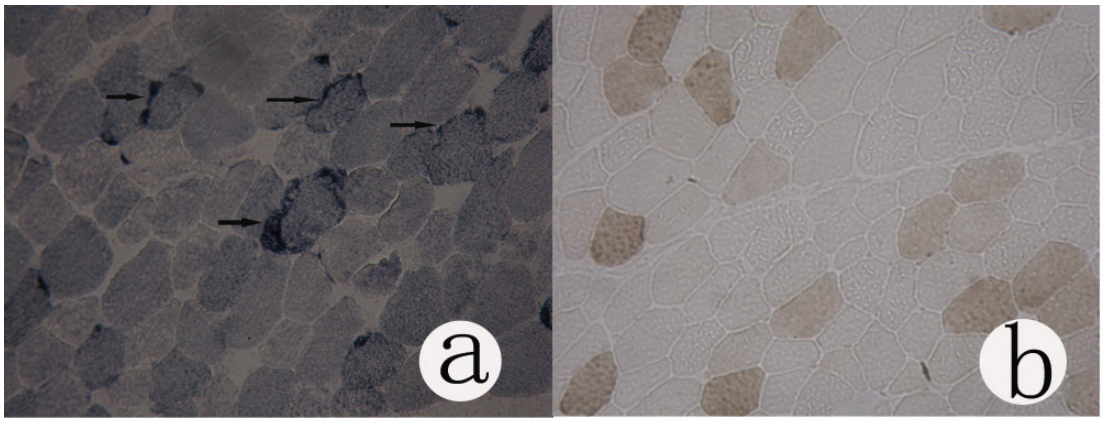

Figure 1. Muscle histology of the daughter. (a) SDH stain, black arrows indicate ragged-red fibres. (b) COX stain, only few COX positive fibers are seen. (×200)

Because there are no controlled studies that compared the efficacy of different AEDs, the seizures associated with MERRF are treated with different AEDs. Between the two patients from this MERRF family, the myoclonus as well as the responses to AEDs exhibited marked differences, which indicate the heterogeneity of this disorder. The daughter failed to improve with monotherapy with several types of AEDs even at sufficient doses; therefore, combination therapy with LEV and TPM was administered. MERRF is a genetically and clinically heterogeneous disease. However, there is no clear correlation between the genotype and clinical phenotype for affected individuals. The clinical phenotype may depend on the mutation site, the relative abundance of mutant mtDNAs, the tissue distribution of the mutant mtDNAs and the threshold effect. Among these factors, variable mutational load and tissue distribution may account for the clinical diversity of MERRF.[20-22] We found that complex IV enzyme activity was markedly decreased in the daughter compared with her mother, which indicated more severe mitochondrial respiratory chain dysfunction in the daughter. It has been reported that COX deficient muscle fibers are due to deletions and point mutations of the mtDNA in segments of muscle fibers[23], and high mutational load correlated with more severe cytochrome c oxidase deficiency.[24,25] Therefore, we expected the daughter to display a higher mutational load compared with her mother. Because they have the same mutation, unequal mutational loads may partly explain the different clinical phenotypes and responses to AEDs in the two patients. Furthermore, it has been reported that mitochondrial respiratory chain defects are one of the important causes of symptomatic epilepsy.[26] The myoclonus recurrence in the daughter may have resulted from a more severe mitochondrial respiratory chain defect in the brain. In addition, the good control of myoclonus in the mother indicated that not all MERRF patients are refractory to AEDs.

VPA is not recommended for seizure control in cases of mitochondrial encephalomyopathy. Moreover, in MELAS, VPA worsens epilepsy.[6,7] However, in our patients, the mother continued to experience only a few myoclonus after years of VPA treatment, and she exhibited no obvious side effects including liver function impairment. The reason for the lack of adverse VPA-mediated effects is unknown. Does MERRF differ from other mitochondrial encephalomyopathies, such as MELAS and CPEO, in terms of VPA's side effects? Further studies and largeer numbers of patients are necessary to address this question.

LEV has been reported to be an ideal medication for myoclonus control in MERRF.[4,8] Nevertheless, in our patients, the daughter still experienced recurrent attacks after LEV was administered at sufficient doses. When TPM was added, the frequency and severity of the myoclonus decreased.

Lactic acidosis is a feature of MERRF, and it may be affected by many types of AEDs.[9-11,13,14] However, the impact of TPM and LEV on lactate level in patients with MERRF remains unknown. In the present study, the daughter revealed no distinct changes in blood lactate acid level while receiving combination therapy with LEV and TPM for 4 months, at a range of TPM doses. This finding indicates that LEV and TPM may not affect lactate acid metabolism short term. However longer follow-up is necessary. It is not known whether there was cerebrospinal

fluid (CSF) lactic acid levels change. Because there was no consent, lumbar puncture was not performed. Lactic acidosis is toxic to all types of cells, particularly if their metabolism is already impaired. The correction of lactic acidosis is a major goal in MERRF treatment, therefore, the use of lactate-lowering agents including riboflavin, succinate and coefficient Q.

In conclusion, the understanding of the underlying biochemical and pathophysiological basis of mitochondrial encephalomyopathies, including MERRF, is very limited. Seizures and myoclonus due to different types of mitochondrial encephalomyopathies, even those of the same phenotype may show varying responses to AEDs[4], as in our cases.

DISCLOSURE

Conflict of interest: None

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