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Case Report

Valproic Acid Treatment for Spinal Muscular Atrophy Patient with Abnormal Electroencephalogram Findings

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Abstract

Background: Valproic Acid (VPA), an anticonvulsant, is a histone deacetylase inhibitor that can increase the Survival Motor Neuron (*SMN*) level in Spinal Muscular Atrophy (SMA) patients and anticipated to be an effective therapeutic agent for SMA.

Patient description: VPA was administered for SMA to a 6-year-old female who showed non-symptomatic abnormal electroencephalogram findings, as well as SMA signs and symptoms. Although the VPA concentration of our patient remained in a low range, electroencephalogram abnormalities disappeared within 10 months after starting administration. On the other hand, clinical signs and symptoms of SMA, respiratory function values, and modified Hammersmith Functional Motor Scale results showed no remarkable changes. We also analyzed survival motor neuron (*SMN*) transcripts and splicing factor transcripts, such as splicing factor 2/alternative splicing factor (SF2/ASF) and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), using quantitative real-time PCR assays performed before and after VPA treatment. The amounts of the FL-*SMN*, total-*SMN*, ASF/SF2, and hnRNPA1 transcripts showed an increasing tendency, whereas that of Δ7-*SMN* was decreased.

Conclusion: Our findings indicate a discrepancy between *SMN* transcript response, and improvements in SMA symptoms and signs following VPA administration in some SMA patients.

Introduction

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by progressive muscular atrophy, as well as weakness of the limbs and trunk derived from degeneration of motor neurons of the spinal cord. The disease is classified into 5 groups; type 0 (prenatal), type 1 (Werdnig-Hoffman disease), type 2 (Dubowitz disease), type 3 (Kugelberg-Welander disease), and type 4 (adult form), based on age at onset and achievement of motor milestones [1].

The survival motor neuron (*SMN*) gene is responsible for SMA. Two highly homologous copies of that gene, namely *SMN1* and *SMN2*, exist within the SMA genetic region on chromosome 5q11.2–13.3 [2]. *SMN1* is homozygously deleted or interrupted in more than 95% of SMA patients, which results in a deficiency of the *SMN* protein [2,3]. *SMN1* and *SMN2* encode the same protein, because of a synonymous nucleotide change, and their difference is only a single nucleotide change, as nucleotide +6 in the coding region of exon 7 in *SMN1* is C and that of *SMN2* is T. However, they also show different splicing patterns [4]. All *SMN1*-derived transcripts contain exon 7, namely, the full-length *SMN* (FL-*SMN*) transcript, while the majority of *SMN2*-derived transcripts lack exon 7 (Δ7-*SMN*), because the C to T change in *SMN2* at nucleotide position +6 in exon 7 induces exon skipping [4]. This genetic mechanism is enhanced by an exonic splicing enhancer (ESE) and exonic splicing silencer (ESS). In *SMN1*, a heptamer sequence motif including C at nucleotide position +6 in exon 7 shapes a splicing factor 2/alternative splicing factor (SF2/ASF) binding site as an ESE, resulting in exon 7 inclusion [5]. On the other hand, the corresponding T nucleotide in *SMN2* disrupts the ESE motif and shapes a heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) binding site as an ESS, resulting in exclusion of exon 7 [6].

The *SMN2* copy number is inversely correlated with disease severity. Although *SMN2* does not fully compensate for loss or dysfunction of *SMN1*, a higher *SMN2* copy number ameliorates the clinical phenotype [1,7]. *SMN2* is thought to compensate for loss of *SMN1* by producing a small amount of full-length *SMN* protein. Thus, treatment strategies for SMA have focused on increasing the expression of *SMN2*. Valproic acid (VPA) is an anticonvulsant used for treatment of epileptic patients. In addition, the agent is also a histone deacetylase (HDAC) inhibitor that can increase *SMN* levels in SMA patients via activation of *SMN2* transcription and splicing correction of *SMN2* exon 7 [8,9]. Thus, VPA is anticipated to be an effective therapeutic drug for SMA [10-12]. Here, we report VPA treatment given to a 6-year-old girl with SMA for electroencephalogram abnormalities. We evaluated changes in electroencephalogram findings, as well as SMA symptoms and signs, and also the *SMN* transcript, ASF-SF2, and hnRNPA1 before and after starting VPA treatment.

Case Report

A 6-year-old girl with SMA came to our hospital for evaluations of respiratory function and general condition. Developmental milestones were delayed, and the patient showed unstable sitting and walking, though mental development was normal. Previous

findings for the patient showed *SMN1* deletion, and that she carried 3 copies of *SMN2* and the neuronal apoptosis inhibitory protein gene (NAIP). Non-invasive ventilation (NIV) was used during sleep at night. Electroencephalogram findings revealed abnormal spikes and waves, with sharp waves frequently occurring in the right temporal and occipital regions, though related clinical symptoms were not apparent (Figure 1). Brain magnetic resonance imaging (MRI) showed no abnormalities (Figure 2). We determined that treatment for the abnormal electroencephalogram findings would be the first objective, thus VPA administration was decided. In addition, we hoped to evaluate its effects on laboratory findings, as well as SMA symptoms and signs.

Ethical Approval

Informed consent was obtained from the patient and her family prior to beginning the study, and the protocol was approved by the local ethical committees of Toneyama National Hospital and Kobe University.

Clinical Course of Patient

VPA was started at 25 mg/day and then gradually increased to 225 mg/day over a period of 10 months. L-carnitine was also administered at 100 mg/day from 5 months after starting VPA treatment. Following initiation of VPA therapy, abnormal waves shown in electroencephalogram findings disappeared. On the other hand, the modified Hammersmith Functional Motor Scale for SMA (MHFMS) before starting VPA was 1/40 and remained at 1/40 after 10 months of VPA treatment, indicating no remarkable change (Figure 3).

For respiratory function, we assessed vital capacity (VC), maximum insufflation capacity (MIC), and cough peak flow (CPF) [13]. VC before starting VPA administration was in a range of 610-700 ml, while that was 530-700 ml after starting treatment, indicating a decrease in VC after starting VPA. Furthermore, MIC before treatment was 850 ml, then slightly decreased to 700 ml after 10 months, while CPF before treatment was 50 l/minute and slightly increased to 70 l/minute after 10 months. VPA concentration was 11-16 μg/ml. There were no abnormal changes in liver function and carnitine fraction also remained normal.

SMN, SF2/ASF, and hnRNPA1 transcript

We analyzed peripheral white blood cell samples obtained from the patient prior to initiating VPA treatment and at 7 months after beginning treatment (125 mg/day). *SMN* transcripts (FL-*SMN*, total-*SMN*, Δ7-*SMN*), SF2/ASF, and hnRNPA1 were examined using quantitative real-time PCR (qRT-PCR) analysis, with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript as the internal reference, with the levels of *SMN*, SF2/ASF, and hnRNPA1 expressed relative to that of GAPDH [14]. Details of the methods utilized for qRT-PCR have been reported [14].

The FL-*SMN* transcript amount showed an increasing tendency after starting VPA treatment, with the value after 7 months reaching a relative ratio of 0.87, an increase of 0.18 points from the pre-treatment value. That also reached 87% of the values for the FL-*SMN* transcript in a healthy control (Figure 4). After starting VPA treatment, the Δ7-*SMN* transcript amount showed a decreasing tendency, while the amount of total-*SMN* transcript showed a slight increasing tendency. Furthermore, the SF2/ASF transcript amount showed an approximately 2-fold increase after 7 months as compared to the pretreatment value, while the hnRNPA1 transcript showed a very slight increase at that time point.

Discussion

In the present patient, electroencephalogram abnormalities disappeared after starting VPA administration at a low concentration, whereas motor function did not change and respiratory function gradually deteriorated. Thus, there was no evident efficacy of administration of a low concentration of VPA in regard to motor
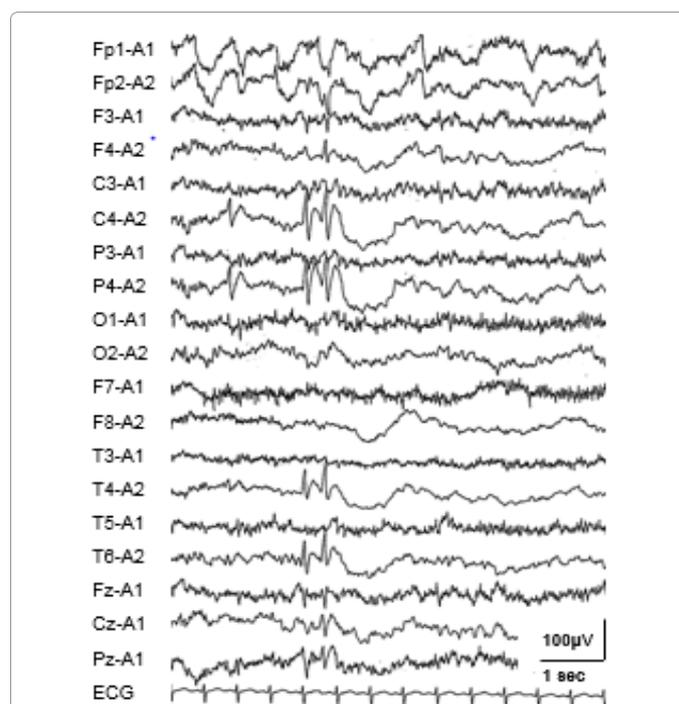

Figure 1: Electroencephalogram obtained before starting VPA treatment. Abnormal spikes and waves, including sharp waves frequently occurred in the right temporal and occipital regions, though clinical symptoms were not apparent valproic acid (VPA).
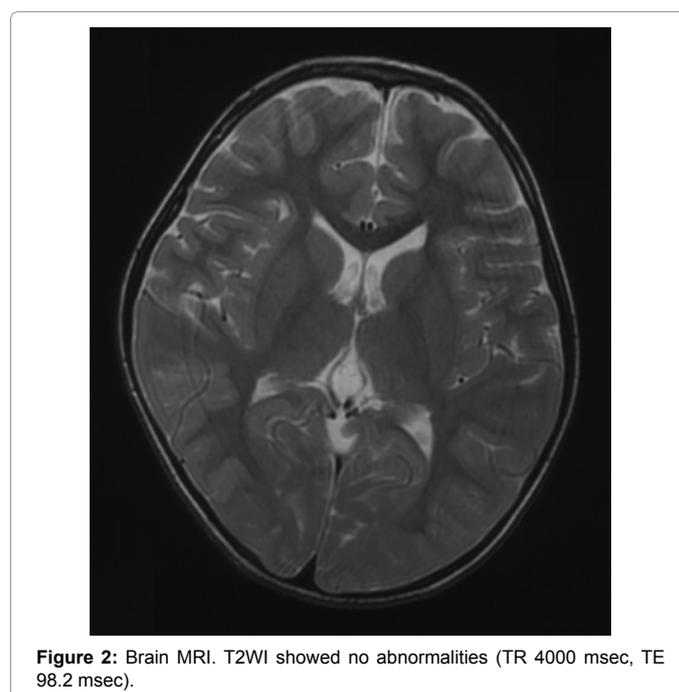

Figure 2: Brain MRI. T2WI showed no abnormalities (TR 4000 msec, TE 98.2 msec).
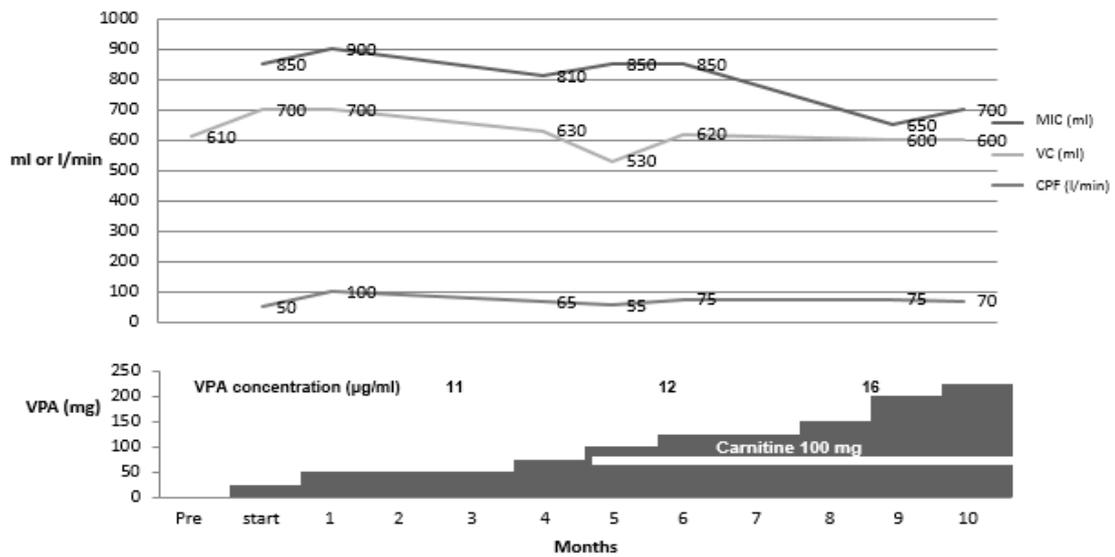
Figure 3: Sequential changes in respiratory function, VPA administration, and serum concentrations of VPA. We assessed vital capacity (VC), maximum insufflation capacity (MIC), and cough peak flow (CPF).
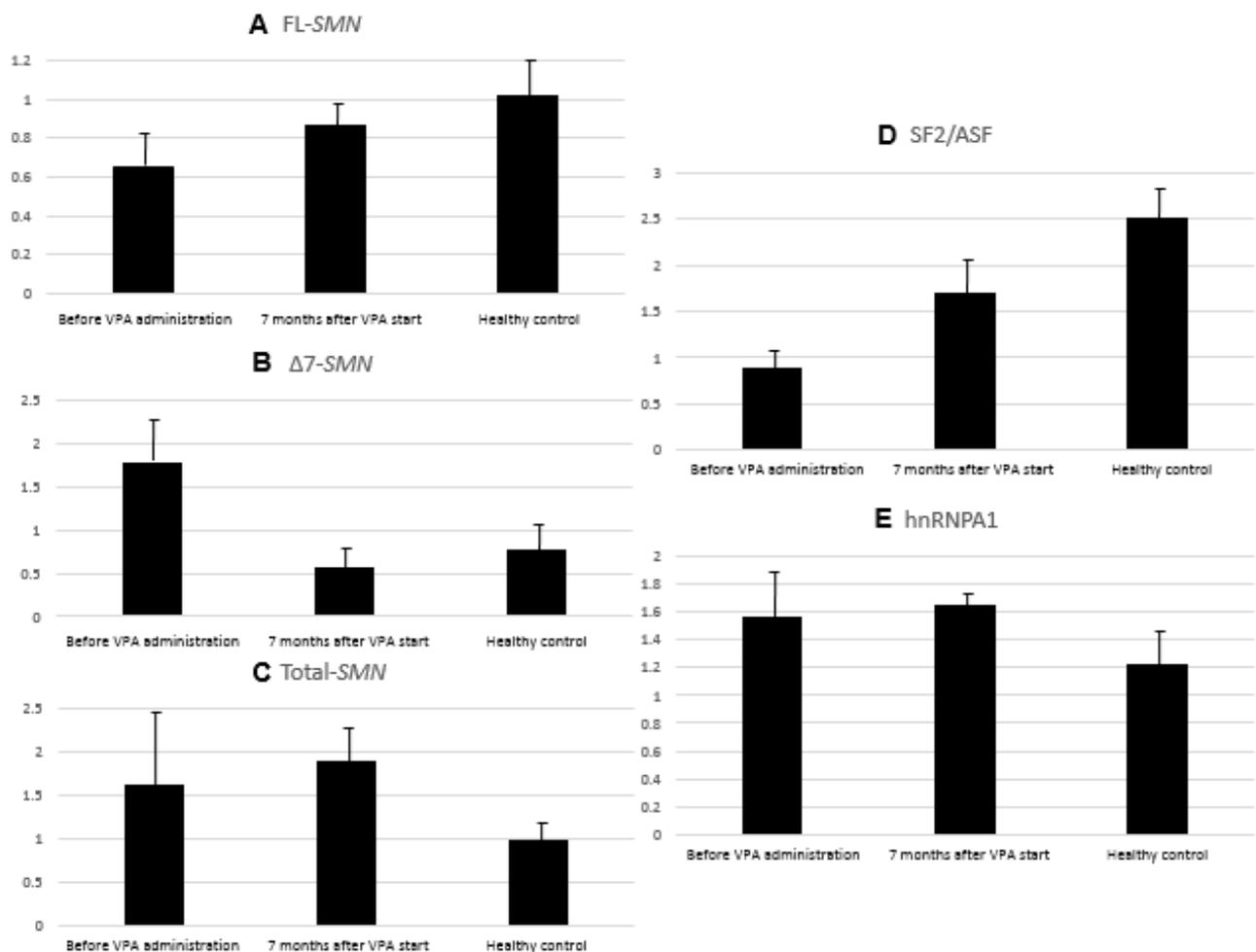

Figure 4: A: FL-SMN, B: Δ7-SMN, C: total-SMN, D: SF2/ASF, E: hnRNPA1. The transcript amounts of FL-SMN, total-SMN, SF2/ASF, and hnRNPA1 showed an increasing tendency, while that of Δ7-SMN was decreased. survival motor neuron (SMN), splicing factor 2/alternative splicing factor (SF2/ASF), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1).

and respiratory functions. On the other hand, the transcription amounts of FL-*SMN*, SF2/ASF, and hnRNPA1 showed an increasing tendency, which was considered to be an effect of VPA, even at a low concentration.

There are some limitations in regard to this study. First, the VPA concentration in our patient was in a low range, 50 to 100 μg/ml, which is considered to be moderate for epilepsy treatment. Although VPA concentration does not necessarily correlate with motor function improvement of MHFMS [12], that in our patient might have been too low to have effects on motor and respiratory functions. In addition, we cannot exclude the possibility that changes in *SMN*, SF2/ASF, and hnRNPA1 transcript amounts in peripheral blood leukocytes may not necessarily correlate with changes in motor neuron functions. VPA, which is an HDAC inhibitor, might cause changes in the *SMN*, SF2/ASF, and hnRNPA1 transcripts in motor neurons in the present case. Even the low concentration of VPA in the present case had effects on the central nervous system, shown by the resolution of electroencephalogram findings.

Finally, subtle changes in clinical signs and symptoms, such as MHFMS and respiratory function, may not have been detected by the evaluation methods employed. In our previous study of VPA therapy for SMA, we found motor function improvement in a child and improvements of respiratory functions in elderly patients who received the drug [12]. However, the efficacy of VPA therapy for SMA patients is controversial, as not all show a response. Appropriate methods to evaluate its efficacy in those patients more precisely are needed. Previous reports have described electroencephalogram abnormalities in SMA patients. The form of SMA has been reported to be related to the ASAH1 gene and the disease is known to be accompanied by progressive myoclonic epilepsy (SMA associated with progressive myoclonic epilepsy; SMA-PME) [15]. Although our patient showed electroencephalogram abnormalities, no epileptic attack was observed. Thus, we did not evaluate the ASAH1 gene in the present case, as the details did not match ASAH1-related SMA.

Conclusion

In conclusion, our findings indicate a discrepancy between response of *SMN* transcripts to VPA, and improvements in signs and symptoms in some SMA patients.

Acknowledgments

The present findings were reported at the 49th Kinki District Meeting of the Japanese Society of Child Neurology (Nara, Japan).

Authors' Contributions

All of the authors significantly contributed to production of this report. TS was the principal investigator, ISKH evaluated and analyzed the *SMN* transcript amounts, TS wrote the first draft of the paper, and HF, KS, and HN reviewed the drafts of the paper. All authors interpreted data, contributed to subsequent versions of the text, and approved the final version of the manuscript.

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