Case F: Condition immutability.
Case G: Condition immutability and sleepiness.

Many of the patients reported no subjective symptoms. Three complained of sleepiness including a dropout case (case A), while a precordial sense of incongruity was noted by three patients, including case A. There was also a complaint of belching, which was thought to be an effect of carnitine administration. There was no liver function abnormality reported and carnitine fractionation was normal. Table 2 shows changes in bodyweight for each patient. In five of the six patients, bodyweight increased by 1–3 kg from pretreatment, whereas that decreased by 1 kg in case G.

Discussion

After a 6-month administration of VPA, many items related to respiratory function were improved in the participants of the present study. Our items used to evaluate respiratory function, such as MIC and CPF, were not utilized in previous reports. Increasing MIC and CPF is important for patients with neuromuscular diseases to maintain good respiratory condition.[23] Our results showed that those improved values indicate the effectiveness of VPA administration to maintain a good respiratory condition even in adult SMA patients who show progression. Although Swobota reported improvements in maximum inspiratory pressures, forced vital capacity and forced expiratory volume in 1 s in patients aged over 5 years in an open label study,[15] there were no changes in any results of pulmonary function testing carried out in a double-blind trial thereafter.[17] Furthermore, no previous studies have reported that respiratory function was clearly improved with VPA administration, except for one that speculated that improved respiratory function might have been a result of growth and development.[18]

As a next step, our evaluation items of respiratory function, such as MIC and CPF, which have not been used in previous studies, should be evaluated as part of a placebo-controlled randomized trial to confirm the effects of VPA on respiratory condition in SMA patients.

In contrast, there was no relationship between VPA blood level and change in FL-*SMN* transcription products. Also, the level of VPA in blood in cases with improved MHFMS was less than an optimal level. Thus, improvement at the study end-point was not necessarily associated with VPA blood level or FL-*SMN* transcription product.

Past reports of VPA administration in SMA patients are summarized in Table 4, with most of those cases being SMA type 2 and 3.[13–20] The VPA dose in each of those reports is assumed to have been in accordance with the dose or blood level when used as an anticonvulsant. In four reports, carnitine administration was combined.[15,17–19] Two of those were designed as a double-blind study, whereas the others were open label.[17,20] In six reports, either *SMN* transcription level or SMN protein level was evaluated.[14–18,20] As an evaluation of motor function, the original Hammersmith Functional Motor Scale (HFMS) or MHFMS was used in four reports,[15,17–19] whereas a muscle strength test was also used in five reports.[13,17–20] One study found increased levels of *SMN* mRNA in association with VPA administration,[14] and another noted increased levels of SMN protein with VPA administration.[16] However, in three reports, there was no evident change in *SMN* transcription level with VPA administration[15,17,18] In the report by Weihl, motor function efficacy was noted in SMA type 3 and 4 patients,[13] whereas Darbar reported that HFMS improvement in SMA type 3 was not observed.[19] Kissel reported no statistically significant differences regarding changes in maximum voluntary isometric contraction in ambulatory SMA adults.[20] Also, Swoboda reported that children aged under 5 years,[15] furthermore, those aged 2–3 years with SMA type 2 showed MHFMS improvement with VPA administration.[17]

So the effects of VPA on SMA patients are controversial. Based on the present results, we expect that respiratory function in adult patients with progression, as well as motor function in younger children, has a possibility to improve after VPA administration. However, our open study was limited by the number of cases analyzed, and establishment of a control was difficult. We cannot conclude that the change in end-point after VPA administration is exclusively related to VPA administration. In particular, in the MHFMS of case E, growth development could have influenced our evaluation of clinical manifestations. The effects of growth development on motor functional evaluation should be evaluated in a placebo-controlled randomized trial.

As for the effects of VPA on SMN, promotion of *SMN2* gene transcription by activation of the *SMN2* gene promoter (production increase of full length type *SMN2* mRNA and Δ7-*SMN2* mRNA), splice progress of the *SMN2* gene exon 7 by gene activation to encode a splicing related protein and a combination of these two mechanisms have been considered.[11,12] An increase in FL- and Δ7-*SMN* transcription product quantity is expected to occur with VPA administration. Furthermore, VPA is a multifunctional drug that is expected to have a neuroprotective effect.[25] Therefore, it is also speculated that VPA blood level, FL-*SMN* transcription product quantity and improvement in outcome are not necessarily linked.

Regarding the change in quantity of the *SMN* transcription product after VPA administration, we considered the effects of fluctuations in the system of measurement. Whether an increase in SMN protein in peripheral blood leukocytes reflects an increase in that in ventral horn cells remains unknown. It is also not clear if an increase in SMN protein in the ventral horn cells is directly associated with clinical manifestation improvement. In addition, if an imperceptible change in motor function occurs, it might not be possible to detect the difference using the method of evaluation utilized in the present study. Thus, subtle changes in clinical signs and symptoms might not be detected by the present evaluation method.

It was also difficult to evaluate the effects of physiotherapy on motor and respiratory functions in a comprehensive manner because of variations in each patient. In the present cases, uniform physiotherapy was not possible because of functional differences among our patients, the therapeutic

Table 4 Summary of reports related to administration of valproic acid in patients with spinal muscular atrophy

Author	Clinical trial phase	VPA	Carnitine	SMA type	*n*	Age	Duration	Evaluation				Results	Conclusion	Year	Ref
								Motor function	PFT	SMN	Others				
Weihl *et al.*	Open	Administration 500–1000 mg/day Mean serum level 87 μg/mL	–	3,4	7	17–45 years (mean 17 years)	1–15 months (mean 8 months)	Muscle strength	–	–	–	Improvement of motor strength and subjective benefit	VPA treatment is efficacious in adult SMA type 3/4	2006	13
Brichta *et al.*	Open	Administration 1200–1800 mg/day Serum level 70–100 mg/L	–	Carrier	10	50.0 ± 10.9 years	>5 weeks	–	–	SMN protein analysis SMN2 messenger RNA (blood)	–	Increased SMN messenger RNA and protein levels in seven carriers	Long-term clinical trials in SMA patients that correlate SMN expression in blood with individual motor function tests are required	2006	14
		Serum level 38–99 mg/L		1	5	1.6 ± 0.9 years	>4 weeks			SMN2 messenger RNA (blood)		Elevated SMN2 messenger RNA levels in seven patients			
		Serum level 47.9–98.3 mg/L		2	11	10.3 ± 7.1 years						Unchanged or decreased in 13 patients			
		Serum level 58.5–99.0 mg/L		3	4	20.8 ± 6.9 years									
Swoboda *et al.*	Open	Serum level 50–100 mg/dL	Administration 50 mg/kg/day	1	2	2–3 years	6 months	MHFMS	FVC, FEV1, MEP, MIP (over 5 years)	Quantitative assessment of SMN mRNA	CMAP, MUNE, DEXA	Increased mean score on the MHFMS scale in SMA 2	The study provides good evidence that VPA can be used safely in SMA subjects over 2 years of age in the setting of close monitoring of carnitine status	2009	15
				2	29	2–14 years						However, significant improvement restricted to SMA 2 participants under 5 years of age			
				3	11	2–31 years						Some items improved in PFT			
												Unchanged Full length SMN levels			
												Significantly reduced Δ7-SMN levels			
Piepers *et al.*	Open	Serum level 70–100 mg/mL	–	2,3	6	1.6–16.5 years	4 months	–	–	SMN protein concentration of l ymphocyte	–	Significantly increased SMN protein levels: five of six	SMN protein quantification by ELISA is a useful tool for evaluating the effects of treatment in SMA	2010	16

Table 4. Continued

Author	Clinical trial phase	VPA	Carnitine	SMA type	*n*	Age	Duration	Evaluation: Motor function	Evaluation: PFT	Evaluation: SMN	Evaluation: Others	Results	Conclusion	Year	Ref
Swoboda *et al.*	Double blind	Serum level 50–100 mg/dL	Administration 50 mg/kg/day (maximum of 1000 mg)	2,3	30	1.8–8.7 years (mean 4.3 years)	6+6 months	MHFMS, Myometry measurement	FVC, FEV1, MEP, MIP (over 5 years)	Quantitative assessment of SMN mRNA	CMAP, DEXA, PedsQL	Children ages 2–3 years that received 12 months treatment had significantly improved MHFMS scores No change of QOL, CMAP, myometry measurements, and SMN Treatment not associated with changes in the PFT outcomes (over 5 years) Excessive weight gain was the most frequent drug-related adverse event	No benefit of treatment with VPA and L-carnitine in young non-ambulatory SMA	2010	17
		Placebo	Placebo	2,3	31	2.1–7.9 years (mean 4.4 years)	Placebo 6 months + VPA 6 months								
Kissel *et al.*	Open	Serum level 50–100 mg/dL	Administration 50 mg/kg/day (maximum of 1000 mg)	2,3 standers and walkers	33	2.8–16.3 years (median 6.9 years)	12 months	MHFMS-Extend, TTF, FMM, Myometry measurement (over 5 years)	FVC, FEV1, MEP, MIP (over 5 years)	Quantitative assessment of SMN mRNA	CMAP, DEXA, PedsQL	Weight gain of 20% above body weight occurred in 17%. No significant change of MHFMS-Extend, TTF, FMM, PedsQL and SMN transcript level FVC, FEV1 showed improvement at one year as expected with normal growth	VPA is not effective in improving strength or function in SMA children	2011	18
Darbar *et al.*	Open	Administration 20 mg/kg/day	Administration 100 mg/kg/day	2,3	22	2–18 years (mean 5.5 years)	1 year	MRC method, HFMS	–	–	Barthel Index	Gained no muscle strength SMA 2 presented significant gain in HFMS, but not type 3 Improvement of Barthel Index	VPA may be a potential alternative to ameliorate the progression of SMA	2011	19
Kissel *et al.*	Double blind, cross over	Administration 10–20 mg/kg/day Trough levels of 50–100 mg/dL	–	Ambulant adults with SMA	33	19.9–55.3 years (mean 37.2 years)	Placebo 6 months + VPA 6 months (cross over)	MVICT, SMAFRS, hand-held dynamometer, distance in 6-min walk, time to climb 4 standard stairs	FVC, FEV1, MIP	SMN2 copy number, mRNA levels, and SMN protein levels	CMAP, MUNE, DEXA, QOL	There was no change in outcomes at 6 or 12 months	VPA did not improve strength or function in SMA adults	2014	20

CMAP, maximum ulnar compound muscle action potential; DEXA, dual-energy X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; FEV1, forced expiratory volume in 1 s; FMM, fine motor modules; FVC, forced vital capacity; HFMS, Hammersmith Functional Motor Scale; MEP, maximum expiratory pressure; MIP, maximum inspiratory pressure; MHFMS, Modified Hammersmith Functional Motor Scale for SMA; MHFMS-Extend, Modified Hammersmith Functional Motor Scale-Extend; MRC method, Medical Research Council method; MUNE, motor unit number estimation; MVICT, maximum voluntary isometric contraction testing; PedsQL, Pediatric Quality of Life Inventory; PFT, pulmonary function testing; QOL, quality of life; SMAFRS, modified SMA Functional Rating Scale; TTF, timed tests of function.

environment (hospitalization, outpatient status and so on) and ethical reasons. Thus, variations in physiotherapy should be minimized to better evaluate the effectiveness of VPA in future studies.

We found no serious side-effects caused by VPA administration. However, many of our patients gained bodyweight as compared with pretreatment, which was induced by VPA. Excessive weight gain has negative effects on motor and respiratory conditions in such patients, thus careful administration of VPA is required. As for carnitine administration, we should recognize side-effects including a precordial sense of incongruity and a complaint of belching, which have not been reported in previous reports.

VPA is expected to show good effects as a therapeutic drug for SMA in younger patients for motor functional improvement and even in adult patients for respiratory improvement. To fully elucidate its effectiveness and efficacy in SMA patients, development of an evaluation method to better determine minimal changes in clinical manifestations including respiratory function items, such as MIC and CPF, which were not used in previous reports, as well as introduction of a new biomarker that can be easily evaluated and is able to differentiate responders to VPA treatment from non-responders are required. Although improvements in MHFMS in the young girl and respiratory function improvements in the older subjects were observed in the present study, our results are difficult to interpret because of the open label nature and small scale. The effects of growth development on motor functional evaluation in child cases, respiratory function using MIC and CPF, and efficacy of VPA for SMA should be evaluated using a placebo-controlled randomized trial protocol.

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