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Original Article

A study of oxidative stress and the newer antiepileptic drugs in epilepsy associated with severe motor and intellectual disabilities

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Abstract

Background: Patients with severe motor and intellectual disabilities (SMID) are those who have both severe intellectual disabilities and severe physical disabilities. Intractable epilepsy is often associated with SMID. The purpose of this study was to elucidate the relationship between epilepsy associated with SMID and oxidative stress, and to clarify the safety and efficacy of the newer antiepileptic drugs (newer AEDs), lamotrigine and levetiracetam.
Methods: This study was conducted in 27 SMID patients with epilepsy who were treated with the newer AEDs. The patient characteristics and the safety and efficacy of the newer AEDs were investigated. The reactive oxygen metabolite (d-ROM) and biological antioxidant potential (BAP) levels were measured as indicators of the degree of oxidative stress. The relationship between the investigation results (the patient characteristics, and the safety and efficacy of the newer AEDs) and the results of measurements of the d-ROMs/BAP were analyzed.
Results: All the patients who discontinued the newer AEDs had abnormal plasma d-ROM levels. In addition, all the patients who developed adverse events also had abnormal d-ROM levels. Furthermore, there was a trend toward a lower response rate in patients with higher plasma d-ROM levels.
Conclusion: The results of this study suggested that d-ROM levels are useful for predicting the safety and efficacy of the newer AEDs (lamotrigine, levetiracetam) in SMID patients with intractable epilepsy. Therefore, d-ROMs could be important biomarkers for determining the safety and efficacy of drug therapy in SMID patients with epilepsy.

Keywords: antiepileptic drugs; children with disabilities; epilepsy; oxidative stress

1. Introduction

The disease entity of severe motor and intellectual disabilities (SMID) is characterized by the presence of both severe intellectual disabilities and severe physical disabilities, and the concept of SMID is similar to the globally recognized concepts of "profound intellectual and multiple disabilities" (PIMD)[1] and "profound and multiple learning disabilities" (PMLD).[2]

There are an estimated 40,000−50,000 SMID patients in Japan.[3] SMID is a condition in which the central nervous system is damaged during the developmental stages in the perinatal period and infancy, and the primary disease or symptoms do not bate with age. In a previous study, we demonstrated a statistically significant association between SMID and epilepsy, which suggests that epilepsy is one of the characteristic manifestations of SMID.[4] Although the older antiepileptic drugs (older AEDs) have long been used in drug therapy for epilepsy in Japan, newer antiepileptic drugs (newer

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AEDs) have received approval and been introduced in the market one after another since 2006. However, there have been few reports exclusively addressing the efficacy of these drugs against epilepsy associated with SMID. Furthermore, although Tanuma et al[5] conducted an investigation on the relationship of SMID with respiratory disturbance and oxidative stress based on measurement of plasma 8-hydroxy-2′-deoxy-guanosine (8-OHdG), there are no reports of studies on the relationship between epilepsy associated with SMID and oxidative stress. Therefore, we conducted this study to clarify the usefulness and safety of the newer AEDs for epilepsy in patients with SMID using oxidative stress markers, namely, the oxidative stress [plasma levels of reactive oxygen metabolites (d-ROMs)] and antioxidant activity [biological antioxidant potential (BAP)] levels.

Table 1
Background of patients ($n = 27$).

Sex	Male	20 (70.8)
	Female	7 (29.2)
Age (y)	Average ± SD	27.0 ± 7.3
	Maximum	39
	Minimum	10
	Median	27.0
Main disease	Cerebral palsy	12 (44.4)
	Hypoxia encephalopathic aftereffects	3 (11.1)
	Epileptic encephalopathy aftereffects	3 (11.1)
	Cerebral hemorrhage aftereffects	2 (7.4)
	Lissencephaly	2 (7.4)
	CFC syndrome	1 (3.7)
	Acute encephalopathic aftereffects	1 (3.7)
	Theophylline encephalopathic aftereffects	1 (3.7)
	Meningitis aftereffects	1 (3.7)
	Dentatorubral-pallidoluysian atrophy	1 (3.7)
Renal damage	Available	0
	None	27
Liver damage	Available	0
	None	27
Comorbidities[a]	Available	0
	None	27
The number of use AEDs	1 agent	0 (0.0)
	2 agents	1 (3.7)
	3 agents	12 (44.4)
	4 agents	12 (44.4)
	5 agents ≤	2 (7.4)
Use of old AEDs[b]	Valproate sodium (VPA)	21 (32.3)
	Phenobarbital (PB)	8 (12.3)
	Zonisamide (ZNS)	8 (12.3)
	Phenytoin (PHT)	6 (9.2)
	Clonazepam (CZP)	6 (9.2)
	Carbamazepine (CBZ)	5 (7.7)
	Clobazam (CLB)	4 (6.2)
	Ethosuximide (ESM)	3 (4.6)
	Nitrazepam (NZP)	2 (3.1)
	Clorazepete dipotassium	1 (1.5)
	Acetazolamide	1 (1.5)

Data are presented as n or n (%).
AEDs = antiepileptic drugs; CFC = cardio-facio-cutaneous; SD = standard deviation.
[a] Hypertension, diabetes, hyperlipidemia, kidney disease, infectious disease.
[b] There is overlap in a value ($n = 65$).

2. Methods

2.1. Patients

Among the SMID patients who were hospitalized and treated at the Japanese Red Cross Tokushima Hinomine Rehabilitation Center for People with Disabilities, Tokushima, Japan between January 1, 2009 and December 31, 2015, 27 who manifested epilepsy and had been initiated on treatment with the newer AEDs were included in this study. After obtaining consent from the parents for this clinical study, random samples were collected from these patients.

2.2. Drugs evaluated

Two drugs, lamotrigine (LTG) and levetiracetam (LEV),[6,7] which are AEDs that received approval for use in Japan in and after 2006 and are highly recommended worldwide, were evaluated as the newer AEDs. AEDs that had received approval for clinical use prior to 2006 were defined as the older AEDs.

2.3. Items investigated

Medical records were investigated to collect the following characteristics of the patients: sex, age, primary disease, having organ disorder, and the number and types of used AEDs.

Table 2
Continuation rate and safety of LTG and LEV.

Drug continuation rate	LTG	LEV
No. of cases	11	16
Withdrawal No. of cases	2 (18.2)	3 (18.8)
(Withdrawal reason)		
Safety (adverse event)		
Drug rash	1 (9.1)	0
Mood change/edema	0	1 (6.3)
Exacerbation of the seizure	0	1 (6.3)
Efficacy		
Seizure No. of times immutability	1 (9.1)	1 (6.3)
Adverse event[a]	**LTG**	**LEV**
No. of cases of the adverse event expression	2 (18.2)	4 (25.0)
No. of cases of the adverse event expression	2 (18.2)	5 (31.2)
(total) (Contents of adverse events)		
Psychiatric disorders		
Mood change	0	1 (6.3)
Nervous system disorders		
Exacerbation of the seizure	0	1 (6.3)
Somnolence	1 (9.1)	2 (12.5)
Skin & subcutaneous tissue disorders		
Drug rash	1 (9.1)	2 (12.5)
General disorders and administration site conditions		
Edema	0	1 (6.3)

Data are presented as n or n (%).
LEV = levetiracetam; LTG = lamotrigine.
[a] Withdrawal number of cases by adverse event: LTG 1 (9.1%), LEV 2 (12.5%).

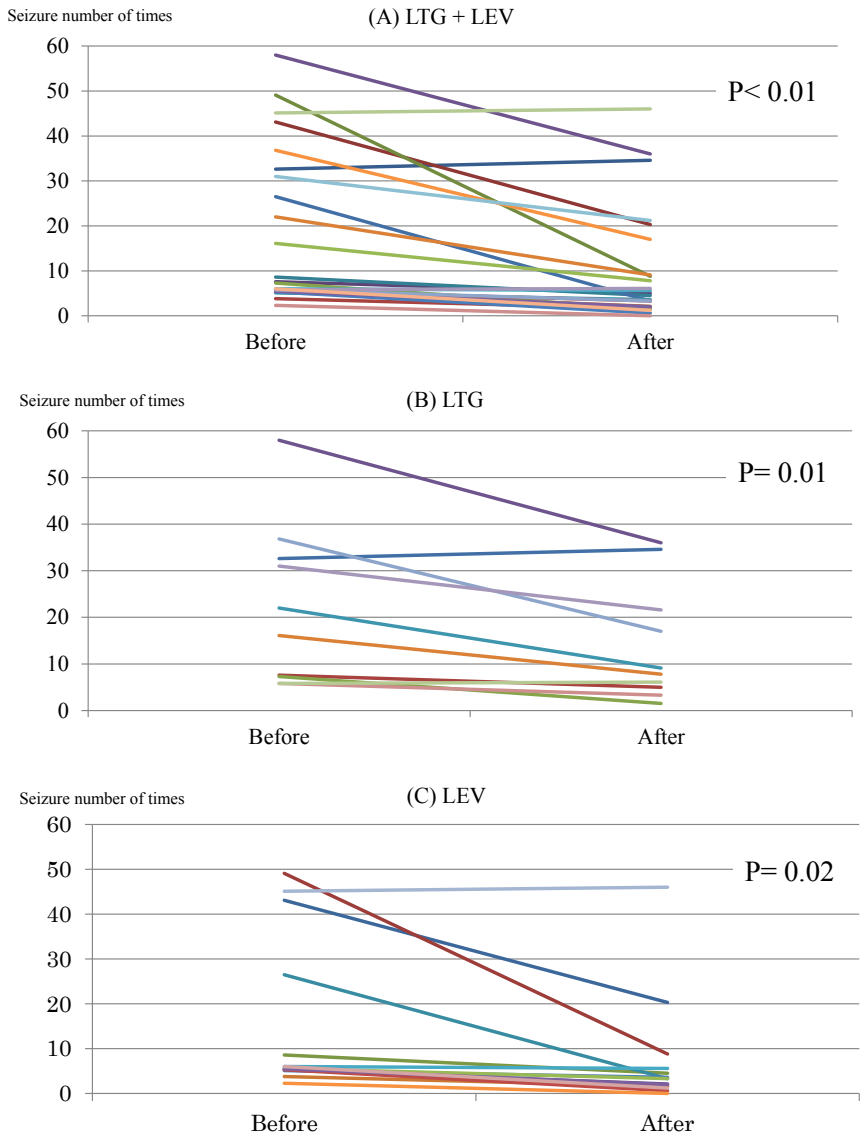

Fig. 1. Change in the 6-month epilepsy seizure number of times prior to and after new AED addition. (A) LTG + LEV. (B) LTG. (C) LEV. AED = antiepileptic drug; LEV = levetiracetam; LTG = lamotrigine.

For the safety assessment, the drug continuation rate, reasons for drug discontinuation and incidence and details of adverse events were investigated. Changes in the number of epileptic seizures and overall improvement from 6 months before to 6 months after addition of the newer AEDs were investigated as the primary efficacy endpoints. The overall improvement was assessed as follows: (1) marked improvement: response rate $\geq$ 75%; (2) improvement: response rate $<$ 75% but $\geq$ 25%; (3) no change: response rate $<$ 25%; (4) aggravation: increase in the number of epileptic seizures after addition of the newer AEDs.

2.4. Items measured

Blood samples were collected from the patients prior to breakfast and prior to administration of the drug (at 6 AM), but not during or immediately after epileptic seizures or during any infection. In those cases, blood was drawn the next time as noted above. Additionally, blood was drawn approximately 1 week prior to and approximately 6 months after the addition of newer AEDs. The blood samples were centrifuged at 1500g for 10 minutes using the Table-top centrifuge model 2410 (Kubota Corporation, Tokyo, Japan) to separate the plasma. The heparinized plasma samples were measured for the following items using a free-radical analyzer (FREE CARRIO DUO; Diacron International, Grosseto, Italy).

2.4.1. Measurement of the oxidative stress level (d-ROMs test)

Twenty microliters of plasma was added to pH 4.8 buffer in a cuvette and mixed by inversion (Fe^{2+} and Fe^{3+} were separated from the blood proteins) to allow the Fe^{2+} and Fe^{3+} radicals to catalyze the degradation of hydroperoxides in the blood into alkoxyl and peroxy radicals. Next, 20 μL of a

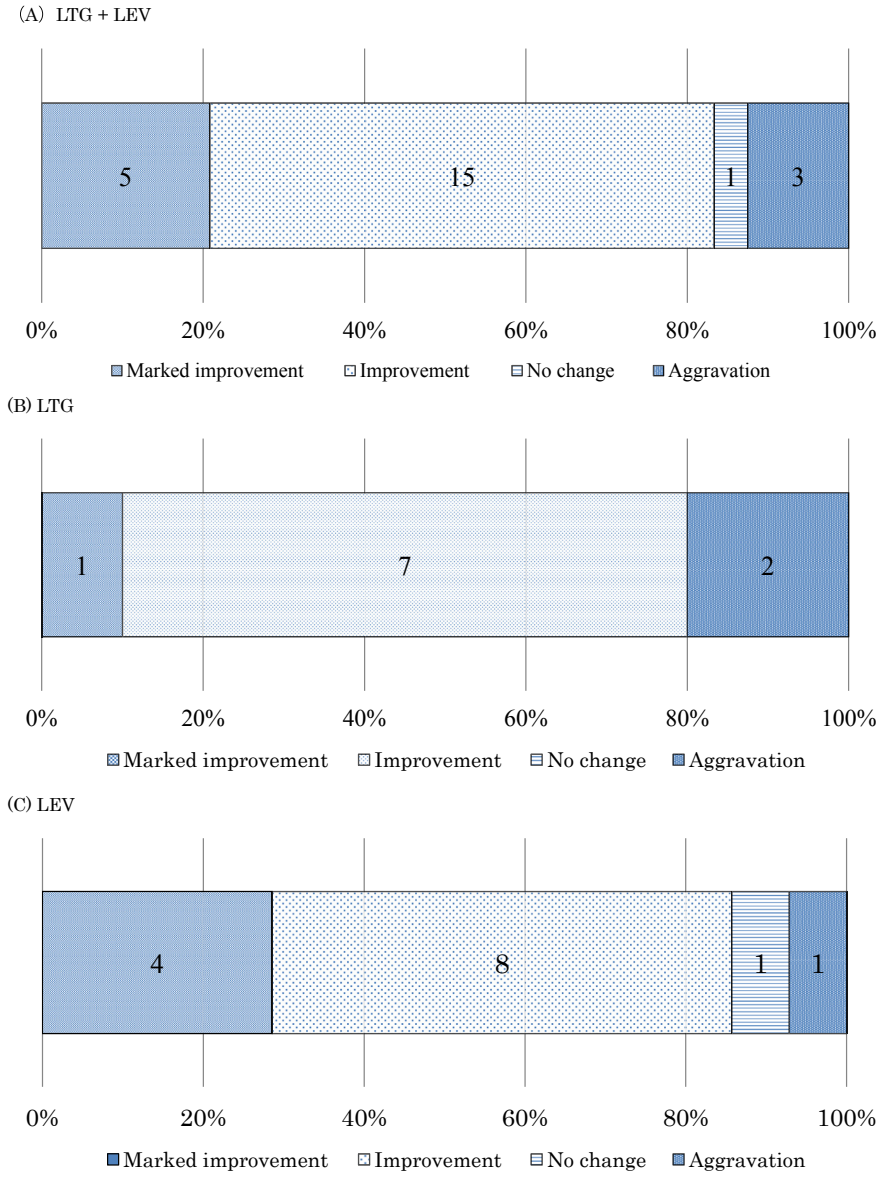

Fig. 2. The overall improvement degree. (A) LTG + LEV. (B) LTG. (C) LEV. LEV = levetiracetam; LTG = lamotrigine.

coloring chromogen (*N*,*N*-diethyl-paraphenylenediamine) was added to the solution, so that the free radicals oxidized the chromogen substrate to yield red-colored radical cations. Then, the solution was mixed by inverting, and the cuvette was placed in a photometer for optical measurement at 505 nm.

2.4.2. Measurement of the antioxidant activity (BAP test)

Fifty microliters of a chromogen for BAP (Fe^{3+}-containing reagent) was added to the cuvette to make the solution red. After mixing the solution by inversion, the color density was measured by photometry. Next, 10 μL of plasma was added to the cuvette and mixed. Thereafter, the cuvette was placed in a thermostat, and the reaction was allowed to proceed for 5 minutes. Finally, the cuvette was placed in a photometer for optical measurement at 505 nm.

In a physically unimpaired person, normal d-ROMs values are < 320 U.CARR, and normal BAP values are > 2000 μmol/L. Accordingly, we adopted these standard values, and those values outside the normal range were defined as abnormal.

2.5. Statistical analysis

The relationship between the data obtained on the safety and efficacy of the newer AEDs and the measured d-ROM and BAP values was analyzed. The statistical analysis software IBM SPSS Statistics, version 21, was used. The two-sided level of significance was set at 5%, and $p < 0.05$ was considered as indicative of a significant difference between the two.

2.6. Ethical considerations

This study was conducted with the approval of the Ethics Committee of the Japanese Red Cross Tokushima Hinomine Rehabilitation Center for People with Disabilities. As it is

difficult for SMID patients themselves to express their will, a written explanation was given to the families of the SMID patients and their consent was obtained.

3. Results

3.1. Patient characteristics

In regards to patient sex, males were proportionally higher, and the mean age of the patients was 27.0 ± 7.3 years. Regarding the main disease entity, cerebral palsy was the most common. The patient characteristics are shown in Table 1.

Overall, there were no patients with liver or renal damage, and in all SMIDs, there was C-reactive protein (CRP) in 0.03–0.33 μg/dL. Concerning the number of AEDs administered per patient, ≥ 80% of the patients were treated with three or four AEDs, and valproate sodium was the most frequent concomitantly used older AED.

3.2. Drug continuation rate and adverse events

The drug continuation rate is shown in Table 2. The adverse events were mild and improved with discontinuation of the drug. All adverse events were mild and improved spontaneously without treatment.

3.3. Changes in the number of epileptic seizures and overall improvement from 6 months before to 6 months after addition of the newer AEDs

Three patients could not take it continuously and were excluded. The results are shown in Figure 1.

The number of epileptic seizures significantly decreased after the addition of the newer AEDs ($p < 0.01$). Analysis by drug showed that both LTG ($p = 0.01$) and LEV ($p < 0.01$) significantly reduced the number of seizures.

The mean new AED response rate to the newer AEDs was 49.0%, and analysis by drug showed that the mean response rates to LTG and LEV were 37.9% and 56.9%, respectively.

The overall improvement is shown in Figure 2.

The criteria for assessment of the overall improvement were defined as follows: (1) marked improvement: response rate ≥ 75%; (2) improvement: response rate < 75% but ≥ 25%; (3) no change: response rate < 25%; (4) aggravation: increased in the number of seizures after addition of the newer AEDs.

When "marked improvement" and "improvement" were defined as "effective," the newer AEDs were effective in 83.3% of the patients. Analysis by drug also showed that LTG and LEV were effective in 80.0% and 85.7% of the patients, respectively.

3.4. d-ROMs and BAP values

The criteria for evaluation of d-ROMs and BAP and the measurement results prior to and after the newer AEDs addition are shown in Table 3 and Figure 3. d-ROMs values were as follows: normal values were "before" 4 (14.8%) "after" 6 (22.2%), and abnormal values were "before" 23 (85.2%) "after" 21 (77.8%).

Table 3
Measurement evaluation standard value of d-ROMs test and BAP test and the result before and after LTG, LEV addition. (A) d-ROMs test, (B) BAP test.[a]

Evaluation	Standard value[b]	Results	
		Before	After
d-ROMs test			
Normal range	250–300	2 (7.4)	5 (18.5)
Border range	301–320	2 (7.4)	1 (3.7)
Low-level oxidative stress	321–340	3 (11.1)	5 (18.5)
Intermediate level oxidative stress	341–400	4 (14.8)	5 (18.5)
High-level oxidative stress	401–500	11 (40.7)	5 (18.5)
Very high-level oxidative stress	>500	5 (18.5)	6 (22.2)
BAP test			
Normal range	>2200	19 (70.4)	18 (66.7)
Border range	2000–2200	7 (25.9)	8 (29.6)
Slight lack state	1800–1999	0	0
Lack state	1600–1799	0	0
Severe lack state	1400–1599	1 (3.7)	1 (3.7)
Very severe lack state	<1400	0	0

Data are presented as *n* (%).
BAP = biological antioxidant potential; d-ROM = reactive oxygen metabolites; LEV = levetiracetam; LTG = lamotrigine.
[a] Comparing before and after LTG, LEV addition, the d-ROM values decreased in predominance statistically after addition (paired *t* test, $p > 0.01$), whereas BAP values did not a change (paired *t* test, $p = 0.97$)
[b] Unit: d-ROMs, U.CARR; BAP, μmol/L.

BAP values: anteroposterior no change, normal values were 26 (96.3%), abnormal value alone was 1 (3.7%). When distributed an oxidation stress degree were evaluated, d-ROM values decreased only in the high-level oxidative stress group.

3.5. Relationship between the continuation rate and plasma d-ROM levels

Comparative analysis between patients with normal and abnormal plasma d-ROM levels revealed that although there were no significant differences between the two groups ($p = 0.55$), all patients with adverse events had abnormal plasma d-ROM levels. In particular, in both individuals who discontinued the newer AEDs not because of adverse events but because of a lack of efficacy, the oxidative stress levels were very high. The results of the analysis are shown in Figure 4A.

3.6. Relationship between adverse events and the plasma levels of d-ROMs

Comparative analysis between patients with normal and abnormal plasma d-ROM levels revealed that although there were no significant differences between the two groups ($p = 0.28$), all patients with adverse events had abnormal plasma d-ROM levels. In particular, one participant who discontinued the newer AEDs owing to the development of seizure aggravation as an adverse event showed a very high level of oxidative stress. The results of the analysis are shown in Figure 4B.

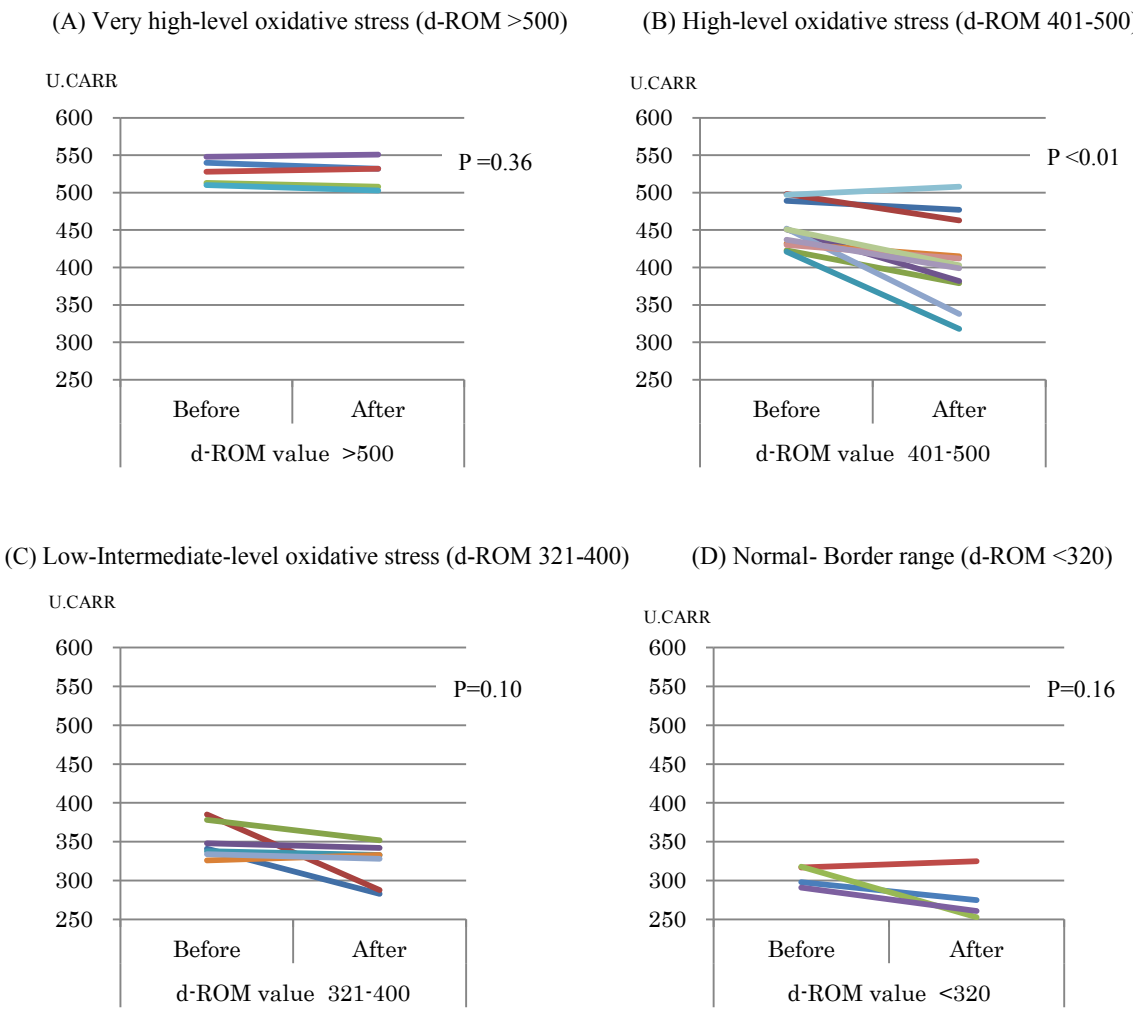

Fig. 3. Change of the d-ROM level prior to and after the new AED addition (paired *t* test). (A−D) Comparison was done according to an oxidation stress category. In (B) the high-level oxidative stress group (d-ROM 401−500), a d-ROM level decreased in predominance. In the other patient groups, there were few changes. AED = antiepileptic drug; d-ROM = reactive oxygen metabolite.

3.7. Relationship between the efficacy (response rate) and plasma d-ROM levels

The results of the analysis are shown in Figure 5.

The findings revealed a trend toward a lower response rate in patients with higher plasma d-ROM levels. All three patients who were assessed as having seizure aggravation/no change in the seizure activity, and discontinued the newer AEDs that showed very high levels of oxidative stress.

4. Discussion

Because epilepsy associated with SMID is intractable, multiple AEDs tend to be used simultaneously. Although concomitant use of multiple AEDs certainly has an effect on the seizure activity, it is associated with several problems: (1) it cannot be identified as to which AED is effective; (2) the higher incidence of adverse events leads to a decrease in the quality of life. In particular, because SMID patients cannot express themselves, they cannot complain of the adverse events that they are experiencing. Therefore, AEDs with high safety and efficacy, and development of objective indicators to predict the clinical course after drug administration are required.

As compared to the older AEDs, the newer AEDs that were recently approved have different mechanisms of action [LTG: inhibition of N-type Ca^{2+} channels and inhibition of glutamate release; LEV: binding to synaptic vesicle protein 2A (SV2A) to modulate neurotransmitters] and have characteristics that are substantially different from those of the older AEDs, in that the frequency of adverse events and efficacy are independent of the blood drug concentrations.

Analysis of the 6-month newer AED continuation rate revealed that, as compared to general epilepsy patients reported in previous studies, the LTG continuation rate was higher (81.8% vs. 65−70%[8,9]) and the LEV continuation rate was somewhat lower (81.2% vs. 92.2%[10]) in our patients, suggesting that the newer AEDs can be continued for a longer period even in SMID patients with epilepsy.

Analysis of the safety of the newer AEDs revealed that as compared to patients with general epilepsy in drug use-results surveys, the incidence of adverse events associated with LTG

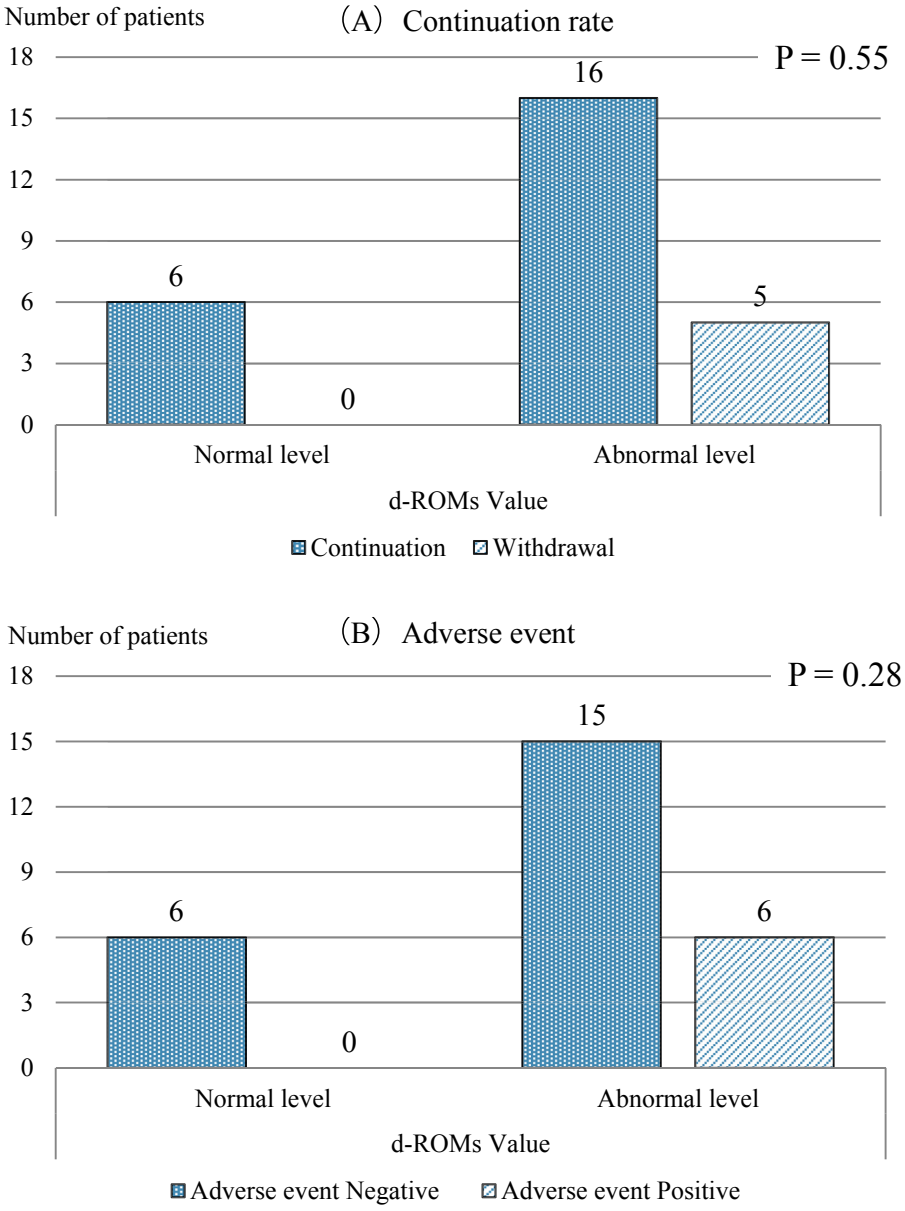

Fig. 4. Relations of (A) continuation rate, (B) adverse event, and the d-ROM values (Fisher's exact test). d-ROM = reactive oxygen metabolite.

was somewhat lower (18.2% vs. 21.9%[11]), and the incidence of adverse events associated with LEV was somewhat higher (25.0% vs. 13.7%[12]) in our patients. However, in the present study, there were no serious adverse events, and all the adverse events resolved spontaneously without treatment. Based on these findings, the newer AEDs, in general, can be said to be highly safe even in SMID patients with epilepsy. However, dizziness, for example, which occurs at a high incidence in patients taking these drugs, is detected based on the patient's own complaints; therefore, its incidence cannot be determined in SMID patients as these patients cannot express themselves or report the symptoms that they are experiencing. Thus, predictors of such adverse events are required in SMID patients receiving drug treatment.

Analysis of the efficacy of the newer AEDs for epilepsy associated with SMID revealed that the addition of the newer AEDs generally decreased the number of epileptic seizures. This is speculated to be attributable to the differing mechanisms of action of the newer AEDs as compared to the older AEDs.

In clinical practice, oxidative stress markers are used in various ways, and the frequently used marker 8-OHdG, which is measured in the urine, shows diurnal variations.[13] However, SMID patients cannot pass urine spontaneously, and it is difficult to collect urine samples from these patients. However, because the plasma d-ROM levels can be measured rapidly at a fixed time using micro blood samples (30 μL) collected from the fingertip in a minimally invasive manner, plasma d-ROM levels are considered a useful marker of oxidative stress in SMID patients.

Plasma levels of oxidative stress markers increase with age.[13,14] In addition, comorbidities (hypertension, diabetes,

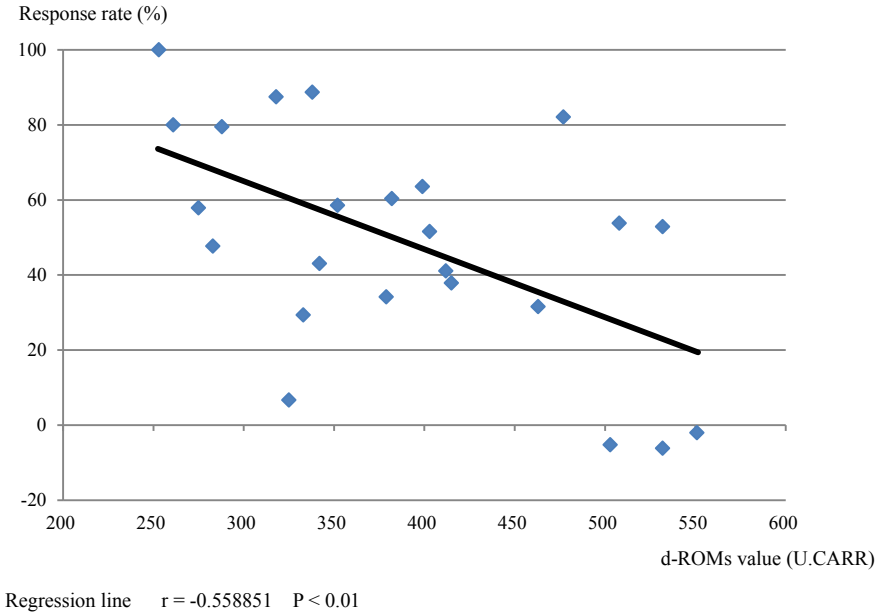

Fig. 5. Relation of the effectiveness (Response rate) and d-ROMs level. (A) Relation between d-ROM values and BAP values. (B) Distribution of d-ROMs/BAP. BAP = biological antioxidant potential; d-ROMs = reactive oxygen metabolites.

renal dysfunction, etc.) are also known to increase the plasma d-ROM levels.[15–17] Comparisons with healthy individuals matched for age[13,14] revealed that the plasma levels of d-ROMs were higher in our patients than in healthy individuals. Analysis of comorbidities also revealed that none of the participants had any factors affecting the plasma d-ROM levels in the present study, and that the high plasma d-ROM levels were likely to be caused by the epileptic seizures in the SMID patients.

In addition, in regard to the BAP values, carnitine replacement therapy is used for hypocarnitinemia associated with high-dose valproate sodium treatment or tube feeding in SMID patients with epilepsy.[18,19] Carnitine is known as an antioxidant, and carnitine administration has been reported to increase the expression of superoxide dismutase, catalase, glutathione peroxidase, etc.,[20] which may lead to maintenance of the antioxidant activity.

We compared d-ROMs and BAP values, prior to and after the newer AEDs addition.

The d-ROMs values decreased in predominance after addition, but the BAP values did not change. LTG and LEV do not have the chemical structure to provide radical removal such as edaravone. Therefore, it is believed that the d-ROM values might decrease because seizure decreased arising from the newer AEDs dosage.

In Figure 3, seizure number of times and d-ROM values did not change in the Very high-level oxidative stress group. However, in the High-level oxidative stress group, attack number of times and d-ROM values decreased. Thus, progress of newer AEDs may suppose the measurement of d-ROMs prior to the addition of newer AEDs.

The d-ROM values were different in each patient. However, the BAP values showed similar levels. In this study, it is thought that the internal antioxidant capacity of the SMID epilepsy patient remained. The d-ROMs/BAP ratio was not substantially changed and depended on the d-ROM values (Fig. 6). We paid attention to the d-ROM value. In the safety assessment of the newer AEDs through analysis of the drug continuation rate and incidence of adverse events, all patients who discontinued the newer AEDs or developed adverse events had abnormal plasma d-ROM levels. All adverse events were mild. Why do adverse events tend to occur in patients with high plasma d-ROM levels? There have been no studies using oxidative stress markers to reliably predict the adverse events of drugs, and the detailed underlying mechanisms are unknown. However, it has been reported that high preoperative plasma d-ROM levels increase the risk of complications after cardiac surgery,[21] and that mutations or increased expression of oxidative stress-related genes increase the risk of development of adverse effects of the drugs.[22] Furthermore, it was also reported that a glycation marker, pentosidine, which is an indicator of aging as well as of oxidation, is an effective predictor of cisplatin cytotoxicity and renal dysfunction.[23] Therefore, measurement of the plasma d-ROM levels prior to the start of treatment allows the probability of adverse events to be predicted, thereby increasing the possibility of early detection of adverse events. We think that the plasma d-ROM levels could be very useful as a predictive marker, especially in SMID patients who cannot express what they are feeling.

Various studies have reported that LTG and LEV exert neuroprotective and anti-inflammatory effects.[24–27] Therefore, it was investigated using electron spin resonance as to whether LTG and LEV possess free-radical scavenging activity, and the results revealed that neither drug had any free-radical scavenging activity, suggesting that LTG and LEV *per se* do not reduce oxidative stress *in vivo*.

The d-ROMs test measures the concentration of hydroperoxides generated by free radicals. Hydroperoxides are known

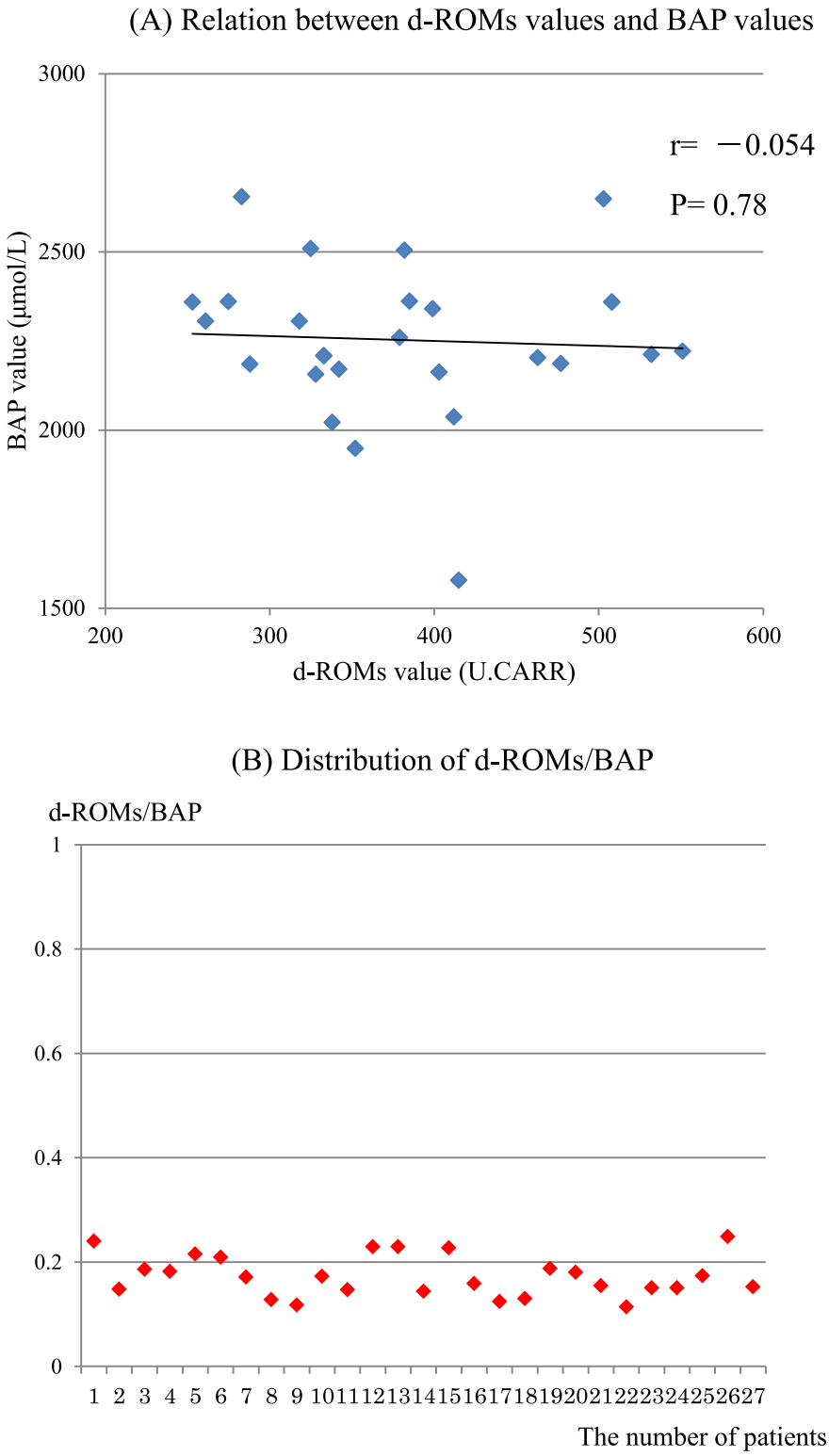

Fig. 6. (A) Relation between d-ROMs value and BAP value and (B) distribution of d-ROMs/BAP. BAP = biological antioxidant potential; d-ROM = reactive oxygen metabolite.

to generate free radicals under certain conditions, such as the presence of metal ions, and are therefore not only oxidative stress markers, but also, possibly, free-radical amplifiers.[28] Accordingly, hydroperoxides may be responsible for the increase in the incidence of adverse events and decrease in the seizure-suppressive effect of the newer AEDs in the presence of high levels of oxidative stress.

Hydroperoxides are generated by reactions between their precursors, peroxy radicals, and unsaturated fatty acids. These reactions can be inhibited by the peroxy radical trapping

activity of vitamin E.[29] Therefore, it has been suggested that prior administration of vitamin E could reduce the plasma levels of d-ROMs and increase the safety and seizure-suppressive effect of LTG and LEV in patients with extremely high plasma d-ROM levels. Vitamin C is also widely known as an antioxidant vitamin; however, water-soluble vitamin C is likely to be poorly effective, because hydroperoxides are lipophilic.

The limitations of this study were the insufficient number of measurements/analyses owing to the small total number of patients classified as having SMID. We think that it is necessary to carry out a study with a larger number of cases of SMID patients with epilepsy in the future. Another future task is to carry out the investigation in patients with PIMD and PMLD, the pathogenetic backgrounds of which are similar to the pathogenesis of SMID.

Our study findings suggested that plasma d-ROM levels may serve as an important biomarker for individualized drug therapy of epilepsy in patients with SMID.

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