Journal of the Neurological Sciences 466 (2024) 123245

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Efficacy of a mitochondrial drug cocktail in preventing acute encephalopathy with biphasic seizures and late reduced diffusion

Taku Omata [a,*], Hiromi Aoyama [b], Kei Murayama [c], Masaki Takayanagi [d], Risa Kawaguchi [b], Ryo Fujimoto [b], Jun-ichi Takanashi [a]

[a] Department of Pediatrics, Tokyo Women's Medical University, Yachiyo Medical Center, Yachiyo, Japan
[b] Division of Child Neurology, Chiba Children's Hospital, Chiba, Japan
[c] Diagnostics and Therapeutics of Intractable Diseases, Intractable Disease Research Center, Graduate School of Medicine, Juntendo University, Tokyo, Japan
[d] Department of Physical Therapy, Faculty of Health Care and Medical Sports, Teikyo Heisei University, Chiba, Japan

ARTICLE INFO

Keywords:
AESD
Mitochondrial cocktail
Febrile seizures
Acute encephalopathy
Mitochondrial dysfunction

ABSTRACT

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is difficult to differentiate from prolonged febrile seizures during the acute phase. Mitochondrial dysfunction-induced energy depletion is among the key mechanisms underlying acute encephalopathy. Therefore, this study aimed to examine the efficacy of a "mitochondrial cocktail" in preventing AESD. We retrospectively studied children experiencing status epilepticus associated with fever lasting more than 30 min, focusing on those who received the mitochondrial cocktail between February 2016 and December 2020, and those who did not receive it within 24 h between February 2012 and January 2014. The mitochondrial cocktail contained vitamins B1, C, and E; biotin; coenzyme Q10; and L-carnitine. AESD occurred in 1 of 41 (2.4 %) patients in the administration group and 7 of 39 (17.9 %) patients in the non-administration group. The incidence of AESD was lower in the administration group than in the non-administration group, with a significant difference ($p = 0.027$). The incidence of encephalopathy, including cases classified as AESD and unclassified, was 7/41 (17.1 %) and 7/39 (17.9 %) in the administration and non-administration groups, respectively, with no significant difference. However, the number of cases with worsening pediatric cerebral performance category scores was significantly lower in the administration group compared to the non-administration group ($p = 0.015$). In conclusion, early administration of the mitochondrial cocktail may help prevent AESD. Some encephalopathy cases do not progress to a biphasic state or develop AESD. Thus, the mitochondrial cocktail should be administered as early as possible to prevent AESD.

1. Introduction

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is clinically characterized by prolonged febrile seizures (PFC) lasting for >30 min as the initial neurological symptom on day 1, followed by secondary seizures (commonly in a cluster of focal seizures). AESD is associated with the deterioration of consciousness on days 4 to 6. Most patients experience persistent disturbance in their level of consciousness between biphasic seizures, although approximately 30 % of patients have normal, clear consciousness [1,2]. Therefore, it is initially difficult to distinguish AESD from PFC.

We previously reported that early (<24 h) drug cocktail administration (mitochondrial cocktail: vitamin B1, vitamin C, biotin, vitamin E, coenzyme Q10, and L-carnitine) can help improve the prognosis of acute encephalopathy [3]. Vitamins act as coenzymes in mitochondrial metabolic pathways, and supplementation can prevent mitochondrial dysfunction [4,5]. Administering antioxidants is necessary to prevent free radical generation due to mitochondrial dysfunction [4]. Depleting energy caused by mitochondrial dysfunction is considered one of the pathological conditions of acute encephalopathy; therefore, administering mitochondrial cocktails may be effective [6]. Therefore, this study aimed to examine whether early administration of a mitochondrial

Abbreviation: AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; GABA, gamma-aminobutyric acid; GCS, Glasgow Coma Scale; JCS, Japan Coma Scale; PFC, prolonged febrile seizures; MEEX, clinically mild encephalopathy associated with excitotoxicity.

* Corresponding author at: Department of Pediatrics, Tokyo Women's Medical University, Yachiyo Medical Center, 477-96 Owadashinden, Yachiyo-shi 276-8524, Japan.
E-mail address: takuoma@hotmail.co.jp (T. Omata).

https://doi.org/10.1016/j.jns.2024.123245
Received 5 June 2024; Received in revised form 12 September 2024; Accepted 15 September 2024
Available online 17 September 2024

cocktail can suppress the onset of AESD in cases with status epilepticus with fever.

2. Material and methods

Based on their medical records, patients with status epilepticus associated with fever lasting for >30 min who received a mitochondrial cocktail between February 2016 and December 2020 were included in this study. During this period, the mitochondrial cocktail was administered to all eligible cases as a part of a standardized treatment protocol, ensuring uniformity in the approach to treatment. Patients with status epilepticus associated with fever lasting for >30 min who did not receive the mitochondrial cocktail within 24 h between February 2012 and January 2014 were also included. In this earlier period, the administration of the mitochondrial cocktail was not standardized and was given at the discretion of the attending physician. Therefore, only cases that did not receive the treatment within 24 h were selected for comparison. The mitochondrial cocktail contained vitamin B1 (10 mg/kg), vitamin C (100 mg/kg), biotin (0.5 mg/kg), vitamin E (10 mg/kg), coenzyme Q10 (5 mg/kg), and L-carnitine (30 mg/kg). The dosage was adjusted based on the weight of each patient; it was not increased beyond the dosage appropriate for the body weight of 20 kg. All six drugs were administered orally and via a nasogastric tube for cases of severe disturbance of consciousness. The duration of administration was 5 days or until the fever subsided and the condition of the patient stabilized.

The ages, durations from onset to medication, final diagnoses, and AESD prediction scores (a system designed by Tada et al. [Tada score] [7]) of the patients were obtained from the medical records. The Tada score is a score based on the consciousness level (12–24 h after convulsion), age, duration of convulsions, presence of mechanical ventilation management, and blood tests (aspartate transaminase, blood glucose, and creatinine) at admission. The maximum score is 9, and a total score of 4 or higher indicates AESD. The final diagnosis was classified as acute encephalopathy or PFC.

Within the category of acute encephalopathy, cases were further classified as either AESD or as "unclassified" acute encephalopathy.

Cases that exhibited a systemic inflammatory response indicative of a cytokine storm were excluded from this study. These exclusions were made because such cases are likely to represent encephalopathies with distinct pathophysiological mechanisms different from AESD, such as acute necrotizing encephalopathy or hemophagocytic syndrome with encephalopathy. We defined acute encephalopathy based on the following specific criteria: (a) acute onset of severe and sustained impairment of consciousness after an infection; (b) exclusion of central nervous system inflammation, level of consciousness of ≤11 on the Glasgow Coma Scale (GCS) or ≥ 20 on the Japan Coma Scale (JCS), and duration of impairment of >24 h. These diagnostic criteria are established by the Japanese research committee on influenza encephalopathy [8]. Patients who did not meet the criteria for acute encephalopathy with no sequelae were classified to have PFC. A pediatric neurologist assessed the classification of acute encephalopathy based on the clinical course and brain imaging. Outcomes were assessed using the pediatric cerebral performance category (PCPC) score (Zaritsky et al.) at hospital discharge [9]. We investigated cases diagnosed with acute encephalopathy, comparing the PCPC scores before onset and at discharge. Specifically, we analyzed the proportion of cases with a pre-onset PCPC score of 1 that worsened to a score of 3 or higher by discharge, comparing the administration and non-administration groups.

For statistical analysis, the Student's *t*-test was used to compare the ages and the Tada scores of the patients in the administration and non-administration groups at admission. Fisher's exact test was used to compare both the AESD incidence between the prescription and non-prescription groups and the number of cases with PCPC worsening among acute encephalopathy patients in each group.

For this study, informed consent was obtained using the opt-out format. This study was reviewed and approved by the Ethical Committee of Chiba Children's Hospital (2024–007).

3. Results

3.1. Summary of patient characteristics

Forty-one patients who received the mitochondrial drug cocktail within 24 h and 39 patients who did not were included. The ages of the patients ranged from 10 to 114 months (mean: 31.3 months) in the administration group and from 7 to 88 months (mean: 32.7 months) in the non-administration group. The ages in both groups were not significantly different ($p > 0.05$). The time from the seizure onset to medication use was 2–19 h (mean: 8.57 h) (Table 1).

The final diagnoses for the administration group were acute encephalopathy (7 cases) and PFC (34 cases). One case of acute encephalopathy was classified as AESD and six were categorized as "unclassified." Seven cases of acute encephalopathy and 32 cases of PFC were observed in the non-administration group, and all acute encephalopathy cases were AESD. No adverse events related to mitochondrial drug cocktail use were observed.

3.2. AESD predictive scores (Tada score)

The AESD predictive scores ranged from 1 to 9 (mean: 2.64) and from 0 to 8 (mean: 2.92) in the administration and non-administration groups, respectively. No significant difference was observed between the two groups.

In the administration group, AESD predictive scores of 4/9 and higher were observed for 10 cases; 1 patient developed AESD, while the other 9 did not. In the non-administration group, AESD predictive scores of 4/9 and higher were observed for 13 cases; 7 developed AESD, although the other 6 did not. The positive predictive value (PPV) was significantly lower in the treatment group compared to the non-treatment group, with the treatment group showing a PPV of 1/10 (10 %) and the non-treatment group showing a PPV of 7/13 (53.8 %). Both groups had a negative predictive value of 100 %.

3.3. Comparison of the mitochondrial cocktail administration group and the non-administration group

AESD occurred in 1 of 41 (2.4 %) patients in the administration group and in 7 of 39 (17.9 %) patients in the non-administration group. The AESD incidence was significantly lower in the administration group than in the non-administration group ($p < 0.05$) (Table 1). The incidence

Table 1
Comparison of patient characteristics and final diagnosis.

	Administration group	Non-administration group	*p*-value
Age (months)	10–114 (mean:31.3)	7–88 (mean: 32.7)	0.79
Time from onset to medication (hour)	2–19 (mean: 8.57)	N/A	N/A
Tada score	1–9 (mean: 2.64)	0–8 (mean: 2.92)	0.40
Final diagnosis			
• AESD	1	7	
• Others	6	0	
• PFC	34	32	
AESD vs. PFC + others			
• AESD	1	7	0.027
• PFC + others	40	32	
AESD+others vs. PFC			
• AESD+others	7	7	1.00
• PFC	34	32	

AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; N/A, not applicable; PFC, prolonged febrile seizures.

of encephalopathy, including AESD and unclassified, was 7/41 (17.1 %) and 7/39 (17.9 %) in the administration and non-administration groups, respectively, with no significant difference (Table 1). In the non-administration group, 7 patients were diagnosed with acute encephalopathy, and 4 had a pre-onset PCPC score of 1. All four (100 %) patients had a PCPC score of 3 or higher at discharge, indicating the presence of sequelae. In contrast, in the administration group, 7 patients were diagnosed with acute encephalopathy, all with a pre-onset PCPC score of 1; however, only 1 (14.2 %) had a PCPC score of 3 or higher at discharge, while the remaining 6 maintained a PCPC score of 1. A significant difference ($p = 0.015$) was observed (Table 2).

4. Discussion

We assessed the efficacy of a drug cocktail containing vitamins (mitochondrial cocktail) in preventing AESD. According to the results of magnetic resonance spectroscopy (MRS) studies, delayed neuronal cell death due to excitotoxicity is considered to cause AESD [10].

Glutamate, an excitatory neurotransmitter released into synapses, is taken up by astrocytes and converted (detoxified) into glutamine. However, in AESD, excess glutamate overwhelms this process, causing calcium influx through the activation of *N*-methyl-D-aspartate receptors in postsynaptic cells. As a result, the intracellular Ca2+ concentration increases, resulting in mitochondrial dysfunction. This increases the concentrations of reactive oxygen species and apoptosis-inducing factors. The mechanism that ultimately leads to cell death is speculated [11]. Therefore, immediate administration of the mitochondrial cocktail to patients with status epilepticus associated with fever that can lead to acute encephalopathy may reduce mitochondrial dysfunction and prevent AESD onset several days later.

Table 3 shows the assumed mechanism of action of the six vitamins we administered. The effects of pyridoxine (vitamin B6) use have also been reported. Vitamin B6 accelerates the conversion of glutamate to gamma-aminobutyric acid (GABA). In this situation, the glutamate level decreases, and the level of GABA, which suppresses nerve excitation, increases, showing that vitamin B6 may be effective against AESD [12,13].

In this study, the average duration from seizure onset to medication administration was 4.84 h, and the drugs could be administered early. Previously, we reported that the administration of mitochondrial cocktail drugs within 24 h may improve prognosis. In this study, we could administer the cocktail within 24 h for all cases.

The AESD prediction score by Tada et al. [7] incorporates the levels of consciousness after 12 to 24 h. The consciousness level (12–24 h after convulsion) is categorized as GCS 15 or JCS 0: no points; GCS 14–9 or JCS 1–30: 1 point; and GCS 8–3 or JCS 100–300: 3 points. The authors reported that if the sum of the above scores is 4 or higher, the sensitivity and specificity of AESD prediction are 88.7 % and 90 %, respectively.

In the treatment group, 10 patients had predictive scores of 4/9 or higher, only 1 out of these 10 patients (10 %) developed AESD. Whereas, in the non-treatment group, 7 out of 13 patients (53.8 %) with predictive scores of 4/9 or higher developed AESD. This marked disparity in PPV—10 % for the treatment group compared to 53.8 % for the non-treatment group—suggests that the mitochondrial cocktail may be effective in reducing the risk of AESD development among high-risk individuals.

In this study, the incidence of AESD was significantly lower in the administration group than in the non-administration group ($p = 0.026$).

Table 2
Number of cases of PCPC worsening among acute encephalopathy.

	PCPC worsened by 2 or more	p-value
Administration group ($N = 7$)	1	0.015
Non-administration group ($N = 4$)	4	

PCPC, pediatric cerebral performance category.

Table 3
Mechanism of action of the components of the mitochondrial drug cocktail.

Components*	Mechanism of action
Vitamin B1	Vitamin B1 activates pyruvate metabolism and the tricarboxylic acid cycle, promoting electron transfer to the mitochondrial electron transport chain.
Vitamin C, Biotin, and Vitamin E	These are commonly incorporated antioxidants in various mitochondrial cocktail drugs along with coenzyme Q10 and work as free radical scavengers.
Coenzyme Q10	It is used as a bypass of the electron transport system and is expected to improve flow. It also has an antioxidant effect. It is believed not to pass through the blood-brain barrier, although it reduces lactate and pyruvate concentrations in the central nervous system. Thus, coenzyme Q10 may be indirectly involved.
Carnitine	Carnitine binds to long-chain acyl-CoAs in the cytoplasm and transports them into the mitochondria. Insufficient carnitine suppresses β-oxidation in the mitochondria and impairs energy metabolism.

* All components are involved in metabolic pathways within the mitochondria.

The incidence of encephalopathy, including cases classified as AESD and unclassified, was 7/41 (17.1 %) and 7/39 (17.9 %) in the administration and non-administration groups, respectively, with no significant difference. This suggests that early administration of the mitochondrial cocktail offers preventive benefits against AESD in selected cases. Additionally, some individuals diagnosed with encephalopathy do not progress to a biphasic state and develop AESD. All individuals with encephalopathy who did not progress to a biphasic state and were categorized as "unclassified" were observed to have a PCPC score of 1 at the time of discharge and had a good prognosis. The analysis of PCPC scores demonstrated a significant difference between the administration and non-administration groups in terms of neurological outcomes at discharge. Therefore, we believe that the mitochondrial cocktail should be administered as early as possible to prevent AESD.

AESD is the most common type of acute encephalopathy in Japan [14]. However, in some mild acute encephalopathies, which did not show abnormal findings on magnetic resonance imaging and were considered the "unclassifiable" type, MRS showed a transient increase in glutamine levels. These mild acute encephalopathies are presumed to be on the mild spectrum of an excitotoxic acute encephalopathy and are clinically termed mild encephalopathy associated with excitotoxicity (MEEX) [15,16]. MRS was not performed in this study, although it is possible that the 6 patients in the administration group who were diagnosed with encephalopathy but did not develop AESD and were categorized into the unclassifiable group may have had MEEX. By examining glutamate and glutamine in patients treated with mitochondrial cocktail drugs, it may be possible to examine changes in excitotoxicity due to treatment and evaluate the efficacy of drugs when the final diagnosis is PFC or "unclassifiable."

The limitations of the study include the difficulty in evaluating the effectiveness of the mitochondrial cocktail. This is because, if effective, the final diagnosis of a patient who would have developed AESD without treatment could change to encephalopathy classified as "unclassifiable" or PFC. In contrast, some patients were diagnosed with PFC without administration of the mitochondrial cocktail. Previous studies have shown that "unclassifiable" types of acute encephalopathy are the second-most common type of acute encephalopathy in Japan after AESD, and it cannot be determined whether the mitochondrial cocktail was effective for the six cases categorized as unclassifiable in the administration group. In this study, we showed that the mitochondrial cocktail may be effective, although it was impossible to present the efficacy of this cocktail with sufficient ground. Thus, examining a large number of people is necessary to demonstrate the effectiveness of the mitochondrial cocktail for AESD. Furthermore, examining the pathophysiology of excitotoxicity through techniques such as MRS may serve

as a basis for treatment.

5. Conclusions

Various vitamins act as coenzymes in the mitochondrial metabolic pathway, and supplementation can prevent mitochondrial dysfunction with AESD. The administration of antioxidants is necessary to prevent free radical generation due to mitochondrial dysfunction. AESD can be difficult to distinguish from PFC at the time of hospitalization. Given the pathophysiology of AESD, the mitochondrial cocktail should be administered as early as possible to prevent AESD. In this study, AESD incidence was significantly lower in the administration group, and the changes in PCPC scores at discharge tended to be lesser in the administration group than in the non-administration group; however, evidence for the effectiveness of mitochondrial cocktails remains inadequate and requires further investigation.

Funding

This study was funded in part by a Grant-in-Aid for Research on Measures for Intractable Diseases No. 24FC1010 (JT) from the Japanese Ministry of Health, Labor, Welfare and JSPS KAKENHI Grant Numbers JP23K07192 (JT) and JP24K10919 (TO), and the Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and development (AMED) JP22ek0109468 (KM), 24ek0109625 (KM).

Ethical approval

The local Ethics Committee of Chiba Children's Hospital approved this study.

Declaration of competing interest

None.

Data availability

The datasets analyzed in the current study are available from the corresponding author upon reasonable request.

Acknowledgments

We would like to thank Editage (www.editage.jp) for English language editing.

References

[1] J. Takanashi, H. Oba, A.J. Barkovich, H. Tada, Y. Tanabe, H. Yamanouchi, S. Fujimoto, M. Kato, M. Kawatani, A. Sudo, H. Ozawa, T. Okanishi, M. Ishitobi, Y. Maegaki, Y. Koyasu, Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy, Neurology 66 (2006) 1304–1309, discussion 1291, https://doi.org/10.1212/01.wnl.0000210487.36667.a5.

[2] J. Takanashi, Two newly proposed infectious encephalitis/encephalopathy syndromes, Brain and Development 31 (2009) 521–528, https://doi.org/10.1016/j.braindev.2009.02.012.

[3] T. Omata, K. Fujii, J.I. Takanashi, K. Murayama, M. Takayanagi, K. Muta, K. Kodama, Y. Iida, Y. Watanabe, N. Shimojo, Drugs indicated for mitochondrial dysfunction as treatments for acute encephalopathy with onset of febrile convulsive status epileptics, J. Neurol. Sci. 360 (2016) 57–60, https://doi.org/10.1016/j.jns.2015.11.043.

[4] G. Pfeffer, K. Majamaa, D.M. Turnbull, D. Thorburn, P.F. Chinnery, Treatment for mitochondrial disorders, Cochrane Database Syst. Rev. 2012 (2012) CD004426, https://doi.org/10.1002/14651858.CD004426.pub3.

[5] K. Abe, Y. Matsuo, J. Kadekawa, S. Inoue, T. Yanagihara, Effect of coenzyme Q10 in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): evaluation by noninvasive tissue oximetry, J. Neurol. Sci. 162 (1999) 65–68, https://doi.org/10.1016/s0022-510x(98)00296-2.

[6] M. Mizuguchi, H. Yamanouchi, T. Ichiyama, M. Shiomi, Acute encephalopathy associated with influenza and other viral infections, Acta Neurol. Scand. 115 (2007) 45–56, https://doi.org/10.1111/j.1600-0404.2007.00809.x.

[7] H. Tada, J. Takanashi, H. Okuno, M. Kubota, T. Yamagata, G. Kawano, T. Shiihara, S. Hamano, S. Hirose, T. Hayashi, H. Osaka, M. Mizuguchi, Predictive score for early diagnosis of acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), J. Neurol. Sci. 358 (2015) 62–65, https://doi.org/10.1016/j.jns.2015.08.016.

[8] S. To Chiryo-sha, Tokyo, Committee for the Compilation of Guidelines for the Diagnosis and Treatment of Acute Encephalopathy in Childhood, Guidelines on the Diagnosis and Treatment of Acute Encephalopathy in Childhood in the Japanese Society of Child Neurology (Ed.) 2016, 2016.

[9] A. Zaritsky, V. Nadkarni, M.F. Hazinski, G. Foltin, L. Quan, J. Wright, D. Fiser, D. Zideman, P. O'Malley, L. Chameides, R.O. Cummins, Recommended guidelines for uniform reporting of pediatric advanced life support: the pediatric Utstein style, in: A statement for healthcare professionals from a task force of the American Academy of Pediatrics, the American Heart Association, and the European Resuscitation Council, Pediatrics 96, 1995, pp. 765–779, https://doi.org/10.1161/01.CIR.92.7.2006.

[10] J.I. Takanashi, M. Mizuguchi, M. Terai, A.J. Barkovich, Disrupted glutamate-glutamine cycle in acute encephalopathy with biphasic seizures and late reduced diffusion, Neuroradiology 57 (2015) 1163–1168, https://doi.org/10.1007/s00234-015-1573-x.

[11] M.A. Tarnopolsky, The mitochondrial cocktail: rationale for combined nutraceutical therapy in mitochondrial cytopathies, Adv. Drug Deliv. Rev. 60 (2008) 1561–1567, https://doi.org/10.1016/j.addr.2008.05.001.

[12] K.O. Fukui, M. Kubota, H. Terashima, A. Ishiguro, H. Kashii, Early administration of vitamins B1 and B6 and l-carnitine prevents a second attack of acute encephalopathy with biphasic seizures and late reduced diffusion: a case control study, Brain and Development 41 (2019) 618–624, https://doi.org/10.1016/j.braindev.2019.02.015.

[13] T. Akiyama, S. Toda, N. Kimura, Y. Mogami, Y. Hanaoka, C. Tokorodani, T. Ito, H. Miyahara, Y. Hyodo, K. Kobayashi, Vitamin B6 in acute encephalopathy with biphasic seizures and late reduced diffusion, Brain and Development 42 (2020) 402–407, https://doi.org/10.1016/j.braindev.2020.02.002.

[14] A. Hoshino, M. Saitoh, A. Oka, A. Okumura, M. Kubota, Y. Saito, J.I. Takanashi, S. Hirose, T. Yamagata, H. Yamanouchi, M. Mizuguchi, Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes, Brain and Development 34 (2012) 337–343, https://doi.org/10.1016/j.braindev.2011.07.012.

[15] N. Hirai, D. Yoshimaru, Y. Moriyama, K. Yasukawa, J.I. Takanashi, A new infectious encephalopathy syndrome, clinically mild encephalopathy associated with excitotoxicity (MEEX), J. Neurol. Sci. 380 (2017) 27–30, https://doi.org/10.1016/j.jns.2017.06.045.

[16] J.I. Takanashi, Y. Murofushi, N. Hirai, K. Sano, E. Matsuo, K. Saito, K. Yasukawa, H. Hamada, Prognostic value of MR spectroscopy in patients with acute excitotoxic encephalopathy, J. Neurol. Sci. 408 (2020) 116636, https://doi.org/10.1016/j.jns.2019.116636.