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An open label trial of a standardized extract of cultured *Lentinula edodes* mycelia (ECLM) in children with refractory epilepsy

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ABSTRACT

Objectives: This open-label trial was performed to assess the immune markers in a cohort of children with refractory epilepsy in order to identify diagnostic and therapeutic markers and to also investigate the effect of an immunologically active supplement derived from mushroom, a standardized extract of cultured *Lentinula edodes* mycelia (ECLM), on the clinical status and on the immunological markers in the subjects.

Methods: Eighteen pediatric patients diagnosed with refractory epilepsy in which antiepileptic therapy was not effective were enrolled. All subjects were also treated with anticonvulsant medications for their seizures. Patients were administered 0.6 g/day (< 3 years old) or 1.2 g/day (≧ 3 years old) of ECLM (fine-granule equivalent) orally for one month. Immunological markers were assessed at baseline and after one month. Video electroencephalogram (EEG) was taken twice, once before the trials and once at the end of ECLM treatment. Seizure frequency and

behavioral symptoms were measured by a questionnaire survey of the parents. A two-month follow-up was also performed for surveillance of secondary infections.

Results: The subjects treated with ECLM showed significant elevation of several immune parameters including immunoglobulin G (IgG), CD3, CD4, and CD20 lymphocytes, and phagocytic index. Six of the 18 children on stable anticonvulsant drugs had fewer seizures reported during the ECLM treatment. The ECLM-treated patients also had less sharp and spike activity in the EEG measurement. At the two-month follow-up, the ECLM-treated subjects had developed fewer infections and viral syndromes compared to their condition before the trial. The children's family reported other subjective behavioral improvements, leading to improved quality of life (QOL).

Conclusion: ECLM administration to the pediatric patients with refractory epilepsy contributed to improvement of epileptic manifestation and decrease of epileptic seizure, possibly by suppressing the reactivation of herpes virus, as well as improvement of QOL. In addition, it was demonstrated that ECLM is a safe supplement for infants and children.

Keywords: ECLM, epilepsy, children, pediatric, mushroom

BACKGROUND

According to the global campaign against epilepsy [1], more than 3 million people in North America have epilepsy. The incidence of epilepsy in North America is estimated to be 50 per 100,000 people per year, with higher incidence in children younger than 5 years old and the elderly. Epilepsy is a neurological condition caused by several etiologies. Although the clinical seizure may be stereotyped in patients, the underlying cerebral pathology may differ. Two-thirds of cases have no identifiable condition and insult (cryptogenic) or are a result of a genetic abnormality (idiopathic). The remaining one-third of epilepsy occurs in patients with identifiable insults or cerebral abnormality such as neurodegenerative disorders, intracranial infections, tumors, vascular accidents, and traumas [1, 2]. Ninety percent of epilepsy patients in the U.S. are taking antiepileptic drugs (AEDs). However, only 68% of patients are satisfied with these medications as they have side effects and do not always control the seizures [1]. Refractory epilepsy, in which medication does not bring the seizures under control, occurs in 3-5% of the population worldwide with the highest incidence among children. Approximately 10-40% of children with epilepsy will continue to have seizures despite treatment with AEDs [3]. Therefore, other therapeutic interventions are needed to improve the response of children with refractory seizures to conventional treatment. The incidence of cryptogenic cases of epilepsy and drug-resistance suggests the possibility of other etiologies of epilepsy, including autoimmune mechanisms. Recent research has emphasized the role of innate and adaptive immune systems in epilepsy [4, 5]. Inflammatory triggers have been shown to enhance seizures in animal models, and conversely seizures have also been shown to

induce neuroinflammation. In addition, inflammatory cytokine secretion has been seen in pathological analysis of brain tissue of epileptic patients [5-7].

It has also been evident that an immune etiology for certain types of epilepsy is attributed to specific immunological deficits in epileptic syndromes. For instance, Rasmussen encephalitis is an autoimmune disorder of the central nervous system causing seizures and other symptoms. An antibody to glutamate receptor 3 (GluR3) is contained in the serum of patients suffering from Rasmussen encephalitis, and immunization with GluR3 induces a disorder similar to the human disease in animal models. Circumstantial evidence theoretically demonstrates that autoimmune mechanisms play a central role in Rasmussen encephalitis. There is also a report that T-lymphocyte populations are restricted in the brains of patients with Rasmussen encephalitis. Additionally, the reduction of serum anti-GluR3 antibody level via plasma exchange is associated with lowering the seizure and improving neurologic function [8].

Epilepsy is also a common complication in patients with systemic lupus erythematosus (SLE), especially in those with anti-phospholipid antibodies [8-10]. It is possible that these antibodies cause immune-mediated cortical damage leading to seizures. In terms of expanding the reach of the immunological etiology of epilepsy, investigators have also been interested in two other childhood epilepsy syndromes, West Syndrome (infantile spasms) and the Lennox-Gastaut Syndrome (LGS). Although the pathophysiological characteristics of both syndromes remain to be elucidated, it has been reported that treatment with corticotropin and corticosteroids contributes to better responses compared to conventional AEDs in some patients, especially with West Syndrome [11]. Also, the elevation of immunoglobulin G (IgG) and IgM has been observed in LGS patients, and intravenous immunoglobulin (IVIG) infusions were effective in some cases [8, 10].

There have also been some reports that epilepsy patients have autoantibodies including anti-ganglioside (GM1; a ganglioside found in the nervous system predominantly) and anti-glutamic acid decarboxylase (GAD) antibodies [12, 13] as well as anti-phospholipid antibodies. In animal models, it has been shown that anti-GM1 antibodies exert seizures while GM1 reduces them. Substantial evidence also supports the theory that epileptic pathogenicity is caused by anti-GAD antibodies directed against GAD, which is an enzyme involved in the production of GABA. The animal models and human studies have revealed that decreased concentration of GAD in brain tissue is associated with induction of seizures. It has also been reported that anti-GAD antibodies are observed in some patients with stiff-man syndrome, which is a rare disease of the nervous system and responsible for increased incidence of epilepsy and drug-resistant epilepsy [10].

Landau-Kleffner syndrome (LKS) and Hashimoto's encephalopathy are two other syndromes in which epileptic seizures are a frequent manifestation that have possible immunological etiology. LKS is a childhood disorder characterized by loss of language comprehension and verbal expression and 45% of LKS patients have IgG autoantibodies. In addition, it has been reported that clinical symptoms are ameliorated by IVIG infusions. Hashimoto's encephalopathy is a term used to describe an encephalopathy of autoimmune origin where immunomodulation such as IVIG

infusions and plasma exchange have been found to be effective [10, 14].

It is also reported that refractory childhood epilepsy frequently results from encephalitis induced by herpes simplex viruses such as HSV-1 and HSV-2 [15]. Most HSV-1 infections are initially acquired during childhood [16] and the virus becomes persistent for the entire lifetime where latency and reactivation of the virus are repeated depending on immunological status of the host.

The immunologic mechanisms affecting the pathogenesis of epilepsy are a recent topic of research and studies that support this pro-inflammatory hypothesis will contribute to a better understanding of the disease and open new therapeutic possibilities. There is a reasonable consensus that manipulation of the immune system may benefit individuals with refractory epilepsy [5] and therefore the use of supplements with immune system activity would be a valid target for research.

ECLM is a standardized extract of cultured *Lentinula edodes* mycelia, which has been used as a dietary supplement. ECLM consists of carbohydrates and several ingredients, and oligosaccharides are assumed to confer the biological activities of ECLM. The α-glucan content, as a major constituent of carbohydrates in ECLM, was indicated as 28.9 ± 2.4% (weight) of the ECLM freeze-dried powder. Whereas, the β-glucan content was very low [17-20]. ECLM have been shown to influence the immune system as an immunomodulatory agent [21] and by activation of natural killer (NK) cells [22-25]. The ECLM-related products have been available in the market worldwide.

This research was designed to examine laboratory immune markers in children diagnosed with drug-resistant epilepsy and to identify specific immunological deficiency that would lead to a diagnostic or therapeutic profile. We also assessed the effect of a supplement ECLM with immunological activity, which was administered as an open label intervention in a cohort of subjects.

METHODS

Subjects and administration

This study was an open-label trial to determine the effect of ECLM on immunological markers, seizure frequency, and incidence of secondary infections. The subjects served as their own historic controls. All subjects were enrolled in the study based on the inclusion and exclusion criteria as shown in Table 1, remaining on a stable dose of anticonvulsant during the trial. The clinical study was conducted in accordance with the principles of Good Clinical Practice (GCP) and ethical standards set out in the Declaration of Helsinki of the World Medical Association. Eighteen children (11 males and 7 females) aged 1-7 years old (mean age 4.7) suffering from refractory epilepsy of different types were enrolled in the trial. Patients under 3 years old received 0.6 g/day (2 capsules/day) and patients three years or older received 1.2 g/day (4 capsules/day) of ECLM orally for one month in the form of soft capsules (300 mg ECLM (fine-granule equivalent)/capsule) produced by Amino Up Co., Ltd., Sapporo, Japan.

Table 1. Inclusion and exclusion criteria

Inclusion criteria:
Boys and girls aged 1-7 years old diagnosed with epilepsy in which the antiepileptic therapy was not effective.
Subjects with at least 2 general seizures or partial seizures (with secondary generalization or without it) per week.
Subjects who were considered by the investigator able to complete the trial.
Subjects whose legal representatives signed the informed consent.
Exclusion criteria:
Subjects whose seizures are non-epileptic.
Subjects with an active infection of the central nervous system, demyelinating disease, or any disease of the central nervous system that, in the opinion of the investigator, could progress during the study.
Patients who had taken the current antiepileptic drug for more than 30 days.

Endpoints

Endpoints shown in Table 2 were assessed one month after the ECLM treatment began as well as a two-month follow-up for surveillance of secondary infections. Immunological markers were measured at baseline and after one month, which were immunoglobulin A (IgA), G (IgG), M (IgM), leukocytes, lymphocytes, CD3, CD4, CD8, and CD20 lymphocytes, CD4/CD8 ratio, CD HLA-DR, phagocytic index, and circulating immune complex. EEG was taken twice, once before the trials and once at the end of ECLM treatment. Video EEG lasted about 2-3 hours, including falling asleep, sleep, and awakening.

Table 2. Endpoints

***Primary endpoint parameters*:**
Changes in immunological markers. *
Alterations of video electroencephalogram (EEG). *
Frequency of seizures (the percentage of patients with a reduction in seizures). *
Incidence rate of secondary infections. **
***Secondary endpoint parameters*:**
Improvement of quality of life, including fatigue, sleep, and appetite. ***
Assessment of safety based on the reports of adverse events. ***

*Before and at the end of ECLM treatment;

**during the two-month follow-up period;

***during the study period.

The change in EEG abnormality was analyzed by the specialist and determined by comparing the degree of sharp, spike, and other abnormal activities recorded at baseline to the EEG images at one month. Seizure frequency was determined from counts kept by the subject's family. Secondary infections were checked by the principle investigator during the two-month follow-up observation. An evaluation of the general well-being of the subjects was measured by questionnaire survey throughout the study. Immune markers were measured by a commercial laboratory, Avicenna (Moscow, the Russian Federation), on blood samples collected before the commencement of ECLM intake and after one month.

Statistical analysis

The statistical analysis was carried out, and qualitative variables were summarized using descriptive statistics (mean, median, standard error, the number of cases and percentages). The *p*-values less than 0.05 were considered to be statistically significant.

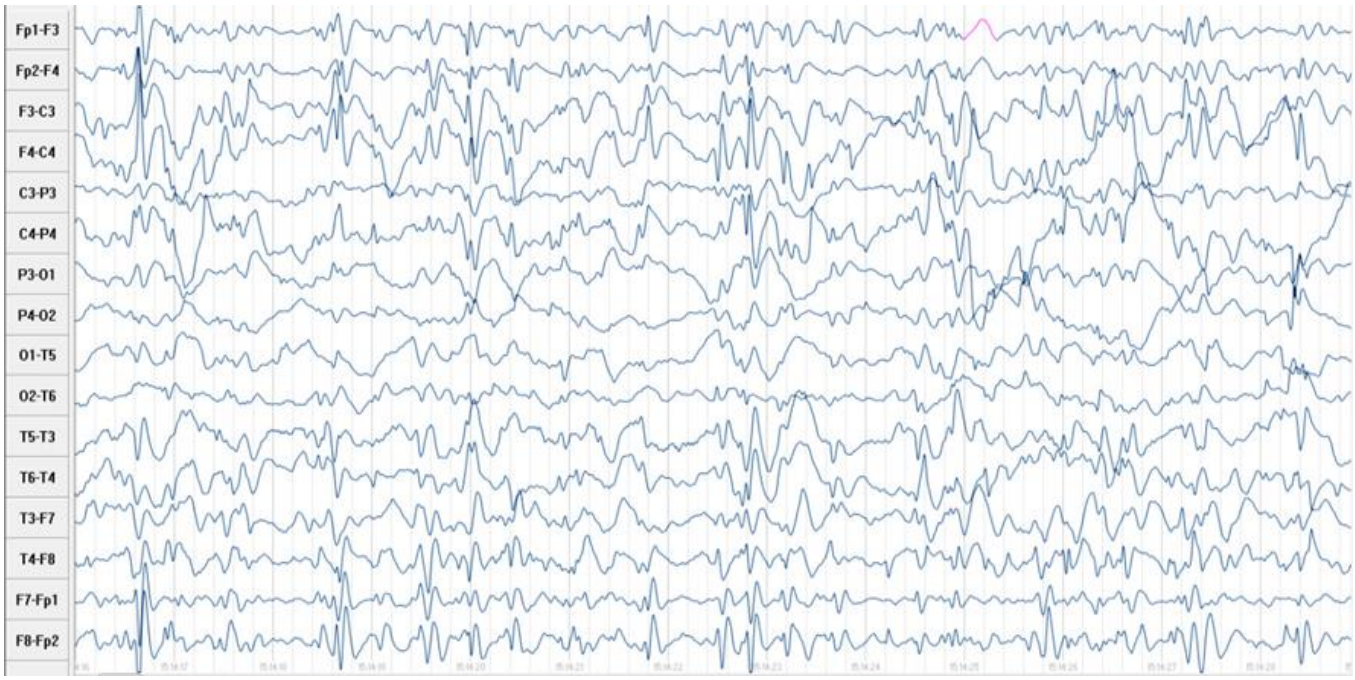
RESULTS AND DISCUSSION

When the pediatric patients suffering from refractory epilepsy received 0.6 or 1.2 g/day of ECLM orally for one month, the value of IgG, the percentages of CD3, CD4, and CD20 lymphocytes, and phagocytic index were elevated significantly compared to baseline (Table 3). The previous studies [21, 22, 26] revealed that ECLM is capable of stimulating the immune system responses in animal models and is involved in induction of T cells and IL-12, enhancement of NK cells, and augmentation of macrophage-dependent immune responses. In West Nile virus-infected mice, ECLM was shown to significantly elevate the production of IgG, IgM and γδ T cells [27]. Furthermore, human trials have demonstrated an effect on the immune system in cancer treatment where ECLM was acting as a biological response modifier [28], enhancing interferon (IFN)-γ and tumor necrosis factor (TNF)-α productions by $CD4^+$ and $CD8^+$ T cells [29]. The results of immune parameters from the current study were supported by the previous findings that ECLM treatment is associated with upregulation of innate and acquired immunity.

Video EEG data were taken twice each before and after ECLM treatment in the 18 subjects. One case exhibiting the most remarkable alteration in EGG was shown in Figure 1. The EEG data was analyzed by the specialist and the results demonstrated that "benign epileptiform discharge," "complex of sharp-slow wave," "sharp wave," and "spike" were found in 12, 16, 18, and 16 children before ECLM administration, respectively. However, the number of subjects showing each signal was drastically decreased after ECLM treatment: benign epileptiform discharge 4 children; complex of sharp-slow wave 11; sharp wave 7; and spike 2. When seizure frequency was counted by the subject's family, 6 of the 18 subjects had decreased seizure frequency and severity from baseline to one-month visit within the trial period. The percentage of patients with a reduction of seizures was 33.3%. The results of EGG measurement and seizure frequency observation obviously suggest that ECLM supplementation might contribute to ameliorating pediatric refractory epilepsy.

Figure 1. ECLM treatment

(A) Before ECLM treatment



(B) After ECLM treatment

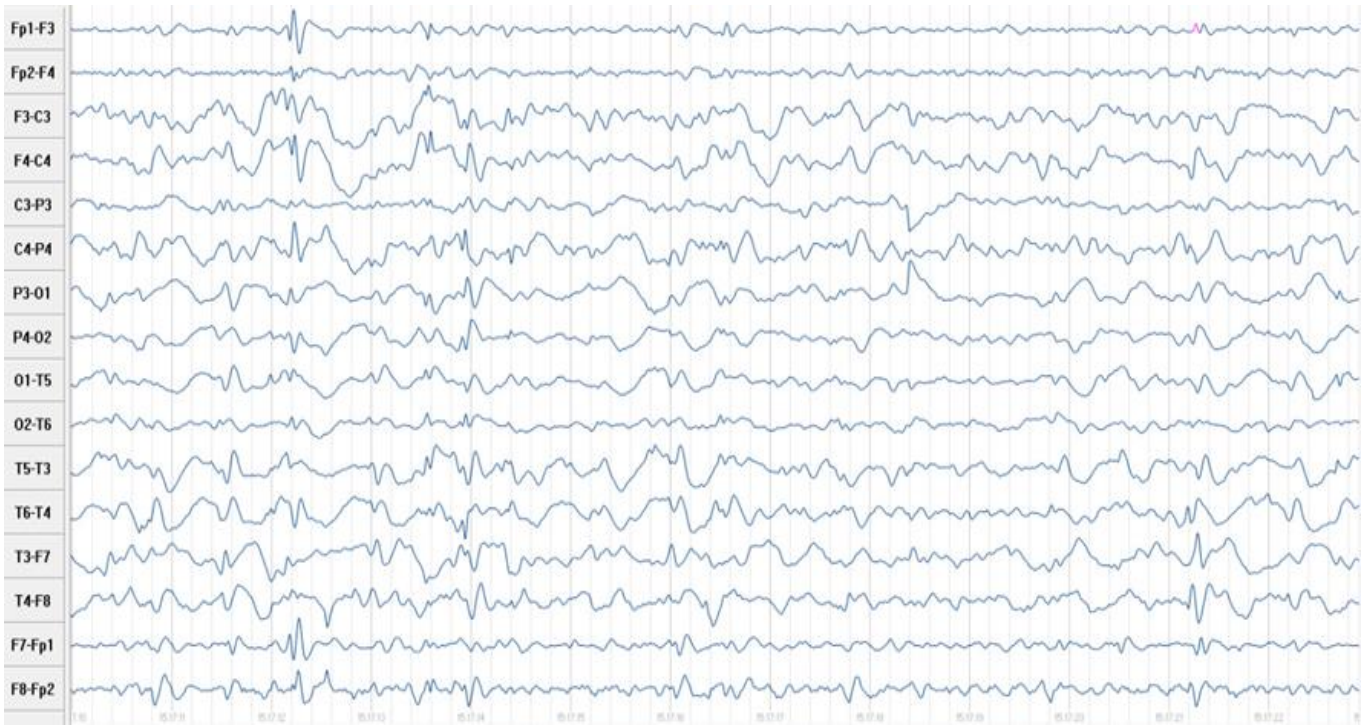


Figure 1. The representative EEG image before ECLM treatment (A) and after ECLM treatment (B) in one subject with the most remarkable change. Video EEG data were taken before and after ECLM treatment in 18 pediatric patients with refractory epilepsy. Video EEG lasted about 2-3 hours, including falling asleep, sleep, and awakening. The change in EEG data was determined by comparing the degree of sharp, spike, and other abnormal activity.

Over the past decade, we have accumulated numerous data showing the relationship of epilepsy to immunological dysfunction that allowed us to refer this type of pathology in a group of immune diseases with progressive course [30]. Early studies of immunologic markers showed that epileptic patients had significantly fewer circulating T4 helper lymphocytes and a significantly greater number of T8 lymphocytes when compared to control subjects [31]. The T4/T8 ratio was also consistently and significantly lower in the epileptic group. The data suggested that there was a derangement of cell-mediated immunity in individuals with epilepsy. In Table 3, ECLM intake significantly increased CD4 T-helper lymphocytes while it did not affect the number of CD8 T-killer lymphocytes, resulting in elevation of CD4/CD8 ratio although it was not significant. Thus, we speculate that the improvement of epilepsy in the present study might be attributable to normalizing and modulating the derangement of immunity by the ECLM treatment.

Another immunological consequence in patients with epilepsy may be a secondary immunodeficiency, which may impair resistance to infectious syndromes such as acute respiratory viral infections, herpetic infections and others [32]. Recurrent infections interfere with treatment and normal activities in patients with seizures. The clinical experience suggests that there are significant neuroimmunological impairments in patients with epilepsy. Understanding the immune deficits in epilepsy would improve management of both the seizures and the secondary infections seen in patients. In addition to the possibility that ECLM might be effective for epilepsy manifestation, plenty of investigations in rodent models have reported that ECLM supplementation improved immune response to influenza infection [33], West Nile encephalitis [27], various kinds of infections with *Klebsiella pneumoniae*, *Candida albicans*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* [34], and surgical wound infections [35]. Thus, these findings under immunocompromised conditions indicate that ECLM could control the secondary infections in epilepsy patients. Recently, it has also been reported that ECLM remarkably decreased salivary human herpes virus (HHV)-6 in cancer patients undergoing continuous and systemic chemotherapy when ECLM was administered orally [36]. In the current trial, the incidence rate of secondary infections of herpes virus was decreased by 30% compared to previous months. Considering the relevance between epilepsy from encephalitis and herpes virus, the suppression of the secondary infections and the reactivation of herpes virus may be recognized as a more-relevant efficacy of ECLM on epilepsy.

When the general well-being of the subjects and the compliance of the supplement were evaluated through the questionnaire survey reported by the parents during the study period, 15 of the 18 subjects stated improvement of overall health. In particular, the survey showed a positive trend in reducing emotional lability, tearfulness, irritability, and fatigue. After 4-6 weeks of the therapy, there were distinct positive changes in the patients' condition, such as improved appetite and resistance to various kinds of mental and physical stress. A significant reduction of troubled sleep and weather sensitivity was also registered. The dietary supplement ECLM was well tolerated and did not cause any side effects. The results from questionnaire survey revealed that ECLM improved QOL in the pediatric subjects with refractory epilepsy although the underlying mechanism of ECLM remains to be elucidated. Lastly, ECLM is safely administered to infants and children as well as adults in terms of the safety.

Table 3. Immune markers of blood samples

Parameters		Baseline	One month	p-value
IgA	(g/L)	1.12 ± 0.12	1.22 ± 0.12	0.140
IgG	(g/L)	8.82 ± 0.23	9.93 ± 0.50*	0.020
IgM	(g/L)	0.86 ± 0.07	0.98 ± 0.08	0.150
Leukocytes	(x 10^3/mL)	7.51 ± 0.47	8.09 ± 0.55	0.056
Lymphocytes	(%)	43.8 ± 2.5	44.3 ± 2.1	0.680
CD3 (T-lymphocytes)	(%)	40.6 ± 1.2	43.0 ± 1.0*	0.047
CD4 (T-helpers)	(%)	22.8 ± 0.8	24.6 ± 0.8*	0.025
CD8 (T-killers)	(%)	21.3 ± 0.9	21.9 ± 0.7	0.378
CD20 (B-lymphocytes)	(%)	15.4 ± 0.5	17.1 ± 0.5**	0.001
CD4/CD8 ratio		1.08 ± 0.04	1.11 ± 0.04	0.258
CD HLA-DR	(%)	16.0 ± 0.8	16.2 ± 0.6	0.819
Phagocytic index	(%)	34.3 ± 2.2	41.4 ± 3.5**	0.007
Circulating immune complex		27.9 ± 1.7	27.1 ± 2.2	0.497

Data represent mean ± SEM. *p<0.05 and **p<0.01 vs baseline.

CONCLUSION

The study demonstrates that ECLM, a supplement with immunomodulating effects, can be safely used in the treatment of refractory epilepsy in children. ECLM administration to the pediatric patients with refractory epilepsy contributed to improving epilepsy manifestation and decreasing the number of epileptic seizures through upregulating the critical immune parameters. These results suggest that ECLM-ameliorated immunological status of the host might suppress the secondary infections and the reactivation of herpes virus as well as improve QOL. Since this study was a preliminary trial using historic controls, the findings need to be confirmed using a longer duration double blind or crossover design.

List of Abbreviations: ECLM, extract of cultured *Lentinula edodes* mycelia; EEG, electroencephalogram; IgG, immunoglobulin G; QOL, quality of life; AED, antiepileptic drug; GluR3, glutamate receptor 3; SLE, system lupus erythematosus; LGS, Lennox-Gastaut syndrome; IVIG, intravenous immunoglobulin; GAD, glutamic acid decarboxylase; LKS, Landau-Kleffner syndrome; NK, natural killer; GCP, Good Clinical Practice; IgA, immunoglobulin A; IgM, immunoglobulin M; IFN, interferon; TNF, tumor necrosis factor; HHV, human herpes virus; HSV, herpes simplex virus.

Competing Interests: The authors, Natalia V. Mikhailichenko and Viacheslav Kulagin, of NEVRON International Medical Center, Vladivostok, declare no conflicts of interest in terms of the research, authorship and/or publication of this article. No competing financial interests exist.

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