

Effects of Cerebrospinal Fluid AMPA Receptor Levels on the Clinical Efficacy of Lamotrigine in Epilepsy Treatment

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Abstract

Background: Epilepsy is one of the greatest health burdens in the world, and it deeply impacts the mental and physical health of the affected population. Some individuals can completely recover through effective treatment, while others have difficulty recovering and even have mortality risks during seizure attacks. Thus, we explored the effects of cerebrospinal fluid AMPA receptor levels on the clinical efficacy of lamotrigine in epilepsy treatment. We believe our work might have implications in epilepsy treatment.

Methods: Seventy cases of epilepsy diagnosed in our hospital were selected for this study from December 2016 to October 2018. The AMPA receptor content of patients in cerebrospinal fluid was determined by enzyme-linked immunosorbent assay. The patients were placed into a high AMPA group (n=34) and a low AMPA group (n=36) according to the median value at 4.08 ng/ml. Clinical efficacy and the incidence of adverse reactions were compared between the two groups.

Results: Before treatment, there was no significant difference in seizure frequency between the two groups ($P>0.05$). After treatment for 6 and 12 months, the seizure frequencies of the two groups were gradually reduced ($P<0.05$). Moreover, the number of seizures in the low AMPA group was significantly lower than that in the high AMPA group ($P<0.05$). The response rate of the high AMPA group was 79.41%, which was significantly lower than that of the low group AMPA ($\chi^2=6.055$, $P=0.048$). The improvement in electroencephalogram in the high AMPA group was 67.65%, which was significantly lower than that in the low AMPA group ($\chi^2=4.686$, $P=0.030$). However, there was no significant difference in the incidence of adverse reactions between the two groups ($\chi^2=0.202$, $P=0.653$).

Conclusions: AMPA receptor plays an important role in the development of epilepsy, and lamotrigine treatment was more efficacious in patients with low AMPA receptor levels.

Background

Epilepsy is characterized by chronic and recurrent transient brain dysfunction and is a relatively common neurological disorder worldwide. A systematic analysis revealed approximately 45.9 million epilepsy patients worldwide, and in 2016, age-standardized rates increased by 5.6% compared with those in 1990. The incidence rates are approximately 25/1000 per capita, which is followed by the incidence rates of stroke^[1]. Although treatment options for epilepsy are increasing, pharmacological treatment remains the first choice to control epilepsy. Lamotrigine (LTG) is a benzotriazine derivative and is a novel broad-spectrum antiepileptic drug (AED) widely used in the treatment of partial and generalized seizures as monotherapy^[2]. LTG can also be an add-on therapy among children older than 2 years and adult epilepsy patients with positive clinical tolerability. LTG is widely used in epilepsy populations because its efficacy and safety have been thoroughly demonstrated after many years of clinical observation. Currently, although some Chinese researchers have devoted themselves to improving epilepsy treatment options, reports on the mechanisms between neurotransmission receptors and AEDs are rare. The alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor is one of the major mediators of glutamate-mediated excitatory neurotransmission, and hyperactivation of the AMPA receptor can provoke many acute neurologic function injuries, which could be the underlying mechanism of seizure onset^[3, 4]. Hence,

we recruited 70 epilepsy patients who sought medical treatment in our hospital, studied the effects of cerebrospinal fluid (CSF) AMPA receptor levels on the clinical efficacy of LTG in epilepsy treatment, and provided clinical evidence to improve epilepsy treatment.

Methods

1 Materials

We recruited 70 epilepsy patients in the encephalopathy department of the first affiliated hospital of Guangxi University of Chinese medicine from December 2016 to October 2018, including 42 male cases and 28 female cases. The patients were between 18 years old and 63 years old, with an average age (42.19 ± 12.80) years old, a disease course between 6 months and 12 years, and an average disease course of 5.29 ± 2.42 years. We classified these patients as generalized onset (46 cases) and partial onset (24 cases) according to the International League Against Epilepsy (ILAE) classification^[5]. All recruited patients met the following criteria: ① initial complete accurate diagnosis of epilepsy based on clinical manifestation, regular electroencephalogram (EEG) and/or video EEG; ② naïve to other AEDs before treatment or inability to take other AEDs due to high adverse effects; ③ no apparent impairment of heart, lung, hepatic and renal organs, as well as the absence of other serious diseases, progressive nervous system disease and mental disease; ④ no history of alcohol dependence and drug abuse; ⑤ no contraindications with the use of LTG or no simultaneous use of any other medicines that can affect the efficacy of LTG; and ⑥ not currently pregnant and lactating (female patients). This study was approved by the Ethics Committees of the First Affiliated Hospital of Guangxi University of Chinese Medicine. All patients and families provided informed consent to participate.

2. Interventions

LTG (trade name: Lamictal, purchased from GlaxoSmithKline, Tianjin, SFDA approval number: J20130026, 50 mg per tablet) was used. The initial dosage was prescribed at 12.5 mg daily and then titrated to a dosage of 25 mg, twice a day, in ten days. During the experiment, dose adjustment was performed according to the seizure frequency, with an increase in dosage of 12.5 mg every ten days until a dosage of 100 mg to 200 mg, twice a day, was reached. The dosage was maintained once patients reached the maximal efficacy. The duration of the treatment course was 12 months. Patients underwent for routine blood, routine urine, hepatorenal function and immunity testing every 3 months. Patients were observed closely for severe adverse effects, such as allergic rash.

3. Evaluation of clinical efficacy

Generally, we depended on no seizures occurring within one year to evaluate the efficacy of LTG^[6]. From another perspective, we can also use EEG to help us evaluate treatment efficacy; based on the *clinical criteria of electroencephalography*^[7], EEG is classified as follows: normal EEG at level one, mild abnormal EEG at level 2, moderate abnormal EEG at level three, and severe abnormal EEG at level four.

Classifications of mild, moderate, and severe abnormal EEG were considered abnormal EEG. If EEG was improved after treatment, treatment was regarded as effective; otherwise, it was regarded as ineffective.

4. Measurement of CSF AMPA receptor content

CSF was collected by lumbar puncture and centrifuged for 10 min at 2,000 rpm immediately after collection. The supernatant was stored in a freezer at temperatures below 80°C to avoid repeat freeze thawing. CSF AMPA receptor expression levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions by laboratory technology employees. The kit was purchased from Shanghai Chaoyan Biotechnology Company Limited which located in Shanghai (article No.SEE803Hu).

5. Statistical analysis

We performed data analyses with SPSS (version 19.0), and quantitative data analyses are reported as the mean \pm standard deviation. Comparisons between two groups were performed with Student's t test (for independent samples). Comparisons between multiple groups were performed with one-way analysis of variance. Numerical data analyses were performed with constituent ratio. Analyses of comparisons between two groups were performed with the Chi-squared (χ^2) test, and the alpha level of the test was $\alpha=0.05$.

Results

1. Outcome of CSF AMPA receptor content measurement

The mean level of AMPA receptor content in all patients was 6.19 ± 2.21 ng/mL. According to the median value of 4.08 ng/mL, patients were divided into the high AMPA group (34 cases, AMPA level >4.08 ng/mL) and low AMPA group (36 cases, AMPA level ≤ 4.08 ng/mL).

2. Basic information compared between the high AMPA group and the low AMPA group

Differences between the two groups of patients regarding age, gender, prodrome, seizure type, intracranial pressure, etc., were not significant ($P>0.05$) (Table 1).

3. Impact of the level of CSF AMPA receptor content on lamotrigine clinical efficacy

The rate of no seizure recurrence in the high AMPA group was 79.41% (27/34) within one year, which was markedly lower than that in the low AMPA group (97.22%, 35/36). This difference was significant ($\chi^2=6.055$, $P=0.048$) (Table 2).

4. Impact of CSF AMPA receptor content on lamotrigine treatment reflected on EEG

The rate of abnormal EEG between the high and low AMPA groups was not different before treatment ($\chi^2=0.005$, $P=0.942$). However, the rate of abnormal EEG in the low AMPA group was 63.89% after treatment, which was markedly lower than that in the high AMPA group (85.29%). The difference was significant ($\chi^2=4.194$, $P=0.041$) (Table 3).

5. Effects of CSF AMPA receptor content on lamotrigine adverse effects

There were 9 cases with adverse effects after treatment with LTG, with 5 cases in the high AMPA group (rate: 14.71%, 5/34) and 4 cases in the low AMPA group (rate: 11.11%, 4/36). The difference was not significant ($\chi^2=0.202$, $P=0.653$).

Discussion

Glutamate receptors represent the majority of excitatory neurotransmission receptors in the central nervous system among mammals, and these receptors are divided into ionotropic and metabotropic^[8]. Specifically, ionotropic glutamate receptors contain N-methyl-D-aspartate (NMDA), AMPA receptors and kainite (KA), which are the main mediators of brain function, such as synaptic transmission, long-term synaptic potentiation, synaptic plasticity and learning/memorization^[9]. According to previous studies, the AMPA receptor also belongs to chemical voltage-gated channel receptors. Activation of AMPA receptors can initiate the immediate opening of the ion channel, causing excessive influx of monovalent cations Na^+ and K^+ and resulting in depolarization of the postsynaptic membrane^[10]. Moreover, activation of AMPA receptors can lead to an influx of extracellular Ca^{2+} , triggering a cascade of biochemical events that can change the characteristics of the membrane and cause excessive generation of long-term potentiation. In research on the pathological mechanism of epilepsy, researchers have discovered that the AMPA receptor channel GluR2 subunit can be replaced by the GluR1 and GluR3 subunits, enhancing neuronal uptake of Ca^{2+} and provoking brain injury in the hippocampal areas CA1 and CA3 that triggers seizure frequency^[3]. Additionally, injection of an AMPA receptor inhibitor before seizure onset can significantly impact neuroprotective function, which can effectively decrease seizure frequency^[11]. All of these studies have fully indicated that the AMPA receptor plays an important role in epilepsy onset and progression. Thus, treatments targeting AMPA receptors might be helpful in controlling seizure attacks.

LTG is a benzotriazine derivative, a currently novel broad-spectrum AED. The mechanism of LTG in controlling epilepsy includes inhibiting the release of glutamate and aspartic acid, followed by selective effects on Na^+ channels and blocking the binding of excitatory glutamate to its receptors^[12]. Our study relied on the median value of CSF AMPA receptor content to assign patients to two groups. After 6 and 12 months of treatment with LTG, seizure frequency gradually decreased in all patients and confirmed the value of LTG in treating epilepsy, which was consistent with previous studies^[13, 14]. Most importantly, our study also discovered that seizure frequency in the low AMPA group was markedly lower than that in the high AMPA group, suggesting that AMPA receptor levels might have an impact during LTG treatment in epilepsy. Moreover, through extensive comparisons of clinical efficacy between the two groups, we found

that the response rate and the rate of EEG improvement were lower in the high AMPA group than in the low AMPA group. This result further indicates that lower AMPA receptor content results in better clinical efficacy of LTG. We hypothesize that these findings might be attributed to less excitatory glutamate with low AMPA receptor content. LTG selectively inhibits neuronal depolarization and high-frequency discharge in seizure lesions, stabilizing neuronal Na⁺ channels and blocking the transmission of overexcited synapses to postsynaptic membranes, thus decreasing the frequency of seizure and producing positive clinical efficacy. In addition, the adverse effects between the two groups were not significantly different, suggesting that AMPA receptor content has no impact on LTG safety and reliability.

As stated above, our study suggests that CSF AMPA receptor content plays a significant role in epilepsy onset and progression and that low AMPA receptor content is better in LTG treatment. The limitations of our study include the small number of cases, short duration and lack of long-term observations. Further high-quality research is needed to fully evaluate the effects of CSF AMPA receptor content on the efficacy of LTG treatment in epilepsy.

Conclusions

As mentioned above, AMPA receptors play an important role in the development of epilepsy, and low AMPA receptor levels in patients increase the efficacy of LTG treatment.

Abbreviations

AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; LTG: lamotrigine; AEDs: antiepileptic drugs; ILAE: International League Against Epilepsy; EEG: electroencephalogram; CSF: cerebrospinal fluid; ELISA: enzyme-linked immunosorbent assay; KA: kainite. NMDA: ionotropic glutamate containing N-methyl-D-aspartate.

Declarations

Acknowledgments

Not applicable

Funding

Not applicable

Availability of data and materials

All data will be secured on the hospital computer and will be available on request from the corresponding author.

Authors' contribution

QJT and YHL conceived the idea, designed the study and produced the first draft of the study. YHL helped with the statistical analysis. TLW, JWY, TBW and HHL have provided intellectual content in the preparation and editing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The First Affiliated Hospital of Guangxi University of Chinese Medicine's Research Ethics Committee approved the study, and all participants provided written informed consent.

Consent for publication

Written informed consent was obtained from the patients for publication of this study and any accompanying data.

Competing interests

Authors declare no financial or nonfinancial interests.

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Tables

	high AMPA group	Low AMPA group	t/χ^2 vaule	P vaule
Age (year)	40.95±13.06	43.51±11.93	0.857	0.395
Gender (male/female)	19/15	23/13	0.467	0.494
Prodrome			0.508	0.917
Fever	22	23		
Abdominal pain and diarrhea	3	4		
Cold sore	2	1		
No symptoms	7	8		
Seizure type			0.827	0.843
Generalized onset	2	3		
Simple partial onset	1	3		
Complex partial onset	1	3		
Partial secondary generalized onset	30	29		
Intracranial pressure			0.183	0.669
normal	13	12		
abnormal	21	24		

Table 1 Basic characteristic comparative between high AMPA group and low AMPA group

case(n)	no recur within one year	rates of no recur within one year	χ^2 value
PA 34	27	79.41(27/34)	6.055 0.048
PA 36	35	97.22(35/36)	

Table 2.Level of CSF AMPA receptors impact on lamotrigine clinical efficacy

	group	case[n]	0	1	2	3	Rate of abnormal[%]
Before treatment	High AMPA	34	3	14	10	7	91.18[31/34]
	Low AMPA	36	3	13	12	8	91.67[33/36]
	χ^2 value						0.005
	<i>P</i> value						0.942
After treatment	High AMPA	34	5	16	8	5	85.29[29/34]
	Low AMPA	36	13	15	5	3	63.89[23/36]
	χ^2 value						4.194
	<i>P</i> value						0.041

Table 3 Impact of CSF AMPA receptors content on lamotrigine’s treatment reflect on EEG