Effects of linoleic acid on generalized convulsive and nonconvulsive epileptic seizures

Fatih EKİCİ1*, Gönül GÜROL2, Nurbay ATEŞ3

1 Department of Physiology, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey
2 Department of Physiology, Faculty of Medicine, Sakarya University, Sakarya, Turkey
3 Department of Physiology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey

Background/aim: To comparatively investigate the effects of linoleic acid on convulsive and nonconvulsive epileptic seizures.

Materials and methods: Rats were divided into 3 groups: convulsive epileptic rats receiving only pentylentetrazole (PTZ) injections (group 1), convulsive epileptic rats receiving PTZ and linoleic acid (group 2), and Wistar Albino Glaxo rats from Rijswijk with genetic absence epilepsy receiving linoleic acid (group 3). The duration and severity of convulsive activity were determined in groups in which convulsive seizures were induced by PTZ. In group 3, intravenous linoleic acid was administered after 1-h baseline electroencephalography (EEG) recordings. The EEG recordings were analyzed.

Results: When groups 1 and 2 were compared, the delay in onset of minor seizures and the decrease in the number of rats developing major seizures were found statistically significant. When the mean spike-wave discharge number and duration values for the rats in group 3 were compared to baseline values, a statistically significant increase was found in the 1st and 6th hours and there was no significant difference in the 24th hour.

Conclusion: While our study shows that linoleic acid may be effective in the treatment of generalized convulsive epilepsy along with conventional antiepileptic drugs used in epilepsy treatment, it reports that linoleic acid is not appropriate in the treatment of nonconvulsive epilepsies.

Key words: Linoleic acid, epilepsy, pentylentetrazole

1. Introduction
Epileptic seizure is the generation of abnormal, excessive, and hypersynchronized activity in the cortical and subcortical neurons due to several factors in the central nervous system. The condition where seizures show a tendency to relapse and become chronic is called epilepsy. Epilepsy, among the common neurological diseases of the brain, affects approximately 1% of the population (1–4). In addition, recurrent seizures are a significant cause of childhood morbidity (5). Generalized seizures can be subdivided into 2 groups: generalized tonic-clonic seizures and generalized absence seizures (6). The focal or generalized convulsive seizures observed in epilepsy are caused by an increase in the excitability in a specific region of brain cortex or subcortical structures or throughout the cortex. The reflections in electroencephalography (EEG) of the excitability increase occurring in the cortex during generalized seizures are EEG segments in the form of spike and spike-wave complexes. The excitability level in the brain neurons depends on the balance between excitatory and inhibitory effects. The excitability increase is theoretically dependent on the increase of excitatory effectiveness or the decrease in inhibitory effectiveness (i.e. disinhibition). The main event in the regulation of excitability at the neuronal level is the alteration of the permeability to Na+, Ca++, K+, and Cl- ions. The excitation at the neuronal level is dependent on the increase in permeability of cell membranes to Na+ and in some neurons to Ca++. The inhibition, however, is caused by an increase in the Cl- or K+ permeability. Convulsive seizures are caused by the dysfunction of a large cell population, which initiates local paroxysmal discharges, as a result of an over-release of glutamate and aspartate as well as increased N-methyl-D-aspartic acid receptor activity. The discharges propagating from the sites of origin to the other regions via abnormal dendritic meshwork cause the generation of focal seizures by firing the neurons without inhibitory mechanisms. The progression of discharge propagation leads to generalized seizures. The excitatory discharges deactivate the inhibitory mechanisms, which are under

* Correspondence: drfatihekici@hotmail.com
the control of gamma-aminobutyric acid (GABA)-ergic neurons (2,3,7).

Absence epilepsy, which is also classically known as petit mal seizure, is a form of nonconvulsive generalized epilepsy and its pathology is different from convulsive seizures. In the classical EEG pattern there are spike-wave discharges (SWDs) accompanying the seizure (2,8). There are many studies showing that GABAA and GABAB receptors are involved in the generation of SWDs (9,10). An increase in GABAergic activity can induce the generation of SWDs by increasing the intensity of the absence seizures in humans and animal models through inhibition and synchronization of neuronal firing. It was also shown that neurotransmitters other than GABA and ion channels could also be effective in the control of intrinsic oscillatory mechanisms (11).

While many new antiepileptic agents have been introduced in recent years, no significant changes have been observed in the distribution of patients not responding to pharmacotherapy. Therefore, there is a need for new approaches in epilepsy treatment.

Essential fatty acids are required for the fluidity of the membrane structure and the synthesis of eicosanoids. The 2 fatty acids essential for humans are linoleic acid, the precursor of prostaglandins, and linolenic acid (12). Recent studies have found that polyunsaturated fatty acids (PUFAs) are useful in epileptic seizures, brain ischemia, depression, and several coronary diseases. It is believed that PUFAs exert their corrective actions on neuronal damage by affecting glutamate receptors, glutamate transporters, and ion channels in brain. In addition, the ability of PUFAs to readily pass through the blood-brain barrier enhances their neuroprotective effects (13–15). It has been shown that dietary lipids not only influence the biophysical state of cell membranes but, via direct and indirect routes, they also act on multiple pathways including signaling and gene and protein activities (16).

The effect of PUFAs on epileptic seizures was investigated in several studies performed by establishing in vitro and in vivo models with controversial results (17). Furthermore, their effects on absence epilepsy have not been completely understood. Therefore, in our study the effects of PUFAs on 2 types of generalized epilepsy were investigated. Pentylentetrazole (PTZ) was used to test the effects of PUFAs on generalized tonic-clonic epilepsy and Wistar Albino Glaxo rats from Rijswijk (W AG/Rij) were used to test the effects of PUFAs on absence epilepsy.

2. Materials and methods

2.1. Animals

In our study, Wistar Albino rats (in the convulsive group) and 9 month old WAG/Rij (in the nonconvulsive group) of 200–300 g in weight, raised in the Kocaeli University (KOU) School of Medicine Experimental Medicine Research Laboratory, were used. All animals had free access to food and water. During the experiments attention was paid to protecting animal rights and the study was initiated after the approval of the KOU Ethics Committee. Two experimental groups were formed: the convulsive seizure group of Wistar rats with PTZ administration and the nonconvulsive (absence) seizure group of WAG/Rij strain rats, genetically predisposed to absence epilepsy (18).

2.1.1. Convulsive epilepsy group (n = 7)

Epileptic seizure activity was assessed for 30 min following intraperitoneal (i.p.) injections of 60 mg/kg PTZ using the scale described by Mares et al. (19). The experimental group (n = 7) that was administered PTZ (i.p.) 30 min after intravenous (i.v.) administration of 100 nmol/kg linoleic acid was also assessed with the same method and statistically compared.

2.1.2. Spontaneous nonconvulsive absence epilepsy group (n = 7)

EEG 1-h baseline recordings were obtained from the WAG/Rij rats, which were implanted with epidural tripolar EEG electrodes under anesthesia (20). In the rats that were administered 100 nmol/kg of i.v. linoleic acid, the mean number and duration (s) values of SWDs were compared with the baseline values.

2.2. EEG recording electrode implantation

In the epilepsy groups, the rats were chronically implanted with tripolar electrodes (Plastic Products Company MS 333/2A) in the skull for EEG evaluation. For this purpose, rats were anesthetized with ketamine (100 mg/kg, i.p.) and chlorpromazine (1 mg/kg, i.p.), placed in a stereotaxic device (Stoelting Model 15600), and held in place by their ears and teeth. The scalp was opened and the lambda and bregma were exposed. The bregma was used as a reference point. The recording electrodes were implanted at the frontal region coordinates of 2 mm anterior and 3.5 mm lateral and the parietal region coordinates of 6 mm posterior and 4 mm lateral, and the reference electrode was implanted in the cortex above the cerebellum and held in place with the help of dental acrylic. After this procedure the animals were allowed to rest for 1 week. The EEG recordings were obtained using the EEG100B Biopac System.

2.3. Epileptic seizure activity assessment

2.3.1. Evaluation of convulsive seizures

Epileptic seizure activity was evaluated for 30 min following i.p. injections of 60 mg/kg PTZ with respect to seizure onset time, seizure severity, and total seizure duration. The scale described by Mares et al. was used for the evaluation of generalized seizure severity (19). According to this scale: 0 = no behavioral changes, 0.5 = atypical behavior, 1
isolated myoclonic jerks, 2 = atypical minimal seizures, 3 = minimal seizures (with preserved righting reflex), 4 = major seizures (without tonic phase), and 5 = complete generalized tonic-clonic seizures (CGTCs).

Abnormal behavior changes, isolated myoclonic jerks, and clonic seizures accompanying the clonus of facial and forelimb were considered as minimal PTZ seizures. The subsequent onset of loss of righting reflex with extension of head, neck, and tail was considered as major PTZ seizure without tonic phase and the prolonged clonuses following tonic flexion or extension as complete major seizure. The seizure onset time was determined by measuring the time to occurrence of the first myoclonic jerk following the PTZ administration. The generalized tonic-clonic seizure latency was determined by recording the time to occurrence of the major seizure. Seizure duration was determined by measuring the major seizure duration and all time values were expressed in seconds (19).

2.3.2. The evaluation of absence seizures

The 1-h baseline EEG recordings and the EEG recordings obtained during the 1st, 6th, and 24th hours after the linoleic acid administration were analyzed and the number and duration (s) of SWDs were assessed.

2.4. Statistical evaluation

The data were expressed as mean ± standard error (SE). In the convulsive epilepsy groups, the delay in the time to onset of minor seizure was evaluated using the Mann–Whitney U test and the decrease in the number of rats developing major seizures was assessed with the chi-square test. The changes in major seizure latency and total major seizure duration were not included in the assessment due to the low number of rats experiencing seizures. In the absence epilepsy group, the comparisons among 1st, 6th, and 24th hour values and baseline values, with respect to the mean number and duration values of SWDs, were performed using the Friedman test and subsequently with the Bonferroni correction Wilcoxon signed ranks test. In all tests, P < 0.05 was accepted as statistically significant.

3. Results

3.1. Convulsive epilepsy group (n = 7)

In the PTZ-administered group, generalized tonic-clonic seizure activity developed in all rats. In this group, the mean time to onset of minor seizure was 91.4 ± 59 s, the major seizure latency was 237.8 ± 104 s, and the major seizure duration was 82.7 ± 18.9 s. In the experimental group, which was administered PTZ 30 min after 100 nmol/kg of linoleic acid administration, no major seizures developed in 5 of the 7 rats and very short-duration CGTC developed in only 2 rats. The mean time to onset of minor seizure was 133.5 ± 5.6 s, the major seizure latency was 430 ± 35 s, and the major seizure duration was 7.5 ± 2.5 s. When these values were compared to the PTZ group, the delay in the time to onset of minor seizure and the decrease in the number of rats experiencing major seizures were found to be statistically significant (P < 0.05) (Figures 1a and 1b). The changes in major seizure latency and total major seizure duration were not included in the assessment due to the low number of rats experiencing seizures.

3.2. Nonconvulsive absence epilepsy group (n = 7)

Baseline activities of WAG/Rij rats were recorded before the administration of linoleic acid (Figure 2a). In the 1-h baseline (0900–1000 hours) EEG recordings, the mean total SWD number and duration values were 16.5 ± 0.9 and 126.2 ± 18.3 s, respectively. In the experimental group (Figure 2b), the mean SWD number and duration values in the EEG recordings following linoleic acid administration were 33.4 ± 3.5 and 253 ± 28.5 s in the 1st hour, 25.1 ± 3 and 202 ± 36.1 s in the 6th hour, and 16.8 ± 0.8 and 112.1 ± 22.1 s in the 24th hour (Figures 3a and 3b). In view of these results, when compared to the baseline values, the mean SWD number and duration values showed a statistically significant increase in the 1st and 6th hours (P < 0.05), but no significant difference was observed in the 24th hour (P > 0.05).

![Figure 1a](image-url) The effects of linoleic acid on the time to onset of minor seizures (*: P < 0.05).

![Figure 1b](image-url) The development of CGTC in the convulsive epilepsy group (*: P < 0.05).
4. Discussion

4.1. In terms of convulsive epilepsy

The use of linoleic acid significantly prevented major seizure development, delayed the onset of seizures, and decreased the major seizure duration. These results are consistent with the results of the limited number of studies on the use of linoleic acid in convulsive epilepsies and the known mechanisms of the effect of linoleic acid. The effect of PUFAs on epileptic seizures was previously demonstrated in some studies conducted by establishing in vitro and in vivo models. In the in vitro model, seizure-like activity was generated using glutamatergic neurons and linoleic acid was shown to block the glutamatergic transmission. In the in vivo model, in rats in which temporal lobe epilepsy was induced by kainic acid, linoleic acid administration was found to prevent epileptiform activity and also decrease the hippocampal damage that occurs during seizures (13). Moreover, in a study where various PUFAs were given to rats in which a cortical stimulation model was established, it was found that PUFAs had an anticonvulsant effect and that this effect lasted for 6 h and had completely disappeared by the following day (21). In the studies mentioned above and in our study, it was shown that PUFAs had an anticonvulsant effect and this effect lasted for 6 h. In particular, in a very recent study conducted in rats with the same PTZ model used in our study, the combined use of linoleic and α-linoleic acid was found to increase the resistance to PTZ seizures (17). With respect to convulsive epilepsy, our study appears to be consistent with the limited number of previous animal studies. PUFAs are thought to exert their neuronal excitability-decreasing effect by inhibiting glutamatergic synaptic transmission by partially inhibiting the voltage-dependent Na⁺ and Ca²⁺ channels as well as by pre- and postsynaptically activating the 2 P-domain channels (TREK-1, TREK-2, TRAAK), which is a recently cloned K⁺ channel. In addition, it can be said that the ability of PUFAs to pass through the blood–brain barrier easily enhances their neuroprotective effects (13–15,17).

Figure 2a. Epileptic activity of WAG/Rij rat group receiving linoleic acid.

Figure 2b. Epileptic activity of convulsive epileptic rat group receiving PTZ and linoleic acid.

Figure 3a. The increases in the numbers of SWDs between the linoleic acid injection and the 1st and 6th hours were found statistically significant (*: P < 0.05).

Figure 3b. The increases in the durations of SWDs between the linoleic acid injection and the 1st and 6th hours were found statistically significant (*: P < 0.05).
4.2. In terms of nonconvulsive epilepsy (absence)
The results we obtained from our study show that linoleic acid increased the number and duration of the absence seizures that developed in the WAG/Rij rats. To date, there are not enough literature data obtained from the use of PUFAs in absence epilepsy that we can use to compare with these results. In studies on the modulation of Ca\(^{2+}\) channels in absence epilepsy, it was found that the T-type channel blockage decreased the SWDs, while the L-type channel blockage increased the SWDs (9). The findings that the Ca\(^{2+}\) channels inhibited by PUFAs are particularly L-type channels show that the inhibition of L-type Ca\(^{2+}\) channels may be an important factor in the development of the SWD-increasing effect of linoleic acid. The Na\(^{+}\) channel-blocking effect of PUFAs results in the development of the SWD-increasing effect of linoleic acid may be effective in the treatment of generalized convulsive epilepsy. We believe that an increase in the number of studies on this subject and the introduction of these agents as combination treatment choices will open new horizons in epilepsy treatment. However, according to our results, the use of linoleic acid in absence epilepsy does not appear to be appropriate. Our study also demonstrates the pharmacological differentiation in convulsive and nonconvulsive epilepsies. In the experimental and clinical studies conducted to date, it has been suggested that, as changes in the balance between excitatory and inhibitory systems cause different effects in the development of these epilepsies, the approaches used in their treatments should also differ. The results of our study have once again demonstrated this fact.

References


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