

Effects of Combined Action of Valproate and *Ginkgo biloba* on Cognitive Function in Penicillin-Induced Developmental Epilepsy of Rat

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ABSTRACT

Background and Aim: The aim of this study is to demonstrate the effect of combined action of Valproate and *Ginkgo biloba* on cognitive function in penicillin-induced developmental epilepsy of rat. **Methods:** Penicillin-induced developmental epilepsy in adolescence rat was established by intraperitoneal injection of penicillin diluted in saline as previously reported. Rats were assigned to 3 groups: Control group (CG), Penicillin-induced seizures group (MG) and Combination group of Valproate and *Ginkgo biloba* (V+G). The effect of combination of Valproate and *Ginkgo biloba* was evaluated through the test of water maze. **Results:** The present study demonstrated that the combination of Valproate and *Ginkgo biloba* improved cognitive dysfunction and resulted in higher therapeutic efficacy. **Conclusion:** Our data provide that combination of Valproate and *Ginkgo biloba* may be useful for treatment of developmental Epilepsy.

Keywords: Developmental epilepsy, Penicillin-induced seizures, Valproic acid, *Ginkgo biloba*, Cognitive function, Morris water maze.

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INTRODUCTION

Epilepsy is a long-term neurological condition characterized by recurrent spontaneous seizures, which are believed to arise from a disruption in the balance between excitatory and inhibitory neuronal activity in certain areas of the brain.^[1] Various hypotheses have been proposed to explain the increased neuronal excitability associated with this disorder. Seizures affect roughly 1% of the population, and in some patients, they remain poorly controlled despite treatment with currently available antiepileptic drugs. Resistance to these medications occurs in approximately 20-30% of cases. Thus, a more rational treatment method is searched and more sophisticated data about the pathophysiologic basis of epilepsy and epileptogenesis is obtained by using experimental models.^[2,3] Different convulsant substances are used to induce epileptiform activity in animal

models. Penicillin-induced epileptiform activity is a commonly employed experimental model of focal epilepsy and has been established in several laboratory species, including mice, rats, and rabbits.^[4-10] In recent years, increasing interest has focused on the hypothesis that seizures occurring during brain maturation may lead to long-term impairment of hippocampal function. Clinical findings suggest that epilepsy is more prevalent in children than in adults, particularly during the first year of life, and that seizures arising in this early postnatal period are associated with a broad range of cognitive deficits.^[11,12] Supporting these clinical observations, numerous experimental studies have demonstrated that rats subjected to recurrent seizures during development show learning impairments and reduced seizure thresholds in adulthood.^[13,14] Valproic acid (2-propylpentanoic acid; VPA), a branched-chain fatty acid, was introduced as an antiepileptic agent in the United States in 1978. Sodium valproate is now widely used as a first-line therapy for childhood epilepsy due to its extensive clinical utility.^[15] Nevertheless, several clinical reports indicate that although VPA is effective in controlling seizures, it can also cause varying degrees of cognitive dysfunction, including deficits in attention and memory, as well as abnormal emotional behavior. These adverse effects may negatively influence quality of life and contribute to treatment discontinuation in some patients.



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Ginkgo biloba, belonging to the family Ginkgoaceae, has been cultivated in Korea for many years. Leaf extracts of *Ginkgo biloba* have traditionally been employed in the management of cognitive disorders and peripheral vascular diseases and are known to modulate platelet function through inhibition of platelet-activating factor.^[16] *Ginkgo biloba* Extracts (GBEs), prepared from *Ginkgo Folium*, contain flavonoids and terpene lactones, which are regarded as the major bioactive components responsible for their therapeutic effects, particularly in cardiovascular disorders.^[17]

Flavonoid glycosides and terpenoids are the most important active ingredients in the extract and those have been shown to exhibit a variety of pharmacological actions.^[18]

The beneficial effects of *Ginkgo biloba* for cure of many diseases may be associated to the scavenging of free radicals and anti-lipid peroxidation, consequently dilated blood vessels, ameliorated microcirculation and inhibited cell apoptosis. In this study, we form a hypothesis that developmental epilepsy may be associated to oxidative stress as well as central nerve degenerative diseases such as Parkinson and Alzheimer's disease. In view of this situation, we aimed to use epileptic drugs that are well defined, have few side effects, have a long-lasting effect after discontinuation of treatment, and have a high efficacy when used with other new-drug antiepileptic.

MATERIALS AND METHODS

Experimental Animals and Groups

Male Wistar rats at Postnatal day 21 (P21) were provided by Laboratory Animal Centre of Chongjin College of Medical Sciences and adapted in a lab environment before experiments for a week. 60 Rats are randomly chosen and during the experiment, feed and water were available to rats at any time. The temperature was maintained at $20\pm 2^{\circ}\text{C}$ and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Chongjin College of Medical Sciences. The Institutional Animal Care and Use Committee of the Chongjin College of Medical Sciences approved the animal protocol used in this study. Rats were randomly divided into 3 groups (n=20/group): Control Group (CG), Model Group (MG) and Combinative Group of Valproate and *Ginkgo biloba* (V+G).

Developmental Epilepsy Model

Penicillin (5,600,000 U/kg dissolved in saline) or saline alone (control) was administered intraperitoneally as previously reported.^[19]

Briefly, penicillin was injected i.p. every other day in eleven consecutive days from Postnatal day 28 (P28).

Morris Water Maze Test

The Morris water maze test was used to assess visuospatial learning and memory abilities. Evaluations were performed on rats at postnatal days 45 (P45) and 66 (P66) after the last administration of penicillin. The detailed protocol of the Morris water maze has been reported earlier.^[20,21] In brief, during the place navigation phase (spatial learning acquisition, SRM), animals completed two trials per day over four consecutive days. For each trial, rats were placed into the pool facing the wall from one of four randomly assigned starting positions (north, east, south, or west). They were allowed up to 120 s to locate the submerged platform. If the platform was not reached within this time, the rat was gently placed on the platform and allowed to remain there for 20 s before being returned to its home cage. Both escape latency (time required to reach the platform) and swim paths were recorded automatically using a computerized video-tracking system. The average latency for each session was determined from the two trials completed by each animal.

For the spatial probe test (SWM), the platform was removed on the sixth day of testing. Each rat was released into the pool from the midpoint of the pool wall in the target quadrant and allowed to swim freely for 120 s. The time spent in the quadrant that previously contained the platform was measured and used as an indicator of spatial memory retention.

Administration of Sodium Valproate (VPA) and *Ginkgo biloba*

VPA and *Ginkgo biloba* extract (27.2% Ginkgo favonolucosides, 6.9% total terpene lactone and Ginkgaolic acid <1PPM) were orally administered at 300 mg/kg (twice daily) and 70 mg/kg (once daily) respectively for 17 days (until postnatal day 66) after injection of penicillin finished.

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS 16.0 and the differences between the means assessed using Dunnett's multiple range test. A p value of < 0.05 or < 0.01 was taken as the level of statistical significance.

RESULTS

Escape Latencies from the Water Maze

The escape latencies from the water maze in rats at postnatal day 45 (P45) and 66 (P66) were shown in Tables 1 and 2. The escape latencies from the water maze were significantly longer in rats of MG than CG. But combination of Valproate and *Ginkgo biloba* showed that this index was significantly shorted compared to MG.

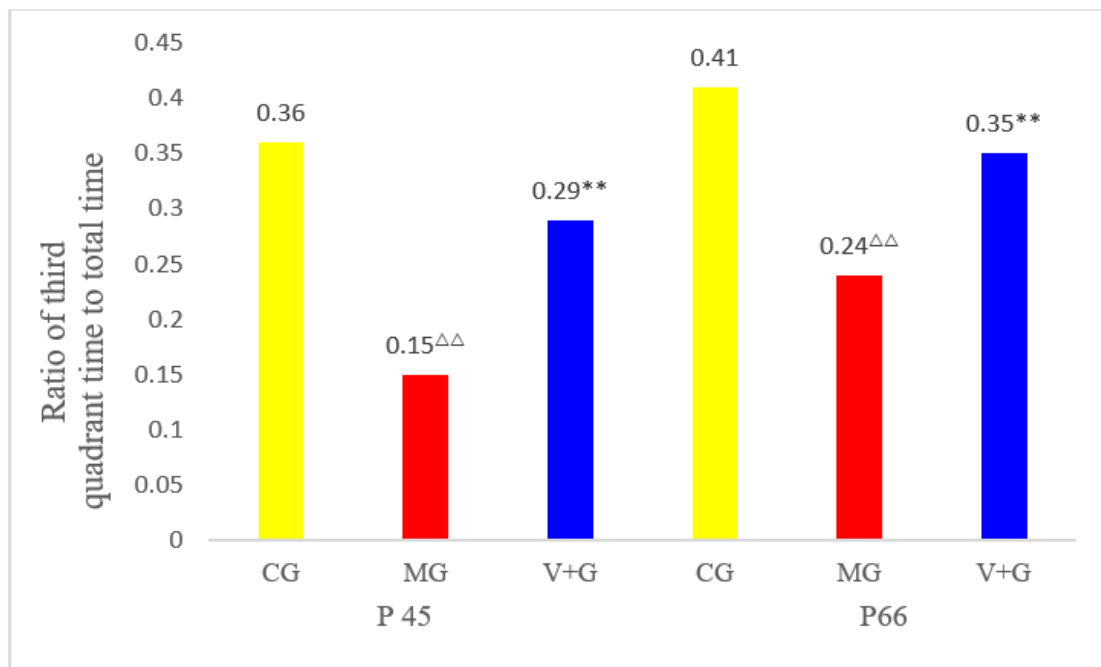


Figure 1: Spatial probe test. Each value represents the Mean \pm SEM of 20 rats per group. ** $p < 0.01$ as compared with MG. $\Delta\Delta$ $p < 0.01$ as compared with CG.

Spatial Probe Test

The results of spatial probe test in rats at postnatal day 45 (P45) and 66 (P66) were shown in Figure 1. In the spatial probe test, the ratios of third quadrant time to total swim time in MGs in rats at postnatal day 45 (P45) and 66 (P66) were significantly lower than CGs ($p < 0.01$). However, combination of Valproate and *Ginkgo biloba* showed the significant effects in this index compared to MGs (respectively $p < 0.01$).

DISCUSSION

Experimental epilepsy models have been developed to assess the pathophysiology of epileptic seizures and to search for new effective anti-epileptic drugs. Among suggested models, because penicillin has structural resemblances to GABA antagonist-bicuculline, experts have been used this anti-biotics for aim of making epilepsy models with GABA alteration. On the other hand one advantage of penicillin-induced epilepsy model is that ways of the penicillin application are diverse.

In this study, developmental epilepsy model was established by through penicillin injection in adolescence rat. In some researchers reported that penicillin-induced recurrent developmental seizures resulted in impairment of visual-spatial learning and memory when the animals were tested weeks later. VPA is widely used to treat partial and generalized seizures, acute mania, and as prophylaxis for bipolar disorder and migraine headaches. Although acute VPA intoxication frequently results in mild, self-limited central nervous system depression, serious toxicity and death have been reported. So that many patients including seizures did not want to continue treatment with VPA

Table 1: The escape latency from the water maze in rats at postnatal day 45 (P45).

Group	Escape latency(s)
CG	36.3 \pm 1.1
MG	48.6 \pm 2.5 $\Delta\Delta$
V+G	40.1 \pm 2.0*

Each value represents the Mean \pm SEM of 20 rats per group. * $p < 0.05$ as compared with MG. $\Delta\Delta$ $p < 0.01$ as compared with CG.

Table 2: The escape latency from the water maze in rats at postnatal day 66 (P66).

Group	Escape latency(s)
CG	27.5 \pm 1.8
MG	51.3 \pm 3.6 $\Delta\Delta$
V+G	38.4 \pm 3.1**

Each value represents the Mean \pm SEM of 20 rats per group. ** $p < 0.01$ as compared with MG. $\Delta\Delta$ $p < 0.01$ as compared with CG.

and try to choose the other drugs except VPA. Due to these current situations a more rational treatment method is needed for the treatment of epilepsy and epileptogenesis. On the other hand, *Ginkgo biloba* is also cultivated for the several benefits such as health foods, medication, etc. in lots of countries. Also, in DPR Korea *Ginkgo biloba* has been used as an herbal medicine for the treatment of many diseases, in particular, ischemic heart disease and is also widely used as a potent source of natural strong antioxidants in our country as well as others. So far, there were no studies to try to use herbal medicine-*Ginkgo biloba* for aim of decreasing VPA toxicity and improving cure efficacy in the treatment of epilepsy. According to our result on rats at Postnatal

day 45 (P45) and 66 (P66), the escape latencies from the water maze and the ratios of third quadrant time to total swim time in the spatial probe test were significantly alternated in MG than CG. But combination of Valproate and *Ginkgo biloba* showed significant improvement compared to MG in these indices. Thus, we suggest that these combinative effects were attributed to the anti-epileptic effects of VPA and the main active component of *Ginkgo biloba* exhibiting free radical scavenging activity. Also, in present study, we form a hypothesis that developmental epilepsy may be linked to production of oxidative stress, because *Ginkgo biloba* exhibits a potential anti-oxidant action.

CONCLUSION

Our results suggest that the combination of VPA and *Ginkgo biloba* has potential for the treatment of developmental epilepsy. However, further studies should confirm the relationship of oxidative stress during epilepsy and also investigate that it plays a definite role in pathophysiology of developmental epilepsy. It is however assumed that this combinative therapy might provide a beneficial effect in the treatment of developmental epilepsy in children.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CG: Control Group; **MG:** Model Group (Penicillin-induced seizures group); **V+G:** Valproate and *Ginkgo biloba* combination group; **VPA:** Valproic Acid (Sodium Valproate); **GBE/GBEs:** *Ginkgo biloba* Extract(s); **SRM:** Spatial Reference Memory; **SWM:**

Spatial Working Memory; **ANOVA:** Analysis of Variance; **SEM:** Standard Error of Mean; **GABA:** Gamma-Aminobutyric Acid.

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