European Journal of Pharmacology 902 (2021) 174099

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Examination of the antiepileptic effects of valacyclovir using kindling mice– search for novel antiepileptic agents by drug repositioning using a large medical information database

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ARTICLE INFO

Keywords:
Epilepsy
Valacyclovir
Large medical information database
Drug repositioning

ABSTRACT

Despite the availability of more than 20 clinical antiepileptic drugs, approximately 30% of patients with epilepsy do not respond to antiepileptic drug treatment. Therefore, it is important to develop antiepileptic products that function via novel mechanisms. In the present study, we evaluated data from one of the largest global databases to identify drugs with antiepileptic effects, and subsequently attempted to understand the effect of the combination of antiepileptic drugs and valacyclovir in epileptic seizures using a kindling model. To induce kindling in mice, pentylenetetrazol at a dose of 40 mg/kg was administered once every 48 h. Valacyclovir was orally administered 30 min before antiepileptic drug injection in kindled mice, and behavioral seizures were monitored for 20 min following pentylenetetrazol administration. Additionally, c-Fos expression in the hippocampal dentate gyrus was measured in kindled mice. Valacyclovir showed inhibitory effects on pentylenetetrazol-induced kindled seizures. In addition, simultaneous use of levetiracetam and valacyclovir caused more potent inhibition of seizure activity, and neither valproic acid nor diazepam augmented the anti-seizure effect in kindled mice. Furthermore, kindled mice showed increased c-Fos levels in the dentate gyrus. The increase in c-Fos expression was significantly inhibited by the simultaneous use of levetiracetam and valacyclovir. The findings of the present study indicate that a combination of levetiracetam and valacyclovir had possible anticonvulsive effects on pentylenetetrazol-induced kindled epileptic seizures. These results suggest that valacyclovir may have an anti-seizure effect in patients with epilepsy.

1. Introduction

Epilepsy is the most prevalent disease of the central nervous system worldwide, with approximately 30% of patients who were reported to have refractory epilepsy resistant to antiepileptic drugs (Banerjee et al., 2009). Various antiepileptic drugs have been developed. However, the detailed mechanisms underlying epilepsy remain unknown, and the development of antiepileptic drugs with novel pharmacological effects

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https://doi.org/10.1016/j.ejphar.2021.174099
Received 6 January 2021; Received in revised form 31 March 2021; Accepted 7 April 2021
Available online 25 April 2021

leaves much to be desired (Wahab, 2010).

In recent years, drug repositioning has attracted attention as a novel strategy for drug discovery and development. Drug repositioning is a method that involves the discovery of new pharmacological effects of existing drugs already used in clinical practice, and the development of these drugs for the treatment of other diseases (Ashburn and Thor, 2004; Morimoto et al., 2004). Currently, a large number of relatively easy-to-develop drugs with excellent safety and pharmacokinetics have already been developed, leaving a decreasing probability for the successful development of new drugs (Langedijk et al., 2015). It takes a considerable amount of time and money to launch new drugs and to get them approved for use in humans; however, compounds identified by drug repositioning are already in clinical use. This is highly advantageous because a large amount of information on human safety and drug pharmacokinetics is known to have a high degree of certainty; pre-existing clinical trial data can also greatly reduce the time and cost required for drug development.

Therefore, we sought to find new antiepileptic drug candidates by searching for drugs with antiepileptic action using information from the representative adverse event spontaneous report database of medicine. Reports from the Food and Drug Administration (FDA) and studies using epilepsy models have shown that the administration of cefepime exacerbates epileptic seizures (Payne et al., 2017; Tanaka et al., 2014). In this study, we first used FDA Adverse Event Reporting System (FAERS) to statistically analyze the effects of cefepime use on epileptic seizures, and to search for drugs that suppress "seizures due to Cefepime side effects." Valacyclovir, one of the drugs found in the database, is used to treat herpes simplex virus infections which exhibits antiviral effects by inhibiting DNA polymerase and DNA strand formation. In this study, we investigated the antiepileptic effects of valacyclovir using a kindling model, an animal model of intractable epilepsy. Repeated application of constant electrical stimulation below the seizure threshold, or a seizure-inducing substance such as pentylenetetrazol, to the brain gradually increases seizure size and eventually leads to generalised seizures (de Almeida Rabello Oliveira et al., 2008). The advantage of this model is that both the effect of the antiepileptic drug in the process of acquisition of epileptogenic life and the pathological mechanism of intractable epilepsy after acquisition can be examined. In this study, we used a medical information database to investigate new antiepileptic drugs, and to clarify their mechanisms based on the concept of drug repositioning.

2. Materials and methods

2.1. FAERS analysis

FAERS datasets from the FDA's website for the third quarter of 2010 to the second quarter of 2015 were downloaded. A total of 4,331,802 overlapping reports were deleted according to FDA recommendations using the most recent CASE numbers, resulting in 3,723,754 eligible reports. A post-duplicate report was used to compare the incidence of seizures between patients who received cefepime and those who did not. In addition, reported cases of "convulsion due to side effects of Cefepime" were extracted, and the concomitant medication, which suppresses convulsion, was comprehensively analyzed in the obtained 2220 reports. Drug names registered as trade names or abbreviations were converted into generic names prior to analysis according to the ICH International Pharmaceutical Glossary (MedDRA)/J ver). The following adverse events, including seizures, convulsions, or epilepsy disease names listed in 19.0, were defined as "seizures" (Table 1).

Table 1
Defined words as "seizures."

Seizure	alcoholic seizure
	partial seizures
	atonic seizures
	partial seizures with secondary generalisation
	autonomic seizure
	psychogenic seizure
	change in seizure presentation
	psychomotor seizures
	complex partial seizures
	seizure
	generalised tonic-clonic seizure
	seizure anoxic
	hyperglycaemic seizure
	seizure cluster
	hypocalcaemic seizure
	seizure like phenomena
	hypoglycaemic seizure
	simple partial seizures
	hyponatraemic seizure
Convulsion	clonic convulsion
	convulsions local
	convulsion
	drug withdrawal convulsions
	convulsion in childhood
	febrile convulsion
	convulsion neonatal
	grand mal convulsion
	convulsion prophylaxis
	tonic convulsion
Epilepsy	atypical benign partial epilepsy
	lafora's myoclonic epilepsy
	benign rolandic epilepsy
	myoclonic epilepsy
	epilepsy
	myoclonic epilepsy and ragged-red fibres
	epilepsy congenital
	petit mal epilepsy
	frontal lobe epilepsy
	post-traumatic epilepsy
	generalised non-convulsive epilepsy
	severe myoclonic epilepsy of infancy
	idiopathic generalised epilepsy
	sudden unexplained death in epilepsy
	juvenile myoclonic epilepsy
	temporal lobe epilepsy

2.2. Animal

A total of 200 four-week-old male ICR mice were used in all animal experiments (Japan Clare). All animals were kept at a temperature of 24 ± 1 °C and humidity of 40–60%. The mice were allowed to acclimate for 1 week after delivery before starting the production of kindling. All mice were allowed free access to food and drinking water. All experiments were conducted in accordance with the Tokushima University Animal Experiment Management Regulations, after being reviewed and approved by the Tokushima University Animal Experiment Committee. (Approval No. T30-85).

2.3. Kindling model

The procedure for kindling seizures was done similar to what was described in previous studies (Takechi et al., 2011; Watanabe et al., 2011). In clinical practice, it is well known that frequent generalised seizures in childhood can cause significant impairment in brain development and are likely to lead to the development of epilepsy. Therefore, 4-week-old mice that could be administered the dose, for an *in vivo* study, were used. Mice were placed in plastic cages, and pentylenetetrazol (PTZ) was administered by intraperitoneal (i.p.) injection at 40 mg/kg every other day to produce kindling mice. After administration of PTZ, the seizure symptoms of the mice were observed for 20 min. The response to kindling was evaluated using the modified Racine's scale - stage 0, no change; stage 1, slight attraction of ears and faces; stage 2, instantaneous attraction of hands and feet; stage 3, repeated contractions and relaxations of forelimbs; stage 4, clonic and rollover; stage 5, tonic-clonic seizures. When convulsions of stage 4 or 5 were induced by PTZ administration, and stages 4 or 5 convulsions were observed three

consecutive times, the kindling process would be deemed as completed, and the mice were used for experiments after a 1-week pause of PTZ administration. The mice were then randomly assigned to one of the different treatment groups. All animals received the same treatment throughout the experiments.

2.4. Valacyclovir monotherapy study

Mice with completed kindling were divided into four groups. In the first group, ICR mice received intraperitoneal PTZ (40 mg/kg) (vehicle group). In the second group, kindling mice received intraperitoneal PTZ (40 mg/kg) (kindling group). In the remaining two groups, kindling mice were orally administered valacyclovir (VACV) (300 mg/kg or 1000 mg/kg), a prodrug of acyclovir, and 60 min later, PTZ (40 mg/kg) was administered by i.p. injection. After completion of PTZ administration in the respective groups, convulsive symptoms were observed and evaluated using Racine's Scale for 20 min. The mortality rate was calculated by observing the number of mice that died during the seizure.

2.5. Effect of valacyclovir on kindling formation

Male ICR mice were divided into two groups so that the mean body weight of each group was comparable, and PTZ (40 mg/kg) was administered every other day in each group. The groups were divided into saline and VACV groups. Saline and VACV were orally administered 60 min prior to PTZ administration in the groups of mice, respectively. After administration of PTZ, convulsive symptoms were observed for 20 min, and were evaluated using the Racine's Scale.

2.6. Combination experiment of valacyclovir and anticonvulsants

Kindled mice were orally administered with VACV, and 30 min later, intraperitoneally injected with various conventional antiepileptic drugs. Thirty min after this, PTZ was administered by i.p. injection (40 mg/kg), and mice were observed for 20 min. The antiepileptic drug dose selections in this study were based on previous reports which used Racine's Scale as an evaluation index (; Mandhane et al., 2007). Four antiepileptic drugs that inhibit neuronal excitability - levetiracetam, gabapentin, lamotrigine, and perampanel - were used. Three inhibitory potentiators of valproic acid diazepam, and phenobarbital sodium salt were also studied.

2.7. Effect of chronic simultaneous use of levetiracetam and valacyclovir on kindled seizures

Kindled mice were orally administered with VACV, and 30 min later, intraperitoneally injected with levetiracetam at a dose of 50 mg/kg daily for 5 days. Thirty min after this, on the final day, PTZ was administered by i.p. injection (40 mg/kg), and mice were observed for 20 min (Fig. 1).

2.8. Changes in c-Fos expression due to administration of valacyclovir and levetiracetam in kindled mice

The mice were divided into four groups, and c-Fos expression was examined by immunostaining. In the first group, saline was administered orally, followed by administration of PTZ 60 min later (control group). In the second group, VACV was administered orally, and PTZ was administered 60 min later (VACV group). Levetiracetam was administered intraperitoneally in the third group and PTZ was

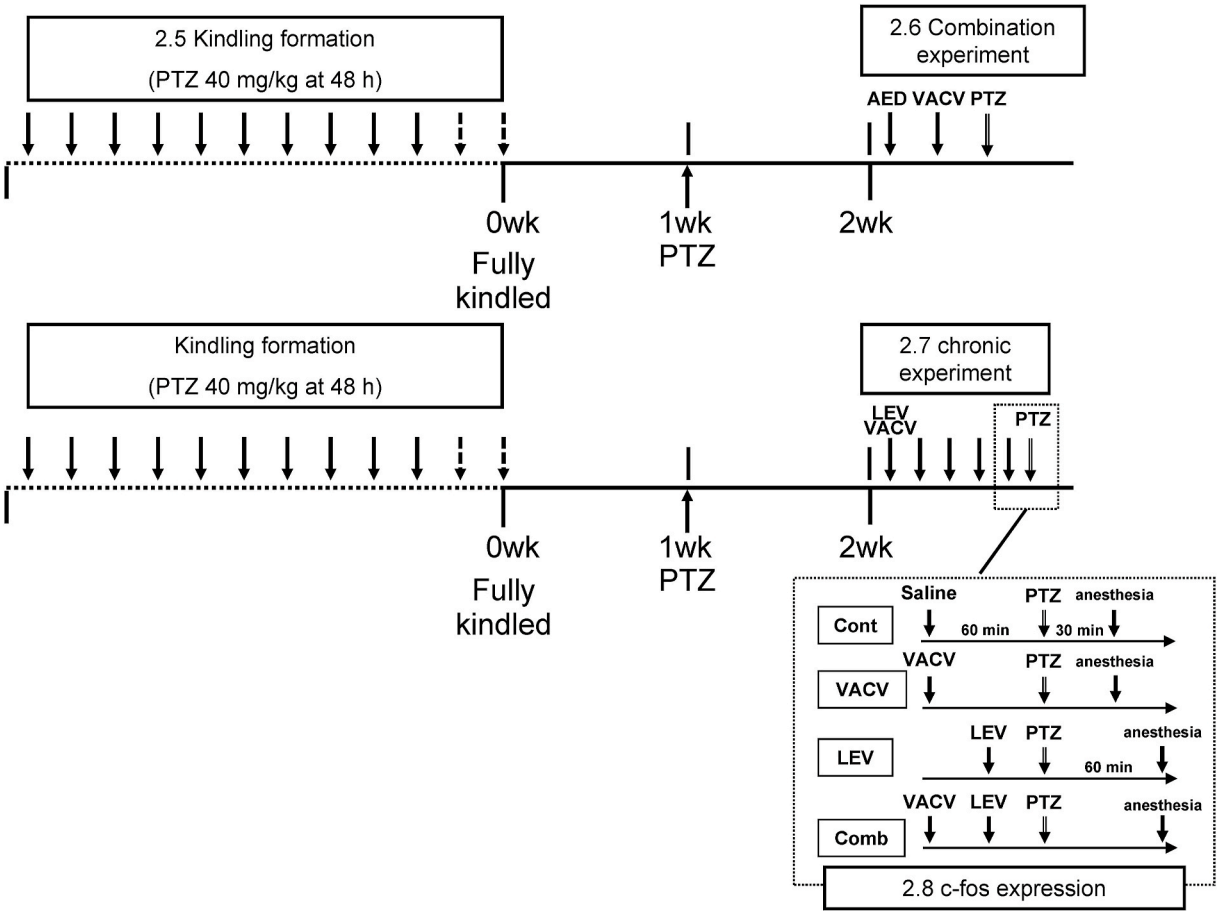

Fig. 1. Schedules for the experiment. Each number is a section of result. PTZ, pentylenetetrazol; AED, antiepileptic drugs; VACV, valacyclovir; LEV, levetiracetam.

Table 2
The relation between cefepime and convulsion.

Drug	Convulsion		ROR (95%CI)	p value
	without cefepime (%)	with cefepime (%)		
Cefepime hydrochloride	50509/3722847 (1.36)	24/907 (2.65)	1.98 (1.32–2.96)	0.002

administered 30 min later (LEV group). In all groups, convulsive symptoms were observed for 20 min after PTZ administration.

Pentobarbital solutions (50 mg/kg) were administered intraperitoneally and deeply anesthetized 30 min after administration of PTZ in the control and VACV groups and 60 min after administration of PTZ in the LEV group. To investigate changes with drug-induced c-Fos expression, the mice were anesthetized 90 min after the last administration of the drug, perfused with 50 ml of saline with 4% PFA, and their brains were extracted afterwards (Fig. 1). The brains were dissected, post-fixed for 3 h, and embedded in paraffin. The paraffin block was sliced into 4 μm sections, and heat treatment was performed with citric acid (pH 6.0) for 40 min to activate the antigen. After blocking with 1% BSA, the primary antibody (mouse anti-c-Fos; 1:200; SCB Santa Cruz Biotechnology, Inc.) reaction was carried out overnight at 4 °C. Antibody binding was visualized with 3, 3-diaminobenzidine (DAB) using the Histofine Mouse Staining Kit (Nichirei Bioscience, Tokyo, Japan). After several washes in PBS, slides were dehydrated in graded concentrations of ethanol and xylene, and then mounted in Eukitt quick-hardening medium (Sigma). After staining, the number of c-Fos-positive cells in the hippocampal dentate gyrus was counted.

2.9. Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.). Statistical analyses were performed using Bell Curve for Excel (Social Survey Research Information Co., Ltd.) and R version 3.2.1 for FAERS analysis for Fisher's exact test. Statistical analyses using Steel-Dwass tests and Mann-Whitney *U* test were performed to assess convulsive symptoms in animals with Fisher exact tests used for mortality. The effects of valacyclovir compare with control on kindling formation were computed by repeated measure analysis of variance using STATA 16. The data was compared by the area under the curve to reduce the number of tests. Levene's test, Kruskal-Wallis with Steel-Dwass *post-hoc* test were used for statistical analysis of c-Fos count in kindled mice in which $P < 0.05$ was considered statistically significant.

3. Results

3.1. Convulsions as side effects of cefepime and their relationship with concomitant drugs

The relationship between cefepime use and convulsion crises was analyzed using FAERS. As a result, 24 cases of convulsions using cefepime and 50,509 cases of convulsions not using cefepime were reported, representing seizure incidences of 2.65% and 1.36%, respectively. RORs of 1.98 (95% CI 1.32–2.96, $P = 0.002$) showed that patients who used cefepime had a significantly higher seizure rate than those who did not. FAERS was then used to search for concomitant drugs that attenuate seizures as a side effect of cefepime (Table 2). Results showed that seizure incidence was significantly lower with concurrent use of acyclovir, indicating the possibility that of its anticonvulsant effect. Table 3 shows the top three cases (P values listed in ascending order) for drugs with an ROR less than 1.

3.2. Investigation of antiepileptic effects on valacyclovir administration

We also examined whether VACV exhibited anticonvulsant action. Administration of VACV (300 mg/kg) did not significantly reduce seizure scores and showed no antiepileptic effects (Fig. 2A). Similarly, administration of VACV (1000 mg/kg) did not show any significant antiepileptic effects (Fig. 2A). There was also a trend toward reduced mortality in the group treated with VACV compared to the kindling mice (Fig. 2B).

3.3. Effects of VACV on kindling formation

The effect of VACV on kindling was also investigated. The increase in seizure scores was slower in the VACV group than in the control group. Comparatively, VACV caused significant retardation of kindling formation (Fig. 3).

3.4. Investigation of antiepileptic effects by combination of antiepileptic drugs and VACV in a kindling model

We also investigated the effect of concomitant use of VACV and pre-existing antiepileptic drugs. When combined with levetiracetam, seizure scores were found to be significantly decreased (Fig. 4A). In contrast, no significant decrease in seizure score was observed in combination with any inhibitory potentiator antiepileptic drug (Fig. 4B).

3.5. Effect of chronic simultaneous use of levetiracetam and valacyclovir on kindled seizures

We examined the effects of continuous administration of VACV and levetiracetam for 5 days in kindled mice. Simultaneous administration of levetiracetam at a dose of 50 mg/kg and valacyclovir at a dose of 300 mg/kg caused significant inhibition of the seizure stage (Fig. 5).

3.6. Changes in neural activity (immunostaining with c-Fos antibodies) due to VACV administration

The number of c-Fos-positive cells in the hippocampal dentate gyrus of the mice was counted, and the neural activity was assessed. The control group had the highest number of c-Fos-positive cells, and the VACV + LEV group had the lowest number of c-Fos cells (Fig. 6B).

Table 3
Search for concomitant drugs that attenuate seizures.

DrugB	Cefepime users Convulsion		ROR (95%CI)	p value
	without Drug B (%)	with Drug B (%)		
Acyclovir	60/1838 (3.26)	5/382 (1.31)	0.39 (0.16–0.99)	0.046
Furosemide	58/1770 (3.28)	7/450 (1.56)	0.47 (0.21–1.03)	0.059
Micafungin sodium	63/1985 (3.17)	2/235 (0.85)	0.26 (0.06–1.01)	0.063

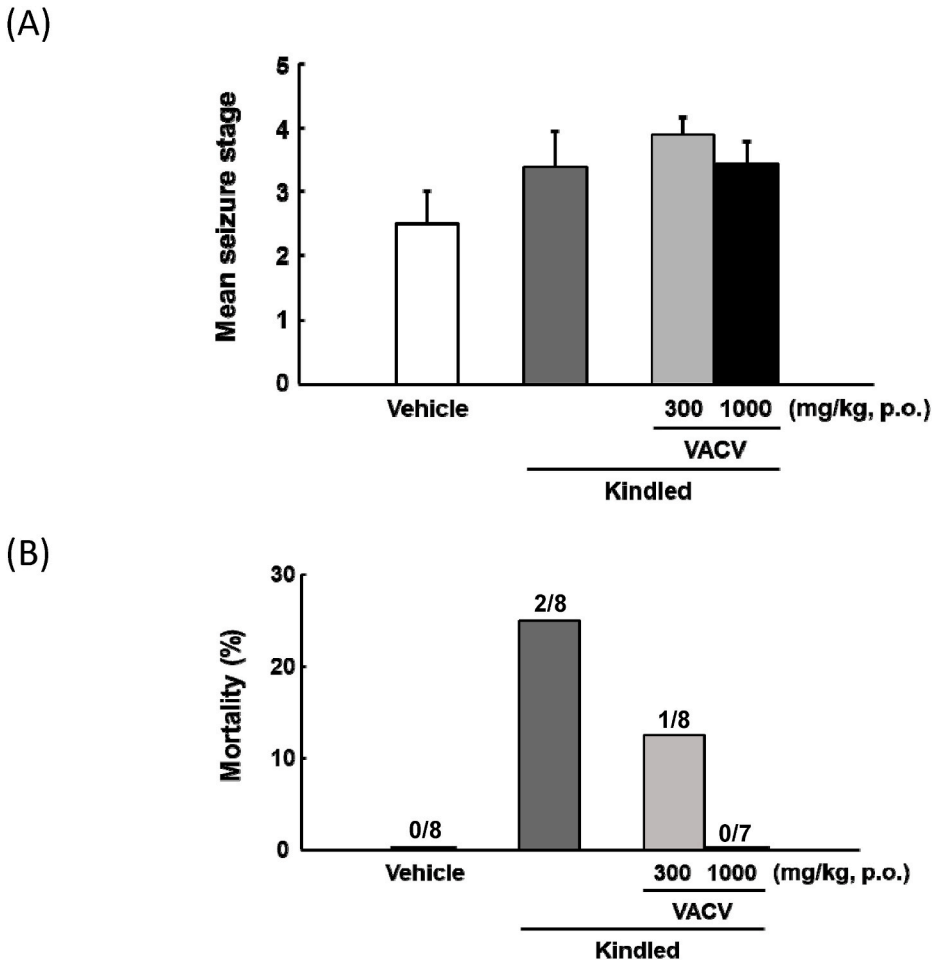

Fig. 2. Effects of valacyclovir monotherapy on kindled mice. VACV: valacyclovir (300 or 1000 mg/kg, p.o.) Each value represents the mean ± S.E.M. of 7–8 mice.

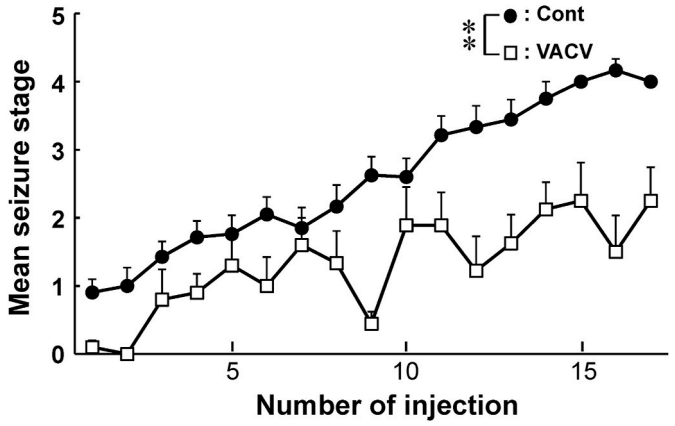

Fig. 3. Effect of valacyclovir on kindling formation. VACV: valacyclovir (300 mg/kg, p.o.) Each value represents the mean ± S.E.M. of 10–21 mice. **P < 0.01 vs. control group.

4. Discussion

Epilepsy is a common disease that affects approximately 1% of the population, and has currently brought about the campaign of the development of various antiepileptic drugs for clinical use. However, refractory epilepsy, a variant that is resistant to therapy even when multiple antiepileptic drugs are used, is found in approximately 30% of all epileptic patients. This leaves the development of antiepileptic drugs with novel pharmacological effects as highly desirable (Banerjee et al., 2009). In this study, existing drugs with antiepileptic actions were searched from the viewpoint of drug repositioning. Our results raised the possibility of an antiepileptic action of acyclovir, as indicated by the database analysis using FAERS. The antiepileptic action of acyclovir was subsequently verified using a kindling model of epilepsy.

Previous FDA reports and studies using epilepsy models have shown that the administration of cefepime exacerbates epileptic seizures (Payne et al., 2017; Tanaka et al., 2014). Here, we first studied the effects of cefepime usage on epileptic seizure crisis using FAERS statistical analysis, and searched for drugs that might suppress “convulsions as a side effect of cefepime.” The analysis showed that patients who used cefepime had approximately twice the incidence of seizures when compared to patients who did not, which was statistically significant. This was found to be statistically significant and was consistent with FDA reports and results from previous studies (Payne et al., 2017; Tanaka et al., 2014). A comprehensive analysis of pharmaceutical products used in conjunction with cefepime revealed that 92 compounds, including furosemide and bumetanide, which had already been reported to have antiepileptic effects, had an ROR of less than 1 in combination (Haglund and Hochman, 2005; Hochman et al., 1995; Koyama et al., 2012).

Following the results of the database analysis, we verified the antiepileptic effect of acyclovir using VACV, its prodrug form, in a single administration experiment, a kindling formation experiment, and a combination experiment with existing antiepileptic drugs. Oral administration was chosen based on the concept of drug repositioning, and the need for repeat administration multiple times due to acyclovir’s low

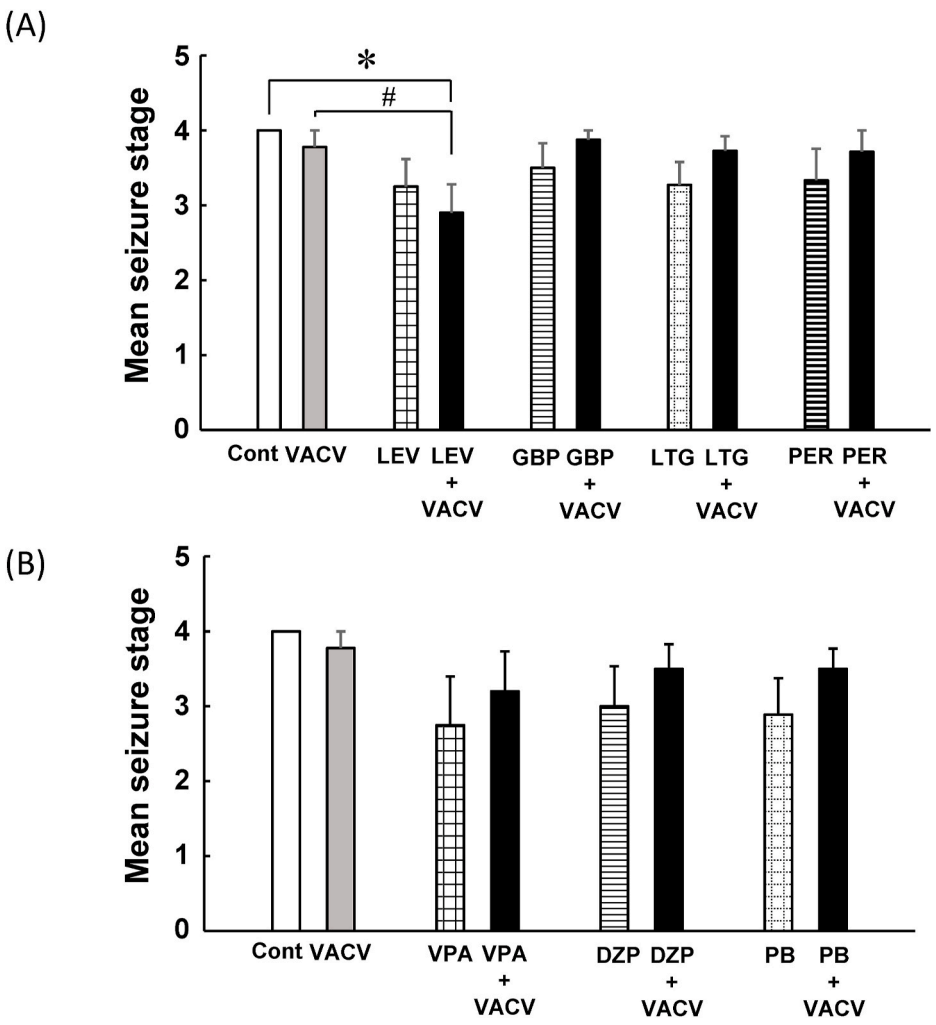

Fig. 4. The antiepileptic effects by combination of antiepileptic drugs and VACV for kindling. VACV: valacyclovir (300 mg/kg, p.o.) (A) Effect of four antiepileptic drugs that inhibit neuronal excitability. LEV: levetiracetam (50 mg/kg, i.p.), GBP: gabapentin (50 mg/kg, i.p.), LTG: lamotrigine (5 mg/kg, i.p.), PER: perampanel (1 mg/kg, i.p.) (B) Effect of three inhibitory potentiators. VPA: valproic acid (500 mg/kg, i.p.), DZP: diazepam (0.2 mg/kg, i.p.), PB: phenobarbital sodium salt (10 mg/kg, i.p.). Each value represents the mean ± S.E.M. of 6–11 mice. *P < 0.05 vs. control group; #P < 0.05 vs. VACV group.

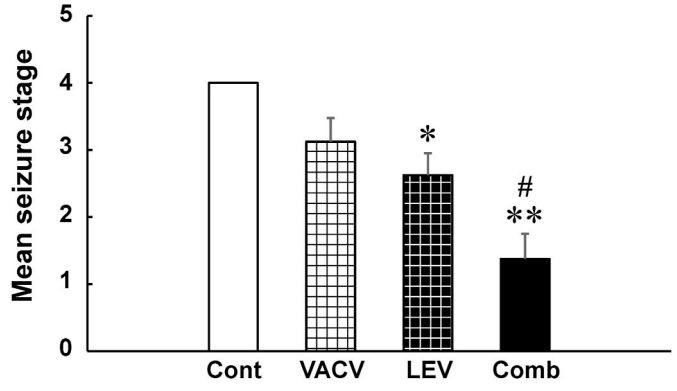

Fig. 5. Effect of chronic simultaneous use of levetiracetam and valacyclovir on kindled seizures. Cont; control, VACV; valacyclovir (300 mg/kg, p.o.), LEV; levetiracetam (50 mg/kg, i.p.), Comb; valacyclovir and levetiracetam Each value represents the mean ± S.E.M. of 8 mice. *P < 0.05 vs. control group; **P < 0.01 vs. control group. #P < 0.05 vs. VACV group.

absorptivity. In addition to this, it was difficult to continue chronic administration in the kindling formation model; therefore, VACV, which requires less frequent administration, was used as an alternative. VACV is rapidly metabolized and converted to acyclovir, a small molecule capable of penetrating the blood-brain barrier, after oral administration; This mechanism explains VACV's superior oral absorbency compared to acyclovir, which increases the quantity of acyclovir reaching the brain upon administration (Laskin, 1983).

First, VACV was tested at two concentrations, 300 mg/kg and 1000 mg/kg, but no significant reduction in seizure scores was observed in either group, indicating that VACV alone had no antiepileptic effect. Previous studies have shown that oral administration of 300 mg/kg of VACV to mice suppresses HSV-1-mediated inflammation in the piriform cortex and the main sensory nucleus of the trigeminal nerve, and that the single dose used in this study may have been adequate to suppress central inflammation, but inadequate to suppress seizures (Wu et al., 2004). However, the VACV dose was set to 300 mg/kg in the subsequent experiments as the very high 1000 mg/kg dose increased the risk of adverse events, such as anaphylactic shock and psychoneurotic symptoms, and because a previous study using VACV used the same dose. Mortality also tended to decrease with the administration of VACV (300 and 1000 mg/kg) in this study.

Chronic administration of VACV was subsequently carried out in kindling mice to examine its effects on kindling formation. We observed that the increase in seizure scores tended to be slower in the VACV group than in the saline group; therefore, we postulated that the chronic administration of VACV might suppress kindling epileptogenicity and delay the severity and refractoriness of epilepsy.

In the experiments combining the use of VACV with existing antiepileptic drugs, we examined the effect of VACV on the antiepileptic action of seven existing drugs, including new ones. Additionally, we investigated the effect of chronic simultaneous use of levetiracetam and valacyclovir on kindled seizures for five days. We found that seizure

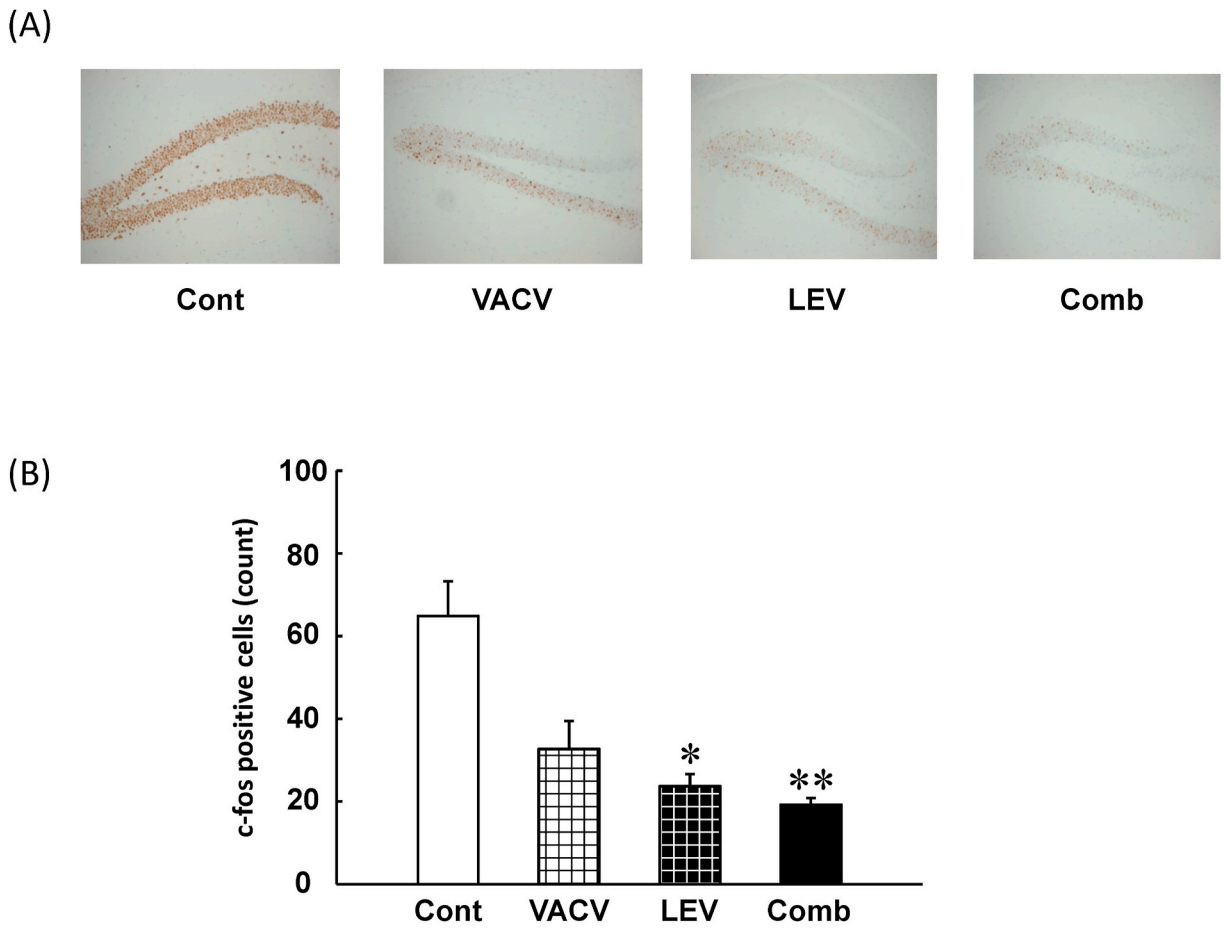

Fig. 6. Changes in neural activity (c-Fos mapping) due to VACV administration. (A) Representative example of the c-Fos in immunohistochemistry. Cont; control, VACV; valacyclovir (300 mg/kg, p.o.), LEV; levetiracetam (50 mg/kg, i.p.), Comb; valacyclovir and levetiracetam (B) The expression of c-Fos in the dentate gyrus was measured. Each value represents the mean ± S.E.M. of 5–8 mice. *P < 0.05 vs. control group; **P < 0.01 vs. control group. Levene's test (P = 0.0073), Kruskal-Wallis with Steel-Dwass *post-hoc* test.

scores were significantly lowered by the combined use of VACV and levetiracetam, indicating the possibility of enhancing acyclovir's antiepileptic action in combination with levetiracetam. All of the existing drugs used in this study, with the exception of levetiracetam, target ion channels and receptors, and exert antiepileptic effects through ion regulation. Levetiracetam exerts antiepileptic effects primarily through its action on the synaptic vesicle protein SV2A (Lynch et al., 2004). SV2A is widely expressed in the brain, particularly in the cerebral cortex, hippocampus, and cerebellum (Bajjalieh et al., 1994). It has been shown that expression specificity is higher in GABA nerves than in glutamate nerves in all regions, and that SV2A is specifically expressed in the GABA-intervening nerves, particularly in the pyramidal cell layer of the hippocampal dentate ileum region and CA1-CA3 regions (Bajjalieh et al., 1993; Bragina et al., 2011; Ohno et al., 2009, 2012; Toering et al., 2009; Tokudome et al., 2016; Wu et al., 2004). It has also been reported that the expression of SV2A is decreased in the hippocampus and cerebral cortex in both human epilepsy patients and in models of epilepsy (Feng et al., 2009; Hanaya et al., 2012; Van Vliet et al., 2009). The decrease in convulsion scores observed with the combination of VACV and levetiracetam was attributed to the binding of levetiracetam to SV2A, which may be promoted by VACV through the alterations of levetiracetam kinetics and blood levels. With previous studies reporting levetiracetam's delay in kindling and antiepileptogenic effect, and this study's report which showed that VACV tended to suppress epileptogenicity, it is highly suggestive that VACV enhanced the epileptogenic inhibitory effect of levetiracetam. Therefore, we surmise that VACV exerts its antiepileptic action through both anticonvulsant and antiepileptogenic actions.

Finally, to examine the mechanism of the antiepileptic action of VACV, changes in c-Fos expression in the brain were examined. c-Fos is widely known as an indicator of neuronal activity, and it has been reported that c-Fos rapidly accumulates in the hippocampal area during seizure development (Herrera and Robertson, 1996). In this study, neuronal activity was assessed by counting the number of c-Fos-positive cells in the hippocampal dentate gyrus following VACV administration. The expression of c-Fos-positive cells in mice treated with VACV and LEV was investigated in the dentate gyrus. We subsequently found that the number of c-Fos-positive cells was highest in kindled mice (control group), while the number of positive cells in the combination of LEV and VACV groups was smaller. Based on these results, we hypothesized that administration of levetiracetam suppressed the excitatory system. Similarly, suppression of the excitatory system was also induced by VACV administration, as evidenced by the decreased number of c-Fos-positive cells observed in the VACV group compared to the control group.

Our drug repositioning study using large-scale medical information databases suggested that the antiherpes drug acyclovir may also have an antiepileptic effect. We further showed that the antiepileptic effect was significantly enhanced with the combined use of VACV, an acyclovir prodrug, and levetiracetam in experiments using the kindling mouse epilepsy model. In addition, since c-Fos-positive cells decreased following administration of VACV, we considered that VACV might suppress nerves of the excitatory system and thereby have an anticonvulsant effect.

Credit author statement

We listed the following researchers as authorship member for this paper.

Shimon Takahashi contributed to study conception and design, acquisition of data, analysis of data, drafting the manuscript and final approval of the version to be published. He agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kenshi Takechi contributed to study conception and design, acquisition of data, analysis of data, drafting the manuscript and final approval of the version to be published. He agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Natsumi Jozukuri contributed to analysis and interpretation of data, revising it critically for important intellectual content and final approval of the version to be published. She agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Takahiro Niimura contributed to analysis and interpretation of data, revising it critically for important intellectual content and final approval of the version to be published. He agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number 17K17922. We would like to thank Editage (www.editage.com) for English language editing.

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