Purpose: This study explored the pharmacodynamic and pharmacokinetic effects of combining perampanel (PER) with commonly co-administered AEDs.

Method: A strong stimulus intensity (three-fold higher than after-discharge threshold) was used to elicit drug-resistant seizures in a rat amygdala kindling model. Vehicle, low-dose PER (0.75 mg/kg), or high-dose PER (1.5 mg/kg), in combination with vehicle, levetiracetam (LEV) 50 mg/kg, lamotrigine (LAM) 20 mg/kg, carbamazepine (CBZ) 20 mg/kg, or valproic acid (VPA) 200 mg/kg, were administered intraperitoneally to groups of 6–13 rats. Seizure score, electroencephalography (EEG) seizure duration, and motor seizure duration were evaluated, with pharmacodynamic interactions determined by two-way analysis of variance (ANOVA). Motor impairment was evaluated by rotarod test and two-way ANOVA.

Results: High-dose PER, but not low-dose PER, LEV, LAM, CBZ, or VPA, reduced EEG seizure duration, motor seizure duration, and seizure score compared with vehicle alone. However, when low-dose PER was administered in combination with LEV, LAM, CBZ, or VPA, seizure severity parameters were reduced compared with the concomitant AEDs alone. These pharmacodynamic interactions were statistically significant in some cases, but the same AED combinations were not associated with statistically significant neurotoxic interactions. Efficacy may have been slightly affected by changes in PER plasma concentrations in the presence of other AEDs: PER plasma concentrations increased with LEV or LAM co-administration, and decreased with CBZ or VPA co-administration.

Conclusion: Overall, these data support published Phase III data demonstrating the efficacy of PER as adjunctive therapy for the treatment of refractory partial-onset seizures in patients aged ≥12 years.
Pharmacodynamic and pharmacokinetic interactions of perampanel and other antiepileptic drugs in a rat amygdala kindling model

Ting Wu, Yoko Nagaya, Takahisa Hanada

Highlights

- Antiepileptic drugs (AEDs) were administered in a rat amygdala kindling model
- At the doses tested, individual AEDs had no effect on seizure severity parameters
- Combination of perampanel with other AEDs reduced seizure severity parameters
- Perampanel plasma concentrations could be affected by the presence of other AEDs
- These findings support Phase III data and provide insight into AED interactions
Pharmacodynamic and pharmacokinetic interactions of perampanel and other antiepileptic drugs in a rat amygdala kindling model

Ting Wu, a Yoko Nagaya, b Takahisa Hanada a,c

aGlobal Biopharmacology, Neuroscience & General Medicine Product Creation System, Eisai Co Ltd, Tsukuba, Ibaraki, Japan; bDrug Metabolism and Pharmacokinetics Japan, Eisai Product Creation Systems Eisai Co Ltd, Tsukuba, Ibaraki, Japan; cCenter for Tsukuba Advanced Research Alliance, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

Author contact details:

Ting Wu, Eisai Co. Ltd, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan; tel: +81 29 847 6824; fax: +81 29 847 2037; email: t2-wu@hhc.eisai.co.jp.

Yoko Nagaya, Eisai Co. Ltd, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan; tel: +81 29 847 5684; fax: +81 29 847 5672; email: y-nagaya@hhc.eisai.co.jp.

Takahisa Hanada (corresponding author), Eisai Co. Ltd, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan; tel: +81 28 847 6944; fax: +81 29 847 5738; email: t-hanada@hhc.eisai.co.jp.

Running title: Perampanel combinations in a rat model (39/40 characters max, incl. spaces)

No. words (main body of article only): 3084 (maximum 4000)

No. figures/tables: 6/6 (plus one supplementary figure)
Abstract

**Purpose:** This study explored the pharmacodynamic and pharmacokinetic effects of combining perampanel (PER) with commonly co-administered AEDs.

**Method:** A strong stimulus intensity (three-fold higher than after-discharge threshold) was used to elicit drug-resistant seizures in a rat amygdala kindling model. Vehicle, low-dose PER (0.75 mg/kg), or high-dose PER (1.5 mg/kg), in combination with vehicle, levetiracetam (LEV) 50 mg/kg, lamotrigine (LAM) 20 mg/kg, carbamazepine (CBZ) 20 mg/kg, or valproic acid (VPA) 200 mg/kg, were administered intraperitoneally to groups of 6–13 rats. Seizure score, electroencephalography (EEG) seizure duration, and motor seizure duration were evaluated, with pharmacodynamic interactions determined by two-way analysis of variance (ANOVA). Motor impairment was evaluated by rotarod test and two-way ANOVA.

**Results:** High-dose PER, but not low-dose PER, LEV, LAM, CBZ, or VPA, reduced EEG seizure duration, motor seizure duration, and seizure score compared with vehicle alone. However, when low-dose PER was administered in combination with LEV, LAM, CBZ, or VPA, seizure severity parameters were reduced compared with the concomitant AEDs alone. These pharmacodynamic interactions were statistically significant in some cases, but the same AED combinations were not associated with statistically significant neurotoxic interactions. Efficacy may have been slightly affected by changes in PER plasma concentrations in the presence of other AEDs: PER plasma concentrations increased with LEV or LAM co-administration, and decreased with CBZ or VPA co-administration.

**Conclusion:** Overall, these data support published Phase III data demonstrating the efficacy of PER as adjunctive therapy for the treatment of refractory partial-onset seizures in patients aged ≥12 years.
Key words: localization-related epilepsy; kindling; EEG; antiepileptic drugs

Abstract word count: 248/250
Introduction

It has been estimated that 20–40% of patients with epilepsy will experience partial-onset seizures that are refractory to current interventions.\(^1\) While combination therapies are commonly implemented, clinical studies have provided limited data to guide the appropriate and effective combination of antiepileptic drugs (AEDs), and currently administered combinations tend to be selected based on their potential for minimal pharmacokinetic or pharmacodynamic interactions rather than their clinical efficacy.\(^2\) The development of rational combination therapies may be facilitated by the identification of AEDs with discrete mechanisms of action and well-established pharmacodynamic profiles.

Perampanel (PER), a noncompetitive \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor antagonist, is a structurally novel, first-in-class AED that is approved in the USA and Europe for the adjunctive treatment of refractory partial-onset seizures in patients aged \(\geq\)12 years.\(^3,4\) The perampanel approvals were based on efficacy and safety data from three Phase III registration trials.\(^5-7\) In a pooled analysis of pharmacokinetic and pharmacodynamic data from the registration trials, a significant relationship was shown to exist between increases in PER plasma concentration (i.e., systemic exposure) and reductions in seizure frequency.\(^8\)

The discrete mechanism of action of PER may be expected to complement the mechanism of action of other currently available AEDs.\(^9,10\) In the Phase III trials, PER efficacy appeared consistent across subgroups of patients receiving any of the four most commonly administered concomitant AEDs (i.e., levetiracetam [LEV], lamotrigine [LAM], carbamazepine [CBZ], and valproic acid [VPA]).\(^11\) However, it is important to note that 86%

---

*Non-standard abbreviations used in the article: CBZ, carbamazepine; LAM, lamotrigine; LEV, levetiracetam; PER, perampanel; VPA, valproic acid*
of patients were receiving at least two concomitant AEDs, which may have included enzyme-inducing AEDs. Consequently, such polytherapy, as well as the complexity of epilepsy, make it difficult to estimate pharmacodynamic interactions between PER and specific AEDs in this clinical setting. A clearer picture may be provided by preclinical studies, where variables can be manipulated in an experimental system.

The rat amygdala kindling model, an experimental system in which the number of repeated brief seizures can be controlled, allows investigation of the progressive nature of epilepsy. As the animals exhibit a plethora of molecular, cellular, and network alterations reflecting those reported in patients, the model is clinically relevant for investigating the effect of treatment with concomitant AEDs on refractory partial-onset seizures. Historically, experience with various AEDs has supported the predictive validity of the amygdala kindling model for the treatment of focal and generalized tonic-clonic seizures in the clinical setting.

Seizures induced in the amygdala kindling model by a strong stimulus intensity are associated with reduced AED efficacy and may, therefore, provide a suitably sensitive model for assessing the pharmacodynamic interactions of AEDs.

Here, a drug-resistant rat amygdala kindling model was used to explore interactions between PER and the four most commonly co-administered AEDs in the Phase III registration trials (LEV, LAM, CBZ, and VPA), by studying the effects of combinations of these AEDs on seizure score, electroencephalography (EEG) seizure duration, motor seizure duration, neurotoxicity and plasma AED concentrations.
Materials and methods

Animals

Male Wistar Kyoto rats (Charles River Laboratories Japan Inc., Kanagawa, Japan), weighing 250–400 g were used for all experiments. Animals were housed in cages in a controlled environment (constant temperature 22 ± 1°C; humidity 50–60%; 12-h dark/light cycle [lights on between 07:00 and 19:00]) and had free access to food (MF; Oriental Yeast Co., Tokyo, Japan) and water. All animal experiments were approved by the Committee for the Welfare of Laboratory Animals of Eisai Co. Ltd.

Stereotaxic surgery

Animals were acclimatized for at least 1 week prior to surgery. On the day of surgery, rats were anesthetized with pentobarbital 65 mg/kg (Somnopentyl; Kyoritsu Seiyaku Co. Ltd, Tokyo, Japan) administered intraperitoneally (i.p.). A tripolar electrode (TN201-059; Unique Medical Co. Ltd, Tokyo, Japan) was implanted into the basolateral amygdala (anterior–posterior: -2.5 mm; lateral: -4.8 mm; depth: -7.5 mm) according to the coordinates of Paxinos and Watson. A reference electrode was placed on the contralateral cortex. Electrodes were fixed to the skull with acrylic dental cement. After electrode implantation, rats were returned to their home cage and allowed to recover.

Amygdala kindling

After at least 1 week of recovery, the after-discharge threshold (ADT) was determined for each rat. To achieve this, the rat amygdala was stimulated using an electronic stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) and the following parameters: 500 μA, 1 ms, monophasic square-wave pulses, 50/s for 1 s. Stimulation was initiated at 0.04 mA and was then increased by 25% every 30 s until the ADT was elicited. The ADT was defined as the
point when abnormal EEG, and a behavioral seizure of at least Racine stage-1, was observed. Racine seizure stage was classified as follows: (1) mouth and facial movements; (2) head nodding; (3) unilateral forelimb clonus; (4) rearing and bilateral forelimb clonus; and (5) rearing and falling.

**Drugs**

PER (Eisai Co. Ltd, Tokyo, Japan), LEV (Tokyo Chemical Industry Co. Ltd, Tokyo, Japan), LAM (AK Scientific Inc., California, USA), CBZ, and VPA (Wako Pure Chemical Industries, Ltd, Tokyo, Japan) were dissolved in a 1:1:1 mixture of water, dimethyl sulfoxide, and polyethylene glycol-200 (hereafter denoted as vehicle). Drugs were administered intraperitoneally 30 minutes (PER, LEV, CBZ, VPA) or 60 minutes (LAM) prior to ADT evaluation.

**Dose selection**

The effects of PER 0–1.5 mg/kg, LEV 0–50 mg/kg, LAM 0–20 mg/kg, CBZ 0–30 mg/kg, and VPA 0–400 mg/kg on ADT were assessed in groups of 5–13 rats. Mean changes in ADT were compared with pretreatment data using one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test (significance level p < 0.05). Doses that significantly elevated ADT were selected for use in the subsequent analyses.

**Pharmacodynamic interaction analyses**

Seizures induced by high-intensity stimuli (three-fold higher than the ADT stimulus [3ADT]) were used to establish a drug-resistant amygdala kindling model. This intensity of stimulation was selected to increase the sensitivity of the study, as some AEDs begin to lose anti-seizure effects at this high intensity.17
Effects of individual AEDs and combinations of AEDs on Racine seizure score, EEG seizure duration (duration from start of spiking EEG activity to the end of continuous spiking activity), and motor seizure duration (duration of Racine score 4–5 seizures) were evaluated using the 3ADT-stimulus rat amygdala kindling model in groups of four to eight rats per treatment group (crossover between treatment groups led to actual sample sizes of 8–38 rats). Mean seizure score was presented as a score ranging from 0 to 5 (0, no seizure behavior; 5, full motor seizure); mean EEG seizure duration and motor seizure duration were presented as percentages of pretreatment values.

For the effects of individual AEDs, changes in seizure parameters from pretreatment were analyzed using a one-way ANOVA (significance level \( p < 0.05 \)).

To explore the pharmacodynamic interactions of LEV, LAM, CBZ, or VPA when combined with vehicle, low-dose PER, or high-dose PER, a non-parametric Steel-Dwass test was used to compare seizure score, and a parametric Tukey’s multiple comparison test was used to compare EEG seizure duration and motor seizure duration (significance level \( p < 0.05 \)).

In addition, the same data set was analyzed for pharmacodynamic interactions between PER and the other AEDs using a two-way ANOVA. These analyses allowed the contributions of perampanel (analysis not specific to low or high dose), and each concomitant AED, to be evaluated as part of the combination, such that the contribution of each AED to the overall anti-seizure effects could be analyzed. A non-parametric ANOVA was used to analyze effects on seizure score, while a parametric ANOVA was used to analyze effects on EEG seizure duration and motor seizure duration. If positive interactions were statistically significant (significance level \( p < 0.05 \)), with greater effects than the sum of the effects achieved with the individual AEDs, then the interactions were defined as significant. Alternatively, if interactions were associated with similar, or almost similar, effects compared
with the sum of those achieved with the individual AEDs, then the interactions were considered non-significant.

**Rotarod test**

A rotarod test was performed to explore the potential for motor impairment, as a form of neurotoxicity, when animals were treated with low-dose PER, LEV, LAM, CBZ, or VPA, alone or in combination.

Rats were introduced to the rotating rod during a three-day training period. On the day of testing, rats were placed on a rod with a constant rotating speed of 6 rpm, and the baseline time to fall for each animal was averaged over two attempts (cut-off: 120 s); those who remained on the rod for a mean time of >90 s were used for the test. During the test, AEDs were administered as previously described to groups of six rats. Twenty minutes after administration, the rats were placed on a rod with a constant rotating speed of 6 rpm and the latency time to fall was recorded.

The effects of individual treatment with low-dose PER, LEV, LAM, CBZ, or VPA were analyzed using Dunnett’s multiple comparisons (significance level p < 0.05). A two-way ANOVA was used to assess the interaction of low-dose PER with the other AEDs (significance level p < 0.05).

**Plasma concentrations and pharmacokinetic analyses**

Groups of four rats each were treated with low-dose PER, LEV, LAM, CBZ, or VPA plus vehicle, or combinations of low-dose PER with each of the other four AEDs. Plasma samples were obtained from each animal, deproteinized with acetonitrile and filtered. They were then diluted 20-fold with 50% acetonitrile and concentrations were analyzed using a Waters LC/MS/MS system (Waters Co., Massachusetts, USA).
For PER, LEV, LAM, and CBZ, ionization mode was positive electrospray ionization. Chromatography was performed using a Chromolith® FastGradient RP-18e (2.0 mm i.d. × 50 mm; Merck, Darmstadt, Germany). The mobile phases were: (A) water containing 0.1% formic acid; (B) acetonitrile containing 0.1% formic acid. The initial condition was (A) 100%, and (B) was increased linearly to 50% or 80% over 3 minutes. The monitoring ions were: PER, 350.2/219.3; LEV, 170.9/126.0; LAM, 256.0/108.9; and CBZ, 237.3/194.0. Flow rate was 0.3 mL/min.

For VPA, ionization mode was negative electrospray ionization. Chromatography was performed using LUNA 5u C18(2) 100Å (2.0 mm i.d. × 50 mm; Phenomenex, California, USA). The mobile phases were: (A) 1 mol/L ammonium acetate/water (1:200 v/v) and (B) 1 mol/L ammonium acetate/water/acetonitrile (1:20:180 v/v/v). The initial condition was (A) 90%, and (B) 10%, increasing linearly to 80% over 3 minutes. The monitoring ion of VPA was 142.9. Flow rate was 0.3 mL/min.

An unpaired t-test was used to compare plasma concentrations of an AED when administered together with a concomitant AED with plasma concentrations when administered with vehicle only (significance level p < 0.05).

Results

Dose selection

All drug effects on ADT were dose-dependent. PER conferred significant changes from the pre-treatment ADT at a dose of 1.5 mg/kg, and LEV, LAM, CBZ, and VPA demonstrated significant effects from doses upwards of 25 mg/kg, 10 mg/kg, 10 mg/kg, and 200 mg/kg, respectively (Table 1). All changes from pre-treatment ADT were below 300%. The doses
selected for subsequent analyses were PER 0.75 mg/kg (low dose), PER 1.5 mg/kg (high
dose), LEV 25 mg/kg, LAM 10 mg/kg, CBZ 10 mg/kg, and VPA 200 mg/kg.

When the 3ADT stimulus was applied, high-dose PER significantly reduced seizure score,
EEG seizure duration, and motor seizure duration compared with vehicle alone (p < 0.0001
for all outcomes; Fig. 1). In contrast, low-dose PER, LEV 50 mg/kg, LAM 20 mg/kg, CBZ
20 mg/kg, or VPA 200 mg/kg did not significantly reduce any of these seizure parameters
(Fig. 1 and 2).

**Pharmacodynamic interaction analyses**

While individually they had no effect on seizure score or duration, the combination of low-
dose PER with each of the four AEDs significantly reduced all seizure parameters compared
with the respective AED alone. In many cases, there were also significant reductions
compared with low-dose PER alone (Fig. 3).

Two-way ANOVA indicated that all interactions were positive (Table 2). Significant
interactions were observed in the parameter of seizure score for PER plus CBZ or VPA, in
the parameter of EEG seizure duration for PER plus VPA, and in the parameter of motor
seizure duration for PER plus LEV, LAM, CBZ or VPA. On the other hand, non-significant
interactions were observed in the parameter of seizure score for PER plus LEV or LAM and
in the parameter of EEG seizure duration for PER plus LEV, LAM or CBZ.

**Rotarod test**

Individual treatment with low-dose PER, LAM, CBZ, or VPA, but not LEV, reduced the
mean latency time to fall from baseline (Supplementary Fig. 1). Low-dose PER, CBZ, and
VPA each conferred statistically significant reductions of over 50% from baseline, compared
with vehicle alone (vehicle, 0.2% reduction). Interactions between low-dose PER and each of the other four AEDs were not statistically significant.

**Plasma concentrations and pharmacokinetic analyses**

Administration of low-dose PER did not influence the plasma concentrations of concomitant LEV, LAM, CBZ, or VPA (Table 3). However, the plasma concentration of low-dose PER was significantly increased when co-administered with LEV 50 mg/kg (+17.2%) or LAM 20 mg/kg (+38.2%), and reduced when co-administered with CBZ 20 mg/kg (-18.2%) or VPA 200 mg/kg (-31.4%).

**Discussion**

The rat amygdala kindling model was used as an appropriate and clinically relevant approach to assess the effects of PER in combination with other AEDs. Of note, drug effects in this model have been shown to be predictive of effects on complex partial seizures with secondary generalization in humans.13 AED doses were selected that significantly elevated the ADT threshold in the conventional amygdala kindling model, but had no effect on seizure parameters when a 3-ADT stimulus was applied to create a condition that would be less sensitive to AEDs.17 This was intended to simulate the clinical situation, where standard doses may improve seizure outcomes in patients with epilepsy (ADT), but have no impact in patients with refractory epilepsy (3-ADT). In accordance with this model, plasma concentrations of the AEDs were within the therapeutic range reported in patients with epilepsy.19 Furthermore, results from the rotarod test indicated motor impairment with all tested AEDs except for LEV, supporting sufficient exposure levels to these AEDs.
Although none of the individual AEDs (low-dose PER, LEV, LAM, CBZ, and VPA) demonstrated efficacy when administered individually in the 3ADT-stimulus model of refractory epilepsy, low-dose PER combined with concomitant LEV, LAM, CBZ, or VPA provided reductions in all seizure parameters. The effects of these combinations were significant compared with the individual effects of the respective concomitant AEDs, and in many cases were also significant when compared with the individual effects of low-dose PER.

These observations are consistent with the mechanism of action of PER complementing the mechanism of action of other currently available AEDs in refractory epilepsy,\(^9,10\) thus supporting its current indication as an adjunctive treatment for refractory partial-onset seizures in patients aged \(\geq12\) years.\(^3,4\) In addition, we suggest that these observations are consistent with the hypothesis that PER has an effect on both seizure initiation and propagation, and that PER and these AED combinations may be effective for secondary generalized seizures by inhibiting propagation of seizures from a remote focus. In contrast, phenytoin has been found to increase the threshold for focal seizures in the rat amygdala kindling model, but with limited efficacy in reducing the spread of seizures.\(^20\)

Statistical analyses indicated positive interactions between PER and the four concomitant AEDs, which were often statistically significant. However, it is important to note that previously reported analyses of pooled Phase III data have indicated that specific concomitant administration of LEV, LAM or VPA, versus other concomitant AEDs, has no impact on the efficacy of adjunctive PER in the treatment of partial-onset seizures in humans.\(^21\) This may be because, as suggested by our experimental results, any pharmacodynamic interactions are not specific to individual AEDs, and therefore no difference is observed between patients receiving one type of concomitant AED and patients receiving another. Certainly, the use of
polytherapy and the complexity of epilepsy complicate the evaluation of specific pharmacodynamic interactions in these trials. Nonetheless, so far there is no clear evidence of pharmacodynamic interactions between PER and other AEDs in patients with epilepsy, and therefore it remains to be determined whether the interactions observed in the rat amygdala kindling model translate to the clinical setting.

Since low-dose PER, LAM, CBZ, and VPA all reduced the latency time to fall in the rotarod test, all were considered to be associated with some degree of neurotoxicity. However, although concomitant administration of low-dose PER conferred an additional latency reduction, beyond that observed with LAM, CBZ, or VPA when administered individually, this interaction was not statistically significant. Overall, the data suggest that adjunctive PER may not be associated with specific neurotoxicity interactions with AEDs with other mechanisms of action,

PER is metabolized by CYP3A4/5 isoenzymes, meaning that its clearance is increased, and plasma concentrations are reduced, in the presence of enzyme-inducing AEDs such as CBZ, oxcarbazepine and phenytoin. In the present study, the plasma concentration of PER was reduced in the presence of CBZ, and also in the presence of VPA, suggesting that any pharmacodynamic interactions were potentially underestimated. In contrast, the plasma concentration of PER was increased by 17.2% when administered in combination with LEV, and by 38.2% in combination with LAM. Of note, the efficacy of the combination of PER and LAM was comparable or even superior to high-dose PER, indicating that a positive pharmacodynamic interaction may exist.

Some seizure models provide reduced sensitivity to some AEDs, but not others, which can be useful in some experimental settings. In addition, isobolography is often used to evaluate specific pharmacodynamic interactions across a dose range and can be applied in the
amygdala kindling model. However, the approach used in this study was selected to produce resistance to a range of AEDs at clinically relevant doses, which conferred behavioral neurotoxicity, so that multiple interactions could be evaluated and, therefore, it was considered unlikely that this approach would overestimate any findings.

**Conclusion**

These data from a rat amygdala kindling model support the efficacy of PER as adjunctive therapy for the treatment of refractory partial-onset seizures when used in combination with LEV, LAM, CBZ, or VPA. Interestingly, they also indicate positive and significantly positive effects of adjunctive PER on seizure severity parameters, without showing specific interactions with any of the individual AEDs in particular. However, these findings are yet to be confirmed in a clinical population, where the assessment of pharmacodynamic interactions is complicated by polytherapy and the complexity of epilepsy.

**Acknowledgments**

We thank David Squillacote, Eisai Inc., for reviewing the content of this manuscript and for providing valuable comments. This study was funded by Eisai Co. Ltd. Kate Carpenter of Choice assisted with development of the outline, with subsequent editorial support provided by Nicole M Kane, PhD, and Hannah FitzGibbon, PhD, of Complete Medical Communications. All professional editorial support was funded by Eisai Inc.

**Role of the funding source**

Eisai Co. Ltd funded the study. All authors are employees of Eisai Co. Ltd and take full responsibility for the study design, the collection, analysis and interpretation of the data, and the decision to submit the article for publication. Eisai Inc. funded professional editorial
support for the development of the manuscript. David Squillacote of Eisai Inc. reviewed the manuscript and provided editorial suggestions.

**Disclosure of conflicts of interest**

T Wu, Y Nagaya and T Hanada are employees of Eisai Co. Ltd.

**Contribution to article**

T Wu, Y Nagaya and T Hanada all contributed to the design and execution of the study and the writing and refinement of the manuscript.
References


Table 1
Effects of individual AEDs on ADT in the rat amygdala kindling model.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose, mg/kg</th>
<th>Mean percentage increase in ADT compared with pretreatment (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PER (n=5–8)</td>
<td>0 (vehicle only)</td>
<td>104.4 (4.0)</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>109.6 (6.1)</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>152.1 (22.0)</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>225.6 (41.4)</td>
</tr>
<tr>
<td>LEV (n=12–13)</td>
<td>0 (vehicle only)</td>
<td>94.3 (3.0)</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>115.0 (6.1)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>124.1 (7.9)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>132.5 (11.1)</td>
</tr>
<tr>
<td>LAM (n=8)</td>
<td>0 (vehicle only)</td>
<td>97.5 (6.2)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>112.5 (4.4)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>140.5 (8.0)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>148.8 (8.6)</td>
</tr>
<tr>
<td>CBZ (n=10–11)</td>
<td>0 (vehicle only)</td>
<td>97.8 (2.2)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>128.5 (5.4)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>143.4 (10.0)</td>
</tr>
<tr>
<td>VPA (n=8)</td>
<td>0 (vehicle only)</td>
<td>100.0 (4.7)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>138.0 (7.4)</td>
</tr>
<tr>
<td>200</td>
<td>188.5 (17.5)\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>219.0 (21.1)\textsuperscript{a}</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}p < 0.05 vs pretreatment

ADT, after-discharge threshold; AED, antiepileptic drug; CBZ, carbamazepine; LAM, lamotrigine; LEV, levetiracetam; PER, perampanel; SEM, standard error of the mean; VPA, valproic acid
### Table 2

Statistical analysis of pharmacodynamic interactions between PER and concomitant LEV 50 mg/kg, LAM 20 mg/kg, CBZ 20 mg/kg, or VPA 200 mg/kg (two-way ANOVA; parametric test used for seizure score, non-parametric test used for EEG seizure duration and motor seizure duration).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Seizure score</th>
<th>EEG seizure duration</th>
<th>Motor seizure duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect</td>
<td>p-value</td>
<td>Effect</td>
</tr>
<tr>
<td>PER alone</td>
<td>Significant</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>LEV alone</td>
<td>Non-significant</td>
<td>0.0811</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Interaction</td>
<td>Non-significant</td>
<td>0.3391</td>
<td>Non-significant</td>
</tr>
<tr>
<td>PER alone</td>
<td>Significant</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>LAM alone</td>
<td>Significant</td>
<td>0.0016</td>
<td>Significant</td>
</tr>
<tr>
<td>Interaction</td>
<td>Non-significant</td>
<td>0.9677</td>
<td>Non-significant</td>
</tr>
<tr>
<td>PER alone</td>
<td>Significant</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>CBZ alone</td>
<td>Significant</td>
<td>0.0008</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Interaction</td>
<td>Significant</td>
<td>0.0403</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Treatment</td>
<td>Significance</td>
<td>p-value</td>
<td>Significance</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>-----------</td>
<td>--------------</td>
</tr>
<tr>
<td>PER alone</td>
<td>Significant</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>VPA alone</td>
<td>Significant</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>Interaction</td>
<td><strong>Significant</strong></td>
<td>0.0072</td>
<td><strong>Significant</strong></td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; CBZ, carbamazepine; EEG, electroencephalography; LAM, lamotrigine; LEV, levetiracetam; PER, perampanel; VPA, valproic acid
Table 3

Plasma concentrations of PER and AEDs administered either alone or in combination.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concomitant AED</th>
<th>Mean plasma concentration of drug (SEM)</th>
<th>Change compared with drug plus vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>PER 0.75 mg/kg (n=4)</td>
<td>Vehicle</td>
<td>218.0 (8.1) ng/mL</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LEV 50 mg/kg</td>
<td>255.4 (15.7) ng/mL</td>
<td>+17.2%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>LAM 20 mg/kg</td>
<td>301.3 (10.8) ng/mL</td>
<td>+38.2%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CBZ 20 mg/kg</td>
<td>178.4 (9.3) ng/mL</td>
<td>-18.2%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>VPA 200 mg/kg</td>
<td>149.5 (10.0) ng/mL</td>
<td>-31.4%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LEV 50 mg/kg (n=4)</td>
<td>Vehicle</td>
<td>41.9 (1.4) μg/mL</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PER 0.75 mg/kg</td>
<td>40.5 (2.4) μg/mL</td>
<td>-3.3%</td>
</tr>
<tr>
<td>LAM 20 mg/kg (n=4)</td>
<td>Vehicle</td>
<td>10.5 (0.3) μg/mL</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PER 0.75 mg/kg</td>
<td>10.5 (0.5) μg/mL</td>
<td>0.0</td>
</tr>
<tr>
<td>CBZ 20 mg/kg (n=4)</td>
<td>Vehicle</td>
<td>6.7 (0.8) μg/mL</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PER 0.75 mg/kg</td>
<td>7.1 (0.3) μg/mL</td>
<td>+6.0%</td>
</tr>
<tr>
<td>VPA 200 mg/kg (n=4)</td>
<td>Vehicle</td>
<td>452.9 (23.8) μg/mL</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PER 0.75 mg/kg</td>
<td>459.8 (32.9) μg/mL</td>
<td>+1.5%</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.05 vs drug plus vehicle

AED, antiepileptic drug; CBZ, carbamazepine; LAM, lamotrigine; LEV, levetiracetam; PER, perampanel; SEM, standard error of the mean; VPA, valproic acid
Figure legends

**Fig. 1.** Anti-seizure effect of PER in the 3ADT-stimulus amygdala kindling model: (A) seizure score; (B) EEG seizure duration; and (C) motor seizure duration (presented as mean ± SEM).

*Footnote:*

****p < 0.0001 vs vehicle alone

ADT, after-discharge threshold; EEG, electroencephalography; PER, perampanel; SEM, standard error of the mean

**Fig. 2.** Anti-seizure effect of AEDs in the 3ADT-stimulus amygdala kindling model: (A) seizure score; (B) EEG seizure duration; and (C) motor seizure duration (presented as mean ± SEM).

*Footnote:*

ADT, after-discharge threshold; AED, antiepileptic drug; CBZ, carbamazepine; EEG, electroencephalography; LAM, lamotrigine; LEV, levetiracetam; SEM, standard error of the mean; VPA, valproic acid

**Fig. 3.** Anti-seizure effect of low-dose (0.75 mg/kg) and high-dose (1.5 mg/kg) PER in combination with concomitant AEDs in the 3ADT-stimulus amygdala kindling model: (A) LEV 50 mg/kg; (B) LAM 20 mg/kg; (C) CBZ 20 mg/kg; and (D) VPA 200 mg/kg (presented as mean ± SEM).

*Footnote:*
*p < 0.05 vs vehicle plus LEV, LAM, CBZ, or VPA; †p < 0.05 vs vehicle plus vehicle;
‡p < 0.05 vs PER 0.75 mg/kg or PER 1.5 mg/kg plus vehicle

CBZ, carbamazepine; EEG, electroencephalography; LAM, lamotrigine; LEV, levetiracetam;
PER, perampanel; SEM, standard error of the mean; VPA, valproic acid

**Supplementary Fig. 1.** Motor impairment induced by low-dose (0.75 mg/kg) PER, LEV 50
mg/kg, LAM 20 mg/kg, CBZ 20 mg/kg, or VPA 200 mg/kg, alone or in combination, as
assessed by rotarod test (presented as mean ± SEM).

*Footnote:*

*p < 0.05 vs vehicle alone

CBZ, carbamazepine; LAM, lamotrigine; LEV, levetiracetam; PER, perampanel; SEM,
standard error of the mean; VPA, valproic acid
Figure 1

A

Seizure score (0 to 5)

PER 0.75 mg/kg (n=38)

PER 1.5 mg/kg (n=38)

B

EEG seizure duration (% of pretreatment)

PER 0.75 mg/kg (n=38)

PER 1.5 mg/kg (n=38)

C

Motor seizure duration (% of pretreatment)

PER 0.75 mg/kg (n=38)

PER 1.5 mg/kg (n=38)
Figure 3

A. LEV 50 mg/kg

B. LAM 20 mg/kg

C. CBZ 20 mg/kg

D. VPA 200 mg/kg
Figure 4

![Graph showing latency time to fall (% of baseline) for different treatments]

Vehicle (n=6) | LEV 50 mg/kg (n=6) | LAM 20 mg/kg (n=6) | CBZ 20 mg/kg (n=6) | VPA 200 mg/kg (n=6)
--- | --- | --- | --- | ---
99.8 | 103.0 | 86.3 | 45.7 | 36.5

PER 0.75 mg/kg (n=6) | LEV 50 mg/kg (n=6) | LAM 20 mg/kg (n=6) | CBZ 20 mg/kg (n=6) | VPA 200 mg/kg (n=6)
--- | --- | --- | --- | ---
| 6.7 | 9.7 | 8.2 |