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The neuroprotective effect of perampanel in lithium-pilocarpine rat seizure model

Ting Wu[a,b,*], Katsutoshi Ido[a], Yoshihide Osada[a], Sadaharu Kotani[a], Akira Tamaoka[c], Takahisa Hanada[a]

[a] Neurology, Tsukuba Research Department, Discovery, Medicine Creation, Neurology Business Group, Eisai Co., Ltd., Japan
[b] Clinical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan
[c] Institute of Clinical Medicine, Department of Neurology, University of Tsukuba, Japan

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ABSTRACT

Purpose: Status epilepticus (SE) causes irreversible neurodegeneration if not terminated quickly. Perampanel (PER), a potent AMPA receptor antagonist, has previously been shown to terminate seizures in the lithium-pilocarpine SE model. In the present study, we assessed whether PER would also prevent neuronal damage in this model.

Methods: SE was induced in rats using lithium chloride and pilocarpine. Initiation of SE was defined as continuous seizures that exhibited as rearing accompanied by bilateral forelimb clonus (Racine score 4). Either PER (0.6, 2, or 6 mg/kg) or diazepam (DZP, 10 mg/kg) was administered intravenously 30 min after SE initiation. Histopathological samples from treated and seizure-naive rats were taken one week after treatment and then stained with an anti–neuronal nuclei (NeuN) antibody. The sections were analyzed by using a pixel-counting algorithm to quantify the amount of staining in the CA1 subregion of the hippocampus, piriform cortex (Pir), and mediodorsal thalamic nucleus (MD).

Results: DZP administration did not suppress seizures or the degeneration of neurons in the examined areas. Seizures were terminated in 100% of rats treated with 6 mg/kg PER (n = 8) and in 47% (7/15) of rats treated with 2 mg/kg PER, and neurons in the analyzed areas of these animals were preserved to the level seen in naive rats. In the eight animals in which 2 mg/kg PER did not terminate the seizures, neuronal loss was partially attenuated in CA1 and Pir, and neurons were fully preserved in MD. Treatment with 0.6 mg/kg PER did not terminate the seizures or significantly preserve neurons. The anti-seizure effect of PER correlated well with the degree of neuroprotection in each analyzed area.

Conclusions: PER exhibited a strong neuroprotective effect in a drug-refractory SE model, and this effect was correlated with its attenuation of seizure.

1. Introduction

Status epilepticus (SE) carries a risk of major morbidity or mortality (Lowenstein and Alldredge, 1998; Rossetti and Lowenstein, 2011). There are many treatments for SE, including benzodiazepines such as diazepam (DZP) and lorazepam (Meierkord et al., 2006, 2010; Brophy et al., 2012). However, 30–43% of SE cases become refractory to drugs (Holtkamp et al., 2005; Mayer et al., 2002). Prolonged seizure results in neuronal injury, neuronal death, and alteration of neuronal networks that can result in death or spontaneous recurrent seizures (SRS) and cognitive impairment that will impact the patient's whole life (Curia et al., 2014; Trinka et al., 2015). Many approved antiepileptic drugs (AEDs) have been examined in various animal models of SE, some of them showed that DZP can terminate seizure (Eslami et al., 2016; Imran et al., 2015; Suchomelova et al., 2006). However, there still few AEDs that produce complete and immediate termination of prolonged seizures (Rossetti and Lowenstein, 2011 Löscher and Brandt, 2010). This suggests a need for AEDs with novel mechanisms to treat drug-resistant SE.

Abbreviations: AED, antiepileptic drugs; AMPA, alpha amino-3-hydroxy-5-methylisoxazole-4-propionic acid; ANOVA, analysis of variance; DAB, diaminobenzidine; DZP, diazepam; EEG, electroencephalogram; GABAA, γ-aminobutyric acid receptor type A; NeuN, neuronal nuclei; PBS, phosphate-buffered saline; PER, perampanel; SE, status epilepticus; SRS, spontaneous recurrent seizures

* Corresponding author at: Neurology, Tsukuba Research Department, Discovery, Medicine Creation, Neurology Business Group, Eisai Co., Ltd., Tokodai 5-1-3, Tsukuba, Ibaraki 300-2635, Japan.
E-mail address: t2-wu@hhc.eisai.co.jp (T. Wu).

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Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonists act as broad-spectrum anticonvulsants in animal seizure models (Rogawski and Donevan, 1999). Continuous infusion of NS1209 and repeated injection of GYKI52466 each terminated prolonged seizures in animal models of SE (Pitkänen et al., 2007; Fritsch et al., 2010). However, neither drug alone was able to stop seizures with a single dose. Perampanel (PER), a noncompetitive AMPA receptor antagonist, is currently approved for the adjunctive treatment of partial-onset seizures, with or without secondary generalized seizures, and for primary generalized tonic-clonic seizure in patients 12 years and older. In a previous report, a single PER treatment terminated DZP-resistant SE on electroencephalography (EEG) when given 30 min after SE initiation (Hanada et al., 2014). This result suggests that PER has a higher potential for suppressing refractory SE than other AEDs and other investigational AMPA antagonists.

Neuronal damage is as important as seizure duration when considering the prognosis of refractory SE, but AEDs used in clinical practice have shown limited effects on reducing neuronal damage in preclinical models (Löscher and Brandt, 2010). NS1209 exerted only mild neuroprotective effects, and no pathological analysis has been performed for GYKI52466 (Pitkänen et al., 2007; Fritsch et al., 2010). The efficacy of PER has only been demonstrated with EEG changes (Hanada et al., 2014), and its impact on neuronal injury has not been explored. The lithium-pilocarpine rat model is known to reproduce clinical and neuropathological features of human SE (Dubé et al., 2006; Clifford et al., 1987; Turski et al., 1989; Curia et al., 2014). In this model, administration of lithium and pilocarpine causes SE, which leads to severe neuronal damage mainly in the hippocampus, piriform cortex, entorhinal cortex, amygdala, and thalamus (Clifford et al., 1987). In this study, we examined whether PER has neuroprotective effects in brain areas that can be severely damaged by SE: the CA1 subregion of the hippocampus, the piriform cortex (Pir), and the mediodorsal thalamus (MD). We also examined the relationship between neuroprotection and seizure suppression.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (6 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The rats were housed with artificial lighting of 12 h light (7 a.m.–7 p.m.), 12 h dark. Rats were provided with pellet food (MF, Oriental Yeast Co., Japan) and tap water ad libitum. All procedures were performed in the animal facility accredited by the Center for Accreditation of Laboratory Animal Care and use Japan Health Sciences Foundation. All protocols were approved by the Institutional Animal Care and Use Committee and carried out according to the Eisai Animal Experimentation Regulations.

2.2. Induction of SE and treatment protocol

SE was induced by the administration of lithium chloride (Wako Pure Chemical Industries, Japan) (3 mEq/kg, i.p.), followed 18–24 h later by scopolamine methyl bromide (Sigma-Aldrich, Japan) (5 mg/kg, i.p.) and pilocarpine (Wako Pure Chemical Industries, Japan) (30 mg/kg, i.p.) (Curia et al., 2008). The test drugs, PER (Eisai Co., Ltd, Kashima, Japan) and DZP (Wako Pure Chemical Industries, Japan), were diluted with a vehicle consisting of distilled water, dimethyl sulfoxide, and polyethylene glycol 300, at 1:1:1 (v/v). In accordance with a previous study (Hanada et al., 2014), three doses of PER were used: 0.6 mg/kg (n = 15), 2 mg/kg (n = 15), or 6 mg/kg (n = 8). In the present study, DZP rather than vehicle was used as the control to reduce mortality. PER or DZP 10 mg/kg (n = 6) were administered (i.v.) 30 min after the initiation of SE, which was defined as continuous seizures with rearing accompanied by bilateral forelimb clonus (Racine score 4; Racine, 1972). The latency to SE initiation was evaluated in all rats and analyzed by group, in accordance with a previous study (Lucchi et al., 2013). A treatment-naive group (n = 9) of rats not administered lithium chloride and pilocarpine to induce seizure was also included.

2.3. Behavioral observation

Seizure was classified in accordance with the Racine score (Racine, 1972) as follows: score 0–no seizure behavior; score 1–immobility, eye closure, twitching of vibrissae, sniffing, facial clonus; score 2–head nodding associated with more severe facial clonus; score 3–clonus of one forelimb; score 4–rearing, often accompanied by bilateral forelimb clonus; score 5–score 4 with loss of balance and falling, accompanied by generalized clonic seizures. The efficacy of drugs on seizure suppression was evaluated by using the Racine score 30 min after drug administration.

2.4. Immunohistochemical procedure

Animals were deeply anesthetized one week after SE induction and perfused with saline, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.0). The brains were removed, post-fixed with 4% paraformaldehyde in PBS, and cryoprotected with 30% sucrose in PBS. Frozen sections were cut at 30 μm and stored in 0.05% NaN_3 in PBS. Slices taken from −3.8 mm relative to bregma were used for immunostaining. These selected floating sections were stained with anti-neuronal nuclei (NeuN) antibody (1:500, Chemicon International, Inc., USA), which was used to determine neuronal loss in CA1, Pir, and MD. Secondary antibody was Biotinylated horse anti-mouse IgG (1:200, Vector Laboratories, USA). It was followed by the addition of avidin-biotin-peroxidase complex (VECTASTAIN Elite ABC HRP Kit, Vector Laboratories, USA). The peroxidase was then developed by the diaminobenzidine (DAB reagent, Vector Laboratories, USA).

2.5. Quantification of NeuN expression

Slides in each group were scanned (Aperio Technologies, USA), and the NeuN-positive cells were detected with a positive pixel-count algorithm (ImageScope, Aperio Technologies, Inc., USA) that quantifies the amount of a specific stain (Liu et al., 2008). In this algorithm, the brown color of DAB on images of the tissues was identified as being weakly positive, positive, or strongly positive. For pixels that meet the color specification, the algorithm counted the number in each intensity range. In the present study, the whole CA1, Pir, and MD regions were analyzed, and the pixels with positive or strongly positive intensity were counted. The average pixel counts of each group across all sections were calculated. All of these analyses were performed by an investigator blinded to the treatment groups. Samples were photographed with a fluorescence microscope (BZ-X700/BZ-710, KEYENCE, Japan) for expressing the images of each treatment group.

2.6. Statistical analysis

The survival rate was analyzed with Fisher's exact test. The latency to SE initiation was analyzed with one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests. Seizure score was analyzed with one-way ANOVA with Dunn's multiple comparison tests. The percentage of pixel counts was compared between the naive and DZP group with Student's *t* test, and between the DZP group and the groups given different doses of PER with one-way ANOVA with Dunnett's multiple comparison tests. The correlation of SE seizure suppression effects and neuroprotective effects was analyzed with Pearson's correlation coefficient analysis. GraphPad Prism software (Version. 6.07, GraphPad Software, Inc., USA) was used for all statistical analysis. The graphs of correlation were drawn with JMP software (Version. 12.2.0, SAS Insutitute Inc., USA). The level of significance was