Epilepsy Research 115 (2015) 73–76

Contents lists available at www.sciencedirect.com

Epilepsy Research

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

Short communication

# Effects of eugenol on granule cell dispersion in a mouse model of temporal lobe epilepsy

Kyoung Hoon Jeong[a,c,*], Dong-Seok Lee[a,b], Sang Ryong Kim[a,b,c,*]

[a] School of Life Sciences, BK21 plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 702-701, Republic of Korea
[b] Institute of Life Science & Biotechnology, Kyungpook National University, Daegu 702-701, Republic of Korea
[c] Brain Science and Engineering Institute, Kyungpook National University, Daegu 700-842, Republic of Korea

ARTICLE INFO

*Article history:*
Received 11 March 2015
Received in revised form 19 May 2015
Accepted 2 June 2015
Available online 5 June 2015

*Keywords:*
Eugenol
Seizure
Granule cell dispersion
Mammalian target of rapamycin complex 1
Hippocampus

ABSTRACT

Granule cell dispersion (GCD), a structural abnormality, is characteristic of temporal lobe epilepsy (TLE). Eugenol (EUG) is an essential component of medicinal herbs and is suggested to exert anticonvulsant activity. However, it is unclear whether EUG ameliorates the abnormal morphological changes in granule cells induced by epileptic insults. In the present study, we examined whether intraperitoneal injection of EUG attenuated increased seizure activity and GCD following intrahippocampal injection of kainic acid (KA). Our results showed that EUG significantly increased the seizure threshold, resulting in delayed seizure onset, and reduced GCD in KA-induced epilepsy. Moreover, EUG treatment significantly attenuated KA-induced activation of mammalian target of rapamycin complex 1 (mTORC1), which is involved in GCD development, in the dentate gyrus (DG). These results suggest that EUG may have beneficial effects in the treatment of epilepsy through its ability to inhibit GCD *via* suppression of KA-induced mTORC1 activation in the hippocampal DG *in vivo*.



## 1. Introduction

Epilepsy is a neurological disease that affects approximately 1% of the population (Fisher, 2015; Thurman et al., 2011). Temporal lobe epilepsy (TLE) is the most common form of partial or localization-related epilepsy in humans; TLE is characterized by pathophysiological features such as hippocampal sclerosis and granule cell dispersion (GCD) (Houser, 1990). GCD is a morphological change defined by granule cell layer (GCL) enlargement and granule cell dispersion within the molecular layer of the dentate gyrus (DG), which is closely related with frequent seizures and status epilepticus (Houser, 1990). Moreover, clinical observation of GCD-related morphological changes could aid in the prognosis of postsurgical seizure outcomes in TLE patients with DG pathology (Na et al., 2014). Therefore, controlling GCD may have clinical relevance (Houser, 1990; Murphy et al., 2012).

Eugenol (EUG, 4-allyl-2-methoxyphenol), a major component of clove oil, has been used in dental care for its antiseptic and analgesic properties (Chaieb et al., 2007). EUG has potential anticonvulsant activity (Müller et al., 2006; Pourgholami et al., 1999); however, it is still unclear how EUG exerts anticonvulsant effects in the epileptic hippocampus. Histological changes such as GCD were observed in the hippocampus of a rodent model of epilepsy in which kainic acid (KA) was injected into the hippocampus (Murphy et al., 2012). In the present study, we examined whether EUG inhibited the development of GCD in KA-induced epilepsy. We also determined if EUG had an inhibitory effect on KA-induced activation of the mammalian target of rapamycin complex 1 (mTORC1) in the hippocampal DG.

## 2. Materials and methods

### 2.1. Intrahippocampal injection of KA

All experiments were performed in accordance with approved animal protocols and guidelines established by the Animal Care Committee of Kyungpook National University (No. KNU 2012-37). Male C57BL/6 mice (8-weeks old; Daehan Biolink, Eumseong, Korea) were anesthetized using chloral hydrate (360 mg/kg; Sigma, St. Louis, MO, USA), and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Mice received a unilateral injection of KA [0.2 μg in 4 μL phosphate-buffered saline (PBS); Sigma] into the hippocampus (AP: −2.0 mm; ML: −1.6 mm; DV: −1.5 mm, relative to the bregma; Franklin and Paxinos, 2004) using a 10-μL

* Corresponding authors at: School of Life Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea. Tel.: +82 53 950 7362; fax: +82 53 943 2762.
*E-mail addresses:* greenquest81@gmail.com (K.H. Jeong), lee1@knu.ac.kr (D.-S. Lee), srk75@knu.ac.kr (S.R. Kim).

http://dx.doi.org/10.1016/j.eplepsyres.2015.06.001
0920-1211/

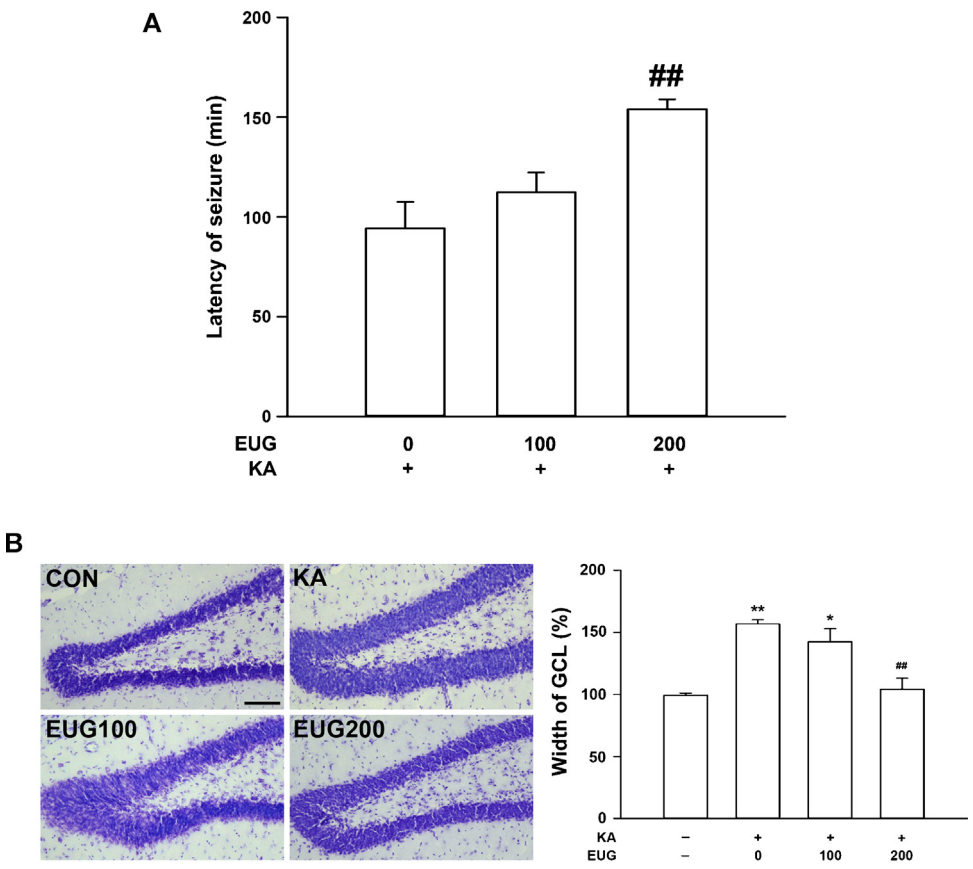


**Fig. 1.** Eugenol (EUG) influences seizure susceptibility and granule cell dispersion (GCD) in the hippocampal dentate gyrus (DG) in a kainic acid (KA)-induced epilepsy model. (A) Seizure onset latency (stage 3) was significantly increased in the 200 mg/kg EUG plus KA group compared with the KA alone group. $^{\#\#}p < 0.001$, significantly different from KA (one-way ANOVA and Tukey's *post hoc* tests; $n = 4$ for each group). (B) Morphologic analysis of dispersed granule cells 7 days after intrahippocampal KA injection. The left panel shows a representative coronal section of the ipsilateral DG following Nissl staining by cresyl violet. Scale bar, 100 μm. CON, phosphate-buffered saline (PBS)-treated group; KA, KA-treated group, EUG100, 100 mg/kg EUG-treated group plus KA; EUG200, 200 mg/kg EUG-treated group plus KA. The histogram results show the quantitative analysis of granule cell layer (GCL) width normalized to the contralateral side for each sample (right panel). The KA-induced increase in GCL width (i.e., GCD) was significantly decreased in the group treated with both 200 mg/kg EUG and KA. $^{**}p < 0.001$ and $^{*}p = 0.005$, significantly different compared with CON, respectively. $^{\#\#}p < 0.001$, significantly different compared with KA alone (one-way ANOVA and Tukey's *post hoc* tests; $n = 4$ for each group). All values are expressed as mean ± standard error of the mean (SEM).

Hamilton syringe needle (30S) attached to a syringe pump (KD Scientific, NewHope, PA, USA). Mice treated with PBS (Sigma) were used as controls; no significant changes in the GCL were observed in the DG of control mice (Supplementary Fig. S1). KA-treated mice were monitored for 6 h to evaluate seizure onset. Seizure stages were determined, as previously described, with some modifications (Macias et al., 2013; Racine, 1972): stage 1, facial movement; stage 2, head nodding and myoclonic twitching; stage 3, forelimb clonus with lordotic posture; stage 4, forelimb clonus with reared posture; and stage 5, tonic-clonic seizure without postural control. In the present study, the mice exhibiting behavior categorized at stage 3 were classified as positive for seizure onset.

### 2.2. Drug administration

As previously described, with modifications (Won et al., 1998), mice received a daily intraperitoneal injection of EUG (100 or 200 mg/10 mL/kg per day; Sigma), suspended in 0.1 M PBS with 0.1% Tween 20 (Sigma). The first EUG injection was administrated 1 h before the KA treatment (Sigma), and the injections were administrated for 6 days after KA treatment. Mice treated with 200 mg/10 mL/kg EUG alone were used as controls; these mice showed no significant change in GCL in the DG (Supplementary Fig. S1). Rapamycin [RA; 10 mg/kg (Jeon et al., 2015); LC Laboratories, Woburn, MA, USA], which was diluted in a vehicle solution (4% ethanol, 5% Tween 80, and 5% PEG 400; Sigma), was used as a positive control for GCD changes mediated by mTORC1 deactivation (Shima et al., 2015).

### 2.3. Staining procedures and GCD measurement

At 7 days after KA treatment, mice were anesthetized using chloral hydrate (360 mg/kg; Sigma), and then they were transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB; Sigma). Using a cryostat (Microm International GmbH, Walldorf, Germany), 30-μm-thick coronal sections were cut. For Nissl staining, sections were mounted on gelatin-coated slides and stained with 0.5% cresyl violet (Sigma). The presence of GCD was determined by measuring the average width of the GCL in the mid and medial one-fourth portions of the upper blade of the DG (Shima et al., 2015). GCD was expressed as a percentage of the GCL width on the ipsilateral side compared with that on the contralateral side. For immunohistochemistry, sections were incubated for 48 h at 4 °C with anti-phospho-4E-BP1 (p-4E-BP1, a phosphorylated form of the mTORC1 substrate 4E-BP1; 1:1.000; Cell Signaling, Beverly, MA, USA), which indicates mTORC1 activation (Jeon et al., 2015). Sections were then incubated with a suitable biotinylated secondary antibody and processed with an avidin-biotin complex kit (Vector Laboratories, Burlingame, CA, USA). The signal was visualized by incubating sections in 0.5 mg/mL 3.3′-diaminobenzidine (DAB;