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Protection by Methanol Extract of Longan (*Dimocarpus Longan* Lour.) Peel against Kainic acid-Induced Seizure

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Abstract – This experiment was undertaken to investigate whether methanol extract of fruit peel of *Dimocarpus longan* Lour. (MEFL) protects against kainic acid (KA)-induced seizure. Oral administration of MEFL (1, 2 and 4 mg/kg) increased KA (50 mg/kg)-induced survival rate and latency of convulsion onset, and deceased seizure scores and weight loss induced by intraperitoneal (i.p) injection of KA in mice. In addition, MEFL protected against cell death in the hippocampus of rat brain after KA-administration as analyzed by using TUNEL assay in rats. MEFL also significantly blocked seizure-form of electroencephalogram (EEG) power spectra induced by KA in rats. MEFL also inhibited elevation of $[Ca^{2+}]i$ and increased $[Cl^{-}]i$ induced by KA in cultured neuronal cells. Therefore, it is suggested that MEFL protects against seizure induced by KA, decreasing $[Ca^{2+}]i$.
Keywords – *Dimocarpus longan,* Kainic acid (KA), Seizure; Hippocampus, Cell death, $[Ca^{2+}]i$.

Introduction

An excitatory amino acid L-glutamate analog, kainic acid (KA), causes neuronal loss and is used to create a model of temporal lobe epilepsy in animals (Alldredge and Lowenstein, 1999). Epilepsy, a central nervous system (CNS) disorder, has in part been ascribed to excessive release or impaired uptake of endogenous excitatory amino acid, such as glutamate, which can lead to neuronal damage, and/or cell loss (McNamara, 1994). The KA receptor referred as non-NMDA receptors, is one of ionotropic glutamate receptors. The activation of NMDA and non-NMDA glutamate receptors results in massive Ca^{2+} influx into neurons. The excess intracellular Ca^{2+} causes structural damage to cytoskeleton and membranes as the result of activation kinases, proteases and free radical production which eventually leads to neuronal damages and death. Epilepticus specially causes sustained elevation of intracellular calcium levels in hippocampal neurons (Choi, 1992; Pal *et al*., 1999).

Longan (fruit peel of *Dimocarpus longan* Lour., Sapindaceae) is a subtropical fruit widely grown in China and South East Asia. It had been consumed in both fresh and widely used for the treatment of anxiety and insomnia in Asia (Sun *et al*., 2007). In modern pharmacological studies, longan is known to contain many pharmacologically active components such as specific amino acids and polyphenolic compounds (Rangkadilok *et al*., 2005; Ryu *et al*., 2003). These compounds have been shown to have multiple pharmacological activities, including antioxidants, anticarcinogenic activities (Hsieh *et al*., 2008; Minakata *et al*., 1985; Rangkadilok *et al*., 2007). Extract of longan was proved to have anxiolytic activity (Okuyama *et al*., 1999). We recently found that methanol extract of longan augmented pentobarbital-induced sleeping through GABAergic systems (Ma *et al*., 2009). However, there is little information on the pharmacological profiles of longan, although longan has been often been used for the treatment of convulsion. Therefore, the present study was aimed to evaluate the anticonvulsant effects of methanol extract of fruit peel of *Dimocarpus longan* Lour. (MEFL) on KA-induced convulsion and investigate possible mechanisms.

Experimental

Animals – ICR male mice (Samtako, Osan, Korea) weighing 22 - 25 g and male Sprague-Dawley rats (Samtako, Osan, Korea) weighing 220 - 250 g were used.

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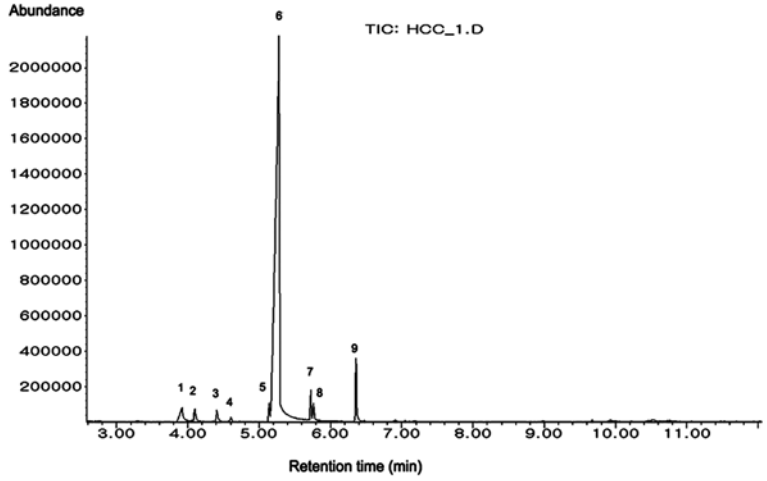

Fig. 1. GC/MS Analysis of methanol extract of longan.

Peak 1: 4-oxo-pentanoic acid, Peak 2: furylhydroxymethyl ketone, Peak 3: pentanoic acid, Peak 4: C-2.C-3-epoxy-methylcyclohept-4-en-1-ol, Peak 5: 2-ethyl-5-propylthiophene, Peak 6: 5-(hydroxymethyl)-2-furaldehyde (MW 126), Peak 7: 3-methyl-2-furoic acid, Peak 8: unidentified, Peak 9: ethyl-4-amino-4H-imidazole-5-carboxylate.

Animals were housed in acrylic cages (45 × 60 × 25 cm) with water and food available *ad libitum* under an artificial 12-h light/dark cycle (lights on at 7:00 am) and at a constant temperature (22 ± 2 °C). To ensure adaptation to the new environment, animals were kept in the departmental holding room for 1 week before testing. This study was performed in accordance with the Chungbuk National University Laboratory Animal Research Center guidelines for the care and use of laboratory animals.

Extraction and GC-MS analysis – Dried fruit peel of *Dimocarpus longan* Lour. was purchased from in a Kyungdong herb market (Seoul, Korea). The air-dried fruit peel material was extracted at 50 °C for 48 hrs with 70% MeOH. The extract was filtered and concentrated using a rotary vacuum evaporator followed by lyophilization. The yield of MEFL was about 12.6% (w/w) (Rangkadilok *et al*., 2007). The dried extract was dissolved in saline before use (Fig. 1). The sample extracts (350 mg) were diluted to 10 ml with methanol, and then a 1-mL aliquot was filtered through a 0.20 μm syringe filter (Sartorius, Germany). An aliquot (1 μl) from the filtrate was used for GC/MS analysis. The analysis was performed with an Agilent 6890N series GC system coupled to an Agilent 5973N mass selective detector (Agilent Technologies, USA). A HP-5MS fused silica column (5% phenyl methyl polysiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm) was used for the peaks separation. The GC oven temperature was programmed from 80 (1 min, isothermal) to 280 °C at 20 °C/min, and finally held for 15 min. The flow rate of carrier gas (high purity helium) was 1.0 ml/min (37 cm/sec) in constant flow mode. Split injection (Injection volume, 1.0 μl) was used with a split ratio of 20 : 1 at the injector temperature, 250 °C. The MSD was operated in full scan mode (40 – 550 amu at 2.89 scan / sec) at 70 eV. Peak identification was performed using the HP Enhanced ChemStation software, G1701DA MSD ChemStation Rev. D.01.00.75.

Assessment of anticonvulsant activity – KA (5 mg/kg i.p) was injected 1 hour after oral administration of the test extract, MEFL. After KA injection, the animals were placed in individual acrylic plastic cages (30 cm width × 30 cm diameter × 30 cm height) and monitored for 30 min. In addition, the number of surviving animals, onset of convulsion (latency time), seizure severity score and weight loss (%) were observed (Sperk *et al*., 1985; Vellucci and Webster, 1984). All experiments were performed between 9:00 am to 6:00 pm in a quiet room with an ambient temperature of 22 + 1 °C.

TUNEL assay – TUNEL analysis of dying cells: To assess dying cells, sections adjacent to those used for cresyl violet staining were processed using an In Situ Cell Death Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany) for analysis of DNA fragmentation, according to the manufacturer's specifications. Briefly, after fixation in 4% paraformaldehyde for 10 min at room temperature, the sections were washed in PBS three times for 10 min. To block endogenous peroxidase activity, the sections were incubated with blocking solution (0.3% hydrogen peroxide in methanol) for 20 min at room temperature and rinsed in PBS. After incubation in permeabilization solution (0.1% Triton X-100 in 0.1%

sodium citrate) for 2 min on ice they were washed in PBS three times for 5 min. The sections were then incubated in a TUNEL reaction mixture in a humidified chamber for 60 min at 37 °C. After washing in PBS, the sections were mounted in DAPI solution containing 90% glycerol, p-phenylene-diamine (1 mg/ml, Sigma), and 4',6'-diamino-2'-phenylindole (DAPI 2 μg/ml) to identify cells showing cell death. Finally the sections were analyzed with a fluorescence microscope (Olympus, Tokyo, Japan) (Vermes et al., 2000).

EEG recording – Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and electrodes were implanted over the cortex using a stereotaxic apparatus (Paxinos and Watson, 1998). The animals were allowed to recover for 7 days after the surgery. An EEG apparatus (DSI, St. Paul, MN, USA) and its corresponding software were used. EEG recording was performed in a plexiglass compartment. KA was injected 1 h after MEFL (2, 4 mg/kg, p.o) administration. Baseline EEG recordings were performed for 15 min before KA (50 mg/kg, i.p) injection. Subsequently, EEGs were recorded continuously and behavioral observation was noted. Upon completion of the 60 min recording sessions, the animal was removed from the recording chamber and returned to its home cage. EEG activity was evaluated using the program *SleepSign* (ver. 2.0). The power spectra were plotted and the integrated energy signals are expressed as mV^2.

Cell culture – Primary cultures of cerebellar neurons enriched in granule cells were prepared from the cerebellum of eight-day-old Sprague-Dawley rats as previously described (Ma *et al*., 2007). Briefly, cells were plated (1×10^6 cells per 0.2 ml) in 96-well microplates or (2×10^6 cells per 2 ml) in 60-mm dishes that had been coated with poly-$_D$-lysine (10 μg/ml) (Sigma, St. Louis, MO, USA). The cells were cultured in basal Eagle's medium (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies), 2 mM glutamine, gentamicin (100 μg/ml), antibiotic-antimycotic solution (10 ml/l; Sigma, USA) and 25 mM KCl. This high concentration of potassium is necessary to induce persistent depolarization, which promotes the survival of granule cells. Cells were incubated for 6 - 9 days in a humidified 5% CO_2/95% air atmosphere at 37 °C. Cytosine arabinofuranoside (final concentration 10 μM) was added to cultures 18-24 h after plating to inhibit the proliferation of non-neuronal cells. Cultured cells were used for measurements after 5 - 6 days.

Measurement of neuronal cell toxicity – KA and muscimol were soluble in the incubation buffer described below. MEFL was dissolved with the buffer. Neurotoxicity testing was performed on neurons grown for 7-8 days *in vitro* on 96-well microplates. The cells were incubated for 30 min in the same medium, the culture medium was removed, and neurons were incubated with a Krebs-Ringer-HEPES buffer (KRH; consists of HEPES 20 mM, NaCl 137 mM, $CaCl_2$ 1.3 mM, $MgSO_4$ 0.4 mM, $MgCl_2$ 0.5 mM, KCl 5.0 mM, KH_2PO_4 0.4 mM, Na_2HPO_4 0.6 mM, $NaHCO_3$ 3.0 mM, glucose 5.6 mM, glycine 0.01 mM) containing 30 μM propidium iodide (Ohashi *et al*., 2007). Neurons were incubated for a further 24 h in the presence of KA (600 μM) at 37 °C. In every step, MEFL (0.25, 0.5, 1 μg/ml) or muscimol (40 μM) was added 30 min prior to the exposure of cells to KA and was present in the incubation buffer during KA exposure. Neuronal death was measured by propidium iodide detection. Propidium iodide (PI) detection: neuronal viability was monitored by PI fluorometry using a multi-well fluorescence scanner (FLUO star 403, BMG Lab Technologies GmbH, Offenburg, Germany). Briefly, fluorescence from each well was measured using excitation and emission wavelengths of 544 nm (25 nm band pass) and 590 nm (35 nm band pass), respectively. For each experiment, an initial fluorescence measurement (Fo) was made 20 min after addition of PI and then at intervals thereafter. At the end of each experiment, cells were killed with 375 μM digitonin to penetrate all cells, and the PI fluorescence obtained after this treatment and 1 h incubation represented 100% cell death (the final fluorescence; Ffin). Cell death was then expressed as a percentage of the final fluorescence (Ffin) minus the background fluorescence (Fo) taken before the injury, as shown in the following equation: % cell death = 100 (Ft-Fo) / (Ffin-Fo) where Ft is the fluorescence of cells measured at after the onset of injury.

Measurement of chloride influx – The intracellular Cl^- concentration of cerebellar neurons was estimated using the Cl^- sensitive fluorescence probe MQAE according to the method of West and Molloy, with a slight modification (West and Molloy, 1996; West *et al*., 1976). The buffer (pH 7.4) contained the following: 2.4 mM HPO^{2-}_4, 0.6 mM $H_2PO^-_4$, 10 mM HEPES, 10 mM $_D$-glucose and 1 mM $MgSO_4$. A variety of MQAE-loading conditions were assessed. The cells were incubated overnight in medium containing 10 mM MQAE (Dojindo, Japan). After loading, the cells were washed three times in the relevant Cl^- containing buffer. The buffer was replaced with buffer with or without the compounds. Then, MEFL (0.25, 0.5, 1 μg/ml) or muscimol (40 μM) was added 30 min prior to the exposure of cells to KA (600 μM) and was present in the incubation buffer during KA exposure. Repetitive fluorescence measurements

Table 1. Anticonvulsant activity of MEFL against KA-induced seizure

Treatment (mg/kg)	Number of Survival (%)	Seizure score (Mean)	Latency to convulsion (s)	Weight loss (%)
Kainate 50 + Saline	11/15 (73%)	5 ± 0.23	890 ± 144	3.1 ± 0.62
Kainate 50 + MEFL 1	13/15 (87%)	4.3 ± 0.29	1290 ± 124.5*	2.1 ± 0.62
Kainate 50 + MEFL 2	15/15 (100%)*	4.4 ± 0.20*	1385 ± 118.5*	2.4 ± 0.59
Kainate 50 + MEFL 4	14/15 (93%)	3.6 ± 0.33***	1467 ± 128.6**	1.7 ± 0.60

Values are Mean ± S.E. *P < 0.05, ** P < 0.01, *** P < 0.005 compared with KA control group.

were initiated immediately using a FLUOstar (excitation wavelength: 320 nm; emission wavelength: 460 nm; BMG Lab Technology, Germany). The data is presented as the relative fluorescence Fo/F, where Fo is the fluorescence without Cl^- ions and F is the fluorescence as a function of time. The Fo/F values are directly proportional to $[Cl^-]i$.

Measurement of $[Ca^{2+}]$i concentration – A confocal scanning microscope (Leica, TC-SP, Heidelberg, Germany) was used to evaluate relative changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) by monitoring Fluo-3 fluorescence after intracellular cleavage of superfused Fluo-3 AM (1 μM; Molecular Probes, Eugene, Oregon, USA). Cells grown on glass cover slides were loaded with 1 μM Fluo 3-AM for 45 min in D-Hanks solution at 37 °C in the CO_2 incubator, and washed with incubation buffer. Baseline was recorded for 120 s prior to the addition of KA (600 μM). To test the effects of MEFL (0.5, 1 μg/ml) and muscimol (40 μM) on KA-induced $[Ca^{2+}]i$ changes, the cells were exposed to these compounds in the incubation buffer for 10 min, after being loaded with Furo 3-AM and washed. The compounds were also present in the incubation buffer during the measurement of KA-induced $[Ca^{2+}]i$ change. This experiment was performed in the dark. Laser scanning was used to obtain a timed series of images with excitation at 488 nm and emission at 510 nm, and an acquisition rate of 1 frame every 30 s. The obtained images were quantitatively analyzed for changes in fluorescence intensities within a cell using Leica confocal software. Data were obtained by evaluating the fluorescence (F) within a cell, subtracting the background fluorescence, and dividing by the fluorescence intensity before KA treatment (Fo), expressed as F/Fo. confocal cultures had incubation buffer added. For the drug treatment groups, MEFL (0.5, 1 μg/ml) or muscimol (40 μM) was added to the incubation buffer 10 min before and during recording.

Statistical analysis – Results are presented as the mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's post hoc multiple comparison test. Survival analysis was done using the Chi-square test (SPSS, Chicago, IL, USA)

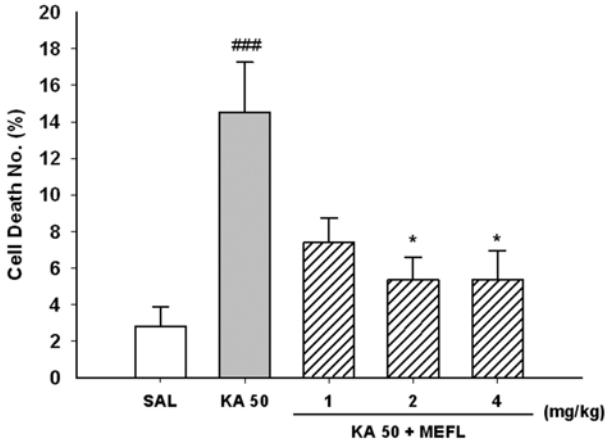

Fig. 2. Protection by MEFL against KA-induced cell death. After observation of 630x, the TUNEL, DAPI positive stained nucleus was counted. DAPI stained nucleus represents viable cells, while TUNEL stained nucleus represents apoptosis cells. Dead cells were counted as (TUNEL) / (TUNEL + DAPI) × 100. $^*P < 0.05$ compared with kainic acid control group, $^{###}P < 0.005$ compared with saline group.

Results

MEFL protects against KA-induced convulsions – KA (50 mg/kg, i.p.) elicited convulsions in 87% of the mice. Administration of MEFL (2 and 4 mg/kg, p.o.) significantly decreased seizure severity score in KA treated mice. M MEFL (1, 2 and 4 mg/kg, p.o.) also significantly increased the latency of seizure onset in KA treated mice in dose dependent manner (Table 1).

MEFL attenuates neuronal cell death of hippocampus – We performed TUNEL staining to visualize the fragmented nuclei characteristic of apoptotic nuclei. Concurrent staining of DNA with 4.6-diamidino-2-phenyl-indole (DAPI) and analysis of stained cells by laser scanning cytometry allows sorting of cell populations. KA treatment produced TUNEL positive cells in the CA3 pyramidal cell layer, with DAPI staining indicating that surviving cells sustained DNA damage. The rate of apoptotic cell staining was 14.5% ± 2.7%, but MEFL reduced it to 5.4% ± 1.2% (Fig. 2).

MEFL decreases seizure-form EEG activity induced by KA – KA induced intense, high-amplitude electrical

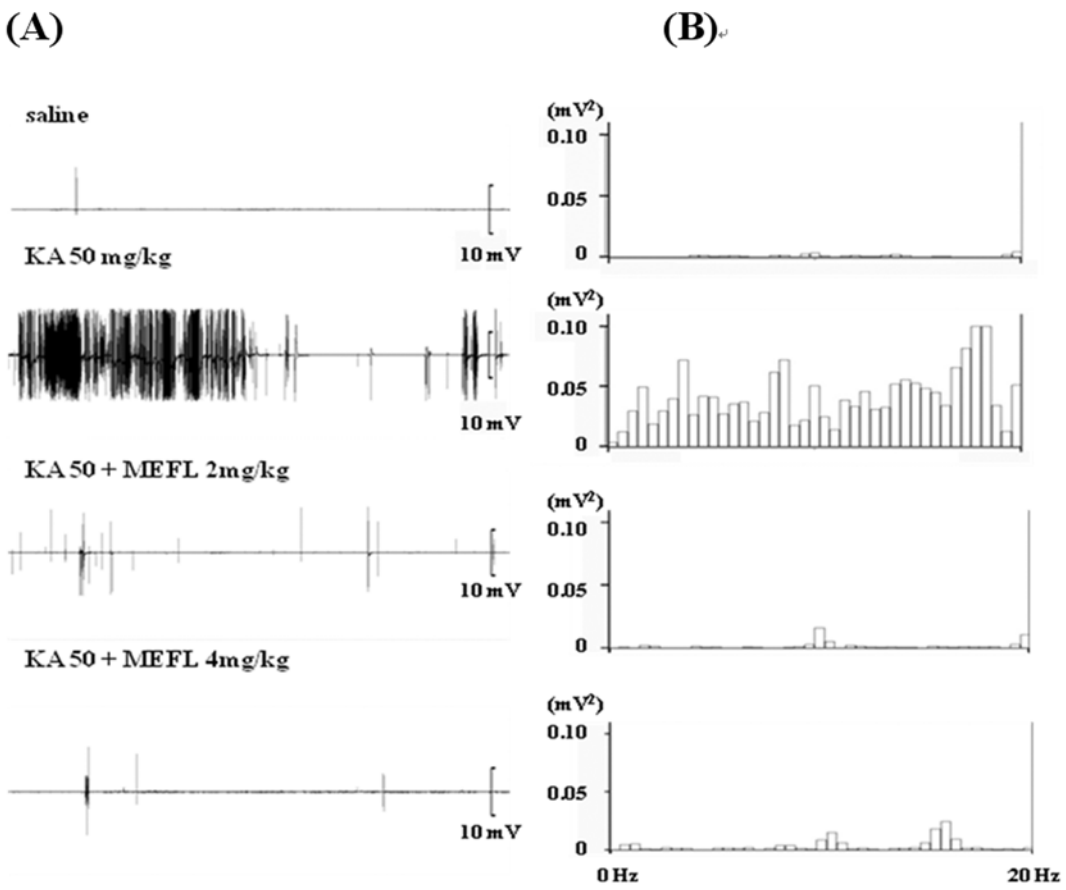

Fig. 3. **(A) Effects of MEFL on electrical seizure discharges and (B) power spectra of EEG in cortex induced by KA.** MEFL and muscimol were administered 1 h before KA injection. Control; KA (50 mg/kg); MEFL (2 mg/kg) + KA (50 mg/kg); MEFL (4 mg/kg) + KA (50 mg/kg). An electrical seizure was measured in the 0-20 Hz range.

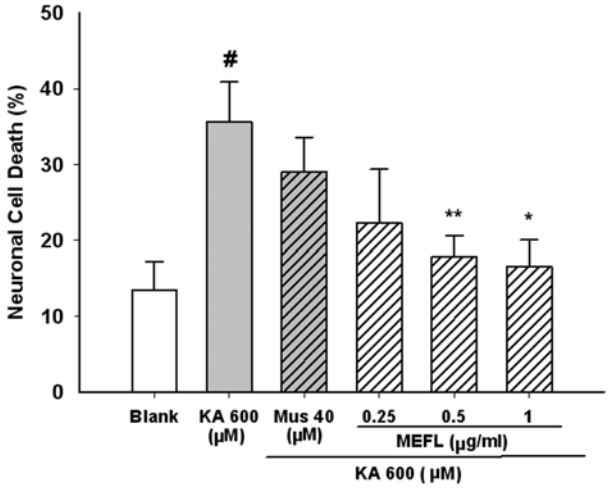

Fig. 4. Effects of MEFL on KA-induced neuronal cell death. Cell toxicity rate of cultured neuronal cells through propidium iodide detection. Neuronal cell death (%) was calculated as 100 (Ft-Fo) / (Ffin-Fo). Ft: Fluorescence of samples at 24 h later; Fo: Initial fluorescence; Ffin: Fluorescence with 375 μM digitonin. $*P < 0.05$, $**P < 0.01$ compared with KA control, $^{\#}P < 0.05$ compared with blank.

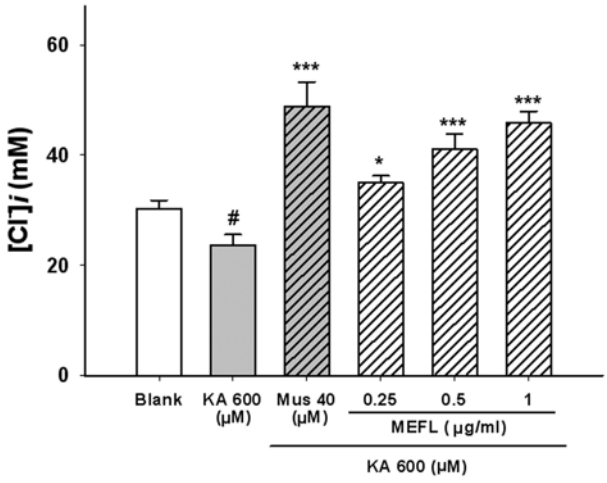

Fig. 5. Effects of MEFL on KA-induced chloride influx in cultured neuronal cells. The cerebellar neuronal cells were cultured for 5 days, incubated with MQAE overnight, and then MEFL (0.25, 0.5 and 1 μg/ml) and muscimol (40 μM) were added 30 min prior to kainic acid (600 μM) treatment. Representative fluorescence measurements were initiated immediately using a FLUO star. The experiment was repeated 3 times. Each column represents the mean ± S.E.M. $*P < 0.05$, $***P < 0.005$, compared with KA control, $^{\#}P < 0.05$ compared with blank.

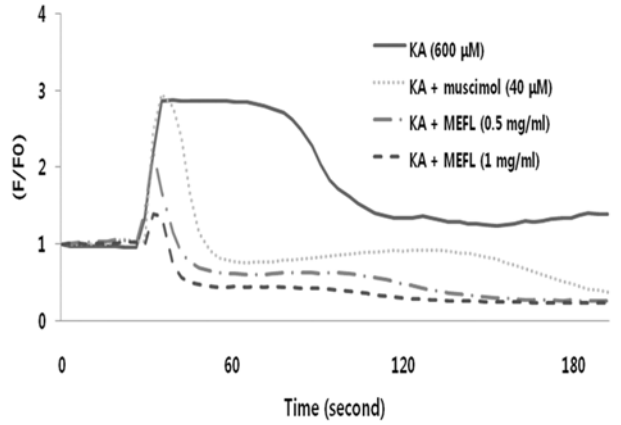

Fig. 6. Change of $[Ca^{2+}]i$ in cultured neuronal cells. Intracellular $[Ca^{2+}]i$ was monitored using a confocal microscope. Results are expressed as the relative change in intensity of Fluo-3 fluorescence (F/Fo). Each trace represents F/Fo value from a representative cell population.

seizure discharges in the anterior cortex, usually simultaneously with behavioral seizures. Continual EEG changes presented in the form of sporadic isolated waves that progressed into a series of synchronized poly-spikes characterized by uninterrupted bursts of high-voltage amplitude spikes (1 - 10 mV) (Fig. 3, A). These changes started gradually 10 - 15 min after KA application. MEFL treatment reduced this electrical seizure activity, with only a few low-amplitude seizure discharges detected (Fig. 3, B).

MEFL inhibits cultured neuronal cell death – KA (600 μM)-induced plasma membrane damage in cerebellar granule neurons, affecting their ability to take up propidium iodide (PI), with a cell death rate of 35.6% ± 5.3%. However, MEFL (0.5, 1 μg/ml) significantly reduced cell death to 17.8% ± 2.7% and 16.6% ± 3.5%, respectively. (Fig. 4).

MEFL increases chloride influx – Resting intracellular Cl^- levels were calibrated using standard 0, 15, 30, 45, 60, or 75 mM Cl^- solutions, with methylsulfate used to replace Cl^-. Thibutyltin chloride (5 μM) and nigericin (5 μM) were present to artificially facilitate the balance between intracellular Cl^- and extracellular Cl^- concentrations. Resting $[Cl^-]i$ in cultured neuronal cells was 30.32 ± 1.51 mM, and $[Cl^-]i$ in KA treated cells was 23.68 ± 1.92 mM. MEFL (0.25, 0.5, 1 μg/ml) increased chloride influx to 35, 41.1, and 45.8 mM, respectively, and muscimol increased chloride influx to 48.90 mM (Fig. 5).

MEFL inhibits KA-induced elevation of intracellular $[Ca^{2+}]_i$ – KA (600 μM) increased intracellular $[Ca^{2+}]i$, peaking at 60 - 90 seconds. Muscimol (40 μM) and MEFL (0.5, 1 μg/ml) treatment blocked this increase in $[Ca^{2+}]i$ but did not affect basal $[Ca^{2+}]i$ (Fig. 6).

Discussion

From these experiments, MEFL inhibited convulsions and neuronal cell death induced by KA, a potent neuroexcitotoxin. MEFL protected against neuronal toxicity, especially apoptotic death in the hippocampal CA3 region. MEFL inhibited the occurrence of EEG seizure-form activity and decreased intracellular calcium levels in cultured neuronal cells. MEFL also inhibited elevation of $[Ca^{2+}]i$ and increased $[Cl^-]i$ induced by KA in cultured neuronal cells

KA is a selective ionotropic glutamate agonist (Giusti *et al*., 1996). KA receptors underlie a seizure triggering mechanism in experimentally elicited seizures. KA works like glutamate and enhances glutamatergic neurotransmission. Elevated extracellular glutamate induces neurodegenerative processes associated with convulsions and other neuropathological conditions (Lothman *et al*., 1981; Regan and Choi, 1991). These glutamate receptors agonists, either peripheral or intracerebral, induce convulsions in rodents (Chapman, 1998). KA is toxic mainly in the hippocampal formation, which has high-affinity KA binding sites (CA3 pyramidal cells). MEFL protects against neuronal toxicity and apoptosis in the CA3 region.

KA also induces excitatory changes in surface EEG activity and increased EEG power spectra (Kriz *et al*., 2003). MEFL prevented this EEG seizure-form activity. MEFL may block glutamatergic transmission by KA receptors to prevent the generation, and propagation of seizure-related neuronal discharges, potentially through inhibitory systems in the CNS.

The cerebellum and hippocampus are closely related to seizure onset (Liu *et al*., 2001). Cultured rat cerebellar granule neurons are a common model for studying seizure mechanisms and glutamate or GABAergic systems. MEFL protected neurons against KA-induced cell death and blocked KA-induced increases in $[Ca^{2+}]i$. MEFL also inhibited KA-induced $[Ca^{2+}]i$ and elevated Cl^- influx, indicating that MEFL may specifically influence Ca^{2+} dependent pathways of KA receptors and Cl^- influx of GABA receptors. Ionotrophic glutamate receptors regulate neuronal survival *in vivo* as well as *in vitro* (Speckmann *et al*., 1989). Inotropic glutamate receptor agonists, such as KA, typically increase cell death. Stimulation of glutamate receptors triggers Na^+ influx and neuronal depolarization, opening of voltage-dependent calcium channels, and stimulation of calcium influx (Park *et al*., 2004). Elevated Ca^{2+} generates reactive oxygen species and induces neuronal injury, leading to Cl^- influx down its electrochemical gradient, cationic influx, and osmotic

lysis, producing neuronal death (Allison and Pratt, 2003; Coyle and Puttfarcken, 1993). Most anticonvulsant drugs work by antagonizing Na^+ or Ca^{2+} channels, inhibiting glutamate receptors, or increasing Cl^- influx, facilitating GABAergic inhibition (Ben-Ari, 2002). MEFL could influence the expression of $GABA_A$ receptor subunits and glutamate acid decarboxylase (GAD) in cultured granule neurons (Ma *et al*., 2009). Our current results further suggest that MEFL inhibits Ca^{2+} influx activated by the KA receptor through direct interaction with glutamate receptors. MEFL may block Ca^{2+} and glutamate, as well as Ca^{2+}-dependent Cl^- channels, or stimulate GABAergic systems. $GABA_A$ receptors transmit Cl^- to produce rapid hyperpolarization and block the excitatory effects of KA (Saija *et al*., 1994). Baclofen blocks presynaptic Ca^{2+} entry in the CA1 (Heinemann *et al*., 1984). GABA reduces pre- and postsynaptic $[Ca^{2+}]_i$ levels. Muscimol prevented seizures and lethality through activating the $GABA_A$ receptor to facilitate inhibitory neurotransmission (Hansen *et al*., 2004). MEFL similar to muscimol, inhibited neuronal death, increased intracellular chloride, and decreased intracellular calcium.

Taken together, MEFL has considerable anticonvulsant effects. We presume that MEFL protects KA-induced seizure indirectly through increase of chloride level. Further investigation is needed to understand the pharmacological actions and possible mechanisms of MEFL.

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