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Asiatic acid and maslinic acid attenuated kainic acid-induced seizure through decreasing hippocampal inflammatory and oxidative stress

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ABSTRACT

Seizure is a neurological disorder including hippocampal oxidative and inflammatory stress, and glutamate toxicity. Thus, any agent(s) that mitigate(s) these events in hippocampus might attenuate seizure severity. The effects of asiatic acid (AA) or maslinic acid (MA) pre-administration at 20 or 40 mg/kg body weight/day upon inflammatory, oxidative and apoptotic injury in hippocampus of kainic acid (KA)-treated mice were examined. KA induced seizure-like behavioral patterns, which was attenuated by AA or MA pre-administration. KA stimulated the release of interleukin (IL)-1beta, IL-6, tumor necrosis factor-alpha and prostaglandin E_2 in hippocampus of mice. AA or MA pre-administration decreased the production of these inflammatory factors. AA or MA also diminished KA-induced increase in hippocampal cyclooxygenase-2 activity and relative NF-κB p50/65 binding activity. KA depleted glutathione content and promoted reactive oxygen species generation. AA or MA pre-administration reversed these alterations. KA lowered Bcl-2 mRNA expression and increased Bax mRNA expression. AA or MA treatments reduced Bax mRNA expression. AA or MA pre-administration enhanced glutamine synthetase activity, decreased glutamate level and increased glutamine level in hippocampus of KA treated mice. In addition, AA or MA pre-treatments at 10 and 20 μM increased viability and decreased plasma membrane damage in KA treated nerve growth factor (NGF)-differentiated PC12 cells. Both agents also lowered the release of calcium ion induced by KA in NGF-treated PC12 cells. These findings support that asiatic acid and maslinic acid are potent nutraceutical agents for seizure alleviation.

1. Introduction

Seizure, a neurological disorder, is characterized by involuntary shaking of partial or entire body, and sometimes causes consciousness loss (Chen et al., 2017). Both experimental and clinical evidence indicate that inflammatory stress is involved in the etiopathogenesis of seizure, especially in the area of hippocampus (Friedman, 2011; Vezzani et al., 2011). The activation of crucial inflammatory mediators such as cyclooxygenase (COX)-2 and nuclear factor kappa B (NF-κB), and the over-production of the down-stream inflammatory factors including interleukin (IL)-1beta, IL-6, tumor necrosis factor (TNF)-alpha and prostaglandin E_2 (PGE_2) contributed to the progression of seizure (Huang et al., 2012; Teocchi et al., 2013). On the other hand, excessive generation of reactive oxygen species (ROS) promotes seizure related to neuronal membrane depolarization, and exacerbates neuron malfunctions (Kovac et al., 2014). Consequently, both inflammatory and oxidative injuries cause the death of brain nerve cells (Murashima et al., 2005). In addition, glutamate excitotoxicity is another important element in charge of seizure induction (Ravizza et al., 2011). Eid et al. (2013) reported that increased extracellular glutamate level in hyperexcitable areas of brain trigged seizure through activating glutamate receptor and genes involved in synaptic plasticity. Glutamine synthetase (GS) could metabolize glutamate to glutamine, and facilitates glutamate clearance (Rosati et al., 2009). Therefore, any agent(s) with the capability to diminish inflammatory, oxidative and/or glutamate toxicity in hippocampus may attenuate seizure severity.

Kainic acid (KA), a glutamate related compound, could induce neuronal excitability and neuronal membrane depolarization by releasing calcium ions to impair nerve impulse transmission (Malva et al., 2003). KA-induced seizure in rodents has been widely used as an experimental seizure model for the associated pathological, preventive and therapeutic researches (Gupta et al., 2002; Huang et al., 2012). Furthermore, KA-induced seizure has been considered as a model of lesional epilepsy because KA causes focal hippocampal lesion (Craig et al., 2008). Asiatic acid (AA) and maslinic acid (MA) are pentacyclic triterpenes (Fig. 1) naturally occurring in many edible plant foods such as centella (*Centella asiatica* L.), olive (*Olea europaea* L.), brown mustard (*Brassica juncea*) and gynura (*Gynura bicolor* DC) (Yin et al., 2012;

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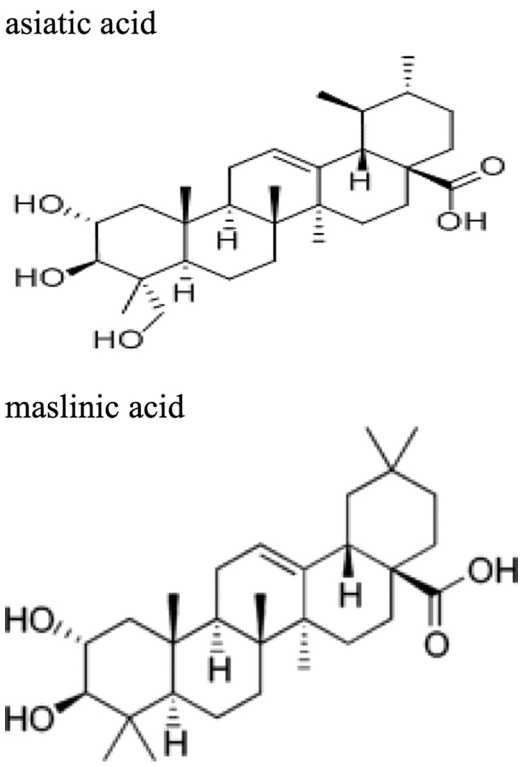

Fig. 1. Structure of asiatic acid and maslinic acid.

James et al., 2013; Sánchez-Quesada et al., 2013). Lee et al. (2014) indicated that AA displayed neuroprotective activities for focal embolic stroke in rats via lowering the release of apoptosis-inducing factor from brain mitochondria. Our previous animal study revealed that dietary AA intake alleviated the progression of Parkinson's disease by suppressing striatal expression of α-synuclein and increasing striatal dopamine level (Chao et al., 2016). Huang et al. (2011) reported that MA limited NF-κB expression and ameliorated inflammatory injury in cortical astrocytes. Qian et al. (2015) indicated that MA benefited synaptogenesis in cerebral ischemia model through activating Akt/GSK-3β. Those previous studies suggest that AA and MA are potent protective agents for brain. Therefore, both animal and cell line studies were designed to evaluate the anti-seizure activities of AA and MA.

In our present study, the effects of AA and MA pre-administrations at various doses upon inflammatory, oxidative and apoptotic injury in hippocampus of KA-treated mice were examined. The impact of these agents upon seizure behavior, and hippocampal variation of inflammatory and oxidative factors, glutamine level and GS activity was measured. In addition, the activities of AA or MA against KA induced apoptosis and calcium release in nerve growth factor (NGF) differentiated PC12 cells were determined.

2. Materials and methods

2.1. Materials

AA (98%), MA (97%), KA (99.5%) and NGF (99.5%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). AA or MA was suspended in 0.8% methyl cellulose (MC). KA was dissolved in phosphate-buffered saline (PBS, pH 7.2). Antibodies were purchased from Boehringer Ingelheim Co. (Ingelheim am Rhein, Germany).

2.2. Animals

Male C57BL/6 mice at 4-week old were obtained from the National Laboratory Animal Center (Taipei City, Taiwan). Mice were housed in cages under a 12-h light/dark cycle and a constant temperature, 22 ± 2 °C. Mice were fed by water and standard diet. Mice with body weight at 24.9 ± 1.4 g were used in all experiments. Use of these mice was approved by China Medical University Animal Care Committee, and the approval number was 103-27-N. All procedures were handled carefully in order to minimize mice suffering within experiments.

2.3. Experimental design

AA or MA at 20 or 40 mg/kg body weight (BW)/day was orally administrated once per day for 7 consecutive days, and followed by KA treatment at 20 mg/kg BW intraperitoneally for seizure induction. Mice in control and KA groups received MC oral administration for 7 days. Then, mice in control groups were treated by intraperitoneal injection of MC, and mice in KA groups were intraperitoneally injected by KA (20 mg/kg BW). Each group had 7 mice. After KA challenge, seizure behavior was monitored and rated according to the modified Racine's scale (Wang et al., 2014): stage 0, normal behavior; stage 1, facial automatisms; stage 2, facial and head clonus; stage 3, forelimb clonus; stage 4, rearing; stage 5, rearing, loss of balance and falling. Seizure score was recorded at 0, 30, 60, 90, 120, 150 and 180 min after KA challenge. One day after the KA treatment, mice were sacrificed by decapitation under CO_2 asphyxia. The whole brain was dissected and the hippocampus of each mouse was collected for analyses. AA, MA or KA treatment did not cause mouse loss during experimental period.

2.4. Measurement of inflammatory factors

Ten mg hippocampus was homogenized in a Tris solution (10 mM, pH 7.4) composted of 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride and 0.01% Tween 80. After centrifuging at 9000 × *g* for 30 min at 4 °C, IL-1beta, IL-6 or TNF-alpha content in supernatant was assayed by cytoscreen kits (BioSource International, Camarillo, CA, USA). PGE_2 level was determined by an EIA kit obtained from Cayman Chemical Co. (Ann Arbor, MI, USA).

2.5. Assay of caspase activity

The activity of caspase-3 and caspase-8 was assayed by fluorometric kits (Upstate, Lake Placid, NY, USA). The values of coefficient of variability for inter-assay and intra-assay were 4.7–6.1% and 4.2–5.3%, respectively. Ten mg hippocampus was lysed by lysis buffer. Lysates were then mixed with reaction buffer and specific fluorogenic substrates for caspase-3 or -8. After 1 h incubation at 37 °C, fluorescence was monitored by a Hitachi fluorophotometer (F-4500, Tokyo, Japan), excitation wavelength was 400 nm and emission wavelength was 505 nm. Protein content was measured by a commercial assay kit (Pierce, Rockford, IL, USA). Activity was showed as fluorescence unit/mg protein.

2.6. NF-κB p50/65 binding activity assay

Nuclear extract was prepared according to the method of Schilling et al. (1999). Ten μg nuclear protein extract was used for measuring NF-κB p50/65 binding activity. NF-κB p50/65 binding activity was assayed by a commercial kit purchased from Chemicon International Co. (Temecula, CA, USA). It was processed by adding the substrate, 3, 3′, 5, 5′-tetramethylbenzidine, a primary NF-κB p50/p65 antibody. After 1 h incubation at room temperature and washing by PBS twice, a second horseradish peroxidase-conjugated antibody was added for another 1 h. The variation of absorbance at 450 nm was monitored by a microtiter plate reader (Model 550, Bio-Rad, Hercules, CA, USA). Data are expressed as optical density (OD) value/mg protein.