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Neuroprotective Effect of *Acanthopanax sessiliflorus* against Toxicity Induced by N-Methyl-D-Aspartate in Rat Organotypic Hippocampal Slice Culture

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Abstract – We investigated that water extract of *Acanthopanax sessiliflorus* roots rescued the N-methyl-D-aspartate (NMDA), agonist of glutamate receptor, -induced toxicity in rat organotypic hippocampal slice culture. When the cell death in NMDA only-treated hippocampal slices was set 100%, *A. sessiliflorus* decreased the cell death to 75.4, 51.6, 48.9, and 40.6% at 1, 10, 50, and 100 μg/ml treatment, respectively. On the basis of these results, the water extract of *A. sessiliflorus* roots may be a preventive agent against NMDA-induced cytotoxicity.
Keywords – *Acanthopanax sessiliflorus*, Araliaceae, N-Methyl-D-Aspartate, Exicitoxicity, Hippocampal slice culture, Neuroprotection

Introduction

After onset of ischemia, ATP was depleted and ion balance between inner- and outer-membrane was devastated, which induced the excessive release of pre-synaptic glutamate (Nicholls and Attwell, 1990). Although glutamate is an important neurotransmitter for the neuronal function, excessive glutamate resulted in over influx of calcium and neuronal cell death. Glutamate receptors are classified into two types: ionotropic and metabolic receptors. Ionotropic receptors are also further divided into three types depending on the agonist-specific preference: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainaite receptors. Since ionotropic receptor is coupled to an ion channel, binding of a ligand stimulate the ion channel opening and influx of calcium. Increased cytosolic calcium had deleterious effect on mitochondrial function and generated the reactive oxygen species (ROS), leading to the neuronal cell death (Gunasekar *et al.*, 1995; Tong *et al.*, 1995). Among glutamate receptor types, NMDA receptor-mediated calcium influx has been reported to contribute to the neuronal cell death by ischemic injury. Therefore, blocking pathological pathway mediated by NMDA receptor is thought to be strategy for the neuroprotective drug development in ischemia.

Pharmacological activities of the genus *Acanthopanax* plants including *A. senticosus* and *A. koreanum* have been extensively studied. Investigations on the compounds from *A. senticosus* have revealed the presence of phenolic compounds such as isofraxidin, eleutheroside E_2, and isomaltol 3-*O*-α-D-glucopyranoside from the roots (Li *et al.*, 2001) and chiisanoside, chiisanogenin and hyperin from the leaves (Lee *et al.*, 2003), etc. Recently we reported the anti-oxidant effect of the water extract from the stem bark of this plant (Lee *et al.*, 2004) and the isolation of constituents from this plant (Ryu *et al.*, 2004). *A. senticosus* showed anti-oxidant, ant-inflammatory, and anti-bacterial effect (Jung *et al.*, 2003; Lee *et al.*, 2003a; Lee *et al.*, 2004). In particular, *A. senticosus* enhanced the generation of noradrenaline and dopamine. Even if *A. sessiliflorus* has been used as a tonic and sedative, biological effects, especially on nervous system, were not investigated relative to other genus *Acanthopanax* plants.

Hippocampal slice culture has been used for an investigation of neurotoxic and gliotoxic effect, such as trauma, ischemia, Alzheimer, and neuroimflamation (Andrius *et al.*, 2001; Bonde *et al.*, 2002; Nicholas *et al.*, 2000). In this study, we found that water extract of *A. sessiliflorus* roots (WAS) protected the neurons from toxcity induced by NMDA in hippocampal slice culture.

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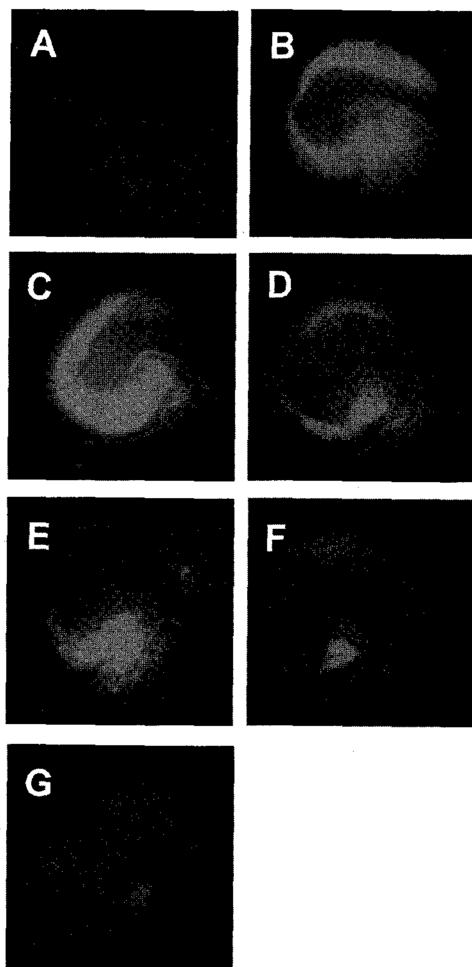

Fig. 1. Water extract of *A. sessiliflorus* roots (WAS) attenuated the neuronal cell death induced by NMDA. After 24 h treatment with NMDA (10 μM) and samples, cell death was determined by using PI staining. MK-801 was used as positive control. **(A)**: vehicle, **(B)**: NMDA (10 μM), **(C)**: NMDA (10 μM) and WAS (1 μg/ml), **(D)**: NMDA (10 μM) and WAS (10 μg/ml), **(E)**: NMDA (10 μM) and WAS (50 μg/ml), **(F)**: NMDA (10 μM) and WAS (100 μg/ml), **(G)**: NMDA (10 μM) and MK-801 (30 μM).

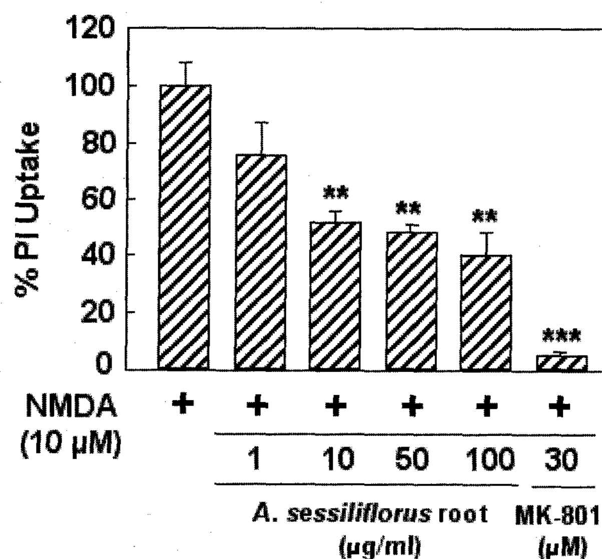

Fig. 2. Cell death of the NMDA-exposed hippocampal slice culture in the presence of WAS (n = 6). Cell death of NMDA only-treated hippocampal slices was set to 100%. Compared with this 100% value, cell death in sample-treated hippocampal slice culture was calculated. The three independent experiments were carried out. The data was expressed as the mean ± S.E.M. The asterisk indicates a significant difference from exposure to NMDA alone.

Experimental

Materials – α-Modified Eagle Medium (α-MEM), penicillin/streptomycin solution, Hank's balance salt solution (HBSS) and horse serum (HS) were purchased from Gibco BRL (Grand Island, NY, USA). Propidium iodide (PI), D-glucose, and all the other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant and extraction – The roots of *A. sessiliflorus* Harms was collected at Kyungsang Province, Yongdong, Korea. The air-dried powder (1 kg) of *A. sessiliflorus* was extracted with distilled H_2O under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 110 g of the residue.

Organotypic hippocampal slice culture – Hippocampus slice culture was prepared and grown according to the modified interface culture method (Noraberg *et al.*, 1999). Sprague-Dawley rats (7 days old) were decapitated. Hippocampus was isolated and dorsal halves were cut in transverse sections at 400 μm by Mcllwain Tissue Chopper (Mickle Laboratory Engineering Co., Surrey, UK). The six tissue slices were in random order placed on insert membrane (0.4 μm in porous, 30 mm in diameter, Millipore Co., Bedford, MA, USA). The inserts were transferred to 6 well culture trays, where each well contained 1 ml culture medium composed of 50% α-MEM, 25% HS and 25% HBSS supplemented with 25 mM D-glucose. The medium was changed every 3 days and experiments were carried out after 14 days. Slices are divided into seven experimental groups: Vehicle treatment, NMDA (10 μM) only treatment, NMDA (10 μM) plus WAS (1 μg/ml), NMDA (10 μM) plus WAS (10 μg/ml), NMDA (10 μM) plus WAS (50 μg/ml), NMDA (10 μM) plus WAS (100 μg/ml), and MK-801 (30 μM). After 48 h, cell death was analyzed. On the basis of a previous report (Kristensen *et al.*, 2001), treated NMDA concentration was determined.

Cell death analysis – Cell death was assessed using the fluorescent exclusion dye PI, which fluorescence was

excited at 514 nm using a confocal laser-scanning microscope (Carl Zeiss, LSM 510, Germany). The digital photos were analyzed directly in public domain NIH image program. Cell death area of NMDA-exposed hippocampal slice culture was considered as 100% cell death. Compared with this 100% value, cell death in sample-treated hippocampal slice culture was calculated.

Statistical analysis – The data is presented as a mean ± S.E.M from three independent experiments. A statistical comparison between the different treatments was done by one-way ANOVA followed by Turkey's test. Differences with P value less than 0.05 were considered statistically significant.

Results and Discussion

A. senticosus was reported that it may act by regulating noradrenaline and dopamine levels in specific brain regions related to stress response and Parkinson's disease (Fujikawa *et al.*, 2002). In this study, we found that *A. senticosus* counteracted the toxicity induced by NMDA in rat hippocampal slices in culture.

Cell death was analyzed by using PI staining. PI uptake was excluded in intact membrane. However, damaged membrane allowed the entrance of PI. NMDA treatment elicited the cell death of hippocampal region including the pyramidal and a granular cell layer. The WAS rescued the cell death in dose dependent manners. In the cultures treated with WAS for 24h, PI uptake was significantly reduced. PI uptake in the groups treated with WAS at the concentrations of 1, 10, 50, and 100 μg/ml was 75.4, 51.6, 48.9, and 40.6% respectively, of the level in the NMDA-treated group. Very low level of PI staining was observed in the cultures treated with MK-801.

Hippocampal slice culture takes advantage for the development of neuroprotective agents: relevance to animal experimental model and economical and easy handling relative to *in vivo* model (Frotscher *et al.*, 1995; Gahwiler *et al.*, 1997). In addition, neuroprotective effect of candidates can be investigated by using hippocampal slice culture under diverse pathological conditions. Since over-stimulation of NMDA receptor triggers the excessive influx calcium and leads to the neuronal cell death in ischemia, examination of neuroprotection in NMDA-treated hippocampal slice is believed to be one of the strategies for the neuroprotective drug development in ischemia. Aniracetam was reported to protect the neuronal cell against NMDA-induced toxicity in hippocampal slice culture (Pizzi *et al.*, 1995). Lee *et al.* demonstrated total methanol extract and alkaloid fraction of *Uncaria rhynchophylla* showed the neuroprotective effect in NMDA-exposed hippocampal slices (2003b; 2003c).

In the present study, results from the hippocampal slices induced the neuronal cell damage by the treatment of NMDA and from the MK-801, antagonist of NMDA receptor, counteracted against the toxicity induced by NMDA, which results are well agreed with previous reports (Kristensen *et al.*, 2001). Likewise, we found that WAS root attenuated the neuronal cell death induced by NMDA in hippocampal slice culture. Although we demonstrated the neuroprotective effect of *A. sessiliflorus* against NMDA, protective pathway of *A. sessiliflorus* was not identified.

Interestingly, *A. sessiliflorus* exhibited a region-specific neuro-protective effect in the pyramidal cell layer containing CA1 and 3 regions, indicating that the event leading to granular cell death in dendate gyrus in different from that in CA1 and 3 regions remain to be elucidated. In conclusion, based on the experimental results, there is a possibility that *A. sessiliflorus* has the neuroprotective activity. We carry out the experiments to investigate that which compounds of *A. sessiliflorus* and which mechanisms are responsible for the reduction of the neuronal cell death.

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