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Original Articles

Effects of *Arisaematics rhizoma* on Ischemic Damage and Cytotoxicity in Brain

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Abstract

(Arisaematis rhizoma) (Arisaematis amurense var. serratum NAKAI) (Udannamsung; UDNS) is a medicinal plant used in Korea for the treatment of various symptoms accompanying hypertension and cerebrovascular disorders. The present study was done to investigate the effects of UDNS on cultured primary neuron cell systems, cell cytotoxicity and lipid peroxidation in Amyloid-β protein treatment conditions. Cell killing was significantly enhanced by addition of increasing concentrations of Amyloid-β protein. Pretreatment of UDNS attenuated in cell killing enhanced by increasing concentrations of A myloid-β protein. MDA level induced by Amyloid-β protein25-35 treatment was significantly increased and the level was slightly reduced by pretreatment of UDNS. The present study showed that Amyloid-β protein strongly increased MDA level and the level was enhanced by addition of increasing concentrations of Amyloid-β protein or by time-related exposure to Amyloid-β protein. Furthermore, the protective effects which the water extracts had on Amyloid- protein peptide-induced neuronal death were also observed by LDH assay using cultured astrocyte cells. Amyloid-β protein-induced cell death was protected by the application of water extract of UDNS in a dose-dependent manner, and concentrations of 30 to 50 g/ml had a significant effect compared to exposure of Amyloid-β protein only. Oral administration of UDNS into mice prevented ischemia-induced learning disability and rescued hippocampal CA1 neurons from lethal ischemic damage. The presence of UDNS in neuron cultures rescued the neurons from NO-induced death. Taken together UDNS may exert its neuroprotective effect by reducing the NO-mediated formation of free radicals or antagonizing their toxicity.

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Key Words: Udannamsung, ischemia, neuroprotective effect, learning disability, hippocampal CA1 neurons.

Introduction

Neurodegenerative disorders are characterized by a progressive cognitive decline resulting from selective neuronal dysfunction, synaptic loss, and neuronal cell death. The well-studied neuropathological features of neurodegenerative disorder showed characteristics such as loss of neurons, formation of intra-neuronal neurofibrillary tangles composed of paired helical filaments of the cytoskeletal protein, and extracellular plaques composed primarily of diffuse or compacted deposits of amyloid-β(Amyloid-β protein) aggregates, with or without a component of dystrophic neurities[1,2].

It has been suggested that glial cells may play a role in the neurodegenerative cascade. Neurodegenerative disorders usually involve the activation of astrocytes and gliosis (microglia). Glial activation involves morphological changes (more spherical cell soma, hypertrophy of nuclei, appearance of extensive cellular processes) and changes in expression of a large number of

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proteins[3]. There are a number of stimuli that cause glial activation. One of the the best inducers of glial activation is neuronal dysfunction or injury.

It was reported that Arisaematis rhizoma(Udan-namsung; UDNS) and its extract are specifically effective for cerebrovascular lesion and aphasia during the treatment of Wind-heat syndrome and heat-phlegm in Oriental medicine[4], although little is yet known about the pharmacological effects or active ingredients. In our recent study, the pharmacological mechanism for UDNS was attributed to anti-aging and sexual-reinforcing activities in experimental in vitro and in vivo systems[5,6]. Mammals respond to oxygen deficiency in many different ways[7]. One strategy for survival of the individual cells under hypoxic conditions is the induction of glycolytic enzymes facilitating ATP production by glycolysis rather than mitochondrial oxidative phosphorylation.

This study reports the effect of UDNS on cytotoxicity of cultured astrocytes in Amyloid-β protein-treated conditions. Also, we report in vivo evidence that UDNS plays an important role in protecting neurons from ischemia-induced cell death. Experimental results on the mechanism underlying the protective effect of UDNS on glutamate-induced neuron death are also reported.

Materials and methods

Animals

Male and female rats, each weighing 20-30g (12 weeks of age), were used.

Materials

The Amyloid- β protein25-35 peptide was synthesized by Applied Biosystem's Protein Synthesizer Model 470A (Peptron Co., LTD, Taejon, Korea). Fetal bovine serum (FBS) and penicillin-streptomycin were obtained from GIBCO-BRL (Grand Island, New York, USA). Dulbeccos Modified Eagles Medium (DMEM), glutamine, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide(MTT), dimethyl sulphoxide (DMSO)were purchased from Sigma Chem. Co. (St. Louis, USA).

Extraction and preparation of UDNS

UDNS (100 g) was obtained from Oriental Medical Hospital, Dongguk University College of Oriental Medicine, and extracted with boiling water for 3 hrs. Then, the extract was evaporated to under reduced pressure by 75%, 85%, 95% ethanol solution. The last extracts were diluted by 0.9% NaCl and filtrated. The extract solution was stored at 4℃.

Cell culture

Cortical astrocyte cultures were prepared from neonatal rat (1-2 day old) pups by the method of Levision and McCarthy[8]. Cerebral cortex was dissected from neonatal day 1-2 Sprague-Dawley rat and dissociated by gentle trituration. Cells were plated in 6-well culture plates (0.2 mg/ml in sodium borate buffer, pH 8.3) at a density of 40,000 cells per well. After overnight incubation in Dulbecco's modified Eagle's medium (DMEM ; Gibco) supplemented with 20% fetal bovine serum, the medium was changed to serum-free defined medium for neurons DMEM supplemented with 2 mM glutamine, 1 mM pyruvate, penicillin-streptomycin-amphotericin B mixture, 5 mM HEPES, 0.5% glucose, 10 μg/ml insulin, 30 nM sodium selenite, 20 nM progesterone, 100 μM putrescine, and 20 μg/ml transferrin). The cultures were incubated at 37℃ in an atmosphere of 5% CO_2/95% room air, and the medium was replaced every other day. Experiments were performed in 6-

7-days-old culture.

Depending upon the experimental group, UDNS was added (at 2% volume in culture medium) to or omitted from flasks. After 16-18 hrs, cells were washed twice with warm phosphate-buffered saline (PBS) and serum-free medium was added to the flask. Then the cells were treated with 1 mM Amyloid-β protein for 2 hr and enzyme activities were measured. Amyloid-β protein was diluted in serum-free medium and added to the cultures.

Determination of cell viability and toxicity assay

Cell viability was determined with 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) to the coloured formazan product by mitochondrial enzymes in viable cells[9]. Cells were cultured in 24 or 96 well culture plates at a density of 10,000 cells per well for lactate dehydrogenase (LDH) assay or 40,000 cells per well for MTT reduction assay. LDH activities in the medium were measured by a Cytotox 96 nonradioactive cytotoxicity assay kit (Promega) according to the manufacturer' s instructions. The results were expressed as percentages of peak LDH release on complete cell lysis (control).

The MTT reduction was measured essentially as described previously[10] with a slight modification. In brief, after incubating cells for 48 hrs with various samples, Amyloid-β protein, MTT (Sigma) solution in PBS was added to a final concentration of 0.5 mg/ml, and the cells were further incubated for 4 hrs at 37℃. After incubation, the plate were centrifuged at 900×g for 10 min. to obtain the resulting insoluble formazan precipitates. To dissolve the crystal precipitates, 150 μl or 600 μl of a 1:1 mix of ethanol and DMSO were added to each well. Each plate was gently shaken for approxima-tely 20 min before reading on the Enzyme-Linked Immuno-sorbent Assay (ELISA) reader (measure-ment 570 nm, reference 620 nm). Absorbance of converted dye was measured. Assay values obtained on addition of vehicle were taken as 100%, and complete inhibition of MTT reduction (0%) was defined as the value obtained following addition of 0.9% Triton X-100.

To examine whether UDNS could attenuate the cytotoxicity of Amyloid-β protein peptide, cultures were pretreated with indicated concentrations of UDNS for 4 hrs. Thereafter, 10 μM Amyloid-β protein was added to cultures and incubated for 48 hrs. LDH activity in the culture medium was determined as described above. To investigate the effect of pretreatment with Amyloid-β protein peptide on the cytotoxicity induced by hydrogen peroxide or glutamate, cells were pretreated with 50 μM Amyloid-β protein peptide for 48 hrs, and then 100 μM hydrogen peroxide or 100 μM glutamate was added to cultures and incubated further for 4 hrs or 1 hr, respectively.

Oral administration of UDNS

UDNS was dissolved in a vehicle consisting of 0.01M PBS (pH 7.5) and 0.1% BSA. UDNS at a dose of 10 (n = 8), 25 (n = 8), 50 (n = 11), 75 (n = 8), or 100 mg/day was orally administrated for 7 days into each normothermic mouse in which 3-min. forebrain ischemia had been induced as described[11-16]; control animals (one group with 3-min forebrain ischemia and one group of sham-operated animals) received vehicle administration (n = 11 in each group). The oral administration of UDNS was started at 8 or 24 hrs before the ischemic insult in the UDNS-treated groups.

Occlusion of the Common Carotid Arteries

Occlusion of the common carotid arteries was

performed as described[13,14,17]. Sham-operated animals were treated in the same manner except that the common carotid arteries were not clamped. During forebrain ischemia, the fall in brain temperature has been shown to differ with animal, thereby affecting the number of viable CA1 neurons after ischemia[11]. To avoid the effect of unstable brain temperature on ischemic neuronal loss, we kept brain and rectal temperatures at 37.0±0.2℃ while clamping the common carotid arteries[11-17]. This enabled us to induce an invariable neuronal damage in the hippocampal CA1 field even after 3-min. ischemic insult[12-17] and to evaluate accurately the in vivo effects of neuroprotective agents on delayed neuronal death.

Estimation of Learning Ability by Passive Avoidance Task

Seven days after forebrain ischemia, the mouses were trained in a conventional step-down passive avoidance apparatus that was divided into a safe platform and a chamber with a stainless-steel grid floor[12-18]. Each animal was placed initially on the safe platform. When the mouse stepped down onto the grid floor, it received a foot shock. Although the mouse moved repeatedly up and down between the platform and the grid, it eventually remained on the platform. This training session lasted 5 min. Twenty-four hours later, the mouse was again placed on the safe platform while the shock generator was turned off, and the response latency, i.e., the time until it stepped down onto the grid floor, was measured. This test session also lasted 5 min. The long response latency indicates better learning ability. The results of passive avoidance task has been shown to correlate well with the number of hippocampal CA1 neurons[12-19].

Culture of Neurons

Hippocampal and cerebral cortical neurons from brains of 19-day old fetal Wistar rats were cultured as described[20]. The cells cultured for 7-10 days in the serum-free B-27/Neurobasal (GIBCO) chemically defined medium were used for experiments.

Statistics

The effects of UDNS on response latency and

Table 1. LDH Activity in the Culture Medium of Rat Cortical Astrocytes with Amyloid-β Protein and Protective Effects of UDNS Treatment

Concentration (μM)	LDH (% of maximal release)		
	Control (60 μg/ml)	Pretreatment of UDNS (60 μg/ml)	Posttreatment of UDNS
0.1	2.5±0.2	2.6±0.2	2.7±0.1
0.5	3.6±0.2	2.6±0.2	2.9±0.2
1.0	4.4±0.3	3.2±0.3	4.7±0.4
5.0	5.3±0.6	3.4 ±0.3	4.5±0.5
10	8.5±0.6	4.6±0.4	7.5±0.4
25	11.4±1.6	6.4 ±0.4*	8.6±0.7
50	36.4±5.4	17.3±1.6**	25.3±3.4*
100	57.3±5.4	38.4 ±4.4**	44.7±4.4*

The data expressed as percentage of maximal LDH release that was obtained on complete cell lysis. Data are mean ± SE values obtained from five culture wells per experiment, determined in three to five independent experiments. *, P<0.05. **, P<0.001.

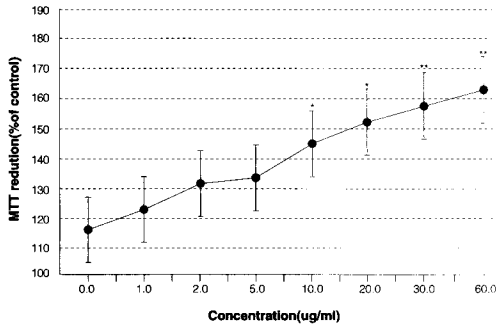

Fig. 1. Effects of various concentrations of UDNS on MTT reduction in cultured rat cortical astrocytes.
Data are mean±SE values obtained from five culture wells per experiment, determined in three to five independent experiments. *, P<0.05. **, P<0.001.

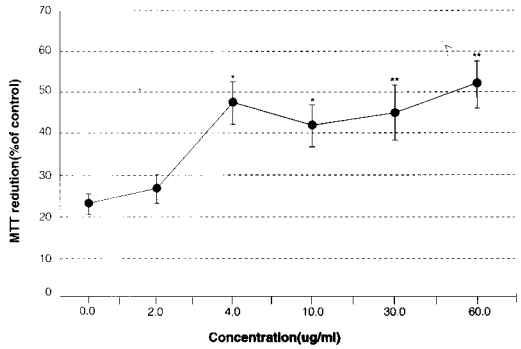

Fig. 2. Effects of UDNS on Amyloid-β protein-induced cytotoxicity of cultured rat cortical astrocytes.
Cultures were treated with 50 μM Amyloid-β protein for 48 hrs before application of various concentrations of UDNS. At 24 hrs after UDNS treatment, MTT reduction was assayed. Data are mean±SEM values obtained from five culture wells per experiment, determined in three to five independent experiments. *, P<0.05. **, P<0.001.

CA1 neuronal density were evaluated by the two-tailed Mann-Whitney *U* test, which enabled us to compare the EPO-treated groups with the corresponding ischemic control groups. All data were represented as mean±SE. Standard procedures were use to calculate means and standard deviation of the mean. Mean values were compared using Duncan' s Multiple Range Test with the SAS program (SAS Institute, Cary, NC) ; $P<0.05$ was considered significant.

Results

1. Effect of Amyloid- β protein on cell killing in cultured astrocytes

Protective and proliferative effects of UDNS on LDH activity in 24 hrs before treatment with indicated concentrations of Amyloid- protein were measured. The pretreatment of 60 g/ml of concentrated UDNS solution (Table 1) reduced the LDH activity by 67% of control group when compared at concentration of 100 M Amyloid- β protein(57.3 vs 38.4). Also, when the protective and proliferative effects of UDNS on LDH activity in 24 hrs after treatment with indicated concentrations of Amyloid-β protein, the posttreatment (60 g/ml) of UDNS solution reduced the LDH activity by 78% of control group of 100 μM Amyloid-β protein (57.3 vs 44.7). This result indicates that the pretreatment of UDNS is much more effective for astrocyte protection than post-treatment of UDNS.

2. Effect of UDNS on Amyloid- β protein-induced cell killing in cultured astrocytes

We measured the protective and proliferative effects of UDNS on MTT reduction in cultured astrocytes at 48 hrs after UDNS treatment (Fig. 1). The treatment of 60 g/ml of concentrated UDNS solution increased the MTT reduction activity by 162.4% of control group. Interestingly, 20 g/ml and 10 g/ml concentrations of the UDNS solution resulted in by 153.7% and 145.5% increase of the control group, respectively, revealing maximal

MTT reduction activity at 60 g/ml. Upon further dilution of the UDNS solution up to 60 g/ml concentration, the reduction activity was higher than that of vehicle. Thus, it could be concluded that UDNS is highly effective for the protection and proliferation of rat cortical astrocytes.

On the other hand, pretreatment of UDNS attenuated in a cell killing enhanced by exposure to increasing concentrations of Amyloid- β protein. This indicates that cells pretreated with UDNS allowed a resistance against the toxic effects of increasing concentrations of Amyloid-β protein peptides. However, gradual dilution of the UDNS concentration increased cell killing activity of Amyloid-β protein. With the pretreatment with 60 g/ml UDNS, about 52.3% of the cells were killed within 48 hrs by 50 M Amyloid-β protein (Fig. 2).

3. Effect of Amyloid- β protein on cell killing in cultured astrocytes

The toxicities of Amyloid- β protein on astrocytes were assessed by LDH assay. Following the appropriate incubation time with various peptides, LDH activities in the medium were measured by a Cytotox96 nonradioactive cytotoxicity assay kit (Promega) according to the manufacturer' s instructions. The results were expressed as a percentage of peak LDH release obtained on a complete lysis. The Amyloid-β protein increased LDH release by 56.7% of the maximal value at 100 μM concentration. Amyloid-β protein induced LDH release by only 7.5 and 36.5%, respectively, even at 20 and 50 μM (Table 2). However, the non-toxic fragment of Amyloid-β protein, had little effect on LDH release up to 100 M. Also, for examination of the effects of UDNS treatment, cells were treated with Amyloid-β protein for 2 hrs and further treated with the indicated concentration of UDNS, and then LDH activities in the culture medium of cultured rat cortical astrocytes were assayed at 48 hrs after treatment with the indicated concentrations of UDNS. LDH releases were severely decreased in the cells, indicating that UDNS treament reduced cell injury and protected the cells against Amyloid-β protein-induced cytotoxicity (Table 2).

The data expressed as percentage of maximal LDH release that was obtained on complete cell lysis. For effects of UDNS treatment, cells were treated with Amyloid- β protein for 2 h and further treated with indicated concentration of UDNS. LDH activities in the culture medium of cultured

Table 2. LDH Activity in the Culture Medium of Rat Cortical Astrocytes with Amyloid- β Protein

Concentration(μM)	LDH (% of maximal release)	
	Amyloid-β protein	Amyloid-β protein+ UDNS (20 μg/ml)
0.1	3.8	2.1
0.5	3.7	2.8
5.0	5.4	2.9
10	6.3	4.3
20	7.5	4.9
40	16.4	10.4*
50	36.5	15.5**
100	56.7	16.7**

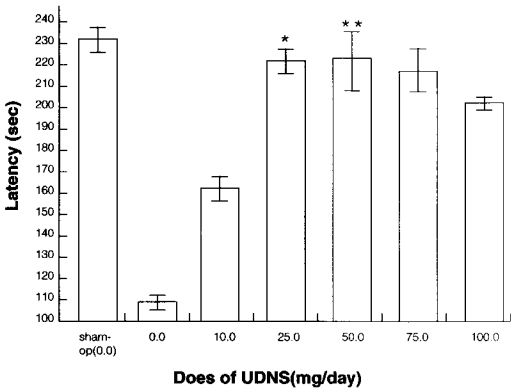

Fig. 3. Effects of oral administration of UDNS on the response latency and hippocampal CA1 region of 3-min. ischemic mouses.
Open columns indicate sham-operated (sham-op) animals and closed columns indicate vehicle- or UDNS-administrated ischemic animals. Each value represents mean SE (n = 7-12). *, P < 0.05 and **, P < 0.01, significantly different from the corresponding vehicle- administrated ischemic group (statistical significance tested by the two-tailed Mann-Whitney U test).

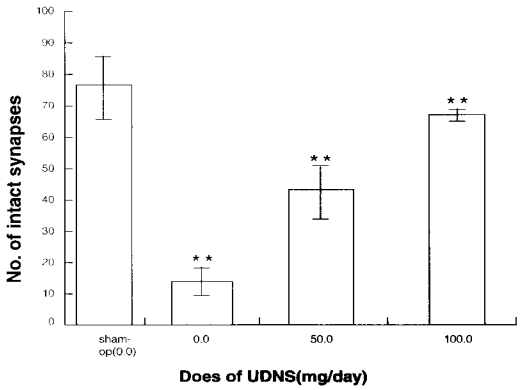

Fig. 4. Effects of oral administration of UDNS on the synapses of stratum lacunosum/radiatum in the hippocampal CA1 region of 3-min. ischemic mouses.
The number of synapses within the stratum lacunosum/radiatum were counted. Open columns indicate sham-operated (sham-op) animals and stippled or solid columns indicate vehicle- or UDNS-administrated ischemic animals. Each value represents mean SE (n=6-8). ** P<0.01, significantly different from the corresponding vehicle-treated ischemic group (statistically significance tested by the two-tailed Mann-Whitney U test).

rat cortical astrocytes were assayed at 48 h after treatment with indicated concentrations of UDNS. Data are mean±SE values obtained from five culture wells per experiment, determined in three to five independent experiments. *, P<0.05. **, P<0.001.

4. Protective effect of UDNS on ischemia-induced neuron death.

The response latency in the step-down passive avoidance task, which is an index of learning ability, and CA1 neuronal density of sham-operated animals with vehicle infusion were 245±21 s and 232±12cells/mm, respectively, while those of 3-min ischemic mouses infused with vehicle alone were 126±5 s and 127±13 cells/mm, respectively. There were significant differences in response latency (U = 0, P < 0.01) and CA1 neuronal density ($U = 0$, P < 0.01) between the two groups (Fig.3,4).

The continuous administration of UDNS at a dose of 10, 25, 50 75, or 100 mg/day for 7 days caused a significant prolongation in response latency time (UDNS vs. vehicle in ischemic mouses: $U = 14.0$, P < 0.05; $U = 12.0$, P < 0.01; $U = 12.0$, P < 0.01) (Fig. 3). Subsequent histological examinations revealed that treatment with UDNS at the dose of 50, 75, or 100 mg/day rescued many ischemic neurons that were destined to degenerate without the treatment (UDNS vs. vehicle in ischemic mouses: $U = 18.0$, P < 0.05; $U = 15.0$, P < 0.01; $U = 11.0$, P < 0.01) (Fig.4). The lower dose of UDNS (10 mg/day) was not significantly effective, although the mean response latency and CA1 neuronal density were higher in 10 mg/ml/day

UDNS-administrated ischemic animals than in vehicle-infused ischemic animals (Fig. 3,4). UDNS at a dose of 500 mg/day or 1000 mg/day was ineffective in preventing the ischemia-induced reduction of response latency and neuronal loss (data not shown).

5. Neuroprotective effect of UDNS on glutamate-induced neuron death.

For better understanding of the molecular mechanism underlying the neuroprotective action of UDNS, we further investigated the effect of UDNS on cultured neurons. A massive increase of intracellular Ca^{2+} concentration evoked by glutamate-induced NMDA receptor activation plays a critical role in triggering intracellular events that elicit cell destruction[23-25]. UDNS protects primary cultured neurons from NMDA receptor-mediated glutamate toxicity[20]. UDNS may exhibit its neuroprotective action by repressing the glutamate-induced increase in Ca^{2+} concentration. Glutamate stimulated the rate of Ca^{2+} uptake when compared with the rate in the presence of MK801 (a potent NMDA receptor antagonist), but UDNS did not reduce the glutamate-mediated stimulation (data not shown). Likewise, glutamate induced an increase in intracellular Ca^{2+} concentration but UDNS failed to repress this increase.

On the other hand, UDNS protected neurons from glutamate-induced death (Fig.5). Me-TC, an inhibitor of NO synthase[26], also neutralized the glutamate toxicity almost completely, suggesting that the glutamate toxicity is mediated by NO. We examined whether or not UDNS could rescue neurons from NO toxicity by using SNP[27], a NO-generating agent. Incubation of neurons with SNP caused significant neuronal loss, but pretreatment of neurons with UDNS resulted in almost complete survival of the neurons (Fig.5). In contrast, UDNS did not protect SNP-induced neuron death when added to the culture medium simultaneously with SNP (data not shown).

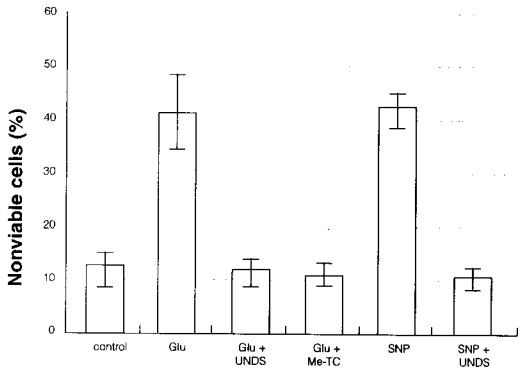

Fig. 5. UDNS protects cultured hippocampal neurons from cell toxicity of SNP-derived NO.
Neurons were incubated with test materials as described in the Materials and Methods. Glu, glutamate; Me-TC, a NO synthase inhibitor. Total and nonviable cell number was counted. Each value is the mean±SD of triplicate experiments.

Discussion

Effects of UDNS on cultured astrocyte cell system in Amyloid-β protein treatment conditions were examined. Cell killing was significantly enhanced by addition of increasing concentrations of Amyloid-β protein. Pretreatment of UDNS attenuated in cell killing enhanced by increasing concentrations of Amyloid-β protein.

On the other hand, we measured the protective and proliferative effects of UDNS on LDH activity in 24 hrs before treatment with indicated concentrations of Amyloid-β protein. The pretreatment of 60 μg/ml of concentrated UDNS solution reduced the LDH activity by 67% of control group when compared at concentration of

100 M Amyloid-β protein25-35 (57.3 vs 38.4). Also, when the protective and proliferative effects of UDNS on LDH activity in 24 hrs after treatment with indicated concentrations of Amyloid- β protein25-35, the posttreatment (60 μg/ml) of UDNS solution reduced the LDH activity by 78% of control group of 100 M Amyloid-β protein (57.3 vs 44.7). This result indicates that the pretreatment of UDNS is much more effective for astrocyte protection than posttreatment of UDNS.

The present study was carried out to investigate the effects of UDNS on cultured astrocyte cell systems, Amyloid-β protein-induced cytotoxicity and antioxidative enzyme activities in Amyloid-β protein treated conditions. Cellular cytotoxicity was significantly enhanced by addition of increasing concentrations of Amyloid-β protein. Pretreatment of UDNS attenuated in cell killing enhanced by increasing concentrations of Amyloid-β protein. These results of cellular cytotoxicity and MDA level by Amyloid-β protein treatment are in good agreement with that of Glascott et al.[21,22]. The study showed that Amyloid-β protein strongly increased the MDA level and the level was enhanced by both a dose- and time- dependent manner of Amyloid-β protein treatment.

Lipid peroxidation was prevented or greatly reduced by addition of antioxidants (Vit E, Vit C, DPPD or deferoxamine)[23]. For example, addition of antioxidants to the cell culture medium significantly reduced cell killing and content of intracellular antioxidants. In the case of GSH, the cells exposed to *t*-BHP promptly decreased, however, the preincubation of the cells with antioxidants (DPPD or deferoxamine) did not lead to change in the level of intracellular GSH or the accumulation of GSSG in the medium after exposure to *t*-BHP[24]. Recently, it was reported that repeated oral administration of deer antler extract showed an inhibitory effect on monoamine oxidase activity, one of the senescence-marker enzymes, reduced MDA level and increased SOD activity in the liver and brain tissues of aged mice[25,26]. Therefore, it was suggested that changes in MDA level are related to the alterations of antioxidative enzyme activity and these biochemical changes were recovered to normal levels by the addition of antioxidants.

Conclusion

Arisaematis rhizoma(Udannamsung; UDNS) is a medicinal plant used for the treatment of various symptoms accompanying cerebrovascular disorders and epilepsy. The present study was done to investigate the effects of UDNS on cultured primary neuron cell systems, cell cytotoxicity in Amyloid-β protein treatment conditions and ischemia-induced cell death in vivo. Cell killing was significantly enhanced by addition of increasing concentrations of Amyloid-β protein. Pretreatment of UDNS attenuated in cell killing enhanced by increasing concentrations of Amyloid- β protein. Furthermore, the protective effect of the water extracts of UDNS on Amyloid-β proteinpeptide-induced neuronal death were also observed by LDH assay using cultured astrocyte cells. Amyloid-β protein-induced cell death was protected by the application of water extract of UDNS in a dose-dependent manner, and concentrations of 30 to 50 g/ml had a significant effect compared to exposure of Amyloid-β protein only. Here we report in vivo evidence that UDNS protects neurons against ischemia-induced cell death.

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