Inhibitory Effect of *Acanthopanax* Extract on the Dopaminergic Neuroblastoma Cells against Rotenone-induced Toxicity

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Abstract

During screening of Korean indigenous medicinal herbs with anti-neurodegenerative activities, we found that the extract of *Acanthopanax divaricatus vat. albeofructus* could inhibit rotenone-induced DNA and cell damage in neuroblastoma cells. A simple in vitro model developed for the study of possible mechanisms underlying neurodegeneration in Parkinson’s disease includes the administration of rotenone to the human dopaminergic neuroblastoma cells, SH-SY5Y. In this investigation, rotenone induced oxidative DNA damage of lymphocytes as expected. However, the oxidative DNA damage was inhibited in vitro upon treating *Acanthopanax* extract. Moreover, *Acanthopanax* extract resulted in the inhibitory activities against cell damage, ROS generation and chromatin condensation by rotenone.

Keywords: *Acanthopanax*, Rotenone, Toxicity, Neuroblastoma cells

Introduction

Parkinson’s disease (PD) is a neurodegenerative movement disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta¹. A classic model developed to investigate possible mechanisms underlying neurodegeneration in PD consists in the administration of the pesticide rotenone, to the human dopaminergic neuroblastoma cell line, SH-SY5Y. Rotenone, a specific inhibitor of mitochondrial complex I, could induce apoptosis in SH-SY5Y cells and in mesencephalic dopaminergic cells by the generation of reactive oxygen species (ROS)². Rotenone can be used not only in in vivo models of PD but also in an in vitro system for search new materials with neuroprotective properties³.

Oxidative stress has been suggested to play an important role in the etiology and progression of PD. Oxidative stress due to ROS generation led to several damaging effects as they can attack proteins, enzymes, carbohydrates, lipids and DNA in cells and tissues. They induce membrane damage, protein modification, DNA damage, and cell death by DNA fragmentation and lipid peroxidation⁴. In vitro and in vivo studies of PD models have shown that natural and endogenous antioxidants such as polyphenols, coenzyme Q10, and vitamins A, C, and E might have protective effects against oxidative stress-induced neuronal death⁵.

*Acanthopanax* is a widely used oriental medicinal herb that enhances the strength, energy and general well-being for humans. The major active constituents of *Acanthopanax* were reported to be eleutheroside, acanthoside, daucosterine, β-sitosterol, sesamine, and savinine⁶. It has been reported to treat cirrhosis, chronic bronchitis, hypertension, ischemic heart disease, gastric ulcer, rheumatism, diabetes and allergic inflammation⁷. However, it remains unclear whether *Acanthopanax* could suppress the DNA and cell damage in neuroblastoma cells by rotenone. Therefore, the present work was conducted to determine to what extent *Acanthopanax divaricatus vat. albeofructus*, an indigenous herbal medicine in Korea, modulates the toxicity of rotenone against DNA and cells in human neuroblastoma cell line, SH-SY5Y cells.

Results

Cytoprotective Effects Assessed by MTT Assay

To investigate the cytoprotective effect of *Acanthopanax divaricatus vat. albeofructus* by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay⁸, various concentrations of rotenone were treated in neuroblastoma cells SH-SY5Y cells for 24 h (Figure 1). Rotenone exposure reduced the cell viability in a concentration-dependent manner. The expo-
sure of SH-SY5Y cells to 200 nM rotenone alone resulted in approximately 20% decrease in cell viability. After the addition of 400 nM rotenone, the cell viability significantly dropped to approximately 64% of the control, while the addition of 0.8 μg/mL of the extract increased the cell viability to 76.4% of the control (Figure 2). These data indicate a partial protection of the deleterious effects of rotenone on SH-SY5Y cells by the addition of the extract. Moreover, the addition of the extract on the 200 nM rotenone treated cells produced an even higher reduction of the cytotoxic effect of rotenone, since the cell viability increased to 92% of the control condition. This result shows that A. divaricatus var. albeofructus extracts have cytoprotective effect against rotenone-induced cell damage in vitro.

Suppressive Effects on the Rotenone-induced DNA Damage

The single-cell gel electrophoresis assay (comet assay) is a well-established genotoxicity test for estimating oxidative DNA damage, such as DNA single and double strand breaks at the individual cell level, both in blood and in cells. Cells that have damaged DNA migrate much further and appear as fluorescent comets with tails of DNA fragmentation or unwinding, whereas intact undamaged DNA moves minimally due to its large size. As shown in Figure 3, lymphocytes treated with rotenone showed notable DNA damages, evaluated by the olive tail moment in a comet assay. The olive tail moment at 70 μM rotenone was about 32 ± 1.51, compared with 8 ± 0.38 in the DMSO-treated control, indicating a severe DNA damage with rotenone. However, upon adding Acanthopanax extract, the oxidative DNA damage caused by rotenone was inhibited as demonstrated by the reduction of the olive tail moment. The olive tail moment at 5 μg/mL Acanthopanax was reduced up to 13 ± 2.91, a similar level to the control. Moreover, the similar suppressive effect on the rotenone-induced DNA damage using SH-SY5Y cells were also found (data not shown). The result suggests that A. divaricatus var. albeofructus extract could suppress the rotenone-induced oxidative DNA damage in vitro.

Suppressive Effects on the Intracellular ROS Generation

The intracellular ROS scavenging activity of Acanthopanax extracts was evaluated by 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) assay as shown in Figure 4. Nonfluorescent dichlorofluorescein (DCF) can rapidly diffuse through the cell membrane and is hydrolyzed by intracellular esterases to an oxidative sensitive form, 2′,7′-dichlorofluorescein (DCF). This serves as a substrate for intracellular oxidant to gen-