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Effects of *Bambusae concretio* Salicea (Chunchukhwang) on amyloid β-induced cell toxicity and antioxidative enzymes in cultured rat neuronal astrocytes

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Abstract

Bambusae concretio Salicea (BCS; plant family name: *Phyllostachys bambusoides* Siebold et Zuccarinii) is a medicinal plant used in Korea for the treatment of various symptoms accompanying hypertension and cerebrovascular disorders. Previously, it was shown that BCS is an effective protectant against oxidative glutamate toxicity in the murine neuroblastoma cells and human neuroblastoma cells. Treatment with BCS increased the secretion of the non-amyloidogenic amyloid precursor protein fragment, and decreased the secretion of amyloid-β (Aβ) peptides from neuronal cells [Jeong, J.C., Seo, Y.J., Kim, H.M., Lee, Y.C., Kim, C.H., 2003. Inhibitory effects of *Bombusae concretio* Salicea on neuronal secretion of Alzheimer's β-amyloid peptides, a neuro-degenerative peptide. Neurochemical Research 28, 1785–1792.]. To further examine the pharmacological activity of BCS, we studied the protective effect of the water extracts on Aβ25-35 peptide-induced neuronal death by microscopic observation and lactate dehydrogenase (LDH) assay, and action on antioxidative enzymes using cultured astrocyte cells. Ten μM Aβ25-35-induced cell death was protected by the application of water extract of BCS in a dose-dependent manner, and concentrations of 1–10 μg/ml had a significant effect compared to exposure to Aβ25-35 only. When antioxidative enzyme activities such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST) were assayed after Aβ25-35 treatment, the enzymes were decreased in a similar fashion. However, those activities were enhanced by BCS treatment and this may have resulted from the potentiation of antioxidative ability by BCS. The ability of BCS to reduce cellular cytotoxicity induced by 10 μM Aβ25-35 suggests that BCS may be a protective agent for free radical generating compounds such as Aβ25-35, and that Aβ25-35 is not only a potent lipid peroxide inducer, but also causes changes in antioxidative enzymes. From the results, it was concluded that BCS has a protective effect on Aβ-induced neuronal death in cultured astrocyte cells through the inhibition of lipid peroxidation and protection of antioxidative enzymes.

Keywords: Astrocyte; Alzheimer's disease; Amyloid-β; *Bombusae concretio* Salicea (Chunchukhwang); Antioxidative enzyme

Abbreviations: BCS, *Bambusae concretio* Salicea water extract; Aβ, amyloid-β; LDH, lactate dehydrohenase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GST, glutathione-*S*-transferase; AD, Alzheimer's disease; APP, amyloid precursor protein; FBS, fetal bovine serum; DMEM, Dulbecco's modified eagle's medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide; DMSO, dimethyl sulphoxide; TBA, 2-thiobabituric acid; TEP, 1,1,3,3-tetraethoxypropane; EDTA, ethylenediamine tetraacetic acid; SOD, superoxide dismutase; XOD, xanthine, xanthine oxidase; NBT, nitro blue tetrazolium; DETAPAC, diethylene triamine pentaacetic acid; GSH, reduced glutathione; GSSG-reductase, oxidized glutathione-reductase; NADPH, nicotinamide adenine dinucleotide phosphate; CDNB, 1-chloro-2,4-dinitrobenzene; NaN_3, sodium azide; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive cognitive decline resulting from selective neuronal dysfunction, synaptic loss, and neuronal cell death. The well-studied neuropathological features of AD show compacted deposits of amyloid-β (Aβ) aggregates (Selkoe, 1991; Terry, 1996). Aβ is 39–43 amino acids long and proteolytically derived from an integral membrane protein termed amyloid precursor protein (APP) (Kang et al., 1987; Kim and Suh, 1996), although the mechanism for APP processing is still unknown. There are many in vitro studies demonstrating that Aβ is directly neurotoxic and increases neuronal susceptibility to other toxic agents (Cotman et al., 1996; Kim and Suh, 1996; Yankner, 1996). The toxic effect of Aβ is correlated with its ability to form aggregates (Pike et al., 1995). Both oxygen species (Goodman et al., 1994) and excessive Ca^{2+} influx (Mattson et al., 1993) are also implicated in the mechanism of Aβ neurotoxicity. In contrast, it was also reported that Aβ promotes neurite outgrowth under certain culture conditions instead of having a toxic action (Koo et al., 1990).

On the other hand, AD could be induced by surrounding free radicals and also be protected by enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST) (Seifter et al., 1988; Halliwell, 1994). Antioxidants scavenge and minimize the formation of oxygen-derived species and inhibit oxidative damage induced by free radicals. They also recover the level of intracellular antioxidants (vitamins, methionine, glutathione and glutathione-related minerals) (Ip et al., 1991; Schrauzer, 1992). Hence, these antioxidants may be particularly important in diminishing cumulative oxidative damage. Recently, several reports have suggested that natural dietary plants may play an antioxidative role in the prevention of aging and carcinogenesis and may offer effective protection from lipid peroxidative damage in vitro and in vivo (Ruch et al., 1989; Tsuda et al., 1994). Therefore, much attention has been focused on natural antioxidants (Wang et al., 1988a,b).

In particular, it was reported that the extract of *Bambusae concretio* Salicea (Chunchukhwang in Korean; *Phyllostachys bambusoides* Siebold et Zuccarinii in plant family name; BCS) is specifically effective for cerebrovascular lesions and aphasia during the treatment of wind-heat syndrome and heat-phlegm in oriental medicine (Lee, 1986). BCS has been long being used in Traditional Korean Medicine for clinical treatment of degenerative neuronal disorders (Lee, 1986; Kosuwon et al., 1994). Previously, the pharmacological mechanism for BCS was attributed to anti-aging and sexual-reinforcing activities in experimental in vitro and in vivo systems (Chen et al., 1992; Lee and Chung, 1998). Recently, to delineate the mechanisms of action of BCS in experimental systems of oxidative neuronal cell death, we have investigated the neuroprotective effects exhibited by in different concentrations of BCS (Jeong et al., 2003). The idea of a novel and neuroprotective function of BCS had arisen because BCS showed comparable neuroprotection (Jeong et al., 2003).

This study was carried out to investigate the effect of BCS on cultured astrocytes, lipid peroxidation and antioxidative enzyme activities following Aβ25-35 treatment.

2. Materials and methods

2.1. Materials

The Aβ25-35 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). Dulbecco's Modified Eagle's Medium (DMEM), glutamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide (MTT), dimethyl sulphoxide (DMSO), 2-thiobabituric acid (TBA), 1,1,3,3-tetraethoxypropane (TEP), ethylenediamine tetraacetic acid (EDTA), superoxide dismutase (SOD: from bovine liver), xanthine, xanthine oxidase (XOD), nitro blue tetrazolium (NBT), catalase (from bovine liver), diethylene triamine pentaacetic acid (DETAPAC), reduced glutathione (GSH), oxidized glutathione-reductase (GSSG-reductase), and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chem. Co. (St. Louis, USA). 1-Chloro-2,4-dinitrobenzene (CDNB) and sodium azide (NaN_3) were obtain from Aldrich Chem. Co. (Milwaukee, WI).

2.2. Bombusae concretio Salicea and the extraction

Three hundred grams of BCS (*Phyllostachys bambusoides* Siebold et Zuccarinii in plant family name) (OHC-B-3 in herbarium record) was obtained from Oriental Herbal Center (OHC), Oriental Medical Hospital, Dongguk University College of Oriental Medicine, and extracted with 500 ml of boiling water for 3 h. After the extract was centrifuged at 7500 rpm for 30 min, the supernatant was lyophilized. For direct use, the extract solution was stored at 4 °C in aliquots.

2.3. Cell culture and treatment of BCS

Cortical astrocyte cultures were prepared from neonatal rat (1–2 day old) pups by the method of Levision and McCarthy (Levision and McCarthy, 1991). Cerebral cortex was dissected from neonatal day 1–2 Sprague–Dawley rats and dissociated by gentle trituration. Cells were plated in six-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 DMEM 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,