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BRAIN RESEARCH

Research Report

Characterizing the neuroprotective effects of alkaline extract of *Lycium barbarum* on β-amyloid peptide neurotoxicity

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ABSTRACT

Lycium barbarum is an oriental medicinal herb that has long been used for its anti-aging and cell-protective properties. Previous studies have shown that aqueous extracts from *L. barbarum* exhibit neuroprotection via inhibiting pro-apoptotic signaling pathways. Other active components can also be accomplished by novel alkaline extraction method, which may give different profiles of water-soluble components. We hypothesize that another active component obtained by alkaline extraction method exerts different biological mechanisms to protect neurons. In this study, we aim to examine the neuroprotective effects from the alkaline extract of *L. barbarum*, namely LBB, to attenuate β-amyloid (Aβ) peptide neurotoxicity. Primary cortical neurons were exposed to Aβ-peptides inducing apoptosis and neuronal cell death. Pretreatment of LBB significantly reduced the level of lactate dehydrogenase (LDH) release and the activity of caspase-3 triggered by Aβ. "Wash-out" procedures did not reduce its neuroprotective effects, suggesting that LBB may not bind directly to Aβ. We have further isolated three subfractions from LBB, namely LBB-0, LBB-I and LBB-II. LBB-I and LBB-II showed differential neuroprotective effects. Western blot analysis demonstrated that LBB-I and LBB-II markedly enhanced the phosphorylation of Akt. Taken together, our results suggested that the glycoconjugate isolated from novel alkaline extraction method can open up a new avenue for drug discovery in neurodegenerative diseases.

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1. Introduction

Alzheimer's disease (AD) is one of the most common causes of dementia, affecting more than 4.5 million people in the US. With the rapid expansion of aged population, it is estimated that the number of AD patients will increase by almost 3-fold in 2050 (Hebert et al., 2003). It is likely to be an important public issue that brings great financial burden to our society. The presence of senile plaques is one of the pathological hallmarks in AD (Mattson, 2004; Selkoe, 2001), in which aggregations of beta-amyloid peptide (Aβ-peptide) can be identified. It has been shown that soluble, oligomeric or fibrillar forms of Aβ exhibit certain extent of neurotoxicity. Previous experiments have demonstrated that Aβ-peptides are toxic to cultured neurons (Busciglio et al., 1992; Estus et al., 1997; Grace et al., 2002). Evidence from *in vivo* experiments also showed that Aβ is one of the pathological factors leading to neuronal loss, tau phosphorylation and activation of microglia (Geula et al., 1998; Maurice et al., 1996). The mechanisms of Aβ to impose toxicity on neurons have been studied extensively. It has been suggested that the activation of caspase (Harada and Sugimoto, 1999; Troy et al., 2000), stress kinases, and induction of oxidative stress (Folin et al., 2006) are involved in the apoptotic processes.

Although many studies had been directed to AD treatment, there is still yet promising intervention for curing the disease. The cholinesterase inhibitor is the most commonly used class of drug that had been approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD (Doody, 2003; Giacobini, 2000). Neuroprotection is the attempt to preserve normal cellular interaction in the brain and minimize loss of neuronal functions in pathological conditions. Currently, much attention has been focused on the potential of using natural herbs as neuroprotective agent.

The fruits of *Lycium barbarum*, also called *Fructus lycii* or Gouqizi, have long been used in oriental medicine for the nourishment of the kidney, liver and eyes. Recent research has documented its anti-aging properties. There are many studies showing its beneficial effects on age-related conditions such as reduction of free radicals in our body (Li et al., 2007). The most common components of *L. barbarum* found in the aqueous extract of the fruit are polysaccharides and polysaccharide proteins. For instance, the polysaccharides of *L. barbarum* protect rat testes from heat-induced damage and protects mouse testicular cells from H_2O_2-induced DNA damage (Luo et al., 2006). Its beneficial effects can be found in irradiation, chemotherapy, or mitomycin C-induced mice myelosuppression by promoting the recovery of peripheral white blood cell and red blood cell count in these mice models (Gong et al., 2005; Hai-Yang et al., 2004). Apart from the above protective effects, extracts of *L. barbarum* are also a potential agent for treatment of diabetes since its polysaccharides can reduce blood glucose, triglyceride and total cholesterol levels in diabetic or hyperlipidemic rabbits (Luo et al., 2004). Polysaccharides from *L. barbarum* also demonstrate their hypoglycemic effects in a model of non-insulin-dependent diabetes mellitus (NIDDM) rats (Zhao et al., 2005b).

Since *L. barbarum* has protective functions on different cell types and can ameliorate an age-related disease (Chan et al., 2007), this laboratory was the first to carry out studies to investigate its neuroprotective properties from a water extract of the fruit. Our data suggested that the water-soluble extracts can protect cultured neurons from β-amyloid-induced neurotoxicity by suppression of the c-Jun N-terminal (JNK) signaling pathway (Yu et al., 2005). Its aqueous extract can also protect neurons from dithiothreitol (DTT)-induced endoplasmic reticulum reducing stress (Yu et al., 2006a). In spite of the fact that aqueous extracts elicit neuroprotection, there are other active components that make *L. barbarum* to be known as anti-aging herbal medicine. We hypothesize that other active components can be found by novel alkaline extraction method. This provides a novel way in drug discovery of neuroprotective agent. In the present study, we aim to characterize any potential neuroprotective effects of the alkaline extract of

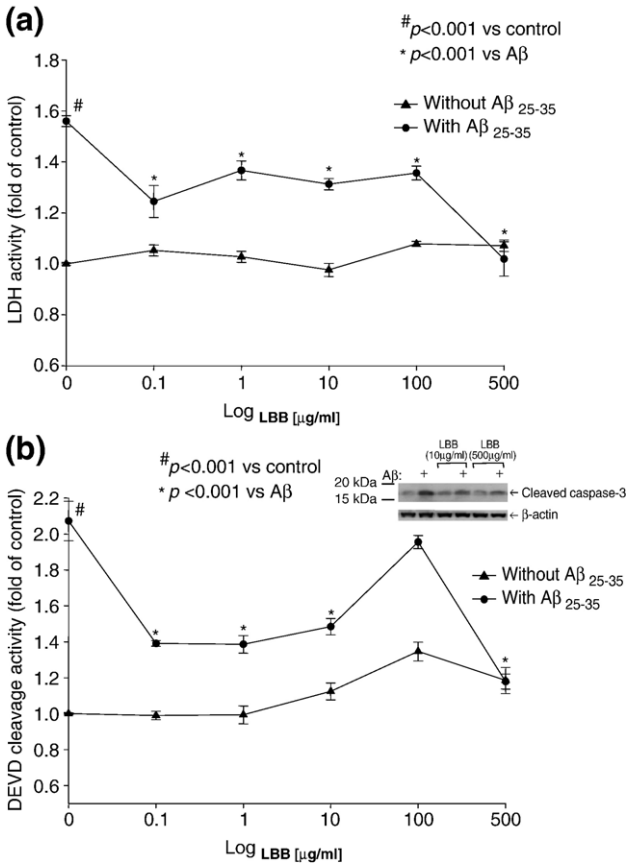

Fig. 1 – Protective effects of the alkaline extract LBB on Aβ-peptide-triggered neurotoxicity. Rat primary cortical neurons were pretreated with different dosages of LBB for 1 h, neurons were exposed to $A\beta_{25-35}$ peptide (25 μM) for 24 h. (a) The level of general cell death was assayed by LDH assay which detected the release of LDH in culture medium. (b) Caspase-3 like activity was determined by the colorimetric caspase-3 like assay. DEVD cleavage was expressed as fold of control, which was calculated as: ($s.a._{\text{of Aβ-treated/LBB-treated}}/s.a._{\text{control}}$). Results were expressed as mean ± SE from at least 3 independent experiments. Data were analyzed by one-way ANOVA for multiple comparison and Student–Newman–Keuls test as post hoc test. $^{\#}p<0.001$ relative to control. $^{*}p<0.001$ relative to cultures treated with Aβ only.