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BRAIN RESEARCH

Research Report

The protective effects of chitooligosaccharides against glucose deprivation-induced cell apoptosis in cultured cortical neurons through activation of PI3K/Akt and MEK/ERK1/2 pathways

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ABSTRACT

Chitooligosaccharides (COSs), the biodegradation product of chitosan, possess a wide range of biological activities. In this study, we investigated the influences of COSs on primary cultured cortical neurons exposed to glucose deprivation (GD). The cell viability assessment by MTT assay, in couple with cell apoptosis analysis by Hoechst 33342 and TUNEL staining, indicated that GD-induced cell apoptosis in cultured cortical neurons was attenuated by COSs co-treatment in a dose-dependent manner. Light micrography following tetramethylrhodamine methyl ester staining revealed that COSs protected cultured cortical neurons from GD insult through the stabilization of mitochondrial membrane potentials. COSs co-treatment also led to the increase in Bcl-2/Bax protein ratio and the inhibition of caspase-3 activation in cultured cortical neurons exposed to GD insult. We further found that COSs were able to transiently cause the activation of Akt and ERK1/2 proteins, and anti-apoptotic effects of COSs could be blocked by chemical inhibition of PI3K and MEK. Taken together, the results suggest that COSs hold a promise to serve as a potential neuroprotective agent for treating cerebral ischemic stroke and neurodegenerative diseases.

1. Introduction

In the pathogenesis of cerebral ischemia and stroke, partial or total obstruction of blood flow to the brain leads to a deficient supply of glucose, oxygen, serum, and nutrient that are indispensable for energy generation, and depletion of cellular energy sources is responsible for neuronal insult. In many studies, neuronal insult induced by oxygen and/or glucose deprivation usually serves as an in vitro model for investigating the molecular mechanisms of neuronal damage during brain ischemia (Moley and Mueckler, 2000).

Chitosan, a polysaccharide made up of D-glucosamine units (Fig. 1A), is derived from deacetylation of chitin which can be largely obtained from crustacean shells and fungi. Chitosan has proven to be a good biomaterial with a wide range of biomedical applications (Suh, 2000). The biodegradation (chemically called hydrolysis) product of chitosan is low molecular weight chitooligosaccharides (COSs) with different polymerization degrees (from 2 to 10). Many studies have revealed a wide range of biological activities of COSs, including anti-oxidative activity (Park et al., 2003a), anti-tumor activity (Xu et al., 2008b), anti-inflammatory effects (Lee et al., 2009), anti-microbial activity (Fernandes et al., 2008), anti-hypertensive (Park et al., 2003b), and hepatoprotective activity (Xu et al., 2008a). Recently, it has been shown that COSs protected primary culture of hippocampal neurons against glutamate-

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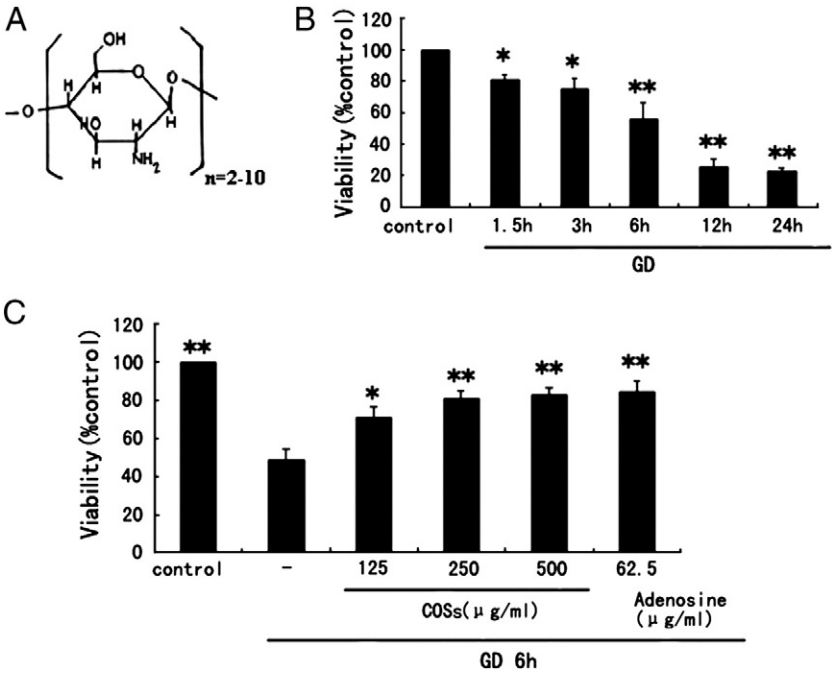

Fig. 1 – GD-induced insult to cultured cortical neurons and protective effects of COSs. (A) Chemical structure of chitooligosaccharides. (B) Time-dependent effects of exposure to GD on the cell viability of cultured cortical neurons. *P<0.05 or **P<0.01 versus control, which represented no cell treatment. (C) Protective effects of COSs on GD-induced cell insults in cortical neurons. Cells were subjected to GD stimulation and co-treatment with 125, 250, or 500 μg/ml COSs or 62.5 μg/ml adenosine for 6 h. *P<0.05 or **P<0.01 versus exposure to GD alone. The cell viability (B and C) was expressed as the percent (%) of control value as measured by MTT assay. The data are presented as means±SD of three independent experiments (each in triplicate).

induced neurotoxicity (Zhou et al., 2008), influenced neuronal differentiation of PC-12 cells in vitro (Yang et al., 2009), and promoted the peripheral nerve regeneration in the rat nerve crush injury model in vivo (Jiang et al., 2009). To further expand the potential therapeutic applications of COSs, this study was aimed to investigate the effects of COSs against glucose deprivation (GD)-induced neurotoxicity in primary culture of rat cortical neurons and to explore the possible involvement of some signaling pathways.

2. Results

2.1. *COSs protected cortical neurons against GD-induced cytotoxicity*

Cultured cortical neurons were exposed to GD for 1.5, 3, 6, 12, and 24 h, respectively, and cell survival was assessed by MTT assay. As compared to cultured cortical neurons that underwent neither GD insult nor other treatments (control), significant decreases were observed in cell viability of cultured cortical neurons that underwent GD insult for different times, and these decreases showed a time-dependent pattern (Fig. 1B). Exposure to GD for 6 h, which resulted in 56.1±3.8% of relative cell viability, was used to induce cell insult to cultured cortical neurons in all subsequent experiments.

As compared to cell viability decrease induced by exposure to GD alone for 6 h, simultaneous treatment (called co-treatment) with COSs at concentrations of 125, 250, and 500 μg/ml led to significant restoration of cell survival to different degrees. In other words, COSs co-treatment significantly attenuated GD-induced decrease in cell viability of cultured cortical neurons in a dose-dependent manner. And the neuroprotective effect of COSs at 500 μg/ml was similar to that of adenosine at 62.5 μg/ml without significant difference between each other (Fig. 1C). Since adenosine, a purine nucleoside, has the function of protecting cells against ischemia induced injury via the preservation of cellular ATP, it was used for comparison with COSs in this study (Fowler, 2006; Goldberg et al., 1988).

Morphological observation indicated that cultured cortical neurons exposed to GD alone for 6 h displayed a significant morphological change, including disappearance of cellular processes and decrease of cellular refraction, and the cell damage was improved by co-treatment with COSs or adenosine (data not shown).

2.2. *COSs protected cortical neurons against GD-induced cell apoptosis*

Since MTT assay failed to distinguish between necrosis and apoptosis, we carried out other procedures to determine the mode of cell death induced by GD. Hoechst 33342 staining showed that after GD insult for 6 h, 29.35±4.31% of cultured cortical neurons displayed typical apoptotic morphology as featured by chromatin condensation, nuclear shrinkage, and formation of a few apoptotic bodies. Co-treatment with 500 μg/ml COSs or 62.5 μg/ml adenosine, however, significantly reduced GD-