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Catechin and epicatechin from Smilacis chinae rhizome protect cultured rat cortical neurons against amyloid β protein (25-35)-induced neurotoxicity through inhibition of cytosolic calcium elevation

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Objectives

Catechins have attracted significant attention recently, because of their potential neuroprotective activities with powerful antioxidant properties. Several studies in animals and cell culture models suggest that catechins may affect several potential targets associated with Alzheimer disease (AD) progression. However, these reports have focused on the antioxidant effect of catechins, as a major contributable property for the neuroprotection. In a recent study, we reported that methanol extract of *Smilacis chinae* rhizome protected Aβ (25-35)-induced neuronal cell damage in cultured rat cortical neurons (Ban et al., 2006). Here we report that catechin and epicatechin isolated from *Smilacis chinae* rhizome reduce Aβ (25-35)-induced neuronal damage mainly through the inhibition of cytosolic Ca^{2+} ($[Ca_{2+}]_c$) elevation, followed by inhibition of reactive oxygen species (ROS) generation, glutamate release and then apoptosis in primarily cultured rat cortical neurons.

Materials and Methods

Primary cortical neuronal cultures were prepared from the forebrains of 15-day-old fetuses from pregnant SD rats. The 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and the number of apoptotic nuclei, evidenced by Hoechst 33342 staining, were tested for the measurement of neuronal cell death 24 h after the treatment with 10 μM Aβ (25-35). $[Ca^{2+}]_c$ and ROS generation induced by 10 μM Aβ (25-35) were detected with fluorescent dye, Fluo-4 AM and H_2DCFDA, respectively, by laser scanning confocal microscopy. The amount of glutamate secreted into the medium was quantified by HPLC with ECD. Caspase-3 protein expression was detected by western blot analysis.

Results and Discussion

Catechin and epicatechin from *Smilacis chinae* rhizome inhibited 10 μM Aβ (25-35)-induced apoptotic neuronal cell death at a concentration of 10 μM (Fig. 1, 2). Catechin and epicatechin inhibited 10 μM Aβ (25-35)-induced elevation of $[Ca^{2+}]_c$. Catechin and epicatechin also inhibited glutamate release into medium induced by 10 μM Aβ (25-35), generation of reactive oxygen species (ROS) and activation of

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caspase-3 (Fig. 3, 4). These results suggest that catechin and epicatechin prevent A β (25-35)-induced neuronal cell damage by interfering with the increase of $[Ca^{2+}]_c$, and then by inhibiting glutamate release, generation of ROS and caspase-3 activity. A β is believed to play a central role in the pathophysiology of AD. Although it is still controversial whether increased Aβ formation is sufficient to cause nerve cell degeneration in AD, neurotoxic effects of Aβ have been demonstrated in both in vitro and in vivo. Catechin and epicatechin completely blocked Aβ (25-35)-induced neuronal cell death in the present study. These results suggest a further evidence of the possibility of *Simlacis chinae* rhizome, which contains catechin and epicatechin as major active components, having neuroprotective effect in AD brains with the prevention of the disease progression.

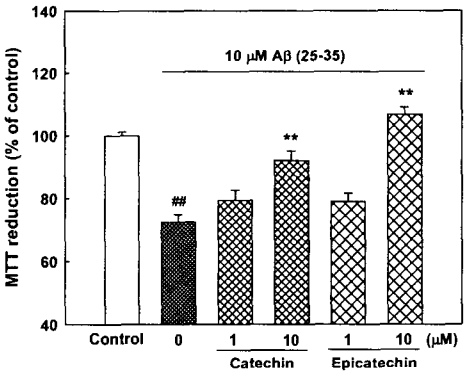

Fig. 1. Inhibitory effects of catechins on Aβ -induced cell death in cultured cortical neurons.

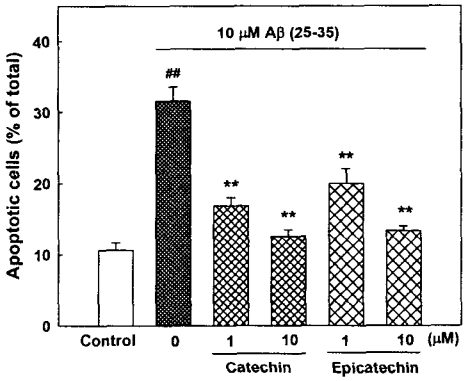

Fig. 2. Inhibitory effect of catechis on Aβ -induced apoptosis of cultured cortical neurons as measured by Hoechst 33342 staining.

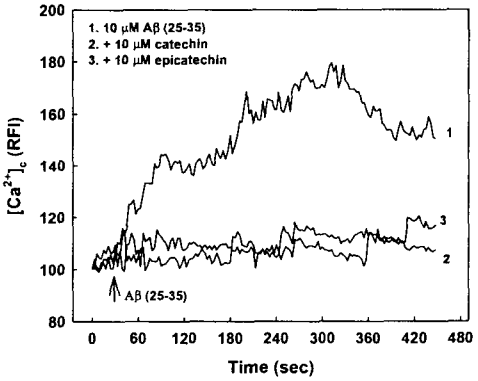

Fig. 3. Change of $[Ca^{2+}]_c$ in response to Aβ in the presence or absence of catechins in cultured cortical neurons.

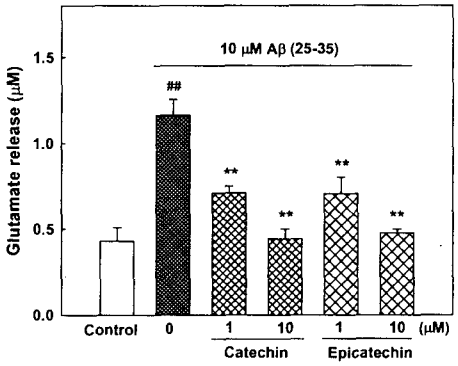

Fig. 4. Inhibitory effect of catechins on Aβ -induced glutamate release in cultured cortical neurons.