[14:50～15:30]

S-Allyl-L-cysteine, a Garlic Compound, Selectively Protects Cultured Hippocampal Neurons from ER Stress-induced Neuronal Death.

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Abstract

We have assessed amyloid β-peptide (Aβ)-induced neurotoxicity in primary neurons and organotypic hippocampal slice cultures (OHC) in rat. Exposing cultured hippocampal and cerebellar granule neurons to Aβ resulted in a decrease of MTT reduction, and in destruction of neuronal integrity. Treatment of these neurons with tunicamycin, an inhibitor of N-glycosylation in the endoplasmic reticulum (ER), also decreased MTT reduction in these neurons. S-allyl-L-cysteine (SAC), an active organosulfur compound in aged garlic extract, protected hippocampal but not cerebellar granule neurons against Aβ- or tunicamycin-induced toxicity. In the hippocampal neurons, protein expressions of casapse-12 and GRP 78 were significantly increased after $A\beta_{25\text{-}35}$ or tunicamycin treatment. The increase in the expression of caspase-12 was suppressed by simultaneously adding 1 μM SAC in these neurons. In contrast, in the cerebellar granule neurons, the expression of caspase-12 was extremely lower than that in the hippocampal neurons, and an increase in the expression by $A\beta_{25\text{-}35}$ or tunicamycin was not detected. In OHC, ibotenic acid (IBO), a NMDA receptor agonist, induced concentration-dependent neuronal death. When Aβ was combined with IBO, there was more intense cell death than with IBO alone. SAC protected neurons in the CA3 area and the dentate gyrus (DG) from the cell death induced by IBO in combination with Aβ, although there was no change in the CA1 area. Although protein expression of casapse-12 in the CA3 area and the DG was significantly increased after the simultaneous treatment of Aβ and IBO, no increase in the expression was observed in the CA1 area. These results suggest that SAC could protect against the neuronal cell death induced by the activation of caspase-12 in primary cultures and OHC. It is also suggested that multiple mechanisms may be involved in neuronal death induced by Aβ and Aβ in combination with IBO.

Key words: ER stress, caspase-12, CHOP, organosulfur compound

Introduction

S-allyl-L-cysteine (SAC), one of the organosulfur compounds found in aged garlic extract, has been shown to possess various biological effects including neurotrophic activity. in cultured neurons (Moriguchi *et al.*, 1997a; Moriguchi *et al.*, 1997b). SAC has been also shown to inhibit neuronal damage induced by ischemia in rat brain (Numagami and Ohnishi, 2001).

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that causes progressive loss of cognitive abilities. The pathology of AD is characterized by progressive

formation of neuronal fibrillary tangles and secretion of amyloid β-protein (Aβ). It has been demonstrated that Aβ has cytotoxic effects on cultured neurons (Yao *et al.*, 1999). In our previous experiments, we showed that SAC could protect against Aβ- and tunicamycin (an inhibitor of N-glycosylation in the ER)-induced cell death, but not against 4-hydroxynonenal (HNE)-induced cell death in nerve growth factor-differentiated PC12 cells (Ito *et al.*, 2003a). In the study described here, therefore, we characterized the neuronal death induced by Aβ and tunicamycin whether SAC could prevent these types of neuronal death in cultured rat hippocampal neurons. In addition, mechanistic differences between the Aβ-induced neuronal death that is observed in cultured hippocampal neurons and that observed in cerebellar granule neurons have also been investigated.

The organotypic hippocampal culture (OHC) is a system that is useful when studying the mechanisms of neurodegeneration. This is because several features of hippocampal circuitry are preserved, and the preparation is well suited for prolonged pharmacological treatment and recovery, which would be difficult to perform in an intact animal experiment. Therefore, we have also assessed Aβ-induced neurotoxicity, with and without added ibotenic acid (IBO), a potent NMDA agonist, in an organotypic hippocampal slice culture (OHC).

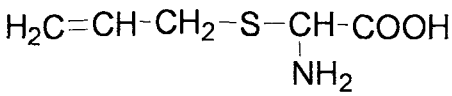

Figure. 1. Chemical structure of S-allyl-L-cysteine (SAC).

Materials and Methods

Cell cultures and determination of cell viability

Hippocampal neurons: Primary cultures of hippocampal neurons were prepared from 18-day-old embryos of Wistar rats as described by Brewer *et al.* (1993). Cell viability was determined by a double-staining procedure using Hoechst 33258/PI. The MTT reduction assay, which has been widely used for measuring cell viability, was also performed according to the method described previously (Ito *et al.*, 2003a).

Cerebellar granule neurons: Cultures rich in cerebellar granule neurons were prepared from 8-day-old Wistar rats as reported previously (Ito *et al.*, 1995).

Organotypic hippocampal slice culture (OHC): The OHC was prepared as described by Bruce *et al.* (1996). The effects of Aβ, IBO, S-allyl-L-cysteine and other drugs were tested in mature cultures, which had been maintained for 21-22 days in vitro (DIV), unless otherwise specified. A 100-μL aliquot of Aβ, IBO, or Aβ in combination with IBO (Aβ+IBO) was applied directly to the top of the slices. The degree of hippocampal neuronal death was evaluated by microscopic observation of PI uptake. Sections were photographed and an analysis of specific areas was done, initially by selecting the areas of the hippocampal regions (CA1-CA3, DG) on the image.

Results and discussion

1. Aβ- and tunicamycin-induced neurotoxicities in primary neurons and SAC

Exposing cultured hippocampal and cerebellar granule neurons to Aβ resulted in a decrease of MTT reduction, and in destruction of neuronal integrity. Treatment of these neurons with tunicamycin also decreased MTT reduction in these neurons. An active organosulfur compound in aged garlic extract, SAC, protected hippocampal but not cerebellar granule neurons against Aβ- or tunicamycin-induced toxicity (Fig. 2). In the hippocampal neurons, protein expressions of casapse-12 and GRP 78 were significantly increased after $A\beta_{25\text{-}35}$ or tunicamycin treatment. The increase in the expression of caspase-12 was suppressed by simultaneously adding 1 μM SAC in these neurons. In cerebellar granule neurons, protein expressions of CHOP and GRP 78 were significantly increased after these treatments. Moreover, the expression of caspase-12 was extremely lower than that in the hippocampal neurons, and an increase in the expression by $A\beta_{25\text{-}35}$ or tunicamycin was not detected. These results suggest that the mechanism underlying Aβ toxicity in cerebellar granule neurons is different from that in hippocampal neurons, and thus SAC exhibits a selective neuroprotective effect on Aβ toxicity in hippocampal neurons, but not in cerebellar granule neurons (Kosuge *et al.*, 2003).

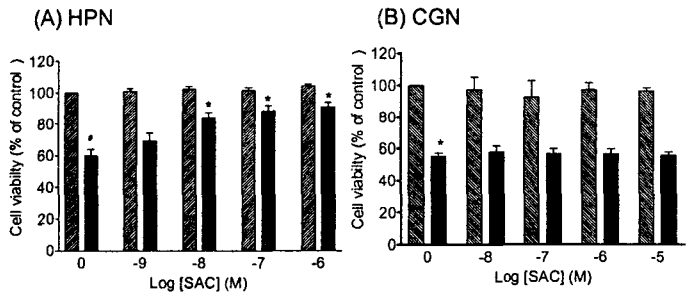

Figure 2. S-allyl-L-cysteine (SAC) protects against Aβ-induced neuronal death in cultured hippocampal neurons (HPN) but not in cerebellar granule neurons (CGN). Various concentrations of SAC were added simultaneously with 5 μM $A\beta_{1\text{-}40}$, and levels of MTT reduction were examined 24 h after these treatments. $^{\#}P<0.01$ as compared with control. $^{*}P<0.01$ as compared with 5 μM Aβ alone.

2. Aβ-induced neurotoxicity in OHC and SAC

In the OHC cultured for 3 weeks, there was little neurotoxicity after treatment with $A\beta_{25\text{-}35}$ (25 or 50 μM) alone for 48 h. However, with IBO alone neuronal death was observed in the pyramidal cell layer at low concentrations, and there was dramatic neuronal death at concentrations of 65 μM or more. Exposure of OHC to Aβ in combination with IBO (Aβ + IBO) induced severe cell death when compared with the effect of IBO alone (Ito *et al.*, 2003). SAC protected neurons in the CA3 area and the dentate gyrus (DG) from the cell death induced by Aβ + IBO with no change in the CA1 area. Therefore, we investigated region specificity of cell death induced by Aβ + IBO in the OHC. When CA1 area and the other area

(CA3 area and DG) were dissected out from the OHC after exposure to Aβ + IBO and investigated by Western blot analysis, the immunoreactivity of active form of caspase-12 is increased in the CA3 and the DG but not in the CA1 area. These results suggest that the mechanism by which neuronal death is induced by Aβ+IBO in combination in the CA3 and the DG differs from that induced in the CA1 area, and thus SAC exhibits a selective neuroprotective effect on toxicity induced by Aβ+IBO in combination in the CA3 and the DG, but not in the CA1 area.

Conclusions

Our findings indicate that SAC could provide a significant neuroprotective effect against Aβ-induced toxicity, not only in dissociated PC 12 cells and hippocampal neurons but also in neurons in specific areas of the OHC in the presence of IBO. It is also suggested that SAC could selectively protect neurons from caspase-12-dependent cell death but not from CHOP-dependent cell death that is triggered by ER dysfunction.

Acknowledgement

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S16 15:40~16:20 November 12, 2004 (Fri)

Characteristics of Neuronal Dysfunction induced by Thiamine-Deficient Feeding

Takeshi Tadano (Tohoku Pharmaceutical University, Japan)

S17 16:20~17:00 November 12, 2004 (Fri)

Assessment of Drug Dependence Potential in Rhesus Monkeys and Rats

Yoshio Wakasa (Ina Research Inc., Japan)