Prevention of the Neurotoxicity of the Amyloid β Protein by Genipin

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Genipin, which was shown in our previous investigation to have prominent neuritogenic activity in paraneurons such as PC12h cells, was studied to determine whether it could prevent the toxicity of Alzheimer's amyloid β protein ($A\beta$) in cultured hippocampal neurons. Increased release of lactate dehydrogenase from hippocampal neurons after 2 d of $A\beta$ 25-35 administration was prevented dose dependently by the addition of genipin 20–40 μ M. Morphological observations and trypan blue staining of cells confirmed the protection of hippocampal neurons from $A\beta$ toxicity by genipin. Geniposide had less effect in preventing $A\beta$ toxicity.

Key words amyloid β protein; hippocampal neuron; genipin; neurotoxicity; neuroprotection

It is now generally documented that the cause of Alzheimer's disease is progressive deposition (histopathologically identified as senile plaque) of the amyloid β protein ($A\beta$) ($A\beta$ 1-42 and $A\beta$ 1-40) with aging or due to genetic background in vulnerable brain regions, including the hippocampus and cortex. This deposition is strongly suspected to contribute to disruption of the neuronal network as a result of ubiquitous apoptotic cell death induced by $A\beta$.^{1,2} The addition of $A\beta$ and its hydrophobic core fragments such as $A\beta$ 25-35 (a monodecapeptide with an amino acid sequence identical to that from 25 to 35 of $A\beta$) has been reported to induce significant cell death in approximately one week in primary cultures of rat hippocampal neurons.³⁻⁵

Geniposide and genipin, iridoid compounds found in many plants such as *Gardenia jasminoides*, have been demonstrated in our laboratory to have neurotrophic, nerve growth factor (NGF)-like activity in their neuritogenesis and signaling promotion in PC12h cells, with genipin having the

stronger effect.^{6,7} Administration of genipin counteracted the trauma- and scopolamine-induced impairment of memory in mice.⁸ In some reported experiments, NGF and other neurotrophic factors reversed the deficit in spatial learning and memory in aged rats.^{9,10} We investigated whether these two iridoids can affect the neuronal toxicity of $A\beta$ in cultured hippocampal neurons.

Hippocampal neurons from 18-d rat embryos were prepared as described previously.¹¹ Hippocampal cells were maintained for 2 d in 48-well culture plates with Dulbecco's modified Eagle medium (DMEM) supplemented with 10% precolostrum new-born calf serum, selected for neuronal populations by incubation with cytosine- β -D-arabino-furanoside 10 μ M for 24 h in Ca^{2+} , Mg^{2+} -free phosphate-buffered saline, pH 7.4 (CMF-PBS), and then treated with $A\beta$ 25-35 (40 μ M) in CMF-PBS for 2 d without serum. $A\beta$ 25-35 was prepared using a peptide synthesizer according to the method described previously.¹¹ It was dissolved in CMF-PBS at a concentration of 1.8 mM (most of the $A\beta$ was in fine suspension).

Toxic effect on the cultures was evaluated by estimation of lactate dehydrogenase (LDH) release during 2 d of culture in the presence of $A\beta$ 25-35 with and without test compounds as specified below and according to the method described previously.¹²

Geniposide was kindly provided by Toyo F.C.C. Co. (Tokyo, Japan) and genipin was purchased from Wako Pure Chemical Industries (Osaka, Japan). They were dissolved in distilled water and refrigerated until use.

The addition of $A\beta$ 25-35 40 μ M to the hippocampal neuron cultures markedly increased LDH release (Fig. 1, $A\beta$ 25-35 only). Our colleagues previously found in cultured hippocampal cells that $A\beta$ 25-35-induced plasma membrane impairment was blocked by the addition of the endonuclease inhibitor aurintricarboxylic acid, indicating that the cell degeneration was a result of apoptotic cell death.¹³ The neurotoxicity of $A\beta$ was effectively attenuated by the addition of genipin (20 μ M or more) to the medium (Fig. 1A). Geniposide at any concentration used had little effect on $A\beta$ neurotoxicity, however. Enzymatic cleavage of the glucoside bond of geniposide has been shown to reinforce the biological activity of the glucoside in PC12h cells.⁶ The aglycon genipin

Fig. 1. Prevention of $A\beta$ 25-35 Cytotoxicity by Genipin and Geniposide in Rat Hippocampal Neurons

Culture wells containing neurons were treated with varying concentrations of genipin (A) or geniposide (B) in the presence or absence of $A\beta$ 25-35 40 μ M for 48 h as indicated. LDH activity in an aliquot of culture medium in each well was determined by the rate of oxidation of NADH after treatment. Values represent mean $\pm$ S.E.M. (A, $n=3$; B, $n=5-6$). * $p<0.01$ vs. no addition; # $p<0.01$ vs. $A\beta$ 25-35 alone. Differences between groups were assessed by analysis of variance (ANOVA) and Bonferroni's test.

Fig. 2. Morphological Images of Rat Hippocampal Neurons Showing the Effect of Genipin on A β 25-35-Induced Cytotoxicity

Cultures of neurons were treated with or without genipin 40 μ M and A β 25-35 40 μ M for 48 h as follows. A, vehicle; B, genipin; C, A β 25-35; D, genipin and A β 25-35. Scale bar=100 μ m.

is thus assumed to be the specifically active structure of geniposide. The results of microscopic investigation of the effect of genipin on cell death induced by A β 25-35 is shown in Fig. 2. The cultured hippocampal neurons had conspicuously de-

generated on day 2 of treatment with A β 25-35 40 μ M, but the degeneration did not occur to the same extent in the presence of genipin 40 μ M. Trypan blue staining of cells supported the above result, suggesting that the viability of the cells depends on the presence of genipin (20, 40 μ M) in cell cultures treated with A β 25-35 (data not shown). Increased LDH release induced by treatment with A β 25-35 in hippocampal neurons was also prevented by the addition of the enzymatic hydrolysate of aucubin (more than 10 μ M as aucubin, unpublished data), which was effective in promoting neuritogenesis in PC12h cells.⁶⁾ We have shown in paraneuronal PC12h cells that genipin is capable of inducing activation of the mitogen-activated protein kinase cascade, which is an important signaling pathway implicated in preventing apoptosis.¹⁴⁾ Yamamoto *et al.* reported that genipin, but not geniposide, suppressed the Fas-induced apoptosis in cultured mouse liver cells *via* inhibition of both caspase activation and reduction of mitochondrial membrane potential.¹⁵⁾ These observations suggest the possible mechanism of the protective effect of genipin against A β 25-35-induced cytotoxicity. These results all indicate that genipin and its congeners are potential candidates for the lead compound for treatment of Alzheimer's disease, and that they are unique in their ready accessibility to cellular target(s) across cell membranes (cf. ref. 7) and direct protection of hippocampal neurons from the toxicity of A β .

REFERENCES

- 1) Selkoe D. J., *J. Biol. Chem.*, **271**, 18295—18298 (1996).
- 2) Yankner B. A., *Neuron*, **16**, 921—932 (1996).
- 3) Yankner B. A., Duffy L. K., Kirschner D. A., *Science*, **250**, 279—282 (1990).
- 4) Lorenzo A., Yankner B. A., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 12243—12247 (1994).
- 5) Cotman C. W., Anderson A. J., *Mol. Neurobiol.*, **10**, 19—45 (1995).
- 6) Yamazaki M., Chiba K., Mohri T., *Biol. Pharm. Bull.*, **19**, 791—795 (1996).
- 7) Yamazaki M., Chiba K., Mohri T., Hatanaka H., *J. Neurochem.*, **79**, 45—54 (2001).
- 8) Imakura K., Yamazaki M., Mohri T., *J. Trad. Med.*, **13**, 173—179 (1996).
- 9) Fischer W., Sirevaag A., Wiegand S. J., Lindsay R. M., Bjorklund A., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 8607—8611 (1994).
- 10) Markowska A. L., Koliatsos V. E., Breckler S. J., Price D. L., Olton D. S., *J. Neurosci.*, **14**, 4815—4824 (1994).
- 11) Takadera T., Sakura N., Mohri T., Hashimoto T., *Neurosci. Lett.*, **161**, 41—44 (1993).
- 12) Koriyama Y., Yamazaki M., Chiba K., Mohri T., *Jpn. J. Pharmacol.*, **82**, 301—306 (2000).
- 13) Takadera T., Mohri T., Sakura N., Hashimoto T., *Neuropathol.*, **14** (Suppl.), 56 (1994).
- 14) Gjertsen B., Døskeland S. O., *Biochim. Biophys. Acta*, **1269**, 187—199 (1995).
- 15) Yamamoto M., Miura N., Ohtake N., Amagaya S., Ishige A., Sasaki H., Komatsu Y., Fukuda K., Ito T., Terasawa K., *Gastroenterology*, **118**, 380—389 (2000).