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Aβ 17–42 in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis

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Summary

The p3 peptide [amyloid β-peptide (Aβ) 17–40/42], derived by α- and γ-secretase cleavage of the amyloid precursor protein (APP), is a major constituent of diffuse plaques in Alzheimer's disease and cerebellar preamyloid in Down's syndrome. However, the importance of p3 peptide accumulation in Alzheimer's disease and its toxic properties is not clear. Here, we demonstrate that treatment of cells with Aβ 17–42 leads to apoptosis in two human neuroblastoma cell lines, SH-SY5Y and IMR-32. Aβ 17–42 activated caspase-8 and caspase-3, induced poly(ADP-ribose) polymerase cleavage, but did not activate caspase-9. Selective caspase-8 and caspase-3 inhibitors completely blocked Aβ 17–42-induced neuronal death. Aβ 17–42 moderately activated c-Jun N-terminal kinase (JNK); however, overexpression of a dominant-negative mutant of SEK1, the upstream kinase of JNK, protected against Aβ 17–42 induced neuronal death. These results demonstrate that Aβ 17–42 induced neuronal apoptosis via a Fas-like/caspase-8 activation pathway. Our findings reveal the previously unrecognized toxic effect of Aβ 17–42. We propose that Aβ 17–42 constitutes an additional toxic peptide derived from APP proteolysis and may thus contribute to the neuronal cell loss characteristic of Alzheimer's disease.

Keywords: Alzheimer's disease; p3 peptide; apoptosis; caspase; JNK

Abbreviations: Aβ = amyloid β-peptide; APP = amyloid precursor protein; ELISA = enzyme-linked immunosorbent assay; JNK = c-Jun N-terminal kinase; PARP = poly(ADP-ribose) polymerase

Introduction

Alzheimer's disease is a neurological disorder associated with memory loss, spatial disorientation and deterioration of intellectual capacity. A prominent pathological feature of Alzheimer's disease is the presence of extracellular senile plaques that are composed of structurally abnormal neuronal processes and amyloid β-peptide (Aβ). Aβ peptides are derived from the longer amyloid precursor protein (APP) by two mutually exclusive proteolytic pathways. In one, sequential cleavage of APP by β- and γ-secretases generates the amyloidogenic fragments Aβ 1–40/42. In the second, cleavage by α- and γ-secretases generate purported non-amyloidogenic Aβ 17–40/42 peptides, also known as p3 fragments (Iversen *et al*., 1995; Selkoe, 2001). In addition to Aβ 1–40/42, plaques also contain p3 fragments. Immunohistochemical studies have shown that p3 is prevalent in selected areas of Alzheimer's disease brains and in a subset of dystrophic neurites, but it is absent or sparse in age-matched non-Alzheimer's disease brains (Higgins *et al*., 1996). Numerous studies have demonstrated that Aβ 1–42 is toxic to neurones and activates microglia and astrocytes (Akama *et al*., 1998; Combs *et al*., 2001; Selkoe, 2001). However, the potential pathological role of p3 fragments in Alzheimer's disease aetiology has not been studied extensively.

p3 is a major constituent of cerebellar preamyloid and also is present in neuritic plaques in Down's syndrome (Gowing *et al*., 1994; Lalowski *et al*., 1996). Down's syndrome is a genetic disease caused by the presence of an additional copy of chromosome 21. Thus, Down's syndrome patients have three copies of the APP gene, overexpress APP in the brain and develop Alzheimer's disease at an early age (Petronis, 1999; Neve *et al*., 2000). In Alzheimer's disease and Down's syndrome, it is believed that disruption of the normal function of APP including overexpression or altered processing of APP is the most likely explanation for amyloid plaque

formation and subsequent neuronal loss and dementia (Petronis, 1999). However, the importance of p3 accumulation in Alzheimer's disease and Down's syndrome and its function are not clear. In this study, we examined the toxic effects of p3 and investigated the signal transduction pathway activated by p3. The results of our experiments demonstrate that treatment of cells with the p3 fragment Aβ 17–42 induced c-Jun N-terminal kinase (JNK) phosphorylation, activated a Fas-like/caspase-8 cascade and led to neuronal apoptosis. The results suggest that activation of JNK and caspase-8 play an important role in Aβ 17–42-induced neuronal apoptosis.

Material and Methods

Materials

Media and N2 supplements for cell culture were from Invitrogen (Carlsbad, CA, USA). p3 and Aβ peptides including Aβ 17–40, Aβ 17–42, Aβ 25–35, Aβ 1–40, Aβ 1–42 and Aβ 42–1 were purchased from Biosource International (Camarillo, CA, USA). Aβ 17–40 and Aβ 17–42 peptides were dissolved in 10 mM HCl at 1 mM and used immediately. Aβ 1–40, Aβ 1–42 and Aβ 42–1 were dissolved in hexafluoroisopropanol and dried under a stream of argon gas. The dried peptides were then dissolved in water at 1 mM and incubated at 37°C for 48–72 h before use. Aβ 25–35 was dissolved in water and incubated at 37°C for 24 h before use. Hoechst 33342 was obtained from Molecular Probes (Eugene, OR, USA). Anti-caspase-3, anti-caspase-8 and anti-poly(ADP-ribose) polymerase (PARP) antibodies were purchased from BD PharMingen (San Diego, CA, USA); anti-cleaved-caspase-3, anti-cleaved PARP and anti-caspase-9 were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-JNK1 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho stress activated protein kinase (SAPK)/JNK was purchased from Promega (Madison, WI, USA). The caspase inhibitors z-VAD-fmk, DEVD-fmk, IETD-fmk and LEHD-fmk were purchased from Enzyme Systems Products (Livermore, CA, USA).

Cell cultures and transfection

Undifferentiated SH-SY5Y human neuroblastoma cells were grown in 50% Eagle's minimum essential medium (MEM)/50% F-12 nutrient mixture with 10% fetal bovine serum (FBS), 1 × non-essential amino acid solution and 1 × antibiotic-antimycotic (100 units/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml Fungizone) at 37°C under 5% CO_2/95% air. IMR-32 human neuroblastoma cells were grown in Dulbecco-modified essential medium (DMEM) with 10% FBS and 1 × antibiotic-antimycotic. Cells were treated with various peptides in serum free media containing N2 supplements. Transfections were performed with LipofectAmine (Invitrogen) according to the manufacturer's protocol. SH-SY5Y cells were transfected with either pcDNA3.zeo or pcDNA.zeo-SEK1-AL constructs, kindly provided by Dr James R. Woodgett, Ontario Cancer Institute, Toronto, Ontario, Canada. SEK1-AL encodes a dominant negative form of SEK1 containing a double mutation (S220A and T224L) (Yan *et al*., 1994). Pooled cells stably expressing pcDNA3.zeo or pcDNA.zeo-SEK1-AL were selected in media containing 200 μg/ml Zeocin (Invitrogen) for 2 months.

Assessment of cell viability and apoptosis assays

Cell viability was evaluated using Trypan blue exclusion—counting the number of dead (blue) and live cells in the cultures after p3 peptide treatment for 48 h. A cell death detection enzyme-linked immunosorbent assay (ELISA) kit (Roche Molecular Biochemicals, Indianapolis, IN, USA) was used to detect apoptosis after p3 peptide treatment of cells for 48 h. The assay is based on a quantitative sandwich ELISA principle using antibodies directed against DNA and histones to detect mono- and oligonucleosomes in the cytoplasm of cells undergoing apoptosis. The ELISA was carried out according to the manufacturer's protocol. For Hoechst staining, cells were treated with p3 peptides for 48 h. Fresh media containing 10 μM Hoechst 33342 was added for 20 min before the cells were photographed by fluorescence microscopy. Apoptotic cells were identified by the appearance of condensed and fragmented nuclei.

Immunoblot analysis

Cells were harvested in 300 μl of lysis buffer [20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM β-glycerolphosphate, 1% Triton X-100, 10% glycerol, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM Na_3VO_4 and 5 mM NaF]. The resulting lysates were resolved on 4–12% NuPAGE Bis–Tris gels (30 μg/lane; Invitrogen) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked in TBST (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% non-fat milk and then probed with different antibodies. Proteins were detected by using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Measurement of cellular caspase activity

Treated cells were harvested in cell lysis buffer (50 mM HEPES, pH 7.4, 1 mM DTT, 0.1 mM EDTA, 0.1% CHAPS and 0.1% Triton X-100). IETD-p-nitroanilide and LEHD-p nitroanilide were the substrates for caspase-8 and caspase-9, respectively. The experiments were performed according to the manufacturer's protocol (Biosource International).

Results

Induction of neuronal apoptosis by the p3 peptide Aβ 17–42

To determine the toxicity of p3 peptides, SH-SY5Y and IMR-32 cells were treated with Aβ 17–40 or 17–42 for 48 h and cell death was measured by Trypan blue exclusion. Aβ 17–42 dose-dependently killed cells with a maximum cell death of 40% at 30 μM; Aβ 17–40 was less toxic (~20% cell death at 30 μM) in this assay (Fig. 1A and B, upper panels). A cell death detection ELISA, which measures cytoplasmic oligonucleosomes, was used to evaluate apoptosis. Aβ 17–42 treatment caused ~4-fold increase in absorbance values while Aβ 17–40 only increased absorbance by ~50% (Fig. 1A and B, lower panels). The amount of cell death caused by Aβ 17–42 treatment of SH-SY5Y cells was similar to the amount caused by equivalent concentrations of Aβ 1–42 and 25–35 (Fig. 1C). Aβ 25–35 is a non-physiological fragment of full-length Aβ peptides. It has toxic properties similar to full-length peptides. In contrast, control peptides Aβ 42–1 (Fig. 1C) and Aβ 35–25 (data not shown) were not toxic. For subsequent experiments therefore, we focused on the effects of Aβ 17–42.

SH-SY5Y cells exhibit a pyramidal shape with several processes extending from each cell when grown in complete media or serum-free media containing N2 supplements. After treatment with Aβ 17–42, the cells exhibited a shrunken morphology with rounded cell bodies and retracted processes (Fig. 1Db). Cell cultures treated with Aβ 17–42 exhibited numerous highly condensed and fragmented nuclei as measured by Hoechst 33342 fluorescent labelling, while nuclei of untreated cells were more homogeneously labelled (Fig. 1Dd). These results suggest that Aβ 17–42 caused neuronal cell death mainly by apoptosis.

Involvement of caspase activation in Aβ 17–42-induced apoptosis

The general caspase inhibitor z-VAD was used to determine whether Aβ 17–42-induced cell death involves activation of a caspase cascade. Treatment of cells with 100 μM z-VAD completely prevented apoptotic cell death induced by Aβ 17–42 (Fig. 2A). Furthermore, z-VAD also prevented the cleavage of PARP induced by Aβ 17–42 (Fig. 2B). The apoptotic cell death induced by Aβ 1–42 was also prevented by z-VAD, suggesting that both peptides induced cell death in a caspase-dependent manner.

Caspase-3 is a key executioner caspase in apoptotic cell death (Fernandes-Alnemri *et al*., 1994; Strasser *et al*., 2000). We examined whether Aβ 17–42 treatment of SH-SY5Y cells activated caspase-3 by using western blotting with an antibody specific for the activated/cleaved fragment of this protein. We detected a time-dependent increase in cleaved fragments of caspase-3 after treatment of the cells with 20 μM Aβ 17–42 (Fig. 3A). Treatment of cells with a specific caspase-3 inhibitor, DEVD, prevented cell death induced by Aβ 17–42 (Fig. 3B) and blocked both caspase-3 and PARP cleavage (Fig. 3C).

Caspase-8 and caspase-9 are two key initiators of distinct caspase signalling pathways (Nijhawan *et al*., 2000). In order to investigate their involvement in Aβ 17–42-induced apoptosis, caspase-8 and caspase-9 activities were measured colorimetrically using IETD-p-nitroanilide or LEHD-p-nitroanilide as substrates, respectively, in lysates of cells treated with Aβ 17–42. Caspase-8 activity increased ~2-fold after 18 h treatment with Aβ 17–42 (Fig. 4B). Western blot analysis showed a decrease in full-length caspase-8 protein after 18 and 24 h treatment with Aβ 17–42 (Fig. 4A). IETD, a specific inhibitor of caspase-8, protected cells completely from Aβ 17–42-induced cell death (Fig. 4C). In contrast, we did not detect either significant increases in caspase-9 activity (Fig. 4B) or decreases in full-length caspase-9 protein (data not shown) in cells treated with Aβ 17–42. The specific caspase-9 inhibitor, LEHD, exhibited ~30% protection against Aβ 17–42-induced cell death (Fig. 4C), possibly reflecting non-specific inhibition of other caspases. These results suggest that caspase-8 activation plays an important role in Aβ 17–42-induced apoptosis in SH-SY5Y cells.

Involvement of JNK activation in Aβ 17–42-induced apoptosis

JNK is a stress-activated protein kinase (Hibi *et al*., 1993). Activation of JNK plays an important role in apoptosis induced by several types of cell stress (Estus *et al*., 1994; Sanchez *et al*., 1994; Ham *et al*., 1995; Mesner *et al*., 1995; Xia *et al*., 1995; Namgung and Xia, 2000). In order to evaluate the role of JNK activation in Aβ 17–42-induced apoptosis, lysates of Aβ 17–42-treated cells were analysed by western blot with a specific phospho-JNK antibody. Aβ 17–42 treatment of cells rapidly induced JNK phosphorylation in a biphasic manner with an initial peak at 5 min and a second peak at 1.5–6 h (Fig. 5A). To determine whether JNK activation was important in Aβ 17–42-induced cell death, we measured the cell viability in SH-SY5Y cells stably transfected with HA-tagged SEK1-AL, a dominant interfering form of SEK1, the kinase upstream of JNK (Sanchez *et al*., 1994). Expression of SEK1-AL was confirmed by western blot analysis (data not shown). Overexpression of this dominant negative form of SEK1-AL protected cells from Aβ 17–42-induced cell death by 50–60% compared with cells transfected with vector alone (Fig. 5B). These results are consistent with the view that JNK activation is an important and early event in the induction of apoptosis by Aβ 17–42.

Discussion

This study examined the toxic properties of p3 fragments derived from the so-called 'non-amyloidogenic pathway' of APP processing. We demonstrated that treatment with the p3 fragment Aβ 17–42 caused the death of SH-SY5Y and IMR-

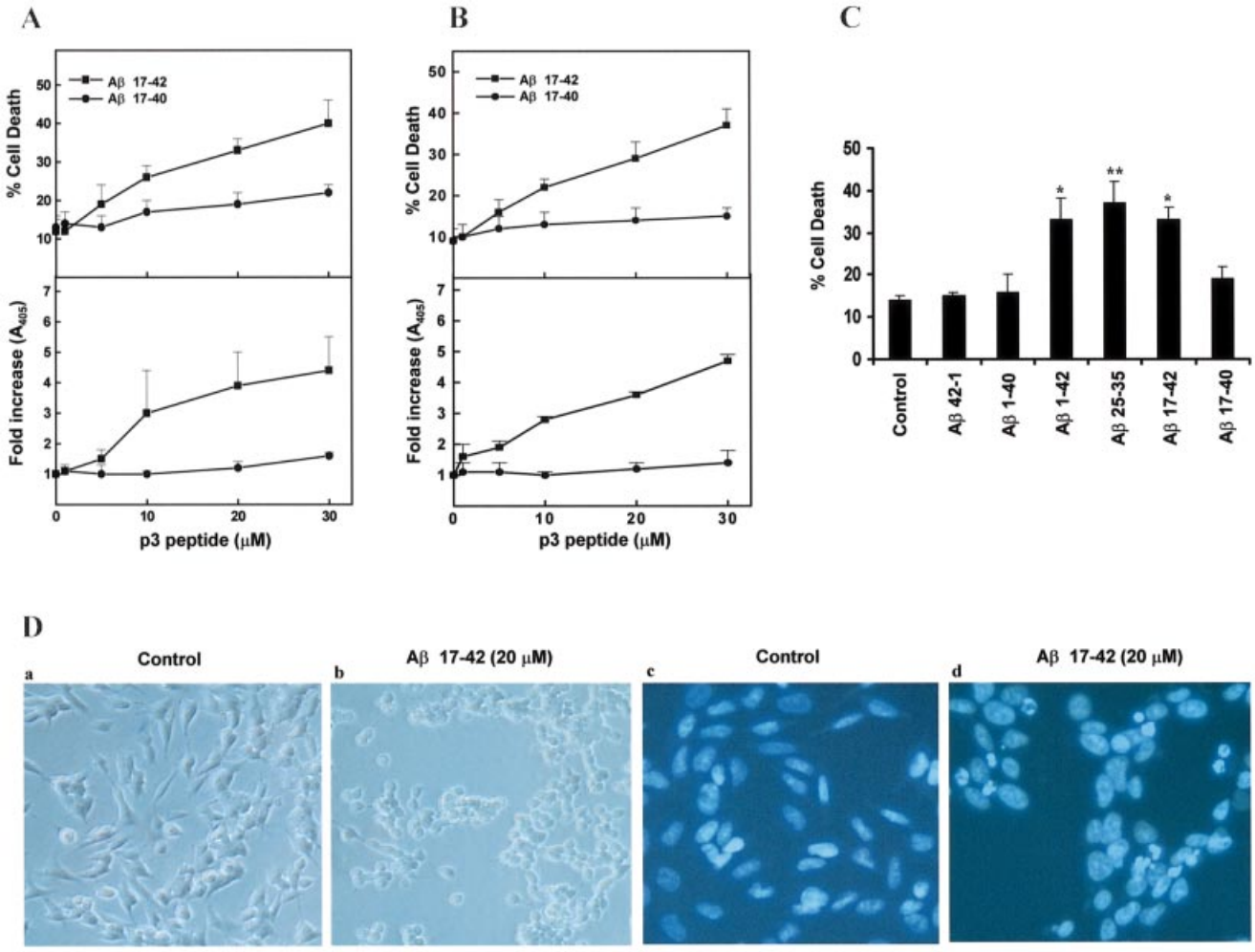

Fig. 1 Toxicity of p3 peptides. (**A**) SH-SY5Y and (**B**) IMR-32 cells were treated with p3 peptides for 48 h; Trypan blue exclusion (upper panels) and ELISA (lower panels) were used to determine cell death. Data are means ± SEM of three separate experiments. (**C**) SH-SY5Y cells were treated with various peptides at 20 μM for 48 h. Cell death was measured by Trypan blue exclusion. The results are presented as the mean ± SEM percentage cell death for three or four independent experiments each performed in duplicate. Significant differences between control and peptide treatment were assessed by Student's *t*-test. $*P < 0.05$, $**P < 0.01$. (**D**) SH-SY5Y cells were untreated or treated with 20 μM Aβ 17–42 for 48 h. Light micrographs show (a) the morphology of the control and (b) SH-SY5Y cells treated with 20 μM Aβ 17–42. Hoechst labelling shows (c) untreated cells and (d) nuclear condensation and fragmentation in 20 μM Aβ 17–42 treated cells. Cells were labelled with 10 μM Hoechst 33342 for 20 min after exposure to Aβ 17–42 for 48 h.

32 human neuroblastoma cells. This death was apoptotic by several criteria including shrunken cell bodies, condensed and fragmented chromatin, and increased amounts of cytoplasmic oligonucleosomes as measured by ELISA. The potency of Aβ 17–42 was similar to that of Aβ 1–42 and Aβ 25–35, while Aβ 17–40 was less toxic to cells (similar to Aβ 1–40). Our results suggest that a caspase cascade consisting of the initiator caspase-8 and the executioner caspase-3 is an important pathway in Aβ 17–42-induced apoptosis. Furthermore, JNK activation was an early, important event in Aβ 17–42-induced death. These results suggest that Aβ 17–42 has *in vitro* toxic properties and thus may partially contribute to neuronal loss in Alzheimer's disease and Down's syndrome.

Like Aβ, p3 is secreted from cells and deposited in brain parenchyma, accumulating predominantly in fleecy, diffuse amyloid deposits (Motte and Williams, 1989), and also in some senile plaques (Higgins *et al*., 1996). Aβ 17–42 is also the major component of amyloid plaques in the cerebellum of Down's syndrome patients. In an aged canine model of Alzheimer's disease, Aβ 17–42 and other N-terminal truncated Aβ peptides terminating at amino acid 42 are the major components of preamyloid (Wisniewski *et al*., 1996). The brain levels of p3 and similarly N-truncated Aβ peptides relative to full length peptides are not known. In familial forms of Alzheimer's disease, mutations in presenilins and APP lead to an increase in the relative amount of Aβ peptides ending at amino acid 42, including the p3 fragment Aβ 17–

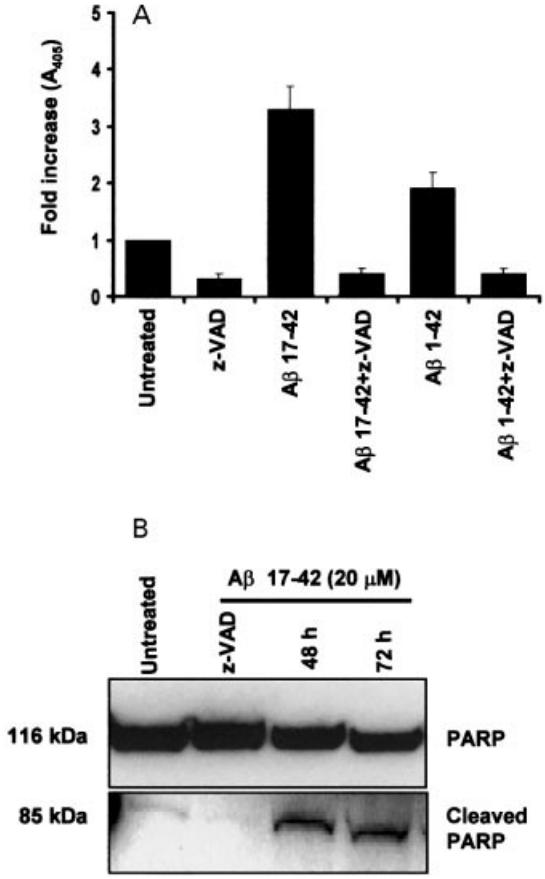

Fig. 2 Aβ 17–42 activated caspase cascade in SH-SY5Y cells. (**A**) Cells were untreated or treated for 48 h with: 100 μM z-VAD; 20 μM Aβ 17–42; 100 μM z-VAD + 20 μM Aβ 17–42; 20 μM Aβ 1–42; or 100 μM z-VAD + 20 μM Aβ 1–42. Apoptotic cell death was measured by ELISA. Data are means ± SEM for three separate experiments. (**B**) Western blot analysis shows full length PARP and cleaved PARP in cells either untreated, or treated with 100 μM z-VAD + 20 μM Aβ 17–42 at 72 h, or 20 μM Aβ 17–42 at 48 and 72 h.

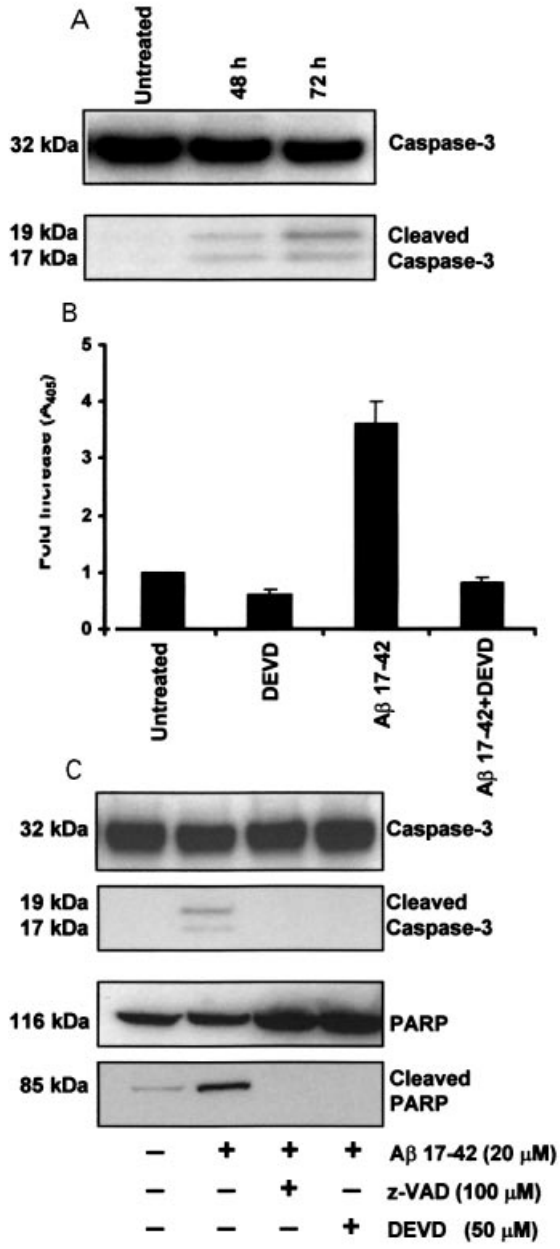

Fig. 3 Aβ 17–42 induced caspase-3 activation in SH-SY5Y cells. (**A**) Western blot analysis shows reduced procaspase-3 and increased cleaved caspase-3 in cells treated with 20 μM Aβ 17–42 for 48 and 72 h. (**B**) Cells were untreated or treated for 48 h with: 50 μM DEVD; 20 μM Aβ 17–42; or 50 μM DEVD + 20 μM Aβ 17–42. Apoptotic cell death was measured by ELISA. Data are means ± SEM for three separate experiments. (**C**) Western blot analysis shows the effects of 100 μM z-VAD and 50 μM DEVD on caspase-3 and PARP cleavages induced by 20 μM Aβ 17–42 for 48 h.

42. *In vitro*, the p3 fragment Aβ 17–42 can form a β-sheet conformation and is highly fibrillogenic (Pike *et al*., 1995). Our demonstration that Aβ 17–42 is toxic *in vitro* is in keeping with an earlier report (Pike *et al*., 1995) showing that an N-terminal deleted Aβ peptide lacking the first 16 amino acids (i.e. p3) enhanced aggregation of the peptide and caused death of primary hippocampal neurones. However, in that report, the type of cell death caused by p3 was not determined. Our results showed that Aβ 17–42 caused apoptosis in both SH-SY5Y and IMR-32 cells. Interestingly, a recent study has shown that p3 also induces cytokine production in mouse microglial cells similar to Aβ 1–42 (Szczepanik *et al*., 2001). Taken together, these findings strongly suggest that p3 peptides have a role in neuronal death and in the enhanced inflammatory response in Alzheimer's disease and Down's syndrome.

Several reports have shown that caspase activity is increased in animal models of Alzheimer's disease and in brains of Alzheimer's disease patients (Selznick *et al*., 1999; Masumura *et al*., 2000). A growing body of evidence suggests that caspase activation plays an important role in Aβ-induced cell death (Harada and Sugimoto, 1999; Giovanni *et al*., 2000; Allen *et al*., 2001; Troy *et al*., 2001). However, the

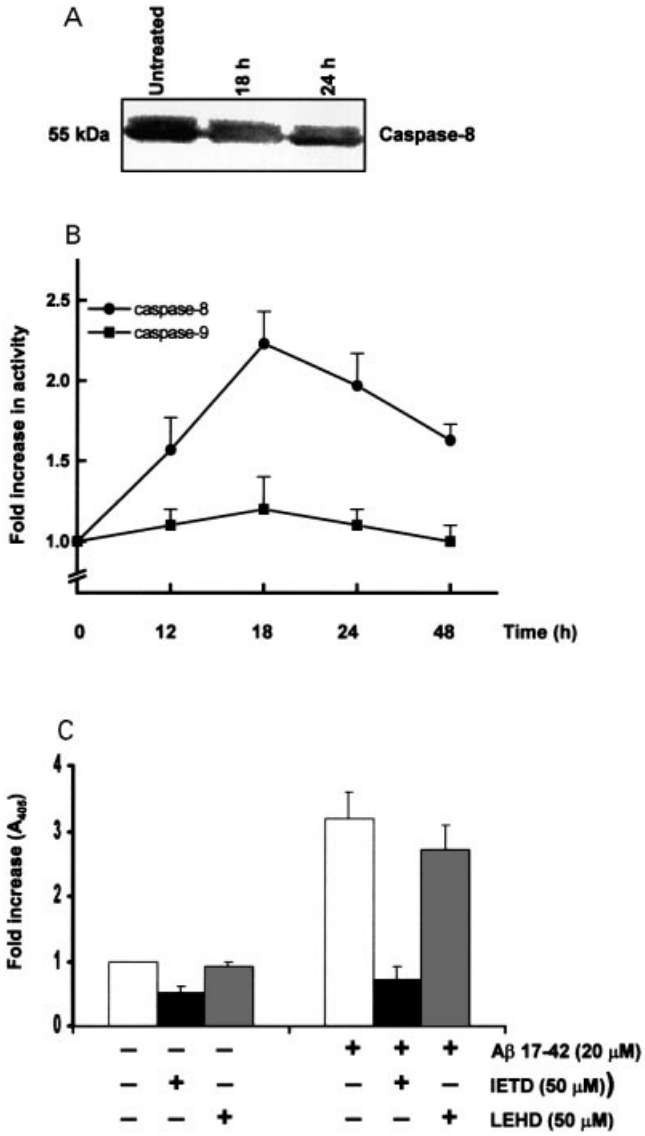

Fig. 4 Aβ 17–42 induced caspase-8 activation in SH-SY5Y cells. (**A**) Western blot analysis shows reduced procaspase-8 in cells treated with 20 μM Aβ 17–42 for 18 and 24 h. (**B**) Cells were treated with 20 μM Aβ 17–42 for the indicated times. Activity of caspase-8 and caspase-9 in cell extracts was measured colorimetrically by the cleavage of IETD-pNA and LEHD-pNA, respectively. Results are means ± SEM from five independent experiments. (**C**) Cells were untreated or treated for 48 h with 50 μM IETD or 50 μM LEHD, with or without 20 μM Aβ 17–42. Apoptotic cell death was measured by ELISA. Data are means ± SEM for three separate experiments.

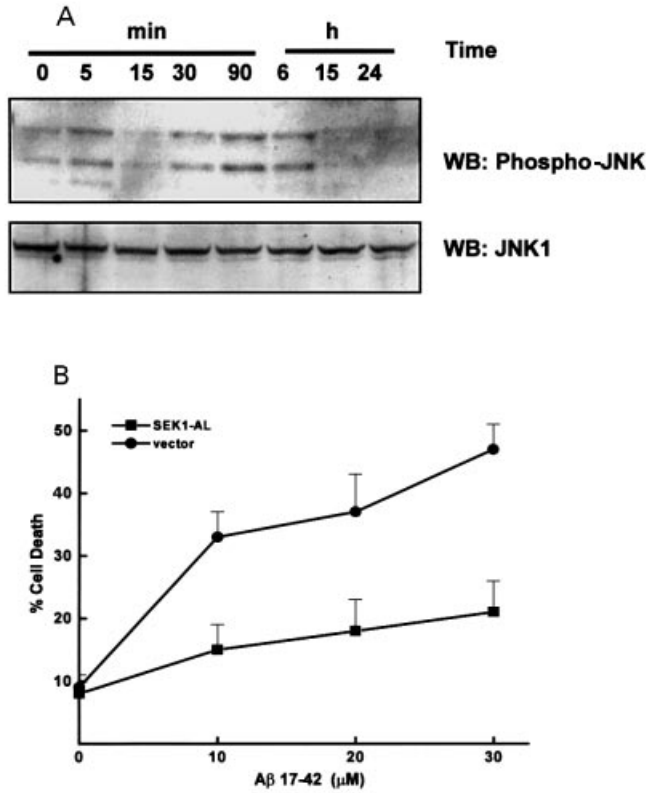

Fig. 5 Effect of Aβ 17–42 on JNK phosphorylation in SH-SY5Y cells. (**A**) Western blot analysis shows JNK activation in cells treated with 20 μM Aβ 17–42 for the indicated times by using anti-phospho-SAPK/JNK antibody (top panel) and anti-JNK1 antibody (bottom panel). Results are representative of three separate experiments. (**B**) Pooled cells stably expressing dominant-negative SEK1-AL exhibited reduced Aβ 17–42-induced cell death compared with SH-SY5Y cells stably transfected with vector alone. Cell death was measured by Trypan blue exclusion after 48 h treatment. The results are the mean ± SEM percentage cell death for three or four independent experiments each performed in duplicate.

caspase cascade involved in p3-induced neuronal death is unclear. Our results showed that Aβ 17–42-induced apoptosis in SH-SY5Y cells was mediated predominantly by a caspase-8 and caspase-3 pathway. Importantly, specific inhibitors of these caspases completely prevented Aβ 17–42-induced apoptosis, strongly suggesting that these caspases are critical in SH-SY5Y cell death. We were unable to detect caspase-9 activity after a 48 h treatment period, suggesting that Aβ 17–42 mainly activated a Fas–Fas ligand/caspase-8 signalling pathway rather than a mitochondrial cytochrome *c*/caspase-9 pathway leading to neuronal apoptosis. These findings are in good agreement with the recent observation by Allen and colleagues (Allen *et al*., 2001) that Aβ-induced neuronal death was mediated by caspase-8 and might involve a Fas-like death receptor. However, our results do not exclude the possibility that other caspases may be involved in Aβ 17–42-induced neuronal apoptosis.

Neuronal cellular signalling cascades activated by p3 treatment had not been previously reported. This is the first demonstration that Aβ 17–42 treatment of SH-SY5Y cells leads to early activation of JNK (within 5 min and a second peak of activity at 1.5–6 h). This rapid activation of JNK suggested that p3 may act through a receptor-mediated response. The delayed activation of JNK may be caused by reactive oxygen species induced by Aβ 17–42, since oxidative stress is known to activate JNK. Overexpression of a dominant-negative SEK1-AL (MEKK4) protected cells from Aβ 17–42-induced cell death by ~60%. These results

suggest that JNK activation plays a critical role in p3-induced neuronal apoptosis. We recently showed that Aβ 1–42, like Aβ 17–42, also activates JNK in SH-SY5Y cells. Overexpression of a dominant-negative SEK1 blocked Aβ 1–42-induced cell death, suggesting that both Aβ 17–42 and Aβ 1–42 have similar modes of action (Wei *et al*., 2002). Other evidence has also shown that JNK and c-Jun are activated in degenerating and apoptotic neurones in Alzheimer's disease brain (Anderson *et al*., 1996; Marcus *et al*., 1998; Shoji *et al*., 2000; Zhu *et al*., 2001). JNK is potently and preferentially activated in response to a variety of stress signals including growth factor withdrawal, γ-irradiation and TNF-α (tumour necrosis factor alpha) or Fas ligand treatments (Chen *et al*., 1996). JNK activation also is associated with the induction of apoptosis (Estus *et al*., 1994; Ham *et al*., 1995; Verheij *et al*., 1996; Le Niculescu *et al*., 1999; Namgung and Xia, 2000;). Thus, these findings suggest that Aβ 17–42 activated JNK may lead to upregulation of apoptosis-associated gene expression and trigger a caspase cascade leading to apoptotic cell death.

In summary, we have demonstrated that p3 peptide is toxic to neurones *in vitro*. The results suggested that p3 fragment Aβ 17–42 should be considered a potential toxic product of APP α-secretase processing. The results also raise a cautionary note about shifting APP processing to the α-secretase pathway as a method of decreasing β-secretase amyloidogenic processing of APP as a therapeutic approach in Alzheimer's disease.

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