Electron microscopic study of the effect of p75NTR extracellular domain on the amyloid β protein assembly

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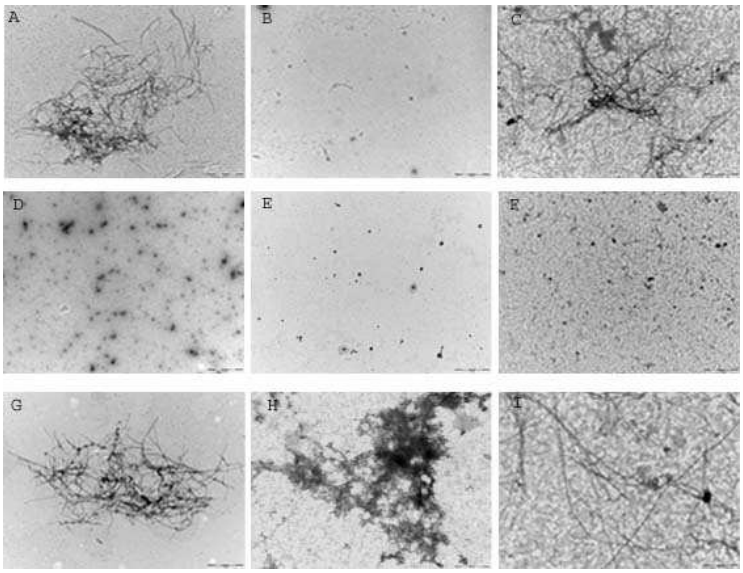
Accumulation of toxic amyloid-β in the cerebral cortex and hippocampus is a major pathological feature of Alzheimers disease (AD). The p75 neurotrophin receptor (p75NTR) is the common receptor for neurotrophins which can mediate cell death and neurite degeneration. Previous studies showed that the expression of p75NTR is increased in AD brain and activation of p75NTR by Aβ and nerve growth factor (NGF) does not always promote neuronal death but can promote survival of human neurons (Zhang *et al.*, 2003). In this study, we investigated the effect of p75NTR extracellular domain fused with Fc fragment of human IgG (p75/Fc) on the Aβ assembly using transmission electron microscopy (TEM). Aβ peptide purchased from American Peptide (Sunnyvale,CA) was dissolved in DMEM at the final concentration of 22μmol/l. Samples were divided into 4 experimental groups and incubated at 4°C and 37°C respectively for 24 hours: 1, Aβ prepared immediately after dissolution without incubation; 2, Aβ incubated alone; 3, Aβ incubated with p75NTR (molar ratio 1:0.5) and 4, Aβ incubated with human IgG (molar ratio 1:0.5).



Electron micrographs of samples of incubates. Scale bars 1μm.

We found that Aβ prepared immediately after dissolution without incubation showed only amorphous material under TEM. After Aβ was incubated alone at 37°C for 24 hours, numerous fibrils were seen (Figure A). However, when Aβ was incubated together with p75/Fc, only short fibrils can be observed and the number of fibrils remarkably reduced (Figure B). Addition of Human Ig G into the Aβ solution did not alter the morphology and number of fibrils obviously (Figure C). After incubation at 4°C, numerous oligomers formed (Figure D). Addition of p75/Fc to the Aβ remarkably reduced the number of oligomers (Figure E) but addition of human IgG did not change the morphology and number of oligomers (Figure F). We further investigated whether the p75/Fc can disaggregate the preformed Aβ fibrils. When preformed Aβ fibrils were incubated with p75/Fc for additional 3 days, most fibrils disassembled and became aggregates, but these fibrils showed no change when incubated with human IgG (Figure H). In conclusion, p75/Fc can inhibit the oligomerization and fibrillation of Aβ. Furthermore, p75/Fc can also disaggregate the preformed Aβ fibrils. Thus, p75 NTR may be a potential therapeutic agent for AD.

Zhang Y, Hong Y, Bounhar Y, Blacker M, Roucou X, Tounekti O, Vereker E, Bowers WJ, Federoff HJ, Goodyer CG, LeBlanc A. (2003) p75 neurotrophin receptor protects primary cultures of human neurons against extracellular amyloid beta peptide cytotoxicity, *Journal of Neuroscience* **23:** 7385-7394.