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아밀로이드베타로 유도된 알츠하이머성 치매 모델에서 BF-7의 보호 효과

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The Protective Effect of BF-7 on β-amyloid-indeuced Alzheimer's Disease Model.

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Objectives

BF-7, *Bombyx mori* extracts, is known to have cell protective effect against various insults. But so far, the protective mechanism is largely unknown. We determined whether BF-7 protected against β-amyloid induced neurotoxicity and learning impairment in vivo models.

Materials and Methods

Aβ fibril preparation Peptides corresponding to amino acids 1-42 of human Aβ protein were purchased from BioSource (Camarillo, CA, USA). To fibrillize, Aβ peptides were resuspended in sterile dH_2O (final concentration, 9 mg/ml) followed by incubation for 24 h at 37℃.

Animal and Surgical Procedure Young adult male ICR mice were caged individually at least 1 week prior to the start of the experiments and kept on a normal laboratory diet and tap water ad libitum in an air-conditioned room (21 ± 2℃) with a 12-h light cylcle. The animals were anethetized intraperitoneally (i.p) with ketamine and mounted in a stereotaxic frame. All efforts were made to minimize animal suffering throughout the experiments. The administration of Aβ was performed as followed. Briefly, each mouse was injected at the bregma with a 50 ㎕ Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.2mm. The ingection volume was 5 ㎕.

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Passive Avoidance performance On day 7 after Aβ injection, mice were trained on a one-trial step-through passive avoidance task. The passive avoidance box was divided into two compartments, one illuminated and one dark, equipped with a grid floor. During the training trial, each mouse was placed in the lighted compartment; as soon as it entered the dark compartment, the door was closed and the mouse received and inescapable shock (0.1 mA, 3s). In the testing trial, given 1 day after the trainig trial, the mouse was again placed in the lighted compartment and the time until it re-entered the dark compartment was measeured (the step-through latency maximum tesing limit was 90 s).

Immunohistochemistry and Histochemistry On day 14 after Aβ injection, animals were anethetized with ketamine and perfused transcardially with 4% paraformaldehyde (0.1 M phosphate buffer, pH.7.4). The dissected brains were cryoprotected in PBS containing 20% sucrose for 1 day,, coronary cut into consecutive 40 ㎛ sections with a freezing microtome. The sections were stained with cresyl violet, which stains the Nissl bodies in the perikarya (Nissl staining). Assessment of apoptosis in the hippocampus by Aβ was carried out using ApopTag, Apoptosis detection kit.

Results

The results may be summarized as follows:

1. Aβ induces neuronal cell death both in vitro and in vivo.
2. BF-7 attenuates loss of learning and memory function induced by Ab.
3. BF-7 prevents increase of bax and decrease of bcl-xl in hippocampus, thus elevates neuronal survival in mice.
4. BF-7 inhibits caspase activity and neuronal cell death although couldn't diminish mitochondrial malfunction in vitro system. This result suggests that BF-7 inhibits the decrease of bcl-xl, which prevents the conformational change of bax.