Differential expression of proteins of caspases and Bcl-2 families in the brain of mice

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Abstract. Apoptosis is an important process in the variety of different biological system including cell death and embryonic development. Inappropriate apoptosis is implicated in many human diseases such as Alzheimer's disease. Central component of the machinery of apoptosis program in neurons of patients with Alzheimer's disease includes proteins of caspases and Bcl-2 families. We examined whether endogenous protein levels of caspases and Bcl-2 families are expressed in a differential manner during the embryonic and postnatal development of BDF1 strain. Here, all four proteins with caspases-3, -9, Bcl-2 and Bax were highly expressed between embryonic day 19 and 1 week age of early postnatal development, but thereafter the expression dramatically declined. These patterns are needed to compare the proteins in the brains of APPsw-transgenic mice that are expected to be expressed highly in the brain of adult mice. Thus, the results are useful to understand fundamentally the mechanisms of the apoptotic changes during the embryonic and postnatal development of Alzheimer's model mice.

Introduction

Apoptosis is an important process in the variety of different biological system including cell death and embryonic development. Inappropriate apoptosis is implicated in many human patients with Alzheimer's disease (AD), Huntington's disease, and ischemic damage (1,2). Caspases, the executioners of apoptosis, is an aspartate-specific cysteine class of protease and is a central component of the machinery of apoptosis. Among the caspase family, caspase-3 is a potent effector of neuronal death during nervous system development and normally exists in the cytosolic fraction of cells as an inactive precursor that is activated by enzymatic cleavage in early apoptosis. Upon activation by an apoptosis-inducing death signal, caspase-3 is cleaved into p18 (amino acids 29-175) and p12 (amino acids 176-277) subunits, which are derived from the 32-kDa pro-enzyme (procaspase-3, CPP32) (3). Also, caspase-3 has been shown to be significant in nervous system development (7,8) and in AD brain (9,10). Interestingly, caspase-3 levels were high from embryonic day 19 to 2 weeks in the brain of Wistar rat (11). In addition, Bcl-2 family protein including Bax are involved in inducing apoptosis and have channel activity *in vitro*. As Bax can heterodimerize with Bcl-2 when they are in their compact α-helical bundle conformation in environment, these proteins produce heterodimeric channels when integrated into membranes. In particular, ratio of Bcl-2 and Bax is critical; excess Bax paradoxically promotes cell death (4) and Bcl-2 exhibits cytoprotective functions in cellular contexts (5,6).

In this study, we examined whether endogenous protein levels of caspases-3/-9 and Bcl-2/Bax families in BDF1 strain are regulated in differential manner during the embryonic and postnatal development of BDF strain.

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Materials and methods

BDF1 strain of mice. BDF1 strain of mice used for production of transgenic mouse expressing neuron-specific enolase (NSE)-controlled APPsw in this laboratory were handled in an accrediated Korea FDA animal facility in accordance with the AAALAC International Animal Care Policy (Accredited Unit-Korea Food and Drug Administration: Unit Number-000936) and were maintained in a specified pathogen-free state. Mice were housed in cage under a strick light cycle (light on at 06:00 h and off at 18:00), and were given a standard irradiated chow diet (Purina Mills Inc.) *ad libitum*.

Western blot analysis. Protein was prepared from the brains at various stages of embryonic and postnatal development BDF1 strain. Protein was then separated in a 10% poly-

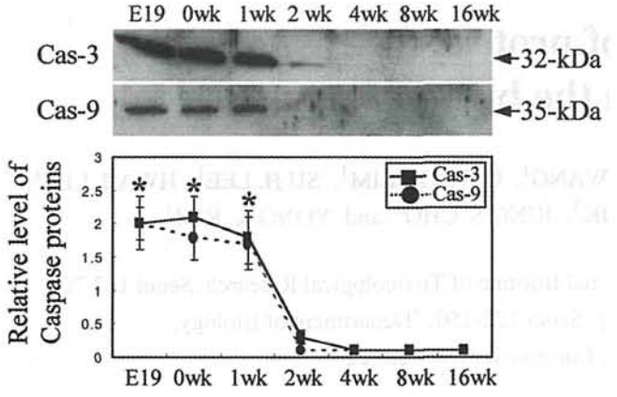

Figure 1. Western blot analysis of caspase-3 and -9 proteins. Lysates were prepared from the brains at indicated stage. Proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. Caspase-3 and -9 were detected with antibodies raised against mouse caspase-3 and -9 followed by anti-rabbit-HRP visual. Protein levels in various stage of development were quantified by a Kodak Electrophoresis Documentation and Analysis System. Three mice per age group were assayed in triplicate on Western blot analysis. Median value and SD are shown. *p<0.05 versus 2-, 4-, 8-, and 16-weeks old mice.

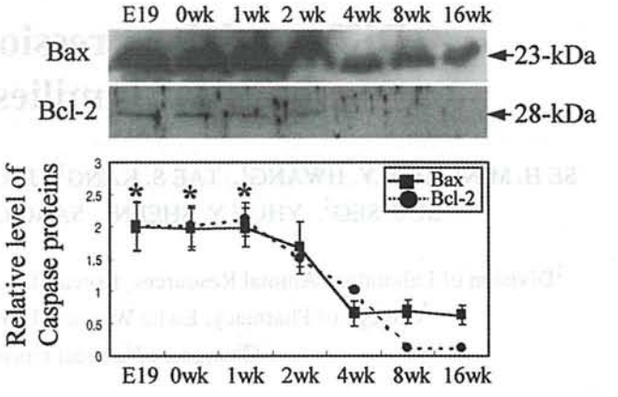

Figure 2. Western blot analysis of Bcl-2 and Bax proteins. Proteins in the brain were separated by 10% SDS-PAGE and transferred into nitrocellulose membrane. Bcl-2 and Bax were detected with mouse antibodies against Bcl-2 and Bax. Protein levels in various stages of development were quantified by a Kodak Electrophoresis Documentation and Analysis System. Three mice per age group were assayed in triplicate on Western blot analysis. Median value and SD are shown. *p<0.05 versus 2-, 4-, 8-, and 16-weeks old mice.

acryamide gel electrophoresis for 3 h and transferred to a nitrocellulose membrane using an electroblot for 2 h. Each membrane was incubated separately with the primary rabbit polyclonal anti-caspase-3 antibody (1:500), rabbit polyclonal anti-caspase-9 antibody (1:500), goat polyclonal Bcl-2 antibody (1:250), and goat polyclonal anti-Bax anbody (1:250). After blocking, the membranes were washed with washing buffer and then incubated with secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymede) at a 1:1,000 dilution at room temperature for 2 h. Specific proteins were detected by an enhanced chemiluminescent substrate (ECL, Pharmacia).

Results

Differential expression of caspase-3 and -9. Expressions of caspase-3 prepared from the brains in various stages of embryonic and postnatal development were examined by Western blot analyses. As shown in Fig. 1, the level of mouse caspase-3 protein was highly expressed between 1 and 4 weeks of age, and thereafter it dramatically declined. The pattern of caspase-9 expression is also similar to those of caspase-3, which was not seen in the brain of Wistar rat (11).

Differential expressions of Bcl-2 and Bax proteins. We examined the Bcl-2 and Bax expression to test whether both levels are also regulated in a differential manner. Protein (50 μg) prepared from the brain of mice were transferred to a nitrocellulose membrane, and the Western blot analysis was carried out using rabbit anti-Bcl2 and Bax antibodies. As shown in Fig. 2, the levels of both Bcl-2 and Bax were significantly high at neonate and low at the adult during the postnatal development, but intensity of Bax bands were much stronger than those of Bcl-2.

Discussion

We found that both caspase-3 and -9 proteins were highly expressed in embryonic and early postnatal development, but not in the late postnatal development. First, it is interest to compare these differential patterns between this result and the results of APP-transgenic line, because caspase-3 expression can be observed in the brain of Alzheimer's disease (13-15) and possibly APPsw transgenic mice. In fact, there is a link between caspase-3 and APP in neuronal cell death. It has been demonstrated that APP protein is a substrate for caspase-3 (12) and their cleavage of APP with caspase-3 results in reduced Aß secretion (16). Also, caspase-3 is involved in APP processing consistent with elevation of Aß formation in neuron of AD (10). Second, it is possible that up-regulation in the embryonic and early postnatal development may reflect a compensative mechanism to maximize the positive effects of caspases-3 and -9 activities which could be necessary for cell-death during the embryonic and early development. Third, factors-regulating element within the caspase-3 and -9 promoter may be the target-binding site for an as-yet-unidentified early-developmental factor. To test this, we constructed a fusion gene containing human caspase-3 promoter-linked to the *lacZ* coding sequence. The fusion gene will be transfected into the embryonic and adult neuron cells to compare the *lacZ* gene expression between two cell lines.

We also observed high level expression of Bcl-2 and Bax proteins in the brain of embryonic early and late postnatal development. Since the ratio of Bcl-2 and Bax is important (4), over-expression of Bcl-2 expression at embryonic and early postnatal development lead to cell-death. Bax protein might mediate or contribute to the protection of neuronal cells against cell death, and thus it was highly expressed between embryonic day 19 and 2-weeks of age. The result is

required to compare the expressions of Bcl-2 family with Bax in the brain of APP-transgenic mice. Thus, the results are useful to understand fundamentally the mechanisms of the pathological changes underlying AD and differential regulations of caspase-3 and -9, and Bcl-2 and Bax proteins families. Currently, we are preparing the production of transgenic mice expressing NSE-controlled APPsw using BDF1 strain of mice.

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