β-amyloid 42 accumulation in the lumbar spinal cord motor neurons of amyotrophic lateral sclerosis patients

Noel Y. Calingasan,* Junyu Chen, Mahmoud Kiaei, and M. Flint Beal

Department of Neurology and Neuroscience, Weill Medical College of Cornell University, 525 East 68th Street, F-610, New York, NY 10021, USA

Received 26 October 2004; revised 28 December 2004; accepted 12 January 2005
Available online 23 February 2005

Amyotrophic lateral sclerosis (ALS) is characterized by a progressive loss of large motor neurons in the brain and spinal cord. Amyloid precursor protein (APP), the transmembrane precursor of β-amyloid (Aβ), accumulates in the anterior horn motor neurons of ALS patients with mild lesions. APP undergoes an alternative proteolysis mediated by caspase-3, which is activated in motor neurons in a mouse model of ALS. The ALS spinal cord motor neurons also show evidence of increased oxidative damage, which is thought to alter APP processing. We sought to determine whether Aβ42, the more pathogenic Aβ species, accumulates in the postmortem lumbar spinal cord of ALS patients. While there was little or no Aβ42 labeling in control spinal cord tissues, elevated Aβ42 immunoreactivity occurred in ALS motor neuronal perikarya and axonal swellings in the anterior horn. A few Aβ42-positive neurons exhibited thioflavine S staining. No extracellular Aβ42 deposits were found. Aβ42 coexisted with the oxidative damage markers malondialdehyde, 8-hydroxydeoxyguanosine, heme oxygenase-1, and nitrotyrosine in abnormal neurons. The neurons with intracellular Aβ42 accumulation also displayed robust cleaved caspase-3 immunoreactivity. Very little Aβ42 immunoreactivity occurred in motor neurons of both control and ALS. These results suggest that aberrant accumulation of Aβ42 in ALS spinal cord motor neurons is associated with oxidative stress, and may play a role in the pathogenesis of neurodegeneration in ALS.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Amyotrophic lateral sclerosis; β-amyloid 42; Oxidative stress; Spinal cord; Neurodegeneration; Immunohistochemistry; Motor neurons

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, lethal neuromuscular disease characterized by a progressive loss of large motor neurons in the brain and spinal cord. Approximately 10% of ALS cases have missense mutations in the gene encoding for the Cu/Zn superoxide dismutase, while the majority of ALS patients are sporadic. The exact mechanism of motor neuron degeneration in ALS remains obscure, although several hypotheses have been proposed including apoptosis, (Martin, 1999; Przedborski, 2004), oxidative stress (Carri et al., 2003; Ferrante et al., 1997; Hall et al., 1997; Sasaki et al., 2000), and cytoskeletal abnormalities (Julien, 2001).

Amyloid precursor protein (APP), the transmembrane precursor of β-amyloid protein (Aβ), is increased in the spinal cord anterior horn neurons of ALS patients with short clinical courses, or with mild motor neuronal loss (Sasaki and Iwata, 1999). Interestingly, enzyme-linked immunosorbent assays revealed increases in Aβ in the skin of ALS patients compared to controls (Tamaoka et al., 2000). APP undergoes an alternative proteolysis mediated by caspases, a family of cysteine proteases that specifically cleave protein substrates after Asp residues (Salvesen and Dixit, 1997). Caspase-3 (CPP-32, Apoaptain, Yama, SCA-1) is the predominant caspase involved in APP cleavage (Gervais et al., 1999). APP is directly cleaved by caspase-3 during apoptosis, thereby enhancing Aβ formation (Gervais et al., 1999). Increasing evidence supports the role of caspase-3 activation in the neurodegenerative process in a transgenic mouse model of ALS (Li et al., 2000; Pasinelli et al., 2000; Vukosavic et al., 1997).

APP processing can be altered by oxidative stress, facilitating the cellular accumulation of Aβ (Mazur-Kolecka et al., in press; Misonou et al., 2000; Ohyagi et al., 2000). Conversely, Aβ can trigger oxidative stress (Butterfield and Bush, 2004; Hensley et al., 1994). Thus, Aβ accumulation is tightly linked to oxidative stress in neuronal and non-neuronal systems in studies related to Alzheimer’s disease, although a direct causal relationship remains to be elucidated. In ALS, there is evidence for increased oxidative damage in spinal cord motor neurons as detected biochemically and immunohistochemically (Carri et al., 2003; Ferrante et al., 1997; Hall et al., 1997; Sasaki et al., 2000). However, whether or not oxidative damage is associated with Aβ accumulation in these neurons has not yet been explored.

Owing to the APP accumulation, caspase-3 activation and oxidative damage in ALS, we sought to determine whether Aβ...
accumulates in the lumbar spinal cord of ALS patients. We report here that Aβ42 but not Aβ40 immunoreactivity was increased in the lumbar spinal cord motor neurons of ALS patients compared to controls. Such increases occurred in the neurons that were also strongly immunoreactive for markers of oxidative damage, as well as for caspase-3.

Materials and methods

Tissue preparation

ALS (n = 6) and control (n = 6) lumbar spinal cord tissues were obtained at autopsy in the Department of Pathology of Columbia Presbyterian Medical Center. All ALS patients fulfilled the clinical and pathological criteria of the disease, with one patient identified as having an I113T mutation to superoxide dismutase. All controls had non-neurological diseases including cardiac disease, peritoneal hemorrhage, diabetes, and leukemia. ALS patients had a mean age of 59 years (ranging from 27 to 69; 4 males and 2 females), while controls had a mean age of 65 years (ranging from 54 to 73; 3 males and 3 females).

The tissues were fixed with 10% neutral buffered formalin and processed for paraffin embedding. Serial coronal sections (7 μm thick) were cut and mounted onto gelatin-coated glass slides.

Immunohistochemistry

The tissue sections were deparaffinized, rehydrated, and treated with formic acid for 5 min. After rinsing in 0.1 M phosphate-buffered saline (PBS), the sections were processed for immunohistochemistry using a modified avidin–biotin–peroxidase phosphate-buffered saline (PBS), the sections were processed for paraffin embedding. Serial coronal sections (7 μm thick) were cut and mounted onto gelatin-coated glass slides.

Materials and methods

Tissue preparation

ALS (n = 6) and control (n = 6) lumbar spinal cord tissues were obtained at autopsy in the Department of Pathology of Columbia Presbyterian Medical Center. All ALS patients fulfilled the clinical and pathological criteria of the disease, with one patient identified as having an I113T mutation to superoxide dismutase. All controls had non-neurological diseases including cardiac disease, peritoneal hemorrhage, diabetes, and leukemia. ALS patients had a mean age of 59 years (ranging from 27 to 69; 4 males and 2 females), while controls had a mean age of 65 years (ranging from 54 to 73; 3 males and 3 females).

The tissues were fixed with 10% neutral buffered formalin and processed for paraffin embedding. Serial coronal sections (7 μm thick) were cut and mounted onto gelatin-coated glass slides.

Immunohistochemistry

The tissue sections were deparaffinized, rehydrated, and treated with formic acid for 5 min. After rinsing in 0.1 M phosphate-buffered saline (PBS), the sections were processed for immunohistochemistry using a modified avidin–biotin–peroxidase technique (Hsu et al., 1981). The sections were pretreated with 3% H2O2 in PBS for 30 min and then rinsed with PBS 3 times. The sections were incubated sequentially in (a) 1% bovine serum albumin (BSA)/0.2% Triton X-100 for 30 min, (b) primary antibody for 18 h, (c) appropriate biotinylated secondary antibody (1:200; Vector Laboratories, Burlingame, CA) for 1 h, and (d) avidin–biotin–peroxidase complex (1:200 in PBS; Vector) for 1 h. The immunoreaction was visualized using 3,3'-diaminobenzidine tetrahydrochloride dihydrate (DAB) as the chromogen. The primary and secondary antibodies were diluted in PBS/0.5% BSA. All incubations and rinses were performed at room temperature. Sections were dehydrated, cleared in xylene, and coverslipped. The two Aβ42 antibodies used were an affinity-purified rabbit antibody specific to the C-terminus of Aβ42 (5 μg/mL; Chemicon, Temecula, CA) and a mouse anti-Aβ42 clone 12F4 (1:1000; Upstate, Lake Placid, NY). The Aβ42 antibodies have been characterized previously, and do not cross-react with APP or Aβ40 (Dominguez et al., 2001; Kamal et al., 2001). A rabbit antibody against the C-terminus of Aβ40 (20 μg/mL; Chemicon) was also employed. Aβ refers to Aβ peptides in general unless stated otherwise.

A mouse anti-SMI-32 antibody (1:3000; Sternberger Monoclonals, Lutherville, MD) was used as a marker of motor neurons (Tsang et al., 2000). For detection of oxidative damage, the antibodies used were rabbit anti-malondialdehyde (1:1000) (Hall et al., 1997), goat anti-8-hydroxydeoxyguanosine (1:200; 8-OHdG; Chemicon), rabbit anti-nitrotrosyline (1:50; Upstate, Charlotteville, VA), and rabbit anti-heme oxygenase-1 (1:1000; Stressgen Biotechnologies, British Columbia, Canada). A rabbit polyclonal antibody against cleaved caspase-3 (1:200; BD Biosciences, San Diego, CA) was used to detect caspase-3 activation.

Competition experiments confirmed the immunological specificity of Aβ42 staining. The sections were incubated in antibody that was preadsorbed for 4 h at 37°C with 1 μM Aβ (aa. 1–42) peptide (Oncogene Research products, La Jolla, CA). As a methodological specificity control, the sections were incubated in PBS/BSA in place of the Aβ42 antibody. As a positive control, Alzheimer brain sections known to exhibit Aβ42-immunoreactive senile plaques were processed in parallel with the ALS sections.

Coexistence of Aβ42 with other markers was performed by immunostaining adjacent sections. Since motor neurons are large (up to 100 μm in diameter), overlap of different immunoreactivities in the same motor neuron can be determined in adjacent 7-μm-thick sections. Careful microscopic examination of anatomical landmarks such as blood vessels common to two adjacent sections was used as a reference guide to verify coexistence. We did not employ double-label immunofluorescence since this technique is often complicated by the autofluorescence of neural tissues due mostly to the fluorescent pigment lipofuscin. This pigment accumulates in the cytoplasm of neurons with age thereby carrying the risk of false results. Furthermore, double-label immunofluorescence requires two different host species for the antibodies to be colocalized. In this study, two of the Aβ antibodies, as well as most of antibodies for detection of oxidative stress used were all raised in rabbit.

Thioflavine S histochemistry

Sections adjacent to those stained with Aβ42 were processed for thioflavine S histochemistry (Guntern et al., 1992). Briefly, the sections were incubated sequentially in 0.25% KMnO4 for 20 min, 1% K2SO4 for 2 min, and 0.125% thioflavine S in 50% ethanol for 5 min. Alzheimer brain sections known to contain senile plaques were used as a positive control.

Detection of apoptosis

In situ demonstration of apoptotic cells was performed using the ApopTag Peroxidase Apoptosis Detection Kit (Chemicon). In this technique, apoptotic cells are labeled by modifying genomic DNA utilizing terminal deoxynucleotidyl transferase. Sections from a normal female rat mammary gland known to show apoptotic cells served as a positive control. After staining for apoptosis, the sections were immunostained for Aβ42 using Vector VIP substrate (Vector) to determine whether apoptotic cells exhibit Aβ42 accumulation.

Semi quantitative histological analysis

The numbers of Aβ42- or SMI-32-immunoreactive neurons in the anterior horn per section were determined. For each patient, three serial transverse sections through the lumbar spinal cord (70 μm apart) were analyzed. Only intensely stained neurons, regardless of size were counted within the anterior horn below a horizontal line drawn through the central canal encompassing layers 7–9. The numbers of Aβ42-positive neurons per section, as well as the proportion of SMI-32-immunoreactive motor neurons with Aβ42 accumulation per
section were estimated. An unpaired *t* test was used for statistical analysis.

**Results**

All ALS tissues showed the hallmark neuropathological features of the disease. A dramatic loss of SMI-32-labeled motor neurons was evident in the anterior horn of all ALS tissues compared to controls (46% of control). Some of the residual motor neurons in ALS spinal cord exhibited eosinophilic Lewy body-like inclusions (data not shown). These observations are consistent with a previous study (He and Hays, 2004), which used tissues from the same patients reported here.

Immunohistochemistry using antibodies specific to the C-terminus of Aβ42 revealed very little or no Aβ42 immunoreactivity in motor neurons in the control tissues (Fig. 1). In contrast, an intracellular accumulation of Aβ42 in the anterior horn motor neurons was evident in all ALS spinal cord tissues, including the case with an I113T mutation to superoxide dismutase. Aβ42 accumulation was found in neurons labeled with the motor neuron marker SMI-32 (data not shown). A robust immunostaining occurred mainly in the perikarya (Fig. 2). In apparently normal motor neurons, focal accumulations of Aβ42 immunoreactivity were evident in neuronal processes (Fig. 2A) or in the perikarya (Fig. 2B). The most intensely stained motor neurons showed atrophy, chromatolysis, and lack of intact nuclear morphology (Figs. 2C, D). The posterior horn of the lumbar spinal cord also contained a few darkly stained

![Fig. 1. Low (top) and high (bottom) magnification photomicrographs showing Aβ42 immunoreactivity in the anterior horn of the lumbar spinal cord sections from control and ALS patients. Very little or no immunoreactivity was found in motor neurons of control sections. In contrast, Aβ42 immunoreactivity was markedly elevated in many of the remaining neurons with abnormal morphology in ALS.](image1)

![Fig. 2. High magnification photomicrographs of the ventral horn of ALS lumbar spinal cord showing Aβ42 accumulation in motor neurons with varying degree of morphological abnormalities (A–D). An apparently normal neuron shows an accumulation in a neuronal process (A) while another neuron with increasing focal immunoreactivity has abnormal nucleus (B). Atrophied neurons (C, D) show the most intense immunoreactivity.](image2)
neuronal perikarya and processes. The cervical and thoracic spinal cord sections, which were available for some patients, also showed similar Aβ42 accumulations as the lumbar spinal cord sections. The white matter of either ALS or control spinal cord tissues did not show any dramatic increase in Aβ42 immunostaining. No extracellular Aβ42 deposits reminiscent of Alzheimer senile plaques were found in either ALS or control tissues. The two Aβ42 antibodies (rabbit antibody specific to the C-terminus of Aβ42 and a mouse anti-Aβ42 clone 12F4) revealed identical patterns of immunoreactivity.

The majority of the Aβ42-immunoreactive motor neurons in ALS lacked thioflavine S staining (Figs. 3A, A'). However, a few Aβ42-positive neurons displayed thioflavine S labeling (Figs. 3B, B') indicative of a β-pleated sheet conformation of the intraneuronal Aβ42. Alzheimer brain sections stained simultaneously with the ALS spinal cord sections revealed typical Aβ42-labeled senile plaques (Fig. 3, inset).

Competition experiments supported the specificity of the Aβ42 immunoreactivity. Incubation of the ALS tissue sections in antibody preadsorbed with synthetic Aβ1-42 peptide, or in PBS/BSA abolished the staining (Fig. 4). No apparent Aβ40 accumulation was detected in either control or ALS (data not shown).

A semiquantitative histological analysis showed a significant increase in the number of neurons with intense Aβ42 staining in ALS anterior horn compared to controls ($P < 0.001$; Fig. 5). In controls, only 7% of motor neurons in the anterior horn displayed intense immunoreactivity, while in ALS, 68% of residual motor neurons showed strong labeling.

Given that there is evidence for increased oxidative damage in both sporadic and familial ALS (Carri et al., 2003; Ferrante et al., 1997; Hall et al., 1997; Sasaki et al., 2000), and that Aβ is associated with oxidative damage (Butterfield and Bush, 2004; Hensley et al., 1994), we examined the spatial relationship between accumulations of Aβ42 and markers of oxidative stress in ALS

Fig. 3. Adjacent sections through the ALS lumbar spinal cord stained with Aβ42 (A, B) or thioflavine S (A', B') showing Aβ42-positive neurons lacking thioflavine S staining (A, A'), and cells with both Aβ42 and thioflavine S labeling (B, B'). Neurons with the same number indicate the same cell. Inset shows thioflavine S labeled Alzheimer senile plaques as positive control.

Fig. 4. Adjacent sections stained with Aβ42 (A) or with Aβ42 that was preincubated with synthetic Aβ1-42 peptide (B) showing abolition of staining by synthetic Aβ1-42 peptide competition. Neurons with the same number indicate the same cell. Inset shows an Aβ42-immunolabeled Alzheimer senile plaque as a positive control.
Fig. 5. Semiquantitative analysis of Aβ42 accumulation in control and ALS lumbar spinal cord. The number of Aβ42-stained motor neurons/section (left) and the proportion of SMI-32 positive motor neurons with Aβ42 accumulation (right) are shown. The number of intensely stained neurons within the anterior horn encompassing layers 7–9 was determined. Values represent the means ± SEM (n = 6 per group). ***P < 0.001.

Fig. 6. Coexistence of Aβ42 (A–D) with markers of oxidative damage (A'–D') in motor neurons of ALS. Aβ42-stained sections (left panel) are adjacent to sections stained with respective oxidative markers (right panel) to show colocalization in the same cell. Cells labeled with the same number indicate the same neuron. Virtually all Aβ42-labeled neurons also showed increases in immunoreactivity for malondialdehyde (A'), 8-hydroxydeoxyguanosine (B'), heme oxygenase-1 (C'), and nitrotyrosine (D'). Although cytoplasmic 8-hydroxydeoxyguanosine staining is shown, other neurons with both nuclear and cytoplasmic labeling occurred (not shown), consistent with published results (Ferrante et al., 1997).
spinal cord. The markers used were: (1) malondialdehyde, a marker of lipid peroxidation; (2) 8-hydroxydeoxyguanosine, a marker of oxidative damage to DNA; (3) heme oxygenase-1, an enzyme that cleaves heme to produce CO and biliverdin, and is known to be induced by oxidative stress; and (4) nitrotyrosine, a marker of peroxynitrite-mediated protein nitration. Peroxynitrite is a reaction product of nitric oxide and superoxide and a potent oxidant. Careful examination of adjacent serial sections immunostained with different antibodies revealed that Aβ42 coexisted with key markers of oxidative damage. Virtually all motor neurons with Aβ42 accumulation exhibited strong immunoreactivity for malondialdehyde, 8-hydroxydeoxyguanosine, heme oxygenase-1, and nitrotyrosine (Fig. 6). The staining pattern for markers of oxidative damage was identical to the previous report in both sporadic and familial ALS (Ferrante et al., 1997). Some 8-hydroxydeoxyguanosine-positive neurons displayed both nuclear and cytoplasmic staining (not shown). Most of the double-labeled motor neurons showed abnormal morphology.

The neurons with Aβ42 accumulation also displayed robust caspase-3 immunoreactivity (Figs. 7A, A'). Apoptotic cells were extremely rare in ALS tissues (Fig. 7B). These cells were atrophied, and showed intense Aβ42 immunoreactivity.

**Discussion**

This study is the first demonstration that Aβ42 accumulates in the lumbar anterior horn motor neurons of ALS patients. These observations extend further the previous report of increased APP immunoreactivity in ALS motor neurons (Sasaki and Iwata, 1999). APP accumulation is thought to result from impaired axoplasmic transport, or from enhanced APP synthesis in response to neuronal damage (Sasaki and Iwata, 1999). Consonant with this hypothesis is the ultrastructural demonstration of a marked accumulation of membrane-bound cytoplasmic organelles in the proximal axons, particularly in the axon hillock of ALS motor neurons (Sasaki and Iwata, 1996).

While an elevation of APP may be a component of an early nervous tissue response to injury, Aβ42 accumulation may reflect a late event with more deleterious consequences. The selective neurotoxicity of intracellular Aβ is well documented. Intracellular microinjections of Aβ1-42 peptide or a cDNA-expressing cytosolic Aβ1-42 rapidly induce cell death of primary cultured human neurons, while Aβ1-40, Aβ40-1, or Aβ42-1 peptides are not toxic (Zhang et al., 2002). In contrast, primary astrocytes are resistant to intracellular microinjections of Aβ. The intracellular Aβ1-42-induced neuronal death is mediated by the proapoptotic Bax protein, suggesting the involvement of an apoptotic pathway (Zhang et al., 2002). Morphological studies suggest that the intraneuronal Aβ42 accumulation in Alzheimer’s disease is an initial cause of neuronal dysfunction and death. For example, intracellular accumulation of Aβ42 appears to precede other neuropathological lesions (D’Andrea et al., 2001; Gouras et al., 2000). Moreover, intraneuronal Aβ42 accumulation is associated with synaptic abnormalities before the appearance of senile plaques (Takahashi et al., 2002). A recent study in a novel Alzheimer transgenic model reported an extensive neuronal loss (>50%) in the CA1/2 hippocampal pyramidal cell layer that correlates with strong accumulation of intraneuronal Abeta and thioflavine S-positive intracellular material but not with extracellular Aβ deposits (Casas et al., 2004). Thus, accumulating evidence points to the crucial role of intraneuronal Aβ as an initial step of a fatal cascade in Alzheimer’s disease (Wirths et al., 2004 for review). It is possible that the neuropathological consequences of intraneuronal Aβ42 accumulation in Alzheimer’s disease also occur in ALS except for the extracellular deposition of Aβ42.

A large body of evidence, mostly from studies related to Alzheimer’s disease, underscores the link between Aβ accumu-
lation and oxidative stress. Aβ is a potentially important source of oxidative damage. Aβ causes lipid peroxidation, protein oxidation, free radical formation, and cell death in neurons (Butterfield and Bush, 2004; Hensley et al., 1994). In Alzheimer’s disease, the interaction between Aβ and Aβ-binding alcohol dehydrogenase promotes leakage of reactive oxygen species, mitochondrial dysfunction, and cell death (Lustbader et al., 2004).

In vitro studies show that Aβ can increase intracellular H2O2 and lipid peroxides, and induce the activity of NfL (a transcription factor that is regulated by oxidative stress (Behl et al., 1994a)). Conversely, oxidative stress may alter APP processing and facilitate intracellular accumulation of Aβ in smooth muscle cells (Mazur-Kolecka et al., in press; Misonou et al., 2000; Ohyagi et al., 2000). Increases in APP can occur in response to a variety of factors including oxidative stress (Wilcockson et al., 2002). The redox interactions between APP, Aβ, and redox bioactive metals appear to be a critical mechanism by which Aβ amyloidosis and oxidative stress promote each other (Huang et al., 2004). Previous studies support a key role of oxidative damage in the pathogenesis of both sporadic and familial ALS (Carri et al., 2003; Ferrante et al., 1997; Hall et al., 1997; Sasaki et al., 2000). It is conceivable that the interrelated events involving Aβ and oxidative stress in Alzheimer’s disease also occur in ALS. The present results provide a neuroanatomical basis for the possible link between intraneuronal Aβ42 accumulation and oxidative damage in ALS.

The current observations do not preclude the possibility that Aβ42 elevation is a protective response to oxidative stress in the residual motor neurons in ALS. Contrary to the popular view that Aβ is neurotoxic, a protective function for Aβ has been proposed recently (Lee et al., 2004). While in vitro studies demonstrate the neurotoxicity of intracellular or extracellular Aβ, studies in Alzheimer’s disease patients suggest that increases in Aβ may be a protective adaptation to the disease (Nunomura et al., 2001). If Aβ can function as a potent antioxidant like superoxide dismutase as suggested previously (Cuajungco et al., 2000), the elevated Aβ42 in the remaining motor neurons in ALS may account for the survival of these neurons in spite of the concomitant oxidative stress.

The coexistence of Aβ with cleaved caspase-3 in ALS motor neurons is consistent with the production of Aβ resulting from caspase-3 cleavage of APP. This observation is similar to the reported overlap of caspase-3 and Aβ in many Alzheimer senile plaques (Su et al., 2001). APP can be directly cleaved by caspase-3 during apoptosis resulting in an elevated Aβ formation (Gervais et al., 1999). Caspase-3 is one of the major proteases responsible for the degradation of key cell constituents in many types of neuronal apoptosis (Salvesen and Dixit, 1997). The cleavage of APP by caspase-3 appears to occur not only in disease, but also during normal development. The lumbar spinal cord motor neurons of the chick embryo undergo a period of naturally occurring programmed cell death requiring activation of caspases. In vitro studies using chick lumbar spinal cord motor neurons revealed increases in protein levels of intracellular Aβ and APP, as well as activation of caspase-3 in dying motor neurons but not in healthy cells (Barnes et al., 1998). APP may serve as a substrate for caspase-3, thereby generating a potentially neurotoxic intracellular Aβ during normal development (Barnes et al., 1998). It is tempting to speculate that events of developmental apoptosis in the lumbar spinal cord may be reactivated during early stages of neurodegeneration in disease states such as ALS.

Apoptotic cells were rarely found in ALS spinal cord. One possible explanation is that apoptosis probably occurred at an earlier stage of the disease. Furthermore, caspase-3 activation does not necessarily lead to apoptotic cell death. Co-expression of caspase-3 inhibitory molecules such as inhibitors of apoptosis proteins (IAPs) in these motor neurons may also play a role. For example, axotomy leads to apoptosis of motor neurons in neonates but not in adults. Perrelet et al. (2004) recently showed that the resistance of adult rat lumbar spinal cord motor neurons to apoptosis is related to the ratio between X-linked inhibitor of apoptosis proteins (XIAPs) and its inhibitor, XIAP-associated factor 1. Thus, these inhibitory proteins could prevent apoptotic neuron loss but still lead to neuronal dysfunction. This idea may explain the presence of many residual neurons with Aβ42 and caspase-3 accumulations and abnormal morphology, but lacking signs of apoptosis. Another possible explanation is that Aβ predominantly induces necrosis rather than apoptosis (Behl et al., 1994b). A previous report did not find evidence for apoptosis as a major mechanism of motor neuronal cell death in sporadic ALS (Embacher et al., 2001).

In conclusion, intracellular Aβ42 accumulation is not unique to AD and Down’s syndrome. In ALS, aberrant intraneuronal Aβ accumulation without extracellular deposition occurred selectively in the spinal cord motor neurons with increased markers of oxidative damage. The elevation of Aβ42 within motor neurons may reflect either a neuroprotective response to oxidative stress, or a neurotoxic factor that contributes to neuronal dysfunction and death.

Acknowledgments

We are grateful to Dr. Arthur P. Hays for providing the postmortem spinal cord tissues. This work was supported by a grant from the ALS Association (MFB) and the Weill Medical College of Cornell University’s Center for Clinical Care and Aging Research (NYC).

References


the redox-silencing and entombment of Aβ by zinc. J. Biol. Chem. 275, 19439–19442.


