ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 26 (2007) 165–173

Differential regulation of inhibitors of apoptosis proteins in Alzheimer's disease brains

Lori-Ann Christie,[a,*] Joseph H. Su,[a] Christina H. Tu,[a] Malcolm C. Dick,[a] Jun Zhou,[b] and Carl W. Cotman[a]

[a]*Institute for Brain Aging and Dementia, 1113 Gillespie Neuroscience Research Facility, University of California, Irvine, CA 92697, USA*
[b]*Department of Medicine, University of California, Irvine, CA, USA*

Received 9 October 2006; revised 7 December 2006; accepted 9 December 2006
Available online 3 January 2007

Neuronal degeneration linked to apoptosis can be inhibited by a family of proteins known as inhibitors of apoptosis proteins (IAPs). We examined three members of the IAP family that are implicated in the regulation of neuronal death. We assessed NAIP, XIAP, and cIAP-2 protein levels in the entorhinal cortex of non-demented, cognitively impaired and Alzheimer's disease cases. Levels of paired helical filament-1 (PHF-1), a marker of neurofibrillary tangles, were assessed to determine their relationship to IAP levels. NAIP was decreased in AD cases compared to mildly impaired and unimpaired cases, and this decrease was associated with increased PHF-1 levels. Low NAIP levels were associated with higher Braak and Braak tangle stage and cognitive dysfunction. XIAP levels were higher in AD cases and cIAP-2 levels did not vary with clinical status. Our data suggest that decreased NAIP may place neurons at risk for the development of tangles and apoptosis.

Keywords: Inhibitor of apoptosis; Neurodegeneration; Alzheimer's disease; NAIP; XIAP; cIAP-2; Caspase activation; Tau; Neurofibrillary tangles; MMSE

Introduction

Alzheimer's disease (AD) is characterized by a progressive accumulation of extracellular β-amyloid (Aβ) in the form of senile plaques and intracellular tau in the form of neuropil threads and neurofibrillary tangles (NFTs). In addition, neuronal and synaptic loss occur during the progression of AD (for reviews, see Cotman, 1998; Mattson, 2004), and this degeneration is linked to the presence of Aβ plaques (Allen et al., 2001; Song et al., 2006; Takuma et al., 2004) and NFTs (Broe et al., 2001, and reviewed in Dickson, 2004; Gomez-Isla et al., 1997; Rohn et al., 2002b).

Recent evidence suggests that neuronal degeneration and loss in AD may result in part from the activation of apoptotic-related pathways or apoptosis itself. In AD and other neurodegenerative disorders, neurons induce a series of proteases including caspases, a class of cysteine proteases, which are key molecules in the execution of apoptosis (reviewed in Prunell and Troy, 2004). Caspases act to either initiate (initiator caspases) or advance (executioner caspases) apoptosis and result in the characteristic morphological changes of cell death such as shrinkage, nuclear fragmentation, and membrane blebbing. Activated initiator and effector caspases are elevated in the brains of AD patients (Guo et al., 2004; Rohn et al., 2001, 2002a; Su et al., 2001, 2002; Zhao et al., 2003) and exhibit a high degree of co-localization with NFTs, granulovacuolar degeneration, and dystrophic neurites (Guo et al., 2004; Newman et al., 2005; Rissman et al., 2004; Rohn et al., 2001). An accumulation of caspase cleavage products including cleaved cytoskeletal proteins (e.g., actin, spectrin, tau), amyloid precursor protein (APP), glial fibrillary acidic protein (GFAP), and poly (ADP-ribose) polymerase (PARP) (for reviews, see Marks and Berg, 1999; Rohn et al., 2002b) is also found in the AD brain.

Caspase activation may provide a mechanistic link between Aβ and NFT pathologies in AD (Cotman et al., 2005). In the AD brain, Aβ plaques co-localize with cells showing classical apoptotic morphology and caspase activation (reviewed in Cotman et al., 2005). This is consistent with in vitro studies showing that aggregated Aβ triggers caspase-dependent apoptosis (Lesne et al., 2005; Loo et al., 1993). Several recent studies report that Aβ-mediated caspase activation may contribute to the development of tangle pathology and neuronal compromise. Caspase-cleaved tau co-localizes with intracellular Aβ, activated caspase-3, and paired helical filament-1 (PHF-1), a marker of mature tangle pathology, and is detected in patients with mild cognitive impairment but not within control brains, suggesting that caspase cleavage of tau may occur early in the progression of AD (Rissman et al., 2004). Consistent with this hypothesis, Aβ exposure leads to the formation of paired helical tau-like filaments in vitro (Ferrari et al., 2003; Gamblin et al., 2003), which occurs via Aβ's activation of caspases (Ivins et al., 1999; Loo et al., 1993) that in turn cleave

* Corresponding author. Fax: +1 949 824 2071.
E-mail address: lori@uci.edu (L.-A. Christie).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2006.12.017

tau (Gamblin et al., 2003; Guo et al., 2004; Rissman et al., 2004). Further, caspase cleavage of tau leads to a conformational change that is recognized by markers of early tangle epitopes, like MC1 (Rissman et al., 2004). The resulting cleavage product, ΔTau or truncated tau, accelerates the aggregation of full-length tau filaments in vitro, suggesting that it may initiate and/or catalyze NFT formation (Gamblin et al., 2003; Rissman et al., 2004; Rohn et al., 2002a).

Taken together, these findings suggest that activation of apoptotic-related caspase pathways and/or apoptosis itself contribute to the progression of neuropathological changes and cell death in AD. If fully activated, indications of apoptosis in the AD brain should be obvious yet there are contrasting and conflicting reports on the existence of DNA fragmentation, nuclear apoptotic bodies, and other classical apoptotic morphology in the AD brain (for example, Woodhouse et al., 2006). Furthermore, caspase activation can occur within a cell without resulting in classical apoptosis, and prolonged caspase activation sometimes occurs without cell death (reviewed in Cotman et al., 2005). Therefore, there must be counteractive anti-apoptotic mechanisms acting to limit cell death whose downregulation could leave neurons susceptible to apoptotic death and upregulation could inhibit caspase activation and arrest apoptosis.

A family of structurally related endogenous proteins termed inhibitors of apoptosis (IAPs) are key in acting to restrain the activity of both initiator and effector caspases (reviewed in Liston et al., 2003). Eight mammalian IAPs have been identified to date, and include: XIAP, cIAP-1, cIAP-2, NAIP, Livin, TsIAP, Survivin, and Bruce (Prunell and Troy, 2004). Of these, NAIP, XIAP, and cIAP-2 are implicated in the regulation of neuronal death in several disease models (reviewed in Prunell and Troy, 2004). NAIP directly inhibits caspase-3, -7, and -9 (Maier et al., 2002; Davoodi et al., 2004) and XIAP directly inhibits caspase-3 and caspase-7 (Deveraux et al., 1997). cIAP-2 may inhibit caspase activation and proteolytic processing of pro-caspase-3 (Roy et al., 1997, but see Eckelman and Salvesen, 2006). Elevated neuronal expression of NAIP reduces ischemic damage in the rat hippocampus (Xu et al., 1997), and is protective in a 6-hydroxydopamine model of Parkinson's disease (Crocker et al., 2001). Consistent with these findings, in vitro studies report that upregulation of NAIP and decreased interaction of NAIP with its endogenous inhibitor, Smac, by the presence of neurotrophin-3, protect neurons from Aβ induced death (Lesne et al., 2005). Transgenic XIAP-overexpressing mice show reduced caspase-3 activation, fewer cells with DNA fragmentation, reduction in infarct size, and better neurological outcome after transient cerebral ischemia (Trapp et al., 2003). Following axotomy in neonates, overexpression of cIAP-2 is protective (Perrelet et al., 2000), and increases in cIAP-2 are detected following traumatic brain injury (Keane et al., 2001).

Few studies have examined how IAPs are regulated in human neurodegenerative disorders or if their expression in the AD brain is related to the presence of the disease's hallmark pathological features or cognitive decline (Seidl et al., 1999). In the present investigation, we sought to determine the levels of NAIP, XIAP, and cIAP-2 in a region of the brain vulnerable to AD pathology, the medial temporal lobe, of nondemented aged individuals compared to patients with dementia of varying severity. We also assessed levels of a marker of mature hyperphosphorylated tau (PHF-1) in order to determine the association between NAIP, XIAP, and cIAP-2 and tangle pathology. Finally, we investigated the ability of IAP level to predict global cognition and Braak and Braak tangle and plaque staging. We report that (1) NAIP levels are decreased in AD patients compared to mildly impaired and control cases, (2) NAIP levels are inversely related to PHF-1 levels, and (3) low NAIP and high XIAP immunoreactivity are associated with impaired cognitive function and increased tangle pathology. Our data suggest that the expression of at least one apoptotic inhibitory protein, NAIP, may be protective against the development of tangle pathology and cognitive decline in AD.

Materials and methods

Subjects

Participants were recruited to enter the Alzheimer's Disease Research Center (ADRC) at the University of California, Irvine and completed a standard neuropsychological test battery. Measures of global cognitive status from the Mini-Mental State Examination (MMSE; Folstein et al., 1975) and recent verbal memory from the CERAD Word List (CWLT; Morris et al., 1989) were used to examine the relationship between IAP immunoreactivity and cognitive ability. Scores from the final tests prior to death were used in statistical analyses.

Brain tissue from participants was collected at autopsy from the Institute for Brain Aging and Dementia Tissue Repository for Western blot and immunohistochemical analysis. Entorhinal and hippocampal tissue from 15 cases with short postmortem intervals was examined. Table 1 summarizes demographic information for these cases, which were categorized into three groups based on their clinical diagnostic status at time of death as (1) non-demented individuals (NL, $N=4$), (2) age-matched individuals who were diagnosed as cognitively impaired but not demented (CIND), mild cognitive impairment (MCI) or mild AD (CIND/MCI/mild AD, $N=3$), and (3) age-matched AD cases (AD, $N=8$) (Morris et al., 1989). AD cases were further selected to include a range in severity of cognitive decline and Braak and Braak (1991) staging for tangles and plaques.

Antibodies

Details regarding the antibodies used in the present study are listed in Table 2. Briefly, rabbit anti-human NAIP antibody raised against amino acids 473–490 of human NAIP was purchased from R&D Systems (Minneapolis, MN). Monoclonal anti-XIAP generated from human IAP-like protein was purchased from BD Biosciences (San Diego, CA) and rabbit polyclonal anti-c-IAP2 raised against amino acids 94–178 of human cIAP-2 was purchased from Santa Cruz Biotechology (Santa Cruz, CA). A well-characterized monoclonal antibody, anti-PHF-1 (a generous gift from Peter Davies, Yeshiva University, Bronx, NY), was used to detect the presence of hyperphosphorylated tau. Monoclonal anti-β-Actin (mouse IgG2a isotype, Sigma-Aldrich, Saint Louis, MO) was used as a protein loading control for Western blots.

Western blot analysis

Samples of entorhinal cortex (~100 mg) prepared from fresh frozen brain tissue were homogenized in 1:10 volume extraction buffer (10 mM Tris, 10 mM EDTA, 150 mM NaCl, 0.1% mSDS, 1% Triton, 0.5% NP40, 0.2 mM Na_3VO_4, 0.2 mM PMSF, 50 mM NaF, and a Protease Inhibitor cocktail from Sigma P-8340). The