Results

In cases of FAD, 8OHG immunoreactivity was prominent in the neuronal cytoplasm in the superior/middle frontal gyrus (Figs. 1A–C). Neuronal 8OHG immunoreaction was widely distributed throughout the cortical layers (Fig. 1A), while in controls, staining was very low (Fig. 1E). Pyramidal neurons of larger size in the superior/middle frontal gyrus tended to show higher immunointensity of 8OHG in each case of FAD, although individual variation of immunointensity among FAD cases was observed. Moderately positive immunoreaction of neuronal 8OHG was observed in a presymptomatic case carrying a PS-1 gene mutation (Fig. 1D).

To investigate whether the immunoreaction with the 1F7 antibody was derived from oxidized RNA or oxidized DNA or both, we performed nuclease treatment before immunostaining with 1F7. The immunoreaction in the sections of FAD was diminished greatly by DNase-free RNase pretreatment (Figs. 2A and B), but only slightly by the RNase-free DNase pretreatment (Figs. 2C and D), as we demonstrated in sections of sporadic AD and DLB (Nunomura et al., 1999, 2002). Therefore, not only in sporadic AD and DLB but also in FAD is RNA a major site of nucleic acid oxidation.

Relative scale measurements of 8OHG immunoreactivity using a computer-assisted image analysis system demonstrated that the increase was significant in FAD when compared with a control group (Fig. 3A). Because 8OHG immunoreactivities tend to show an age-dependent increase in non-demented individuals (Nunomura et al., 1999), the significant increase in 8OHG immunoreactivity in FAD cases (mean age, 59 years) compared with controls (mean age, 66 years) cannot be explained by the difference in age of subjects between the groups. Neither, these results cannot be explained by neuronal shrinkage, because the average cell profile area remained unchanged between FAD cases (161 μm^2) and controls (148 μm^2). Similar levels of relative 8OHG were demonstrated between the PS-1 and the AβPP FAD (the average of the relative 8OHG = 10.2 (arbitrary units) and 9.6, respectively). Interestingly, a presymptomatic case carrying a PS-1 gene mutation showed a considerable level of relative 8OHG (Fig. 3A). Levels of the relative 8OHG immunoreactivity were not related to postmortem intervals among FAD cases ($P > 0.9$ by linear regression analysis) as well as among controls ($P > 0.9$). Furthermore, an agonal state before death also failed to alter the relative 8OHG immunoreactivity. We found similar average values for the relative 8OHG immunoreactivity in controls who died from internal malignancy ($n = 9$, relative 8OHG = 4.7), leukemia ($n = 2$, relative 8OHG = 7.9), heart failure ($n = 3$, relative 8OHG = 5.9), and unknown ($n = 1$, relative 8OHG = 4.9), as we showed in other series of controls (Nunomura et al., 1999).

When we investigated relationship between percentage area of Aβ 42 or Aβ 40 burden and relative 8OHG levels in FAD, we found a significant inverse correlation between percentage area of Aβ42 burden and relative 8OHG levels, but no significant correlation between percentage area of Aβ40 burden and relative 8OHG levels (Figs. 3B and C), as we observed in Down syndrome (Nunomura et al., 2000). In controls, only seven cases showed Aβ42 burden and only three cases showed Aβ40 burden. No apparent relationship between percentage area of Aβ42 or Aβ40 and the levels of neuronal 8OHG was detected in control subjects (data not shown).

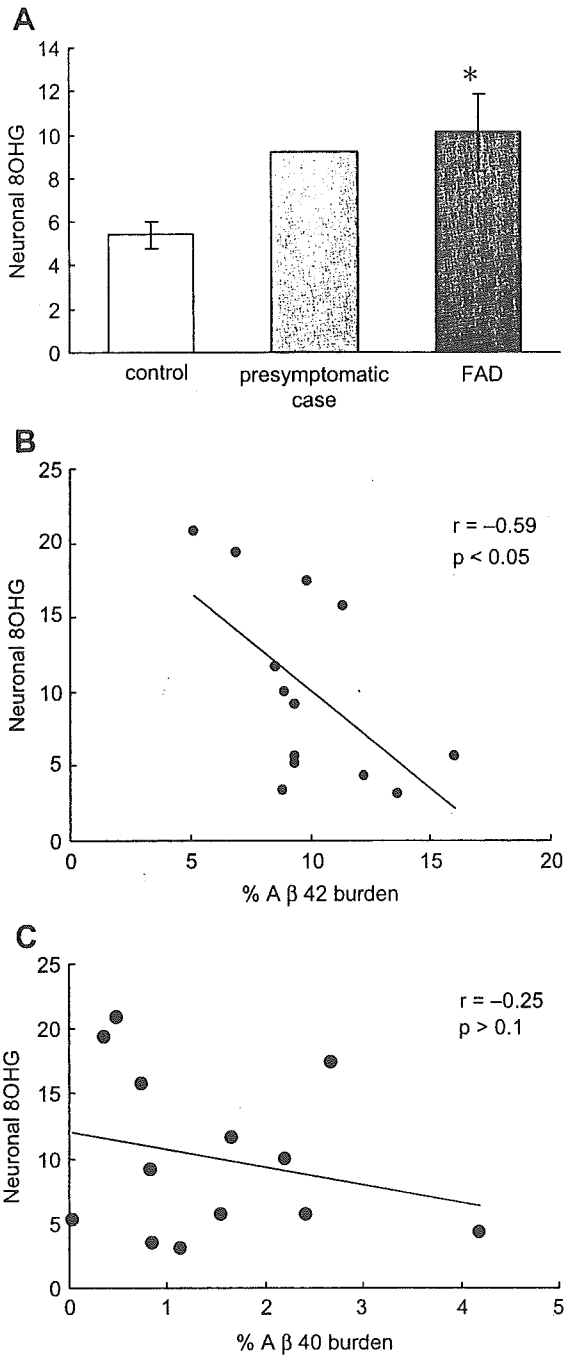

Fig. 3. Increased levels of neuronal 8OHG immunointensity and an inverse relationship between percentage area of Aβ42 burden and neuronal 8OHG immunointensity in the neocortex of FAD. Relative scale measurements reveal that the levels of 8OHG immunoreactivity are significantly increased in FAD cases ($n = 13$, average age 59 years) compared with controls ($n = 15$, average age 66 years) (*$P < 0.05$ by Mann–Whitney U test). A presymptomatic case with a PS-1 gene mutation (51 years old) shows a similar level of neuronal 8OHG immunointensity to the average of the FAD group. Values shown are the averages with SE (A). In FAD cases, there is a significant inverse correlation of percentage area of Aβ42 burden (B), but not percentage area of Aβ40 burden (C), with the levels of 8OHG immunoreactivity by linear regression analysis.

Discussion

Recently, an increasing number of in vitro and in vivo studies have suggested that oxidative stress is involved in the pathogenesis of AD and has an involvement in FAD with AβPP, PS-1, or PS-2 gene mutation. Indeed, increased oxidative stress, elevated vulnerability to oxidative stress-induced cell death, and reduced antioxidant defenses have been demonstrated in (i) cell lines expressing mutant human AβPP, PS-1, or PS-2 (Eckert et al., 2001; Guo et al., 1997; Hashimoto et al., 2002; Marques et al., 2003), (ii) transgenic mice expressing mutant human AβPP or PS-1 as well as knock in mice expressing mutant human PS-1 (Guo et al., 1999; LaFontaine et al., 2002; Leutner et al., 2000; Matsuoka et al., 2001; Praticò et al., 2001; Smith et al., 1998; Takahashi et al., 2000), (iii) fibroblasts and lymphoblasts from FAD patients with AβPP or PS-1 gene mutation (Cecchi et al., 2002), and (iv) cerebral cortex of autopsied brain samples from patients with AβPP gene mutation (Bogdanovic et al., 2001). The findings presented here represent the first evidence of increased oxidative damage to RNA in the cerebral cortex neurons of FAD, a finding previously made for the cerebral cortex neurons in sporadic AD and DLB (Nunomura et al., 1999, 2001, 2002) as well as for the substantia nigra neurons of Parkinson's disease (Zhang et al., 1999). Therefore, RNA oxidation is a common phenomenon in vulnerable neurons of sporadic and familial types of AD as well as some disorders classified in the category of synucleinopathy. A recent biochemical study has revealed that some mRNA species are selectively oxidized in the cerebral cortex of AD, and as a biological consequence, abnormal processing of proteins occurred to the oxidized mRNAs when they are expressed in cell lines (Shan et al., 2003). These findings suggest that RNA oxidation itself is directly associated with neuronal deterioration instead of harmless epiphenomenon during the process of neurodegeneration.

Interestingly, a presymptomatic case carrying a PS-1 mutation, whose autopsied cerebral cortex exhibited a substantial amount of Aβ42 deposition but no Aβ40 deposition (Lippa et al., 1998), showed a considerable level of neuronal RNA oxidation. This observation clearly suggests an early involvement of oxidative stress in the pathological cascade of FAD, which corresponds with our previous finding in Down syndrome cases where neuronal RNA oxidation precedes Aβ deposition (Nunomura et al., 2000). The early involvement of oxidative stress in FAD is also supported by experiments examining transgenic mice expressing human AβPP with FAD mutation and showing increased lipid peroxidation before Aβ plaque formation (Praticò et al., 2001).

Furthermore, we found a significant inverse correlation of Aβ42 burden, but not Aβ40 burden, with neuronal RNA oxidation. Again, this observation is completely coincident with the results of our previous study on Down syndrome (Nunomura et al., 2000). Because Aβ 42 deposition is an upstream event in the pathological cascade of FAD (Iwatsubo et al., 1994; Kalaria et al., 1996; Lippa et al., 1998), early involvement of oxidative stress is suggested by the association of Aβ42 burden with the levels of neuronal RNA oxidation. We may be able to explain the inverse correlation between Aβ42 burden and neuronal RNA oxidation when we consider roles of transition metals such as copper and iron, efficient catalysts of oxidation, and zinc, a redox-inert antioxidant. These transition metals are significantly elevated in the neocortex and especially enriched in Aβ plaques of individuals with AD (Lovell et al., 1998). Indeed, Aβ1–42 possesses high affinity for these transition metals and the binding promotes assembly of Aβ (Atwood et al., 1999). The inverse correlation may reflect a possible antioxidant property of the Aβ peptide that chelates copper and iron to keep these transition metals in a redox-inactive form (Kontush, 2001; Zou et al., 2002). Another possible explanation is that the inverse correlation may reflect zinc elevation as a homeostatic antioxidant response to oxidative stress with subsequent abundant Aβ plaques formation (Cuajungco et al., 2000). Because recent studies have suggested that prefibrillar Aβ, but not the Aβ fibril, shows toxicity (Lambert et al., 1998; Walsh et al., 1999), Aβ plaques themselves may represent a fraction of total Aβ in the brain that has been condensed and neutralized and no longer contributes to neurotoxicity. Further investigations on the relationship between intraneuronally accumulated Aβ (Gouras et al., 2000) and oxidative stress markers are necessary to elucidate whether intraneuronal Aβ peptide has pro- or antioxidant property.

Conclusion

We observed prominent nucleic acid oxidation marked by 8OHG immunoreactivity in FAD patients with PS-1 or AβPP gene mutation. 8OHG was mainly restricted to cytoplasmic RNA of vulnerable neurons in FAD as we observed in sporadic AD. Early involvement of RNA oxidation in the pathological cascade of FAD was suggested by a presymptomatic case who carried a PS-1 mutation and showed a considerable level of neuronal RNA oxidation. An inverse correlation of Aβ42 burden with neuronal RNA oxidation in FAD, which was also demonstrated in Down syndrome, might suggest a link between the process of Aβ plaque formation and an effective tissue protective response to oxidative stress.

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References

Atwood, C.S., Huang, X., Moir, R.D., Tanzi, R.E., Bush, A.I., 1999. Role of free radicals and metal ions in the pathogenesis of Alzheimer's disease. Met. Ions Biol. Syst. 36, 309–364.

Bogdanovic, N., Zilmer, M., Zilmer, K., Rehema, A., Karelson, E., 2001. The Swedish APP670/671 Alzheimer's disease mutation: the first evidence for strikingly increased oxidative injury in the temporal inferior cortex. Dement. Geriatr. Cognit. Disord. 12, 364–370.

Cecchi, C., Fiorillo, C., Sorb, S., Latorraca, S., Nacmias, B., Bagnoli, S., Nassi, P., Liguri, G., 2002. Oxidative stress and reduced antioxidant defenses in peripheral cells from familial Alzheimer's patients. Free Radic. Biol. Med. 33, 1372–1379.

Cuajungco, M.P., Goldstein, L.E., Nunomura, A., Smith, M.A., Lim, J.T., Atwood, C.S., Huang, X., Farrag, Y.W., Perry, G., Bush, A.I., 2000. Evidence that the β-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of Aβ by zinc. J. Biol. Chem. 275, 19439–19442.

Eckert, A., Steiner, B., Marques, C., Leutz, S., Romig, H., Haass, C., Muller, W.E., 2001. Elevated vulnerability to oxidative stress-induced cell death and activation of caspase-3 by the Swedish amyloid precursor protein mutation. J. Neurosci. Res. 64, 183–192.

Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F.,

Greenfield, J.P., Haroutunian, V., Buxbaum, J.D., Xu, H., Greengard, P., Relkin, N.R., 2000. Intraneuronal Aβ42 accumulation in human brain. Am. J. Pathol. 156, 15–20.

Guo, Q., Sopher, B.L., Furukawa, K., Pham, D.G., Robinson, N., Martin, G.M., Mattson, M.P., 1997. Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: involvement of calcium and oxyradicals. J. Neurosci. 17, 4212–4222.

Guo, Q., Sebastian, L., Sopher, B.L., Miller, M.W., Ware, C.B., Martin, G.M., Mattson, M.P., 1999. Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid β-peptide toxicity: central roles of superoxide production and caspase activation. J. Neurochem. 72, 1019–1029.

Hashimoto, Y., Niikura, T., Ito, Y., Kita, Y., Terashita, K., Nishimoto, I., 2002. Neurotoxic mechanisms by Alzheimer's disease-linked N141I mutant presenilin 2. J. Pharmacol. Exp. Ther. 300, 736–745.

Hyman, B.T., Marzloff, K., Arriagada, P.V., 1993. The lack of accumulation of senile plaques or amyloid burden in Alzheimer's disease suggests a dynamic balance between amyloid deposition and resolution. J. Neuropathol. Exp. Neurol. 52, 594–600.

Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., Ihara, Y., 1994. Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). Neuron 13, 45–53.

Jenner, P., 1998. Oxidative mechanisms in nigral cell death in Parkinson's disease. Mov. Disord. Suppl. 13 (1), 24–34.

Kalaria, R.N., Cohen, D.L., Greenberg, B.D., Savage, M.J., Bogdanovic, N.E., Winblad, B., Lannfelt, L., Adem, A., 1996. Abundance of the longer Aβ 42 in neocortical and cerebrovascular amyloid β deposits in Swedish familial Alzheimer's disease and Down's syndrome. NeuroReport 7, 1377–1381.

Kontush, A., 2001. Amyloid-β: an antioxidant that becomes a pro-oxidant and critically contributes to Alzheimer's disease. Free Radic. Biol. Med. 31, 1120–1131.

LaFontaine, M.A., Mattson, M.P., Butterfield, D.A., 2002. Oxidative stress in synaptosomal proteins from mutant presenilin-1 knock-in mice: implications for familial Alzheimer's disease. Neurochem. Res. 27, 417–421.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., Klein, W.L., 1998. Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U. S. A. 26, 6448–6453.

Leutner, S., Czech, C., Schindowski, K., Touchet, N., Eckert, A., Muller, W.E., 2000. Reduced antioxidant enzyme activity in brains of mice transgenic for human presenilin-1 with single or multiple mutations. Neurosci. Lett. 292, 87–90.

Lippa, C.F., Nee, L.E., Mori, H., St George-Hyslop, P., 1998. Aβ-42 deposition precedes other changes in PS-1 Alzheimer's disease. Lancet 352, 1117–1118.

Lovell, M.A., Robertson, J.D., Teesdale, W.J., Campbell, J.L., Markesbery, W.R., 1998. Copper, iron and zinc in Alzheimer's disease senile plaques. J. Neurol. Sci. 158, 47–52.

Markesbery, W.R., Carney, J.M., 1999. Oxidative alterations in Alzheimer's disease. Brain Pathol. 9, 133–146.

Marques, C.A., Keil, U., Bonert, A., Steiner, B., Haass, C., Muller, W.E., Eckert, A., 2003. Neurotoxic mechanisms caused by the Alzheimer's disease-linked Swedish amyloid precursor protein mutation: oxidative stress, caspases, and the JNK pathway. J. Biol. Chem. 278, 28294–28302.

Matsuoka, Y., Picciano, M., La Francois, J., Duff, K., 2001. Fibrillar β-amyloid evokes oxidative damage in a transgenic mouse model of Alzheimer's disease. Neuroscience 104, 609–613.

Nunomura, A., Perry, G., Pappolla, M.A., Wade, R., Hirai, K., Chiba, S., Smith, M.A., 1999. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. J. Neurosci. 19, 1959–1964.

Nunomura, A., Perry, G., Pappolla, M.A., Friedland, R.P., Hirai, K., Chiba, S., Smith, M.A., 2000. Neuronal oxidative stress precedes amyloid-β deposition in Down syndrome. J. Neuropathol. Exp. Neurol. 59, 1011–1017.

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E.K., Jones, P.K., Ghanbari, H., Wataya, T., Shimohama, S., Chiba, S., Atwood, C.S., Petersen, R.B., Smith, M.A., 2001. Oxidative damage is the earliest event in Alzheimer disease. J. Neuropathol. Exp. Neurol. 60, 759–767.

Nunomura, A., Chiba, S., Kosaka, K., Takeda, A., Castellani, R.J., Smith, M.A., Perry, G., 2002. Neuronal RNA oxidation is a prominent feature of dementia with Lewy bodies. NeuroReport 13, 2035–2039 (Erratum: 2003 NeuroReport 14, 93).

Perry, G., Castellani, R.J., Hirai, K., Smith, M.A., 1998. Reactive oxygen species mediate cellular damage in Alzheimer disease. J. Alzheimer Dis. 1, 45–55.

Praticò, D., Uryu, K., Leight, S., Trojanoswki, J.Q., Lee, V.M., 2001. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J. Neurosci. 21, 4183–4187.

Shan, X., Tashiro, H., Lin, C.L., 2003. The identification and characterization of oxidized RNAs in Alzheimer's disease. J. Neurosci. 23, 4913–4921.

Smith, M.A., Hirai, K., Hsiao, K., Pappolla, M.A., Harris, P.L., Siedlak, S.L., Tabaton, M., Perry, G., 1998. Amyloid-β deposition in Alzheimer transgenic mice is associated with oxidative stress. J. Neurochem. 70, 2212–2215.

Smith, M.A., Rottkamp, C.A., Nunomura, A., Raina, A.K., Perry, G., 2000. Oxidative stress in Alzheimer's disease. Biochim. Biophys. Acta 1502, 139–144.

Sternberger, L.A., 1986. Immunocytochemistry, third ed. Wiley, New York.

Takahashi, M., Dore, S., Ferris, C.D., Tomita, T., Sawa, A., Wolosker, H., Borchelt, D.R., Iwatsubo, T., Kim, S.H., Thinakaran, G., Sisodia, S.S., Snyder, S.H., 2000. Amyloid precursor proteins inhibit heme oxygenase activity and augment neurotoxicity in Alzheimer's disease. Neuron 28, 461–473.

Walsh, D.M., Hartley, D.M., Kusumoto, Y., Fezoui, Y., Condron, M.M., Lomakin, A., Benedek, G.B., Selkoe, D.J., Teplow, D.B., 1999. Amyloid β-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J. Biol. Chem. 274, 45–52.

Yin, B., Whyatt, R.M., Perera, F.P., Randall, M.C., Cooper, T.B., Santella, R.M., 1995. Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. Free Radic. Biol. Med. 18, 1023–1032.

Zhang, J., Perry, G., Smith, M.A., Robertson, D., Olson, S.J., Graham, D.G., Montine, T.J., 1999. Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am. J. Pathol. 154, 1423–1429.

Zhang, Y., Dawson, V.L., Dawson, T.M., 2000. Oxidative stress and genetics in the pathogenesis of Parkinson's disease. Neurobiol. Dis. 7, 240–250.

Zou, K., Gong, J.S., Yanagisawa, K., Michikawa, M., 2002. A novel function of monomeric amyloid β-protein serving as an antioxidant molecule against metal-induced oxidative damage. J. Neurosci. 22, 4833–4841.