In Vitro and *in Vivo* Protein Oxidation Induced by Alzheimer's Disease Amyloid β-Peptide (1-42)

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Amyloid β-peptide (Aβ) is thought by many researchers to be central to the pathogenesis of Alzheimer's disease (AD) (reviewed in Ref. 1). In addition, oxidative stress, manifested by protein oxidation and lipid peroxidation, is apparent in AD brain.[2,3] Our laboratory developed a comprehensive hypothesis for neurotoxicity in AD brain that unites these two observations and provides a testable framework for much of the AD literature. We proposed an Aβ-associated free radical oxidative stress model for neuronal death in AD brain[2] (FIG. 1). In AD brain, the predominant forms of Aβ are Aβ(1-40) and Aβ(1-42). Consistent with our model and in ways completely inhibited by free radical scavengers (antioxidants), Aβ leads to lipid peroxidation[4,5] and protein oxidation[6–8] in various brain membrane systems; generates reactive oxygen species (ROS);[7,8] inhibits hippocampal neuronal and cortical synaptosomal membrane ion-motive ATPases, including Na^+/K^+-ATPase and Ca^{2+}-ATPase; blocks glutamate uptake and inhibits the activity of glutamine synthetase (both of the latter Aβ-induced alterations have the effect of increasing excitotoxic glutamate levels); causes intracellular Ca^{2+} levels to increase dramatically;[8] and leads to neurotoxicity in hippocampal neuronal or astrocytic cultures (reviewed in Ref. 2).

A prediction of the Aβ-associated free radical oxidative stress model for neurotoxicity in AD brain is that Aβ(1-42), the predominant form of Aβ found in AD, will induce protein oxidation. A key marker of protein oxidation is protein carbonyl content.[9] Previous studies showed increased antioxidant-inhibited protein oxidation in hippocampal neuronal cultures induced by Aβ(1-40)[8] and Aβ(25-35).[6,7] In the current study, we provide evidence for Aβ(1-42)–induced ROS generation *in vitro* and protein oxidation *in vitro* and *in vivo*. In agreement with our model (FIG. 1), 10 μM Aβ(1-42) added to cultured hippocampal neurons led to ROS formation that was inhibited by vitamin E (Fig. 2A) and induced significantly greater protein oxidation than in controls (Fig. 2B). In addition to the *in vitro* studies, *in vivo* studies were carried out. We reported earlier that AD brain regions rich in Aβ-containing senile plaques had significantly increased protein oxidation but Aβ-poor cerebellum did

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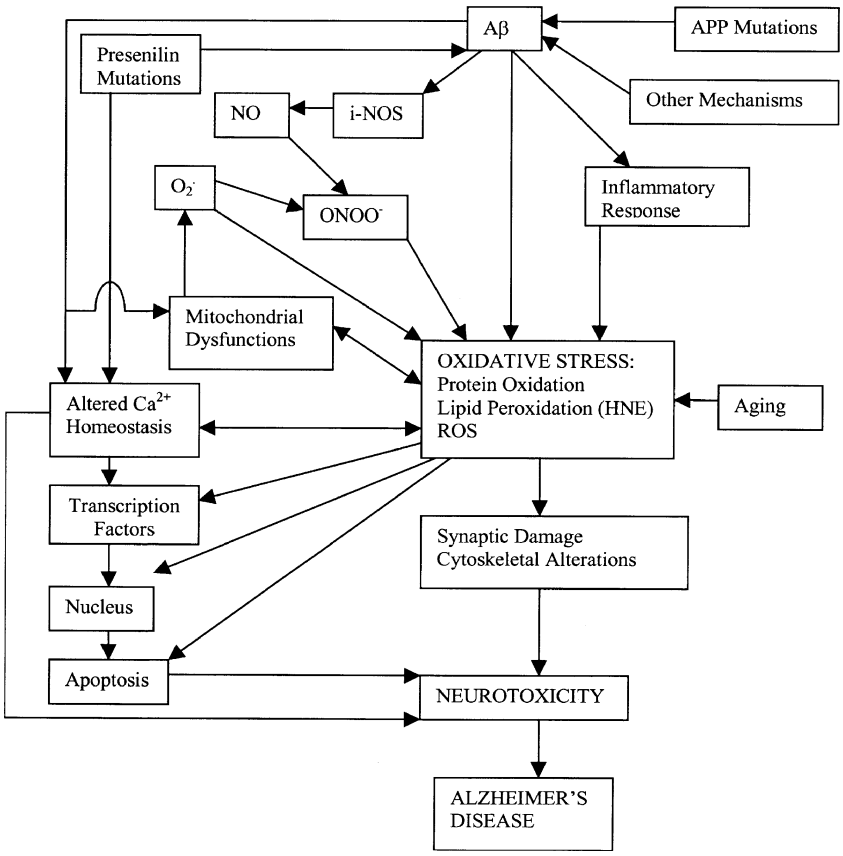

FIGURE 1. Flow diagram of our comprehensive model for Aβ-associated free radical oxidative stress–induced neurotoxicity in Alzheimer's disease brain. See Ref. 2 for a review and greater details.

not.[10] If our model is correct, then one may predict that transgenic animals overexpressing Aβ(1-42) should show increased protein oxidation *in vivo*. *Caenorhabditis elegans (C. elegans)* transgenic animals expressing full-length Aβ(1-42) were produced,[11] and protein oxidation was determined. In agreement with predictions of our model and with our earlier studies in AD brain,[10] Aβ(1-42)–expressing animals had significantly increased protein oxidation *in vivo* (FIG. 2C). To gain some insight into potential molecular mechanisms by which Aβ(1-42) led to protein oxidation *in vivo*, methionine was mutated to cys in this *in vivo* model of Aβ(1-42) expression. Consistent with previous *in vitro* studies of methionine substitution in Aβ(25-35) and Aβ(1-40) (2,13), no *in vivo* protein oxidation was found.

These findings are consistent with the Aβ-associated free radical oxidative stress model of neurotoxicity in AD brain[2] (FIG. 1). Other sequelae of Aβ(1-42)–induced *in vitro* and *in vivo* oxidative stress and their inhibition by antioxidants are currently

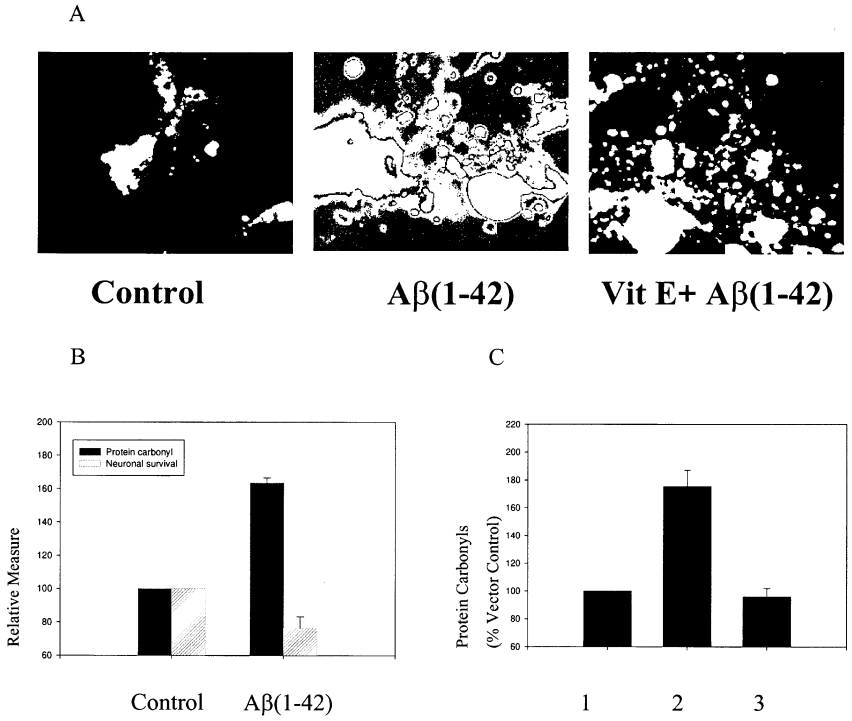

FIGURE 2. A. Reactive oxygen species production in cultured hippocampal neurons to which Aβ(1-42) had been added. ROS are assessed by fluorescence of 2,7-dicholorofluorescein, formed by reaction of peroxyl radicals or hydrogen peroxide to the DCF dye employed. **B.** Protein carbonyls *(dark bars)*, a measure of protein oxidation, and cell survival *(lighter bars)* of hippocampal neurons to which Aβ(1-42) had been added. Percent increased protein carbonyls in Aβ(1-42)–treated neurons over that of controls; mean ± SEM: 163 ± 2%, $p < 0.01$, $n = 3$. Percent cell survival was decreased significantly in Aβ(1-42)–treated neurons (76.3% of control cells, $p < 0.01$, $n = 3$). **C.** *In vivo* protein oxidation was found in *C. elegans* transgenic animals expressing full-length Aβ(1-42). (1) Protein carbonyls in vector control animals were assigned a value of 100%, $n = 5$. (2) Percent increased protein carbonyls over that of vector control; mean ± SEM: 176 ± 3%, $p < 0.001$, $n = 5$). (3) Protein carbonyls in transgenic animals in which methionine residue 35 in Aβ(1-42) was mutated to cysteine were equal to those of vector controls—e.g., no increase in protein oxidation was found.

under investigation. These current and ongoing studies may provide additional insight into AD pathogenesis and therapeutic strategies.

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