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

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Amyloid -peptide (A) 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 -associated free radical oxidative stress model for neuronal death in AD brain[2,3] (FIG. 1). In AD brain, the predominant forms of A are A (1-40) and A (1-42). Consistent with our model and in ways completely inhibited by free radical scavengers (antioxidants), A leads to lipid peroxidation[4,5] and protein oxidation[6-8] in various brain membrane systems; generates reactive oxygen species (ROS);[7,8]

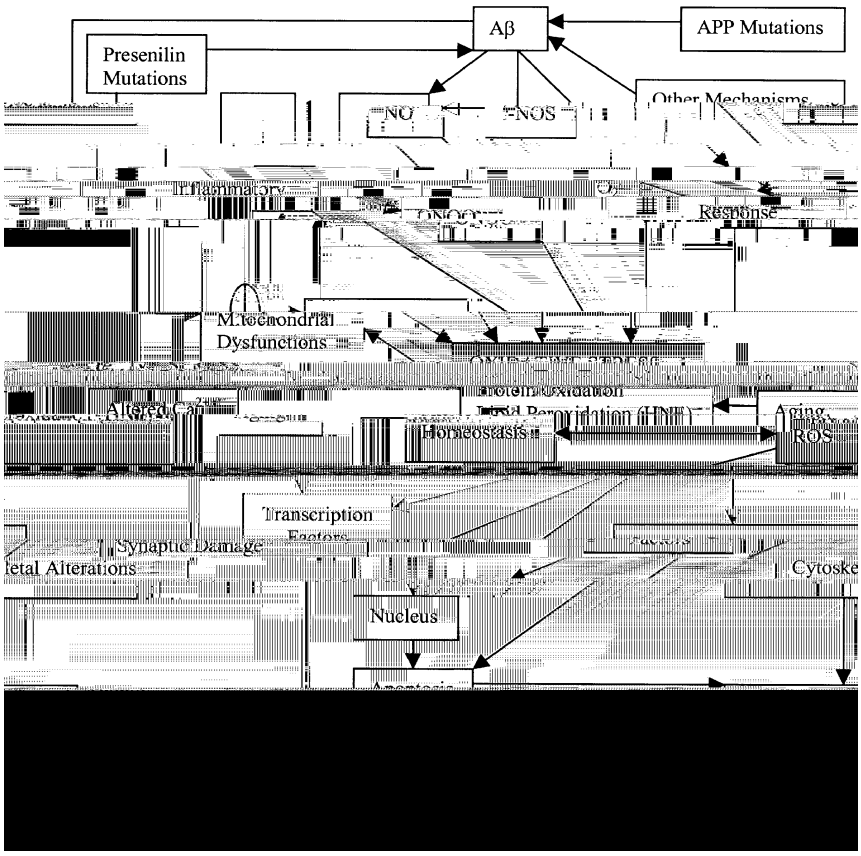

FIGURE 1. Flow diagram of our comprehensive model for A -associated free radical oxidative stress–induced neurotoxicity in Alzheimer's disease brain. See Ref. 2 for a review and greater details.

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

These findings are consistent with the A -associated free radical oxidative stress model of neurotoxicity in AD brain[2] (FIG. 1). Other sequelae of A (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 A (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 A (1-42) had been added. Percent increased protein carbonyls in A (1-42)–treated neurons over that of controls; mean SEM: 163 2%, p 0.01, n 3. Percent cell survival was decreased significantly in A (1-42)–treated neurons (p 76.3% of control cells, p 0.01, n 3). **C.** In vivo protein oxidation was found in C. elegans 4Ci6. Per9i.0212 0exp0 01.56J/F10 1lfull-lengt(In vivo)]TJ/F10 1 2r/F7 1 Tf e

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

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