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*Invited Review*

# Neurofibrillary pathology and aluminum in Alzheimer's disease

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**Summary.** Since the first reports of aluminum-induced neurofibrillary degeneration in experimental animals, extensive studies have been performed to clarify the role played by aluminum in the pathogenesis of Alzheimer's disease (AD). Additional evidence implicating aluminum in AD includes elevated levels of aluminum in the AD brain, epidemiological data linking aluminum exposure to AD, and interactions between aluminum and protein components in the pathological lesions of AD, i.e., neurofibrillary tangles (NFTs) and senile plaques (SPs). As most of this evidence is circumstantial and some of it is not consistent in all reports, the role of aluminum in the pathogenesis of AD has remained controversial. However, the interaction of aluminum with altered forms of τ in the paired helical filaments (PHFs) of neurofibrillary lesions is highly likely to contribute to the formation of NFTs because (1) aluminum and abnormally phosphorylated τ (known as PHFτ) are colocalized in NFTs, and (2) aluminum is known to preferentially interact with such phosphorylated proteins. Recently, we demonstrated that aluminum binds selectively to PHFτ, induces PHFτ to aggregate, and retards the *in vivo* proteolysis of PHFτ. These data suggest that aluminum could serve as co-factor in the formation of NFTs by interacting with PHFτ. This review summarizes current understanding of how aluminum might contribute to the formation of neurofibrillary lesions from PHFτ in neurons of the AD brain.

**Key words:** Alzheimer, Aluminium

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder which clinically manifests itself by progressive deterioration of memory and cognition. The major histopathological abnormalities that characterize the brains of patients with AD include an excess of neurofibrillary lesions and senile plaques (SPs), and the massive loss of telencephalic neurons (for reviews, see Kosik, 1992; Lee and Trojanowski, 1992; Goedert, 1993; Trojanowski et al., 1993a,b; Trojanowski and Lee, 1994). These intraneuronal neurofibrillary lesions include neurofibrillary tangles (NFTs) dystrophic neurites associated with SPs, and neuropil threads (NTs). When viewed by electron microscopy, these lesions contain abnormal filamentous structures called paired helical filaments (PHFs) which are formed from hyperphosphorylated adult central nervous system (CNS) τ proteins known as PHFτ or A68 (for reviews, see Goedert, 1993; Trojanowski et al., 1993a,b; Trojanowski and Lee, 1994). In contrast, extracellular SPs contain other abnormal filaments called amyloid fibrils which are formed from 39-43 amino acid long ß-amyloid peptides (Aß) derived from much larger, alternatively spliced amyloid precursor proteins (APPs) encoded by a gene on chromosome 21 (for reviews, see Hardy, 1993; Mullan and Crawford, 1993; Selkoe, 1993). In addition to these major structural elements, a number of other protein components selectively accumulate in NFTs and SPs including ubiquitin (Mori et al., 1987; Perry et al., 1987; Shaw and Chau, 1988) $\alpha_1$-antichymotrypsin (ACT) (Abraham et al., 1988; Gollin et al., 1992), heparan sulphate proteoglycans (HSPGs) (Snow et al., 1988; Perlmutter et al., 1991; Perry et al., 1991), and apolipoprotein E (ApoE) (Namba et al., 1991; Wisniewski and Frangione, 1992; Strittmatter et al., 1993).

Elucidation of mechanisms leading to the generation and deposition of Aß/amyloid fibrils and PHFτ/PHFs is a central focus of AD research. Since Aß can be detected immunohistochemically in AD brains, the Aß that accumulates in SPs was considered to be derived from the abnormal metabolism of APPs in the AD brain. However, generation of Aß from APPs was recently shown to occur under normal conditions. Aß can be detected intracellularly in cultured cells (Wertkin et al., 1993), is constitutively secreted from cultured cells, and is present in normal cerebrospinal fluid (Haass et al., 1992; Shoji et al., 1992; Wertkin et al., 1993). Moreover, cultured cells expressing APP with double amino acid

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substitutions (Lys-Met to Asn-Leu at position 670/71) found in a Swedish pedigree with early-onset familial AD (FAD), secrete elevated levels of Aß (Cai et al., 1993; Citron et al., 1992). Further, Aß secreted from cells expressing APP with the FAD amino acid substitution at Val 717 is composed on longer Aß peptides (42-43 amino-acids) (Suzuki et al., 1994), which are known to be highly amyloidogenic compared to the shorter Aß peptides (40 amino acids) (Jarret et al., 1993; Jarret and Lansbury, 1993). Transgenic mice containing human sequences encoding the APP cDNA produced Aß deposits in the mouse brain (Quon et al., 1991), and transgenic mice containing human APP with the FAD 717 mutation produced more extensive and advanced (congophilic) deposits of Aß (Games et al., 1995). Thus, although missense mutations in APP have been demonstrated only in a limited number of FAD cases, it is highly likely that these mutations are responsible for the deposition of Aß in these hereditary variants of AD.

PHFs in NFTs consists of CNS τ proteins that are abnormally phosphorylated at specific residues (for reviews, see Goedert, 1993; Trojanowski et al., 1993a,b; Trojanowski and Lee, 1994), and all six CNS τ isoforms contribute to the formation of PHFs (Goedert et al., 1992b). *In vitro* studies showed that recombinant τ fragments comprised of nonphosphorylated microtubule binding repeats as well as full length τ were able to form PHF-like structures (Crowther et al., 1992; Wille et al., 1992). PHFτ has been reported to be «hyper-phosphorylated» or «abnormally» phosphorylated at sites that are not found in normal adult human τ isolated from postmortem brain samples (Lee et al., 1991; Hasegawa et al., 1992; Goedert et al., 1993). These and other studies (reviewed in Goedert, 1993; Trojanowski et al., 1993a,b; Trojanowski, 1994) have led to the hypothesis that the excessive or abnormal activity of kinases may be responsible for the phosphorylation of τ, and that this might be a key event in the transformation of normal adult τ into PHFτ during the progression of AD. Several kinases have been shown to phosphorylate τ *in vitro*, including extracellular signal-regulated kinases (ERKs) or microtubule-associated protein (MAP) kinases (Drewes et al., 1992; Goedert et al., 1992a), glycogen synthase kinase-3 (GSK3) (Hanger et al., 1992; Mandelkow et al., 1992) or τ protein kinase I (Ishiguro et al., 1988), CDK5 (Bauman et al., 1993; Kobayashi et al., 1993; Paudel et al., 1993) or τ protein kinase II (Ishiguro et al., 1992), CDC2 kinase (Ledesma et al., 1992), and proline-directed kinases (Vulliet et al., 1992). Recent insights into the mechanisms leading to the generation of PHFτ have also come from studies of the phosphatases responsible for the dephosphorylation of τ. Protein phosphatase 2A and 2B were shown to dephosphorylate τ proteins *in vitro* (Goedert et al., 1992b; Drewes et al., 1993; Harris et al., 1993), and other studies demonstrated that inhibition of these phosphatases by okadaic acid induced the hyper-phosphorylation of normal adult τ which resembled PHFτ (Furiya et al., 1993; Harris et al., 1993; Vandermeeren et al., 1993). Subsequently, we demonstrated that normal τ in living neurons already possesses phosphate groups at many of the same sites as PHFτ by analyzing τ proteins prepared from normal fragments of cortex resected with seizure foci from epileptic patients (Matsuo et al., 1994). However, following progressively longer postsurgical intervals, biopsy-derived τ was dephosphorylated to attain the same phosphorylation state as normal adult τ prepared from postmortem brains. Since PHFτ remains highly phosphorylated in the AD brain even after a long postmortem interval, the dephosphorylation of PHFτ must be inhibited in neurons of the AD brain. Significantly, these data suggest that there is an impairment in the activity of brain phosphatases or that the expression of these phosphatases is reduced. Thus, impaired activation or reduced levels of protein phosphatases, as well as increased activation of kinases could contribute to the generation of maximally phosphorylated PHFτ in the AD brain.

The pathobiological effects of the extracellular deposition of Aß on surrounding neurons in the AD brain is unknown. Aß was shown to possess toxic effects on neurons in primary cultured neurons (Yankner et al., 1990a,b) and in experimental animals which received intracerebral administration of synthetic Aß or AD-derived Aß (Frautschy et al., 1991; Kowall et al., 1991). However, the neurotoxicity of Aß has not been replicated in several other studies (Kosik and Coleman, 1992). In fact it is well recognized that the massive deposition of Aß in SPs which meets the criteria of AD can be observed commonly in non-demented aged individuals (Roses, 1994). In contrast, the intracellular deposition of PHF-τ correlates well with the dementia in AD (reviewed in Trojanowski and Lee, 1994). PHFτ is generated and accumulates in neuronal perikarya as NFTs and in the processes of neurons as neuropil threads (NTs) and SP-associated dystrophic neurites. PHFτ losses the ability to bind microtubules (MTs), and this could lead to the depolymerization of MTs, the disruption of axonal transport, and the dysfunction and/or degeneration of neurons in AD. The accumulation of PHFs in neurons could exacerbate this process by sequestering normal τ and by physically blocking the transport of proteins and organelles in neuronal perikarya, axons, and dendrites. These events could then result in neuronal death, and this may explain why the abundance of NFTs correlates better with the dementia in AD than the amount of SPs (Tomlinson et al., 1970; McKee et al., 1991).

It is possible that the formation and maturation of NFTs and SPs may be linked mechanistically (Perry and Smith, 1993). For example, the intracerebral administration of synthetic Aß into rat brain (Kowall et al., 1991) or treatment of primary cultures of rat neurons with synthetic Aß (Takashima et al., 1993) induced neuronal expression of τ proteins recognized by Alz50 monoclonal antibody. Further intracerebral administration of AD PHFτ into rat brain formed proteolysis-resistant aggregates which induced co-

deposits of Aß, ubiquitin, ACT and ApoE (Shin et al., 1993, 1994). *In vitro* studies showed that an APP domain C-terminal to Aß interacts with τ proteins (Perry et al., 1993) or with pronase-treated PHFs (Caputo et al., 1992). The assembly of synthetic Aß into amyloid fibrils was promoted by HSPGs in rat brain which received chronic infusion of Aß with HSPGs (Snow et al., 1994), or by ApoE and ACT *in vitro* (Ma et al., 1994). Thus, interaction between τ/PHFτ, Aß/APPs and other protein constituents might contribute to the formation of NFTs and SPs.

Accumulating data from various lines of research over the last decade support the notion that AD is a heterogeneous disorder. Numerous risk factors or etiologies for AD have been suggested, including genetic and environmental factors. FAD is linked to chromosome 21, 19, and 14 (Hardy, 1993; Mullan and Crawford, 1993; Selkoe, 1993), while the ApoE ε4 allele on chromosome 19 is a risk factor for both FAD and sporadic AD (Corder et al., 1993; Saunders et al., 1993; Schmechel et al., 1993). Mutations in APP have been detected in a few families with FAD (Hardy, 1993; Mullan and Crawford, 1993; Selkoe, 1993), which were shown to favour deposition of Aß as described above. Among environmental risk factors, aluminum has received the most attention. The implication of aluminum in AD is based on several observations (for a recent review, see Markesbery and Ehmann, 1993): 1) aluminum causes neurofibrillary lesions in experimental animals; 2) aluminum is elevated in the AD brain; 3) epidemiological studies link aluminum to AD; and 4) aluminum interacts with various components of the pathological lesions found in the AD brain, such as τ and Aß. Since most of this evidence is circumstantial, and some is contradicted by other studies, the aluminum hypothesis has remained controversial. However, the observed interaction of aluminum with τ is highly likely to contribute the formation of NFTs because 1) aluminum and τ are colocalized in NFTs and 2) τ is highly phosphorylated to form PHFτ, and aluminum is known to preferentially bind to such phosphorylated proteins. Recently, we demonstrated that aluminum could serve as a co-factor in the pathogenesis of NFTs (Shin et al., 1994). Specifically, we showed that aluminum binds selectively to PHFτ, induces PHFτ to aggregate and retards the proteolysis of PHFτ. Our data strengthen the idea that aluminum plays a role in the pathogenesis of AD. Here we review the evidence for the possible link between aluminum and AD with emphasis on the pathobiological significance of the interaction between aluminum and τ.

## Evidence for a possible link between aluminum and AD

The notion that there is a mechanistic association between aluminum and AD began with observations made by Klatzo et al. (1965) and by Terry and Pena (1965) that the administration of aluminum into the CNS produced neurofibrillary lesions in experimental animals that resembled AD NFTs. These workers and others (Wisnieski et al., 1967; Crapper et al., 1973, 1980; De Boni et al., 1976; Gilbert et al., 1992) described prominent, widespread, filamentous accumulations which developed in perikarya following the introduction of aluminum salts directly into brain parenchyma or into the cerebrospinal fluid. These experimentally-induced filamentous changes were originally considered to be comparable to the human NFTs seen in AD brains. Despite the similarities at the light microscopic level, the filamentous lesions seen in the brains of aluminum-injected animals are ultrastructurally and biochemically different from AD NFTs (Terry and Pena, 1965; Wisniewski and Soifer, 1979; Wisniewski et al., 1980a,b; Selkoe et al., 1979). Specifically, the aluminum-induced tangles are composed of straight bundles of neurofilaments (NFs), which are composed of a triplet of NF subunit proteins, while human NFTs in the AD brain are composed of PHFs (Kidd, 1963) formed from hyperphosphorylated τ proteins referred to as PHFτ (Lee et al., 1991). Aluminum also induces neurofilamentous tangles in cultured neurons from the rat brain (Langui et al., 1988, 1990) and in neuroblastoma cells (Miller and Levine, 1974; Shea et al., 1989), while aluminum causes aggregation of isolated NF proteins *in vitro* (Diaz-Nido and Avila, 1990; Nixon et al., 1990; Shea et al., 1992). As no definite evidence has been reported to date for the involvement of NF proteins in NFTs or related neurofibrillary lesions, interactions between aluminum and NF proteins probably do not play a mechanistic role in the pathogenesis of these lesions in AD.

The experimental induction of AD-like neurofibrillary degeneration in rabbits following CNS injections of aluminum salts led to a series of analytical studies designed to investigate the aluminum content of brain tissue from patients with AD compared to controls. Extensive studies by Crapper-McLachlan (Crapper et al., 1973, 1976; McLachlan et al., 1991) have repeatedly demonstrated increased levels of aluminum in neocortical samples from AD patients that contain a large number of NFTs. The results were confirmed by other workers (Ducket and Galle, 1976; Trapp et al., 1978; Perl and Brody, 1980a,b). However, other reports failed to replicate these findings and several investigators found no significant difference in aluminum level of AD and age-matched control brains (McDermott et al., 1979; Markesbery et al., 1981; Traub et al., 1981; Jacobs et al., 1989; Xu et al., 1992; Lovell et al., 1993). These discrepancies, which may be due to differences in the analytic techniques and sample size of the various studies, remain unresolved.

To overcome limitations in assays of the bulk aluminum content of tissue which may fail to identify focal increase in aluminum associated with specific AD lesions such as NFTs and SPs, sensitive microprobe techniques have been used to monitor aluminum concentrations at cellular level, especially in NFTs and SPs. To define the specific location of aluminum in the AD brain, numerous microprobe studies have been

performed using a variety of different techniques. Perl and Brody (1980a) reported the results of an investigation employing scanning electron microscopy in conjunction with energy-dispersive X-ray spectrometry to evaluate the elemental content of NFT-bearing and NFT-free hippocampal neurons in the AD brain. This study demonstrated the presence of aluminum in the nuclear region of NFT-bearing neurons in AD. Using the same technique, aluminum was reported to accumulate in the nuclei and the cytoplasm of NFT-bearing neurons in Guam ALS-Parkinsonism-dementia complex (Perl et al., 1982). The accumulation of aluminum in NFT-bearing neurons of Guam ALS-Parkinsonism-dementia complex was confirmed using a different technique, i.e., wavelength-dispersive X-ray analysis (Garruto et al., 1984). However, other investigators failed to find elevated levels of aluminum in neurons of the AD brain using the same techniques (Jacobs et al., 1989; Chafi et al., 1991). Subsequent application of laser microprobe mass analysis (LAMMA) revealed an accumulation of aluminum localized to the tangles themselves in hippocampal neurons of AD brains (Good et al., 1992). Since LAMMA provides much more precise information on the location, concentration and nature of trace elemental abnormalities in CNS tissues (Heinen et al., 1980; Perl et al., 1986; Perl and Good, 1992), these data suggest that aluminum may accumulate in AD neurons where it becomes concentrated in NFTs. In contrast to NFTs, investigations of the aluminum content in SPs have shown conflicting results. Candy et al. (1986) showed increased levels of aluminum in the cores of SPs in the form of aluminosilicates using scanning electron microscopy in conjunction with energy-dispersive X-ray spectrometry. However, subsequent studies using the same analytical approach (Mori et al., 1988; Jacobs et al., 1989; Chafi et al., 1991), LAMMA (Stern et al., 1986), and nuclear microscopy (Landsberg et al., 1992) have failed to confirm the presence of aluminum in SPs.

Evidence providing a link between the levels of aluminum in drinking water and the incidence of AD is derived from epidemiological studies that compared rates of the disease in populations with access to water containing different amounts of aluminum. A statistically significant association between the concentrations of aluminum in drinking water and the number of cases of AD has been shown in several studies performed in five countries (Flaten, 1990; Martyn et al., 1989; Michel et al., 1991; Neri and Hewitt, 1991). The relative risk of AD in geographical regions characterized by high concentrations of aluminum in the drinking water ranges between 1.5 and 4 compared to regions with low concentrations of aluminum in the drinking water. Although epidemiological studies of this type should be interpreted cautiously, the association between the level of aluminum in the drinking water and the incidence of AD may prove to be an important clue into the pathogenesis of neurofibrillary lesions in AD.

If aluminum has a genuine role in the pathogenesis of these lesions, then removal of aluminum might slow or halt the progression of AD. Indeed Crapper-McLachlan et al. (1991) reported that the sustained administration of desferrioxamine (DFO), a chelating agent with a high affinity for aluminum, would slow the progression of dementia in AD. In a randomized two-year prospective, single-blind clinical trial, a structured performance test measuring daily living skills was videotaped in the home of subjects, and the tapes were analyzed at random by trained behaviour rates who were unaware of the purpose and protocol of the study. The average two-year decline in the DFO-injected group was practically and statistically significantly slower than that observed for the control no-treatment group. Accordingly, the authors concluded that DFO may slow the clinical progression of dementia associated with AD, and that aluminum is an important factor in the complex series of events associated with AD.

Thus a number of the reports summarized above suggest that aluminum may have a role in the etiopathogenesis of AD. Indeed, studies showing that the concentration of aluminum is increased in NFTs provide the most direct evidence for a link between aluminum and AD. The demonstration of aluminum in NFTs, however, does not necessarily indicate a primary pathogenic role for aluminum in the formation of these neuropathological lesions. For example, studies of chronic renal dialysis patients (Candy et al., 1992) using imaging secondary ion mass spectrometry (SIMS) revealed numerous focal accumulations of aluminum associated with pyramidal neurons, while silver staining and immunocytochemical procedures failed to detect NFTs in the cerebral cortex. Thus, this finding suggests that elevated levels of aluminum do not irrevocably lead to the formation of NFTs. However given the consistent presence of elevated amounts of aluminum in NFTs, other factors may work in conjunction with aluminum, thereby leading to the formation of NFTs in the AD brain.

### The interaction of aluminum with PHFτ is involved in the formation of NFTs

The notion that aluminum is implicated in the pathogenesis of AD rests primarily on data showing an association of this element with NFTs (Good and Perl, 1993). Elucidation of the mechanisms for the concentration of aluminum in NFTs and the biological effects that aluminum exerts in NFTs should lead to a better understanding of the role of aluminum in the pathogenesis of AD. A number of studies have examined the effects of aluminum on τ proteins, the major protein component of NFTs. For example, the long-term exposure of cultured neurons to aluminum resulted in the formation of τ-immunoreactive aggregates in cell bodies and cell processes (Kawahara et al., 1992). Also, isolated bovine τ protein, recombinant τ, and AD PHFτ were shown to aggregate *in vitro* following treatment with aluminum (Scott et al., 1993). In addition, enhancement of τ immunoreactivity was observed in aluminum-

treated human neuroblastoma cells (Mesco et al., 1991), and in rat brains injected with aluminum salts (Shigematsu and McGeer, 1992).

Phosphorylation of τ proteins was induced in aluminum-treated neuroblastoma cells (Guy et al., 1991), while similar phosphorylation of τ was not observed in cultured rat and human neurons (Mattson et al., 1993). Aluminum inhibited the activity of protein phosphatase 2A and 2B, both of which are known to dephosphorylate τ proteins *in vitro* (Goedert et al., 1992a; Drewes et al., 1993; Harris et al., 1993). As a result, aluminum increased the phosphorylation of τ (Yamamoto et al., 1990). Aluminum catalyzed the non-enzymatic phosphorylation of τ proteins and other proteins (Abdel-Ghany et al., 1993), but this process is not known to be relevant to the phosphorylation of τ proteins in AD. Intracerebral co-injection of PHFτ and aluminum salts induced neurons near the injection sites to acquire PHFτ-like properties as monitored with antibodies that recognize defined PHFτ epitopes containing phosphoserine residues (Ser202, Ser396, Ser404) (Shin et al., 1994). Harrington et al. (1994) studied the brains from patients with renal failure who were expoxed to aluminum during dialysis in order to investigate AD-like changes in τ proteins. Immunohistochemical analysis failed to show a significant amount of NFTs, whereas immunochemical analysis revealed that normal τ was depleted and that insoluble hyperphosphorylated τ was increased in association with elevated aluminum concentrations. This parallels observations showing that the production of hyperphosphorylated PHFτ is associated with reduced levels of normal τ proteins in areas of the AD brain with abundant neurofibrillary pathology (Bramblett et al., 1992; Shin et al., 1992). Thus, studies of the brains of renal dialysis patients with high levels of aluminum revealed abnormal proteins similar to those seen in AD but these abnormal τ did not become integrated into NFTs. These observations suggest that aluminum plays a role in the phosphorylation of τ, but it remains to be clarified how aluminum contributes to the generation of PHFτ in AD.

Recently we demonstrated that aluminum appears to interact with PHFτ (Shin et al., 1994) *in vitro* and *in vivo*.

### (1) Aluminum preferentially binds to PHFτ in NFTS

By using immunohistochemical procedures we examined whether exogenous aluminum binds to constituent proteins of NFTs and SPs. Hippocampal sections from AD brains were preincubated in 10mM $AlCl_3$/0.1M Tris, pH 6.5 at 37 °C overnight, followed by immunostaining with a wide range of monoclonal and polyclonal antibodies against a variety of protein components of NFTs and SPs. With and without incubation in aluminum salts there was no change in immunoreactive structures recognized by antibodies to Aß, ubiquitin, ACT, ApoE and HSPG. In contrast, there was a marked reduction in the staining of NFTs, SP neurites and neuropil threads by antibodies specific for epitopes containing a phosphorylated Ser202, Ser396 and Ser404 (AT8, T3P and PHF1) in τ. However, antibodies to phosphate-independent epitopes in τ (Alz50, T14, and T46) did not show remarkable changes in the aluminum-treated sections of AD brains. These findings prompted use to speculate that this significant reduction in immunoreactivity could be due to the selective binding of aluminum to specific sites in PHFτ, i.e., regions that include Ser202, Ser396 and Ser404 in a phosphorylated state.

### (2) Aluminum preferentially induces PHFτ to aggregate

We further examined the interaction of aluminum with PHFτ by immunoblot analysis. Crude extracts containing both soluble PHFτ and normal τ prepared from postmortem AD brain and equivalent extracts containing only normal τ from postmortem control adult brain were used. The extracts were incubated with aluminum salts at 37 °C for 1 hour, followed by centrifugation. The resulting supernatants and pellets were subjected to SDS-PAGE and immunoblot analysis using the monoclonal antibodies PHF1 (which recognizes PHFτ but not normal τ) and Tau1 (which recognizes normal τ but not PHFτ). *In vitro* incubation with aluminum salts had no effect on the solubility of normal adult τ, while increasing concentrations of aluminum salts induced PHFτ to aggregate at the top of the stacking gel, or not even enter the gel. Although PHFτ and normal τ in the AD brain extracts exist under identical conditions, aluminum appears to have a higher binding affinity for PHFτ than for normal τ. This property of aluminum may explain why this metal induces the selective aggregation of PHFτ.

### (3) Aluminum retards the *in vivo* proteolysis of PHFτ

Finally, we examined the *in vivo* effect of aluminum of PHFτ by co-injecting PHFτ and aluminum salts into the rat brain. When PHFτ alone was injected into the rat brain, it formed proteolysis-resistant aggregates. However, co-injection of PHFτ with aluminum dramatically enhanced the resistance of PHFτ to *in vivo* degradation as evidenced by the prolonged persistence of PHFτ aggregates at the injection site. In summary, aluminum selectively binds to PHFτ, induces PHFτ to aggregate, and retards the proteolysis of PHFτ

Aluminum is known to bind avidly to phosphate groups in proteins, and aluminum can modify the conformation of proteins to which it binds (Webb et al., 1973; Siegel and Haug, 1983; Birchall and Chapell, 1988). Thus, the observations described here may be explained by the occurrence of aluminum-induced conformational changes in PHFτ that result from the binding of aluminum to specific epitopes in PHFτ that contain phosphorylated residues (e.g., Ser202, Ser396, and Ser404). Taken together with data demonstrating increased levels of aluminum in NFTs, it is plausible to assume that aluminum may be a co-factor in the

formation of neurofibrillary lesions in AD brains. Specifically, in neurons vulnerable to the abnormal hyperphosphorylation of τ, aluminum may bind selectively to PHFτ and promote the aggregation of individual PHFs (which are built from PHFτ) and/or change the molecular conformation of PHFτ so that it is less accessible to proteolyzing enzymes, thereby leading to the formation and maturation of NFTs. A schematic summary for current understanding of how aluminum contributes to the formation of neurofibrillary lesions in AD is shown in Fig. 1.

## Conclusions

Despite long and extensive investigations to clarify the role of aluminum in AD, the aluminum hypothesis has remained controversial. However, recent studies of the interactions between aluminum and PHFτ strongly implicate aluminum as a potential co-factor in the pathogenesis of neurofibrillary lesions in AD. The pathobiological effects of aluminum on PHFτ include the selective binding of aluminum to PHFτ (i.e., abnormally hyperphosphorylated forms of τ) and the aggregation of PHFτ. These effects are highly likely to contribute to the formation of NFTs and dystrophic processes in AD. Once abnormally phosphorylated PHFτ is generated, aluminum may contribute to the formation of NFTs by inducing aggregation of PHFτ. Thus, aluminum may not be a primary cause of AD, but it may serve as a cofactor to accelerate the formation of NFTs (Markesbery and Ehmann, 1993). Since the amount of NFTs is closely linked to the dementia in AD,

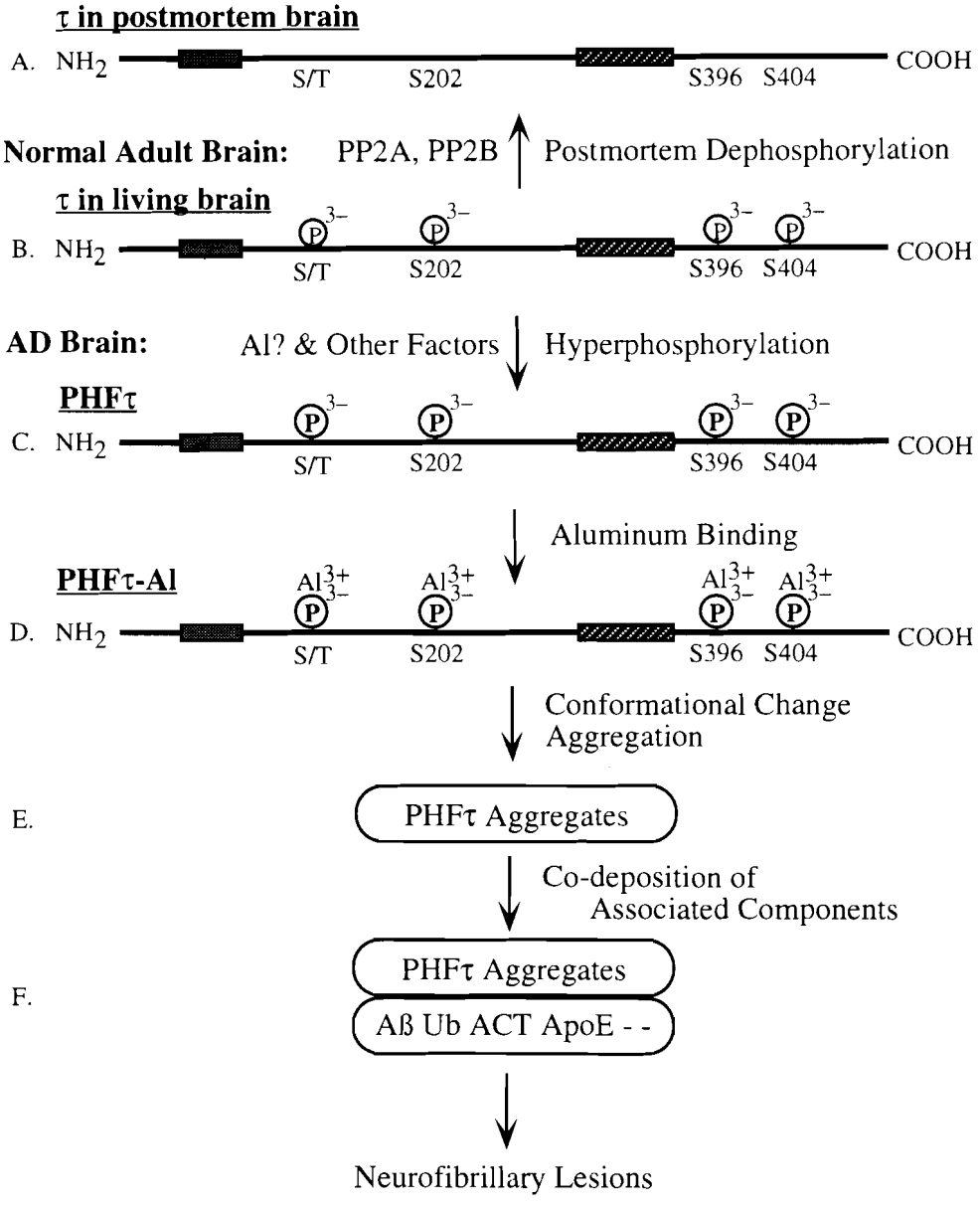


**Fig. 1.** Schematic illustration of how interactions between τ/PHFτ and aluminum could be part of a hypothetical sequence of events that lead to the formation of neurofibrillary lesions in AD. in the normal adult brain, τ freshly prepared from biopsy brain samples (τ in living brain) is already phosphorylated at many of the same sites as PHFτ, though not the same degree (B), and the τ in the living brain is rapidly dephosphorylated by protein phosphatase A (PP2A) and B (PP2B) to the same phosphorylation state as τ prepared from postmortem brains (τ in postmortem brain) (A). In the AD brain, τ is more phosphorylated due to impaired activation of phosphatases as well as excessive activation of kinases, and it is maximally phosphorylated so that it becomes PHFτ (C). A series of these steps might involve phosphorylation of τ by aluminum. After PHFτ is generated, aluminum avidly binds to these abnormally phosphorylated proteins (D), and thereby induces the aggregation and delayed proteolysis of PHFτ (E). The PHFτ aggregates serve as nidus for co-deposition of other associated components such as Aß, ubiquitin, ACT and ApoE (F), leading to the formation and maturation of neurofibrillary lesions in AD. Only selected phosphorylation sites are illustrated here (i.e., S/T, Ser202, Ser396, Ser404) that have been shown to distinguish postmortem normal τ from PHFτ.

further studies of the mechanisms whereby aluminum contributes to the formation of neurofibrillary lesions will shed light on the pathogenesis of AD.

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