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A REVIEW

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ABSTRACT

Aluminum is environmentally ubiquitous, providing human exposure. Usual human exposure is primarily dietary. The potential for significant Al absorption from the nasal cavity and direct distribution into the brain should be further investigated. Decreased renal function increases human risk of Al-induced accumulation and toxicity. Brain Al entry may involve transferrin-receptor mediated endocytosis and a more rapid process transporting small molecular weight Al species. There appears to be Al efflux from the brain, probably as Al citrate. There is prolonged retention of a fraction of Al that enters the brain, suggesting the potential for accumulation with repeated exposure. Al is a neurotoxicant in animals and humans. It has been implicated in the etiology of sporadic Alzheimer's disease (AD) and other neurodegenerative disorders, although this is highly controversial. This controversy has not been resolved by epidemiological studies, as only some found a small association between increased incidence of dementia and drinking water Al concentration. Studies of brain Al in AD have not produced consistent findings and have not resolved the controversy. Injections of Al to animals produce behavioral, neuropathological and neurochemical changes that partially model AD. Aluminum has the ability to produce neurotoxicity by many mechanisms. Excess, insoluble amyloid β protein (Aβ) contributes to AD. Aluminum promotes formation and accumulation of insoluble Aβ and hyperphosphorylated tau. To some extent, Al mimics the deficit of cortical cholinergic neurotransmission seen in AD. Al increases Fe-induced oxidative injury. The toxicity of Al to plants, aquatic life and humans may share common mechanisms, including disruption of the inositol phosphate system and Ca regulation. Facilitation of Fe-induced oxidative injury and disruption of basic cell processes may mediate primary molecular mechanisms of Al-induced neurotoxicity. Avoidance of Al exposure, when practical, seems prudent.

Running head: The toxicology of aluminum in the brain

Key words: Aluminum, Alzheimers's disease, amyloid-β protein, neurofibrillary tangle, neurotoxicity

I. Aluminum is common in the environment.

It is the third most common element and the most common metal of the earth's crust. Although most Al exists as insoluble aluminosilicates and Al oxides, it does present the opportunity for human exposure and provides ubiquitous contamination.

II. Usual human exposure is primarily dietary.

The primary Al sources for the human are food, water, antiperspirants and airborne contaminants. Foods with high Al content include tea, herbs and spices, grain products, processed cheeses and salt (Pennington, 1987). Typical adult dietary Al intake is ≈ 5-10 mg/day (Pennington and Schoen, 1995), nearly all from food. Drinking water provides about 1% of normal daily Al intake. It has been suggested that Al bioavailability from water is greater than from food, based on the presence of ligands in food that might complex Al, to possibly inhibit its oral absorption. However, one study suggests comparable Al bioavailability from food and water (Stauber *et al.,* 1999). This needs to be more rigorously assessed. Recent studies in humans consuming ^{27}Al in drinking water and rats dosed with ^{26}Al under conditions that model drinking water consumption suggest oral Al bioavailability from water is ≈ 0.3% and is not influenced by food in the stomach or water hardness (Stauber *et al.,* 1999; unpublished results).

To determine Al absorption from topical application of antiperspirants, ^{26}Al was incorporated into aluminum chlorohydrate and applied to the underarms of two adults (Flarend *et al*., 2000). Approximately 0.02% of the applied ^{26}Al appeared in the urine.

Pulmonary Al absorption is dependent on particle size. Pulmonary Al absorption from small particles in an industrial setting was estimated to be 1-2% (Gitelman *et al*., 1995; Pierre *et al*., 1995).

III. It has been suggested that Al may be absorbed from the nasal cavity.

Substances in the nasal cavity might be absorbed into systemic circulation, into the perineural space surrounding the olfactory neuron, and directly into the brain through the olfactory neurons (Roberts, 1986). Many substances are well absorbed into systemic circulation from the nasal cavity (Landau *et al*., 1994), from which they could enter the brain through the blood-brain barrier (BBB). This would likely result in fairly uniform distribution throughout the brain. The perineural space that ensheaths neurons is continuous with the CSF compartment that surrounds the brain (reviewed by Jackson *et al*., 1979). Substances can rapidly appear in CSF after application to nasal mucosa (Czerniawska, 1970), and in the nasal cavity after introduction into CSF (Faber, 1937). Brain access via CSF would be expected to produce the highest concentration on the brain surface, at least initially. Olfactory neuron processes terminate in the nasal mucosa, where they have a naked cell membrane. This is the only site where the CNS is exposed to the environment. These neurons synapse with pathways in the olfactory bulb that subsequently connect with many brain regions (Tjälve and Henriksson, 1999), providing the opportunity for substances to enter the brain if they can enter the olfactory neuron and can then distribute across synapses. Absorption by this route would be expected to produce highest concentrations in the olfactory bulb initially with later increases in the brain.

Inhalation or instillation of inorganic Cd, Hg and Ni into the rat nasal cavity resulted in their appearance in the olfactory bulb. Significant metal distribution was not seen into other brain regions. In contrast, Mn was found in several brain regions after its intranasal application, suggesting its ability to distribute across synapses. For a review see Tjälve and Henriksson (1999).

Perl and Good (1987) implanted Gelfoam® saturated with 15% Al lactate or 5% Al chloride into the nasal recess of rabbits. Neuropathological changes and elevated Al were seen in the olfactory bulb, pyriform cortex, hippocampus and cerebral cortex 1 month later, suggesting Al absorption and trans-synaptic distribution. Preliminary results using PIXE analysis revealed Al in brain stem nuclei in rats exposed to Al chlorohydrate by inhalation, but not in non-Al exposed controls, suggesting olfactory nerve uptake and trans-synaptic distribution (Divine *et al.*, 1999). Rats exposed to a lipophilic Al species under conditions designed to maximize inhalation via the nasal-olfactory system

had elevated brain Al (Zatta *et al.*, 1993). However, the Al distribution was not consistent with absorption by the olfactory nerve. The extent of brain Al uptake via the olfactory nerve should be determined.

IV. Some humans are at increased risk of Al-induced accumulation and neurotoxicity.

Aluminum is primarily excreted in the urine. Dialysis is a substitute for renal function. However, it does not efficiently clear phosphate. Phosphate excretion can be enhanced by Al, which forms insoluble Al phosphates in the gastrointestinal tract. However, when massive amounts of Al are consumed to bind phosphate, or as an antacid, in the absence of adequate renal function, Al accumulation and toxicity can result. Al accumulation can develop in renal-impaired people when Al is present in dialysis fluids or is given parenterally. The neurological manifestations of Al accumulation in renal-impaired humans are characterized by the dialysis encephalopathy syndrome (DES) (Alfrey *et al.*, 1976). Al is significantly elevated in brain and neurons in DES (Alfrey *et al.*, 1980; Reusche, 1997). Neurofibrillary tangles (NFTs), senile plaques and cerebrovascular amyloid, which are seen in AD, are not routinely seen in DES. Although generally well recognized and avoided, DES still occurs due to contamination of dialysis fluid (Simoes *et al.*, 1994; Berend *et al.*, 2000) and consumption of Al-containing drugs (Reusche, 1997). Aluminum-induced anemia and bone toxicity occur with lower, more prolonged, exposure.

Toxicity does not appear to result from oral intake of massive amounts of Al by humans who have adequate renal function. However, neurotoxicity can occur in renal-intact humans after alum instillation into the urinary bladder to stop hemorrhaging (Phelps *et al.*, 1999) and has been suggested to result from neurosurgical implants of Al-containing biomaterials (Renard *et al.*, 1994).

V. Brain Al entry may involve transferrin-receptor mediated endocytosis and a more rapid process transporting small molecular weight Al species.

The primary site of distribution between blood and brain is the BBB (Pardridge, 1997). The

anatomical basis of the BBB is the tight junctions between the cells surrounding the microvessels that perfuse the brain (visit http://www.sfn.org/briefings/blood-brain.html). The predominant Al species in plasma is Al-transferrin. It has been suggested that Al enters the brain through the BBB via transferrin-receptor mediated endocytosis (TfR-ME) (Roskams and Connor, 1990). We administered iv ^{26}Al-transferrin to rats and determined the brain and blood ^{26}Al concentrations 1 day later (unpublished results). The amount of Al in the brain (0.005% of the dose) could have been transported into the brain by TfR-ME in < 1 hr if Al transfer clearance from blood to brain is the same as for Fe (Morris *et al.*, 1992: Ueda *et al.*, 1993). The primary small molecular weight Al species in plasma is Al-citrate. When Al-citrate was administered iv, the rate of brain Al appearance was too rapid to be due to diffusion or TfR-ME, suggesting another mechanism of brain Al entry (Allen and Yokel, 1992). The mechanism of this influx is unknown.

VI. Al appears to be actively effluxed from the brain, probably as Al citrate.

When constant Al concentrations were achieved in blood and brain extracellular fluid (ECF), determined by microdialysis sampling, the brain/blood Al ratio was ~ 0.15 (Allen *et al.*, 1995). This suggests a carrier-mediated process effluxing Al from brain ECF. It is unlikely TfR-ME mediates brain Al efflux, due to very low or no Al-transferrin in brain ECF and the inadequate rate of TfR-ME to mediate brain Al influx under these conditions, much less a more rapid efflux. The predominant Al species in brain ECF is probably Al-citrate, due to the much lower transferrin and higher citrate concentrations in brain than blood ECF. Transferrin free sites in CSF are ≤ 0.25 μM versus 50 μM in plasma. CSF citrate is 180 μM versus 100 μM in plasma (Martin, 1997). We suggested that monocarboxylate transporter isoform 1 (MCT1) mediates Al-citrate efflux from brain ECF (Ackley and Yokel, 1997; 1998). Recent studies do not support this (unpublished results).

VII. A fraction of Al that enters the brain is retained for some time.

The positive correlation between brain Al and human age (McDermott *et al.*, 1979;

Markesbery *et al.*, 1984) suggests Al persistence in the brain. We assessed this by giving ^{26}Al-transferrin iv to rats and euthanizing them 0.2 to 128 days later. Brain Al did not significantly decrease, suggesting a rat brain Al half-life > 100 days (unpublished results).

VIII. Aluminum is a known neurotoxicant.

The initial observation of Al-induced neurotoxicity was of Al tartrate-induced neuronal degeneration in rabbits and hindlimb weakness and convulsive movements in dogs (Siem, quoted by Döllken, 1898). Al phosphate injection intracerebrally or into the cisterna magna of rabbits produced ataxia, convulsions and neurofibrillary degeneration (NFD) approximately 9-14 days later (Klatzo *et al*., 1965). The route of exposure, abrupt onset, and profound convulsions do not mimic AD or its progression. Al-induced NFD is characterized by orderly bundles of 10 nm filaments; not modeling the paired, 10-nm wide, helix wound filaments found in AD, the amyotrophic lateral sclerosis parkinsonism dementia complex (ALS-PD), and other neurodegenerative disorders (Wisniewski *et al.*, 1970). Some feel that the differences in neuropathology produced by intracerebroventricular (icv) Al versus those seen in AD disprove any link between Al and AD. However, repeated subcutaneous Al injection of rabbits also produced NFD (De Boni *et al.*, 1976). Symptom onset was delayed, compared to icv Al, and was Al dose dependent, producing an animal model more amenable to prolonged study that more closely mimics AD.

VIII. A. Aluminum has been implicated in the etiology of the amyotrophic lateral sclerosis parkinsonism dementia complex

This syndrome has been reported in the indigenous residents of 3 Western Pacific rim islands. One theory attributes the cause to the high Al and Mn and low Ca concentrations in the soil, and presumably food and water of these people (Yasui *et al*., 1991). The syndrome produces early death, with NFTs that mimic AD, but not the senile (neuritic; amyloid) plaques or cerebrovascular amyloid seen in AD.

VIII. B. Aluminum has been implicated in the etiology of sporadic forms of Alzheimer's disease.

Alzheimer's disease is a progressive deterioration of brain function, characterized initially by cognitive deficits including loss of recent memory and impairment of orientation, language, problem solving and abstract thinking. The disease progresses, to include psychiatric and behavioral disturbances and eventually loss of the ability to perform activities of daily living. Hallmark neuropathological signs include NFTs and senile plaques, particularly in the hippocampus and temporal and parietal cortices, and cerebrovascular amyloid. Early onset AD is usually familial, due to one of several gene mutations which result in increased secretion of neurotoxic amyloid β protein (Aβ). Aβ is constituitively formed by normal cells, including brain cells, by cleavage from amyloid precursor protein (APP), a family of membrane proteins (Figure 1). Excessive Aβ protein can be neurotoxic, as discussed below.

No specific gene mutations have been associated with late-onset/sporadic forms of AD (Price *et al.*, 1998), which account for 85-95% of AD cases (Jones and Oorschot, 1998). Factors that increase the risk of AD include age, brain trauma with loss of consciousness, history of dementia in relatives, lower educational attainment and socioeconomic status, possession of the APO E ε4 allele, and apparently environmental factors. Environmental factors are likely to interact with other factors to cause this disease (Cotton, 1994). The main concern about Al accumulation is that it might contribute to AD.

VIII. C. Some epidemiological studies showed a positive association between drinking water Al and Alzheimer's disease.

There have been numerous reports from Norway, Great Britain, France, Canada and Switzerland investigating the association between AD, dementia and similar endpoints compared to the drinking water Al concentration where the subjects live. The water Al concentration data range from a single sample per location to 10 year records, but none overlap the lifespan of the subjects. Al

was most commonly quantitated by atomic absorption spectroscopy (Table 1). The results of most studies (Table 2) suggest a small non-significant increased risk of dementing illnesses for people living in areas of higher water Al concentration. There are several reports of negative studies. Two groups originally reported increased risk that their subsequent findings failed to support (Martyn *et al.,* 1989 and 1997; Michel *et al.*, 1990 and Jacqmin *et al.*, 1994). Further analysis of the data suggests an influence of fluoride, silicon and water pH on the risk (Jacqmin *et al.*, 1994; Forbes *et al.*, 1995).

Both the Canadian and United States governments are attempting to obtain further research data to help determine if guidelines for Al in drinking water should be established. In Canada, the body that sets national drinking water guidelines, the Federal-Provincial Subcommittee on Drinking Water, established operational guidance limits based on the precautionary principle. These are 100 μg/l for conventional treatment plants using alum coagulation and 200 μg/l for a small number of plants that use lime softening or direct filtration (visit http://www.hc-sc.gc.ca/ehp/ehd/catalogue/bch_pubs/dwgsup_doc/aluminum.pdf). Amendments to the 1996 Safe Water Drinking Act require the US EPA to publish a list of contaminants which are not subject to regulation in drinking water but are known or anticipated to occur in public water systems and which may require regulation under this Act. Al is one of 50 chemicals that are the priority contaminants for EPA's drinking water program. Al was included because of new developments and research on Al epidemiology indicating a potential link between Al and adverse neurological effects. The EPA identified a need for further health and treatment research on Al (EPA, 1998).

VIII. D. Studies have not consistently found an elevation of Al in Alzheimer's disease.

Bulk brain Al has been reported to be ~ 0.018 mM (wet weight) in normal humans (Yasui *et al.*, 1991: Lovell *et al.*, 1993). It has been reported to be 0.10 mM in AD brain (Ward and Mason, 1987), 0.18 in DES (Alfrey *et al.*, 1980) and 0.34 in ALS-PD (Yasui *et al.*, 1991). Al concentration in the cytoplasm of NFT-bearing neurons was reported to be 0.11 mM (Lovell *et al.*, 1993; and

Table 5).

Numerous studies have been conducted to assess the observation that Al is higher in AD than age matched non-AD brains at autopsy (Crapper *et al.*, 1973). Table 3 provides a summary of studies of bulk brain Al in AD, and some comparisons to control subjects. Some studies found higher brain Al in AD; some did not. These inconsistent findings contribute to the controversies concerning the role of Al in the etiology of AD and the possible ability of AD neuropathology to cause neuronal Al accumulation. Similarly, some microprobe studies have demonstrated the presence of Al in senile plaques and NFTs of AD (Tables 4 and 5). The results of some negative studies have not been published. There is considerable variability in the number of observations and completeness of these reports. They cannot all be considered scientifically equal. Unfortunately, these studies of Al concentration in AD brain, determined by bulk brain, microprobe and staining techniques, have yielded inconsistent results. One source of the inconsistencies might be the different detection limits of the methods used (Table 1). Another source of positive results is thought by some to be contamination. Some have interpreted the results as supporting a role for Al in AD; others have interpreted them as refuting that association. Even if brain Al is elevated in AD it would not prove a cause-effect relationship, as AD might produce plaques and NFTs that may then bind Al. These studies have failed to resolve this controversy.

IX. Aluminum injections to animals produce effects that partially model Alzheimer's disease.

A 5-fold elevation of brain Al was associated with NFD in the rabbit (De Boni *et al.*, 1976), consistent with some reports of small elevations of brain Al in AD. Al given icv produced neuropathological changes in the rabbit that partially modeled those seen in AD brain (Forrester and Yokel, 1985: Katsetos *et al.*, 1990). Repeated parenteral Al injections to the adult rabbit, which produce Al accumulation, resulted in decrements in eyeblink reflex acquisition (Yokel, 1983; 1989; Pendlebury *et al.*, 1988). Similar behavioral deficits were seen in AD subjects (Woodruf-Pak *et al.*, 1990; Solomon *et al.*, 1991; Yokel *et al.*, 1994).

Al-induced NFD and AD NFTs positively respond to silver stain and SMI 31, an antibody to phosphorylated neurofilaments, suggesting some similarities in protein composition. However, antibodies against tau and microtubule-associated protein 2 strongly stained AD brain, but not rabbit brain with icv Al chloride-induced NFD (Kowall *et al.*, 1989), demonstrating differences between Al-induced NFD and AD.

Aluminum can produce accumulation of Aβ protein and hyperphosphorylated tau protein, below, modeling changes seen in AD. Injection of Al chloride into the rat brain resulted in staining with Alz50 (an antibody raised against an AD-specific protein), although no staining of ubiquitin, tau or PHFs (Shigematzu and McGeer, 1992). However, Al maltolate injection into rabbit brain produced NFD that contained normal and phosphorylated tau protein and other proteins found in AD brain, including APP, Aβ, α_1-antichymotrypsin, and ubiquitin (Savory *et al.*, 1995; Huang *et al.*, 1997).

X. Aluminum produces neurotoxicity by many mechanisms.

Many of the manifestations of Al neurotoxicity have been previously reviewed (Meiri *et al.*, 1993; McLachlan, 1995). Following is a review of selected neurotoxic actions of Al that may relate to neurodegenerative diseases.

X. A. Aluminum promotes formation and accumulation of insoluble amyloid β protein.

Various mechanisms have been reported to mediate Al-induced promotion of Aβ (Figure 1). Intracerebral administration of 0.04 μmoles Al increased brain APP (Shigamatsu and McGeer, 1992), although Neill *et al. (*1996) found no change in APP mRNA and APP levels in Al-loaded cells and Al-injected rats. Based on the ability of Al to activate serine proteases, it has been suggested that it can increase formation of Aβ from APP (Clauberg and Joshi, 1993). Among 12 metals, Al was the only one that caused APP aggregation at 1 mM (Chong and Suh, 1995), a supraphysiological Al concentration.

Aβ is the 39-42 amino acid C terminus of APP. It is cytotoxic in mM concentrations. Aβ is

produced in most, if not all cells, and released as a soluble protein, in α-helical, random coil and other conformations. Amyloidosis is amyloid protein accumulation. In AD there is Aβ accumulation in blood vessels (cerebrovascular amyloidosis) and in the brain. Aβ is released from brain cells into brain extracellular space.

Senile plaques are extracellular structures consisting of an Aβ core surrounded by degenerative and regenerating neuronal axons and dendrites that may contain paired helical filaments (PHFs), glial cells and perhaps aluminosilicates. Senile plaques occur in the brain of non-demented elderly humans, but are more abundant in AD brain.

Soluble Aβ can be degraded by Ca-dependent metalloproteinases. Al was found to inhibit Aβ degradation by erythrocyte lysates, apparently by displacing Ca, suggesting it could increase brain Aβ exposure (Banks *et al.*, 1996). Several factors can increase the β-sheet conformation of Aβ, a polymer of Aβ filaments held together by hydrogen bonding which has a tendency to continue to aggregate. The β conformation is associated with amyloid aggregation and neurotoxicity. Al (≥ 50 μM) decreased α-helix and β-sheet conformations (Exley *et al.*, 1993), demonstrating its ability to change the secondary/tertiary structure of Aβ. Further studies showed Al, concentration dependently from 0-150 μM Al or at a 1-2 to 1 molar ratio of Al to Aβ, increased the β-sheet conformation and Aβ aggregation, (Vyas and Duffy, 1995; Fasman *et al.*, 1995; Laczko *et al.*, 1996). Aluminosilicates caused Aβ aggregation, suggesting they can serve as nucleation sites (Candy *et al.*, 1992). Similarly, 20 μM Al enhanced phosphate-induced formation of aggregated β sheets of an amyloid peptide fragment, suggesting Al phosphate may promote this process (Bondy and Truong, 1999). Al, 250 μM, was one of a few cations that caused Aβ aggregation and its deposition on cell surfaces (Mantyh *et al.*, 1993; Kawahara *et al.*, 1994).

A second protein in the plaque, non-Aβ component of AD amyloid, which appears to complex with Aβ, was found to be aggregated by Al. Its cleavage was inhibited by 1-4 mM Al (Paik *et al.*, 1997), potentially disrupting normal protein turnover and producing abnormal protein degradation.

Aβ protein, presumably after it polymerized, formed a cation channel in an artificial membrane, which was blocked by 10-20 μM Al (Arispe *et al.*, 1993). The 25-35 amino acid fragment of Aβ (Aβ 25-35) also formed an ionophore that allowed Ca influx. It was inhibited by 10 μM Al (Sanderson *et al.*, 1997) and was believed to be different from that described by Arispe *et al.* These channels allow Ca influx into the cell, increasing intracellular free Ca, potentially producing cytotoxicity.

Reactive oxygen species (ROS) increased Aβ aggregation (Dyrks *et al.*, 1992). Aβ and Aβ 25-35 increased ROS production (Hensley *et al.*, 1994; van Rensburg *et al.*, 1997). Aβ-induced ROS generation was increased by Fe plus 500 μM Al (Bondy *et al.*, 1998). As discussed below, Al, in the presence of Fe, can increase ROS production. This raises the possibility that Al might cause Aβ aggregation by ROS production, augment the ability of aggregated Aβ to generate ROS, thereby enhancing Aβ aggregation to further promote amyloidosis-induced cytotoxicity.

X. B. Aluminum promotes aggregation of hyperphosphorylated tau protein.

Tau is a microtubule assembly protein in neurons that promotes tubulin polymerization and stabilizes microtubules. Abnormally phosphorylated tau is the principal protein subunit of the PHFs that comprise the NFTs of AD (Singer *et al.*, 1997; Drewes *et al.*, 1998).

In AD, there is formation of an abnormal, hyperphosphorylated tau. This forms aggregated, insoluble PHFs, which persist, suggesting resistance to dephosphorylation. These PHFs appear in the NFTs of AD (Figure 2).

Aluminum has been shown to act on a number of processes that promote the formation of PHFs. Al-EDTA, 250 μM, promoted phosphorylation of tau in neuroblastoma cells (Guy *et al.*, 1991), although 200 μM $AlCl_3$ did not produce this in cultured neurons (Mattson *et al.*, 1993). Increased hyperphosphorylated tau associated with elevated brain Al was seen in renal dialysis patients (Harrington *et al.*, 1994). Al, 50-300 μM, increased phosphorylated, aggregated tau protein *in vitro* (Abdel-Ghany *et al.*, 1993; Scott *et al.*, 1993; Shin *et al.*, 1994; Savory *et al.*, 1995), as well as

phosphorylated neurofilaments *in vivo* (Muma and Singer, 1996). This may result from Al inhibition of hyperphosphorylated tau proteolysis (Yamamoto *et al.*, 1990; Shin, 1997). It increased the antibody response to NFTs, including Tau-2, PHF-1 and Alz-50 (Jones and Oorschot, 1998; Savory *et al*., 1998). Alz-50 is believed to indicate conformational changes that precede or accompany the progressive modification and polymerization of tau proteins into PHFs. Murayama *et al.* (1999) provided evidence that Al binds to phosphorylated, but not dephosphorylated, tau protein. Al produces NFD in the rabbit, composed of 10 nm filaments containing tau and abnormally phosphorylated neurofilaments, not the PHFs seen in AD (Singer *et al.*, 1997). Several mechanisms have been reported to mediate the Al promotion of NFTs and NFD (Figure 2).

The above effects of Al on Aβ and tau protein suggest mechanisms by which Al may contribute to AD.

X. C. Alzheimer's disease is associated with deficits of cortical cholinergic neurotransmission, which are mimicked by Al.

The primary neurotransmitter disruption in AD is a reduction of cholinergic function, although numerous other neurotransmitter systems are affected. A decrease in high affinity choline uptake appears to occur in some AD victims (Pascual *et al*., 1991) and was produced by 500 μM Al (Johnson and Jope, 1986; Zubenko and Hanin, 1989). A decrease in cortical choline acetyltransferase activity is seen in AD, and the Al-exposed animal (Beal *et al.*, 1989; Gulya *et al.*, 1990). Using microdialysis, acetylcholine release was found to be lower in Al-loaded rabbits before and during acquisition of the eyeblink reflex (Meyer *et al.*, 1996). Each of these changes reflects effects detrimental to cholinergic neurotransmission.

X. D. Aluminum increases iron-induced oxidative injury

Aluminum is not a transition metal and does not undergo redox reactions *in vivo.* Alone, it has usually been found to not increase oxidative injury. However, increased ROS in the absence of added

Fe was found and attributed to electron leakage due to Al-enhanced mitochondrial activity and increased electron chain activity (Campbell *et al.*, 1999). Al, 100-400 μM, increased Fe-induced oxidative injury *in vitro* (Gutteridge *et al.*, 1985; Toda and Yase, 1998) and *in vivo* (Fraga *et al.*, 1990: Bondy and Kirsten, 1996). Brain oxidative injury is thought to contribute to neurodegenerative disorders, including AD (Christen, 2000). Aluminum increased generation of the hydroxyl radical, a ROS (Xie *et al.*, 1995). ROS attack almost all cell components, including membrane lipids, producing lipid peroxidation, a form of oxidative stress. Al, 500 μM, increased Fe-induced oxidative stress in cultured rat hippocampal neurons, reducing their survival (Xie *et al.*, 1996). It increased Fe-induced ROS, at 100 μM, and glutamate-mediated intracellular ^{45}Ca and free Ca in cerebellar granule cells, at 10 and 300 μM (Mundy *et al.*, 1997). Aluminum and Fe promoted Aβ-induced ROS generation, as noted above (Bondy *et al.,* 1998). Aluminum enhancement of Fe-induced oxidative stress is of concern in light of Al and Fe co-localization in NFTs (Good *et al.*, 1992a) and in Parkinson's disease (Hirsch *et al.*, 1991). Recent observations of Al-induced oxidative damage and the Al uptake into neurons, astrocytes, oligodendrocytes and related cells suggest greater Al uptake by glial cells and greater glial than neuronal toxicity. This suggests glial cells may be the primary target of Al-induced neurotoxicity (Campbell *et al.,* 1999; Golub *et al.,* 1999; Suarez-Fernandez, *et al.,* 1999).

X. E. Aluminum toxicity to plants, aquatic life and humans may share common mechanisms.

Mathematical modeling and studies with phosphatidylserine vesicles suggested Al would displace Ca from membrane surfaces (DeLeers, 1985). Al displaces Ca from the plasma membrane of dividing root cells of plants and from fish gills, causing membrane disruption (reviewed by Kochian and Jones, 1997 and Sparling *et al.*, 1997). Al affects other neuronal Ca regulatory proteins, including Ca/Mg-ATPase (Mundy *et al.*, 1994) and Ca currents (Koenig and Jope, 1987: Busselberg *et al.*, 1994). The inositol phosphate signaling system of plants and animals is adversely affected by Al. The primary manifestation appears to be inhibition of PIP_2 hydrolysis (Shafer and Mundy, 1995; Kochian and Jones, 1997). A proposed mechanism of this inhibition is Al substitution at the Ca binding

domain on phospholipase C (Kochian and Jones, 1997), suggesting a basic mechanism of Al toxicity.

X. F. The Al-induced increase in iron-induced free radical generation and disruption of calcium regulation may constitute some of the primary molecular mechanisms of Al-induced neurotoxicity.

The mechanisms of neurotoxicity for Cd, Pb, Hg, Mn, Hg and Sn involve multiple sites and insults to basic processes of cell function, such as ROS production and interference with Ca function (Atchison and Hare, 1994; Bressler and Goldstein, 1991; Koczyk, 1996; Silbergeld, 1992; and several chapters in Chang and Dyer, 1995). As elemental ions, this might be expected. It is likely that a metal would have multiple sites and mechanisms of toxicity, many due to disruption of some of the multiple functions of essential elements, such as Ca. It is less likely that metal toxicity would be at a specific receptor, disrupting specific processes, as seen with more structurally complex organic toxicants. It is probable that Al has multiple mechanisms of toxicity that impact on basic processes, rather than limited sites and mechanisms of toxicity. Therefore, it might be expected that these basic mechanisms of Al-induced toxicity occur over a wide range of animal and vegetable species.

XI. Should aluminum be avoided?

Al plays no essential role in mammals; therefore there is no risk of Al deficiency. It is harmful in those with reduced/no renal function, including the very young human and end-stage renal dialysis patient. It may be one of many factors contributing to non-familial AD and other neurodegenerative disorders, although this has not been satisfactorily demonstrated. As concluded by Health Canada and the United States Environmental Protection Agency, more research will be necessary to understand the potential of Al to cause neurotoxicity. Considering the potential for Al-induced neurotoxicity and the lack of a deficiency syndrome, it seems prudent to avoid Al exposure when practical.

Table 1. Aluminum detection methods

METHOD	DETECTION LIMIT
Instrumental neutron activation analysis (INAA)	1 ppb (μg/kg)
Electrothermal atomic absorption spectroscopy (AA)	1 ppb
Electron energy loss spectroscopy (EELS)	> 500 ppm (mg/kg)
Energy dispersive (electron probe) X-ray microanalysis (EDX)	> 20 ppm
Proton probe nuclear microscopy	> 10 ppm
Secondary ion mass spectrometry (SIMS)	1 ppm
Laser microprobe mass spectroscopy (LMMS; LAMMA)	1 ppm
Solachrome azurine stain	20 ppm
Morin stain	? ppb

Table 2. Studies of the relationship between drinking water Al concentration and dementias, including Alzheimer's disease.

REFERENCE[a]	ENDPOINT	DRINKING WATER [Al] in µg/l	NUMBER OF SUBJECTS	RISK
Vogt, 1986	Death from senile dementia	200 vs 20	18,664	1.5*
Wood *et al.*, 1988	Mental test in hip fracture patients	180-250 vs <50	386	N/D
Martyn *et al.*, 1989	Probable AD, based on CT scan	>110 vs <10	1185	1.5
Flaten, 1990	Dementia on death certificate	>200 vs <50	14,727	1.4 *†, females only
Michel *et al.*, 1990	Probable AD	100 vs 10	40	≈ 4*†
Neri and Hewitt, 1991	Diagnosis of AD	≥ 200 vs <10	2344	1.5*
Wettstein *et al.*, 1991	Mini Mental Status Exam (MMSE)	98 vs 4	805	N/D
Jacqmin *et al.*, 1994	Cognitive Impairment (<24 in MMSE)	100 vs 5	3777	1.35 @ pH 7 0.5 @ pH 8.5
Forster *et al.*, 1995	AD vs controls	>149 to <50	109 AD	N/D
Forbes *et al.*, 1995	AD	≥336 vs ≤67	2191	2.4
McLachlan *et al.*, 1996	AD vs non-AD	>100 vs <100	296 AD	1.7
Martyn *et al.*, 1997	AD vs non-AD radiological exam	≥110 vs <15	106 AD	N/D

[a] in chronological order of publication
* statistically significant
N/D not statistically different
† an Al concentration dependent effect

Table 3. Al in the brain of Alzheimer disease subjects, determined by bulk brain analyses.

REFERENCE[a]	METHOD	# OF SUBJECTS		RESULTS
		AD	CONTROL	
Crapper *et al.*, 1973	AA	5	3	Heterogeneous distribution within subjects Some overlap of AD & control values Grand mean AD 4 times controls
Crapper *et al.*, 1976	AA	10	7	Continuation of above 28% of AD values > 3 SD above control value mean
Trapp *et al.*, 1978	AA	4	4	Grand mean AD 1.4 times controls *
McDermott *et al.*, 1979	AA	10	9	No difference AD vs control
Crapper *et al.*, 1980	AA			Al in nuclear and heterochromatin fractions of AD 2 times control values
Yasui, 1980	INAA	3	3	Non-significant, 1.4 times, elevation in AD
Traub *et al.*, 1981	AA	7	4	4 of 7 AD brains in control range, abnormal levels only minimally elevated
Markesbery *et al.*, 1981	INAA	12	28	Mean and median of control 1.3 times AD, maximum AD value > max control value
Yoshimasu *et al.*, 1985	INAA	4	6	Significant elevation in AD
Ward and Mason, 1987	INAA	28 30	30 30	Canadian samples: mean AD 8-10 times controls * UK samples: mean AD 3 times controls *
Jacobs *et al.*, 1989	AA	6	4	Control mean 2 times AD mean
Lukiw *et al.*, 1991	AA			Al in dinucleosome fraction 3 times control values
Xu *et al.*, 1992	AA	10	10	Approx 2 times as much in hippocampus, inferior parietal lobule and temporal gyri of AD than control *
Edwardson *et al.*, 1992	AA	8	8	Control mean 1.2 times AD mean
Andrasi *et al.*, 1995	AA	9	20	10 brain regions: AD 2.4-8.1 times control, overall AD 3.8 times control *
Bjertness *et al.*, 1996	AA	16	14	No difference

[a] in chronological order of publication

* statistically significant

Table 4. Aluminum in plaques, determined by microprobe analysis and staining

REFERENCE [a]	METHOD	# OF SUBJECTS		RESULTS
		AD	CONTROL	
Nikaido *et al.*, 1972	EDX			Al not detected
Duckett and Galle, 1980	EDX	18	3	Highest Al seen in plaques & lipofuscin granules in degenerating cells of all brains
Masters *et al.*, 1985	EDX			Al & Si in isolated, intact plaque cores
Candy *et al.*, 1986	EDX			Al & Si in plaques of AD and mentally normal - Al 4-19% of plaque core
Candy *et al.*, 1986	SIMS	3		Al in *in situ* plaque cores
Stern *et al.*, 1986	LMMS	3		Unable to see Al in purified plaque cores
Mori *et al.*, 1988	EDX			Modest increase in some plaque cores & rims
Jacobs *et al.*, 1989	EDX	7		Al not detected
Larsson *et al.*, 1990	Proton microprobe			Al not detected
Moretz *et al.*, 1990	EDX			No significant difference in AD brain
Chafi *et al.*, 1991	EDX			Al not detected
Landsberg *et al.*, 1992	Proton (nuclear) microscopy techniques	5	2	<10% of plaque cores had Al, Al also detected in background and control tissue; Al not detected in plaque cores of unstained tissue
Senitz and Bluthner, 1990.	Morin	3		Al detected in dense core of plaque
Favarato *et al.*, 1992	Morin	5		Staining of plaque core
Kasa *et al.*, 1995	Solachrome azurine	10	5	Moderate-intense staining of core and/or rim of some, not all, plaques

[a] in chronological order of publication

Table 5. Aluminum in neurofibrillary tangles, determined by microprobe analysis and staining

REFERENCE[a]	METHOD	# OF SUBJECTS		RESULTS
		AD	CONTROL	
Terry and Peña, 1965	EDX	1		Al not detected
Perl and Brody, 1980	EDX	3	3	Al seen in nucleus & cytoplasm of NFT-positive cells in AD (91 & 29%) and controls (90 & 11%), but not in non-NFT-bearing neurons (2 - 6%)
Masters *et al.*, 1985	EDX			Excessive Al seen
Jacobs *et al.*, 1989	EDX	7		Al not detected
Stekhoven *et al.*, 1990	EDX n=5 LMMS n=3	5		Al not detected
Moretz *et al.*, 1990	EDX	3		Unable to demonstrate significant Al
Chafi *et al.*, 1991	EDX SIMS			Al not detected
Landsberg *et al.*, 1991	Proton microprobe			Al not detected
Good *et al.*, 1992b	LMMS	10	4	In neurons with NFTs, Al in NFTs > cytoplasm, nucleus & neuropil
Sparkman, 1993	EDX			Al detected in NFTs, but not consistently in PHFs
Lovell *et al.*, 1993	LMMS	7	5	Grand mean [Al] AD cytoplasm of NFT-bearing neurons (2.9 μg/gm), non-NFT-bearing neurons (2.3); control neuron cytoplasm (1.85) [Al] > 3 σ above control means: AD neurons 9.6-14.3%, control 1.3-1.5%
Bouras *et al.*, 1997	LMMS	4	3	Al & Fe seen in NFTs in hippocampus and inferior temporal cortex, and nuclei of NFT-bearing and NFT-free cells of AD cases
Reusche, 1997	LMMS			Al not detected
Makjanic *et al.*, 1998	Nuclear microscopy			Al seen in neurons and neuropil of fixed, osmicated tissue Al not see in unstained, untreated tissue
Kasa *et al.*, 1995	solachrone azurine	10	5	Weak staining of cortical and hippocampal NFTs

[a] in chronological order of publication

FIGURE CAPTIONS

Figure 1. Amyloid β (Aβ) protein is derived from amyloid precursor protein (APP) in peripheral and central cells. It can aggregate and become incorporated into senile plaques, and form a Ca channel. Al can increase this process at several steps.

Figure 2. Phosphorylated tau protein can aggregate and be incorporated into neurofibrillary tangles (NFTs) in the human and in neurofibrillary degeneration (NFD) in the rabbit. Al can increase this process at several steps.

FIGURE 1

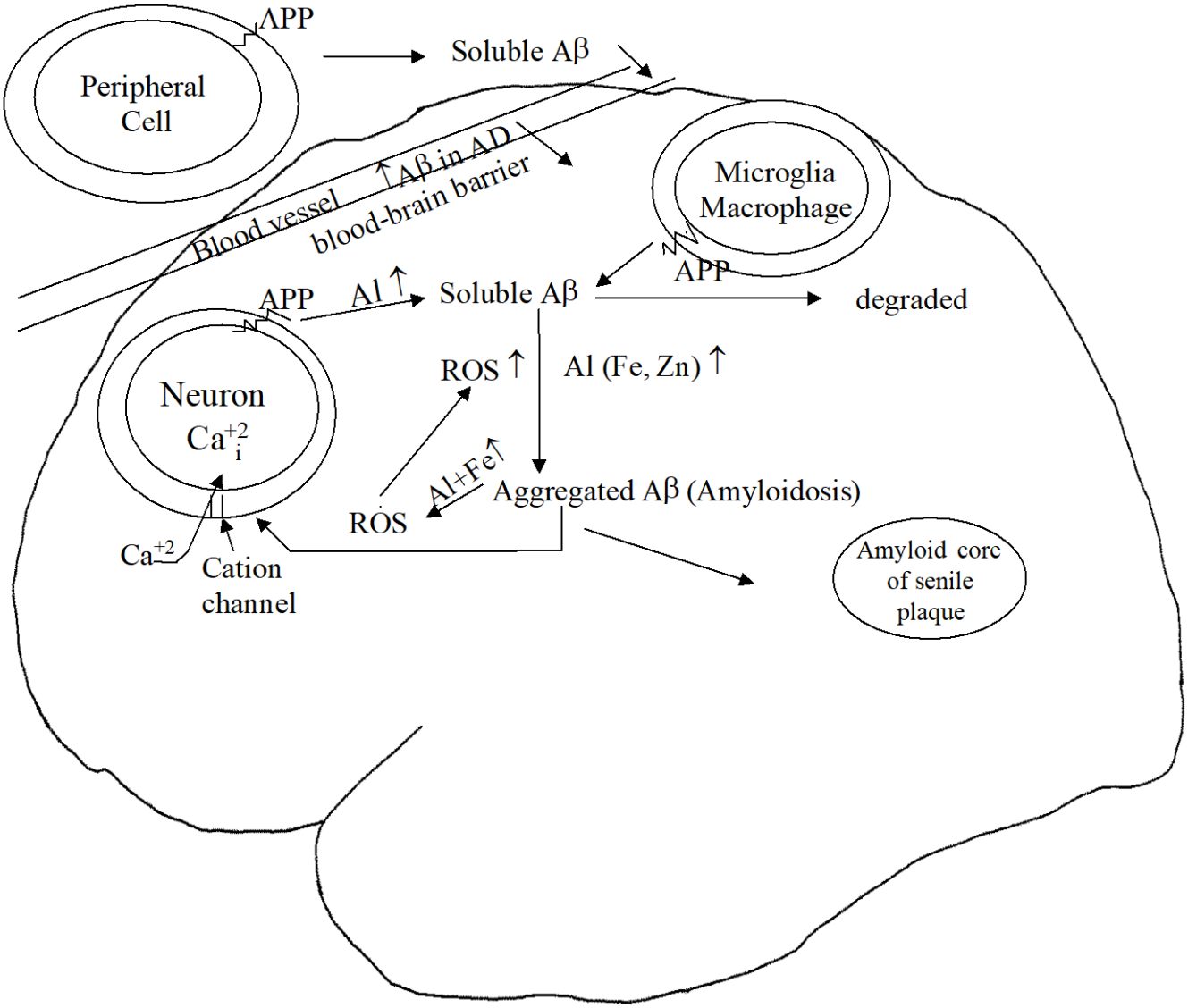
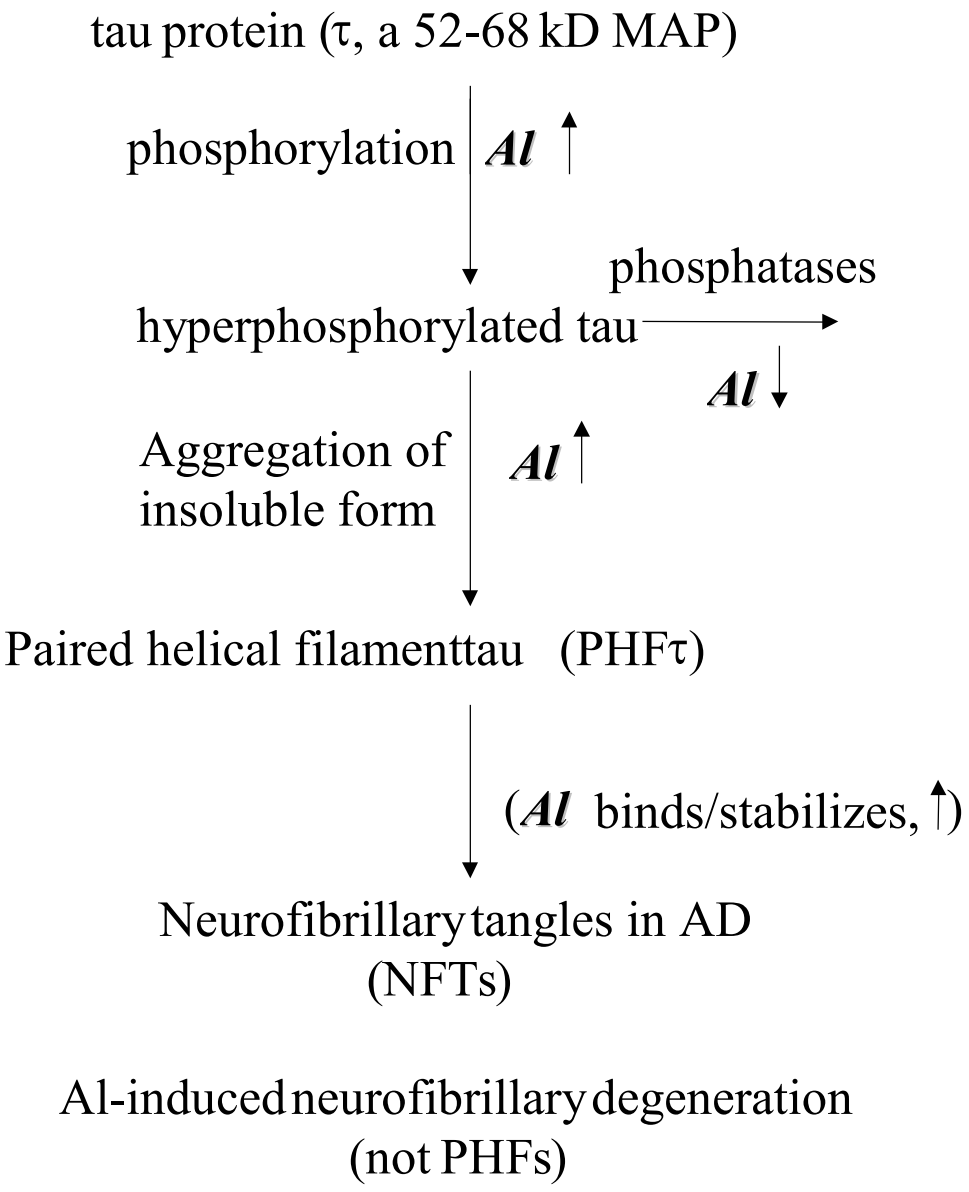

APP
Soluble Aβ
Peripheral Cell
Aβ in AD
Blood vessel
blood-brain barrier
Microglia Macrophage
APP
APP
Al
Soluble Aβ
degraded
ROS
Al (Fe, Zn)
Neuron
Ca+2 i
Al+Fe
ROS
Aggregated Aβ (Amyloidosis)
Ca+2
Cation channel
Amyloid core of senile plaque

FIGURE 2


tau protein (τ, a 52-68 kD MAP)
phosphorylation
Al
phosphatases
hyperphosphorylated tau
Al
Aggregation of
insoluble form
Al
Paired helical filamenttau (PHFτ)
(Al binds/stabilizes, ↑)
Neurofibrillary tangles in AD
(NFTs)
Al-induced neurofibrillary degeneration
(not PHFs)

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