

## Review Article

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# Molecular toxicity of aluminium in relation to neurodegeneration

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**Exposure to high levels of aluminium (Al) leads to neurofibrillary degeneration and that Al concentration is increased in degenerating neurons in Alzheimer's disease (AD). Nevertheless, the role of Al in AD remains controversial and there is little proof directly interlinking Al to AD. The major problem in understanding Al toxicity is the complex Al speciation chemistry in biological systems. A new dimension is provided to show that Al-maltolate treated aged rabbits can be used as a suitable animal model for understanding the pathology in AD. The intracisternal injection of Al-maltolate into aged New Zealand white rabbits results in pathology that mimics several of the neuropathological, biochemical and behavioural changes as observed in AD. The neurodegenerative effects include the formation of intraneuronal neurofilamentous aggregates that are tau positive, oxidative stress and apoptosis. The present review discusses the role of Al and use of Al-treated aged rabbit as a suitable animal model to understand AD pathogenesis.**

**Key words** Aluminium - animal model - Alzheimer's disease - neuropathology

## Introduction

Alzheimer's disease (AD) is a challenging neurodegenerative disorder. Neither AD aetiology nor the onset of AD pathology is totally understood. The major three pathological features, namely the extracellular deposition of the amyloid  $\beta$  protein ( $A\beta$ ), the formation of intraneuronal neurofibrillary tangles (NFTs) and selective neuronal loss are predominantly observed in AD neurodegeneration<sup>1,2</sup>. Although the cause of AD remains poorly understood, multiple factors are reported to influence AD onset. The primary among these, are mutations in the  $A\beta$  precursor (APP) and presenilins 1 and 2 (PS1 & PS2) that lead to increase in the production of the 42-residue  $A\beta$  ( $A\beta_{42}$ ). The E4

allele of apolipo-protein E is the most prevalent risk factor in addition to levels of cholesterol, homocysteine and several minor metal ions such as Al, Cu, Fe, are linked to AD. The contributions of neurotoxicity of Al in experimental animals were first reported in 1897 by Dollken<sup>3</sup>. The modern understanding of the effects of Al in experimental animal initiated by the extraordinary discovery of Klatzo *et al*<sup>4</sup> who reported that injections of Al salts into the rabbit brain leads to the formation of NFTs<sup>5,6</sup>. It is hypothesized that rabbits may be particularly relevant to the investigation of human disease since they belong to the mammalian order *Lagomorpha* that more closely resembles primates than rodents<sup>7</sup>. It has been shown that rabbits may provide a

unique animal system for producing neurofibrillary pathology<sup>4</sup>. Similar observations are reported in cats by Crapper *et al*<sup>8</sup>. Further, there is evidence that Al is neurotoxic, both in humans as well as in experimental animals<sup>9</sup>. It has been also shown that Al salts administered intracerebrally or peripherally in rabbit<sup>4</sup>, cat<sup>8</sup>, mice<sup>10</sup>, rat<sup>11</sup> and monkey<sup>12</sup> induce the formation of neurofibrillary tangles. This is used as a major argument that Al is one of the contributing factors in several neurodegenerative disorders, mainly AD. Further, the molecular understanding of Al neurotoxicity is hampered due to the speciation chemistry of Al.

### Al speciation chemistry

Al speciation chemistry is a very complex phenomenon. In solution, Al undergoes hydrolysis at pH 7.0. It undergoes precipitation to form Al(OH)<sub>3</sub> at pH 7.0 which makes the preparation of Al stock solutions difficult. Al solubility is enhanced under acidic or alkaline condition. In aqueous solution at pH < 5.0, Al exists as an octahedral hexahydrate, [Al(H<sub>2</sub>O)<sub>6</sub>]<sup>3+</sup>, usually abbreviated as Al<sup>3+</sup>. As the solution becomes less acidic, [Al(H<sub>2</sub>O)<sub>6</sub>]<sup>3+</sup> undergoes successive deprotonations to yield different species such as [Al(OH)]<sup>2+</sup>, [Al(OH)<sub>2</sub>]<sup>+</sup> and Al(OH)<sub>3</sub><sup>13,14</sup>. Neutral solutions give a Al(OH)<sub>3</sub> precipitate that re-dissolves, owing to the formation of tetrahedral aluminates, [Al(OH)<sub>4</sub>]<sup>-</sup>, the primary soluble Al (III) species at pH > 6.2, the biological pH<sup>15</sup>. Hence, one cannot compute the soluble Al concentration of the solution simply by adding a known quantity of an Al compound to water, without taking hydrolysis reactions into account. For example, when Al inorganic salts such as chloride, sulphate, hydroxide or perchlorate are dissolved in water at a calculated concentration of 10 mM, the exact Al concentration after pH adjustment and filtration is about 50 µM. The use of Al-lactate or Al-aspartate, however, increases the soluble Al concentration to about 55-330 µM, and use of Al-maltolate or gluconate increases the soluble Al concentration to 4000-6000 µM<sup>16</sup>.

The role of Al in AD still remains a mystery even after many decades of research. This is because of its intrinsic difficulties in understanding the role of chemical speciation in biological systems. Hence to understand the toxicity of Al, an appropriate Al compound is necessarily important. The electroneutral Al-maltolate (Al(mal)<sub>3</sub>) complex<sup>17</sup> looks to be ideal, since this compound can deliver a significant amount of free aqueous Al at physiological pH<sup>13</sup>. Unlike, most other Al salts, such as AlCl<sub>3</sub>, produce insoluble complexes at neutral pH<sup>13</sup>. Al-maltolate increases the

soluble Al concentration from 4-6 mM compared to other organic Al salts like Al-lactate or Al-aspartate (soluble Al concentration is ~55-330 µM). Al-maltolate is soluble and stable from pH 3.0 to 10.0, possesses hydrolytic stability at pH 7.0 and it has no speciation chemistry problems<sup>13</sup>. Al-maltolate is suitable over other Al compounds because of its very high metal solubility at pH 7.0, and prominent kinetic restrictions to ligand exchange reactions in neutral solution<sup>18,19</sup>. Based on our own contributions and those of Savory *et al* it is understood that Al-maltolate is suitable compound for toxicological studies and neuro-pathology in relevance to AD<sup>20,21</sup>.

### Al loading in humans

Al is ubiquitous, but its bioavailability is limited due to its insoluble nature. Because of the insoluble nature of Al, naturally occurring surface and subsoil water is extremely low in Al content and biosystems have little exposure to soluble Al<sup>22</sup>. This has played a key role in keeping a low Al burden in biosystem. However, in pathological conditions, an increased amount of Al has been found in biological systems. Al is widely used in our day-to-day life. One of the possible major sources of human Al consumption is through food, drinking water, beverages and Al-containing drugs<sup>23</sup>. Al-sulphate is used extensively as a flocculation agent to remove organic substances. It is estimated that the dietary intake of Al can be from 3 to 30 mg/day. Al is naturally present in tea leaves. The reported concentration of Al is 0.3 per cent Al in older leaves and about 0.01 per cent in younger ones<sup>24,25</sup>. Typical tea infusions contain 50 times as much as Al as do infusions from coffee. Levels of Al in brewed tea are commonly in the range of 2-6 mg/l<sup>25</sup>. The other sources of Al are food additives, containers, cookware, utensils and food wrappings. Dietary intake of Al from food is small compared with the amounts consumed through the use of Al containing antacids that may provide doses of 50-1000 mg/day<sup>26,27</sup>.

A very recent study showed that glue sniffing is an important problem among teenagers<sup>28</sup>. The investigators determined the serum levels of Al in glue-sniffing adolescents in comparison with healthy subjects. Also, they computed Al levels of different commercial glue preparations (*i.e.* metal and plastic containers). The Al level in serum was 63.29 ± 13.20 and 36.7 ± 8.60 ng/ml in glue-sniffers and in control subjects, respectively. The average Al level in the glues was 8.6 ± 3.24 ng/g in the preparations stored in metal containers, and it is

$3.03 \pm 0.76$  ng/g in plastic containers<sup>28</sup>. The study substantiates the potential Al toxicity in humans. Yet, another study clearly showed that occupational Al exposure could cause neurobehavioural changes. Further, a definite relation was observed between urinary Al concentrations of  $135 \mu\text{g/l}$  and cognitive performance<sup>29</sup>.

#### Al in human brain and cerebrospinal fluid (CSF):

We<sup>30</sup> reported the levels of trace metals concentration of Fe, Zn and Cu in moderate and severely affected AD brain samples<sup>30</sup>. The levels of Fe, Zn, Cu in frontal cortex of control human brain were  $0.9 \pm 0.01$ ,  $0.1 \pm 0.001$ ,  $0.1 \pm 0.01$  ( $\mu\text{g/g}$ ) respectively. And the concentration of Fe, Zn and Cu in moderately affected AD brain were  $6.3 \pm 0.68$ ,  $7.7 \pm 1.1$  and  $0.02 \pm 0.01$  respectively<sup>30</sup>. But in the case of severely affected AD brain, Fe concentration was  $240 \pm 14$ , but Zn and Cu levels were  $0.08 \pm 0.001$  and  $0.03 \pm 0.01$  respectively<sup>30</sup>. But in the case of severely affected AD brain, Fe concentration was  $240 \pm 14$ , but Zn and Cu levels were  $0.08 \pm 0.001$  and  $0.03 \pm 0.01$  respectively. The concentration of Al in the hippocampus region of moderately and severely affected AD was  $7.6 \pm 0.96$  and  $9.22 \pm 18$  respectively which was higher compared to ( $4.2 \pm 0.7$ ) control. The concentration ( $\mu\text{g/g}$ ) of metals like Fe, Cu and Zn in senile plaques of AD brain was  $52.4 \pm 14.5$ ,  $25.0 \pm 7.8$  and  $69.0 \pm 18.4$  respectively<sup>31</sup>. Recently, Walton<sup>32</sup> clearly showed the presence of Al in hippocampal neurons in AD brain and further indicated the subcellular localization using a new staining technique.

Normal and AD-CSF samples were analyzed for Al, S, Na, Mg, Fe, Co, Cu, Mn, Cr, K, Ca, Zn and P using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES). The results showed that Al, Mg, Mn and Ca levels do not show change between normal and AD-CSF<sup>33</sup>. However, K, P, and S were significantly decreased in AD-CSF over normal, while Na level was significantly increased in AD-CSF. Mole percentage ratio of selected elements namely, Na/Fe, Ca/Fe, Al/Fe, Mg/Fe, Na/P, Na/K, Na/S, K/P, Ca/P, K/S, Ca/K, Co/Fe, Ca/S, Al/P, Al/K, Mg/P, Mg/S, Al/Zn, Fe/Cu, Fe/S, Zn/Cu showed a definite increase in AD-CSF over normal. The comparative assessment of the total percentage of charge distribution between normal and AD-CSF indicated that in AD-CSF, the percentage charge distribution of divalent and trivalent ions was moderately decreased, while monovalent charge distribution was moderately increased compared to normal<sup>33</sup>. The comparison of these CSF results with AD

and normal brain showed definite relations (direct or inverse) for selected elements, and these findings are new and novel.

#### Differences in Al compounds in inducing AD neuropathology

Treatment with different Al-compounds to induce neuropathology have yielded several interesting observations. The studies on a variety of Al salts such as Al lactate,  $\text{AlCl}_3$ , AlF and  $\text{AlSiO}_4$  on aged rabbits, showed that neurofibrillary aggregates (NFAs) are most striking in the nucleus *motoris medialis* and *substantia grisea intermedia*: the large neurons of the nucleus of the motoris lateralis are minimally involved<sup>34</sup>. These results indicate that Al-inorganic complexes do not mimic AD neuropathology in its distribution of pathology. However, Al-organic and Al-inorganic complexes administered to different animal groups like cats, ferrets and dogs also did not mimic the AD neuropathology. But, Al-maltolate treated aged rabbits displayed NFTs in the axons imaged in hippocampal neurons, which follows the distribution of these lesions in AD<sup>35</sup>. Other studies also reported that Al-maltolate is comparatively more efficient than the other Al-complexes. The mRNA fraction obtained from the brain polysomal RNA is more active in Al-maltolate exposed compared to Al-lactate and the control young rabbits<sup>36</sup>. Al-maltolate enhances the bioavailability of Al in the brain. Thus, it is quite reasonable to speculate that some positively charged constituents such as Al aid in the formation and stabilization of the NFA's, both in AD and in experimental (Al-maltolate induced rabbits) induced NFAs.

#### Assessment of NFT in Al-induced neurodegeneration

Intraventricular administration of Al-maltolate to rabbits developed widespread neurofibrillary degeneration involving pyramidal neurons of the isocortex and allocortex, projection neurons of the diencephalon, and nerve cells of the brain stem and spinal cord<sup>37</sup>. Perikarya and proximal neurites are especially affected. Bundles of 10 nm filaments are frequently present. The animals treated intravenously for 12 wk or longer displayed NFAs in the occulo-motor complex and in the pyramidal neurons of the occipital cortex. These findings indicate that intraventricular Al-maltolate produces similar, but more widespread degeneration of projection-type neurons than the less water-soluble Al compounds as reported by Katsetos *et al*<sup>37</sup>. NFD has been compared with those of senile dementia of the Alzheimer type (SDAT) and motor neuron disease<sup>37</sup>. Widespread argyrophilic NFAs are

seen in a number of brain regions in Al-treated aged and young rabbits. Moreover, quantitatively the aged animals are affected to a much greater extent suggesting that an active mechanism is involved in suppressing Al-maltolate toxicity that is diminished in the ageing brain. NFAs are observed mostly in the superior cortex, lateral and inferior cerebral cortices, at the level of the superior and the inferior hippocampus also the striatum pyramidal subiculum, superior and inferior segments of hippocampus<sup>38,39</sup>. Using monoclonal antibody (mAb) PHF-1, robust positivity of the NFD is observed in the inferior segment of hippocampus and in cerebral cortical neurons of aged Al-treated rabbits<sup>38</sup>. Savory's group have reported that intracisternal Al administration induces NFD most strikingly in the medulla and upper spinal cord<sup>38-40</sup>. The brain regions are less affected in the case of Al-maltolate treated young rabbits compared to aged ones.

Garruto *et al*<sup>41</sup> carried out imaging of Al in NFT-bearing neurons within Sommer's sector of the hippocampus in Guamanian patients, using a method of computer-controlled electron beam X-ray microanalysis and wavelength dispersive spectrometry<sup>41</sup>. Al is distributed in cell bodies and axonal processes of NFT-bearing neurons. The elemental images showed that Al deposits occur within the same NFT-bearing hippocampal neuron, suggesting this element involvement in NFT formation<sup>41</sup>. No prominent concentrations of Al were imaged in non-NFT-containing regions within the pyramidal cell layer compared to control cases.

The interesting work carried out by Savory and his co-workers on the quantitation of Al in the brain and spinal cord and its effects on neurofilament protein expression and phosphorylation provided new evidence for the involvement of Al in AD<sup>42</sup>. When aged rabbits were treated with Al-maltolate, differential accumulation was observed yielding about 10 µg/g dry tissue in the brain and spinal cord but only 2.1 µg/g dry tissue in the lumbar cord. In addition, argyrophilic tangles were observed in perikarya and proximal neurites of neurons as far distal as the lumbar and sacral cord areas<sup>42</sup>. Immunoblot studies failed to detect changes in three neurofilament protein isoforms, and also no significant alterations in the total phosphate content of these proteins were observed, the genes encoding for the 200 and 68 KDa neurofilament proteins also were unaffected upon Al-maltolate treatment<sup>42</sup>.

In the case of Al-maltolate treated aged rabbits, amyloid precursor protein (APP), A $\beta$ , neurofilament

protein like unphosphorylated tau,  $\alpha$ -1 antichymotrypsin and ubiquitin are observed, while, in AD in addition to the above features neurofilament protein is hyperphosphorylated<sup>40,43</sup>. Abnormally phosphorylated tau<sup>44</sup> present in these NFAs are quantified using a variety of monoclonal antibodies that recognize both nonphosphorylated and phosphorylated tau<sup>40,46</sup>. Immunostaining with Tau-1, Tau-2, AT8, PHF-1 and Alz-50 indicated that both nonphosphorylated and phosphorylated tau are present. Moreover, these aggregates are detectable by silver staining within 24 h of Al-maltolate administration, and neurofilament proteins predominate. Tau is also detectable by 72 h, although the characteristic epitopes of AD as recognized by mAbs, AT8 and PHF-1 are most distinct at 6-7 days following Al injection. It is also proposed that phosphorylation of cytoskeletal proteins drives the formation of the NFAs particularly in AD<sup>46</sup>. Based on thermodynamics, one would predict that hyperphosphorylation and the associated negative charges will lead to the destabilization of these aggregates. Thus, it is quite reasonable to speculate that some positively charged constituents such as metal ions aid in the formation and stabilization of the NFAs, both in AD and in experimental Al-maltolate induced NFAs. In the latter, Al is an obvious candidate for this role<sup>47</sup>. Thus there are marked differences in the composition of the intraneuronal lesions seen in AD and in experimental Al neurotoxicity; hence, Al-induced lesions and those found in AD are originally surmise.

#### **Characteristics of tangles associated with Al treated aged rabbits in comparison with AD**

Al induced NFTs in rabbits do not share all morphologic and biochemical features with the neurofibrillary tangles of AD, but these nevertheless exhibit noteworthy similarities. Although Al induced tangles differ from those of AD in their distribution at both gross and ultrastructural levels, while both types of tangles are found in the cortex and hippocampus<sup>4,48,49</sup>. Al induced tangles are found in the perikaryon and proximal parts of the dendrites and axon<sup>50,51</sup>. In contrast, AD tangles are found throughout the neuron, including the entire length of the dendrites and throughout the axons including the terminals. Al induced tangles are made up of straight 10nm diameter neurofilaments. The protofilament building blocks of Al tangles also differ from those of AD with the diameter of the former 2.0 nm and the latter 3.2 nm. The peptide composition of Al-induced tangles is chiefly neurofilament protein whereas AD paired helical filaments are composed

primarily of hyperphosphorylated tau (a microtubule associated protein) and ubiquitin<sup>12,38,52</sup>. Further, subsequent work carried out by Klatzo *et al*<sup>4</sup> showed that the similarities between Al induced tangles in rabbits and those of AD are more apparent<sup>4</sup>. Furthermore as reviewed by Wisniewski *et al*<sup>50,53,54</sup> Al induced tangles and AD pathology appeared similar only if the tissue is treated with silver staining.

### Al induced neurochemical changes

Some of the cellular processes like oxidative stress, apoptosis and NFT formation, involved in neurodegeneration were induced by Al-maltolate in aged New Zealand white rabbits through intravenous administration. Based on the recent literature data available on the Al-maltolate induced neuropathology have focused on the neuronal injury resulting in the understanding of neuropathogenesis in relevance to AD.

*Oxidative stress:* Al induces oxidative stress. The time and extent of oxidative changes overlap in both Al-maltolate treated and in AD. These are important observations and have important implications in our understanding of the pathogenesis of neurodegeneration in AD. Savory *et al*<sup>43,55</sup> showed that oxidative stress products are released in the *striatum pyramidale hippocampi* and *nucleus lateralis dorsalis thalami* region. We hypothesized that there will be diminished vesicular transport due to Al-maltolate injection which leads to reduced microtubule transport and in turn decrease in axonal mitochondria with increased turnover in the cell body. Also, there may be disruption of the Golgi and reduction of synaptic vesicles. The oxidative products released in the neurons are as follows, malondialdehyde, carbonyls, peroxy nitrates, nitrotyrosines, and enzymes like SOD, haemoxygenase-I, *etc.*<sup>56</sup>. Al levels and its relation to oxidative stress has been reported in glia, astrocytes, microglia, *etc.*<sup>57</sup>. The possible potential mechanism may be the nitration of tyrosine residues in cytoskeletal proteins such as tau mediated by peroxy nitrite breakdown leading to NFT formation. Good *et al*<sup>58,59</sup> demonstrated the presence of nitrotyrosine in neurons in AD, indicating that it is involved in the oxidative damage in AD. Al being a non-redox active metal, is believed to cause a lot of havoc via increasing the redox active iron concentration in brain. This is mainly through a Fenton reaction. Al is simultaneously an activator of SOD and an inhibitor of catalase, therefore superoxide radicals are readily converted to H<sub>2</sub>O<sub>2</sub> and the breakdown to H<sub>2</sub>O and O<sub>2</sub> by catalase is slowed down<sup>56,58,59</sup>, leading to the production of hydroxyl radicals. Thus, Al significantly

plays a role in neurodegeneration through oxidative stress.

*Apoptosis:* Some of the important biochemical events attributed to cell death associated with AD are decreased levels of Bcl-2<sup>60</sup>, increased levels of Bax, and high concentrations of peroxy nitrite products<sup>56,61,62</sup>. Several lines of evidences suggest that cell death induced by Al is apoptosis mediated<sup>63-66</sup>. Apoptosis is believed to be the general mechanism of Al toxicity to the cells. Al treatment induces the characteristic features of the apoptotic mechanism, which includes shrinkage of cell bodies, hypercondensed and irregularly shaped chromatin and extensive fragmentation of chromatin and DNA<sup>67-68</sup>. Al-maltolate and AlCl<sub>3</sub> induce chromatin condensation and DNA ladder formation in PC12 cells. Nerve growth factor (NGF) prevented both chromatin condensation and DNA laddering independently of ROS production<sup>69</sup>. Al induces apoptosis in the astrocytes further leading to the neuronal death by loss of the neurotrophic support<sup>70</sup>. Savory *et al*<sup>43</sup> have focused on the time course and the mechanism of apoptosis in both Al-maltolate treated and in AD brain, which resulted in the understanding of neuropathogenesis in relevance to AD. There is also an effect of Al-maltolate on the mitochondrial-mediated apoptosis pathway. Apoptosis, or programmed cell death, plays a critical role in normal development, maintenance of tissue homeostasis and is also a process by which brain cells die in neurotoxic situations. Mitochondrial changes following cytotoxic stimuli represent a primary event in apoptotic cell death. The apoptogenic factor, cytochrome c, is released from mitochondria into the cytoplasm where it binds to another cytoplasmic factor, Apaf-1. The formed complex then activates the initiator, caspase-9, that in turn activates the effector caspase – caspase-3. Release of cytochrome c from the mitochondria has been shown to involve three distinct pathways<sup>49</sup>. (i) Opening of the mitochondrial transition pore (MTP), (ii) Translocation of mitochondria of the pro-apoptogenic Bax which can form the channel by itself, and (iii) Interaction of Bax with the voltage dependent anion channel (VDAC) to form a larger channel which is permeable to cytochrome c.

Al has been demonstrated to accumulate in neurons following cell depolarization, where it inhibits Na<sup>+</sup>/Ca<sup>2+</sup> exchange and thereby induces an excessive accumulation of mitochondrial Ca<sup>2+</sup><sup>71</sup>. Increase in intra-mitochondrial Ca<sup>2+</sup> levels leads to an opening of the MTP with cytochrome c release and subsequent apoptosis resulting from activation of the caspase family of proteases. Studies have shown that the

intracisternal administration of Al-maltolate results in cytoplasmic cytochrome c translocation, Bcl-2 downregulation and bax upregulation and caspase-3 activation<sup>72,73</sup>. These results indicate that Al targets the mitochondria. Furthermore, it has been demonstrated that the release of cytochrome c, which is inhibited by cyclosporin A, (a specific inhibitor of the MTP opening), implicates the opening of the mitochondrial transition pore as the process by which cytochrome c translocates to the cytoplasmic space from mitochondria<sup>73-75</sup>. The use of pharmacological agents that prevent or reverse the apoptotic effects of Al can provide valuable mechanistic information on the effects of Al on cellular protein targets. Studies showed that chronic treatment of rabbits with lithium in the drinking water results in inhibition of the Al-induced cytochrome c release<sup>43</sup>, enhances levels of the anti-apoptotic proteins Bcl-2 and Bcl-XL, prevents the redistribution of the pro-apoptotic protein bax levels and inhibits caspase-3 activation and DNA fragmentation<sup>48,73-75</sup>. Al induces apoptosis in Neuro-2a cells with increased expression of p53, which shifts the Bcl/Bax ratio towards apoptosis<sup>76</sup>. Also recent studies showed that *Bacoppa* protects cells against Al toxicity<sup>77,78</sup>.

Although mitochondrial alterations may represent an important step in the mechanisms underlying neuronal cell death induced by Al-maltolate, studies by Dewitt *et al*<sup>79</sup> provided evidence suggesting that the endoplasmic reticulum (ER) also plays an important role in regulating this cell death. The ER is an important subcellular site, since it is the major storage location for calcium and contains members of the Bcl-2 family of proteins, Bcl-2 and Bcl-XL. The stress induced by Al-maltolate in the ER has also been shown to result in a specific type of apoptosis mediated by caspase-12 and is independent of mitochondrial-targeted apoptotic signals. Al-maltolate induces a redistribution of the apoptosis-regulatory proteins, with Bax being present at higher levels in the ER than in the cytosol and with decreased amounts of Bcl-2 in the ER<sup>79,80</sup>. It has also been reported that Al induces stress in the ER, as demonstrated by the activation of gadd 153 and its translocation into the nucleus<sup>79,80</sup>. Still, it remains unclear which signaling mechanisms lead to perturbation of ER homeostasis by Al-maltolate.

### Genotoxicity of Al

Al, being a non-physiological metal accumulates in the body, is dispersed in different regions of the cell. The major sites of localization are mitochondria,

lysosomes and nucleus in the cell<sup>81</sup>. The mechanism of Al toxicity to cells still remains unclear. Since Al is a Lewis base, it might bind to oxygen donors generated in the cell. It binds to biomolecules like nucleic acids, phosphate group of ATP and phosphorylated proteins and carboxylic groups of the molecules<sup>82</sup>. Walton<sup>32</sup> showed that Al is centrally localized in the nuclear region compared to other intracellular organelles. Thus, we emphasize the DNA damaging potential by Al and its possible mechanisms. Al acts as a pro-oxidant in the cells. Al induces DNA damage in the human peripheral blood lymphocytes at a concentration of 10 µg/ml. An increase in oxidized bases is observed in DNA at this concentration of Al as validated by digestion with formamido-pyrimidine DNA glycosylase. This indicates that the mechanism of DNA damage is oxidatively linked<sup>83</sup>. Al treatment results in the accumulation of lymphocytes in the S-phase of cell cycle. In the S-phase of cell cycle, DNA replication and chromatin unfolding occurs; making the DNA more susceptible to damage. Further, serum levels of acetyl cholinesterase, glutathione, and catalase and superoxide dismutase are reduced in Al treated rats. Al promotes oxidative stress in rat hippocampus and melatonin prevents this oxidative damage by an increase in the levels of antioxidant enzymes<sup>84,85</sup>. AlCl<sub>3</sub> treatment induces gaps and breaks in the chromosomes with higher frequency<sup>86,87</sup>. Antioxidant related enzyme levels were decreased in Al treated mice<sup>88</sup>. Al in PCD12 cells induces DNA strand breaks by the generation of reactive oxygen species (ROS), thus leading to apoptosis<sup>89</sup>. Al also plays a significant role in altering DNA repair mechanism. It inhibits DNA repair process by inhibiting the effect of DNA repair linked enzymes. It is also known that Al downregulates the DNA ligase gene<sup>83</sup>. Overall, Al induced oxidative DNA damage and apoptosis are interlinked, suggesting that the former precedes the latter and leads to neuronal cell death.

### Al-induced DNA conformational changes

Al causes unwinding of DNA. Al complexes with DNA showing altered melting temperature (T<sub>m</sub>) profiles<sup>82,90</sup>. Al-fluoride stimulates the glycation of Histone H1 at its nucleotide-binding site affecting its chromatin organization ability<sup>91</sup>. Al at very low concentration unwinds the supercoiled DNA irreversibly<sup>90</sup>. It was found that Al at high concentration decreases the rate of replication<sup>92</sup>. Al binds to the phosphate groups of the DNA backbone and at the N-7 position of guanine in GC rich base pairs<sup>93</sup>. Al at low concentration, enhance the T<sub>m</sub> of oligonucleotides

d(GCCCATGGGC) and d(CCGGGCCCGG). It also induces conformation (which is a rare phenomenon) in these oligonucleotides<sup>94</sup>. Our studies<sup>95</sup> showed an evidence for altered DNA conformation in the hippocampus of Alzheimer's disease affected brain. The circular dichroism spectra of severely affected AD DNA showed a typical left-handed Z-DNA conformation; whereas normal, young, and aged brain DNA have the usual B-DNA conformation. Moderately affected AD DNA has modified B-DNA conformation (B-Z intermediate form)<sup>95</sup>. Furthermore, studies from our laboratory also showed that Al levels are elevated in the serum samples of fragile X syndrome and also provided evidence for the interaction of aluminum with (CCG)<sub>12</sub> repeats which is involved in fragile-X-syndrome<sup>96</sup>. Circular dichroism spectroscopic studies of (CCG)<sub>12</sub> indicated B-DNA conformation, and in the presence of Al (10(-5) M) CCG repeats attained Z-DNA conformation<sup>96</sup>. It is interesting to mention that Al-induced Z-conformation is stable even after the total removal of Al from CCG by desferoximine, a chelating drug. Al-D-aspartate induces a topological change in supercoiled DNA converting native B-DNA to unusual C-DNA, a condensed form of DNA<sup>97</sup>. Thus the conformation changes induced by Al enhance susceptibility to DNA damage and gene expression changes that might lead to neuronal cell death in AD.

#### Effect of Al on gene expression

Al is also known to affect gene expression by altering the expression of cerebral proteases leading to cell death<sup>98</sup>. Al activates monoamine oxidase isotypes in rat brain<sup>99</sup>. The levels of mRNA of endogenous antioxidant enzymes have been decreased by Al treatment, indicating that Al affects the gene expression<sup>88</sup>. Al treated rats showed elevation of glial cell marker TNF alpha and glial fibrillary acidic protein (GFAP)<sup>100</sup>. Al treated brain rotation-mediated aggregate cultures revealed decrease gene expression of NGF, brain derived neurotrophic factor (BDNF) and decreased expression of TNF alpha<sup>101</sup>. Mouse brain overloaded with Al showed increased levels of Alzheimer's disease specific protein APP and A $\beta$ . There are increased levels of COX-2 mRNA and decreased levels of choline acetyl transferase protein<sup>102</sup>. Al induces the expression of NF- $\kappa$ B subunits, interleukin-1 beta precursor, phospholipase A2 and DAXX that are involved in the pro-inflammatory and pro-apoptotic signaling mechanisms<sup>103</sup>. AlCl<sub>3</sub> at 1  $\mu$ M concentration downregulated mitochondrial cytochrome c oxidase III, suggesting mitochondrial gene alteration<sup>104</sup>. Trace

amounts of aluminium decreased the RNA poly II activity inhibiting the transcription<sup>105</sup>. The probable mechanism of altering the gene expression is by binding to proteins involved in the gene expression. Al binds to transcription factor IIIA in the zinc finger domain and inhibits its promoter binding<sup>106</sup>. Aluminium sulphate upregulated a specific set of micro-RNAs (mi-RNAs) in the human brain cell cultures which are also found up-regulated in AD brain. These miRNA might effect the pathogenic gene expression changes leading to cell death<sup>107</sup>.

#### Role of Al on cell mediated excitotoxicity

It is now clear that accumulation of metals in AD brain may play a role in neuronal loss. Al, with an ionic radius of 54 ppm, could compete with other metal ions in binding with biomolecules, hence having the ability to replace other essential metals in biomolecules. Martin<sup>13</sup> showed that Al is likely to replace Ca, Mg and Zn. Our laboratory clearly showed that when Al and Fe concentrations are elevated in AD brain, the levels of other elements such as Na, K, Cu, Mg, Zn and Ca are decreased. The co-localization of Fe and Al may be attributed to the similar ionic radius to charge ratio of Al and Fe (Al 0.16: Fe 0.169).

#### Does Al acts through Fe-mediated oxidative stress

Al causes the mitochondrial damage leading to the generation of highly reactive oxy and hydroxy free radicals. Al enhances oxidative stress through enhanced iron-mediated Fenton reactions by increasing the redox-active iron concentration. Al may also cause accumulation of H<sub>2</sub>O<sub>2</sub><sup>108</sup>. And also Al activates superoxide dismutase, while it inhibits catalase. The increased H<sub>2</sub>O<sub>2</sub> pool enhances the presence of redox active iron either from loosely bound Fe or by modulating the electron transport chain<sup>108,109</sup>. This favours the enhancement of Fe-mediated oxidative stress. All these events lead to the generation of hydroxy free radicals and results in neuronal cell death by way of damage to DNA, proteins and lipids. Al promotes the iron induced ROS in the cells<sup>107</sup>.

#### Does Al enhance A $\beta$ production through oxidative stress linked pathways?

Al precipitates A $\beta$  *in vitro* which are distinct fibrillar structures composed of beta-pleated sheets of peptide. The aetiology of their association *in vivo* is not known. Al is known to increase the brain A $\beta$  burden in experimental animals and this might be due to a direct influence upon A $\beta$  anabolism or direct or indirect affects upon A $\beta$  catabolism<sup>110</sup>. It is difficult to rationalize from

an evolutionary perspective the precipitation and persistence of A $\beta$  *in vivo*. However, Al has not been subject to the same evolutionary pressures as A $\beta$ . It is an addition to the biotic environment and its precipitation of A $\beta$  may have only been subjected to natural selection in the recent past. The involvement of Al in the pathogenesis of AD cannot be discarded, especially when there is ample number of reports suggesting the role of Al linked to the amyloid dogma of AD<sup>108,110</sup>.

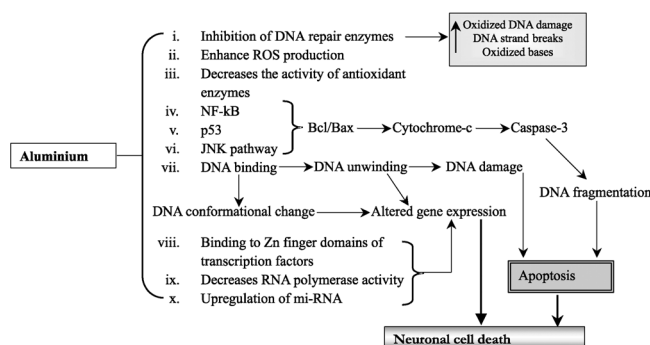
Further, whether oxidative damage increases A $\beta$  peptide production or vice versa is still a debatable issue. Several studies indicate that A $\beta$  and oxidative stress are inextricably linked to each other and Al enhances A $\beta$  production leading to aggregation<sup>108</sup>. Our own studies<sup>20</sup> indicated that Al first elevates oxidative stress, followed by redox active iron, apoptosis, NFT and A $\beta$  immunoreactivity. Since both Al and A $\beta$  peptide lead to increased production of H<sub>2</sub>O<sub>2</sub>, this favours redox-active iron, leading to oxidative stress and cell death. Both metal and A $\beta$  may be co-acting in cell death events. Experiments with aged rabbits showed that Al-maltolate is able to develop oxidative stress in hippocampal neurons leading to apoptosis<sup>111</sup>. The studies further indicated that Al-treated aged rabbit hippocampal neurons first express Bcl-2 (anti-apoptotic) protein in the first 3 h. Later, however, Al favours expression of Bax (pro-apoptotic) protein, accumulation of redox-active iron, presence of oxidative stress and final cumulative apoptosis<sup>39,112</sup>. We also critically analyzed the prospects of Al-amyloid cascade studies and other evolving lines of evidence that might shed insights into the link between Al and AD. Whether AD is also part of this ongoing selection process remains to be elucidated.

## Summary

Regardless of the circumstantial and sometimes ambiguous evidence on the hypothetical involvement of Al in the aetiology and pathogenesis of AD, and several lines of evidence have strongly supported the involvement of Al as a secondary aggravating factor or risk factor in the pathogenesis of AD. The lack of sensitivity to Al neurotoxicity in transgenic mouse models of AD has not allowed the system to be used to explore important aspects of this toxicity. Rabbits are particularly sensitive to Al neurotoxicity and develop severe neurological changes that are dependent on dose, age and route of administration. In this review, we discussed data from our laboratory and others, on the effects of Al on behaviour, neurologic function and morphology, using Al-maltolate administered to rabbits

via intracisternal route. We also focused on the similarities and dissimilarities between Al-induced neurofibrillary degeneration and paired helical filaments from AD and the prevalence of AD. We concluded that Al causes neurotoxicity in multifaceted way by modulating (i) Inhibition of DNA repair enzymes, (ii) Enhancement of ROS production, (iii) Decrease in the activity of antioxidant enzymes, and (iv) Alterations in NF-kB, p53 and JNK pathways. Al also binds to Zn finger domains of transcription factors, thereby decreasing RNA Polymerase activity and upregulating micro-RNA. All these events lead to genomic instability and cell death (Fig.).

A major question remains on whether APP plays a role in maintaining the homeostasis of metals such as Fe and Al, which can induce a variety of insults. Some of these insults lead to weakening of the cellular mechanisms for turnover of proteins and for prevention of aggregate accumulation. The intricate and complex biochemical events in the cell are highly regulated with many checks and balances. These may however be overcome by chronic or acute exposure to several environmental insults. We expect that some of the toxic consequences of metal ions like Fe and Al such as induction of oxidative stress are shared by peptides like amyloid oligomers. The biogenesis of neurofibrillary tangles remains an important question in understanding AD, but an important consideration is that NFTs are associated with a number of diseases such as dementia pugilistica while other causes remain unclear. Common mechanisms such as failure of protein folding and surveillance pathways have not yet been fully explored but remain important considerations.



**Fig.** Aluminium (Al) causes neurotoxicity in multifaceted way by modulating, (i) Inhibition of DNA repair enzymes, (ii) Enhance ROS production, (iii) Decreases the activity of antioxidant enzymes, (iv) altering NF-kB, p53, JNK pathway, DNA binding. Al also binds to Zn finger domains of transcription factors, thereby decreases RNA polymerase activity and upregulation of mi-RNA. All these events lead to genomic instability and cell death.

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