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BRAIN RESEARCH REVIEWS

Review

A new insight on Al-maltolate-treated aged rabbit as Alzheimer's animal model

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ABSTRACT

Lack of an adequate animal model for Alzheimer's disease (AD) has limited an understanding of the pathogenesis of the disease and the development of therapeutic agents targeting key pathophysiological processes. There are undoubtedly few satisfactory animal models for exploring therapies targeting at amyloid beta (Aβ) secretion, deposition, aggregation, and probably the inflammatory response. However, an understanding of the complex events – tau, Aβ, oxidative stress, redox active iron, etc. – involved in the neuronal cell loss is still unclear due to the lack of a suitable animal model system. The use of neurotoxic agents particularly aluminum–organic complexes, especially Al-maltolate, expands the scope of AD research by providing new animal models exhibiting neurodegenerative processes relevant to AD neuropathology. Examination of different species of aged animals including the rapidly advancing transgenic mouse models revealed very limited AD-like pathology. Most other animal models have single event expression such as extracellular Aβ deposition, intraneuronal neurofilamentous aggregation of proteins akin to neurofibrillary tangles, oxidative stress or apoptosis. To date, there are no paradigms of any animal in which all the features of AD were evident. However, the intravenous injection of Al-maltolate into aged New zealand white rabbits results in conditions which mimics a number of neuropathological, biochemical and behavioral changes observed in AD. Such neurodegenerative effects include the formation of intraneuronal neurofilamentous aggregates that are tau positive, immunopositivity of Aβ, presence of redox active iron, oxidative stress and apoptosis, adds credence to the value of this animal model system. The use of this animal model should not be confused with the ongoing controversy regarding the possible role of Al in the neuropathogenesis, a debate which by no means has been concluded. Above all this animal model involving neuropathology induced by Al-maltolate provides a new information in understanding the mechanism of neurodegeneration.

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Contents

1. Introduction

AD is a complex neurodegenerative disorder comprising complex neurobiochemical and neuropathological events, characterized by three typical pathological features, namely the extracellular deposition of Aβ (Selkoe, 1989, 1991; Hardy and Selkoe, 2002; Hardy and Higgins, 1992), the formation of intraneuronal neurofibrillary tangles (NFTs) (Doll, 1993; Perry and Perry, 1985; Perl and Brondy, 1980; Lovell et al., 1993; Wisniewski and Sofer, 1979), and selective neuronal loss. However, it is still unclear which of these pathological features is the primary event in the initiation and progression of this disease. The etiological factors of AD include genetics, head trauma, oxidative stress, infectious agents, and environmental factors including aluminum (Al) toxicity. The pioneering studies on neurotoxicity of Al in experimental animals were first reported in 1897 by Dollken (1897). Many scientific studies have brought to light the potential toxicity of Al in experimental animal models and in humans under different clinical conditions (Spafforth, 1921; McLaughlin et al., 1962). But the usage of Al in experimental animal came to light following the extraordinary discovery of Klatzo et al. (1965) who showed that injections of Al-salts into rabbit brain led to the formation of NFTs which appeared similar to the NFTs of AD (Klatzo et al., 1965; Terry and Peña, 1986). Later, these results were replicated in cats by Crapper et al. (1973). The complex chemistry of Al and the fact that there was no readily available radioisotope for experimental purposes thus hindered the clarification of this element's involvement in the etiology of AD. However, studies by Priest (2004) on humans and animal using the ^{26}Al radioisotope (Yumoto et al., 2001) have demonstrated that Al can indeed enter the central nervous system following systemic administration (Walton et al., 1995). In addition, there is documented evidence that Al is neurotoxic, both in human disease, as well as in experimental animals (Wills and Savory, 1983). Studies by Wen and Wisniewski (1985) histochemically localized Al in rabbit CNS further supported by Uemura (1984) illustrated intranuclear Al accumulation in chronic animals in turn led to neurofibrillary changes. Thereby Al salts administered intracerebrally or peripherally in rabbit (Klatzo et al., 1965), cat (Crapper et al., 1973), monkey (Games et al., 1995), rat (Brining et al., 1996), and dog (Uno et al., 1999) induce the formation of neurofibrillary aggregates (NFAs) which has contributed to the argument that Al is one of the contributing factor to several neurodegenerative disorders, mainly AD. However, this hypothesis remains controversial.

Although understanding of the complex events involved in neuropathogenesis and neurobiochemical events in AD requires the availability of suitable animal model systems. Understanding the neurodegeneration pathways in relationship to Aβ deposition, NFT and neuritic plaque formation using human tissue is limited since only a single time point, an intrinsic limitation resulting from the use of human

autopsy tissue. To date, examination of different species of aged animals including transgenic mice have revealed very limited AD-like neuropathology (Sugaya et al., 1997). Recently, Bishop and Robinson (2000) stated that, "Mice are not Humans," and they could have a much different response to the presence of a neurotoxin. Thereby rabbits may be particularly relevant to the investigation of human disease since they belong to the mammalian order *Lagomorpha* (Graur et al., 1996), a group reported to closely resemble primates than rodents (Graur et al., 1996) and provide a unique animal system for the consistent production of neurofibrillary pathology (Klatzo et al., 1965; Yokel and O'Callaghan, 1998). Expansion of the use of new animal models is obviously needed; hence, rabbits have been considered the most widely used experimental animal for such studies because of its vulnerability to Al and its availability (Klatzo et al., 1965; Yokel and O'Callaghan, 1998). Moreover rabbits, along with cats, develop intraneuronal NFAs in response to the intracerebral administration of Al salts, whereas rodents do not develop these lesions (Yokel and O'Callaghan, 1998). Chronic intracisternal or intracerebral injection of minute quantities of Al into experimental animals, especially rabbits, induces progressively severe neurologic signs associated neuropathologic features of neurodegeneration (Hof et al., 1992), particularly the production of intraneuronal argyrophilic protein aggregates (Klatzo et al., 1965) that bear biochemical similarities to the NFTs observed in AD. Studies by Savory et al. (1994, 1995, 1996b, 1999, 2001, 2003) and Rao et al. (2000) employed *New zealand aged white rabbits* as the experimental animal (4 years old). Besides intraneuronal neurofilamentous changes in the hippocampus, cerebral cortex, brainstem, and spinal cord, which demonstrate many biochemical features, are in common with as seen in AD (Hof et al., 1992), intracisternal administration of Al-maltolate to rabbits also leads to biochemical changes suggestive of apoptosis similar to AD (Savory et al., 1999). Here, in this review, we have tried to convey that this Al/rabbit model system helps to unravel the events associated against the fatal Al neurotoxicity in relevance to AD (Ghribi et al., 2001d). In precise Al-maltolate-treated aged rabbits could be reliable and a sensitive animal model for understanding AD neuropathology.

2. Al-maltolate as novel compound for inducing AD like pathology in aged rabbits

Although Al, still remains as a mystery, even after many decades of research because of its intrinsic difficulties in understanding the role of chemical speciation in biological systems. Hence, to understand the mechanism of Al induced neuropathology, the selection of an appropriate Al compound is important. Scientists have employed the electroneutral Al-maltolate ($Al(mal)_3$) complex (Bertholf et al., 1987) on experimental animals since this compound can deliver a significant amount of free aqueous Al at physiological pH (Martin, 1986). In contrast, most other Al salts, such as $AlCl_3$, produce insoluble complexes at neutral pH (Martin, 1986). The uniqueness of Al-maltolate compound is that this Al-complex 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 from pH 3.0 to 10.0, possesses hydrolytic stability at pH 7.0, and does not have speciation chemistry problems (Martin, 1986). Al-maltolate is preferred over other Al compounds because of its following properties: (a) very high metal solubility at pH 7.0, (b) prominent kinetic restrictions to ligand exchange reactions in neutral solution (Corain et al., 1994; Finneagan et al., 1986), hence suitable for toxicological studies and also to understand the neuropathology.

Administration of different Al compounds to a certain extent induces AD neuropathology, but compared to other compounds, Al-maltolate seems to be more effective. To resolve some of the questions related to the designing of the animal model, investigators have studied a variety of Al salts – Al lactate, $AlCl_3$ (He and Strong, 2000), AlF, and $AlSiO_4$ (Garruto et al., 1984) – on aged rabbits. Certain Al-organic and Al-inorganic complexes administration to different animal groups like cats, ferrets, and dogs also did not mimic the AD neuropathology, though the NFAs were prominent in the CA1 of the hippocampus, the subiculum cortex and in the posterior cingulated gyrus (Strong et al., 1991a; Strong and Garruto, 1991b; Wakayama et al., 1993). Whereas in case of Al-maltolate-treated aged rabbits NFAs formation or paired helical filaments (PHF) were observed in the axons imaged in hippocampal neurons (Fig. 1) (Maccioni and Cambiazo, 1995; Geula et al., 1998; Rao et al., 2000). Studies from Nicholls et al. (1991) also support this concept that Al-maltolate is comparatively more efficient than the other Al-complexes. They reported that when young rabbits (infants) were given s.c. (subcutaneous) injections 3 times weekly of low doses of Al-maltolate (0.5–1.5 mg Al/kg body wt) or Al-lactate (8 mg Al/kg body weight) from 5 or 10 days of age to 14 or 22 days of age. The cell-free protein synthesizing system in the brain exhibited increased activity in Al exposed infants. The mRNA fraction obtained from the brain polysomal RNA were more active in Al exposed

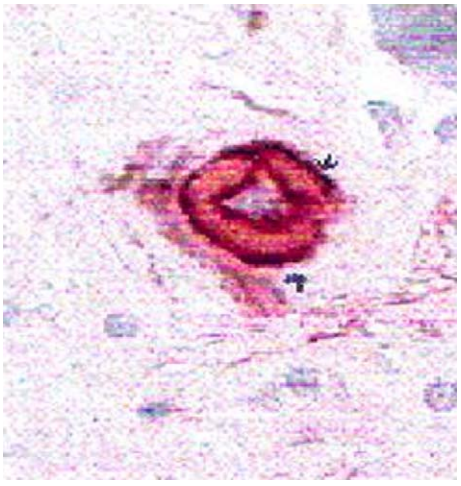

Fig. 1 – Represents the localization of Aβ (Vascular region) in the Al-maltolate-treated aged rabbits which is also observed in the vascular region of demented people.

compared to Al-lactate and the control young rabbits (Nicholls et al., 1991). Hence, Al bound to maltolate, a ligand soluble in lipids as well as in water, was considerably more detrimental to brain protein synthesis also than Al bound to lactate (Nicholls et al., 1991). The animal model presented here thus emphasizes that neutral, water compatible Al (III)-Tris maltolate complex compared to other Al-complexes can be considered as an experimental tool as it offer many advantages (listed beneath) to study human pathologies with relevance to AD.

3. Why choose aged v/s young rabbits

Aged (4–5 years old) and young (8 months old) female rabbits are injected with 25 μl of 25 mM Al-maltolate/kg body weight (13.5 μg/kg of elemental Al). Similarly aged old and young females are treated with an equivalent volume of maltol. Female rabbits are used for consistency in the experimental design and also because the incidence of AD has been reported to be as much as twice as high in women compared to men (Tomlinson, 1992). The youngest rabbits are considered juveniles, since females of this strain reach breeding age by 6 months (Harkness and Wagner, 1989). All animals were sacrificed on days 5 to 7, by which Al-treated group had developed severe neurological symptoms. The time-based studies showed that the development of events is progressive.

3.1. *Susceptibility of aged rabbits in inducing AD neuropathology compared to young ones*

Evidence from clinical and animal model studies demonstrated that brain Al content increases with age, suggesting increased exposure with age or a decreased ability to remove Al from the brain with age (Markesberry, 1994) confirmed by laser probe analysis (Lovell et al., 1993). A very detailed study by Savory et al. (1999) showed that aged rabbits are more susceptible to Al toxicity compared to young rabbits. In young rabbits, the foci of NFT in the hippocampus are not affected (Savory et al., 1999). However, using aged (4–5 years old) rabbits, the hippocampus is mainly affected following Al administration, as demonstrated by NFTs, oxidative stress damage and apoptosis; these events are rare or not observed in the Al-maltolate-treated young rabbits. Al induced oxidative damage, redox-active iron (Fe) accumulation and their relationship to apoptosis were studied extensively by Savory et al. (1999) and Rao et al. (2000), which revealed that the anti-apoptotic Bcl-2 and the pro-apoptotic Bax proteins respond in Al-maltolate-treated aged rabbits (Savory et al., 1999, 2001). The response of these two proteins could constitute a key defect in aged neuron, leading to increased susceptibility to oxidative damage and apoptosis (Rao et al., 2000) as observed in AD, suggesting that Al-maltolate-induced aged rabbits mimics AD pathology. Young animals (which were never found to exhibit apoptosis) have an increased Bcl-2 response, with minimal Bax immunopositivity. Hence, the aged rabbits are considered to be more susceptible in reproducing AD neuropathology compared to young ones.

3.2. *Assessment of neurofibrillary degeneration based on neuroanatomical susceptibility of Al-induced neurodegeneration*

Intraventricular administration of Al-maltolate to rabbits, developed widespread neurofibrillary degeneration (NFD) involving pyramidal neurons of the isocortex and allocortex, projection neurons of the diencephalon, and nerve cells of the brain stem and spinal cord (Katsetos et al., 1990). Perikarya and proximal neurites were especially more affected. Bundles of 10-nm filaments were frequently present in animals treated intravenously for 12 weeks or longer displayed NFAs in the oculomotor complex and in the pyramidal neurons of the occipital isocortex. 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 others. The NFD lesions are compared with those of senile dementia of the Alzheimer type (SDAT) and motor neuron disease (Katsetos et al., 1990). Widespread argyrophilic NFAs were found in a number of brain regions in Al-treated aged and young rabbits, quantitatively the aged animals are affected to a much greater extent. Using 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 (Garruto, 1991; Hof et al., 1992). Studies from Savory's group (Savory et al., 1995, 1996a, 2001) have reported that intracisternal Al administration induces NFD most strikingly in the medulla and upper spinal cord, as similar to regions affected in AD. The brain regions are less affected in the case of Al-maltolate-treated young rabbits compared to aged ones.

4. Strong evidences supporting Al-maltolate/rabbit model for AD

I. Al–Tris (maltolate) aluminum (III) when given i.v. (intravenous) to New zealand white rabbits for a period of time ranging from 5 to 63 weeks. Initially, they were injected 3–5 times a week with 1 ml of 7.5 mM Al $(malt)_3$ and one rabbit with a dose 10 times higher after 14 weeks of treatment. When chemoclinical analysis (glucose, urea, creatinine, cholesterol, bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl-transferase, lactate-dehydrogenase, creatine-kinase, etc., total protein, triglycerides, and Ca^{2+}) were performed, it gave no variation in Al-maltolate-treated animals with respect to the control (Fontana et al., 1991). But the toxicological data showed a moderate systemic general toxicity at doses far higher than those used in earlier experiments using Al $(acac)_3$ (acac = 2,4 pentanedionate), a hydrolytically stable and more lipophilic Al (III) complex. Hence, the diversity of behavior is imperative in terms of metal speciation as well as respect to the thermodynamic and kinetic properties of the two complexes in aqueous solution. (Fontana et al., 1991).

II (a) Kihira et al. (1995) revealed the retrograde transport of Al as a possible mechanism of pathogenesis of AD. Al (as aluminum chloride or maltol), when injected into the subepineurial space of the sciatic nerve with subsequent morphological evaluation of the neurotoxic effect on spinal motor

neurons in rabbits—spheroid/globules, and peripheral chromatolysis, and neuronal degeneration were observed in the spinal anterior horn of Al-maltolate-treated aged rabbits. The soma and dendrites of neurons in the anterior horn of Al-treated rabbit showed marked edematous change, fragmentation of granular endoplasmic reticulum, increased accumulation of neurofilament, and accumulation of free ribosomes and lipid-droplet-like structures.

(b) The above findings indicate that the retrograde transport of Al into spinal motor neurons via the peripheral nervous system may exacerbate neuronal degeneration in ALS immunohistochemically in adult New zealand white rabbits after intraventricular (subacute) and intravenous (chronic) administration of a water-soluble aluminum compound, Al-maltolate (Katsetos et al., 1990; Kihira et al., 1995; Liwincz et al., 1974).

III. Al-maltolate induces cytochrome *c* translocation into the cytosol as early as 3 h in aged but not in young rabbit hippocampus. Pretreatment with cyclosporin A, an inhibitor of the mitochondria permeability transition pore (MTP), blocks cytochrome *c* release. Therefore, it appears that aluminum maltolate-induced cytochrome *c* release results from opening of the MTP. This effect implicates aging as a prerequisite factor, since the MTP does not open in young animals. Mitochondrial injury thus may represent a primary initiator of neurodegeneration (Ghribi et al., 2001c). Recent reports by Ghribi et al. (2002b) showed that pretreatment of Al-maltolate for 14 days with 7 mm of lithium carbonate in drinking water prevents aluminum-induced translocation of cytochrome *c* and upregulates Bcl-2 and Bcl-X(L), downregulates Bax, abolishes caspase-3 activity, and reduces DNA damage. The regulatory effect of lithium on the apoptosis-controlling proteins occurs in both the mitochondria and endoplasmic reticulum (ER) and inhibits the Aβ induced stress in ER of rabbit hippocampus (Ghribi et al., 2003). We propose that the neuroprotective effect of lithium involves the modulation of apoptosis-regulatory proteins present in the subcellular organelles of rabbit brain (Ghribi et al., 2002b, 2003).

IV. Ghribi et al. (2002a) put forth that rabbits treated intracisternally with Al-maltolate had higher levels of procaspase in the cytosolic fractions, whereas p17, the active caspase-3 localized in the ER. This distribution was supported immunohistochemically for the colocalization of p17 with calnexin, a specific marker of the ER, these observation are in accordance with the biochemical changes seen in AD patients.

V. Garruto et al. (1988) 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 micro-analysis and wavelength dispersive spectrometry. Al was 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. No prominent concentrations of Al were imaged in non-NFT-containing regions within the pyramidal cell layer compared to control cases.

VI. The extraordinary work carried out by Savory et al. (1993) on the quantitation of Al in the brain and spinal cord and its effects on neurofilament protein expression and phosphorylation gave a new proof for the involvement of Al in AD. When Al-maltolate was treated to aged rabbits, decreasing concentration was observed (~10 μg/g dry tissue) in the brain and spinal cord, whereas in lumbar cord (~2.1 μg/g dry tissue), argyrophilic tangles were observed in perikarya and proximal neurites of neurons as far distal as the lumbar and sacral cord areas (Savory et al., 1993). 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-kDa and 68-kDa neurofilament protein also were unaffected on Al-maltolate treatment (Savory et al., 1993).

VII. Studies were carried out on neuronal culture system by Hewitt et al. (1991) to evaluate the neurotoxic effects of Al-maltolate on rabbit fetal midbrain sections containing the oculomotor nucleus. Cultures were treated with 5, 7, 9, 11, 13, and 15 μmol/l Al-maltolate, or 39 and 45 μmol/l maltol (molal equivalents to 13 and 15 μmol/l Al-maltolate), at the same control cultures were maintained. The number of tangles produced in Al-maltolate-treated cultures was counted and compared to untreated controls, a total of 7% of neurons following treatment with 11, 13, 15 μmol/l Al-maltolate respectively and none in the controls. Immunohistochemical studies show that NFTs were immunoreactive with MAbs to phosphorylated (SMI-31), nonphosphorylated, phosphorylation dependent (SMI-32) and phosphorylation independent (SMI-33) epitopes of the high (–H) and middle (–M) molecular weight neurofilament subunits (NF-H/M). By contrast these lesions were nonreactive with MAbs recognizing tau, MAP2 or different beta-tubulin isotypes (Muma and Singer, 1996; Hewitt et al., 1991). The above neurocytoskeletal changes observed as seen similar to AD may aid in the assessment of the possible role of Al in the etiology of AD.

VIII. AD is associated with changes affecting numerous neurotransmitter systems (Nordberg, 1992). Of the systems affected in AD, the cholinergic system shows the greatest changes demonstrating a decrease in high affinity choline uptake (HACU); a deficit in activity of acetylcholinesterase (AChE) (Dai et al., 2002); choline acetyl transferase (ChAT) (Gibson and Peterson, 1981); and a decrease in acetylcholine (Ach) concentrations (Slotkin et al., 1990); monoamines, and their precursors. These cholinergic changes are observed in frontal and temporal cortex of postmortem brains of AD (Langlais et al., 1993). It has been hypothesized that to a certain extent cholinergic abnormalities might contribute to cognitive decline in AD (Terry and Buccafusco, 2003; Bartus, 2000).

Studies of the cholinergic system of animals showed an Al-induced decrease in HACU by rat synaptosomes (Lai et al., 1980). It has been shown that AChE decline in mouse brain (Zatta et al., 2002), increase in monoamine oxidase activity in rat brain (Zatta et al., 1999), and decrease in ChAT activity in rabbit brain (Hofstetter et al., 1987). Neurotransmitter system changes in the Al-intoxicated rabbit model mimic those seen in AD (Beal et al., 1989). Lavond et al. (1993) reported that there is an interlink between the Ach overflow and CRs. Al-treated rabbits showed a delay in conditioned eyeblink acquisition and greatly attenuated Ach overflow (Yokel et al., 1994). Thus Al-induced attenuation of Ach

overflow might in turn contribute to the Al-induced learning deficit which mimics the similar observations made in AD (Gron et al., 2005; Yokel et al., 1994). Furthermore, the neurotransmitter alterations which in turn accompany Al neurofibrillary degeneration play a significant role in mediating long term potentiation, a synaptic model of learning (Yokel et al., 1994). There was a significant reduction in acetylcholinesterase activity in entorhinal cortex and hippocampus as well as significant reductions in cortical concentrations of serotonin and norepinephrine in the Al-treated rabbits (Nordberg, 1992). Significant reductions in glutamate, aspartate, and taurine were found in frontoparietal and posterior parietal cortex. However, the concentrations of gamma-amino butyric acid were unchanged in cerebral cortex. Both substance P and cholecystokinin immunoreactivity were significantly reduced in entorhinal cortex, but there were no significant changes in somatostatin and vasoactive intestinal polypeptide in Al-treated rabbit (Nordberg, 1992; Yokel et al., 1994). These findings show a parallence analogous between Al-treated rabbits to AD in terms of neurotransmitter changes.

IX. Inflammation is found to be a key player in the onset of neurodegeneration in AD. In AD patients, there is an upregulation of pro-inflammatory genes (McGeer and McGeer, 1999; Colangelo et al., 2002), and levels of cytokines like interleukin-1 (IL-1), IL-1β, IL-6, tumor necrosis factor and neurotrophins were elevated in the microglia (Zhao et al., 2003) as well as in cerebrospinal fluid and plasma (Sun et al., 2003). There are few significant reports on the role of Al in neuroinflammation. Campbell et al. (2002) have reported that Al increases cell proliferation, cytokine secretion, and nuclear factor-κB (NF-κB) activation in human glioblastoma cells (Campbell et al., 2002), interleukin-1β precursor, cytosolic phospholipase A_2 (Lukiw et al., 2005). Further, inflammation is always correlated with the duration of exposure and the amount of Al accumulated (Dale et al., 1991). There is limited direct evidence of Al induced inflammatory events in the CNS. Glial fibrillary acidic protein (GFAP) has been shown to be associated with gliosis, a generic response of the CNS to neural injury. Further Tsunoda and Sharma (1999) reported that the level of tumor necrosis factor-α, another cytokine implicated in neuronal damage, was significantly increased in the cerebrum of mice exposed to Al compared with controls. (Demircan et al., 1998). Yokel and O'Callaghan (1998) reported that Al increases the frontal cortical GFAP increased (approximately twofold above control) in Al-treated rabbits; whereas hippocampal and cerebellar GFAP concentrations were not affected. Thus, Al-treated rabbits might act as a model system in reproducing AD related inflammatory changes.

X. AD is characterized by impairment in working memory (Baddeley et al., 1991; Germano and Kinsella, 2005), visuoperception, attention, semantic memory, and episodic memory (Scahill et al., 2005; Hodges et al., 1990). To evaluate this, Starr et al. (2005) carried out a study on 9 AD patients (mean age 73.6) and 10 healthy control (mean age 71.8) subjects, who underwent an fMRI memory paradigm. Healthy control subjects activated the right parahippocampal gyrus, whereas subjects with AD activated the right superior frontal gyrus and left uncus. Further, Nordahl et al. (2005) reported that mild cognitive impairment in AD defined as episodic memory impairment is associated with hippocampal atrophy. Hence, cognitive deficits associated with AD still needs to be operationalized both in human subjects and in animals.

In case of Al-treated rabbits, there is a progressive decline in specific memory functions such as deficits in short-term memory and response acquisition (Crapper and Dalton, 1973; Petit et al., 1980; Rabe et al., 1982). The neurobehavioral toxicity studies of Al-treated rabbits were based on the procedure developed by Gormezano (1996) to measure learning and memory. Yokel et al. (1994) has made in-depth study on the Al-induced behavioral toxicity. They studied the rate of acquisition and the retention of the classically conditioned (Pavlovian conditioning) eyeblink reflex, measured as the extension of the third eyelid (nictitating membrane) was determined in a differential and delayed conditioning procedure. A retention and extinction session were studied for 10 days after the last conditioning session by presentation of 100 trials of each tone alone. Eyeblinks initiated within 550 ms after tone onset were considered conditioned responses (CRs). The comparison of the learning curves of rabbits exposed to Al early in development reveals some initial Al-associated improvement in CR acquisition versus Al-induced inhibition of CR acquisition in the adult and aged rabbits (Yokel et al., 1994). The Al-induced inhibition of CR acquisition seen in adult and aged, but not in younger rabbits, suggests that the mature mammalian brain is more susceptible to Al-induced neurobehavioral toxicity. Further, Clark and Squire (1998) suggested that the study of memory processing in the hippocampus might lead to insights about consciousness. They also suggested that hippocampal lesions do not prevent rabbits from learning to blink in response to a tone when they were trained on a conventional protocol in which the tone begins before and then slightly overlaps with an air puff to the eye (Eichenbaum, 1999). But rabbits with hippocampal damage fail to learn on a variant of this protocol in which the tone and air puff are separated by a half-second 'trace' interval (Eichenbaum, 1999). (This gap is probably too brief for rabbits to forget the tone, judging by the short-term/working memory capacity retained by humans with similar damage). The Al-treated rabbit can be acquainted as an animal model for studying behavioral features particularly learning and memory in relevance to neurodegeneration.

XI. The first contribution on therapeutic potential of chelation on Al was reported by Mclachlan et al. (1991). Further Janson (2001) reported that low dose of the injectable desferrioxamine (DFO), to remove Al from the brain of elderly patients were benefited to 50%. Savory et al. (1994) demonstrated the partial reversal of Al-induced neurofibrillary degeneration by DFO in rabbits. Recently, Gong et al. (2005) showed the protective effects of *Ginkgo biloba* extract on Al-induced brain dysfunction. These findings indicate a partial reversal of Al-induced neurodegeneration by DFO and Ginkgo. Further iron-specific chelator like deferiprone is also found to be effective in the chelation of Al (Janson, 2001). The chelation therapy of Al potentially indicates that the rabbit model can be used for research on chelators.

Based on the above observations and evidences, there is a need to obtain meaningful mechanistic information on AD, it is also important to select a relevant animal model system and a well-defined Al-salt for conducting Al neurotoxicologic studies. It is clear that aged rabbits might represent a sensitive animal system for carrying out such toxicity studies, and that Al-maltolate offers many advantages over the other Al-complexes. From the epidemiologic and experimental encephalopathy studies reported, there is ample evidence suggesting that Al might play a role in neuropathology of AD; further mechanistic approach has to be established whether Al is indeed an important factor in the etiology of this devastating disorder, keeping apart the controversies of Al involvement in AD raised by Chafi et al. (1991) and Landsberg et al. (1992), many others. Finally, the ultimate significance of these paradigms/evidences should lead to comprehensively evaluate and synthesize the growing body of relevant scientific data to recognize and develop new models from nature (Rao et al., 1998; Garruto et al., 1984).

5. Contradiction and paradox in Al-maltolate-induced neuropathology in comparison with Alzheimer's disease

The neuropathological features associated with Al-maltolate-treated aged rabbits and AD is summarized in Table 1.

Table 1 – Neuropathological features associated with Al-maltolate treated rabbits and Alzheimer's disease

Characteristics	Al-maltolate induced AD in aged rabbits	Alzheimer's disease
Protein composition	APP, Aβ, unphosphorylated Tau	APP, Aβ, hyperphosphorylated Tau
Biochemical features	Aβ deposition, NFT formation, oxidative stress, apoptosis (Bax ↑ Bcl-2 ↓)	Aβ deposition, NFT formation (Tau), oxidative stress, apoptosis (Bax ↑ Bcl-2 ↓)
Neurochemical features	Alterations in NP-Y, NAAG and its precursor NAL, D-amino acids	Alterations in NP-Y, NAAG and its precursor NAL, D-amino acids ie Asp, Glu, Ser
Immunohistochemical features	Amyloid precursor protein, Aβ, unphosphorylated Tau, α-1-antichymotrypsin and ubiquitin	Amyloid precursor protein, Aβ, hyperphosphorylated Tau, PHF-1, α-1-antichymotrypsin and ubiquitin
Behavioral characteristics	Forward head tilting, photophobia, tremor, passive movement of the extremities, hemipelgic gait, loss of appetite, splaying of the extremities, paralysis and eye blinking classical conditioning	Tremor, passive movement of the extremities, hemipelgic gait, loss of appetite, splaying of the extremities, paralysis and eye blinking classical conditioning, dyspraxia myoclonus, praxis and language (Word finding and Comprehension)

5.1. *Behavioral features*

In the administration of Al-maltolate, rabbits acquire symptoms as early as second day in adults, and it takes certain period of time in infants (Petit et al., 1985; Yokel, 1989), almost all Al-treated animals develop progressive behavioral symptoms consisting of forward head tilting, photophobia, tremor following touch or passive movement of the extremities, hemipelgic gait, seizures, loss of appetite, splaying of the extremities, and paralysis (Kowall et al., 1989; Yokel, 1989). In case of AD all the above features are also observed, in addition to that Dyspraxia myoclonus, praxis and language (word finding and comprehension) exists (Woodruff-Pak and Trojanowski, 1996a; Woodruff-Pak and Papka, 1996b; Woodruff-Pak and Li, 1994). Woodruff-Pak and Li (1994) also reported that behavioral properties like eye blink classical conditioning (EBCC) converge between aged New zealand white rabbit and patients with AD. The similar mechanisms for learning EBCC in rabbits and with impairment of EBCC in AD, disrupted hippocampus, this behavioral paradigm might ameliorate cognitive function in AD.

5.2. *Immunohistochemical features*

In case of Al-maltolate-treated amyloid precursor protein, Aβ protein, neurofilament protein like unphosphorylated tau, α-1 anti-chymotrypsin and a microtubule associated protein ubiquitin are observed (Muma and Singer, 1996), while in AD in addition to the above features neurofilament protein is hyperphosphorylated (Savory et al., 1995, 1996a; Savory and Garruto, 1998). Abnormally phosphorylated tau (Huang et al., 1997), present in these NFAs, were quantified using a variety of monoclonal antibodies (mAbs) that recognize both nonphosphorylated and phosphorylated tau (Savory et al., 1995). Among the mAbs used for immunostaining were Tau-1, Tau-2, AT8, PHF-1, and Alz-50, indicating that both nonphosphorylated and phosphorylated tau are present. It also indicates that 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 (Kosik et al., 1986, Singer et al., 1997; Grundke-Iqbal et al., 1985; Savory and Garruto, 1998). It was also proposed that phosphorylation of cytoskeletal proteins drives the formation of the NFAs particularly in AD (Grundke-Iqbal et al., 1985). Because the aggregates are hyperphosphorylated, phosphorylation alone would render these protein accumulations unstable due to the preponderance of negative charges on the phosphate groups. Thus, immunohistochemical studies are quite reasonable to speculate that some positively charged species constitute an inherent factor in the formation and stabilization of the NFAs, PHFs, and neurofilament proteins both in AD (Su et al., 1996) and in experimental Al-maltolate-induced NFAs in the latter, Al is an obvious candidate for this role (Savory et al.,

1996b). Thus, there are few "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 surmised. Besides behavioral and immunohistochemical features certain neuropsychiatric characteristics (Aarsland et al., 2001; Cummings, 2000) are also prevalent in Al-maltolate/rabbit as observed in AD.

6. Characteristics of tangles associated with Al-maltolate-treated aged rabbits in comparison with AD

Al-maltolate-induced NFT in rabbits do not share all morphologic and biochemical features with the neurofibrillary tangles of AD, they nevertheless exhibit noteworthy similarities. The similarities and differences between Al-maltolate-induced tangles in New zealand aged white rabbits and the neurofibrillary lesions of AD are summarized in Table 2. Although Al-maltolate-induced tangles differ from those of AD in their distribution at both gross and ultrastructural levels, while both types of tangle are found in the cortex and hippocampus, only Al induced pathology is also found in the spinal cord (Garruto, 1991). Indeed, Al-maltolate-induced tangles are found in the perikaryon and proximal parts of the dendrites and axon (Klatzo et al., 1965; Kowall et al., 1989; Savory et al., 2003; Wisniewski et al., 1982; Hof et al., 1992). While AD tangles are found throughout the neuron including the entire length of the dendrites and throughout the axons including the terminals (Binder et al., 1985). Al-maltolate-induced tangles are made up of straight 10-nm 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, while AD paired helical filaments are composed primarily of hyperphosphorylated tau, a microtubule associated protein (Muma and Singer, 1996; Gomez-Isla et al., 1997; Yankner, 1996; Lovestone and Reynolds, 1997), and ubiquitin (Perl and Brondy, 1980; Terry and Peña, 1986; Huang et al., 1997). Although a few researchers have reported that tau is also found in the Al-maltolate-induced rabbits (Kosik et al., 1986; Singer et al., 1997). But majority of investigators fail to confirm the presence of Tau and found the protein primarily existed in unphosphorylated form. However, subsequent work carried out by Klatzo et al. (1996) showed that the similarities between Al-maltolate-induced tangles in rabbits and those of AD are more apparent. Furthermore as reviewed by Wisniewski et al. (1967, 1980, 1982, 1984) and Wisniewski and Sofer (1979) Al-maltolate-induced tangles and AD pathology appeared similar only if the tissue was treated with silver staining.

Table 2 – Characteristics of tangles associated with Al-maltolate treated aged New zealand white rabbits and AD

Tangle characteristics	Aluminum induced AD in aged rabbits	Alzheimer's disease
Protein composition	Neurofilament protein, Tau (unphosphorylated)	Hyperphosphorylated Tau, a microtubule associated protein and ubiquitin
Configuration	Single straight filaments	Paired-helical filaments
Regional localization	Forebrain, spinal cord	Forebrain
Intraneuronal localization	Cell body	Proximal portion of the dendrites and axons entire neuron
Diameter	10 nM	20–24 nM

7. How do the abeta fibrillar deposition and NFTs evolve?

Huang et al. (1997) and Savory et al. (1995) reported that intracisternal administration of Al-maltolate into aged rabbits will produce NFTs firstly, with many immunohistochemical similarities to those observed in AD, which includes the argyrophilic lesions containing abnormal tau, hyperphosphorylated neurofilament protein, amyloid precursor protein, Aβ, ubiquitin and α_1-antichymotrypsin. In this system, hyperphosphorylation of protein constituents of NFTs appears to be secondary process (Savory et al., 1996a) and not the primary event as suggested by other investigators (Iqbal et al., 1994; Matsuo et al., 1994). NFTs 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 pyramidale subiculum, superior and inferior segments of hippocampus (Garruto, 1991; Hof et al., 1992; Klatzo et al., 1965). A few foci of NFTs are present in the hippocampus in the midbrain, including the nucleus nerve occulomotorius and nucleus ruber. The sectioning of the entire cerebrum is performed from the frontal pole to the occipital pole, as well as sectioning the brainstem, thus provide a detailed distribution pattern of NFTs in regions which are significantly involved in AD pathology. Recent studies from Walton (2006) have shown that the autopsy-confirmed cases of AD hippocampus contain substantial amounts of Al in cells and subcellular tissues by using a staining method. All pyramidal neurons including the nucleolus and cytoplasm were also stained with Al, above all the formation of NFTs were observed in Al-rich cytoplasm in AD (Walton, 2006).

Rao et al. (2000) evidenced that Al-maltolate trigger Aβ immunoreactivity in hippocampal region of aged rabbits. Since experimental duration is short, no plaques were observed. Probably longer duration may yield the presence of plaques. Regarding the early phase events, Rao et al. (2000) clearly showed that matured NFTs are formed in neuron as evidenced by single neuron imaging technique. Hence, it is clear that NFT are formed earlier to Aβ plaques formation.

8. Similarities and differences in degenerative aspects in Al-maltolate-treated rabbits

Some of the cellular processes like oxidative stress, apoptosis, and NFT formation that are involved in the neurodegeneration induced by Al-maltolate are carried out on aged New zealand

white rabbits through intravenous administration. Based on the recent literature, data available on the Al-maltolate-induced neuropathology in relevance to AD have focused on the neuronal injury resulting in the understanding of neuropathogenesis in relevance to AD.

8.1. *Oxidative stress*

Oxidative changes seen in Al-maltolate-treated aged rabbits are similar to oxidative changes observed in AD (Markesberry, 1994; Smith et al., 1995, 1996a,b, 2005). The time and extent of oxidative changes overlap in both Al-maltolate-treated and in AD, arguing the fact that the regulatory changes can occur without loss of a function. These are novel observations which may have important implications for aiding in our understanding of the pathogenesis of neurodegeneration in AD. Recently, Savory et al. (1999) experimented that oxidative stress products are released in the Stratum pyramidale hippocampi, nucleus lateralis dorsalis thalami region (Fig. 2). An hypothetical mechanism of potential neuron of Al-maltolate-treated aged rabbits compared with that of AD neuron involved in cell death. We hypothesized that there will be diminished vesicular transport due to Al-maltolate injection leads to reduced microtubules and in turn decrease in axonal mitochondria with increased turnover in the cell body. Also associated with the disruption of the golgi and reduction of synaptic vesicles. The oxidative products released within the neurons are as follows: malondialdehyde, carbonyls, peroxynitrites, nitrotyrosines, and enzymes like superoxide dismutase (SOD), hemeoxygenase-I, etc. (Markesberry, 1994). Smith et al. (1996a,b) developed a specific immunocytochemical technique with in situ 2, 4 dinitro phenylhydrazine labeling linked to an antibody system against DNP to detect the carbonyl reactivity in Al-treated rabbits, AD and in control hippocampus (Smith et al., 1994, 1996a,b, 2005). Smith et al. (2005) demonstrated the presence of carbonyls in both aged rabbits and in AD in NFTs, glia, and non-NFT bearing neurons, as Al levels are also estimated in glia, astrocytes, microglia, etc. (Yokel and O'Callaghan, 1998) and enhance the production of carbonyls. Furthermore, morphometric studies have shown that activated microglia (Carpenter et al., 1993) are strikingly increased in the neocortex of Al-treated aged rabbits and also similar case with AD. A potential mechanism in the nitration of tyrosine residues, such as cytoskeletal proteins in NFT, mediated by peroxynitrite breakdown is observed (Good et al., 1992). Good et al. (1996) demonstrated nitrotyrosine in neurons in AD indicating that it is due to oxidative damage in AD (Good et al., 1992, 1996; Good and Perl, 1993).

Al, being a nonredox active metal, is believed to cause lot of havoc as mentioned in the above paragraph via increasing the redox active iron concentration in brain. This is mainly through Fenton reaction. Al is an activator of SOD and an inhibitor of catalase at the same; therefore, superoxide radicals are readily converted to H_2O_2, and the breakdown to H_2O and O_2 by catalase is slowed down (Markesberry, 1994; Good et al., 1992, 1996; Good and Perl, 1993) and further leading to the production of hydroxyl radicals. These findings encourage a compensatory role for Al-maltolate, whose role in AD still needs to be understood. Hence, oxidative damage instead of being the culprit in AD may in reality play an important role in regulatory process (Su et al., 1994). Since this animal model mimics AD pathology to certain extent, whether Al-maltolate-treated aged rabbits be the human brain's attempt to compensate for designing animal models for AD? This is an attempt to understand the complete neuropathological mechanism using this model. Based on these, we need to develop a reliable model which live up initial hopes, that reliably results in reproducing the neuropathology of AD.

8.2. *Apoptosis*

The foremost event that has to be elucidated is apoptosis, some of the important biochemical events attributed to cell death associated with AD are decreased levels of Bcl-2 (Kitamura et al., 1998), increased levels of Bax (Kitamura et al., 1998), high concentrations of peroxynitrite products (Smith et al., 1994, 1995, 1996a,b; Markesberry, 1994). Investigations from Savory et al. (2001) have focused on the time course and the mechanism of apoptosis in both Al-maltolate-treated and in AD which results in the understanding of neuropathogenesis in relevance to AD. The following aspects highlight on apoptosis mechanism induced by Al-maltolate in aged rabbits which can be used as a parameter to understand apoptotic mechanism in AD.

8.2.1. *Effect of Al-maltolate on the mitochondrial-mediated apoptosis pathway*

Apoptosis or programmed cell death plays a critical role in the normal development and maintenance of tissue homeostasis and is also a process by which brain cells die in

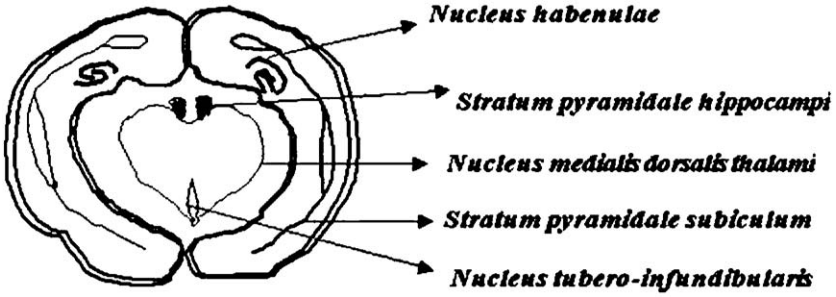

Fig. 2 – Reveals evidence for oxidative stress in aged rabbits on Al-maltolate treatment. The arrow indicates the region of the pyramidal layer of the hippocampus, which is more prone to oxidative damage on Al-maltolate treatment, which reproduces 95% of the homology comparable to oxidative stress of AD neuropathogenesis.

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, and the formed complex 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 (Fig. 3).

(a) Implicates the opening of the mitochondrial transition pore (MTP),
(b) By the translocation of mitochondria of the pro-apoptogenic Bax which can form the channel by itself.
(c) Interaction of Bax with the voltage-dependent anion channel (VDAC) to form a larger channel which is permeable to cytochrome c.

In contrast to Bax, the anti-apoptotic Bcl-2 has the ability to block the release of cytochrome c from mitochondria by mechanisms such as a direct blockade of the MTP opening or by functioning as a docking protein (Adams and Cory, 1998; Eskes et al., 1998). Al has been demonstrated to accumulate in neurons following cell depolarization, where it inhibits Na^+/Ca^{2+} exchange and thereby induces an excessive accumulation of mitochondrial Ca^{2+} (Van Ginkel et al., 1993). Increases in intra mitochondrial Ca^{2+} levels lead to an opening of the MTP with cytochrome c release and subsequent apoptosis resulting from activation of the caspase family of proteases. We have shown that the intracisternal administration of Al-maltolate results in cytoplasm cytochrome c translocation, Bcl-2 downregulation and bax upregulation, as well as caspase-3 activation (Ghribi et al., 2001c, 2002a; Griffioen et al., 2004). These results indicate that Al targets the mitochondria. Furthermore, the fact that we can demonstrate the release of cytochrome c, which is inhibited by cyclosporin A, a specific inhibitor of the MTP opening, implicates opening of the mitochondrial transition pore as the process by which cytochrome c translocates to the cytoplasmic space from mitochondria (Ghribi et al., 2001a,b,c,d, 2002a,b). 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. We have demonstrated that the glial cell-line derived neurotrophic factor (GDNF) protects rabbit hippocampus from the neurotoxic effect of Al but does not prevent the release of cytochrome c as the sole trigger of Al induced apoptosis, at least in this animal model system. However, GDNF treatment

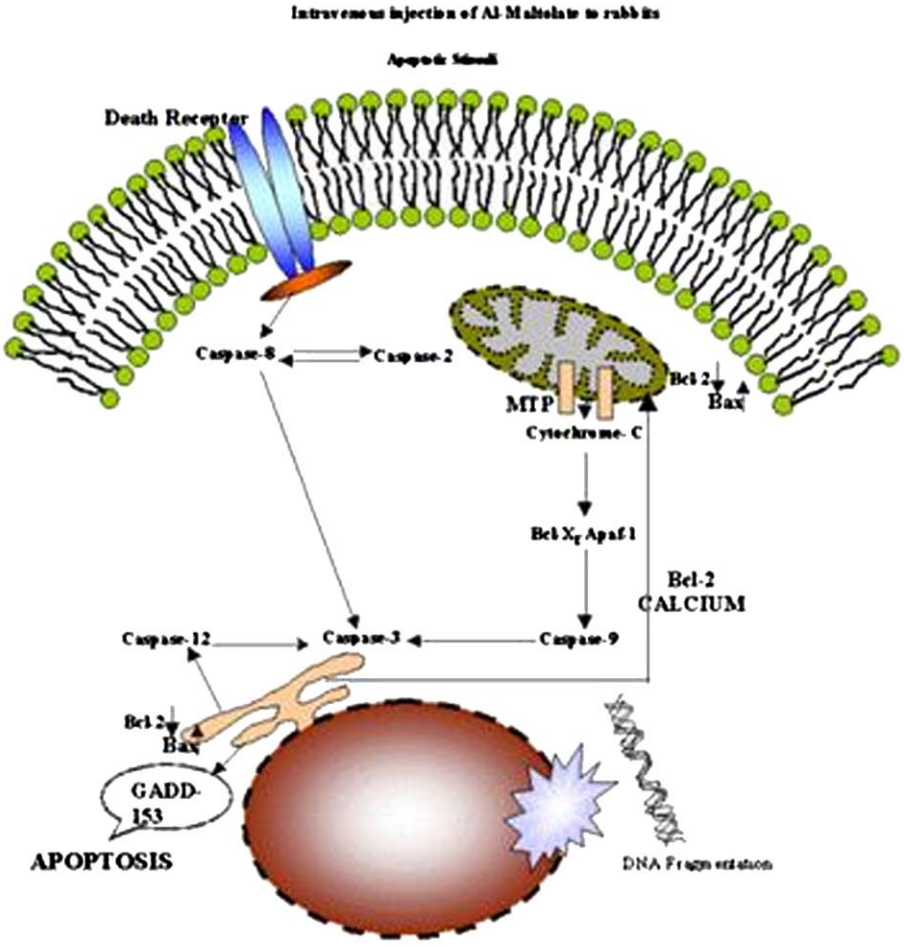

Fig. 3 – Proposed model on Al-maltolate-induced neuronal apoptosis by its effects on functioning of both endoplasmic reticulum and mitochondria in aged rabbits.

increases the level of the anti-apoptotic protein, Bcl-XL, which when over-expressed, has the ability to sequester Apaf-1, and thereby to inhibit Apaf-1-dependent caspase-9 activation. Recent studies from Savory et al. (2001) showed that chronic treatment of rabbits with lithium in the drinking water results in inhibition of the Al-induced cytochrome *c* release, 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 (Ghribi et al., 2001a,b,c,d, 2002a,b; Kowall et al., 1989) and DNA fragmentation (Mecocci et al., 1994, 1997; Kadioglu et al., 2004; Lyras et al., 1997) as observed in AD.

8.2.2. *Effect of Al-maltolate on apoptosis-regulatory proteins that mediate endoplasmic reticulum*

Although mitochondrial alterations may represent an important step in the mechanisms underlying neuronal cell death induced by Al-maltolate, studies from Savory et al. (2003) provided evidence suggesting that the 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 (D'mello et al., 1994). It is also been reported that Al induces stress in the ER, as demonstrated by the activation of gadd 153 and its translocation into the nucleus. The gadd 153 gene is specifically activated by agents that disturb ER function. Although we have demonstrated the effect of Al-maltolate on ER function, it remains unclear which signaling mechanisms lead to perturbation of ER homeostasis by Al-maltolate. Al-maltolate may disturb the Ca^{2+} homeostasis or protein processing in the ER. Severe insult results in sustained depletion of Ca^{2+} stores might be the one of the causes for apoptotic cell death (Savory et al., 2003).

8.3. *NFT formation*

Aging of the animals has a pronounced effect on rendering neurons in certain brain regions susceptible to Al induced neurofibrillary aggregates (NFT), in areas also affected in AD. These NFT typically develop in Al-treated aged rabbits in the cerebral cortex, hippocampus, thalamus, and midbrain, all regions of the brain that are frequently involved or that may be involved in AD (Rao et al., 2000; Savory et al., 1996a). The neurofibrillary aggregates are argyrophilic and are immunostained with mAbs recognizing the abnormal tau present in the neurofibrillary tangles of AD. Young adult rabbits treated with Al form only a few of these intraneuronal lesions. In the early 1986, Terry and Peña (1986) showed an ultrastructurally distinct type of twisted or PHFs (Kosik et al., 1986; Maccioni and Cambiazo, 1995; Geula et al., 1998) quite distinct from the wispy, thinner, single filaments found in plaque amyloid. This difference in structure reflects the fact that tau protein and not the Aβ is found in plaques, a principal component of tangles.

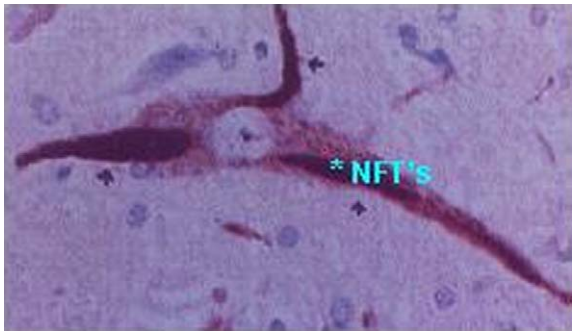

Fig. 4 – NFT in axons imaged in a single neuron from hippocampal region of Al-treated aged rabbits.

Because tangles are found in dementia pugilistica (Dale et al., 1991), they have also been reported in a number of different conditions (Wisniewski et al., 1984) as they are found in AD. In particular, aged rodents, dogs and primates did not develop NFT (Wisniewski et al., 1982). Various attempts were made to induce NFTs in most of the animal models. But in the case of Al-maltolate-treated aged rabbits, the two neuropathological hallmarks of Alzheimer's disease are senile plaques, which are of Aβ and neurofibrillary tangles, made of abnormal twisted strands of the protein, tau. But how tau and amyloid deposits form, or how they cause cells to die in AD is still unanswered. That the two may form independently of one another or one before the other leads to the arousal of the question that how the above two proteins are able to produce disease in the brain, and also how tau deposits accumulate? As NFTs are localized in the proximal portion of dendrites and the axons of entire neurons, but NFTs and apoptosis were colocalized within the same neurons. This is in agreement with the observations of Sugaya et al. (1997), who demonstrated colocalization of NFTs in hippocampal neurons of AD brain. No oxidative stress, apoptosis, NFT formation or PHF was observed in Al-maltolate-treated aged rabbits. NFTs were observed by confocal imaging of axons in hippocampal neurons (Fig. 4) from an Al-treated aged rabbits (Rao et al., 2000). The neurofibrillary tangles and neurofilamentous accumulation induced in rabbits by intracisternal administration of $AlCl_3$, though superficially resemble those seen in aged human brain, these are relatively less compact, rich in neurofilament protein, made up of 12-nm straight filaments (Wisniewski et al., 1980) unlike irreversible nature of NFT, ultra-structurally made up of paired helical filaments and rich in phosphorylated tau and polymerized ubiquitin. It is also essential to realize that AD like illness can be seen in the absence of NFT and senile plaques and centurians with numerous NFT and senile plaques may not manifest cognitive deficits characteristic of AD, hence Al could be one of the "flag poles " in the path of evolution of human disease.

9. Neurochemical features observed in Al-maltolate-treated aged rabbits with that of AD

Al-maltolate-treated aged rabbits revealed certain neurochemical features like reduction in memory molecules like Neuropeptide Y (NPY), neurotransmitters like *N*-acetyl-L-

aspartyl-L-glutamate (NAAG), which resembles the characteristic features of AD.

9.1. *Alterations in the levels of NPY*

NPY present in the hippocampal region is responsible for neurochemical behavior and food intake. NPY is a 36-amino-acid, C-terminal amidated peptide and is widely distributed in the central and peripheral nervous systems. NPY concentration is reduced in cerebrospinal fluid, plasma and also in cerebral cortex, hippocampus, and hypothalamus regions of the AD brain (Chang et al., 1998). Recently, Rao et al. (2000) showed that there is significant similarity between Al-maltolate-treated aged rabbits and have a close correlation with that of AD. Al reduces NPY levels in the hippocampal region of Al-maltolate-treated aged rabbits (Rao et al., 1999). Also studies from Rao and group showed that Al alters the structure of NPY (Rao et al., 1999), this could explain the abnormality in feeding behavior as seen in the patients with AD (Gerald et al., 1996).

9.2. *Imbalances in the levels of N-acetyl-aspartyl-glutamate (NAAG) and its precursor N-acetyl-L-aspartate (NAL)*

Neuropeptides namely NAAG and its precursor NAL levels were found to be low in the AD brain, and it has been reported that these neuropeptides play a significant role in neural transmission. Reduced levels of NAAG and NAL are observed with Al-maltolate-treated aged rabbits as seen in the case of AD (Ramesh et al., 2001). In vivo studies indicated that Al decreased the levels of NAAG and NAL. Jaarsma et al. (1994) reported that NAA and NAAG levels were significantly reduced in hippocampus (by 38% and 24%) and amygdala (28% and 22%) regions of brain but not in cerebral cortex and olfactory bulb. Passani et al. (1997) reported that NAA and NAAG and the activity of NAALADase levels were significantly decreased in AD.

9.3. *D-Aspartate levels in AD/DNA alterations in AD*

In normal human brain, proteins and free amino acids pools predominantly exist in L-forms, while in neurodegenerative brains the amount of racemized forms of amino acids, in particular D-aspartate (D-Asp) and D-glutamate (D-Glu) are relatively in large proportion in core amyloid plaques and NFTs and D-Asp promotes aggregation and fibril formation of the Aβ peptide (Vyas and Duffy, 1995). Recently, Latha et al. (2001) showed that Al-maltolate favors racemization from L-Asp and L-Glu to D-Asp and D-Glu respectively in the aged rabbit brain. Furthermore, Anitha et al. (2002) and Hegde et al. (2004) showed DNA helical changes in hippocampal region in AD brain from B-form to Z-Form, whether similar observations are seen in the case of Al-maltolate-treated rabbits still remains elusive.

A detailed description on Al-maltolate-treated aged rabbits mimicking AD neuropathology (~90%) is as shown in Table 1.

10. Why other animal models including transgenic fails to reproduce total AD neuropathology?

Scientists have succeeded in producing hallmark features of AD in a laboratory animal model. A number of animal models, such as the transgenic mice (Games et al., 1995) and among others rat, monkey, and dog, have been proposed to aid in the understanding of AD neuropathology (Brining et al., 1996; Uno et al., 1999). The much-anticipated transgenic mouse model is generally engineered with the human gene encoding for a form of the brain protein tau/amyloid. Transgenic mouse model enable us to study tau-containing lesions in a number of brain disorders, including the insoluble tau-containing tangles that build up and form one of the key pathological features of AD. Coleman and Greenberg (1996) inserted human tau genes into mice which later developed masses of abnormal tau filaments in nerve cells within the spinal cord,

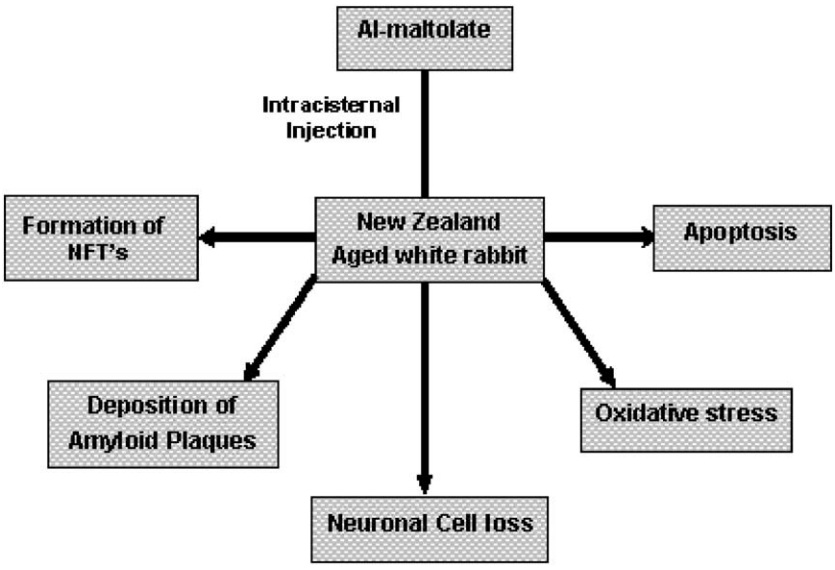

Fig. 5 – Schematic representation of Al-maltolate-treated aged rabbit brain mimicking Alzheimer's disease neuropathology.

cortex, and brainstem, three critical regions of the central nervous system. As the mice aged, insoluble masses of tau filaments grew in number. The transgenic animals also showed evidence of nerve cell degeneration and impaired movement, unlike their littermates lacking the inserted tau gene. Transgenic mice have also been used mainly to examine the process of Aβ deposition (Games et al., 1995), while individual events such as apoptosis and NFT formation have been explored in other animals (Brining et al., 1996; Uno et al., 1999). Coleman and Greenberg (1996) have suggested that the transgenic animal system may aid in an understanding of AD, only with reference to Aβ deposition, single event expression. Hence, researchers need to develop an animal model which would demonstrate all the following events namely, Aβ deposition, PHF formation, neuronal death, cholinergic deficits, inflammatory processes, and cognitive deficits in order to understand the spectrum of AD neuropathology which aids in developing new drug therapies for AD (Savory et al., 1999; Smith and Perry, 1997; Wisniewski and Sofer, 1979; Wisniewski et al., 1980, 1982, 1984). The transgenic mice do not completely mimic AD pathological features, but they closely resemble the other human brain disease affected by neurodegeneration. Thus, the Al-maltolate-treated aged rabbit enables the demonstration of Aβ deposition, and the colocalization of NFT, PHF1, oxidative stress with apoptosis in hippocampal neurons, this brain region is usually and selectively involved in AD pathology (Grundke-Iqbal et al., 1985; Markesberry, 1994; Smith et al., 1996a,b; Su et al., 1994, 1996).

The understanding of AD neurochemistry and neuropathology is a big challenge due to unavailability of a suitable animal model, which mimics AD pathology. Recently, Savory et al. (2003) indicated that aged rabbits after Al-maltolate treatment mimic AD-like neuropathology in terms of neurofibrillary tangles, β-amyloid deposition, oxidative stress and apoptosis in forebrain, hippocampus and midbrain regions (Fig. 5). Besides Savory's contribution in developing animal model for AD, Prof. Rao (2000) has made significant piece of work in providing circumstantial evidences on the neuropathological features in Al-maltolate-treated rabbits as similar in the case of AD. Hence, this animal model might be a promising model for pursuing further studies related to AD without involving the ongoing controversies of Al.

11. Conclusion and future perspectives

It is clear that the existing animal models tested so far fall short in providing reliable and valid information on the neuropathology of AD, irrespective of whether their purpose is for analysis of the disease or developing more effective therapies than those that are presently available. Several models have intrinsic limitations, and on the whole, they do not reproduce the pathogenetic process and are unlikely to help in the development of effective neuroprotective therapies. The genetic, particularly transgenic, technologies that appeared to offer greater construct validity have so far failed to live up initial hopes and do not reliably result in reproducing the neuropathology of AD. It is not yet been resolved whether the molecular basis in developing animal models are incorrect or fundamental speciation differences in Al leading to cellular processing of normal and abnormal proteins. The extent and causes of the neurodegeneration and behavioral deficits seen in these animal models require additional study and may involve higher inputs in this regard. Transgenic mice with the focus on APO-E4, presenilins and tau genes are continuing to be developed. However, Al-maltolate-treated aged rabbits ameliorate AD pathology in behavioral, neurochemical, and immunohistochemical features, but further refinements are required to develop an effective animal model. Al-maltolate-treated aged rabbits might act as a reliable and efficient system in understanding the neuropathogenesis among the currently available ones. This opens up new avenues in developing therapeutic strategies for treating this tragic, devastating disease.

Acknowledgments

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This review is dedicated to Prof. John Savory, University of Virginia, Dr. Mary Herman, NIMH/NIH, with due respect for their pioneering contribution on Al neurotoxicity.

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