Introduction
INTRODUCTION

Humans are exposed daily to low concentration of metal that are released into the environment by both natural and industrial processes. Recently, concern has been raised about the acute and/or chronic exposures of humans to concentration of these metals that are below the threshold level established by the federal regulatory agencies. Many of these metals get accumulated in various tissues and over a period of time are responsible for several diseases of liver, kidney and brain. Indeed brain is known to be a concentrator of metals where potentially toxic levels of copper, iron, aluminium, lead, zinc and mercury could accumulate. Research currently points towards the involvement of metallochemical reactions in Alzheimer’s disease, prion disease, mitochondrial disorder, Parkinson’s disease and Huntington’s disease. Neurotoxic metals usually resemble or take advantage of the vital metals used in normal metabolism. Molecular mimicry often explains why these metals have access to certain tissues and can replace or block their vital processes. It is obvious that several targets can be and are attacked. Cells have specific means for reacting to such attacks. The ultimate response is to get rid of the damaged cell by apoptotic cell death, if the cell is capable to execute the process. It may be advantageous for the tissue, but in the brain it causes irreversible damage and may be the cause of the onset of neurodegenerative diseases. Therefore, it is essential to find out which events precede the major damages. To gain this knowledge, diverse cellular mechanisms, also interrelating ones need to be studied.

Mounting evidence in the recent years has suggested aluminium to have severe toxic manifestations in the central nervous system. Various animal studies have shown that aluminium exposure causes neuropathological, neurobehavioral, neurophysical and neurochemical changes resulting in impaired learning ability (Miu et al., 2003; Colomina et al., 2002). It is an etiopathogenic factor in diseases related to long-term dialysis treatment, and it has been controversially invoked as an aggravating factor or cofactor in Alzheimer’s disease (AD) (Sinczuk-Waleczak, 2001) as well as in other neurodegenerative diseases like Parkinson’s disease (PD) (Uversky et al., 2002; Fahn, 2003) and amyotrophic lateral sclerosis (ALS) (Flaten, 2001;
Exley and Esiri, 2006; Drago et al, 2008). Dave et al (2002) found significant alterations in tissue acetylcholinesterase (AChE) following long-term aluminium exposure. It was observed that the workers working in the welding unit that contain aluminium fumes showed symptoms of aluminium neurotoxicity (Buchta et al; 2003). Infact, the studies by Shirabe et al (2002) depicted excess amount of aluminium among foundry workers in an autopsy case of aluminium encephalopathy. Polizzie et al (2002) also detected early neurotoxic symptoms linking aluminium to Alzheimer’s disease. The neurotoxic symptoms following aluminium exposure include speech disturbances, dysparaxia, tremors, psychosis, partial paralysis and finally death (Spofforth, 1921).

Aluminium is the third most abundant element, which comprises about 8% of the earth’s crust. Though aluminium is non-essential, still we are constantly exposed to it through rapid industrialization (Turgut et al, 2007). The estimated average human exposure of aluminium through food and water is between 2-30 mg/day. It gains entry into the body, as it is a constituent of cooking utensils, medicines and drinking water (Ochmanski and Barabasz, 2000). Aluminium sulfate used for purifying water (Flaten, 2002) is also an important source of aluminium burden in the body. Though poorly absorbed it has an easy access to brain via transferrin mediated carriers, which are only partially occupied by the iron and are available for other metals like aluminium. The brain is also capable of effluxing aluminium via monocarboxylate transporter system, which is compromised during high aluminium exposure.

Aluminium has been shown to accumulate in all the regions of rat brain following chronic exposure, maximum being in hippocampus, which is the site of memory and learning (Lal et al., 1993; Julka and Gill, 1996; Kaur et al., 2006). In humans the aluminium content in brain increases with age (Markesbery et al., 1981). It has been detected in both senile plaques and neurofibrillary tangle (NFT) bearing neurons in the brains of AD patients (McLachlan et al., 1996), which suggests the role of this metal in AD. Recent studies of Walton (2007) on experimental model of AD depicted oxidative damage, neuronal degeneration & hyperphosphorylated tau. Miu et al (2003) observed that long-term exposure of aluminium to adult rats depicted neuropathological modifications such as distorted cerebrovasculature with thickening of the wall of capillaries and associated amyloid deposits. Although, epidemiological studies have indicated a link between increased aluminium
concentration in potable water and Alzheimer’s disease but the precise molecular mechanism of the intervening biochemical events by which aluminium exerts its toxic effects is still not completely understood (Praticò et al, 2002).

Aluminium has no redox capacity in biological system, yet the metal is known to act as a pro-oxidant both in vitro and in vivo conditions (Exley, 2004). Its interaction with active oxygen species and membranes has been reported in the literature (Kong et al, 1992). Aluminium has been shown to enhance \( \text{O}_2^- \) mediated oxidation in different systems generating oxidant species, such as xanthine/xanthine oxidase, the autoxidation of melanin (Meglio and Oteiza, 1999). The other mechanism through which aluminium can cause oxidative damage to neurons is through its interaction with iron (Zatta et al, 2002). It has been shown that aluminium stabilizes ferrous (Fe\(^{2+}\)) ion by reducing its rate of oxidation. Fe\(^{2+}\) is potent in promoting the generation of oxidative species, as it actively catalyses the Fenton reaction (Ellen et al, 1999). Further, aluminium has been shown to disturb the antioxidant enzyme system notably the superoxide dismutase that is activated and catalase, which gets inhibited. It is also known to disturb the glutathione homeostasis (Esparza et al, 2003; Yousef, 2004; Gomez et al, 2005; Nehru and Bhalla, 2006b; Nehru and Bhalla; 2006a). Hence, both in vitro and in vivo studies indicate that aluminium is definitely a promoter of oxidative stress. Evidence of an oxidative stress has been found to be associated with most neurodegenerative disorders in which aluminium is present in relatively high amounts (Berthon 1996; Corain et al., 1996; Becaria et al, 2002; Yokel 2002).

Apart from free radical-induced damage aluminium-induced neurotoxicity has further been related with its mechanism of induction of nitric oxide (NO) (Kaneko et al, 2007). Both apoptosis and necrosis are suggested to be the mechanisms involved in cellular death resulting from aluminium toxicity (Brenner, 2002). Suarez-Fernandez et al (1999) have reported DNA fragmentation and changes in nuclear morphology after aluminium exposure. Mutagenic effects of aluminium toxicity have also been observed in various laboratories (Trippi et al, 2001; Elmore, 2003; Synzynys et al, 2004; Varella et al, 2004).

Aluminium has been shown to cause adverse effects on neurotransmission, either by directly inhibiting the enzymes responsible for the synthesis and/or utilization of neurotransmitters, or by affecting the physical properties of synaptic membranes. The effect of aluminium on learning and memory are important and
specific too since other trivalent toxic metals like boron, chromium and gallium etc have not been shown to illicit the sequences of clinical signs that follow aluminium administration (Idem, 1974). Impairment of cognitive functions along with alterations in cholinergic neurotransmission has been reported after aluminium treatment in both clinical and experimental studies (Szutowicz et al, 2000; Bielarczyk et al, 2003; Szutowics et al, 2006; Rodella et al, 2006). Aluminium exposure to rats has been shown to cause a significant increase in the excitatory amino acids glutamine and glutamate, accompanied by a decrease in GABA content, which suggest a role for this metal in glutamate mediated excitotoxic neuronal injury (El-Rahman, 2003; Nayak and Chatterjee, 2003).

Mitochondrial dysfunction has been implicated in many neurodegenerative diseases associated with increased levels of free radicals and oxidative damage. Toxic consequences of electron transport chain dysfunction may ensue mitochondrial damage including oxidation of mitochondrial DNA, proteins, and lipids, which may lead to the opening of mitochondrial permeability transition pore, an event that is linked to cell death in neuronal model systems (Halliwell, 1992). In vitro studies have shown changes in the mitochondrial structure and function in aluminium exposed rat neuronal cells (Niu et al., 2005). It seems that the alterations in mitochondrial structure and function may play an important role in elucidating various aspects of aluminium neurotoxicity. Therefore, present study was designed to delineate the various cellular events following chronic aluminium exposure in different regions so that it may help in the designing of better therapeutic measures in future.

Despite the lack of successful neuroprotection in various neurotoxic/neurodegeneration, there is a great deal of excitement in drug development targeting compounds against the death of grey and white matter. For a better management it is proposed that the therapeutic intervention should be mechanism based, targeting specific injury cascade since most neurodegeneration that follows after various insults/exposure is not due to primary mechanical insult but rather to secondary injury events. Further the neuroprotection should not be limited only to the post injury setting alone but the compound should be administered during the course of insult and therefore it should be safe, and the therapeutic window should be well documented. However the experimental data may not be relevant in clinical settings since the result of experimental data are determined by the histopathological and behavioural parameters whereas the clinical investigators depend largely on the
functional outcomes. Thus there is a need for screening of herbal medicines for neurodegenerative disorders and their complications (Balasubramanyam et al., 2003). Most neurodegenerative diseases involve multi-step cascade that induce slow degeneration and could best be dealt with long term prevention strategies that are less expensive and safe.

The significance of curcuma longa Linn (Turmeric) in health and nutrition has changed considerably since the discovery of the anti-oxidant property of naturally occurring phenolic compounds, curcuminoids (Balasubramanyam et al., 2003). Curcumin has numerous pharmacological activities like anti-inflammatory activity, anti-mutagenic activity and hepatoprotective activity (Sandur et al., 2007). Earlier studies have shown that curcumin inhibits ROS production (Bishnoi et al., 2006) as well as lipid peroxidation in liver microsomes, erythrocyte membranes and brain homogenates. It can also affect different other cellular processes such as activation of apoptosis, inhibition of platelet aggregation, inhibition of inflammatory cytokine production, inhibition of cyclooxygenase and lipoxygenase isoenzyme (Balasubramanyam et al., 2003). Its importance in neurodegeneration needs to be worked up.

Many of the direct targets of curcumin are regulatory components of intracellular signaling pathways. Curcumin has been shown to regulate phosphoinositol/protein kinase C pathway among others (Hsu et al., 2008). Further, signaling pathways are involved in the regulation of complex neurobiological functions such as cognition, appetite, sexual arousal, sleep patterns, weight and response to hormones, all of which are altered in mood disorders and likely have effects on rodents behaviors (Manji et al., 2003). Studies have also suggested curcumin-metal interaction especially with divalent and trivalent cations. Though there are no reports available with aluminium and curcumin interactions, but according to authors (Daniel et al., 2004) the deleterious effects of aluminium on the activity of AChE may be due to protein-metal interaction, with the metal acting directly on the active site of the enzyme, thus bringing about alterations in the activity of the enzyme. The strong chelating ability of diketones has been widely investigated towards a great number of metal ions and therefore suggests the importance of curcumin in the chelating treatment of metal intoxication and overload. With this background in mind the present study was designed to evaluate the therapeutic potentials of curcumin in aluminium induced neurodegeneration.
Lipid peroxidation has been conclusively demonstrated to be the key mechanisms in neurodegeneration and has therefore prompted the search of neuroprotective strategy/agents aimed at antagonising oxygen radical induced lipid peroxidation. Studies by Peixoto et al. (1999) have shown the increase in lipid peroxidation as the first major event during the ROS injury that occurs within the 30 min of the insult. It occurs both at neuron and blood vessel level impairing neuron function and causing micro vascular changes. Excessive production of free radicals during oxidative stress results in a) vascular damage leading to altered BBB permeability .b) endothelial dysfunction and inflammation c) oxidative stress is also linked with apoptosis which has different relevance with respect to CNS.

Recently, a family of steroid compounds, 21-aminosteroids (Lazaroids), was developed. Although this family was derived from glucocorticoids, it lacks glucocorticoid and mineralocorticoid activities (Jacobsen et al., 1990). These compounds were shown to scavenge lipid peroxyl radicals and to inhibit iron-dependent lipid peroxidation (Braughler et al., 1988a; Braughler and Pregenzer, 1989). However, apart from the pharmacological characteristics of specific compounds, these lazaroids represent very interesting molecules with important pharmacological features not yet explored. They have been effectively employed in animal models of head and spinal cord trauma, sub-arachnoid haemorrhage, neuronal hypoxia (Vignes and Hugon, 2006) and global ischemia in the CNS. Further, they also partially protect from an ischemic lesion or from an inflammatory reaction caused by endotoxins. Lazaroids also increase the survival of transplanted dopaminergic neurons and improves the behavioural recovery in rats (Nakao et al, 1994). Therefore, the present study was further directed to study the role of this synthetic lipid peroxidation inhibitor towards aluminium-induced neurotoxicity.

Thus, it appears that there are several controversies regarding aluminium neurotoxicity and the greatest is with regards to its linking role with Alzheimer’s disease. Several epidemiological studies have failed to reveal a connection with the intake of aluminium and AD suggesting that this may not be the causative agent but may initiate events leading to the disease or accentuate the pathogenesis. Further, there is no consistency with regards to neurofibrillary tangles (NFTs) also. NFTs were never seen in patients with dialysis encephalopathy. There are differences in aluminium speciation where aluminium is found to be neurotoxic even at picomolar concentrations. There are several overlapping mechanisms by which aluminium is
Role of Antioxidants in Al Induced Neurotoxicity

Introduction

found to be neurotoxic. It disrupts the cytoskeleton by inducing the hyperphosphorylation of tau resulting in the formation of bridges and the formation of tau aggregated which would have varied implications in the function of neurons.

Chronic aluminium exposure may result in increased levels of oxidative stress, metabolic compromise, mitochondrial dysfunction, activation of apoptotic cascades, perturbed calcium regulations and abnormal protein processing and degradation. All these occur as a result of multiple yet overlapping cascades and so it is important to study the role of curcumin, which is projected to have phototropic effects such as anti-inflammatory, anti-oxidative, anti-apoptotic and anti-carcinogenic. Further, recent literature has also suggested its importance in stimulation of neurogenesis, enhanced phagocytic clearance, COX-2 induction via AP-1 and NF-kB, potentiation of HSPs, congo red mimetic as well as simultaneously decreases Aβ-production by suppressing cholesterol and BACK-1 induction.

There are two major outcomes of lipid peroxidation i.e structural damage to membrane and generation of bioactive secondary products, which in turn can bring about alterations in biophysical properties of membrane. The use of lazuroids can help us to delineate the importance of altered membrane function and lipid peroxidation during aluminium exposures.

OBJECTIVES

❖ To experimentally induce neurotoxicity by subjecting the animals to aluminium chloride exposure orally for a period of two months.
❖ To develop peripheral markers for aluminium neurotoxicity.
❖ To study the role of glial cells in the management of oxidative stress in aluminium induced neurotoxicity.
❖ To elucidate the effect of aluminium on the neurochemical alterations in cholinergic, dopaminergic and serotonergic system with emphasis on the neurobehavioral deficits observed, during the course of the study.
❖ To undertake the behavioural studies so as to assess the short-term memory, cognitive behaviour, locomotor activities, muscular activity, spatial memory and psychomotor dysfunction of the animals (active avoidance test, Passive avoidance test, Total locomotor activity, Rota rod test, Elevated plus maze and Morris water maze).
Introduction

To elucidate the possible link between aluminium neurotoxicity and oxidative stress parameters such as Lipid peroxidation, Antioxidant defence system enzymes (Superoxide dismutase, Catalase and Glutathione homeostasis), Nitric oxide synthase activity, and levels of L-citrulline and the estimation of ROS.

To elucidate the role of aluminium in energy metabolism by estimating lactate dehydrogenase, succinate dehydrogenase, hexokinase, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, glycogen content and glycogen phosphorylase.

A detailed in vivo investigation of the toxic effects of aluminium on mitochondrial function by studying mitochondrial complexes, ATP synthesis, ATP hydrolysis, ATP levels and cytochrome levels.

To elucidate the role of aluminium in neuronal cell death by dissecting out the apoptotic pathway by studying caspase-3, caspase-9, DNA fragmentation and comet assay.

To elucidate the role of aluminium in neuronal focal inflammation by studying genes involved in inflammation such as NF-kB and TNF-α and by different staining techniques.

To study structural changes at light microscopic level by subjecting the tissues to histological examination using different stains such as H&E, Solochrome and Gallocyanin.

To investigate the effect of natural antioxidant i.e. curcumin on aluminum-induced neurotoxicity and associated behavioral, biochemical, neurochemical and molecular alterations.

To study the mechanism involved in the neuroprotective role of U-74500A on aluminium-induced neurotoxicity.