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Neuroprotective effect of carvedilol against aluminium induced toxicity: possible behavioral and biochemical alterations in rats

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Abstract:
Aluminium, is a trace element available in the Earth's crust naturally and has a toxic potential for humans. It has been suggested as a contributing factor in the pathogenesis of Alzheimer's disease. β-Adrenoceptor blocking agents (β-blockers) have been established as therapeutics for the treatment of patients with hypertension, ischemic heart diseases, chronic heart failure, arrhythmias and glaucoma. Over the years, however, β-blockers have been associated with an incidence, albeit low, of central nervous system (CNS) side effects. In addition, noradrenergic receptors play a modulatory role in many nerve functions, including vigilance, attention, reward, learning and memory. Therefore, the present study has been designed to explore the possible role of carvedilol, an adrenergic antagonist against aluminium chloride-induced neurotoxicity in rats. Aluminium chloride (100 mg/kg) was administered daily for six weeks that significantly increased cognitive dysfunction in the Morris water maze and oxidative damage as indicated by a rise in lipid peroxidation and nitrite concentration and depleted reduced glutathione, superoxide dismutase, catalase and glutathione S-transferase activity compared to sham treatment. Chronic aluminium chloride treatment also significantly increased acetylcholinesterase activity and the aluminium concentration in brain compared to sham. Chronic administration of carvedilol (2.5 and 5 mg/kg, *po*) daily to rats for a period of 6 weeks significantly improved the memory performance tasks of rats in the Morris water maze test, attenuated oxidative stress (reduced lipid peroxidation, nitrite concentration and restored reduced glutathione, superoxide dismutase, catalase and glutathione S-transferase activity), decreased acetylcholinesterase activity and aluminium concentration in aluminium-treated rats compared to control rats ($p < 0.05$). Results of this study demonstrated the neuroprotective potential of carvedilol in aluminium chloride-induced cognitive dysfunction and oxidative damage.

Key words:
aluminium, Alzheimer's disease, oxidative stress, carvedilol, neuroprotection

Introduction

Metal dyshomeostasis has been implicated in many neurodegenerative disorders. Aluminium is the most abundant metal in nature but has no known biological function. In humans, aluminium plays a causal role in dialysis encephalopathy, osteomalacia and microcytic anemia [2]. Numerous studies have demonstrated that concentrations of aluminium are elevated in the brains of patients suffering from Parkinson's Disease [9], amyotrophic lateral sclerosis [29] and senile dementia of Alzheimer's type [28]. In addition, aluminium is a well-known neurotoxicant reported to accelerate oxidative damage to biomolecules. Furthermore, aluminium salts have been reported to cause cell deple-

tion in the hippocampus, isocortex [27] and degeneration of cholinergic terminals in the cortical areas. It accumulates in the cingulated bundle and thereby induces learning deficits [30]. Though the molecular mechanism underlying aluminium-induced memory impairment is not clear, it has been suggested that it interferes with glutamatergic neurotransmission [32] and impairs hippocampal long-term potentiation by disrupting the glutamate-NO-cyclic guanosine monophosphate signaling pathway [5].

Aluminium (Al), oxidative stress and impaired cholinergic function have been all related to cognitive dysfunction. Aluminium crosses the blood brain barrier *via* the specific high affinity receptors for transferrin (TfR) [34]. Upon entering the brain it affects the slow and fast axonal transports, induces inflammatory responses [4], inhibits long-term potentiation, and causes synaptic structural abnormalities, thereby resulting in profound memory loss. In addition, aluminium induces protein misfolding and self-aggregation of highly phosphorylated cytoskeletal proteins such as neurofilaments or microtubule-associated proteins and Aβ, which have been implicated in Alzheimer's disease (AD) [17]. It has already been reported that aluminium has been found in both senile plaques and neurofibrillary tangle (NFT)-bearing neurons in the brains of AD patients [26]. Moreover, it is a potent cholinotoxin and causes apoptotic neuronal loss, which is a characteristic symptom of neurodegeneration associated with AD [14].

Carvedilol is a nonselective β-adrenoceptor blocker with multiple pleiotropic actions including antioxidant, α_1-adrenoceptor blocking, vasodilatation, inhibition of apoptosis [35], anti-inflammatory [37], mitochondrial protective [1], non-competitive inhibition of NMDA receptor and calcium channel blocking [24]. Carvedilol has also been shown to exert neuroprotective effects in several models of transient focal stroke, and a cardioprotective effect in several models of cardiovascular ischemia and reperfusion [35]. These effects are related to its antioxidant and free radical scavenger properties. The antioxidant activity of carvedilol has been attributed to its carbazole moiety and it is approximately 10-fold more potent as an antioxidant than vitamin E. It is interesting that we found carvedilol alleviated D-galactose-induced ageing despite having no effect on hyperglycemia [19]. Based on this background, the present study was carried out to investigate the neuroprotective effect of carvedilol in aluminium-induced cognitive impairment and oxidative stress in rats.

Materials and Methods

Animals

Male Wistar rats, (180–200 g) procured from the Central Animal House, Panjab University, Chandigarh, were used. Animals were acclimatized to laboratory conditions at room temperature prior to the experiments. Animals were kept under standard conditions of a 12-hour light/dark cycle with food and water *ad libitum* in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 15.00 h. The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

Drugs and experimental design

Aluminium chloride (CDH, India) and carvedilol (Sigma Chemicals Co., St. Louis, MO, USA) solutions were made freshly at the beginning of each experiment. For oral administration, aluminium chloride and carvedilol were dissolved in sterile water and normal saline respectively and administered orally at a dose of 0.5 ml/100 g body weight. Animals were randomized into five groups consisting of 7 animals in each.

Group 1: Naive (received vehicle for aluminium chloride and carvedilol)

Group 2: Aluminium chloride (100 mg/kg) + vehicle for carvedilol

Group 3: Carvedilol (5 mg/kg) + vehicle for aluminium chloride

Group 4: Carvedilol (2.5 mg/kg) + aluminium chloride (100 mg/kg)

Group 5: Carvedilol (5 mg/kg) + aluminium chloride (100 mg/kg)

The study was carried out for a period of 42 days (6 weeks). The doses of aluminium chloride and carvedilol were selected based on earlier studies done in our laboratory [19, 31].

BEHAVIORAL ASSESSMENT

Assessment of cognitive performance by the Morris water maze task

The acquisition and retention of memory was evaluated using the Morris water maze [31]. The Morris

water maze consisted of a large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1°C) divided into four equal quadrants with the help of two threads fixed at right angles to each other. The pool was placed in an illuminated room with several colored light clues. These external clues are remained unchanged throughout the experimental period and used as the reference memory. A circular platform (4.5 cm diameter) was placed in one quadrant of the pool, 1 cm above the water level during the acquisition phase. The same platform was placed 1 cm below the water level for the retention phase. The position of the platform was not changed in any quadrant during assessment of both phases. Each animal was subjected to four consecutive trials with a gap of 5 min. The animal was gently placed in the water of the pool between quadrants facing the wall of the pool, with the drop location changed for each trial. The animal was then allowed 120 s to locate the platform. Next, the animal was allowed to stay on the platform for 20 s. If the animal failed to reach the platform within 120 s, it was guided to the platform and allowed to remain there for 20 s.

Maze acquisition phase (training)

Animals received two consecutive daily training sessions on day 19 and 20. During the acquisition phase, each rat was put into the water in any one of four starting positions, the sequence of which was selected randomly. The latency to reach the visual platform (acquisition latency) was measured.

Maze retention phase (testing for retention of the learned task)

Twenty-four hours (day 21) and 21 days (day 42) after the acquisition phase, each animal was released randomly from one of the edges facing the wall of the pool to assess memory retention. Time latency to find the hidden platform on day 21 and 42 following the start of aluminium chloride administration was recorded and termed the first retention latency (1^{st} RL) (21^{st} day) and the second retention latency (2^{nd} RL) (42^{nd} day), respectively.

BIOCHEMICAL ASSESSMENT

Biochemical tests were conducted after performing the last behavioral task. The animals were sacrificed by decapitation. Brains were removed and rinsed with ice-cold isotonic saline. Brains were then homogenized with ice-cold 0.1 mmol/l phosphate buffer (pH 7.4). The homogenates (10% w/v) were then centrifuged at 10,000 × g for 15 min and the supernatant so formed was used for the biochemical analysis.

Measurement of lipid peroxidation

The extent of lipid peroxidation in the brain was determined quantitatively by performing the method described by Wills [36]. The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid (CDH, New Delhi) at 532 nm using a Perkin Elmer Lambda 20 spectrophotometer. The values were calculated using the molar extinction coefficient of the chromophore (1.56×105 $(mol/l)^{-1}cm^{-1}$).

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined by a colorimetric assay with the Griess reagent (0.1% N-(1-napththyl)ethylenediamine dihydrochloride, 1% sulfanilamide and 5% phosphoric acid) (CDH, New Delhi) [13]. Equal volumes of the supernatant and the Griess reagent were mixed and incubated for 10 min at room temperature in the dark. The absorbance was then measured at 540 nm using a spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve.

Estimation of reduced glutathione

Reduced glutathione was estimated according to the method described by Ellman et al. [6]. A 1 ml aliquot of supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested for 1 h at 4°C. The samples were then centrifuged at 1,200 × g for 15 min at 4°C. To 1 ml of the supernatant obtained, 2.7 ml of phosphate buffer (0.1 mmol/l, pH 8) and 0.2 ml of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (SRL, Chandigarh, India) were added. The yellow color developed was measured at 412 nm using a spectrophotometer. Results were calculated using the molar extinction coefficient of the chromophore (1.36×10^4 $(mol/l)^{-1}cm^{-1}$).

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed by the method of Kono [18]. The assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM nitroblue tetrazolium (SRL, Chandigarh, India). In the cuvette, 2 ml of the above mixture, 0.05 ml of hydroxylamine and 0.05 ml of the supernatant were added and autooxidation of hydroxylamine was measured for 2 min at 30 s intervals by recording absorbance at 560 nm.

Catalase activity

Catalase activity was assessed by the method of Luck [23], wherein the breakdown of H_2O_2 is measured. Briefly, 3 ml of H_2O_2 phosphate buffer and 0.05 ml of the supernatant of the tissue homogenate were mixed. The change in absorbance was recorded for 2 min at 30 s intervals at 240 nm. The results were expressed as micromoles of hydrogen peroxide decomposed/min/mg of protein.

Glutathione S-transferase activity

The activity of glutathione S-transferase was assayed by the method of Habig and Jakoby [15]. Briefly, 2.7 ml of phosphate buffer, 0.1 ml of reduced glutathione, 0.1 ml of 1-chloro-2,4-dinitrobenzene (SRL, Chandigarh, India) as substrate and 0.1 ml of supernatant were combined. The increase in the absorbance was recorded at 340 nm for 5 min at 1 min intervals. The results were expressed as nanomoles of CDNB conjugated/min/mg of protein.

Estimation of acetylcholinesterase (AChE) activity

AChE is a marker of the loss of cholinergic neurons in the forebrain. The AChE activity was assessed by the Ellman method [7]. The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (SRL, Chandigarh, India) (Ellman reagent). The change in absorbance was measured for 2 min at 30 s intervals at 412 nm. Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed/min/mg of protein.

Protein estimation

The protein content was estimated by the biuret method [12] using bovine serum albumin as standard.

Aluminium estimation

The aluminium was analyzed by the wet acid digestion method of Zumkley [40] in the hippocampus and cortex of the brain. A mixture of 2.5 ml of perchloric acid/nitric acid (CDH, New Delhi) (1:4 by volume) was added to the tissue and then placed in a sand bath at 40°C to 50°C for 44 h until the point where a white ash or residue was obtained. Residues were then dissolved in 2.5 ml of 10 mM nitric acid. Next, this sample (in liquid form) was placed in the sample holder of an atomic absorption spectrophotometer. The total concentration of aluminium was calculated in μg/g of tissue.

Statistical analysis

Values are expressed as the mean ± SEM. The behavioral assessment data were analyzed by a repeated measures two-way analysis of variance (ANOVA). The biochemical estimations were separately analyzed by one-way ANOVA. *Post-hoc* comparisons between groups were made using Tukey's test. $p < 0.05$ was considered significant.

Results

Effect of carvedilol on memory performance in the Morris water maze task in aluminium-treated rats

Aluminium treated rats significantly delayed acquisition latency to reach the visual platform compared to the naive group, indicating memory deficits. Carvedilol treatment significantly improved this memory performance (i.e., shortened mean acquisition latency) on day 19 and 20 ($p < 0.05$) in the aluminium treated group. Following training, the visual platform was hidden. Aluminium treatment was then found to significantly delay mean acquisition latency (on day 20) and retention latencies (1^{st} and 2^{nd} RL on day 21 and 42, respectively) to escape onto the hidden platform compared to the naive group. These results suggested

that aluminium chloride caused significant cognitive impairment. Further, chronic carvedilol treatment (2.5 and 5 mg/kg) significantly improved memory performance (increased memory retention) for the 1st and 2nd RL on days 21 and 42, respectively, compared to the aluminium chloride treated rats. However, carvedilol (2.5 and 5 mg/kg) treatment only did not influence acquisition latency compared to naive animals (Tab. 1).

Effect of carvedilol on lipid peroxidation, nitrite, reduced glutathione, glutathione S-transferase, superoxide dismutase, and catalase activity in whole brains of rats treated with aluminium chloride

Chronic administration of aluminium chloride significantly raised MDA and nitrite concentration, depleted reduced GSH, and decreased glutathione S-transferase, superoxide dismutase, and catalase activities

Tab. 1. Effect of carvedilol (CAR; 2.5 and 5 mg/kg) on memory performance in the Morris water maze paradigm for aluminium chloride treated rats

Treatment (mg/kg)	Day 20 (IAL)	Day 21 (1st RL)	Day 42 (2nd RL)
Naive	76 ± 2.4	15 ± 2.7	10 ± 2.6
$AlCl_3$ (100)	77.6 ± 2.3	82.8 ± 2.6[a]	81.3 ± 2.3[a]
CAR (5.0)	68.0 ± 3.8	14.5 ± 2.4	11.5 ± 2.5
CAR (2.5) + $AlCl_3$ (100)	69.3 ± 2.7	47.5 ± 1.7[b]	46.0 ± 2.0[b]
CAR (5.0) + $AlCl_3$ (100)	67.5 ± 2.7	30.33 ± 2.4[b,c]	27.8 ± 1.7[b,c]

The initial acquisition latencies (IAL) on day 20 and retention latencies on days 21 (1st RL) and 42 (2nd RL) following $AlCl_3$ concurrent treatment were observed. Values are the means ± SEM. [a] $p < 0.05$ as compared to naive group; [b] $p < 0.05$ as compared to $AlCl_3$ treated group; [c] $p < 0.05$ as compared to CAR (2.5) + $AlCl_3$ group (repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons)

Tab. 2. Effect of carvedilol (CAR; 2.5 and 5 mg/kg) on oxidative stress parameters under aluminium chloride treatment

Treatment (mg/kg)	MDA nmol MDA/mg of protein (% of control)	Nitrite µmol/mg of protein (% of control)	Reduced glutathione nmol/mg of protein (% of control)	Catalase µmol of hydrogen peroxide decomposed/min/mg of protein (% of control)	Superoxide dismutase Units/mg of protein (% of control)	Glutathione S-transferase nmol of CDNB conjugated/min/mg of protein (% of control)
Naive	0.1585 ± 0.04 (100)	226.8 ± 30.06 (100)	0.0653 ± 0.005 (100)	0.709 ± 0.04 (100)	48.25 ± 3.19 (100)	97.9 ± 5 (100)
AlCl3 (100)	0.6965 ± 0.054[a] (338.63)	655.83 ± 28.32[a] (279.44)	0.015 ± 0.0034[a] (39.203)	0.147 ± 0.018[a] (20.73)	13.87 ± 2.11[a] (28.75)	37.8 ± 5.3[a] (38.61)
CAR (5.0)	0.147 ± 0.043 (97.82)	225 ± 37.4 (100.55)	0.0603 ± 0.004 (99.23)	0.702 ± 0.041 (99.15)	41.293 ± 2.189 (97.86)	99.9 ± 5.2 (102.04)
CAR (2.5)+ $AlCl_3$ (100)	0.417 ± 0.024[b] (247.88)	475 ± 29.64[b] (210.33)	0.032 ± 0.001[b] (62.78)	0.36 ± 0.028[b] (43.58)	22.22 ± 2.14[b] (45.44)	47.67 ± 2.2[b] (48.69)
CAR (5.0) + $AlCl_3$ (100)	0.24 ± 0.026[b,c] (157.8)	346.6 ± 30.2[b,c] (163.2)	0.04 ± 0.002[b,c] (78.1)	0.506 ± 0.02[b,c] (60.22)	34.39 ± 2.117[b,c] (74.34)	64.7 ± 1.95[b,c] (65.98)

Values are the means ± SEM. [a] $p < 0.05$ as compared to naïve group; [b] $p < 0.05$ as compared to aluminium treated group; [c] $p < 0.05$ as compared to CAR (2.5) group + $AlCl_3$ (100) group (repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons)

in the whole brain compared to naive rats ($p < 0.05$). However, chronic carvedilol (2.5 and 5 mg/kg) administration to the rats significantly attenuated oxidative damage (as indicated by reductions in MDA, nitrite concentration and reduced GSH, and decreased glutathione S-transferase, superoxide dismutase, and catalase activities) as compared to control rats (Tab. 2). Furthermore, carvedilol (2.5 and 5 mg/kg) treatment alone did not significantly influence these parameters compared to naive rats.

Effect of carvedilol on aluminium concentration in aluminium chloride treated rats

Aluminium chloride treatment significantly increased the aluminium concentration in the hippocampus and cortex of rats compared to control. However, chronic carvedilol (2.5 and 5 mg/kg) treatment significantly attenuated the aluminium concentration in the hippocampus and cortex compared to control ($p < 0.05$) (Fig. 1).

Effect of carvedilol on acetylcholinesterase (AChE) activity in aluminium chloride treated rats

Chronic aluminium chloride treatment significantly increased the whole brain AChE activity compared to naive rats. However, chronic carvedilol (2.5 and 5 mg/kg) treatment significantly attenuated AChE activity, as compared to the control rats ($p < 0.05$) (Fig. 2).

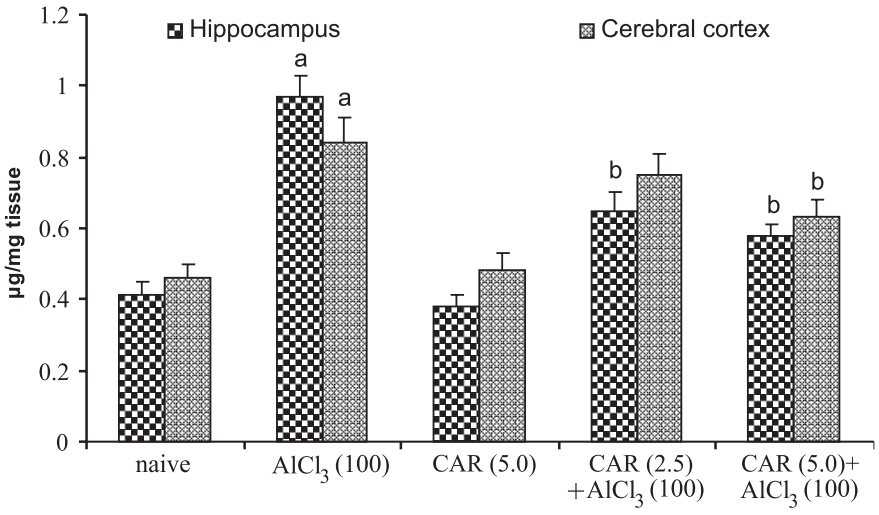

Fig. 1. Effect of carvedilol (2.5 and 5 mg/kg) on the concentration of aluminium in the hippocampus and cortex of aluminium chloride treated rat brains. Values are the means ± SEM. Concentration of Al: µg/mg tissue. [a] $p < 0.05$ as compared to naive group; [b] $p < 0.05$ as compared to $AlCl_3$ treated group (repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons)

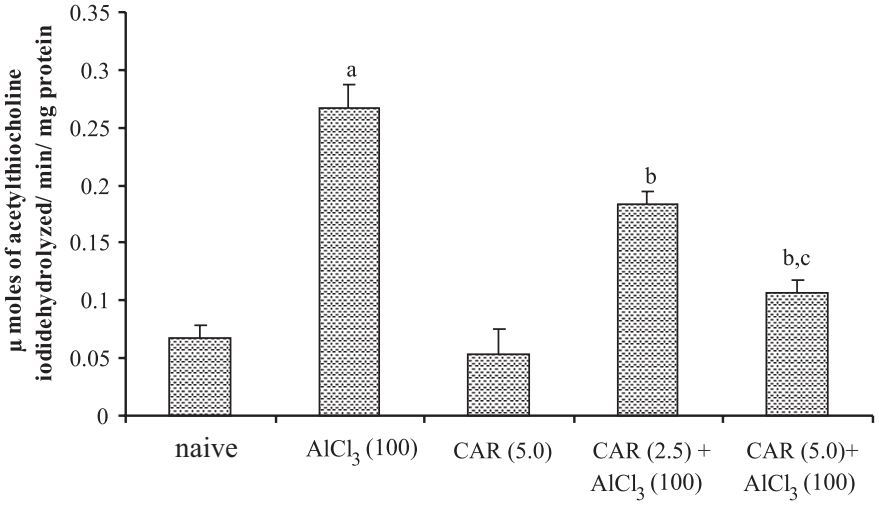

Fig. 2. Effect of carvedilol (2.5 and 5 mg/kg) on acetylcholinesterase levels in aluminium chloride treated rats. Values are the means ± SEM. [a] $p < 0.05$ as compared to naive group; [b] $p < 0.05$ as compared to aluminium chloride treated group; [c] $p < 0.05$ as compared to CAR (2.5) + aluminium chloride group (repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons)

Discussion

Aluminium is a ubiquitous metal and has been implicated in the etiology of neurodegenerative disorders and cognitive dysfunction, where it exacerbates brain oxidative damage [20], causes neuronal inflammation and induces impairment in working memory, visuoperception, attention and semantic memory [30]. Aluminium also functionally alters the blood brain barrier and produces changes in the cholinergic and noradrenergic neurotransmission [38]. It causes impaired glucose utilization, increased free-radical generation and lipid peroxidation as well as changes in phosphoinositide metabolism and protein phosphorylation, thereby causing severe neurotoxicity.

The results of the present study indicated that chronic administration of aluminium chloride resulted in progressive deterioration of spatial memory as determined by Morris water maze task paradigms. Experimentally, it was demonstrated that intracerebral administration of aluminium chloride caused learning deficits in the Morris water maze task in rabbits [33], which was in agreement with our findings. This phenomenon could be attributed to the ability of aluminium to interfere with downstream effector molecules, such as cyclic GMP, involved in long-term potentiation [5]. This disruption could then explain the memory impairment and neurobehavioral deficits observed.

Aluminium was previously found to be a potent pro-oxidant known to enhance lipid peroxides in the cortex and hippocampus [20]. It also caused alterations in iron homeostasis, resulting in excessive free iron ions, which undergo the Fenton reaction and cause oxidative damage, finally culminating in neurodegeneration. Furthermore, it also targets mitochondria, causing the release of cytochrome c and the activation of pro-apoptotic proteins like bax and caspase-3, which trigger neuronal apoptotic death [11].

As oxidative damage is mediated by free radicals, it was necessary to investigate the status of endogenous antioxidant enzymes like catalase, superoxide dismutase and glutathione, which are the first line of defense against free radical damage under oxidative stress conditions. In our study, chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by increases in lipid peroxidation and nitrite concentration, as well as decreases in reduced glutathione, catalase, superoxide dismutase and glutathione S-transferase activity. These changes could have been due to the reduced axonal mitochondria turnover, disruption of the golgi or reduction of synaptic vesicles induced by aluminium treatment, all of which result in the release of oxidative products like malondialdehyde, carbonyls, and peroxynitrites, and of enzymes like superoxide dismutase, within the neurons [3]. Under oxidative stress conditions, SOD presents the first line of defense against superoxide as it dismutates the superoxide anion to H_2O_2 and O_2. Catalase protects SOD by converting H_2O_2 to water and oxygen. Catalase is present in the peroxisomes of mammalian cells, and probably serves to destroy H_2O_2 generated by oxidase enzymes located within these subcellular organelles [41].

Aluminium is a potent cholinotoxin [14]. It has a biphasic effect on acetylcholinesterase activity, with an initial increase in the activity of this enzyme during the first 4–14 days of exposure followed by a marked decrease. This biphasic effect has been attributed to the slow accumulation of aluminium in the brain [22]. This would explain the increase in acetylcholinesterase activity observed in the aluminium chloride treated rats.

Because oxidative stress and cognitive dysfunction are strongly correlated, agents that modulate reactive oxygen species may be potentially useful as antidementia agents. Chronic administration of carvedilol was found to improve not only the memory retention but also reduced oxidative damage induced by chronic aluminium administration. Carvedilol treatment also attenuated the rise in MDA and NO concentration of aluminium treated rats. In fact, it has been reported in the literature that carvedilol acts as a NO quenching agent in vascular endothelial cells and in cell-free systems [39]. It has also been shown to inhibit superoxide ion release from activated neutrophils [25].

Glutathione in its reduced form is the most abundant intracellular antioxidant and is involved in direct scavenging of free radicals or serving as a substrate for the glutathione peroxidase enzyme that catalyzes the detoxification of H_2O_2. It is also known that SOD and catalase are protective enzymes and both function in very close association for the detoxification of highly reactive free radicals. In our study, we demonstrated that carvedilol restored reduced glutathione and increased SOD, catalase and glutathione S-transferase activity in aluminium treated rats. These results were further supported by our previous reports on the impact of carvedilol on D-galactose-induced neuro-

toxicity and colchicine-induced oxidative stress [19, 21]. Carvedilol also has been shown to preserve the endogenous antioxidant system (i.e., vitamin E and reduced glutathione), which is normally consumed when tissues or cells are exposed to oxidative stress [8]. This preservation of endogenous antioxidants may explain why the carvedilol treatment was able to restore reduced glutathione and glutathione S-transferase activity in aluminium treated rats. Moreover, carvedilol has also been reported to protect the expression of many inflammatory mediators and cytokine-like TNF-α and IL-1β, which are mainly responsible for causing oxidative damage.

Cholinergic neurons are positive markers for the evolution of memory and related disorders affecting acetylcholine and resulting in decreased activity of acetylcholinesterase and choline acetyl transferase [10]. Recent findings suggested that administration of aluminium was found to increase acetylcholinesterase in mouse brain [16]. We also demonstrated that chronic administration of aluminium to rats significantly increased acetylcholinesterase, an effect that was attenuated by carvedilol administration.

The efficacy of carvedilol might be attributable to its β-blocking effect (adrenergic blocker), but whether the action of carvedilol on cholinergic neurons could have an additional benefit for AD has yet to be established. Unfortunately, how carvedilol downregulates acetylcholinesterase remains unclear. We speculate that it is associated with oxidative damage to neurons because of the pronounced anti-oxidative effect of carvedilol [19, 21, 31].

In the present study, chronic exposure of aluminium chloride increased aluminium concentrations in the hippocampus and cerebral cortex of rats. Increased concentrations of aluminium have also been observed in the brains of Alzheimer's disease patients who present declines in visual memory, attention concentration, frontal lobe function and vocabulary scores. The results presented here showed that carvedilol was able to attenuate the increased concentration of aluminium in both of these regions of the brain in rats. Therefore, the present study highlights that carvedilol improves behavioral and biochemical function in the aluminium-treated brain, an effect that could be partially correlated with its anti-oxidant properties. However, further cellular studies are required to understand the effect of carvedilol on oxidative stress in different experimental systems.

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