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Exploring neuroprotective, biochemical, and behavioral effects of magnesium threonate in alzheimer's induced by aluminum chloride in rats

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Abstract

Aim: This study investigates the neuroprotective, biochemical, and behavioral effects of magnesium L-threonate (MgT) in a rat model of Alzheimer's disease (AD) induced by aluminum chloride ($AlCl_3$).

Material and Methods: Forty Wistar albino rats were divided into four groups: control (C), Alzheimer's (AD), positive control (Mg), and protective (Pr). AD was induced by daily intraperitoneal injections of $AlCl_3$ for six weeks. Behavioral assessments were conducted using the head-poking test. Biochemical markers, including Tau protein (Tau), amyloid precursor protein (APP), acetylcholinesterase (AchE), glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and albumin, were measured. Histopathological examinations of the hippocampus were also performed.

Results: The AD group exhibited significant behavioral deficits, elevated levels of Tau, APP, AchE, and MDA, and decreased levels of GSH, SOD, and albumin. MgT treatment significantly improved cognitive performance, reduced levels of Tau and APP, and normalized antioxidant enzyme activities. Histopathological analysis revealed severe neuronal degeneration in the AD group, while MgT-treated groups showed reduced neurodegeneration and improved neuronal integrity.

Discussion: In rats, MgT demonstrates significant neuroprotective, biochemical, and behavioral benefits in an $AlCl_3$-induced AD model. These findings suggest that MgT could be a promising therapeutic agent for AD, warranting further investigation and clinical trials.

Keywords

Alzheimer's Disease, Magnesium-L-Threonate, Aluminum Chloride, Neuroprotection

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a decline in memory and cognitive abilities. AD is the most common form of dementia, marked by the accumulation of amyloid-beta peptide (Aβ), which triggers the formation of neuritic plaques and neurofibrillary tangles [1]. Additionally, AD disrupts various neural signaling pathways, particularly the N-methyl-D-aspartate receptor (NMDAR) signaling pathway. NMDAR is a vital calcium channel and glutamate receptor found in neuronal membranes, and its dysfunction is closely associated with AD pathology [2].

In 1906, German neuropathologist and psychiatrist Alois Alzheimer discovered AD. It is the fifth leading cause of death and accounts for 60 to 80 percent of dementia cases [3].

Globally, dementia will impact an estimated 74.7 million people by 2030 and 131.5 million by 2050. While AD can occur in younger individuals, it predominantly affects those over the age of 65 [4].

As a neurodegenerative disease, AD progressively impairs memory and cognition functions, alters behavior, slows thinking, and eventually makes daily tasks unmanageable, requiring full-time care. Early symptoms include difficulties in encoding and recalling new memories, with progressive changes in behavior and cognition as the disease advances [5].

Memory loss, particularly of short-term memory, is often the earliest and most prominent symptom. In later stages, severe memory loss and disorientation occur, impairing even long-term memory. AD typically affects the processing of non-hippocampal memories. In severe late-stage AD, patients often lose the ability to walk, talk, and recognize time and place [6].

The etiology of AD is complex, involving environmental and genetic factors. Key pathological features include amyloid plaques, neurofibrillary tangles (NFTs), and the loss of synapses and neurons [7]. Several hypotheses link mitochondrial DNA mutations or dysfunction to age-related changes and dementia. The oxidative stress hypothesis suggests that amyloid-beta (Aβ) induces oxidative stress, damaging neuronal macromolecules and generating reactive species [8].

The pathogenesis of AD is influenced by a variety of risk factors, including aging, environmental exposures to metals, hereditary factors, cardiovascular diseases, head trauma, and infections [9, 10]. Magnesium, the fourth most abundant element in the human body and the second most abundant intracellular cation, is crucial for numerous biological processes, including brain function. It is a cofactor for more than 600 enzymatic activities, regulates blood pressure and glucose metabolism, and facilitates calcium and potassium transport across cell membranes. Additionally, it is crucial for the conduction of nerve impulses, and its deficiency has been linked to the development of dementia-related disorders, including AD [11].

Adequate magnesium intake, absorption, and metabolism are vital for mental health. MgT from the diet has shown efficacy in increasing brain Mg2+ levels and improving cognitive functions in aged animals. It has demonstrated favorable cognitive effects in animal models of AD [2, 11-12].

Despite numerous efforts to develop treatments for AD, there is currently no cure. However, some medications can manage symptoms and slow the progression of the disease. This study aimed to evaluate the effects of MgT on biochemical, behavioral, and cerebral histopathological changes in rats with AlCl3-induced AD.

Material and Methods

Animals and Experimental Design

Forty Wistar albino rats, aged three months and weighing 200-250 g, were selected from King Fahd Medical Research Center (KFMRC). The rats were housed under standard conditions, with a temperature of 24 ± 1°C, relative humidity of 70%, and a 12-hour light/dark cycle. The study was conducted the ethical guidelines approved by the institutional committee. All rats were provided a standard diet and water ad libitum throughout the six-week experimental period.

The rats were randomly divided into four groups (n = 10 per group):

Control healthy group (C): Rats were injected intraperitoneally (i.p.) with normal saline and received normal saline via oral gavage for six weeks.

AD group (AD): Rats were injected i.p. with AlCl3 (100 mg/kg body weight) [13] daily and received normal saline orally for six weeks.

Positive control group (Mg): Rats received MgT (604 mg/kg body weight/day) [14] daily via oral gavage for six weeks and were treated with normal saline i.p.

Protective group (Pr): Rats were treated with AlCl3 as in the AD group and received MgT (296 mg/kg body weight) orally as in the Mg group for six weeks.

Biochemical Assessment

At the end of the experimental period, all rats were sacrificed by decapitation. Blood samples were collected from the abdominal and thoracic blood vessels. The serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until biochemical analysis. The levels of Tau, APP, AchE, GSH, malondialdehyde (MDA), and superoxide dismutase (SOD) were measured using ELISA kits following the manufacturer's instructions (Glory Science Co. Ltd., USA). Albumin levels were determined using colorimetric methods as described by Lowry et al. [15].

Behavioral Assessment

Head Poking Test

At the end of the six-week treatment period, three rats from each group were subjected to a head-poking behavior study for three days to assess neophilia, anxiety levels and analyze the learning curve [16]. Each rat was placed in the center of an arena and allowed to explore freely. The number of head pokes was recorded, counting consecutive pokes only if the rat fully withdrew its head before poking again. Each session lasted ten minutes.

Histopathological Examination

Three rats from each group were fasted overnight, euthanized, and their brains were removed for histopathological examination. The skull was carefully opened, and the hippocampus was extracted and fixed in 10% neutral buffered formalin. The hippocampal sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope.

Statistical Analysis

Data were expressed as mean ± standard error of the mean

(SEM). Statistical analysis was performed using SPSS version 22 (IBM Corp., Armonk, New York, USA). The Shapiro-Wilk test was used to assess the normality of data distributions. One-way ANOVA followed by Tukey's post hoc test was used for group comparisons. A P-value < 0.05 was considered statistically significant.

Ethical Approval

This study was approved by the Ethics Committee of King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (Date: 2022-08-28, No: 256-22).

Results

Head Poking Test

The head-poking behavior was assessed over three days. On the first and second days, the number of pokes was significantly decreased in the AD and Pr groups compared to the C group ($P < 0.0001$ and $P = 0.015$ for AD on the first day; $P < 0.0001$ and $P = 0.004$ for Pr on the second day). Conversely, the Mg group showed a significant increase in poking numbers on these days compared to the C group ($P = 0.024$ and $P = 0.010$, respectively). Additionally, the AD group had significantly fewer pokes than the Mg group on both the first and second days ($P < 0.0001$ for both).

On the third day, the AD and Pr groups continued to show significantly decreased poking numbers compared to the C group ($P < 0.0001$ and $P = 0.007$, respectively), while the Mg group exhibited a significant increase ($P = 0.036$). Compared to the Mg group, both the AD and Pr groups had significantly fewer pokes ($P < 0.0001$ and $P = 0.023$, respectively).

Biochemical Assessment

As shown in Table 1, levels of Tau and APP were significantly elevated in the AD and Pr groups compared to the C group ($P < 0.0001$ for all). These levels were also significantly higher in the AD group compared to the Mg and Pr groups ($P < 0.0001$ for all). Albumin levels were significantly lower in the AD group compared to the C, Mg, and Pr groups ($P < 0.0001$) and significantly higher in the Mg and Pr groups compared to the C group ($P < 0.0001$ for both).

GSH and SOD levels were significantly reduced in the AD and Pr groups compared to the C group ($P < 0.0001$ for all) and were also significantly lower in the AD group compared to the Mg and Pr groups ($P < 0.0001$ for all). AchE levels were significantly increased in the AD and Pr groups compared to the C group ($P < 0.0001$ for all) and in the AD group compared to the Mg and Pr groups ($P < 0.0001$ and $P = 0.002$) but were significantly decreased in the Mg group compared to the C group ($P = 0.026$). MDA levels were significantly higher in the AD group compared to the C group ($P < 0.0001$ for all).

Histopathological Assessment

Histopathological analysis of the hippocampus from the different study groups (C, AD, Mg, and Pr) revealed significant morphological differences. The control (C) and Mg groups showed a normal hippocampal structure with few degenerated cells. The Pr group exhibited potential therapeutic effects, with some aluminum mobilization from the brain, supporting the biochemical findings. In contrast, the AD group showed pronounced degenerative changes in hippocampal neurons,

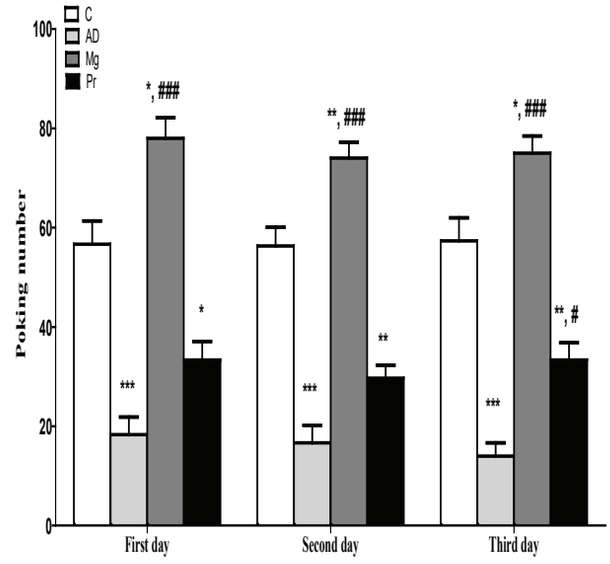

Figure 1. Comparison of head-poking numbers among the groups over three days. Data are expressed as mean ± standard error of the mean (SEM). *: Significant versus group C, ##: Significant versus group AD. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$

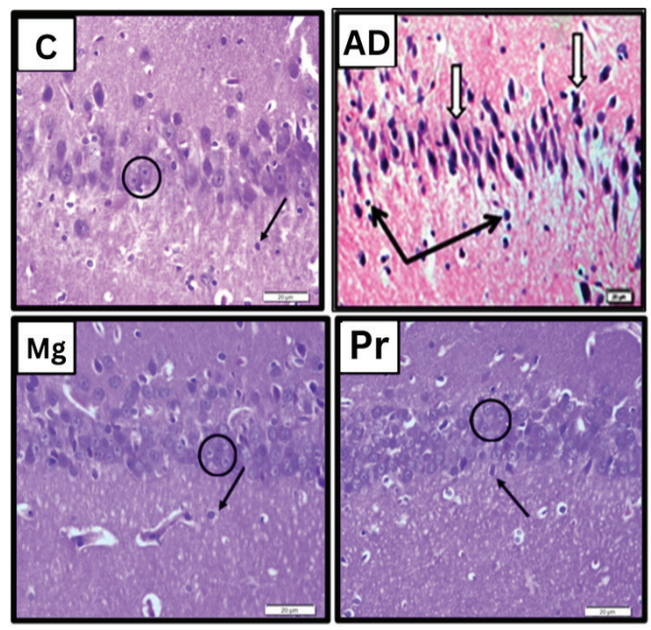

Figure 2. Sections of the rat hippocampus. (C) Control group: photomicrograph (CA1, 400x) of the hippocampus stained with H&E, showing normal neurons (black circle) compactly arranged in the well-delineated neuropil. Small glial cells with dark nuclei are indicated (black arrows). (AD) AD group: significant neuronal shrinkage with dark staining (degeneration or apoptosis). Increased glial cells (thin black arrows). (Mg) Positive control group: normal hippocampal neurons (black circle), most with large vesicular nuclei and regular arrangements. Small glial cells with dark nuclei are indicated (black arrows). (Pr) Protective group: some neurons appear shrunken (black arrows), but potential neuronal protection is observed (black circle)

Table 1. Comparison of biochemical parameter levels in serum between groups

Parameters	Groups (n =10)			
	C	AD	Mg	Pr
Tau (pg/mL)	14.40 ± 0.38	30.92±0.49	15.35 ± 0.19	18.28 ± 0.20
Significance	-	1P <0.0001	1P = 0.999, 2P < 0.0001	2P < 0.0001
APP (pg/mL)	6.80 ± 0.12	13.86±0.09	6.94 ± 0.14	8.70 ± 0.12
Significance	-	1P < 0.0001	1P = 0.830, 2P < 0.0001	2P < 0.0001
AchE (U/mL)	105.18 ± 1.13	116.79 ± 1.14	101.00 ± 0.82	111.33 ± 0.83
Significance	-	1P < 0.0001	1P = 0.026, 2P < 0.0001	1P < 0.0001, 2P = 0.002
Albumin (g/L)	4.13 ± 0.07	1.56 ± 0.03	4.93 ± 0.05	4.63 ± 0.05
Significance	-	1P < 0.0001	1P < 0.0001, 2P < 0.0001	1P < 0.0001, 2P < 0.0001
GSH (mg/ml)	16.92 ± 0.11	3.97 ± 0.05	16.83 ± 0.08	12.01 ± 0.17
Significance	-	1P < 0.0001	1P = 1.000, 2P < 0.0001	1P < 0.0001, 2P < 0.0001
MDA (nmol/ml)	0.57 ± 0.02	1.80 ± 0.02	0.60 ± 0.01	0.51 ± 0.01
Significance	-	1P < 0.0001	1P= 0.612, 2P <0.0001	2P < 0.0001
SOD (U/mg)	181.07±1.28	123.99 ± 0.94	180.40±0.49	171.56 ± 0.67
Significance	-	1P < 0.0001	1P = 0.952, 2P < 0.0001	1P < 0.0001, 2P < 0.0001

Tau: Tau protein, APP: Amyloid precursor protein, AchE; acetylcholine esterase, GSH: Glutathione, GSH: Superoxide dismutase, MDA: Malondialdehyde, SOD: Superoxide dismutase Comparisons between the groups were performed using one-way ANOVA followed by Bonforoni post hoc test. Each data point is representative of 3 independent Data expressed as mean ± standard error of the mean (SEM). 1P: significance versus group C, 2P: significance versus group AD

with cells appearing smaller, shrunken, and deformed, exhibiting dark cytoplasm and small, dark nuclei.

Discussion

This study offers a comprehensive analysis of the neuroprotective, biochemical, and behavioral effects of MgT in an AD model induced by AlCl3 in rats. Aluminum (Al) can cause neurotoxicity by generating free radicals, leading to lipid and protein damage through peroxidation. Chronic exposure to Al has a strong affinity for cell membranes and can stimulate the formation and accumulation of insoluble Aβ plaques and neurofibrillary tangles (NFTs) in the brains of individuals with AD [17].

Recent research has increasingly focused on the role of magnesium (Mg) in dementia and other neurodegenerative diseases such as AD. There are two primary hypotheses regarding Mg's function in dementia. The first hypothesis suggests that Mg directly regulates NMDA receptors, which are ionotropic glutamatergic receptors, by partially blocking the receptor and obstructing calcium channels during neurotransmission. The second hypothesis posits that low levels of Mg2+ stimulate the release of inflammatory substances, such as interleukins, TNF-α, and nitric oxide, which may increase the risk of developing AD [18].

The results of this study highlight the potential of MgT to mitigate the harmful effects of AD through several mechanisms. Mg2+ protects neurons by (1) inhibiting voltage-gated calcium channels and dilating blood vessels; (2) blocking NMDA receptor calcium channels to prevent calcium influx; (3) reducing the production and enhancing the scavenging of free radicals after tissue damage; (4) ameliorating mitochondrial dysfunction, thereby reducing damage caused by impaired energy metabolism; and (5) reducing neuroinflammation by downregulating proinflammatory cytokines [11].

Mg is essential for numerous enzymatic reactions and is critical for synaptic plasticity and neurotransmission. MgT's ability to cross the blood-brain barrier effectively increases brain Mg levels, which is crucial for enhancing synaptic plasticity and improving cognitive functions [11].

Behavioral assessments using the head-poking test demonstrated MgT's impact on cognitive function and anxiety levels. The AD group showed significantly reduced head-poking behavior across all three days of testing, reflecting increased anxiety and cognitive impairment. In contrast, the MgT group showed increased head-poking behavior, indicating reduced anxiety and improved cognitive function. These findings align with previous studies reporting the cognitive-enhancing effects of MgT in AD models [12].

Histopathological examination of the hippocampal neurons revealed significant differences between the treatment groups. The C and Mg groups exhibited normal neuronal structures with few degenerated cells. In contrast, the AD group showed marked neuronal degeneration, characterized by shrunken neurons with dark cytoplasm and nuclei, indicating severe neurodegeneration. The Pr group, which received both AlCl3 and MgT, showed fewer degenerative changes compared to the AD group, suggesting that MgT provides partial neuroprotection even in the presence of AlCl3-induced toxicity. These findings are consistent with previous studies demonstrating the neuroprotective effects of magnesium in neurodegenerative conditions [2].

Biochemical assessments revealed significant alterations in key biomarkers associated with AD. The AD group exhibited elevated levels of APP and Tau protein, hallmark features of AD pathology. These elevations were significantly reduced in the MgT and Pr groups, indicating that MgT can mitigate the pathological accumulation of these proteins. This suggests that MgT may interfere with the amyloidogenic pathway and tau phosphorylation, thereby reducing the formation of amyloid plaques and neurofibrillary tangles [1].

Oxidative stress (OS) markers also showed significant differences between the groups. The AD group had increased levels of MDA, a marker of lipid peroxidation, and decreased levels of antioxidants such as GSH and SOD. These changes indicate enhanced OS in AD. In contrast, the MgT group

exhibited significantly lower MDA levels and higher levels of GSH and SOD, suggesting that MgT has a potent antioxidant effect. This supports the OS hypothesis of AD, which posits that oxidative damage plays a crucial role in the disease's progression [8].

The accumulation of toxic oligomers of Aβ in the brain due to inadequate clearance from interstitial fluid (ISF) may be a cause of AD. Albumin, the primary carrier of Aβ in the blood, changes with aging and neurodegeneration. Low serum albumin is associated with cognitive impairment in the elderly [19]. This supports MgT's ability to improve cognitive function, reduce oxidative stress, and protect neuronal integrity, making it a compelling candidate for further research and clinical trials. Additionally, the safety profile of MgT as a dietary supplement adds to its potential as a feasible and accessible treatment option [12]. The findings of this study have significant clinical implications. Currently, there is no cure for AD, and available treatments mainly focus on symptom management. The identification of MgT as a potential therapeutic agent offers a promising avenue for AD treatment.

Conclusion

In summary, MgT shows substantial neuroprotective, biochemical, and behavioral benefits in an AD model induced by AlCl3 in rats. These results indicate that MgT holds promise as a therapeutic agent for AD, meriting further research and clinical trials. This study contributes to the expanding body of evidence supporting the role of magnesium in brain health and its potential in combating neurodegenerative diseases.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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