Original Article

Hippocampal Beta-Amyloid Accumulation In Aluminium Chloride and D-Galactose-Induced Rats: Establishing a Nontransgenic Alzheimer's Model

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ABSTRACT

Background: Alzheimer's disease (AD) is characterized by tau protein aggregation and beta-amyloid (Aβ) plaques leading to progressive cognitive decline. Nontransgenic models using aluminium chloride ($AlCl_3$) and D-galactose (D-gal) have been established to mimic AD pathology, but beta-amyloid deposition in hippocampal regions remains underexplored. **Objective:** To investigate beta-amyloid accumulation in the hippocampal CA2 region using a nontransgenic rat model of AD made by combined $AlCl_3$ and D-gal administration. **Methods:** Twelve adults male Wistar rats were divided into control (n = 6) and model groups (n = 6). The control group received normal saline orally and water intraperitoneally. The model group received D-gal (60 mg/kg b.w., i.p.) and $AlCl_3$ (200 mg/kg b.w., oral) daily for 10 weeks. Beta-amyloid expression in hippocampal CA2 region was assessed using immunohistochemistry. **Results:** Immunohistochemical analysis revealed marked beta-amyloid immunoreactivity in the model group, with dense extracellular and intracellular deposits distributed throughout the CA2 subfield. The control group showed minimal beta-amyloid staining without plaques, confirming the specificity of the observed pathology. **Conclusion:** Combined $AlCl_3$ and D-gal administration successfully induced significant beta-amyloid accumulation in the hippocampal CA2 region, validating this nontransgenic model for investigating AD pathogenesis. This model provides a valuable platform for studying sporadic AD mechanisms and evaluating potential therapeutic interventions.

KEYWORDS: *Aluminium chloride, Alzheimer's disease, beta-amyloid, D-galactose, hippocampus*

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by decline in cognitive and memory functions. It primarily affects the older persons, with symptoms most commonly appearing after the age of 65.[1] AD is characterized by a multifaceted interaction of elements, chiefly the deposition of amyloid-beta (Aβ) plaques extracellularly and the aggregation of tau protein intracellularly.[2] The accumulation of harmful Aβ oligomers and tau aggregation results in synaptic dysfunction, oxidative stress, mitochondrial damage, and ultimately neuronal degeneration.[3]

Aluminium chloride ($AlCl_3$) and D-galactose (D-gal) are used to develop models for AD due to their capacity to mimic the neurotoxic effects and neurodegenerative

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mechanisms associated with the condition. Aluminium, a metal included in common things such as food packaging foils and utensils, accumulates in the brain, leading to oxidative stress, mitochondrial dysfunction, synaptic impairment, and neuroinflammation. D-gal is a simple sugar. Collectively, these models provide a thorough methodology for investigating the processes of AD and assessing prospective treatments, enabling researchers to analyze pathological changes.[4] Previous studies have established that oral administration of $AlCl_3$ (200 mg/kg b.w) combined with intraperitoneal injection of D-gal (60 mg/kg/b.w) induces cognitive impairment, behavioral alterations, neurodegeneration, glial dysfunction, and changes in biochemical parameters, including tau, acetylcholinesterase, and oxidative stress markers.[5] Nevertheless, these studies did not examine beta-amyloid deposition in the hippocampal region, a defining pathological hallmark of AD. Therefore, the present investigation was undertaken using a comparable dosage to address this gap.

Material and Methods

$AlCl_3$ and D-gal were obtained from Sigma-Aldrich (St. Louis, MO, USA). β-amyloid primary antibodies were obtained from Novus Biologicals (Littleton, USA). Other reagents used were of analytical grade and sourced locally.

Animals

Twelve adults male Wistar rats (2–3 months, 220–250 g) were obtained from a local supplier (Selangor, Malaysia). They were housed under controlled temperature (23 ± 2°C), and a 12 h light/dark cycle, with free access to feed and autoclaved reverse osmosis water. Experimental procedures were approved by the UPM Animal Care and Use Committee (UPM/IACUC/AUP-R071/2020).

Experimental design

Before the experimental period, rats were randomly categorized into two groups of six (6) animals in each group.

Group 1 (G1) is considered the control group, where animals received normal saline through the oral route (oral gavage) and water through the intraperitoneal route (i.p).

Group 2 (G2) is considered a model group in which animals were administered D-gal at a dose of 60 mg/kg/b.w through the i.p. route along with $AlCl_3$ at a dose of 200 mg/kg/b.w/oral.

The dose for the model group was selected based on the preliminary study conducted in our laboratory with different groups, including various graded doses such as D-gal 60 mg/kg/b.w and $AlCl_3$ 100 mg/kg b.w, D-gal 60 mg/kg b.w and $AlCl_3$ 200 mg/kg/b.w and D-gal 60 mg/kg/b.w and $AlCl_3$ 300 mg/kg/b.w for 10 weeks in rats. Based on the results such as behavioral test parameters, neuronal death assessments in three hippocampal subfields, Tau expression in the cerebral cortex and Acetylcholinesterase (AChE) levels in brain tissue, the regimen of D-galactose (60 mg/kg b.w.) and $AlCl_3$ (200 mg/kg b.w.) coadministration for 10 weeks was identified as the most suitable model because the combined alterations across these parameters reliably reflected and cognitive impairments mimicking AD like symptoms in humans.[6]

Sample collection and preparation

After 10 weeks of experiment, the rats were humanely euthanized through decapitation. The brains were carefully removed and rinsed with ice-cold saline to ensure they were clean. The brain samples were preserved in a 10% formalin solution for histological analysis.

Immunohistochemistry for beta amyloid expression in the hippocampal CA2 subregion

Immunohistochemical analysis was performed in the hippocampal CA2 region to evaluate beta-amyloid deposition, a hallmark of AD pathology. Tissue sections were deparaffinized, rehydrated, and subjected to peroxidase blocking and antigen retrieval. Sections were incubated overnight at 4°C with a primary beta-amyloid antibody (MOAB-2, Novus Biologicals; NB-NBP2-13075, supplied by Biomed Global), followed by a biotinylated HRP-conjugated secondary antibody. Immunoreactivity was visualized with DAB and counterstained with hematoxylin. Stained sections were examined under an Olympus phase contrast microscope (Tokyo, Japan) to assess beta-amyloid plaque distribution.

Results

Immunohistochemical analysis of beta-amyloid protein expression in the hippocampal CA2 regions of the brain

Beta-amyloid, a key pathological marker forming extracellular plaques associated with neurodegeneration and cognitive decline, was assessed in the hippocampal CA2 region using immunohistochemistry [Figure 1]. Beta-amyloid plaques appeared as densely pigmented deposits within neuronal layers, indicating abnormal protein accumulation and potential synaptic dysfunction. The model group (D-galactose + $AlCl_3$) showed marked Aβ immunoreactivity, with dense extracellular and intracellular deposits distributed throughout the CA2 subfield. These deposits reflect progressive neurodegeneration within the affected hippocampal region.

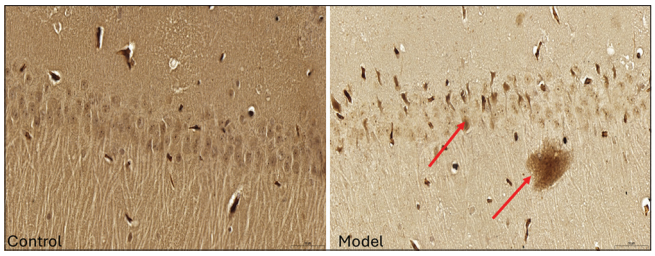

Figure 1: Representative immunohistochemical images of the hippocampal CA2 region showing negligible beta-amyloid staining in controls, while the model group exhibited clearly defined dense plaques

Discussion

This study successfully demonstrated significant beta-amyloid accumulation in the hippocampal CA2 region using a nontransgenic rat model induced by combined $AlCl_3$ and D-gal administration. This finding addresses a critical gap in the characterization of this widely used AD model and validates its utility for investigating amyloid pathology.

The dense beta-amyloid immunoreactivity observed in the CA2 region of treated rats aligns with characteristic AD neuropathology. Beta-amyloid plaques represent cardinal pathological markers of AD and are related with synaptic dysfunction and cognitive decline.[7] The presence of both extracellular and intracellular deposits indicates successful replication of amyloid pathology observed in human AD patients.

The hippocampal CA2 region plays crucial roles in social memory and is particularly vulnerable to amyloid pathology in early AD stages.[8] Our demonstration of significant accumulation in this subfield supports the translational relevance of this model.

The coadministration of $AlCl_3$ and D-gal induces AD like pathology through converging mechanisms. $AlCl_3$ promotes amyloid precursor protein misfolding, enhances gamma-secretase activity and directly binds to amyloid peptides, promoting their aggregation.[9] D-gal contributes through oxidative stress induction and advanced glycation end products formation, which can promote beta-amyloid aggregation and neurotoxicity.[10] The synergistic effects of these compounds likely account for the robust amyloid deposition observed.

Conclusion

Our study validates the utility of the $AlCl_3$ and D-gal model for investigating AD pathogenesis by demonstrating significant beta-amyloid accumulation in the hippocampal CA2 region. This finding, combined with previously reported cognitive and biochemical changes, supports the translational relevance of this model for understanding AD and provides a foundation for future mechanistic studies and therapeutic evaluations.

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Conflicts of interest

There are no conflicts of interest.

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