

Metal mixture-induced non-transgenic animal model of Alzheimer's disease: Pros and cons

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Abstract

Alzheimer's disease (AD) is a multifaceted and heterogeneous age-related disease and represents the most common cause of dementia among the elderly. Over the past two decades, transgenic models of AD appreciably contribute to the understanding of the molecular mechanisms involved in the onset and progression of AD. However, transgenic models generally identify with the familial form of AD that accounts for just 5% of AD cases. Thus, non-transgenic models are also essential to thoroughly understand AD pathophysiology. Environmental exposure to heavy metals has been linked to the pathogenesis of the non-familial, sporadic form of AD. This review summarizes our previously published research that showed a mixture of heavy metals, *i.e.* Arsenic (As), cadmium (Cd) and lead (Pb) at environmentally relevant doses induced AD-parameters and AD-like pathology in the young rats. Our previous findings suggest that the amyloid beta-42 (A β ₁₋₄₂) levels in the As+Cd+Pb-mixture treated Postnatal-90 day rat brain were comparable with the intracerebroventricular A β ₁₋₄₂ infusion rat model, which is well-established non-transgenic model of AD. Additionally, As+Cd+Pb-mixture-induced A β and amyloid precursor protein could be attenuated by known AD-directed drugs, memantine, and donepezil. These findings helped us to conclude that As+Cd+Pb-treated animals could be utilized as a non-transgenic model of AD. This review also summarizes the merits of a non-transgenic animal model of AD, generated through environmental doses of As, Cd and Pb-mixture and its demerits.

Introduction

Alzheimer's disease (AD) is one of the most common forms of progressive dementia globally. According to the recent report of Alzheimer's Association, approximately 4.5 million people in the United States currently suffering from AD.¹ In India, more than 4 million people are affected by some form of dementia.¹ Extracellular senile plaques are considered to be one of the major neuropathological hallmarks of AD.² The key protein component of extracellular plaques is the amyloid beta (A β), a 39 to 43 amino acid peptide, cleaved from amyloid precursor protein (APP) by β -secretase and a putative γ (gamma)-secretase.³ Many lines of evidence suggest that abnormal deposition of neurotoxic A β is associated with a decline in memory and learning ability of affected individuals and contribute to the pathogenesis of AD.⁴

Substantial research has been carried out to develop animal models that exhibit characteristic neuropathological features of AD.⁵ Most of the animal models employed in the AD-directed drug screening include transgenic mice that over-express mutant APP or presenilin genes. However, these transgenic models are developed by genetic manipulation and do not mimic all facets of human AD such as extensive neuronal loss, A β plaque formation or significant memory impairment.^{6,7} Furthermore, transgenic mice do not represent a genuine model for sporadic AD.⁸ Transgenic models are only suitable for studying the familial pattern of AD with genetic links and represent about 5% of all Alzheimer's cases.⁹ A detailed description regarding the limitations of these models is given in Table 1. Therefore, more relevant models bearing AD-like characteristics are essential.¹⁰ Non-transgenic animal models provide alternative approaches to the more widely used transgenic AD models. These models represent the sporadic AD that accounts for 95% of cases.¹⁰ Beside A β deposition, these models also manifest considerable oxidative stress, gliosis, inflammatory reactivity and cognitive impairment.¹¹ Therefore, these models exhibit the complete pathophysiology of AD. Additionally, this type of model is morally accepted by the public and scientific community.¹⁰ However, non-transgenic method of infusing A β peptide¹² or streptozotocin¹³ involves the disadvantage of intracerebroventricular delivery. Moreover, none of the present models are reported to render early-onset AD symptoms.

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Heavy metals contamination and their role in Alzheimer's disease

Majority of the AD cases (approximately 90%) are sporadic, where environmental pollutants act as important risk factors.¹⁴ Environmental exposure to heavy metals such as lead (Pb), mercury (Hg), aluminium (Al), cadmium (Cd) and arsenic (As) have been reported to be involved in AD.¹⁵⁻¹⁸ Arsenic, Cd and Pb are among the leading toxicants detected in the environment globally.^{19,20} These metals have been linked to developmental neurotoxicity and various neurodegenerative disorders.^{21,22}

As+Cd+Pb-mixture treated non-transgenic Alzheimer's disease model

We established a non-transgenic animal model of AD, induced by environmentally relevant doses of heavy metal mixture

through drinking water that showed early signs of AD in young rats.²³ The pregnant Wistar rats were daily gavage-treated with metal mixture (As: 3.80 ppm, Cd: 0.98 ppm, and Pb: 2.22 ppm) dissolved in reverse osmosis-treated water. The treatment of the dams started from gestation day 5 (G-05) until the pups weaned (postnatal day 21, P-21), and the pups from P-22 were directly treated with the metals until P-90²³ (Figure 1).

The metal mixture doses were adjusted according to the comparable body weight of experimental rats. To eliminate confounding consequences of the female reproductive cycle, only male offsprings were used for our study.

This non-transgenic model exhibited the key pathological hallmarks of AD, such as pathological amyloid beta-42 ($A\beta_{1-42}$) and $A\beta_{1-40}$ peptides, APP, rise in oxidative stress, inflammatory markers,²³ and $A\beta$ -mediated neuronal apoptosis in frontal cortex and hippocampus of the brain.²⁴ The model also demonstrated the main manifestation of AD, *i.e.* cognitive impairments²³ (Figure 2). The pathological features were found to closely match the cerebral $A\beta$ -infusion model, which could be attenuated by AD-directed therapeutics.²³ Overall, As+Cd+Pb-exposed Wistar rats displayed typical amyloidogenic features, and satisfied pre-requisites of an early-onset AD model and could be utilized for screening of AD-targeting drugs and therapies.

Merits

This As+Cd+Pb-mixture treated Wistar rats possess many advantages compared to a typical transgenic mice model. More importantly, this model fits into the *amyloid hypothesis*, showing augmented proteolysis of APP towards $A\beta$.²⁵ Here, the diseased state has been developed in a rat model, which is physiologically closer to human compared to a mouse model.²⁶ Additionally,

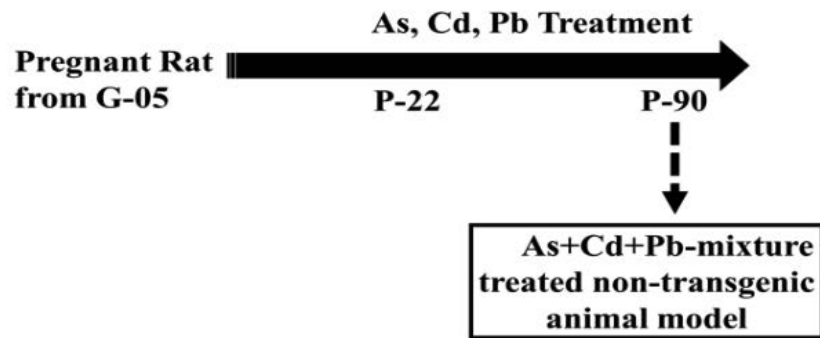


Figure 1. Schematic representation of the As+Cd+Pb-mixture treatment in our non-transgenic model

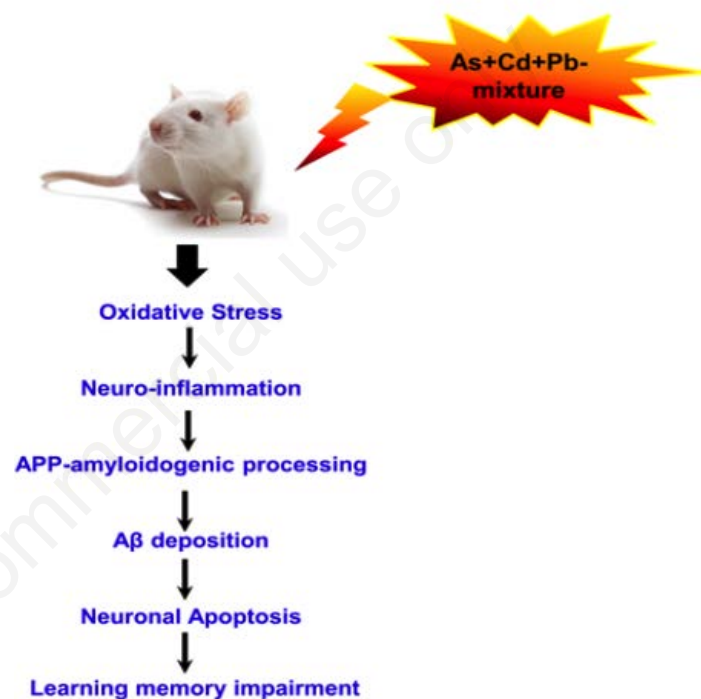


Figure 2. Schematic diagram of the amyloidogenic features in our non-transgenic Alzheimer's disease model.

Table 1. Transgenic models and their limitations.

Category	Pathology	Limitations
Single Transgenic model	APP: $A\beta$ pathology, synaptic dysfunction, cognitive decline and behavioral alterations PS: Increased $A\beta_{1-42}/A\beta_{1-40}$ ratio in some models demonstrates cognitive decline and behavioral abnormalities tau: NFT pathology, neuronal loss, cognitive decline and behavioral alterations	Lacks NFTs and shows limited neuronal loss despite extensive $A\beta$ pathology Lacks NFT, no $A\beta$ pathology and no neuronal loss No $A\beta$ pathology
Double Transgenic model	APP/PS: Accelerated $A\beta$ pathology in APP/PS compared to single transgenic APP model APP/tau: Accelerated tau pathology in APP/tau models compared to single transgenic tau model	No NFT pathology and limited neuronal loss -
Triple Transgenic model	APP/tau/PS: Accelerated tau and $A\beta$ pathology compared to single or double transgenic model. Amyloid plaques, NFT pathology, and cognitive deficit	Limited neuronal loss; No synaptic loss

APP, amyloid precursor protein; NFT, Neurofibrillary tangles; PS, presenilin; $A\beta$, amyloid beta.

this model bears several advantages over the others in terms of its generation. The method of induction is convenient, non-laborious and involves minimum mechanical injury. In comparison, the non-transgenic rodent model for AD, generated through intracerebral injection of A β ,¹² causes non-specific and non-targeted neurodegeneration.^{27,28} Moreover, chronic implantation of the cannula for intracerebral A β delivery leads to thinning of cerebral layers.²⁹ As+Cd+Pb-mixture treated rats, despite being devoid of these shortcomings, expressed A β ₁₋₄₂ and APP at levels comparable to cerebral A β infusion; suggesting suitability as convenient new models for AD.²³ Additionally, rats are larger in size than mice and comparatively easy to treat with test compounds. Therefore, As+Cd+Pb-treated rats bear the essential prerequisites and advantages as an AD model, and hence could be utilized for studying the disease etiology and screening anti-AD therapies.

The time required for generation of our AD model through As+Cd+Pb treatments was much shorter (only 3 months), compared to transgenic models that demand a time-consuming genetic manipulation process. The convenient method of disease induction through oral gavage treatment also proved economical. Therefore, As+Cd+Pb-mixture treated non-transgenic model would enable researchers to conduct quick screening for AD-targeted drugs, saving time and cost.

Most importantly, As+Cd+Pb treatments did not show toxicity of the other vital organs of the rats. Therefore, the heavy metal(s) exposure could be well-claimed as a suitable method to generate potential models for AD.

Demerits

Although this non-transgenic mouse model could be extremely useful for scientific research, several research studies are further required to fully validate this model. Thus, current models require additional modifications to fully replicate the complex conditions of human AD.

Conclusions

Overall, As+Cd+Pb-mixture exposed animal model proposes a suitable new early-onset sporadic animal model for AD, which may be utilized for studying the etiology of AD at an early age and identifying novel compounds targeting the disease. Therefore, in addition to numerous transgenic models, which have been proven to be

powerful tools for understanding the characteristics of the disease, As+Cd+Pb-mixture treated non-transgenic animal AD-model may help enlighten different pathological mechanisms of the disease.

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