The role of toll like receptor 4 in pathogenesis of Alzheimer's disease induced by aluminum chloride

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Abstract*—Amyloid plaques and neurofibrillary tangles are the neuropathological hallmarks of *Alzheimer's disease. β-amyloid peptides deposit as extracellular aggregate and form amyloid plaques that are thought to be toxic to the surrounding neurons. Accumulation of amyloid plaques is reported to be involved in synaptic dysfunction and cognitive impairment in Alzheimer's disease. These deposits are associated with a robust microglial mediated inflammatory response. Toll like receptor 4 (TLR4) is important in the activation of innate immunity. The present study will focus on role of TLR4 in the pathogenesis of Alzheimer's disease. In vivo model of Alzheimer's disease was developed in Sprague Dawley rats by administering aluminium chloride (50 mg/kg) for 28 days by intra peritoneal route. After 28 days, memory test was done in all animals by morris water maze and then all animals were sacrificed and their brains were isolated for TLR4 gene expression using RT-PCR as well as quantification using ELISA kit. Results of the present study show that over expression of TLR4 in the brain of rats as well as loss of memory in rats treated with aluminium chloride. Thus upregulation of TLR4 plays an important role in the pathogenesis of aluminium chloride induced Alzheimer's disease model. This study will be helpful in delaying the disease progression and provide promising therapeutic targets for treatment of Alzheimer's disease.

Index Terms— ***Alzheimer's disease, Toll like receptor 4, Memory loss, Amyloid plaques***

INTRODUCTION

Alzheimer's disease is a common form of dementia, described by German psychiatrist and neuropathologist Alois Alzheimer in 1906. Alzheimer's disease is mostly diagnosed in people over 65 years of age, although early-onset can occur much earlier. Alzheimer's disease being a geriatric disease is proving itself to be the sixth most common cause of deaths worldwide. Its worldwide prevalence is 47.4 million and is expected to rise to 135 million by 2050. Thus, as the world population grows with increased age expectancy the Alzheimer's disease prevalence is to increase [1]. In India according to Delhi consensus about 3.7 million people, i.e., 2.1 million women and 1.5 million men with age 60 and over are suffering from dementia. These values are set to increase to 14.32 million by 2050 [2].

An extracellular plaque of β-amyloid protein, intracellular neurofibrillary tangles, and loss of cholinergic neurons are neuropathologic hallmarks associated with the pathogenesis of Alzheimer's disease [3]. Acetylcholine is a vital neurotransmitter in brain regions involving memory and loss of cholinergic results in cognitive impairment [4]. Glutamate is a useful excitatory neurotransmitter of CNS, although its excessive amount in the brain can lead to cell death. β and γ secretase cleaves the amyloid precursor protein (APP) at different positions and forms insoluble amyloid beta plaques. These insoluble amyloid beta plaques get deposited in the brain, and in turn, induces reactive oxygen species (ROS) production from cortical neurons through activation of NADPH oxidase and cause neuronal damage [5]. Three genes, i.e., APP, presenilin 1 and presenilin 2 are involved with the formation of Aβ [6]. In addition to the above pathogenesis, there are shreds of evidence that there is a role of neuroinflammation and activation of glial cells in Alzheimer's disease, although microglial activation by aggregated Aβ may possess beneficial effects by phagocytic clearance. Microglial activation further leads to progressive neurodegeneration by the production of neurotoxic mediators such as reactive oxygen and nitrogen species, proteolytic enzymes, glutamate, complement factors, and inflammatory cytokines. The interaction of fibrillar forms of Aβ with microglia triggers tyrosine kinase-based cascades involving the Src family kinases and Syk kinase that culminate in the generation of ROS, cytokines, prostaglandins, and phagocytosis. Toll-like receptor 4 (TLR4) is a type of pathogen recognition receptor. TLR4 is vital in the activation of innate immunity and play a key role in neuroinflammation and neurodegeneration in Alzheimer's disease [7, 8].

Currently, only four drugs used for the treatment of Alzheimer's disease are available in the market. These drugs include three cholinesterase inhibitors and on NMDA receptor antagonist. Cholinesterase inhibitors like rivastigmine, donepezil, and galantamine prevent the breakdown of acetylcholine and therefore boost cholinergic neurotransmission, and contribute their clinical benefits. Memantine, an NMDA receptor antagonist, protects neurons from excessive glutamate activity and also decrease tau hyperphosphorylation. These drugs have limitations that they are mainly for symptomatic relief and do not cure Alzheimer's disease. The benefits of cholinesterase inhibitors are temporary, lasting for a maximum of 12–24 months at best and these medications do not slow the rate of decline in cognitive or functional capacities over the long term [9]. As there are only a few current treatments available to treat Alzheimer's disease, there is a need to develop effective therapeutic candidate. The improvement of new therapeutics for the treatment of Alzheimer's disease relies mainly on *in vivo* screening models. Various animal models are used presently for the screening of drugs for the treatment of Alzheimer's disease. Currently, the basic approach of using *in vivo* models that reciprocate the pathogenesis of Alzheimer's disease is based on mutations related to amyloid-β (Aβ) or tau production which are not the etiology of late-onset Alzheimer's disease. Recent investigations suggest that mechanisms like chronic neuroinflammation may occur in the response of amyloid-β and tau pathologies in late-onset Alzheimer's disease. Among various available models, lipopolysaccharide, polyI:C, streptozotocin, and p25 induced immune challenge models are compatible with the neuroinflammation hypothesis of Alzheimer's disease [10]. However, till date, several reports show that $AlCl_3$ is a promising model for the pathogenesis of Alzheimer's disease by amyloid-β (Aβ) or tau production [11, 12], but limited literature is available that give an insight about the role of neuroinflammation and TLR4 in *in vivo* $AlCl_3$ model of Alzheimer's disease.

This study mainly aims to evaluate the role of TLR4 receptors in *in vivo* model of Alzheimer's disease induced by Aluminium chloride. This study will help in preventing disease progression and provide better management of the disease.

I. MATERIALS & METHODS

A. Materials

ELISA kit is procured from Elabscience. All the other chemicals were purchased from HiMedia laboratories Pvt. Ltd., India.

B. Animals

Sprague Dawley rats (n = 30), weighing 200.00 ± 5.00 g, of either sex were used for the study. The animals were housed 3 per cage in polypropylene cages and the environmental conditions of the animal room were as per a specific design. A 10% air exhaust in the air conditioning unit was maintained along with a relative humidity of 60 ±5% and a temperature of 25 ± 3 °C was stabilized. A 12 hour light/dark cycle was also regulated for the experimental animals. Food and water provided *ad libitum* to animal during experimental period. All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee prior to the initiation of the experiment.

C. Experimental Design

In vivo model was developed in Sprague Dawley rats by administering aluminium chloride ($AlCl_3$) (50 mg/kg) for 28 days by intra peritoneal route. After 28 days, memory test was done in all animals by using morris water maze [13]. Lastly, all animals were sacrificed and their brains were isolated for histopathology with congo red staining, TLR4 gene expression using RT-PCR as well as quantification of TLR4 using ELISA kit.

D. Statistical Analysis

Statistical analysis was done using GraphPad Prism 4 (San Diego, CA). All the results were expressed as the mean ± standard error mean (SEM). All the data were statistically analyzed by student t-test (unpaired). $p< 0.05$ was considered statistically significant.

II. RESULTS

A. Evaluation memory performance in $AlCl_3$ treated rats using morris water maze test

Results of morris water maze test show significant increased escape latency of $AlCl_3$ treated rats in comparison to normal rats. Thus memory of $AlCl_3$ treated rats were deteriorated in comparison to normal rats (Figure 1).

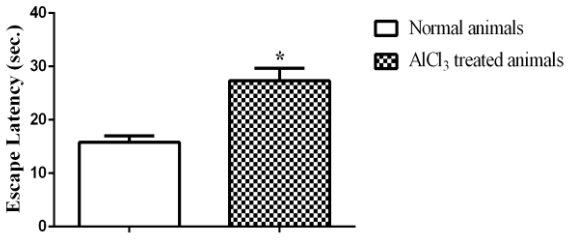

Figure 1: Effect of $AlCl_3$ treatment on memory performance in rats using morris water maze test. Data are expressed as mean ± SEM (n=6). *$p<0.05$ compared to normal animal group using student t-test (unpaired).

B. Histopathology of normal and $AlCl_3$ treated rats

For histology study, sections from brain tissues were prepared from control group and $AlCl_3$ treated group and drug treated group. Sections were stained with congo red. Congo red staining was done in brain sections for detection of amyloid. Congo red specifically stains amyloid and shows dark pink to orange coloration. Figure 2B shows deposition of amyloid in cerebral cortex in $AlCl_3$ treated animals while absent in brain section of rats without $AlCl_3$ treated.

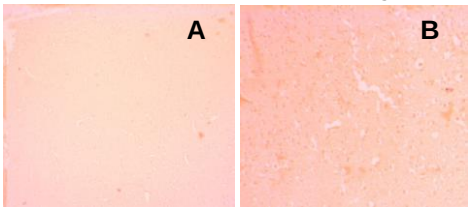

Figure 2: Congo red staining of cortex region of brain sections for detection of amyloid
A= Normal control, B= AlCl3 treated (100X magnification).

C. Level of TLR4 in $AlCl_3$ induced Alzheimer's disease

TLR4 was unregulated in $AlCl_3$ treated rats indicate that $AlCl_3$ treatment causes elevation of TLR4 levels in their brain (Figure 3)

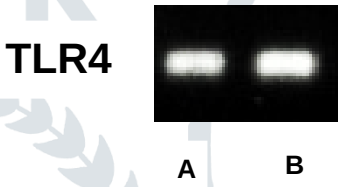

Figure 3: $AlCl_3$ induced changes on TLR4 mRNA expression levels in brain tissues. A= Normal animals, B= $AlCl_3$ treated animals

D. TLR4 expression in $AlCl_3$ induced Alzheimer's disease using ELISA

Results show that expression TlR4 is significantly increased in brains of $AlCl_3$ treated rats in comparison to normal rats (Figure 4).

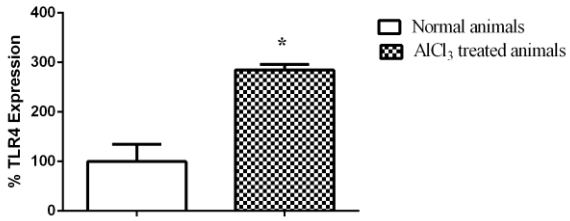

Figure 4: Effect of $AlCl_3$ treatment on % TLR4 Expression in rat's brain using ELISA. Data are expressed as mean ± SEM (n=3). *$p<0.05$ compared to normal animal group using student t-test (unpaired).

III. DISCUSSION

The key findings of the present investigation are as follows: (1) chronic administration of $AlCl_3$ (50 mg/kg, i.p.) in rats for 28 days induced amyloid plaque deposition in histopathological studies and consequently memory loss, (2) chronic administration of $AlCl_3$ (50 mg/kg, i.p.) in rats for 28 days induced overexpression of TLR4 in the brain of rats.

Previous studies have proposed a potential association between the aluminum-induced neurotoxicity and the pathogenesis of Alzheimer's disease. Kawahara et al., [14] reported that chronic exposure of primary cultured neurons of

rat cerebral cortex to aluminum chloride cause marked neuronal death, degeneration of neuritic processes and accumulation of tau protein and β-amyloid protein. Results of congo red stained histopathological slides in the present study also show deposition of β-amyloid protein. Amyloid plaques are composed of insoluble deposits of β-amyloid peptides (Aβ). Patients with familial Alzheimer's disease who have mutations in the amyloid precursor protein (APP) gene have either increased production of Aβ or generate more aggregation-prone forms of Aβ (Aβ42). Aβ42, composed of 42 amino acid residues, aggregates readily and is considered to form amyloid plaque. Aβ plays a central role in the pathophysiology of Alzheimer's disease [15]. The processes of plaque formation by Aluminium are still not very much evident. The previous study postulated that aluminium is a cross-linker and reported to enhance the polymerization of Aβ and thus in turn leads to aggregation of Aβ [14].

Aβ is generally thought to be secreted into the extracellular space and aggregates to form amyloid plaques. These deposits result in a robust microglial-mediated inflammatory response. Activated microglia, innate immune cells in the central nervous system, plays a pivotal role in the progression of the disease: either clearing Aβ deposits by phagocytic activity or contribute to progressive neurodegeneration by releasing cytotoxic substances and pro-inflammatory cytokines and [16]. Toll-like receptor 4 (TLR4) is a type of pathogen recognition receptor and play a chief role in the activation of innate immunity. The result of the present study shows overexpression of TLR4 of rat cerebral cortex and consequently memory loss. Thus, TLR4 plays a vital role in neuroinflammation in $AlCl_3$ induced Alzheimer's disease. Activation of microglial cells stimulates JNK and caspase pathway [17]. Activation of JNK and caspase pathway leads to neuronal cell death and consequently memory loss.

IV. SUMMARY

Aluminium is a cross-linker, and chronic exposure of $AlCl_3$ enhances the polymerization of Aβ and thus, in turn, leads to aggregation of Aβ. Deposition of amyloid plaques and neurofibrillary tangles in the brain causes overexpression of TLR4 on glial cells and neurons and therefore, activation of microglial cells and neurons. Activated microglial cells and neurons, results in the release of releasing cytotoxic substances and pro-inflammatory cytokines as well as stimulation of JNK & caspase pathway that leads to neuronal cell death (Figure 5).

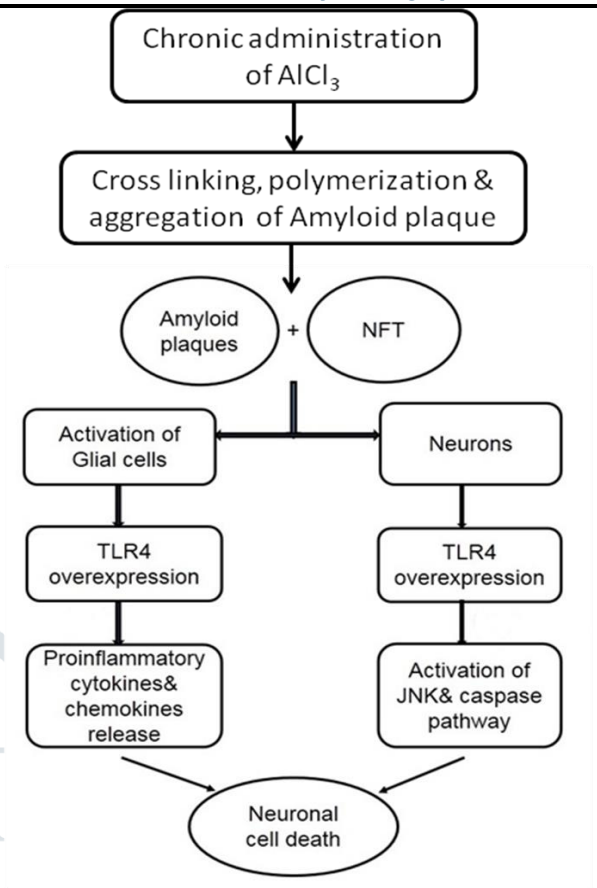

Figure 5: Pathogenesis of $AlCl_3$ induced Alzheimer's disease.

CONCLUSION

TLR4 are unregulated in the brain of $AlCl_3$ treated rats and there is a considerable memory loss in $AlCl_3$ treated rats. Thus, this study will be helpful in delaying the disease progression and provide TLR4 as a promising therapeutic target for treatment of Alzheimer's disease.

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