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EXPERIMENTAL MODELS FOR ALZHEIMER'S DISEASE: A MECHANISTIC VIEW

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ABSTRACT

Alzheimer's disease (AD) is a leading cause of morbidity and mortality and its prevalence is continuously increasing. AD is characterized to varying degrees by Amyloid β, neurofibrillary tangles, gliosis, synaptic and neuronal loss leading to a decline in memory & apraxia agnosia and several neuropsychiatric changes like anxiety and depression etc. Increased age, oxidative stress and neuroinflammation are considered to be the major risk factors implicated in the progression of AD. Various signaling systems, such as vasoconstrictor peptides, inflammatory mediators, growth factors, are involved in the pathogenesis of AD. At present, no promising therapy is available due to lack of understanding of signaling culprits involved in the pathogenesis of AD. Animal models are being developed to better understand the disease pathogenesis and develop drugs for this ailment. In the present review, various common lab animal models for AD are discussed, which has been used, can be used and which will open new vistas for developing new drugs to treat this cognitive syndrome.

INTRODUCTION:

Alzheimer's Disease: Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder that destroys the higher structures of the brain. Prominent neuropathologic features of AD are senile plaques, neurofibrillatory tangles, synaptic, neuronal loss and volume loss (Atrophy) [1, 2, 3, 4]. There are around 35 million patients suffering from Alzheimer's disease all over the world, out of which United States of America alone has around 4.5 million patients [5]. In India AD patents are estimated to be less than 3.5 million [6] and the number is increasing at a fast pace.

After heart diseases, cancer, and stroke it is the fourth leading cause of death in US [7]. Alzheimer's disease is accompanied by psychiatric manifestatations such as psychosis (delusions and hallucinations) and disruptive behaviors (e.g., psychomotor agitation and physical aggression), especially in the later stages of the disease [8, 9, 10]. AD usually begins with difficulties with memory and orientation, with subsequent gradual and progressive decline in visuo spatial skills, language and calculation, praxis (learned motor skills), gnosis (perception), and frontal and executive functions, such as reasoning, judgment, foresight, and insight [11]. Hippocampus, limbic system, and cortex are the primary areas affected in the pathophysiology of AD [1, 12].

A small fraction of patients develop myoclonus, seizures, or spastic paraparesis, and many develop weight loss and extra pyramidal signs. Patients may become lost in their own homes and fail to recognize family members. In latter stages of the disease, basic activities of daily living such as dressing, grooming, bathing, mobility and transfers, toileting, and eating are progressively affected. After years of cognitive and functional decline, patients become vegetative, mute, unresponsive, incontinent, and bed-bound before death ensues- often from pneumonia and overwhelming infection [13, 14].

Although several factors mediate the development and progression of AD, genetic factors (PS1, PS2 and APOE) and associated comorbid conditions, such as age, oxidative stress and hypertension, are considered to be independent risk factors and major determinants in the progression of AD in patients. Various experimental models are employed to induce dementia resembling Alzheimer's disease in order to identify the potential pharmacological targets. However, the literature for animal models of AD is currently inadequate. This review focuses on various common experimentally- developed animal models producing dementia of AD type.

Experimental Models: The animal models for Alzheimer's disease share many features which are common to human disease and have been delineated by targeting oxidative stress, neuroinflammation, cell death, formation of plaques, tangles, cognitive deficits and other behavioral changes.

- **Intracerebroventricular Streptozotocin Induced Dementia:** Streptozotocin is a glucosamine nitrosourea compound (STZ, ($C_8H_{15}N_3O_7$, 2- deoxy- 2 - (3- methyl- 3- nitrosoureido) - D- glucopyranose) discovered in a strain of the soil microbe *Streptomyces achromogene* in 1956 [15,16]. It was classified as an alkylating agent in the

nitrosourea class of anti-cancer drugs and was used to treat cancer of Islets of Langerhans in the pancreas. STZ is toxic to the insulin producing beta cells of the Islets of Langerhans in the pancreas, and is now better known as a diabetogenic agent, it is used to induce diabetes under experimental conditions when injected i.p. in rats at a dose of 45 mg/kg,50, 55 and 60 mg/kg [17,18,19]. The molecular weight of STZ is 265g/mol and the structure is composed of nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other end [20]. The molecular structure was first described by Herr et al. [21].

Interacerebro- ventricular STZ administration has become an established and most appropriate experimental model for Alzheimer's type of dementia [22] as it is characterized by pathological changes similar to those observed in Alzheimer's disease. Streptozotocin, when injected intracerebroventricularly (ICV) in a subdiabetogenic dose (3mg/kg) in rat, causes prolonged impairment in learning and memory [22,23] by exerting profound and long lasting influence on brain biochemistry, metabolism and function [24, 25, 26]. After ICV administration of STZ severe abnormalities in brain glucose and energy metabolism have been found. Glucose utilization reduces in brain areas [24] and the activities of glycolytic key enzymes are decreased markedly [27] causing diminished concentration of the energy rich compound ATP and creatinine phosphate [28,22]. This may form the biological basis for the marked reduction in learning and memory capacities [22].

Tau protein is hyperphosphorylated as a long-term consequence of STZ icv administration [29] and it also causes neuronal damage and cell loss as well as the accumulation of Aβ in the brain [30]. An increase in the total tau protein in the brain and increase in the β amyloid formation enhance inflammatory process and free radical formation [31]. There is gradual increase in the levels of malondialdehyde (MDA) - the end product of lipid peroxidation. An increase in the levels of lipid proxidation in nerve cells results in apoptosis and cell death [31, 32]. There is a simultaneous decrease in glutathione levels too.

The increase in the levels of MDA and the decrease in the levels of glutathione, both directly associated with oxidative stress parallels with the diminution of learning and memory in rats [33]. Activities of glycolytic enzyme are also reduced [27] leading to decreased formation of acetyl CoA and thereby of acetylcholine [34, 35, 36]. Furthermore, ICV STZ application causes the down regulation of gene expression related to insulin signaling such as IGF-1 receptor. In contrast to this down regulation, an upregulation in gene expression related to potassium channels, GABA receptors and glutamate receptors is also observed [37]. After ICV administration, STZ it reaches the fornix and passes into the 3rd ventricle because of the flow of CSF in a rostrocaudal direction [31]. STZ when administered ICV damages the septohippocampal system [38] whereby memory impairment in rat could occur due to direct damage to the system. This is supported by reduced choline acetyl

transferase (ChAT) activity in the hippocampus [35], reduction in the weight of septum by more than 40% [39], decrease in the transport of nerve growth factor from the hippocampus to septum [40], microglial activation and specific damage to myelinated tract in the fornix through generation of oxidative stress. This finally causes disruption in the connection between the septum and hippocampus [41].

- **Intracerebroventricular Administration of Colchicine:** Colchicine, an alkaloid extracted from some plants of the lily family, has been used for centuries to treat acute gouty arthritis. Since 1973, it has been recognized as an effective remedy for propphylaxis of attacks of familial Mediterranean fever [42]. In animal models of central nervous system damage, colchiccine, a microtubule-disrupting agent, is used as a neurotoxin. Following its introduction into the brain, colchicine binds to tubulin, the principal structural protein of the microtubule, and induces microtubular depolymerization and destabilization, with subsequent block of axonal transport and mitosis, resulting in neuronal cell death [43-46].

The neurotoxicity is mediated through free radical production and the resultant oxidative stress [1, 45]. In addition, colchicine causes loss of cholinergic neurons, destruction of cholinergic pathways, and decrease in cholinergic turnover [47]. The distribution of colchicine in the brain is unequal; its concentration in the hippocampus, the area most affected in Alzheimer's disease, is almost three times higher than in other brain regions [48]. The central manifestations of colchicines neurotoxicity in the animal model closely simulate Alzheimer's disease in humans [45, 46, 49]. Both are characterized by oxidative stress, microtubule disruption, decrease in cholinergic activity, and progressive deterioration of cognitive functions [49, 44]. Systemic administration of colchicine in rats also induced cognitive defects similar to those of Alzheimer's and characterized by amnesia of recent learning and loss of formerly established memories [46].

Further, ICV infusion of colchicine significantly impaired the memory with decrease in norepinephrine, Dopamine and serotonin level in cerebral cortex, hippocampus and caudate nucleus [50]. Dopamine, norepinehrine and Serotonin is involved in plural process supporting learning and memory [50, 51, 52]. Decreased level of Dopamine and Serotonin is associated with AD [52, 53, 54]. Central administration of colchicines also causes loss of cholinergic neurons and cognitive dysfunction that is associated with excessive free radical generation [55, 56]. Cyclooxygenase isoforms are differentially regulated following colchicine injection [55] and it increases the expression of cyclooxygenases [57, 58].

Cyclooxygenase isoforms are implicated in acute and chronic neurological diseases including neurodegenerative processes. Expression of cyclooxygenase- 1 and cyclooxygenase-2 is up regulated in cortical and hippocampal pyramidal circuits [59]. Increased expression of cyclooxygenase activity initiates a pathological cascade

associated with elevated levels of inflammatory mediators [60]. Furthermore, Numerous findings suggest that cyclooxygenase and its products might be important mediators of neuronal injury [61, 62]. Furthermore, it has been reported that central administration of colchicines is also associated with an increase in free radical generation and growing body of evidences now further support the concept of reactive oxygen species and its involvement in oxidative pathway of memory impairment [56, 63]. Indeed, Alzheimer's patients produce more glutathione peroxidase, an enzyme that helps to neutralize free radicals, as a defensive reaction against increased production of peroxideswithin the cells and have disturbances in the antioxidant balance which may predispose to increased oxidative stress [64].

Central administration of colchicines also mediates a cascade of actions, particularly elevation of the GLU/GABA ratio in brain cortex [65]. The relative increase in glutamate activates intracellular metabolic events, including excitotoxicity, triggers the generation of free radicals, which overcome antioxidant defenses and provoke oxidative stress [66, 67] and hyperactivation of NMDA receptors leads to massive Ca^{2+} influx that triggers a rapid activation and over stimulation of Ca^{2+} dependent enzymes including phospholipase A2, proteases, protein phosphatases, endonucleases, nitric oxide synthase, protein kinase, xanthine oxidase and cyclooxygenase. This increase cyclooxygenase-2 activity that contributes to the neuronal and synaptic loss associated with neurodegeneration and increased production of oxidative stress and the neurotoxic actions of prostaglandins [68, 69]. These observations are particularly relevant in view of evidences showing that oxidative stress is involved in the development and progression of Alzheimer's disease and that activation of cyclooxygenase- 1, cyclooxygenase- 2 isoforms or both are required for execution of oxidative neuronal death [55].

Colchicine also increases the expression of inducible nitric oxide synthase and cyclooxygenase-2, thereby increasing nitric oxide (NO) and prostaglandin's synthesis, respectively [57, 67]. Nitric oxide, a precursor for free radicals reacts with the superoxide anions produced as a result of excitotoxicity, peroxidase activity of cyclooxygenase and increased oxidative stress after central administration of colchicine that give rise to toxic intermediates (peroxynitrite, nitric dioxide) [66,67] and overproduction of nitric oxide is neurotoxic to cholinergic neurons too [68, 69].

Acetylcholine in nerve terminals in hippocampus, govern vital aspects of memory and other cognitive functions. Central administration of colchicine cause a marked decrease in acetyl cholinesterase activity and the decrease in the acetyl cholinesterase levels that associated with loss of cholinergic neurons in brain might be due to colchicine inhibition of fast axoplasmic flow or a direct toxic effect on cholinergic terminals [70, 71]. It is also plausible that increased free radicals formation causes macromolecular changes in cholinergic neurons and leads to the reduction in acetyl

cholinesterase activity that contributes to learning and memory deficits [55].

- **Ibotenic Acid Lesion of Nucleus Basalis Magnocellularis:** Ibotenic acid lesion of nucleus basalis magnocellularis is an validated model for Alzheimer's disease [72,73]. Learning and memory is closely associated with the functional status of the central cholinergic system. The basal forebrain provides the major source of cholinergic inputs to the neocortex and hippocampus. The main cholinergic pathways in the mammalian forebrain are the projections from the medial septal nucleus and the nucleus of the vertical limb (diagonal band of Broca) to the hippocampus via the fimbria-fornix and the projection from nucleus basalis cellularis (nbm) to the neocortex. The nbm located in the ventromedial region of the globus pallidus accounts for 70-80% of the cholinergic innervation to the cortex [74].

Lesions of the nbm have been proposed as an experimental model for AD, based on the observation that degenerative changes in nucleus basalis of Mynert (nbM), the human counterpart of nbm, are present in patients of AD. In addition, the nbm lesioned rat shows decreases in cholinergic markers, including Ach levels, release and turnover of Ach, choline uptake, ChAt and acetylcholinesterase activity, and number of muscarinic cholinergic receptors, in the frontal cortex [74], similar to what reported in patients of AD [75]. In this model unilateral nbm lesion are induced by injecting ibotenic acid (10 µg/rat), dissolved in 5 µl of Artificial cerebrospinal fluid (CSF), in anaesthetised rats, using the stereotaxic co-ordinates 1.0 mm posterior to bregma, 2.6 mm right lateral and 7.9 mm below the cortical surface [73].

- **Lysophosphatidic Acid induced Tau Hyperphosphorylation:** Microtubules are essential for neurite formation and maintenance. These structures are composed of tubulin and a variety of minor proteins denoted by microtubule-associated proteins (MAPs). The level of MAPs and, more importantly, their phosphorylation level appear to modulate microtubule stability and dynamics. In particular, *Tau*, one of the most abundant axonal MAPs, may play a key role in axonogenesis [3].

Tau phosphorylation is developmentally regulated, being lower in adult than in fetal brain. Additionally, *Tau* hyperphosphorylation occurs in the aberrant structures known as *p*aired *h*elical *f*ilaments (PHFs) that appear in neuronal disorders such as Alzheimer's disease where these structures are a neuropathological hallmark [4, 8]. The bioactive phospholipid lysophosphatidic acid causes growth cone collapse and neurite retraction in neuronal cells. These changes are brought about by the action of a cell surface receptor coupled to specific G proteins that control morphology and motility through the action of a group of small GTPases, the Rho family of proteins. Many studies have focused on actin reorganization modulated by Rho-GTPases, but almost no information has been obtained concerning microtubular network reorganization after LPA-induced Neurite retraction. It has been demonstrated that there is an increase in site-specific

Alzheimer's disease-like *Tau* phosphorylation during LPA-induced neurite retraction in differentiated SY-SH5Y human neuroblastoma cells [76].

- **L- Methioine Induced Dementia:** L-Methionine treatment for 4 weeks, significantly raises serum Homocysteine level [77] and increased levels of homocysteine have been documented to produce changes in structure and function of cerebral blood vessels along with oxidative stress, which play a key role in cerebral vascular dysfunction [78]. Oxidative stress and vascular dysfunction are recognized as important contributing factors in the pathogenesis of AD and other dementia of vascular origin [79]. In AD and other neurodegenerative diseases, structural deformities in the cerebral capillaries lead to impairment of cerebral perfusion and produced a significant impairment of acquisition and retrieval of memory [77] with subsequent neuronal dysfunction and death.

Cerebral vascular endothelial dysfunction has also been shown to enhance progression of dementia of Alzheimer disease (AD) [80]. Enhanced levels of brain AChE activity and oxidative stress have also been noted in patients suffering form dementia of AD and other dementias [81]. Further hyperhomocysteinemia has also been shown to be neurotoxic, and the neurotoxicity may be due to overactivation of N-methyl-D-aspartate receptors or by enhanced vulnerability of hippocampal neuron to excitotoxic insults and amyloid β-peptide toxicity [82, 83]. Moreover, methionine rich diet in rats has been demonstrated to enhance cholesterol concentration in the plasma and liver [84]. Several studies have also revealed high serum cholesterol level as another important risk factor of AD [85] beside oxidative stress and inflammation [86]. Therefore L-Methionine induced memory dysfunction may be attributed to its multiple effects i.e. decrease in serum nitrite level (endothelial dysfunction), rise in oxidative stress level, enhancement of brain AChE activity, serum total cholesterol as well as direct neurotoxicity.

CONCLUSION: The morbidity and mortality due to Alzheimer's disease is continuously increasing worldwide and the therapeutic agents currently available are limited. During the past few decades, the use of animal models has provided new insights into understanding the complex pathogenesis of Alzheimer's disease. Important pathogenic mechanisms still remain active and unmodified by present therapeutic strategies. Identification of signaling culprits involved using various animal models may provide the lead in discovering novel therapeutic agents.

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