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Review Article

In-Vitro & In-Vivo Screening Models For The Neurodegenerative Diseases: A Review

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ABSTRACT

Alzheimer's disease and associated dementias are the second biggest cause of mortality in the United States and other high-income nations, according to the World Health Organisation, and the seventh highest cause of death globally. According to the Alzheimer's Association, the number of deaths linked to Alzheimer's disease grew by 146% between 2000 and 2018. It is projected that the number of Americans alone who have Alzheimer's or another type of dementia would rise from 56 million in 2020 to 88 million by 2050. They discovered that the number of fatalities from AD and other dementias per 100,000 persons grew by 148%, the prevalence of these conditions climbed by 117%. Preclinical protocols of neurodegenerative disorders (NDs) have been produced in order to assess novel therapy approaches and to comprehend the underlying mechanisms of NDs. It concluded, that numerous *in- vivo* screening models including chemical induced(i.e., Amyloid, Colchicine, Scopolamine, Atropine, Aluminium Chloride, High-fat diet (HFD), Kainic Acid, Domoic Acid, Ibotenic acid, and Ethanol), Transgenic Models (i.e., BACE 1,Brain injury induced AD) and spatial memory (i.e., Morris Water-Maze, Radial Arm-Maze, Circular Platform Test) and *in-vitro* screening models include Cells, Tissues, and Molecular simulation model that were reported for the evaluation of NDs including AD and Dementia.

Keywords: Screening models, neurodegenerative diseases, Alzheimer's disease, Dementia, Transgenic animals.

INTRODUCTION

The average life expectancy has improved because of improvements in medical technology and drugs, which have reduced the number of people dying from infections and other illnesses. The number of adults over 80 in the world is predicted to quadruple from 2015 to 2050 [1]. Ageing disorders present a new medical issue as life expectancy rises. Chronic age- related diseases, also known as ageing diseases, are a wide range of illnesses that develop in people as they age. People may experience chronic age-related disorders as they age, which can impair their quality of life and cause them to become incapacitated [2]. Neurodegenerative diseases/illnesses (NDs) areis aging-related illness. Due to their fast-rising frequency NDs i.e., Alzheimer's disease (AD), Parkinson's diseaseare increasingly becoming the focus of pharmaceutical development. Actually, the biggest risk factor for these illnesses is getting older [3]. The general description of NDs is the progressive loss of neurons, which leads to behavioural abnormalities, dementia, altered motor function, and premature mortality [4]. As the population ages, we are more acutely aware of the impact NDs and other ageing illnesses. There are challenges facing the existing medical system because there are few effective medicines and almost no cures. The enormous responsibility of providing care for those afflicted by severe illnesses carries a heavy cost. It can reduce pain, prolong a healthy, disease-free life, and lessen the burden that NDs place on the medical system, given the issue of having few effective medical intervention alternatives.

Preclinical protocols of NDs have been produced in order to assess novel therapy approaches and to comprehend the underlying mechanisms of NDs [5]. Essential insights into the pathophysiology of NDs have

been gained through the use of diverse in vivo models across a range of animals and illness settings. These insights include the characterisation of neuronal patterns and ageing.

Alzheimer's disease/ Dementia

Nowadays, the majority of the world's leading causes of death are age-related illnesses. AD and associated dementias are the second biggest cause of mortality in the United States and other high-income nations, according to the World Health Organisation, and the seventh highest cause of death globally [6]. The deaths attributable to neurological illnesses have increased by 42.1% over the last 40 years [7]. According to the Alzheimer's Association, the number of deaths linked to Alzheimer's disease grew by 146% between 2000 and 2018. In addition to an increase in prevalence, a larger percentage of Italian patients over 65 who participated in ten-year research investigating the prevalence of age-related disorders reported having more than one condition [8]. It is projected that the number of Americans alone who have Alzheimer's or another type of dementia would rise from 56 million in 2020 to 88 million by 2050 [9]. They discovered that the number of fatalities from AD and other dementias per 100,000 persons grew by 148%, the prevalence of these conditions climbed by 117%, and the disability-adjusted life years declined by 121%.

A. *In-vivo* screening models Amyloid-Induced AD

The intracerebroventricular or intrahippocampal routes can be used to deliver amyloid-β42, amyloid-β40, and amyloid-β25-30.These amyloids have been divided into groups according to how many amino acids they each contain. Additionally, they differ in the etiology of AD, with amyloid-β-42 being the most harmful kind.It has been demonstrated that the ICV- Aβinjection causes neurodegenerative processes as well as learning and memory impairment.This could be achieved by bringing the oxidative and nitrosative parameters back to normal [10].Amyloid-β has caused an overabundance of reactive oxygen species (ROS) [11].

Colchicine-Induced AD

The rats who receive a dosage of 15μg in a 5μL vehicle- such as distilled water- experience cognitive impairment.The resulting cognitive impairment is fairly similar to sporadic AD following intracerebroventricular injection.Colchicine aggravates the neuroinflammatory pathways that cause synaptic dysfunction and neurodegeneration and disrupts the oxidative balance andcholinergic pathways [12].Prostaglandin E2 (PGE2), IL-1β, TNF-α, and cyclooxygenase-2 (COX-2) may be involved in the inflammatory response when collagen is produced.

Scopolamine-Induced AD

Since acetylcholine strengthens synaptic connections and is one of the most important neurotransmitters in memory processing, scopolamine-mediated inhibition of cholinergic nervation is frequently utilized as an animal model for Alzheimer's disease. By increasing acetylcholinesterase activity, scopolamine facilitates the breakdown of Ach. The intraperitoneal dosage of scopolamine for an AD model is around 2 mg/kg. Scopolamine breaks the connectivities between several brain regions, including the functional network and the mapping of spatial memory [13].

Atropine-Induced AD

Like scopolamine, atropine enters the cholinergic system and decreases the hypofunction of the muscarinic receptor. It also partially inhibits the nicotinic one. Atropine produced amyloid when administered intraperitoneally (ip) at a dose of 5 mg/kg for 21 days. There may be a connection between amyloidogenesis and the cholinergic system that causes this process.[14][15] Moreover, the decreased acetylcholine release caused by Aβ and vice versa was noted[16].

Aluminium Chloride-Induced AD

When aluminium is present in excess, it can have several hazardous effects. By intraperitoneal injection (i.p.) of aluminium chloride ($AlCl_3$) at a rate of 4mg/kg or 40ml/kg each day for around 40 days[39]. Oxidative stress and mitochondrial malfunction are identified as the major triggers in an $AlCl_3$ model. These factors are reported to manifest by blocking the electron transport chain's NADH dehydrogenase enzyme[17].Additionally, it has been discovered that any aluminium salt might cause AD-associated neurotoxicity at dose(s) of 100mg (1 day)/ 20mg (in 5 days)[18]. Al salts also result in oxidative stress and cholinergic dysfunction, which trigger the apoptotic process[19].

Highfat diet-induced AD

The animals are given a high-fat diet, which causes hypercholesteremia [20]. They have hypercholesteremia since they frequently lack the additional metabolic abnormalities that go along with AD [21]. In HFD-fed rats, dementia is invariably induced during a 3-month period, and at the same time, there are increased Aβ deposits [22]. Research has demonstrated that a high-fat diet (HFD) elevates serum cholesterol levels, which in turn causes an increase in the concentration of cholesterol in the brain [23][24][25]. Aβ deposits can impair cognition by triggering oxidative and nitrosative stress, as well as inflammatory responses. In HFD-fed

mice, this mechanism also results in impaired insulin sensitivity and glucose intolerance, raising the likelihood of AD susceptibility [26]. This model is frequently used to study the significance of various food components in the pathophysiology of AD. But using this model requires an experimental process that takes far too long [27].

Kainic Acid-induced AD

Kainic acid is a glutamate receptor agonistproducing effects similar to those of glutamate. (It is utilised to differentiate amongst glutamate receptors, such as NMDA and AMPA). Research has demonstrated that the administration of KA leads to an elevation in the generation of reactive oxygen species, impairment of mitochondrial function, and programmed cell death in neurons, specifically in the hippocampus(CA1, CA3 areas), as well as in the hilus (dentate gyrus) [28]. Experimental data indicates that when kainic acid is supplied intraperitoneally (i.p.) to rats, it results in minor behavioural symptoms. These symptoms include reduced activity followed by episodes of increased activity, nodding of the head, sudden jerking movements of the forelimbs and jaws, shaking resembling a wet dog, and/or moderate salivation. This information is supported by reference [29].

Domoic Acid-induced AD

Domoic acid (DA) is a neurotoxin that belongs to the group of heterocyclic amino acids. It is an analogue of kainic acid and was first discovered in toxic algal blooms. The presence of this marine biotoxin, which occurs naturally, is responsible for causing amnesic shellfish poisoning. A high dosage of this toxin can lead to symptoms such as vomiting, cramps, coma, and even death. Additionally, it can have neurological effects on people, including hallucinations, disorientation, and memory loss [30]. These compounds are useful for studying neurotoxicity and the anterograde amnesic syndrome that occurs after consuming shellfish contaminated with domoate.. This damage results in a long-lasting loss of memory, which was assessed using the Morris water task [31]. Recent research suggests that the toxicity caused by domoic acid in neuronal cells is a result of an imbalance in the regulation of intracellular calcium levels. Domoic acid inhibits the action of adenylate cyclase, which in turn reduces the level of cAMP, so inhibiting the flow of Ca2+ into brain cells. The excessive accumulation of Ca2+ in neuronal cells causes toxicity, which in turn leads to neurodegeneration and subsequent memory loss [32].

Ibotenic acid-induced AD

It functions as a brain lesioning agent and has demonstrated significant neurotoxicity when directly injected into the brains of mice and rats. Administering ibotenic acid in the medial septum region of the rat brain results in an excitotoxic lesion- degeneration of AchE in hippocampus. These findings suggest that the memory impairment caused by ibotenic acid is due to disruption of the cholinergic network [33].

Ethanol induced-AD

Ethanol, a substance, has been proven to have an impact on spatial learning and memory that is comparable to the effects caused by damage to the hippocampus [34][35]. Administering ethanol through a single i. p. injection at sub-high dose has a significant impact on spatial learning and memory[36]. Furthermore, the injection of ethanol in high dosages has been shown similar effects of damaging the afferents of the hippocampus system [37]. Additionally, it has been shown that the injection of moderate dosages of ethanol acutely results in specific memory impairment in C57BL or 6J mice [38].

❖ Transgenic animals of AD

In instances of familial Alzheimer's disease, the dominant inheritance pattern may lead to functional mutations [39][40]. When Drosophila melanogaster males had their psn function lowered by 50%, they experienced considerable cognitive impairment relative to wild type males [41]. Some strains of transgenic mice possess the genes responsible for producing ApoE4 [42].A protocolof AD has been developed using a combination of APP/PS1 mutations, which results in the expression of both mutant human APP and mutant PS1. This model has demonstrated a deterioration in learning & memory, accompanied by an increased level of amyloid-β and increased phosphorylation (tau protein) [43].

BACE 1

BACE1 enzymatically degrades the APP molecule in the extracellular space, resulting in the cleavage of the protein into two fragments: a soluble peptide found outside the cell and a peptide linked to the cell membrane known as C99 [44]. In a particular study, researchers employed a unique approach involving the use of antibody immunoreactivity to examine the impact of BACE 1 and axonal alterations in relation to plaque development. Researchers conducted a comparison investigation and discovered that the plaques were present alongside elevated levels of BACE1 in older transgenic mice. Young animals also experienced this condition when BACE1 levels increased before plaque formation [45]. However, calpain, a cysteine protease that is dependent on calcium, has a significant function in regulating BACE1. According to reports, in a model of mice with two genetic modifications (APP/PS1), the excessive production of calpastatin, a natural inhibitor of calpain (CAST), controlled by the calmodulin dependent protein kinase II promoter,

stopped the reduction in phosphorylation of CREB molecules[46].The BACE1 knock-in transgenic mice, when combined with the APP transgene, exhibited accelerated neurodegeneration in their brains[47][48].

Brain injury induced AD

The hippocampus is believed to have a crucial function in absorbing information from various sources and integrating them to carry out complex learning tasks [49]. Traumatic brain injury frequently causes cognitive problems due to the death of neurons, either directly or indirectly. The injury was caused by a moderate parasagittal fluid percussion, and it resulted in memory impairment. Experimental studies have demonstrated that targeted mechanical injuries to the hippocampus regions of humans [50], monkeys [51], and rodents [52][53] result in impaired cognitive performance. Several studies indicate that damage to the dorsal hippocampus areas in rodents, which accounts for appr. 40% of the total hippocampal volume, has a considerable negative impact on learning and memory observation [54][55]. According to another report, damaging the small lesions in the dorsal hippocampus of rodents can negatively affect their ability to remember spatial information, while not affecting their performance in tasks that require recognising previously encountered information [56].

❖ Evaluation of spatial memory Morris Water-Maze

The analysis of spatial memory is conducted using the Morris Water Maze, which examines the animal's ability to navigate based on distal visual cues that primarily influence spatial orientation. This modality, created by Morris in 1984, has been widely utilised in numerous neurobehavioral laboratories worldwide [57].

The device consists of a big circular pool filled with water, which has a concealed platform slightly below the water surface. During the trials, rodents are educated to navigate towards the concealed platform in order to escape (water pool). The duration it takes for each rodent to find the escape platform is regarded as escape latency. The amount of time that the animal spends in the quadrant where the platform is located is considered as the retrieval index. Notably, because to its straightforward and uncomplicated experimental approach, this instrument has been extensively utilised to investigate the memory & learning.

Typically, conventional concealed-platform training involves conducting sets of four swimming trials selected at random from four different starting positions. Following every successful trial, the animal generally stays a brief duration on the platform. In particular, mice that exhibit high levels of nervousness or anxiety are physically constrained to prevent them from immersing themselves in water again. Each trial has a duration of 60-120 seconds, at which point it is directed towards the platform. The data collected from the four initial positions are combined to calculate the average values for each trial block. During probing trials, the analysis of spatial memory retrieval involves measuring the amount of time spent in the target quadrant while searching for the platform. A proficiently trained animal often has a strong inclination towards the target quadrant and, on average, allocates around 50% or more of its unrestricted swimming time to observing this quadrant.

The MWM was developed as a particularly sensitive tool to study the consequences of hippocampus injuries in rats [58]. Numerous writers have provided evidence that hippocampus formation has a distinctive and disproportionate role in the spatial features of MWM learning [59]. It has thus been demonstrated that rats with hippocampal lesions exhibit reduced learning in MWM learning on concealed platforms but not on visible ones. Rats with hippocampal lesions were also able to find a hidden platform that was consistently located at the same distance and orientation from an obvious landmark [60], indicating that these animals are still capable of using heading vectors. It's interesting to note that the extent of hippocampal tissue destruction in rats with hippocampal lesions correlates with the impairment of spatial learning[61]. Afterwards, amnesic and anti-amensic medications are used to test [62].

Radial Arm Maze Test

A spatial discrimination test that Olton and colleagues created for mice has been widely used in research on learning and memory. The animal locates the baited arms with efficiency by using the spatial information offered by the distal cues in the room. Studying reference memory and spatial working processes is made possible by this exam. The device is made up of eight radially extending arms fixed to a central platform with a diameter of 26cm. Each arm measures 56 cm in length, 8cm (width), and 5cm (height). Every arm has a guillotine door at the entrance. The maze is raised to a height of 76 cm from the ground and has good lighting. The animal makes advantage of all the additional maze cues that are present in the environment. Food pellet is kept at the end of arm at the starting of trial. All of the guillotine doors are closed when the rat is positioned on the central platform[63].

All the doors reopen after a 60-second pause. The animal's rewards are tallied once this process is completed eight times. Re-entering an arm is regarded as incorrect. The shape, length, and number of arms in the radial arm labyrinth have all been altered, but the driving force behind each modification is hunger. Thus, the primary drawback of this paradigm is that the anorectic impact of some medications, like amphetamine, or hypothalamic lesions change the effect of appetite as a motivating cue. Furthermore, this paradigm requires food deprivation. According to the study, rats with lesions to the retrosplenial cortex performed worse on retention tests in the arm maze and showed no change in their ability to learn place discriminations; in

contrast, rats with lesions to the hippocampus took longer to learn place discrimination tasks and did not show any change in their memory retention [64].

Circular Platform Test

The device is made up of a round, well-lit platform with eighteen identical holes spaced around its edge. Except for one that has a dark tunnel leading to the home cage, all of the holes are blind ended. This goal tunnel maintains a consistent relative position with respect to distant environmental inputs. As an indicator of acquisition and retrieval, the number of mistakes made in locating the hole and the transfer latency time to escape via the goal tunnel are recorded. It is an ecological model that takes into consideration rodents' innate preference for gloomy environments rather than requiring them to go without food or water [65]. The test has been extensively utilised for hippocampal-based spatial memory study since it was first presented by Barnes in 1979 [66][67][68]. When cues from the surrounding environment were removed, mice' memory skills were further demonstrated to be spatial in character. The performance of the animal in this model is likewise impacted by motor impairment.

B. *In-vitro* screening models Tissue models

In a different investigation, compounds that show an affinity for tau plaques in the human brain were found using an HTS technique. After identifying thousands of compounds (less than 500 molecular weight) in a small molecule library, 14 compounds were shown to have a strong affinity for the AD brain. The study also unveiled a brand-new screening method that visualises protein aggregation in the brain by using affinity imaging mass spectrometry (AIMS). Milliliter-scale frozen brain slices were placed in stainless steel microchips and subjected to liquids containing library chemicals. The microchips were manipulated by a robot hand system, which made it possible to create 16,000 slices from a 1 cm3 organ sample. AIMS was then used to visualise the compounds that showed a strong affinity for the structure of the brain [69].

Aβ peptides had a positive effect on undifferentiated neurons, promoting their growth. However, these peptides had a negative effect on mature neurons, causing damage. The extent of the trophic (growth-promoting) and toxic effects of Aβs was determined by the proportion of Aβ25-35 present. Additionally, they demonstrated that the impacts of Aβ can be replicated by tachykinin antagonists and fully counteracted by particular tachykinin agonists [70].

In a prior investigation, Gong et al administered okadaic acid to metabolically active rat brain slices to suppress protein phosphatase 2A. They discovered that AD is associated with the hyperphosphorylation of tau at multiple sites. This model also demonstrated the phosphorylation of MAP protein [71].

In a separate investigation, Li et al provided evidence that memantine effectively suppressed and reversed the aberrant hyper-phosphorylation of tau protein, which is linked with Alzheimer's disease, and the resulting neurodegeneration. This was proven using rat brain slices that were metabolically active. Collectively, the results of these studies indicate that brain slices can be utilized as a biochemical model for AD and to evaluate the effectiveness of prospective treatments [72][73].

Cell Models

This method is highly effective for modeling the disease and discovering new drugs. In Yagi et al's work [74], induced pluripotent stem cells (iPSCs) were obtained from patients with familial Alzheimer's disease who had mutations in PS1 and PS2. These iPSCs showed elevated levels of Aβ42 in all iPSC lines, providing evidence for the involvement of Aβ as a causative component in the development of Alzheimer's disease. Israel et al. successfully generated induced pluripotent stem cells (iPSCs) from both patients with familial Alzheimer's disease (fAD) and individuals with sporadic instances, using the same technique [75]. The study found that the neurons produced from induced pluripotent stem cells (iPSCs) showed notably elevated levels of Aβ40, phospho-tau, GSK-3β, and endosomes. In a prior investigation, the application of docosahexaenoic acid to treat AD neurons derived from induced pluripotent stem cells (iPSCs) resulted in a significant reduction in intracellular Aβ levels in both sporadic AD (sAD) and familial AD neurons[76].

Human neuroblastoma cell lines are utilized as a model to replicate the pathogenesis of Alzheimer's disease. These cells expressed important components of the amyloidogenic cascade, which is also present in AD [77]. Previous research demonstrated that the application of carnosic acid to human neuroblastoma cells exposed to Aβ42 resulted in the reduction of cell death. This effect was achieved by inhibiting the activated caspase cascades caused by the accumulation of Aβ within the cells [78].

Rare sequence variations in the surface receptor microglial TREM2 have been linked to a higher risk of AD. Using the CellomicsArrayScan VTI HCS Reader, targets of small compounds from different chemical libraries were tested on human myeloid cells. It has been found that the greatest and most stable rise in TREM2 protein is seen in inhibitors of kinases MEK1/2. If this finding can be applied therapeutically, it may have major advantages [79].

Molecular simulation model

Denis developed a NADPH oxidase-nitric oxide system as a bio-machinery that acts against bubbles, with the purpose of mimicking the inflammatory cascade observed in Alzheimer's disease (AD). The bio-machinery caused neuronal death by initiating an inflammatory cascade mediated by guanylyl cyclase. Scientists have

documented the creation of computer simulations that model the behaviour of molecules. These simulations involve a peptide called Aβ40 in water, interacting with compounds that disrupt the formation of β-sheets in a specific region of the Aβ40 sequence. The simulations demonstrate that these compounds prevent the aggregation of Aβ40 by stabilizing its natural structure [80].

CONCLUSION

One of the main causes of cognitive impairment, dementia is primarily seen in Alzheimer's patients. Memory is impacted by even normal ageing, which alters how the brain processes information and makes it harder to recall knowledge. Pre-clinical research that is repeatable must serve as the foundation for developing an appropriate diagnostic strategy and treatment plan. Regretfully, the synthetic medications currently prescribed to treat memory loss only alleviate some of the symptoms; they do not correct the underlying molecular deficiencies. Whether using synthetic methods or different herbal concoctions, the outcomes have been encouraging.The pathophysiology of AD and the underlying genetic and metabolic changes have been greatly understood over the past few decades. Every model has distinct advantages as well as unique restrictions. The underlying aims of the study as well as the research goals must be taken into consideration while choosing an experimental model. These models are all especially useful for researching the pathogenesis of AD and assessing possible treatments.
It concluded, that numerous *in-vivo*screening models including chemical induced (i.e., Amyloid, Colchicine, Scopolamine, Atropine, Aluminium Chloride, High-fat diet (HFD), Kainic Acid, Domoic Acid, Ibotenic acid, and Ethanol), Transgenic Models (i.e., BACE 1, Brain injury induced AD) and spatial memory (i.e., Morris Water-Maze, Radial Arm-Maze Test, Circular Platform Test) and *in-vitro* screening models include Cells, Tissues, and Molecular simulation model that were reported for were reported for the evaluation of NDs including AD and Dementia.Animal models can benefit from the extracellular conditions that can be precisely and reliably controlled using *in-vitro* models.

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