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Review

Alzheimer's Disease and Animal Models

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disease that has not yet been cured, and its incidence has been shown to increase with age. As life expectancy in the developed world has been growing over the years, the effects of Alzheimer's has grown to become a social problem, too. Treatments based on the amyloid cascade and tau protein hypotheses, which are the two main pathogeneses of the disease, have not been proving as successful as expected in clinical studies. For this reason, researchers working on new types of medications, first use experimental animal models and tests, as much as needed to make sure they produce results that are efficient enough in treatment. Thus, by trying to develop new approaches, treatment methods are sought. In this review, hypotheses including amyloid deposits, taupathy, increased inflammation, cholinergic loss, oxidative stress and glucose hypometabolism are mentioned in order to better understand the mechanism of developing Alzheimer's disease. Models have been described in experimental animals by intra-cerebroventricular streptozotocin, aluminum chloride administration and cholinergic dysfunction. The models have also been subjected to certain memory tests to proofread the methods used.

Keywords: Aluminum Chloride model, Alzheimer's disease, animal memory tests, cholinergic dysfunction, experimental animal models, intracerebroventricular streptozotocin model.

Dementia is a disorder involving memory loss to a level that the expected cognitive function of the patient is diminished.[1] Today, approximately 24 million people who complain from demantia. Alzheimer's disease, first described in the 1900s, is only the most common type.[2]

Globally, the prevalence of dementia doubles every 20 years. This disease is expected to affect around 81 million people by 2040.[3] AD accounts for 60-80% of all dementia cases and the non-familial type of Alzheimer's disease usually affects people over 65 years of age and has a high observed mortality rate.[4] AD is a neurodegenerative disease that causes a progressive cognitive decline, involving a short - and long - term memory impairments as well as confusion, disruption of verbal communication and sleep-wake cycles, mood fluctuations and various losses of bodily functions[5] which all deeply affect the patient and those around.[6]

The main pathological markers of AD are amyloid plaque and neurofibrillary tangle depositions.[7] Histopathological analysis of the disease show that accumulation of amyloid plaque and neurofibrillary tangles in humans and animal models causes damage in various parts of the brain, such as the forebrain, frontal lobe,[8] hippocampus and the cerebral cortex.[9-11]

Apart from these; inflammation, oxidative damage, glutamate excitotoxicity, insulin resistance, cholinergic loss and synapse loss also cause neurodegeneration.[12]

HYPOTHESES THAT MAY DESCRIBE THE PATHOGENESIS OF ALZHEIMER'S

Amyloid hypothesis

Amyloid plaques are formed by the accumulation of amyloid peptides (Aβ), which are produced by the cleavage of the transmembrane amyloid precursor

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protein by an enzyme called beta secretase. These transmembrane proteins are crucial for the growth and the maintenance of neurons but the amyloid peptides produced by their cleavage, are actually insoluble in human nerve cells.[13-15] Even though this is so, there is no clear correlation between the cortical plaque counts of a patient and their cognitive decline throughout AD; so other factors are surely playing a role in disease progression as well.[16,17]

Tau hypothesis

Abnormal phosphorylation of tau proteins makes them 'sticky', leading to a breakdown in cellular microtubules which impair axonal transport in the cell and thus, neuronal death occurs.[18] A post-mortem study of the human brain found a significant correlation between the density of neocortical neurofibrillary entanglement and the severity of dementia in AD patients.[19]

Inflammation hypothesis

Microglia is a type of brain cell responsible for inflammation and the function of neural maintenance. They are also found to play a role in neurodegenerative diseases. The mechanism of a microglia's response to protein aggregation like the Aβ, contributes to our understanding of Alzheimer's disease. Some studies show that the specific type of microglial cells involved in a reaction actually depends on the presence of amyloid plaque deposition and that the interactions between them can actually contribute to the pathology. Understanding whether the complex interaction between these different microglial functions influences the progression of neurological disease will only be clarified in future studies.[20]

Cholinergic hypothesis

Acetylcholine (ACh) is an important mediator in the processes of various physiological activities such as attention, learning, memory, stress response, wakefulness, sleep as well as the transmission of sensory information.[21] Cholinergic neuron damage is found to be associated with cognitive impairment in AD. This hypothesis has been tested with cholinesterase inhibitors in AD therapy. Although the therapeutics used to inhibit cholinesterase had side effects, they are currently the most preferred method for managing clinical symptoms of AD.[22]

Oxidative stress hypothesis

Oxidative stress is also found to be playing an important role in AD pathogenesis. The brain uses more oxygen than all other tissues and undergoes mitochondrial respiration, which increases its exposure to ROS. AD is thought to be associated with oxidative stress because Aβ is also found to induce oxidative stress.[23] Therefore, an approach in treatment with antioxidant compounds is, in theory, protective against oxidative stress and toxicity. Therefore, it is implicated as part of a standard therapy regimen, because of its potential to arrest the progression of AD on its tracks.[24,25]

Glucose hypometabolism hypothesis

Glucose hypometabolism is associated with cognitive and functional decline in the early stages of AD. Treating the underlying causes of glucose hypometabolism before irreversible damage is important for the treatment of AD.[26,27] In the clinic, there are no specific ways to alleviate glucose hypometabolism, but brain imaging techniques, such as 18FDG-PET (2-deoxy-2-fluoro-18-fluoro-D-glucose with Positron emission tomography), are required for a proper diagnosis of Alzheimer's disease.[28]

COMMONLY USED MODELS IN ALZHEIMER'S DISEASE

In animal models, neuronal dysformation is induced by amyloid deposition and tau hyperphosphorylation. These procedures help mimic the state of AD observed in humans in rodents under laboratory conditions.[18]

Intracerebroventricular Streptozotocin model

A decrease in both oxygen and glucose consumption in the brain is observed in the non-familial type of Alzheimer's disease. This model was proposed upon the observation of a similarity between the clinical courses of human patients with AD and symptoms observed in rats that have been subjected to intra-cerebrovascular (icv) streptomycin (STZ) injections. Even though there was a relatively decreased amount of disproportionate fall in the oxygen consumption of the brain[29] observed, when compared to the actual AD the application of icv STZ is thought to induce a non – transgenic preclinical model of the disease for further scientific inquiries.[30]

The streptozotocin used in these procedures are produced from cultures of Streptomyces achromogenes and is applied bilaterally to the rodent brain. This substance is also a glucosamine-nitrosourea compound that is toxic to the insulin-producing beta cells of the pancreas. STZ application is performed by stereotaxic surgery. 3 mg/kg STZ is administered to both lateral ventricles of the rodents

with a 10µl injector. The same dose of STZ is repeated 48 hours after the first administration. It takes 12 days for the symptoms to be presented and the model to be completed. Later applied passive avoidance tests and the Morris water maze tests confirm whether the model is achieved or not.[31]

The passive avoidance test is performed to test the memory retention deficits of the rodents with icv STZ. Thus, memory based on context and fear conditioning is tested. Mice are expected to learn to stay away from a site in the test associated with a deterrent. A two-compartment setup is used for the test: One of the compartments is large, dark and there, electric shocks are administered to the rodent, while the other compartment is small, bright and there, the animal do not receive electric shocks. The time it takes for the animal placed in the small and bright compartment to pass into the dark compartment is recorded. If the animal placed in the light chamber for the second time realizes that it will receive an electric shock and does not pass into the dark chamber, the test is considered passed.[32,33]

Rats injected with STZ and rats injected with saline are expected to provide different results in the passive avoidance test. Due to their nature, rats prefer a dark environment. Rats passing from the bright zone to the dark zone are shocked and expected to learn that they will be shocked when they move from the bright room to the dark room. Since rats injected with STZ are less inclined to learn that there is an electric shock waiting for them in the dark room, they choose to spend less time in the bright room and move to the dark room (where they previously received electric shocks) in the second test.[34] This is evidence that the STZ model works and produces Alzheimer's in animals.

On the other hand, in the case of the Morris Water Maze Test; a water tank (150 cm in diameter) is used to measure rodents' spatial memory. There is a platform sitting on the tank that will allow to get out of the water. It has been placed submerged 1.5 cm below the water surface at a certain point in the tank and a small black plastic ball is usually placed on the water surface. The rodent is expected to remember about the platform that will allow it to escape from the water which is interlinked with the rodent's capacity of spatial learning. An average of one minute is allowed to complete the test and in case of a delay, memory dysfunction is indicated for the subject in question.

The position of the platform for the test is not changed for the first four days of the test and the delays in the amounts of time the rodents are taking to find the platform are duely noted. Three starting points in the North, East, and West of the platform, are each tried randomly. Each trial is terminated as soon as the rodent achieves to climb the escape platform or after 60 seconds if it was unable to get out. The rodents who could not find the platform in time are put on the platform manually so that they would learn; they are allowed to stay there for 20 seconds so that they can do that. On day 5, a probe trial is performed to assess the rat's capacity to spatially recall of the location of the platform, now that it's been hidden.[35]

As the effects of icv STZ on the Morris water maze test are examined, differences were observed between the saline (control) group rats and the STZ-injected rodents, as expected. When the animals are sent out to find the place of the platform previously taught on day 5; rats with STZ injections are observed unable remember the location of this platform, and the time spent in the close proximities of it, was found to be less than the control group, too.[34]

To exert such an effect, we see that the ICV-STZ injected rodent model includes symptoms of neuroinflammation as well as tau[36] and amyloid[37] pathologies.[38] Immunohistochemical studies show that ICV-STZ mice have early and widespread neuroinflammation characterized by increased astroglial and microglial activation in the hippocampal CA1, CA3 regions, as well as the dentate gyrus. The neuroinflammation caused by STZ is more pronounced than the neuroinflammation observed in transgenic mice who have AD, in the same regions described.[36] As a result, we can test the extent to which any substance selected for use in treatment of Alzheimer's disease symptoms can be tested with this model.[34]

ALUMINUM CHLORIDE IN THE RAT MODEL

Aluminum is a cholinotoxin.[39] It therefore causes cognitive dysfunction, neurodegeneration, and apoptotic neuronal loss.[40] One reason for the decreased cognitive functions of AD patients is aluminum deposition. As evidenced in animal studies, prolonged exposure to aluminum impairs the learning ability of rats. This causes neurochemical, neurobehavioral, and neuropathological changes.[41] In short, Aluminum has an important role in the formation of Alzheimer's disease. Aluminum chloride (AlCl3) is administered to rodents to induce

neurobehavioral and pathological changes in parallel to AD so that a new animal model can be described for the research on disease treatment.[41]

The model is administered 100 mg / kg body weight of AlCl3 and subjected to Morris water maze, open field and new object recognition tests for neurobehavioral evaluation-so that symptoms paralleling AD can be confirmed. Then, the cortex and the hippocampus regions of the rodent brain are examined, so that the integrity of the Alzheimer's model is proven histopathologically, as well. For further inquiries of verification, biochemical tests, an immunohistochemistry panel and a Western Blot are performed. As a result, it is confirmed that the aluminum exposure decreases cognitive and locomotor activities in mice in open field. In the Morris water maze and new object recognition tests, the decrease in memory and learning functions of rats are noted. Histopathological evaluation of the cortex and hippocampus results in the discovery of AlCl3 neuritic plaques and neurofibrillary tangles in the rodent brains. Thus, with the help of the animal model obtained, new approaches in treatment of AD can be explored. Previously, for instance, Epigallocatechin-gallate (EGCG) loaded nanoparticles (nanoEGCG) have been tested in this model as a potential therapeutic.[42]

CHOLINERGIC DYSFUNCTION IN MOUSE MODELS

Alzheimer's disease is characterized, among others, by dysfunctions of cholinergic neurotransmission. Amyloid P (Aβ) peptide accumulation in the brain is found to be a major cause of memory impairment seen in the clinical course of AD. Impairments in cholinergic pathways have also been observed in transgenic mice in which the human Aß Peptide is overexpressed - which further implies the correlation mentioned.[43]

The PDAPP transgenic mice used in this model are homozygous mice containing strains of C57BL/6J, DBA/2J and Swiss-Webster.[44] Young female PDAPP mice and WT controls were used in all in vivo microdialysis studies. WT controls were examined with young PDAPP males to determine outdoor activity in a new setting.[44]

It has been found in the study that the hippocampal ACh flow increases when WT mice are placed in a new environment.[45] In contrast, when PDAPP mice are exposed to the same new environment, the hippocampal ACh release level is increased by more than 2.5-fold over approximately 90 minutes compared to the WT mice. When scopolamine (0.3 mg/kg ip), a pan-muscarinic receptor antagonist, is administered, the level of ACh release in the hippocampus of PDAPP mice is found to be decreased more, when compared to the decrease in WT mice.[45]

With this model, it has been observed that soluble "cholinotoxic" strains of Aß Peptide can damage cholinergic transmission in PDAPP mice and cause memory impairment. To prevent this damage, cholinergic dysfunction can be reversed by treatment with certain anti-Aß Antibodies. Thus, it is thought that memory problems associated with early AD can be corrected. However, more studies are needed to explain the effect of Aβ on cholinergic neurotransmission and neuron damage.[43]

DISCUSSION AND CONCLUSION

Until now, no effective treatment method has been found to fundamentally change the course of AD. Keeping these hypotheses on Alzheimer's disease pathogenesis, more experimental animal models need to be developed in order to find novel approaches in treatment. Of course, this requires choosing the right model, and this can only be made possible by filling in the gaps between human AD pathology and rodent AD models - finding out methods to generate better animal models that perform more in accordance with the actual AD pathology.

That is to say, that an ideal disease model should show lesions and symptoms similar to the real-life disease. Among these Alzheimer's models we have examined, the ICV-STZ model can be preferred by researchers as the most similar model to human non-familial Alzheimer's disease, since it provides amyloid, tau pathology and neuroinflammation; the three most important components that should be observed in AD, until better models are described.

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