A dissertation submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University in the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm)

Project On

Literature review of the role of reactive astrocytes in the pathogenesis of Alzheimer's disease.

Submitted To

The Department of Pharmacy

Faculty of Allied Health Sciences

Daffodil International University

Submitted By

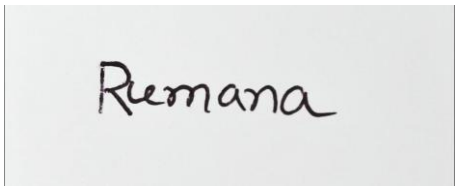
Student ID: 171-29-994

Department of Pharmacy

Faculty of Allied Health Science

Declaration

I, hereby humbly declare that, the declaration work titled **"Literature review of the role of reactive astrocytes in the pathogenesis of Alzheimer's disease"**a requirement for the degree **Bachelor of Pharmacy (B.Pharm)** program under the faculty of Allicd Health Science **Daffodil International University** , Bangladesh was carried out by me under the guidance of my supervisor during the study period of.....



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Approval

This project **"Literature review of the role of reactive astrocytes in the pathogenesis of Alzheimer's disease**" is submitted by Rumana Tabassum Prety Student ID: 171-29-994 Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, has been accepted as satisfactory for as per the requirement for awarding the Bachelor of Pharmacy degree and approved accordingly.

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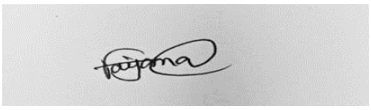

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Dedication

I dedicate this work first and foremost to Almighty Allah and secondly to my family especially my parents and to teachers and my friends.

Abstract

Alzheimer's disease is a type of dementia that affects memory,thinking and behavior that means it is a developing neurodegenerative disorder that slowly weaken memory and in time effect the quality of life. There are many hypothesis concerning the pathophysiology of Alzheimer's diseases. This review work aims to understand the role of reactive astrocytes in the pathogenesis of Alzheimer's disease and its therapeutic mediate.It involves the causes and diagnosis parts of AD as well. As per the hypothesis of AD accumulation of Amyloid beta (Aβ), the main component of the plaques, is the causative agent of Alzheimer's pathology and cell loss, vascular damage, and dementia take place as a result of this deposition. Roles of reactive astrocytes in AD has been explained in this review work that are characterized by the abnormal increase in the number of astrocytes due to infection, ischemia, strokes and neurodegenerative disease. Finally altered glutamate homeostasis, cytokine and growth factor sign, interleukin-6 and Suppressor of Cytokine Signaling 3, cyclooxygenase-2,

TGF- β1 and SMAD3 signaling have been explored here as therapeutic intervention. This work reviews the scholarly articles that shows plausible relation between astrocytes role in triggering CNS inflammation with remarkable inference for diverse CNS disorders particularly Alzheimer’s disease.

Table of content Title

CHAPTER 1

1.1 Historical Background

Alzheimer's disease was first discovered by the doctor Alois Alzheimer and later it was named after him. In 1906, when he conducted an autopsy on a female s brain he precisely recognized a collection of brain cell abnormalities and that patient had trouble with memory issues, confusions and understanding questions. He identified heavy deposits in and out of the nerve cells. He even spotted the existence of tangled bands of fibers in the neurons. This is how this brain disease gets his name . Despite significant improvements in our understanding of AD pathogenesis and the conceptualization of the disease since the first case reported by Alois Alzheimer in 1907, there seem to be no treatments that modify the disease till now.Alzheimer's disease (AD) is the most prevalent cause of dementia and a degenerative brain disease. Dementia results of a loss in attention, vocabulary, difficulties and other cognitive skills that impair the capacity of an individual to conduct day to day tasks. The decline was attributed to the disruption or loss of neurons in the part of the brain involved in cognitive function. During AD neuronal damage and destruction eventually affects other areas of the brain, including those where an individual can perform basic physical functions such as walking and swallowing. The final phase of the disease involves people who are disable and need care every day. Last but not least, Alzheimer's illness is fatal . AD was first identified over 100 years ago.[1] AD is a kind of dementia that slowly deteriorates over time. The disorder is not of a normal aging process and is believed to be causing damage to brain cells via various processes.

1.2 Types of AD

1.2.1 Typical AD

This term refers to the more severe medical syndrome of AD, marked by an early and persistent episodic loss of the predominant memory of the person at later stages and accompanied by other cognitive problems (executive disability, voice, functional and complex visual impairments) and neuropsychiatric shifts. One or more positive biomarkers of Alzheimer's disease in vivo are supported by the diagnosis.[2]

1.2.2 Atypical AD

This term applies to the less severe and well-defined medical phenotypes of Alzheimer's disease. These are predominantly systemic non-influential aphasia, logopicaphasia, frontal AD form and posterior cortical atrophy. According to the findings of in vivo proof for amyloids in the brain (such as the accumulation of different ainyloid marking radio ligands) or CSF (specific variations to Alzheimer's disease of amyloid **β**, tau and phosphotau,concentrations), AD classification is confirmed in existence of all such medical manifestations [2]

1.2.3 Mixed AD

This term refers to patient diagnostics that completely match the diagnostic criteria of traditional AD and additional evidence of other comorbid disorders including cerebral disease or lewy body conditions is medical and brain imaging / biological evidence [2]

1.2.4 Prodromal AD

This term refers to the early symptomatic, pre- dementia stage of AD, when clinical symptoms, such apisodic hippocampal memory loss are present.lt is also known as predementia stage of AD.Nevertheless, never significantly enough to impact everyday instrumental tasks and do not require the diagnosis of dementia and where biomarker CSF data or imaging confirms AD clinical shift. The new AD definition now includes this stage. When AD is seen to be both pre-dementia and dementia stages, it may vanish in the future.[2]

1.3 Overview on dementia

AD is the main cause of dementia and accounts for up to 80% of all dementia cases .lt increases exponentially with age from 0.3 %for the 60-69 age group to 10.3% for the 8089 age group in Europe .[3]Dementia indicates a noticeable decrease in mental capacity, which is

an explanatory word derived from the Latin root demens. A decline of mental function in emotional, cognitive and conative elements is a clinical syndrome of Dementia Dementia has three stages mild, moderate and severe. The various psychological and behavioral symptoms of progressive physical weakness in each stage of the illness require distinct kinds of health facilities. According to a study conducted by The Memory Clinic at the National University Hospital, Singapore, began in 1990 as the foundation for a worldwide research of dementia by the World Health Organization estimated the duration of the phases of dementia. The average mild stage length was 5-6 years, moderate 3-5 years and severe 3-2 years but Data from the severe stage included the death and follow-up figures. The severe stage could therefore be somewhat longer.[3]

1.4 Global scenario

Alzheimer disease is responsible for dementia Global dementia incidence is up to 44 million and is anticipated to quadruple by 2050 [36]. lt was estimated that the worldwide cost of dementia treatment rose from $604 trillion in 2010 to $818 trillion in 2015. The financial expenses increase more quickly than the disease's incidence. Dementia was thus acknowledged as a worldwide public health priority by the WHO and Alzheimer's International Disease. According to a study which was published by the Alzheimer Society in 2010 about 747,000 Canadians suffer from cognitive impairment, including dementia and more than 70,000 people are under 65 years of age and 72 percent of Alzheimer's sufferers are women . The greatest prevalence and occurrence rates of dementia, are believed to be from the people of North America and Western Europe followed by those from Latin America and western-Pacific neighbors.[3]

1.5 Etiology of AD

AD causes neurodegeneration, total cognitive loss and premature death in patients There are many theories concerning the causes of AD. lt is not a goal to discuss all theories but to focus on those that are most likely to participate. Aging is the most common risk factor for the growth of AD and 8 1 % of individuals with AD are 75 years of age or older The chances of growing AD are increasing exponentially with age, doubling after 65 (100) every five

years roughly. In case of age factor we can divided AD according to their onset of actions and they are early-onset familial AD which is rare mutations are responsible here but at least in 3 genes and late onset familial AD, which is the biggest genetic risk factor is the epsilon four allele of the Apo lipoprotein E (APOE) gene for the development of late onset AD. Besides these, cerebrovascular risk factors is significant in the process of development and progression of the disease Other risk factors include genetic factors, previous MCI, risk factors for cardiovascular diseases, education, social and cognitive involvement and tumors brain injury.[19]

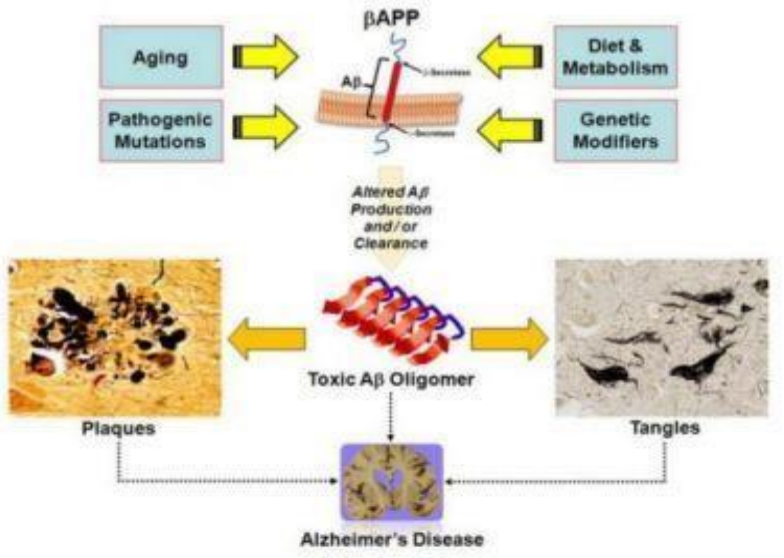


Figure 1: Factors Affecting amyloid accumulation and ad pathology

[(M. Niedowicz, T. Nelson, & Paul Murphy, 2011)]

Neuronal degeneration, plates and tangles are characterized by AD. The amyloid- β precursor protein (APP), which is a type of amyloid- β peptide (Ab), as the central player in disease pathophysiology, has been well established. Different factors (pathogenic genes, genetic modifications, nutrition and metabolism, and even the aging process itself) conspire so that the constant rate of Ab in the AD brain is extremely high. This can occur as a result of elevated APP sounds, improved APP metabolization (more β and γ), reduced ATH catabolism, impaired brain clearance of AQ, [5]or some mixture. High Ab rates, in particular of those prone to

aggregation such as AQ 42 types, result in an increase in oligoineric peptide (consisting of two or more molecules, A β) forms. At least temporarily, these intermediated occur in a toxic soluble state, possibly inside as well as outside the cell. Oligomeric AQ destroys the nerves, resulting in neurofibrillary tangles, and ultimately develops extremel y insoluble fiber that gradually is deposited in the brain as plaque parenchym. There is some evidence of this. Oligomeric A β also has other adverse effects on neuronal activity, of which only a handful have so far been described. While AD is the best known amyloidopathy, other neurodegenerative diseases may have similar mechanisms (acting on proteins other than APP). Understanding the specific pathways that contribute to these disease processes can at least partly lead to therapeutical approaches to neurodegeneration in general.[5]

1.6 Clinical Symptoms

The most common form of dementia is Alzheimer's disease which currently affects 2.5 million Americans. This is the major cause of mental illness in elderly persons, and represents a large number of admissions to care homes and others. [7] Alzheiiner's clinical symptoms often are complex and variations with either depressie or delusional features are recognized by existing medical classifications as distinct subtypes . Converging proof from the genetic risk cohorts and from the clinically regular elderly persons indicates that the Alzheimer's disease pathophysiologic process starts years, if not centuries, before clinical dementia diagnosis. Neuroimaging, CSF and other biomar kers have recent ly progressed and are now able to identify proof of the pathophysiological AD process in vivo [8] The most frequent disorders which may trigger dementia include thyroid, pernicious anemia, luetic brain disease, and other chronic nervous system diseases, and Huntington's, among others. Manic-depression, Parkinson's disease, multi-infract dementia and drug intoxication are among the diseases excluded; and tuinors in brain.[9]

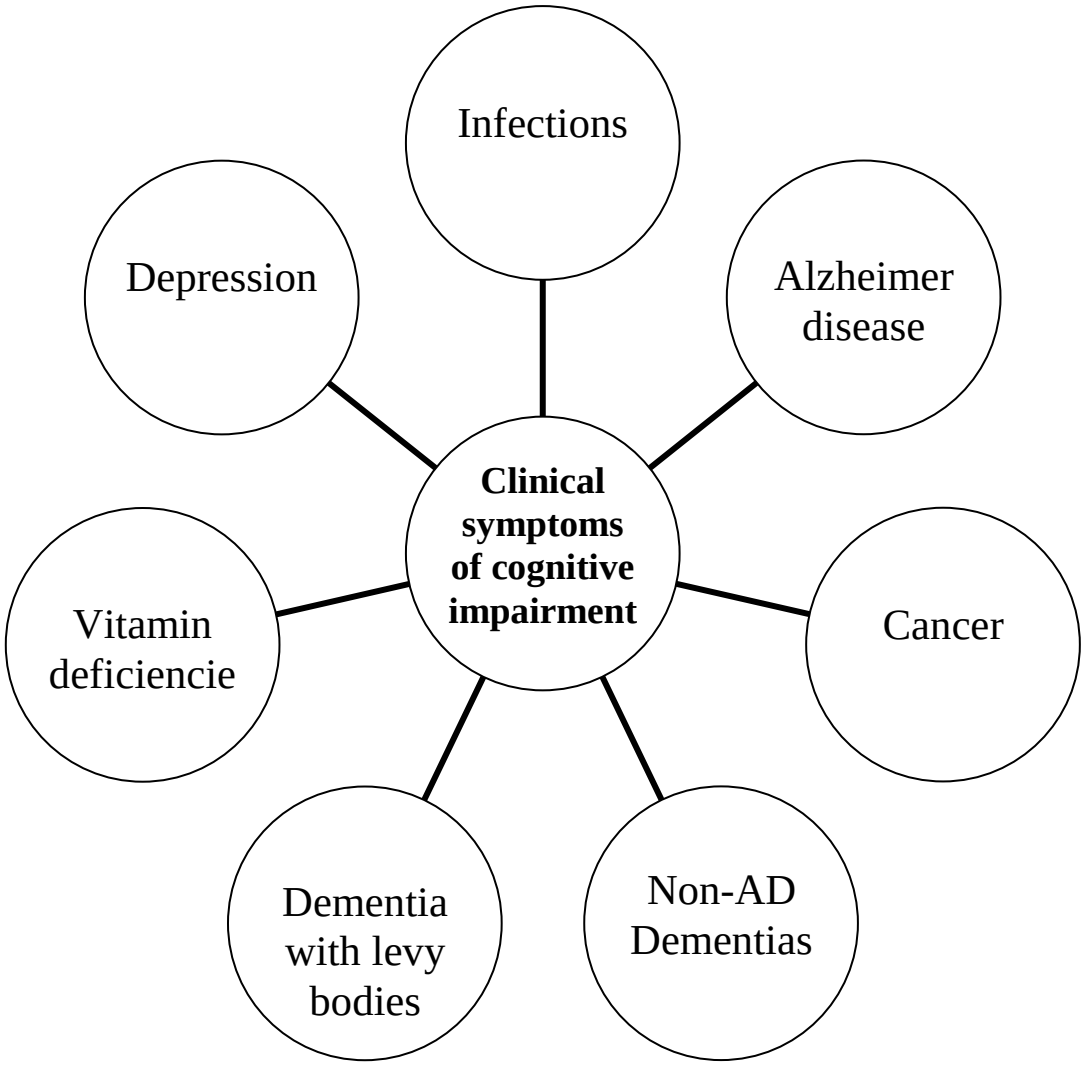


Figure 2 : clinical symptoms Of Alzheimer disease

[(Medical chemistry, volume 14,Issue 1-2018)]

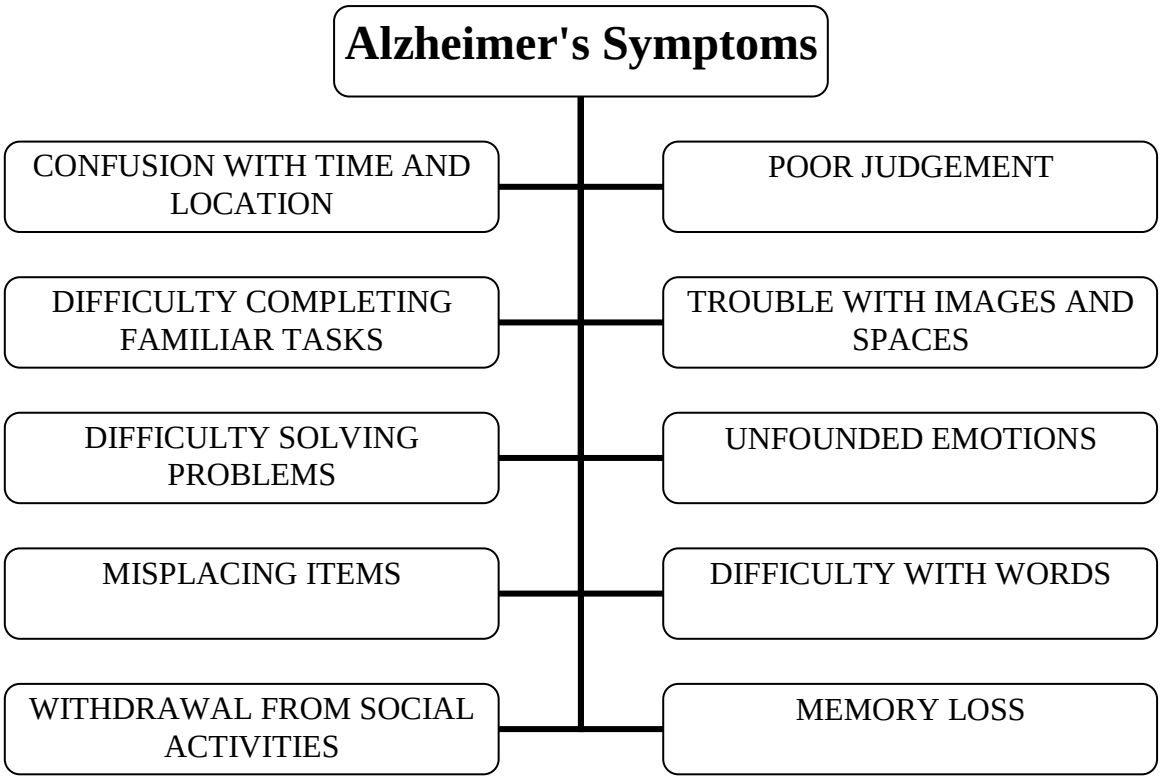


Table 1 : Alzheimer's Symptoms

Chapter 2

Probable Mechanism 2.1 Neurofibrillary tangles

Neurofibrillary tangles and senile plaques are the foundation of the neurofibrillation diagnosis of Alzheimer's disease. The number of neurofibrillary tangles is strongly related to the level of dementia, and the neurofibrillary dysfunction formation is more directly linked. N FT are plugged with the neuronal pericarya, dendrites and axons of abnormal filaments
Their presence means that the neuron does not retain its cytoskeleton correctly,[10]

which is necessary to support its various processes extremely complicated shape of branching. A few neurofibrillary tangles are a common result of aging. The increase in the amount and architect ural distribution of tangles, however, is the primary cause of neuronal mortality in AD and promotes the cardinal pathology and defines the stage of disease as outlined by Braak and Break NFT is traditionally recognized by silver staining techniques but is also shown by its green birefringence following the Congo red staining and fluorescence following the thiof1astin S staining Classic NFT consists of tight packaged bundles which fill and expand to dendrites a more or less important part of the cell body. Death of neurons follows a partial NFT decomposition which has a lr›nser aspect. Extracellular tanks which reflect neuronal loss are presumably persisting for a long period due to partial proteolysis resistance but lack the N-terminal tau domain.[11]

2.2 Senile plaques

Senile plaques (SP) may be the most distinctive histopathological characteristic of Alzheimer's illness. They are heterogeneous in structure and are distributed in the brain which is nonuniform Molecular biological studies of SP and NFT play an important role in the deselopment of hypotheses regarding AD pathogenesis. The finding of AJ as the main molecular of SP leads to the Amyloid Cascade One of the most influent ial molecular pathology models of AD. ACH suggests that the first pathogenic event in the disease leading to the formation of NFT, celestial death and eventual[12] dementia is AJ deposition. Arnyloid precursor protein and presenilin mutations are related to familial variants of AD (FAD) through the development of pathological AQ peptides genes. The existence of AQ in SP is therefore known to be residual of the consequence of a pathogenic gene mutation which contributes to cell death by the accumulation of harmful, insoluble AQ peptides. Gene mutation experiments have had an important impact on the hypothesis of pathogenesis of AD broadly as the pathologic form of familial AD (FAD) is similar, except the age of onset to that of sporadic AD (SAD). Chemical SP and NFT research in AD shows a diverse and diversified structure. At least three forms of influencers (Figure 2) could influence the molecular biology of SP and N FT. Firstly, the residue of a pathogenic gene mutation will shape a molecular constituent and thus be directly connected with the central aetiology. Furthermore, cell degeneration could be a symptom and therefore the outcome of the disease process. Thirdly diffusion and molecular binding to established proteins SP and NFT may acquire new molecular constituents.[13]

SP are much more complicated; they composed of extracellular amyloid deposits and are linked with dystrophic neurite-induced swollen neuron treatments complex sugar elements are regarded as critical for the assembly, like amyloids elsewhere in the body. The cerebral amyloid is characterized by d-amyloids, a brief 40—42 fragment of amino acid of the transmeinbrane protein, a precursor protein of /-amyloids. Beginning from the fifth decade of life, more and more people develop cortical senile plaques until about 75% of the population is influenced by the eighth decade.[14]We have various morphological forms of SP including diffuse, primitive, classic, and compact-type plaques. A wide range of AJ peptides are found among these plaques and as a result of the secretase cleavage of the transmernbrane glycoprotein APP. AJ42/43 is most prevalent in SP whereas the more soluble A/40 is discovered also in combination with blood vessels and may later grow in the illness .[39]

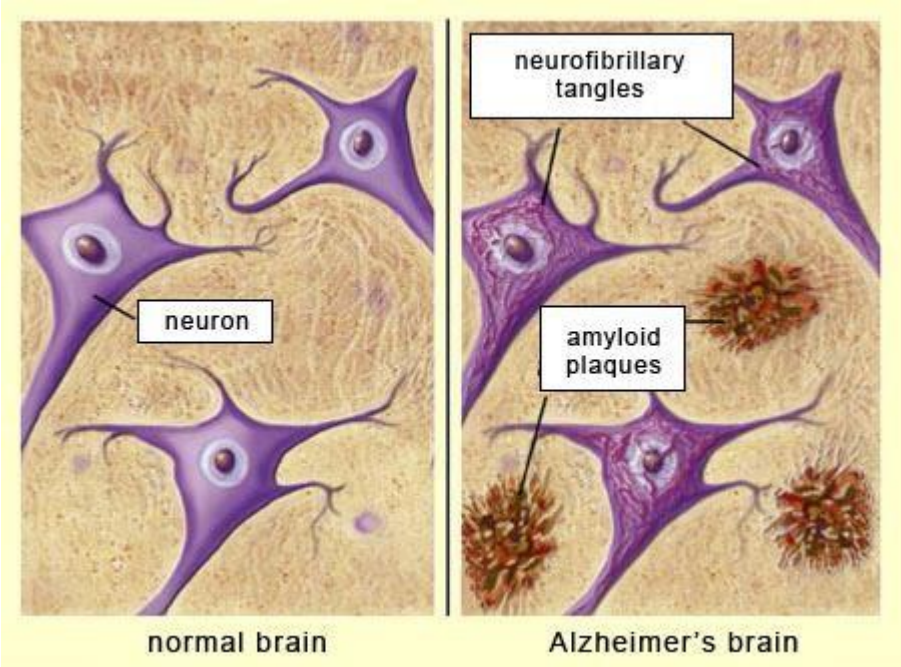


Figure 3:Normal brain vs Alzheimer's brain

Amyloid plaques consist primarily of abnormally folded Ab with 40 or 42 (Ab40 and Ab42) amino acids (ApP metabolizing by-products). Due to its greater fibrillation and insolubility rate, Ab42 is more frequently found in plaques than Ab40. Amyloid deposition not always follows a stereosypical progression pattern but generally takes place in the isocortex and impacts subcortic structures only later on. In contrast to NFTs, amyloid plaques are less often associated with entorhinal cortex and hippocampal formations. Various stage schemes for Braak and B raak, Thal criteria and the Alzheimer's Registry Consortium (CERAD) are used for Ab.[15]

2.3 Tau hypothesis

The presence of aberrant phosphorylation of the protein tau correlated with microtubules in the brains of individuals who are affected is the main feature of the development AD the tau theory offers the strongest explanation for Alzheimer's diseasecase medical findings .Signified work has centered on the discovery of kinases and the creation of pharmacological agents to inhibit these molecules .Studies into the pathophysiology of the principal molecular factors causing AD offered insights into the structural dynamics of tau tau interactions and the connections between Ab development and Tau hyperphosphorylation. ln keeping with our unified hypothesis based on tau exisition as the final, specific signaling mechanism contributing to degenerative processes, the continuous activity of indixidual sariables such as oxidative

agent, iron overload, lipid metabolism disorders, hyperglycemia, insulin rates deregulation, [17]persistent infections, head traumas and others. These factors can induce anomalous cell signaling in micro glyphic cells and astrocytes to activate endogenous signals .Tau is an alternately separated microtubule protein which bounds primarily to neurons. 1ncreased AD has affected by the unusual aggregation of tan and neurofibrillary tangle (NFT) and the development of amyloid plaques. The tau is predominantly present in the human brain as six distinct isoforms, which vary whether one or two N-terminal acidic replication is present or not and in the presence or absence of the second of the four inicrotubule binding repeats .Tau is a protein correlated with the microtubule which regulates tubulin stabilization. We can find the hu man tau gene on chromosome 17. As a consequence of alternative splicing of mRNA six tau isoforms are located with or without exons

2, 3 and 10 are distributed in the adult human brain .Cell or neuron sensitisily to AQ in vivo› results in a rise in tau phosphorylation at different sites as a consequence of activation of specific kinases. Early studies have shown that neurons with AJ fibrils have enhanced phosphorylated tau's immune reactivity. Exposure of cells or neurons to AQ in situ results in increased phosphor Elation of tau at several locations owing to activation of different kinases. Early studies has shown that the immuoreatisity of phosphorylated tan is increased by neuronswith AQ fibrils, and lithium-sensitive, suggesting GSK3J as the liable kinase, [37]. One of the major component of the MAPs of axons is Low molecular weight microtubular associated protein which is known as MAP tau and it plays an important role in stabilizing and triggering its assembly. Under unhealthy circumstances Tan self-aggregates in PHFs, which become a neuropathological characteristic for AD and tauopathies, N FTs during the course of AD .Recent studies suggest that hyper phosphorylated tau oligomers have neurotoxic effects that disrupt the usual neuronal cytoskeleton communication patterns .Tau Oligomerisation tends to associate dysfunctional with cognitie impairment. Important developments in the area of initro polymerization have helped to explain the molecular mechanisms even if we still have to describe the functional changes from the natural conformation of tan to its neurotoxic polymers. Polyanions that suppons PHF, as well as microtubules, have shown their interactivity by positive charges at the end of tau repeat and the start of the Tau theory in Alzheimer's disease by b-structure forming motifs.[19] PHF aggregation and microtubules which are promoted by xlyanions have been shown to function with tau , at the beginning of repeats 2 and 3 and at b-structures at positive charges near the ends of tan repeats. The binding structure of polyanions continues to support the hypothesis that stable microtubules can prevent

PHF formation by blocking interacting sites of tan pnlyanion which are important in PHF abnormality 1n addition tau oligo-structured oligomers tend to take on a role in early AD.[20]

Chapter 3

3.1 Diagnosis of Alzheimer’s disease

A proper assessment of the AD needs post-mortem evaluation of the brain tissues ; however biomarkers combined with several relatively recent classical criteria can help diagnose living patients with cerebrospinal fluid (CSF) and positron emissions tomography (PET) More than a third of Alzheimer’s patients have shown that they are carrying the affected genes of their families and relatises. This figure has been estimated in a latest study to be 75%.Molecular genetic techniques will be very useful in identifying faulty genes. In few cases of Huntings on's disease, molecular genetic technologies have helped researchers find a faulty gene. A latest molecular biology instrument for the development of a diagnostic marker for AD can be found in the monoclonal antibody.The Alzheimer’s assosiatiation has categorized AD into 3 different stages and they are probable, possible and definite. Ponable AD needs confirmation on clinical and cognitive testing and gradual distortion of functioning and memory. Definite Alzheimer requires histopathology confirmation In the presence of other important diseases. A clinical diagnosis can be made of

the possible disease of Alzheimer's, particularly if on clinical considerations, the more likely cause of progressive dementia is Alzheimer's Alzheimer Currently, only two pharmacological treatment classes are offered for AD patients. Donepezil riastigmine and galantamine inhibitors of cholinesterase are recommended for treating patients with mild, moderate or serious dementia of the AD and Parkinson's disease dementia .[6]

3.2Pharmacotherapy of AD

Alzheimer's disease is the most common cause of dementia (loss of memory and cognitive skills). Alzheimer's disease (AD) is a degenerative disease of the brain, resulting in memory loss, cognitive decline, and personality changes. There's no cure for Alzheimers, but one treatment may potentially delay decline from the disease, and there are drug and non-drug options that may help treat symptoms.So, the disease cannot go away or be cured.

Concerning available options can help seperates living with the disease and their caregivers to cope with symptoms and improve quality of life.Medications called cholinesterase inhibitors are prescribed for mild to moderate Alzheimer's disease. These drugs may help reduce some symptoms and help manage some behavioral symptoms. The medications are Razadyne (galantamine), Exelon (rivastigmine), and Aricept(donepezil).[21]

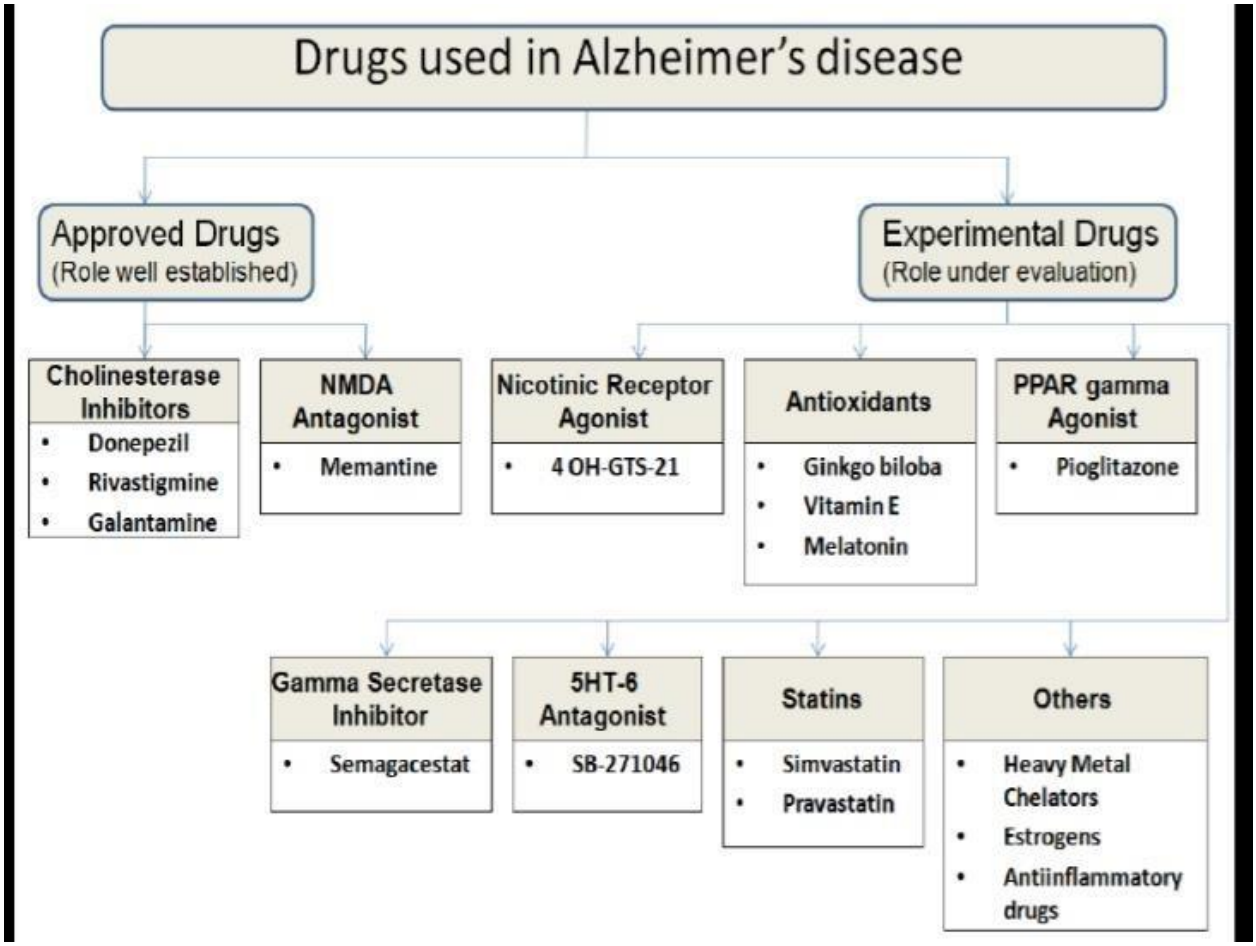


Table 2 :Listed of drugs used in AD

review of the Aduhelm application and the basis for approval. FDA also posted the Concurrence Memorandum by Peter Stein, MD, director of CDERs Office of New Drugs (of which the Office of Neurology is a part) and the Concurrence Memorandum by Patrizia Cavazzoni, MD, director of CDER. The remaining scientific review documents in the Aduhelm action package are not yet available, but will be made available to the public as soon as the internal process of review and redaction is complete.

3.3 Newly approved drugs : Aducanumab (June 2021)

It is an amyloid beta-directed antibody specified to treat Alzheimer's disease. It is accepted under the accelerated approval pathway, which dispenses patients with a serious disease earlier enterance to drugs when there is an expectation of clinical welfare despite some uncertainty about the clinical benefit.Accelerated approval is based upon the drugs effect on a surrogate endpoint an endpoint that reflects the effect of the drug on an important aspect of the disease where the drugs effect on the surrogate endpoint is expected but not established to predict clinical benefit. In the case of Aduhelm, the surrogate endpoint is the reduction of amyloid beta plaque.[22] The accelerated approval pathway requires the company to verify clinical benefit in a post-approval trial. If the sponsor cannot confirm clinical benefit FDA may commence proceedings to withdraw approval of the drug.FDA has posted CDERs Office of Neurologys Summary Review Memorandum describing the agencys substantial[26]

Chapter 4

Reactive astrocytes in Alzheimer's disease 4.1 General description

Astrocytes known as astroglia are characteristic star-shaped glial cells in the brain and spinal cord. They perform many functions, including biochemical support of endothelial cells that form the blood–brain barrier, provision of nutrients to the nervous tissue, maintenance of extracellular ion balance, regulation of cerebral blood flow, and a role in the repair and scarring process of the brain and spinal cord following infection and traumatic injuries. The proportion of astrocytes in the brain is not well defined; depending on the counting technique used, studies have found that the astrocyte proportion varies by region and ranges from 20% to 40% of all glia. Another study reports that astrocytes are the most numerous cell type in the brain.Astrocytes are the major source of cholesterol in the central nervous system.Apolipoprotein E transports cholesterol from astrocytes to neurons and other glial cells, regulating cell signaling in the brain.Astrocytes in humans are more than twenty times larger than in rodent brains, and make contact with more than ten times the number of synapses.[20]

Astrocytes are advanced glial cells that are omnipresent in all CNS areas. Astrocytes are more than 5 times larger than neurons and practically uninterruptedly cut the whole CNS. While neurons were a major focus as mediators of the CN S functions for a long time, an ever-increasing number of evidence suggests that astrocytes and other glia play key roles in health and disease management. In normal, continuous CNS functions astrocytes play a critical role, including blood flow control, neuron energy supply metabolites etc . Astrocytes are heterogeneous in nature and it tiles the entire brain. lt plays an important role in synapse fornation and operation, neurotransmitter release and absorption regulation, trophic factors synthesis and neuron activity .They can be generally divided into astrocytes of white matter, gray matter, ependyrnal astrocytes, radial glia and perivascular astrocytes based on anatomical location[38]. Astrocytes respond in a way that usually involves changes in molecular expressions and morphology to all types of CNS injuries, infection, ischemia and neurodegenerative disease and this process is known as reactive astrocytes [25]

4.2 Characteristics of reactive Astrocytes

Reactive astrocytes (RA) are active forms of astrocytes in response to toxic materials. Astrocytes modify their properties in a morphological, transcriptional and functional manner. As regards morphological changes in toxic materials RA increases its cell size and astrocyte thickness The standard marker of reactive astrocytes has always been Glial fibrillary acidic

protein (GFAP).[40]Other intermediate proteins like vimentin and nest are upregulated in astrocytes with GFAP. Reactive astrocytes have a wide range of heterogeneous morphological .gene expression and functions. Serious injuries and strokes cause astrocytes and extreme reactisity proliferation, while moderately reactive astrocytes do not proliferate from the injury site in distant areas .Reactive astrocyte's proliferation is related to the destruction of astrocyte individual domains. Astrocytes mainly have their distinctive domains, which do not overlap with other domains in the normal brain [26] The state of proliferation and disturbance of the domain are different. Several attempts have been made to examine transcriptional regulation in distilled astrocytes with specific injury or disease models in order to understand the function of responsive astrocytes. Several genes show upregulation in acutely purified astrocytes in LPS and MCAO (Middle Cerebral Artery Occlusion) mouse models accompanying the induction of reactive astrocytes . In order to reproduce astrocytes with reactive effect, various injury models, including stab injury, LPS and MCAO , are used to induce reactive astrocytes. Not only do they induce reactive astrocytes, but they also directly induce microglial activation or neural injury . Nonetheless, the two mechanisms have serious limitations, the first affecting the morphological effects of reactive astrocytes and the latter inflammatory effects of ablate brain cells on the environment. Such systems cannot say the cause and consequences of reactive astrocyte phenomena. Therefore,[27]the design of suitable research models to selectisely induce and control astrocytes in vivo reactivity is undoubtedly necessary to address such limitations. To study the role of reactive astrocytes, it is also essential to understand the process behind the induction of reactive astrocytes.

4.3 Altered characterization of' AD-related reactive astrocytes

The PET tracer I I C deuteriu m-1-depreneyl (1 I C-DED) is used in the brain of a person with an AD for testing monoamine oxidase B in astrocytes . Reactive astrocytes were found in AD animal model also before the development of amyloid plaques. Reactive astrocyes are known to be morphologically, metabnlically and functionally responsive to amyloidbeta. Beta plaques are connected to reactive astrocytes, and beta amyloid causes a GFAP benefit in astrocytes culture. In human patients with AD reactive astrocytes are also demonstrated.[28]

4.4 Oxidative and nitrosative stress

The most common type of dementia in the elderly is AD. Many studies have found that oxidative and stress may be important to Alzheimer's disease pathogenesis (AD), starting

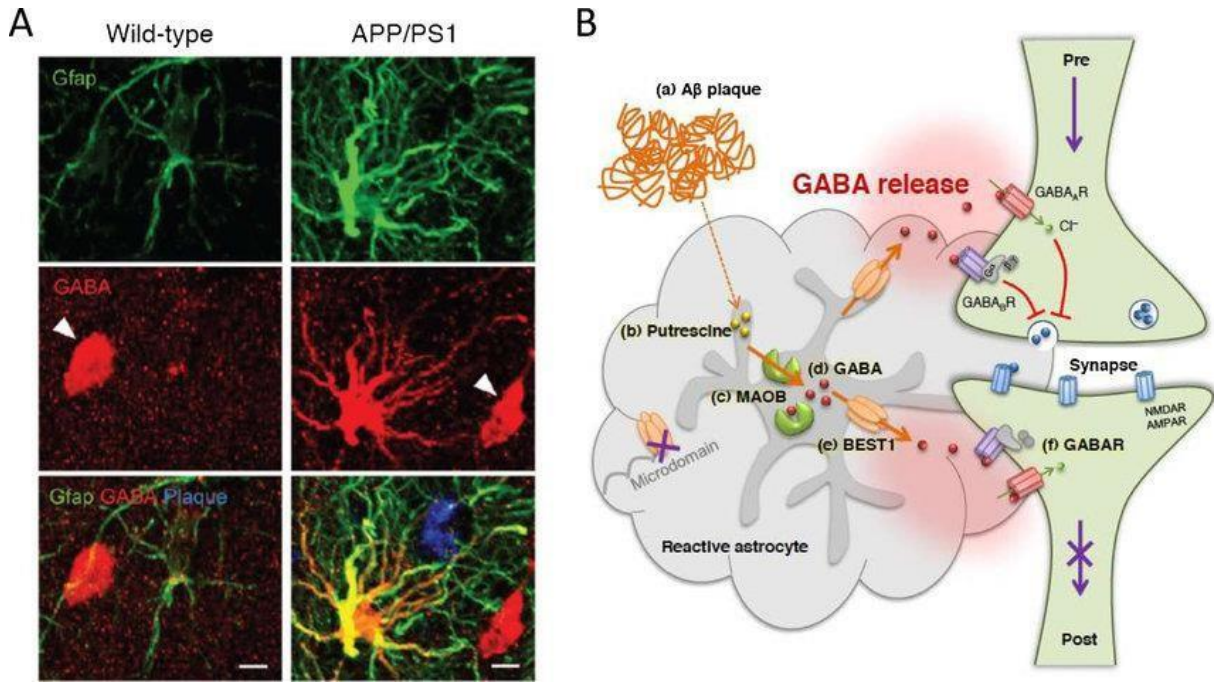
with the most early stage of AD, i.e. mild cognitive impairment (MCI. The deregulation of the redox balance, a failure of antioxidants and/or an overproduction of free radicals leads to oxidativne and nitrosate in biological systems. Free radical attack on lipids, carbohydrates, sugars and nucleic acids occurs in biological materials, the current approach for determining oxidatise nitrogen harm is observed on tissue and liquids. Post-mortem and in vivoexperiments have shown that free radical damage products have collected in the central nervous system and in the external tissues of AD and MCI subjects . ROS are produced and play key roles in CN S functions during normal cellular metabolism, such as cognitive function, secretion of the neurotransmitter, homeostasis and protective brain. ROS levels typically have biphasic impacts on cell viability across physiological ranges. Law levels of ROS stimulating late proliferation of cells, while high salues have harmful effects on enzymes, membrane lipids and DNA leading to cell death. The homeostasis of brain ROS rates is essential for brain functioning. The route across ROS is AD-related. for AD cases, the rate of ROS has been indicated and oxidative stress has been increased. Thus, the inhibition of ROS or enzyrnes correlated with ROS has been a therapeutical approach for AD . ROS and reactive nitrogen species can be harmful, because cells, sugars, proteins and nucleic acids can be damaged and thus inhibit their normal function. Such damage can jeopardize cell viability or lead to cell death via

necrosis and apoptosis. The ROS and RNS exposure are referred to as oxidative (OS) and anitrosate (NS) pressure. These arise in biological systems if redox balance deregulation occurs due to an enzymatic or non-enzymatic antioxidant deficit and/or a spatioteinporal overprrxluction or altered ROS / RNS distribution . Reactive Nitrogen (RNS), a form of which produces nitric oxide synthase (NOS), absorbs nitric oxide. Nitric oxide is not particularly toxic *in vivr›* but can react to the powerful oxidant peroxynitrite through superoxide.[28] For synthesis of NOS, NOS is essential, and three isoforms of NOS, iNOS, eNOS, and nNOS, are usable. Out of the three isoforms of iNOS, pro- inflammatory cytokines and amyloid beta toxins are found in glial cells. iNOS is over- expressed in reactive astrocytes in the AD brain. A study showed that microglia stimulation, which stimulates iNOS, exceeds NO release by microcells and ultimately induces neuronal and immunomodulation harm in AD. A major contributing factor in AD development is the oxidative stress and nitrosatie stress. Nevertheless, major advances in AD care have been shown despite a long background of work in different fields. The causes can include mysterious cell origins of each ROS and the ineffective ROS antioxidants and ROS- generating or decaying enzymes. In this regard, research on the

molecular and cellular mechanisms of the ROS-related pathway in AD pathogenesis would contribute to the understanding of the role of oxidative stress in AD (Chun & Lee, 2018). Myriad Genetics has recently stopped developing Tarenflurbil which is a promising early-stage Alzheimer disease treatment candidate. This was the biggest Phase Ill clinical trial for an AD drug in the world of almost 1 600 North American patients alone. This was the second major medical setback within one year. Even though Pfizer demonstrated an earyromise as an AD treatment, Lipitor B is ineffective in reducing the cognitive decline. Hist ory reported other recent medical deceptions for nonsteroidal anti- inflainmatory medications the substitution of testosterone, and an uncomfortable long period of failure count.[29]

4.5 Gliotransmitters

Astrocytes are not electrically excitable, they have proven to work as active participants in neuronal circuit development and synaptic activity. Astrocytes react to Neurotransmitters by releasing chemical transmitters called gliotransrnitters and contributes to the synaptic awareness treatment. Modern techniques of optical imaging allow to clarify how neurotransmitters cause the release of various gliotransmitters such as glutamate, D-serine and ATP. In addition, recent studies have shown that gliotransmission disturbances result in neuronal dysfunction and abnormal behavior in animal models. Gliotransmission is defined as the quick and regulated exocytosis of activity gliotransmitters and GQ G protein connected receptors in Ca “-an exciting area of research in neuroscience since its first discovery in 1994 . Two key scientific breakthroughs have contributed to a deep understanding of astrocytic behaviors such as gliotransmission: calcium imagining and optical microscopy.[29]



[(Gliotransmitters [(Heejung Chun & C Justin Lee,Dec 2017)]

Figure 4 :Mechanism of resetive astrocyte

4.6 Metabolic Plasticity

Astrocytes are reactive and change their metabolic functions under damage or harmful circumstances, in addition they adjust and improve their metabolism to produce energy in wounded situations. Amyloid beta treated astrocytes have also been observed to switch their profile and increase their use of Glucose . Mechanisms of amyloid beta synthesis and clearance are found to be essential for AD pathogenesis.[28] The response to amyloid plaques is indicated by weakening pathways including autophagy and ubiquitination. lt is well known that defective autophagy is responsible for the development of neurodegeneration, but it is not clear whether autophagy is implicated in AD astrocyte functions. Reactant astrocytes near amyloid plaques have been demonstrated to produce additional putrescine and a type of polyamine degraded by toxic molecules and to degrade putrescin to GABA by means of MAO- B. There could be some

connections between the excess of putrescin and autophagy, but this theory needs to be reviewed in the future. The oxidatie metabolism of monoamines including asbenzylamine, and dopamine is catalyzed by monoamine oxidase B (MAOB) , which is mostly found in astrocytes and is situated in the outer membrane of mitochondria. lt was first reported decades ago that astrocytes expressed MAO-B, and its activity in reactive astrocytes, by 3h-1-deprenyl emulsion autoradiography, was increased. In conjunction with MAO-B, reactive astrocytes are observed using MAO-B probes by PET imaging 1 1 C-DED, an MAO- B inhibitor, have more often been detected in patients with MCI compared to control subjects. In contrast, the elevated rate of I I C-DED was associated with a decrease of MRI-measured gray matter volume in 1 lC-Pl B-positive MCI patients and an improvement in the signal for I I C-PlB -PET amyloid beta fibrils. ln conjunction with astrocyes MAO-B activity, is capable of representing the activity of AD pathogenesis.[29]

Chapter 5

Therapeutic intervention

5.1 General Approach to Reactive Astrocytes as Therapeutic Targets

Research contributed to the simplistic idea that reactive astrogliosis is an all-or-none maladaptive mechanism that accounts for the creation of wounds and that the complete astrogliosis inhibition could be seen as a therapeutical process. Reactive astrogliosis is a finely graded series of modifications which are controlled in scope by different signaling events as a consequence of all CN S insults.[29] The spectrum of modifications in reactive astrogliosis extends from reversible gene expressive adjustments to cell hypertrophy with cellular domains and tissue structure survival after moderate insults to long-lasting cell development with constant tissue structure rearrangements after extreme insults. The changes that occur in reactive astrogliosis that modify astrocyte activities by both increasing and decreasing functions, which can be advantageous and detrimental to neural and nonneural cells surrounding them. Since astrocytes and reactive astrocytes have the capability of essentially affecting all facets of neural activity by controlling blood flow and supply of energy substrates, or by influencing synaptic function and plasticity, it is perhaps not shocking that the mechanisms underlying reactive astrogliosis and scar forming may contribute or may lead to dysfunction. Several studies utilizing transgenic and 1aborator y animal models demonstrate that responsive astrocytes has numerous ways to protect cells and tissues from the use of a range of molecular mechanisms. Nevertheless, reactive astrocytes can also perform a destructise function in injuries or diseases by developing unusual symptoms, such as reactive oxygen species over development (ROS) or many other inflammatory cytokines. Overall, responsive astrocytes can have both positive and negative effects of harm and infection as defined by unique signaling events and molecular effector mechanisms. Taken together, laboratory animal studies demonstrate that the global induction or ablation of reactive astrogliosis is unlikely to be an effective path to treatment, and in many instances is likely to cause more damage than good.[30] Alternatively, therapeutic approaches will concentrate on astrocyte-related molecular mechanisms, affecting both different astrocytes and unique features of responsive astrocytes. There has been substantial progress in discovering molecular mechanisms that control or mediate specific aspects of the reactive astrology. Some of these molecules are generic to many cells (for instance, cytokines, ROS), while others are exclusive to astrocytes and narrowly targetable.

5.2 Altered Glutamate Homeostasis and Alzheimer's disease

The main excitative neurotransmitter Glutamate is carefully regulated by neuronal and glial factors.[30] Astrocyte carries a large majority of extracellular glutamate through excitative transporters of amino acids (EAATs). EAAT2 is highly expressed across the brain and spinal cord of the five subtypes (EEAAT I —EAATS) and is responsible for more than 90°/e of total glutamate uptake. EAAT2 is strongly distributed throughout the five subtypes ' Main and spinal cord (EEAAT I — EAATS) and accounts for more than 90% of the oserall glutamate intake. Glutamate is then converted into glutamine through an enzyme called glutamine synthetase in astrocytes, which is then shuttled back to presynaptic terminals and used for the synthesis of glutamate in the neurotransmitter. This is known as a glutamate glutamine transmission and helps to maintain glutamate hemostasis in the brain. Astrocytes thus disrupt the microenvironment of neurons nearby in a way which damages their ability to feel or respond to increased levels of glutamate, and it triggers NMDA receptor over stimulation, which is responsible for mrxlular cognitie functions of the front cortex.

Normalphysiological aging with decreased NMDA receptors and physiological memory declines their role. But in certain brain areas (prefrontal cortex, hippocampus) these receptors, decreased in number and function due to aging, become overactive so as to compensate for the memory loss that can result from their continuous activation by glutainatergic cortical overactivation, leading to excittoxic neurons damage. Excess extracellular glutamate accumulation and subsequent overstimulation of glutamate NMDA recepters is said to have many neurotoxic effects, including calcium homeostasis dysfunction, increased no output, protease activation, increase in cytototoxic transcription factors and an increase in free radicals. A synaptic dysfunction abnormal glutamate stimulation has been proposed as one of a few mechanisms to damage AD synapses. Evidence shows that the decreased GLT I regulation correlates with the decline in AD. The GLT I knockout mouse models of AD indicated that mental decline had been intensified. Therefore, various studies have shown a reduced level of AD expression for GLT 1. Interestingly recent in-vitro studies suggest that GLT I reduction and mislocation of astrocytes is responsible for AJ species, which results in a significant reduction in the rate of glutamate clearance in extracellular areas. Studies have shown a potential correlation between GLT I expression alterations and astrccytic reactisity.
Mechanical injury astrocyte reactivity has been shown to promote GLT 1 immunoreactivity and reduced activity in glutamate transport which could lead to increasing concentrations of

extracellular glutamate and excitatoxic cell damage. Drugs which increase ex pression and function of astrocytic glutamate transporters thus pose a possible target for neurodegenerative ecitotoxicity- related disorders. For this reason, several chemicals have been tested and have been shown to be effective. An evaluation of I ,040 IDA approved pharmaceutical and nutritional compounds found that over 20 compounds has more than twice as much as untreated controls to improve GLT I safety expression. § Lactam antibiotics, including penicillin and its derivatives, as well as cephalosporin antibiotics, were excessively and [31] fifteen specific, were extremely actise in stimulating the release of GLT I proteins from drug therapy at as early as 48 hour. The study also showed the ability of cefriaxone to improve both GLT I brain and functioning. In chronically cerebral hypoperfused rats, cefriaxone enhanced spatial learning and memory suggesting a role. In contrast, cefriaxone reduced tau disease and strengthened cognitive functions. Certain comxiunds including ampicillin, estrogen, riluzole and insulin also have been discovered to increase GLT1. The second strategy has recently shown the beneficial GLT2 upregulation as the representative LDN / OS U-0212320 lead compound, a synthetic sequence of pyridazine derivatises. ln order to find compounds that increase GLP I translation.[41] has performed high- performance screenings. Seventeen compound classes were found to enable GLT I translation. A pyridazine-based group has been chosen for further research after intensive studies of these compounds and LDN / OSU -021 2320 as the lead compound has been chosen. Pharmacological characterization.[42] showed that LDN/OSU -021 2320 protects cultured neurons from glutamate-mediated excitotoxic injury and death, delays motor function decline. This translational activation is more appealing since the loss of GLT1 protein is most likely at post-transcriptional level due to defects, higher selectivity and rapid effect may be achieved extended lifespan in an animal model of amyotrophic lateral sclerosis via GLTl activation.[30]

5.3 Glutathione

The CNS is regulated by glutathionic astrocytes (GSH) and astrocyte-based GSH performs important roles in the reduction of oxidative stress in the neurons. Cytokine signalization mechanisms correlated with the modulation of specific aspects of astrogli osis affect astrcicyte GSH rates; for example, interru pti ons in STAT3 in astrocytiques dramatically decrease GSH

land enhance oxidative stress GSH levels. S-nitrosoglutathione was described as an enteric astroglia molecule with functions actix'ating the mucosal barrier. The function of 5-

nitrosoglutathione extracted from astroglic in the CN S has not been properly examined. Modulating the development of GSH by reactie astrocytes is an interesting potential goal for oxidatiye stress neuron defense in chronic and acute CN S disorders.[31]

5.4 Cytokine and growth factor signaling

Astrocytes' can secrete and react to a number of important cytokines that affect both the cells around them such as neurons and the astrocytes themselves in cellular condition. Cytokines such may, for instance, be capable of up regulating or decreasing certain pro- and antiinflammatory genes, including the NOS-2 and COX-2 genes. In secreting the trophics such as neurotropic factor (GDNF), brain-derived neurotropic factor (BDNF), nerve factor devlopment (NGF) and basic fibroblast growth factor (bFGF), astrocytes often play an important role. Astrocytes can promote neural and oligrxlendrocytes and promote inyelination in mature oligodendrocytes through secretion of different growth factors. Therefore, astrocyte targeting is very important in order to facilitate the release of the - variable or the regulation of cjtokine release.[32]

5.5 Other cytokines and growth factors

Astrocytes express receptors and respond to a wide range of other factors of growth and cytokines, including but not limited to TNF J, EGF, FGF, endothelins and different interleukins. Such factors may induce the expression of astroc yic-related molecules, such as GFAP, or may have been irrvolved in the spread of astrocytes. Space constraints here restrict thorough consideration, but several factors that come to represent potential therapeutic targets of interest .[32]

5.6 Interleukin-6 and Suppressor of Cytokine Signaling 3

Interleukin-6, is an inflammatory mediator that can be prxtuced by both glia and CNS cells, namely lL- I/, TNFri, and LPS. lL-6 communicates via the gp 130 receptor, which stimulates

the JAK / Stat route and triggers changes in gene expression primarily by triggering the STAT3. STAT3 interleukin-6 is a known source of reactive astrogliosis. Depending on the rodent template (e.g., lL-6 overexpressor or contingent IL-6 o›'erexpressor), the function of lL- 6 can be beneficial or detrimental. STAT3 is an early cause for astroglyis in astrocytes. However, before STAT3 activation in astrocytes (i.e. phosphorylated STAT3, l PSTAT3 j)

and nuclear translocation, events prior to the up regulations of GFAP mRN A and protein expression, gp 130 related cytokines are up regulated in the mouse model with 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP**),** gp130 related cytokines (e.g., lL-6, ciliary neurotropic factor) and nuclear translocations. Since the promoter has consensus sites recognized to be required by a STAT3 promoter to induce GFAP properly, the idea of STAT3 lL-6 as a cause of astrogliosis is no surprise and defined by an u pregling of GFAP expression. STAT3 is not only an astrogliosis cause, but at least in the case of the spinal cord injuries it seems important for sufficient astrogliosis to be present. In specific, Stat3 conditional knockout mice have decreased GAAP expression, reduced the hypertrophy of the astrocytes and lacked proper development of glial scars, in contrast with Stat3 +/+ Tutorial mice. Such hereditary deletion of Stat3 in astrocytes has also resulted in noncell-autonomous results, including decreased micro gliosis and inflainmatory cell invasion, which is a result of increased spinal cord lesion measurements and diminished motor function recovery. A recently published study found that triptolide, an active ingredient in the traditional Chinese herb Tripterygium wilfordi Hook. f., was found to reduce astrogliosis in vitro and in vivo. Nonetheless, because can have an anti-inflammatory effect on other cells, including microglia,155, it is still unclear whether the in vivo phenotype of decreased astrogliosis is induced directly or indirectly by the herb. The study of the signaling pathways lL- 6 and STAT3 should prove to be overall positive in the long run [33]

5.7 Cyclooxygenase-2

The non-steroidal anti-inflammatory agent (NSAIDs) efficacy as well as the prevention ability for neurological conditions and illnesses have been carefully examined. The NSAIDs act to inhibit cyclooxygenase 1 and 2 (COX- 1 and COX-2), enzymes which have specific critical functions in the control of blood flow as well as inflammatory pathways through[34] prostaglandin development. Furthermore, alternate targets are now identified for NSAlDS,

amongst others N F- xB, AP- I and NOS. N SAI DS in the mouse models contributes to lowered reactive astrogliosis, as shown by the transgenic Alzheimer disease system and celebrcoxib in the transgenic ALS model following administration of ibuprofen. However, it is not clear whether these are direct effects or indirect effects for the astroc jtic reactivity of other mechanisms. In-vitro experiments has shown that NSAID acetyl salicylic acid cancels all NFxB behavior and up control in a human astroglial cell line with glial fibril acid (GFAP) mediated hypoxia. Nonetheless, it remains unclear whether any of the beneficial effects of in -vivo NSAI Ds work directly through astrocytes and need more study.[34]

5.8 TGF-β1 and SMAD3 signaling

Transformations of the growth factor /1 is a pleiotropic cytokine usually expressed at small to undetectable levels in the brain, but highly upregulated in a number of neuropathologic conditions and disorders. TGF /1 signals through TGFbRll binding that then heterodimerizes and passes the TGFJ to the signaling receptor TGF ALK 5 or I (ALK5, ALK l), which initiates a cascade of intracellular serin kinase signals.While ALK I SM AD1/5/8 phosphorylates ALK5 SMAD2/3, each contributes to the nuclear translocation of different signaling complexes that result in expression changes. The results of TGF-J I in the brain are common and tend to depend on the nature of the illness or condition studied. MANY studies found that the function of TGL-J I is proinflammatory and neuropathological and has been particularly well established in both in vivo and in vitro rat models of Alzheimer's disease. TGF-§ I has several documented astrocyte effects including genetic expression, such as upregulation of the amyloid precursor protein (APP), regulation by increased expression of GFAP, evoked hypertrophic and on of extracellular matrix molecules, which facilitated the formation of glial scar.

Compared to control microphysicians, mice lacking Smad3, the downstream TGF-/ I signaling effector via ALK5, show an increased rate of wound close following a stab injury to the brain, consistent with the effect on TGF-1 extracellular matrix formation. As all brain, parenchymal cells are capable of separating and reacting to TGL-QR 1, the signaling is recognized both by the ALK I and ALK5 TGFRl receptor.[35] lt is interesting to note. Nonetheless, to date, astrocytes and microglia can only be expressed and signaled via the ALK5 TGFR. This difference alone could be accidental, but microglias and astrocytes have divergent responses to

cytokine at least in part. While there has been improvement in the understanding of differential cell type responses to TGF-J I in the brain, the explanation of control molecules in the future will prove successful in this way .

Chapter 6

Conclusion

The hypothesis excito-toxicity and cholinergic hypothesis are the major objectives in Therapeutic intervention therapy, and they are symptomatic and hardly effective in ad.despite notable changes in understanding of alzheimers diseases pathogenesis, clear and precise confirmation is still lacking on the mechanism of the disease. Nevertheless, the accumulation of evidence on the role of nonneuronal cells, such as astrocytes, has opened up new avenues for research iming to better understand the disease pathology and to identify cell and molecular targets for the production of medicines. Devloping corroboration shows the physiological role of astrocytes in the preservation of normal brain function propose that their altered reactivity functions play a key role in the etiology of ad and in the expansion or production of harmful effects, particular proteins such as mao-b and aqp4 glutamate transports and are effective goals for pathogenesis of ad. Current evidence shows that the role of reactive astrocytes in ad is disputable.to summarize more research are required to establish this hypothesis and based on that to design potential new drugs.

Abbreviation

AD	Alzheimer's disease
TGF	Tissue growth factor
AB	Amyloid beta
MCI	Mild cognitive impairment
APP	Amyloid beta precursor protein
CSF	Cerebrospinal fluid
PET	Positron emission tomography
NFTs	Neurofibrillary tangles
SP	Senile plaques
RA	Reactive astrocytes
GFAP	Glial fibrillary acidic protein
MCAO	Middle cerebral artery occlusion
ROS	Reactive oxide synthase
RNS	Reactive nitrogen synthase
NOS	Nitric oxide synthase
MAOB	Monoamine oxide B
GSH	Antioxidant glutathion
BDNF	Brain-derive neurotropic factor
BFGF	Basic fibroblast growth factor

References

References

1. Kaj Blennow, Mony J de Leon, Henrik Zetterberg
Lancet (London, England) 368 (9533), 387-403, 2006.
2. WG Rosen, RC Mohs, KL Davis,The American journal of psychiatry, 1984.
3.Nature reviews disease primers 1, 15056, 2015.
4.Lennart Mucke
Nature 461 (7266), 895-897, 2009.
5. Zaven S Khachaturian
Archives of neurology 42 (11), 1097-1105, 1985.
6. Mark P Mattson
Nature 430 (7000), 631-639, 2004.
7. Martin Citron
Nature reviews Drug discovery 9 (5), 387-398, 2010.
8. Ashley I Bush
Trends in neurosciences 26 (4), 207-214, 2003.
9. Jie Kang, Hans-Georg Lemaire, Axel Unterbeck, J Michael Salbaum, Colin L Masters, Karl-Heinz Grzeschik, Gerd Multhaup, Konrad Beyreuther, Benno Müller-Hill Nature 325 (6106),733-736, 1987.
10.Nobuya Kitaguchi, Yasuyuki Takahashi, Yasuo Tokushima, Satoshi Shiojiri, Hirataka Ito Nature 331 (6156), 530-532, 1988.
11.Christopher J Phiel, Christina A Wilson, Virginia M-Y Lee, Peter S Klein Nature 423 (6938), 435-439, 2003.
12Norbert Zilka, Michal Novak
Bratislavske lekarske listy 107 (9/10), 343, 2006.
13. Robert Katzman Neurology, 1993.
14. Alzheimer's Association
Alzheimer's & dementia 9 (2), 208-245, 2013.
15. Martin Citron
Nature reviews Drug discovery 9 (5), 387-398, 2010.
16.Wilma G Rosen, Richard C Mohs, Kenneth L Davis The
American journal of psychiatry, 1984.
17Richard Mayeux, Mary Sano,New England Journal of Medicine 341 (22), 1670-1679, 1999.
18.Barry Reisberg, Jeffrey Borenstein, Stacy P Salob, Steven H Ferris The Journal of clinical psychiatry, 1987.
19Alex Martin, Pim Brouwers, Francois Lalonde, Christiane Cox, Norman L Foster, Thomas N Chase,Journal of clinical and experimental neuropsychology 8 (5), 594-610, 1986.
20.Clara C Pratt, Vicki L Schmall, Scott Wright, Marilyn Cleland Family relations, 27-33, 1985.
21. Elena Santana-Sosa, MI Barriopedro, Luis Miguel Lopez-Mojares, M Pérez, A Lucia International journal of sports medicine 29 (10), 845, 2008.
22.Giovanni A Carlesimo, Marlene Oscar-Berman Neuropsychology review 3 (2), 119-169, 1992.
23.Alexei Verkhratsky, Markel Olabarria, Harun N Noristani, Chia-Yu Yeh, Jose Julio Rodriguez
Neurotherapeutics 7 (4), 399-412, 2010.
24.Rodrigo E González-Reyes, Mauricio O Nava-Mesa, Karina Vargas-Sánchez, Daniel Ariza-Salamanca, Laura Mora-Muñoz

Frontiers in Molecular Neuroscience 10, 427, 2017.
25.Heejung Chun, C Justin Lee Neuroscience research 126, 44-52, 2018.
26.Naomi Habib, Cristin McCabe, Sedi Medina, Miriam Varshavsky, Daniel Kitsberg, Raz Dvir-Szternfeld, Gilad Green, Danielle Dionne, Lan Nguyen, Jamie L Marshall, Fei Chen, Feng Zhang, Tommy Kaplan, Aviv Regev, Michal Schwartz Nature neuroscience 23 (6), 701-706, 2020.
27.Amy M Birch
Biochemical Society Transactions 42 (5), 1316-1320, 2014.
28.CJ Garwood, LE Ratcliffe, JE Simpson, PR Heath, PG Ince, SB Wharton Neuropathology and Applied Neurobiology 43 (4), 281-298, 2017.
29.Marc Fakhoury
Current neuropharmacology 16 (5), 508-518, 2018.
30.Vicky Claire Jones, Rebecca Atkinson-Dell, Alexei Verkhratsky, Lisa Mohamet Cell death & disease 8 (3), e2696-e2696, 2017.
31Nobuyuki Sasaki, Sadamu Toki, Hiroshi Chowei, Toshikazu Saito, Norihito Nakano, Yorihide Hayashi, Masayoshi Takeuchi, Zenji Makita Brain research 888 (2), 256-262, 2001.
32.Richard Mayeux, Mary Sano New England Journal of Medicine 341 (22), 1670-1679, 1999.
33.Barry Reisberg, Jeffrey Borenstein, Stacy P Salob, Steven H Ferris
The Journal of clinical psychiatry, 1987.
34. Philip Schelterns, Howard Feldman The Lancet Neurology 2 (9), 539-547, 2003.
35. Peter V Rabins, John S McIntyre
American psychiatric association, Guidelines, 2010.
36.Lane et al, 2018
37.Dolan & Johnson, 2010 38.
Assefa, Gebre, & Altaye, 2018
39.Richard A.Amstrong,2009.
40.Sofroniew,2009.
41.Colton et,al.2010.
42.Kong et,al.2014.