Tau or Amyloid? A critical review of their contribution to the pathophysiology of Alzheimer's disease

Introduction

Alzheimer's disease (AD) is one of the leading causes of age-related dementia in the world, affecting all ethnicities and communities. In the absence of effective therapy, the rate of dementia is predicted to increase four-fold by the middle of this century in developed societies, owing to increased life expectancies (James et al, 2015).

The diagnosis of AD was first published by Dr. Alois Alzheimer (Maurer et al, 1997), based on his diagnosis of his 51-year-old patient, Auguste D. Post-mortem histological examination of the patient's brain revealed the presence neuritic plaques consisting of dystrophic axons and dendrites which contained paired helical filaments (PHF), and star-shaped, extracellular deposits of amyloid fibrils, characteristic of the disease (Dickson, 1997).

Probable AD has been defined by McKhann et al (2011) of the National Institute of Ageing, USA, as a progressive cognitive impairment evidenced from informants' reports and from neuropsychological evaluation or standardised mental status tests (McKhann et al, 2011). The onset is insidious, characterised by a subtle loss of memory, followed by a decline in cognitive skills, personality changes including paranoia, delusions and a decline in social correctness. Deterioration in language skills follow, culminating autonomic and motor dysfunction, manifested as hyperkinetic, hypertonic syndrome. The severity and rate of neurodegeneration varies among individuals (Braak and del Tredici, 2015: p.1). These symptoms develop over several decades as cognitive functions remain intact during the early and middle pre-symptomatic or silent phases. It is only in the later stages of the disease that dysfunctional

behaviour typified by the loss of cognition and coordination become apparent (Didic et al, 2011).

The central nervous system (CNS) is most susceptible to the accumulation of intracellular tangles and extracellular plaques. Cells of the peripheral nervous system (PNS) and enteric nervous system (ENS) appear to be protected. Neurons which are most vulnerable to AD lesions include late-maturing pyramid cells and projection neurons in the prefrontal cortex. These neurons are typically involved in processes of learning, memory and perception. They are unmyelinated or poorly myelinated cells with long, thin-calibre axons and high levels of paraplasmatic pigment granules namely, lipofuscin and neuro-melanin (Braak et al, 1999).

The biochemical nature of amyloid plaques was determined by Glenner and Wong (1984), and identified as extracellular deposits of amyloid β-protein (Aβ). The protein was isolated from amyloid fibres in cerebrovascular plaques associated with AD (Glenner and Wong, 1984). Following its purification, immunological methods became available for the detection of AD-typical plaques in fixed brain tissue. Mouse monoclonal antibodies coupled with the histological stains, Congo Red and Thioflavin-S, are commonly used for pathological examination of post-mortem brain tissue (Rajamohamedsait and Sigurdsson, 2012).

In addition to the Aβ protein, microscopic examination of neuritic plaques also revealed the presence of neurofibrillary tangles (NFTs) within affected neurons. These NFTs, composed of paired helical filaments, were studied by immunocytochemistry. Biochemically, they were identified as phosphorylated β-pleated structures of the microtubule-associated-protein, tau (Kosik et al, 1986).

While both extracellular Aβ deposits and intracellular tau fibrils typify the disease, the relationship between the two proteins is uncertain, and their contribution to the pathophysiology of AD is still being elucidated. Histopathological findings of post-mortem

brain tissue do not always correlate with prior clinical diagnosis as demonstrated by James et al (2015). Studies from 900 different cases showed that 40% of patients diagnosed with non-Alzheimer's dementia had histopathological characteristics typical of AD. In 30% of the cases that were not diagnosed with AD, post-mortem histopathology was consistent with the disease (James et al, 2015).

Currently, ante-mortem pathological tests include the examination of cerebral spinal fluid for the presence of Aβ and tau. Positron emission tomography (PET) is used for the detection of amyloid plaques. Magnetic resonance imaging (MRI) is used for investigating structural dystrophy in key regions of the cortex (McKhann et al,2011). However, these tests are limited by their availability and lack of standardisation and are mainly used in clinical trials. Moreover, like the post-mortem tests, the ante-mortem tests are often ambiguous (McKhann et al, 2011).

In order to provide effective treatment and develop accurate confirmatory tools for diagnosis, it is essential to identify the exact route of the illness, and the molecular processes that lead to its manifestation. This review critically evaluates existing hypotheses pertaining to the pathophysiology of Alzheimer's with a view to suggesting plausible therapies.

Classification of Alzheimer's: an overview

All clinical manifestations of cognitive disorders associated with pathological findings of amyloid plaques and NFTs are subsumed under the blanket definition of AD (Swerdlow, 2007). The disease is categorised as sporadic or familial, based on the genetic predisposition of the patient (Piaceri et al, 2013). While many ambiguous predisposing factors may contribute to the sporadic form of the disease, familial AD is autosomal dominant and attributed to mutations in three well-defined genes. The percentage of familial AD is very small, constituting less than

5% of detected cases (Swerdlow, 2007). The remaining 95% are sporadic AD, the main risk factor being age (Bossers et al, 2010).

Classification of sporadic AD is broadly divided into Dementia of Alzheimer's Type (DAT) which refers to the pre-senile onset as seen in the classical case of Auguste D., manifest before the age of 65, or the Senile dementia of Alzheimer's type (SDAT), where clinical manifestation occurs after the age of 65. Since the only perceptible difference between pre-senile and senile forms of AD is the age of the patient (Katzman, 1976), it is treated as a single disease. Familial Alzheimer's disease (FAD) is autosomal dominant and characterised by a pre-senile or early onset (Swerdlow, 2007).

Stages of Alzheimer's

Based on histopathology of post-mortem brain tissue, the progression of the disease has been divided by Braak and Braak (1991) into six stages., These stages are differentiated by the density and distribution of AD specific lesions, namely NFTs, neuropil threads (NTs) and amyloid plaques. While the density of amyloid deposits was non-conclusive, NFTs and NTs exhibited a pattern consistent with progression of clinical symptoms and permitted the division the disorder into defined stages.

In stages I and II, a few neurofibrillary changes are detected in the brain, but no cognitive changes are apparent. The first two stages are the transentorhinal stage, where low levels of NFTs and NTs are detected in the entorhinal and transentorhinal regions of the perirhinal cortex and in the pyramid cells of the cortical area.

Stages III and IV are the limbic stages with more extensive neurofibrillary changes evident in the transentorhinal layer, the entorhinal cortex and progression into the hippocampus. This is

accompanied by mild cognitive impairment (MCI), which is the earliest recognizable clinical form of the disease (McKhann et al, 2011). MCI is diagnosed when there is evidence of memory loss without discernible impairment of cognitive function or any other sign of dementia (Selkoe, 2001).

In stages V and VI, neurofibrillary changes spread across most of the neocortex. Neuronal degeneration is highlighted by the appearance of ghost tangles, which are extracellular tangles outside dead neurons. The hippocampus is extensively involved and tangles from subicular pyramid cells extend into apical dendrites (Braak and Braak, 1991). Numerous NTs as well as neuritic plaques are seen in the cortical, entorhinal and transentorhinal areas. Ghost tangles are prominent around pyramid cells, as well as regions such as the amygdala. Gradual destruction of the isocortical area is seen, and the diagnosis of dementia is unequivocal (Bossers et al, 2010). In the final stage of AD, the somatosensory regions, thalamus, hypothalamus and lower brain stem are involved. The primary sensory regions are less severely affected (Braak and Braak, 1991).

While six discrete stages of the disease can be differentiated on the basis of the degree and distribution of NFTs and NTs, there is considerable variation in the formation and distribution of Aβ plaques among individuals studied (Braak and Braak, 1991).

The neocortex is the preliminary site for amyloid deposition to occur, with low density deposits of the amyloid plaques in the basal parts of the frontal, temporal and occipital lobes. The amyloid deposits are not always accompanied by NFT or NT, and vice versa, as seen in the early stages of the disease. As the disease progresses, moderate densities of amyloid-β deposits are seen in almost all regions of the neocortex including the frontal and parietal lobes. The hippocampus is slightly affected with globular deposits in the pyramidal layers of the subiculum and fluffy deposits near the surface of the dentate gyrus. Some amyloid deposits

may also be found in the entorhinal cortex. In the final stages of the disease, dense deposits of Aβ-protein are seen throughout the neocortex and some in the hypothalamus and thalamus (Braak and Braak, 1991).

While deposition of amyloid plaques is not clearly correlated with clinical symptoms, it is evident that deposition of Aβ and NFT precede development of clinical symptoms.

The question that has generated a lot of debate among neuroscientists is this: which of the two proteins occur earliest in the disease, and which of the two are pivotal in its pathophysiology?

The case for amyloid β - protein

The amyloid cascade hypothesis explaining the pathophysiology of Alzheimer's has been the most prevalent point of view since the discovery of point mutations in three genes directly linked to familial AD (Hardy and Selkoe, 2002). These are the amyloid precursor protein (APP), presenilin1 (PS1) and presenilin2 (PS2) genes. The detection of high amounts of Aβ - protein in brains of individuals bearing these mutations led to the postulation of the amyloid cascade hypothesis (Stancu et al, 2014).

Essentially, the amyloid cascade hypothesis postulates that improper processing of APP generates the amyloid protein, Aβ. This protein forms extracellular plaques causing neuronal death, leading to neurodegeneration. These degenerative changes are manifested as a loss of cognitive function and clinical dementia symptomatic of AD (Drouet et al, 2000).

APP is a transmembrane glycoprotein occurring in different cell types. It is normally cleaved at the cell surface or the Golgi apparatus to generate a 90-110 kD protein which is secreted, and a 9kD COOH-terminal membrane protein. APP is also cleaved in endosomes and lysosomes where the Aβ protein is potentially generated (Gabuzda et al, 1994).

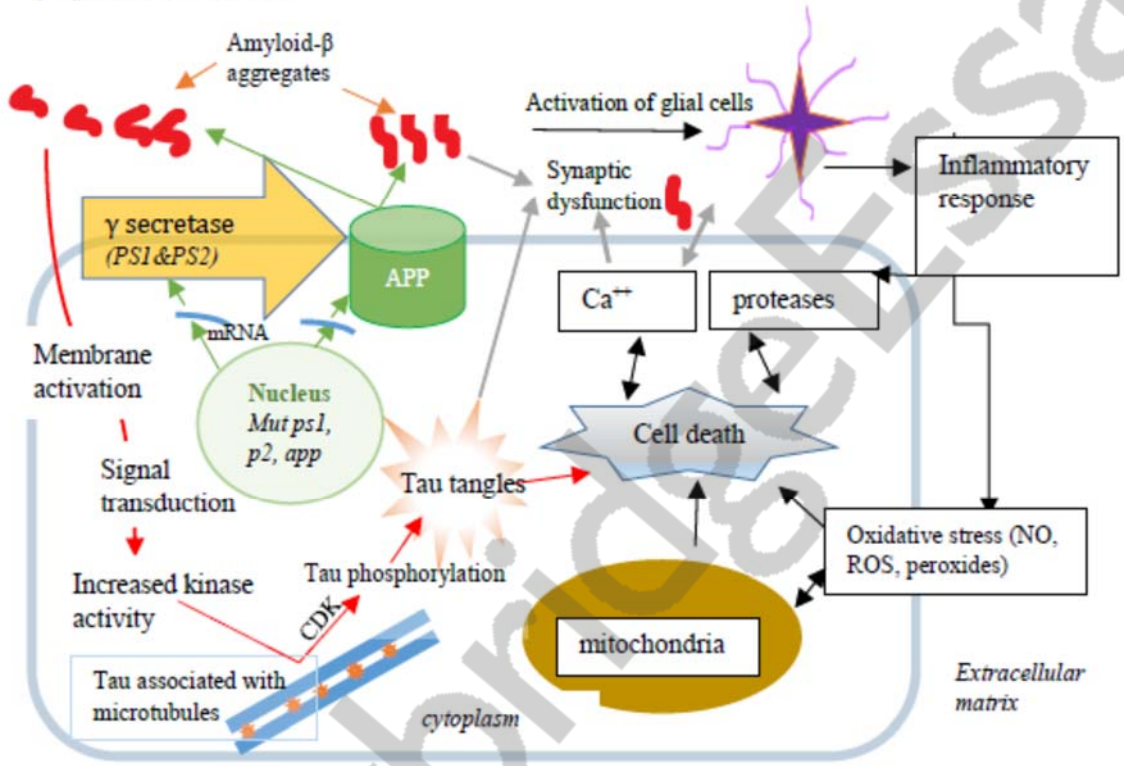
In the native state, the APP protein is believed to be involved in correct neuronal functioning and brain development, but its function is not entirely clear (Zheng et al, 1995).

Mutations in APP or PS lead to an overproduction of Aβ. PS1 and PS2 are subunits of γ-secretase which cleaves APP to generate a 4kD C-terminal peptide consisting of 38-43 amino acids. These are the Aβ40 and Aβ42 proteins that form sticky extracellular plaques of the CNS in AD (Swerdlow, 2007). Aβ binds to membrane lipids and lipoproteins, including the apolipoprotein Apo E, which is a cholesterol transporter (Rodrigues et al, 2012). Drouet et al (2000) suggest that changes in levels of membrane cholesterol due to the adherence of Aβ could alter activity of membrane proteins, including secretases, which cause irregular cleavage of the APP protein.

The Aβ protein aggregates then cause conformational changes from the outside to neuronal membrane proteins, rendering the membrane more permeable to Ca++ ions. Entry of excess Ca++ ions is neurotoxic causing synaptic dysfunction (Drouet et al, 2000). Changes in membrane configuration lead to a signal cascade that activates kinases such as cyclin dependent kinase, CDK and glycogen synthase kinase, GSK3. This results in phosphorylation of tau protein, which then dissociates from microtubules to form paired helical fibrils that tangle into dense insoluble masses. Within neurons these appear as NFTs. In dendrites and axons, they appear as NTs. The dissociation of tau protein from microtubules destabilizes the latter resulting in changes to cell structure, and neuronal transport. Moreover, mitochondrial dysfunction leads to further oxidative stress causing increased levels of nitrous oxide, hydrogen peroxide and lipid peroxidases. Membrane fluidity is also diminished. These combined changes eventually lead to neuronal death. Additionally, glial cells may be activated by the extracellular Aβ triggering the inflammatory process. This results in the release of reactive oxygen species (ROS), nitrous oxide (NO), proteases and complement factors that kill

adjoining neurons. (Rubio-Perez and Morillas-Ruiz, 2012). The amyloid cascade hypothesis is depicted in figure 1 below.

Figure 1. The Amyloid Cascade Hypothesis (adapted from Drouet et al, 2000) Mutations (Mut) in APP, PS1 or PS2 cause improper cleavage of APP. Aβ is formed, leading to a cascade of events resulting in synaptic loss and cell death.



The amyloid cascade hypothesis suggests that AD is a primary manifestation of underlying genetic mutations; non-familial, sporadic AD has been explained by the mitochondrial cascade hypothesis that postulates that AD is a secondary manifestation of a primary root cause.

The mitochondrial cascade hypothesis attempts to explain non-Mendelian factors contributing to late-onset AD. It postulates that it is mitochondrial dysfunction that leads to amyloidosis, tau phosphorylation and cell-cycle re-entry. This is based on Harman's free-radical theory of aging. The theory posits that aging cells undergo damage with the accumulation of oxidative by-products (Harman, 1956). Somatic mutations have been seen to occur in mitochondrial

DNA in experimental animals, and this increases the rate of aging (Wallace,1992). The lowered mitochondrial cytochrome oxidase activity that follows is presumed to account for oxidative stress evidenced in Alzheimer's. Gabuzda et al (1994) demonstrated that in vitro treatment of cells with mitochondrial inhibitors such azide increases the rate of amyloid-β formation, thus increasing the risk of AD. The mitochondrial cascade hypothesis postulates that the essential difference between individuals who suffer from Alzheimer's and others is the inherited difference in the durability of the mitochondrial electron transport chain. Aging mitochondria are prone to oxidative stress resulting in the formation of amyloid-β proteins which affect cell respiration and cause mitochondrial dysfunction as well as tau phosphorylation which brings about de-differentiation of cells and cell-cycle re-entry, eventually leading to neurodegeneration (Swerdlow, 2007).

The case for Tau

While the amyloid cascade hypothesis explains the pathogenesis of AD, this hypothesis has been contested by Braak and del Tredici (2015: p. 2), whose observations led to the postulation that AD begins with the appearance of intracellular NFTs followed by extracellular amyloid plaques in the CNS after about ten years. Neuritic plaques that contain both types of proteins develop only in later stages of the disease. The amyloid cascade hypothesis has also been debated by Hasegawa et al (2016) who argue that, although amyloid plaques are associated with AD, treatment with anti-Aβ antibodies does not relieve symptoms in experimental animals and clinical trials, nor does removing Aβ plaques through vaccination. Further, removal of oxidative stress by anti-oxidants does not reverse symptoms or progression of the disease (Hardy and Selkoe, 2002).

Tau proteins are coded on the long arm of chromosome 17 (17q21) by a single microtubule associated protein tau (MAPT) gene. There are six isoforms of the protein. In the normal state they bind to microtubules and stabilize them thereby supporting the cell structure and guiding the flow of nutrients within the cell (Goedert et al, 1992).

Tau proteins are ubiquitous within neurons, and become localized in axons of mature neurons (Mandelkow and Mandelkow, 2012). In undifferentiated cells, tau is phosphorylated and does not associate with microtubules. As the neuron matures, tau becomes less phosphorylated.

In AD neurons, the tau protein is hyperphosphorylated. There is approximately 4-8 times more phosphorylated tau in AD neurons than in normal brain cells (James et al, 2015). In this state they pair up with other strands of tau to form PHFs which tangle together, disrupting microtubules and neuronal transport. The resultant formation of extremely insoluble tangled aggregates accounts for the loss of axonal transport and neuronal communication, culminating in synaptic dysfunction and cell death (Iqbal et al, 2010).

Cell division is regulated by cyclin-dependent kinases (CDK) and cyclins which are stimulated by external factors that regulate cell division. While adult neurons are terminally differentiated non-dividing cells (Zhu et al, 2007), in AD they are found to re-enter the mitotic phase, although the mitotic cycle is not completed. However, this leads to aneuploidy in neuronal cells seen in AD. Zhu et al (2007) suggest that these abnormalities ultimately contribute to neuronal death.

The cause of tau phosphorylation in AD is not as yet very clear. Mandelkow and Mandelkow (2012) have postulated that age-related proteasome dysfunction or oxidative stress could initiate changes in tau. Cyclin dependent kinases also phosphorylate tau protein. An increase in phosphorylation could be due to the increase in kinase levels with respect to phosphatases.

This may be related to aging, oxidative stress or protein misfolding, as suggested by Mandelkow and Mandelkow (2012).

In the native state tau is unfolded and occurs mainly in the axons. It is believed that phosphorylation of tau leads to its self-assembly (Hasegawa, 2016). In its aggregated form it is resistant to phosphatases. The misfolded β-sheet configuration of tau could seed its own formation and spread in a prion-like manner. This prion-like spread has been demonstrated in vitro in cultures seeded with tau fibrils, and in vivo in mouse models. Neuronal death has been observed in transgenic animal models with alterations in tau, indicating that the protein is sufficient for the disease, independent of Aβ (Zhu et al, 2007).

It is not entirely clear how tau fibrils could spread intracellularly across the CNS. It has been suggested that membrane bound APP may interact with extracellular tau fibrils and recruit them into cells (Hasegawa, 2016). It has also been shown that accumulation of tau is independent of Aβ. Braak and del Tredici (2013) hypothesised that Aβ is secreted from axons of neurons containing tau fibrils, and does not contribute to the primary pathology of the disease. They argue that tau is therefore a prerequisite for amyloid-β formation, and not the other way around (Braak and del Tredici, 2013).

Conclusion and future directions

Clearly, Alzheimer's is a highly complex disorder involving multiple variables, not all of which are well defined. While both Aβ and tau have been found in post-mortem brain preparations of AD patients, neurofibrillary tangles of tau have been found to be more uniformly correlated with clinical symptoms. Both symptomatic and pre-symptomatic cases of the disease contain the tau fibrils in the same type of neuronal cells. The occurrence of tau protein fibrils is consistent with clinical symptoms and shows little variation among different individuals

studied, unlike Aβ which shows a high degree of variation and is inconsistent with the clinical stages of the disease (Braak and del Tredici, 2013).

While this may indicate a more prominent role for tau in the pathogenesis of AD, there are still several gaps in understanding pathways of tau phosphorylation and exact mechanisms of the spread of the disease.

The development of more sensitive and accessible imaging techniques using tracers specific for tau and Aβ would further the understanding of the pathogenesis of AD, and serve as a powerful diagnostic tool in monitoring the disease. The development of therapies targeting tau, such as kinase inhibitors, tau antibodies and drugs would be essential to control progression of the disease. Drugs that retard tau phosphorylation such as valproic acid have proven successful in mice (Swerdlow, 2007). Human clinical trials are ongoing. Recently the drug LMTX that targets tau tangles has shown to slow down the disease, suggesting that tau protein is more directly involved with pathogenesis than amyloid-β (Coghlan, 2016).

It is important to recognise that Alzheimer's is a collective syndrome of multiple causes. Identifying the root cause of the disease would be the key to its cure. Determining a definitive causal and temporal link between tau and Aβ at the molecular level would go a long way in deciphering the pathophysiology of this insidiously degenerative disorder.

List of Abbreviations

Aβ	Amyloid beta
AD	Alzheimer's disease
Apo E	Apolipoprotein E
APP	Amyloid precursor protein
CDK	Cyclin dependent kinase
GSK	Glycogen synthase kinase
PET	Positron emission tomography
PHF	Paired helical fibrils
PS	Presenilin
MCI	Minor cognitive impairment
NO	Nitrous oxide
NFT	Neurofibrillary tangles
NT	Neuropil tangles
ROS	Reactive oxygen species

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