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Review Article

Neurodegeneration and Cytomegalovirus

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Abstract

Introduction: Alzheimer's disease (AD) is the most frequent cause of dementia in the elderly, and it is characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). Several studies have shown clinical correlation between various herpes viruses and neurodegeneration (ND), others have demonstrated coincident markers for AD and Herpes Simplex Virus-1 (HSV-1) infection, but there is yet no study investigating Cytomegalovirus (CMV) and AD.

Human Cytomegalovirus (HCMV) is specie specific, and the scope of mechanistic study is limited; however, because of great similarities in genetics and pathology between HCMV and Murine Cytomegalovirus (MCMV), MCMV will be used as a model system for investigating ND in mice and the result obtained extrapolated to humans.

The study will be carried out to determine the presence of neurodegenerative markers (Aβ42 and high molecular weight tau) in IC-21 macrophage cells and in primary microglial cells following infection with MCMV.

Methods: IC-21 macrophage, 5 x 105 cells will be seeded unto 60 mm dishes and infected 24 hours later with MCMV at a multiplicity of infection (MOI) of 2 plaques forming unit/cell. Cell lysates will be harvested at different time points post infection. Total protein will be quantified, and 20 µg protein loaded into each lane of SDS-PAGE and electrophoresis carried out to separate the proteins. Separated proteins will be transferred to membrane and probed with rabbit polyclonal anti-Aβ42, rabbit polyclonal anti-tau and mouse monoclonal anti-β actin will be the loading control. Indirect immunofluorescence microscopy will be used to detect the presence of the protein of interest. Primary microglial cells will be isolated from P2 brains of mice and homogenized in 2 ml TS buffer (50 mM Tris-HCL) allowing to stabilize. MCMV infection will be done on day 15. Microglial cells will be disrupted with sonicator and centrifuged at 300,000g (450,000 rpm) for 30 minutes.

Conclusion: Presence of high molecular weight tau and Aβ42 following infection of the cells with CMV shows that CMV which belongs to the same family (Herpesviridae) like HSV-1 also induces neurodegeneration.

Keywords: *Neurodegeneration; Alzheimer's: Cytomegalovirus*

Introduction

Neurodegeneration is a chronic pathological disease of the central nervous system (CNS), it is characterized by gradual loss of structure and function of the neurons. Alzheimer's disease (AD) is the most common form of neurodegeneration (ND), clinically it is marked by loss of memory and cognitive impairment, and it is the leading cause of dementia worldwide [1]. Pathological hallmark of the disease

is accumulation of extracellular amyloid plaques which is composed of amyloid β-peptides (Aβ) and intracellular neurofibrillary tangles (NFTs). Neurofibrillary tangle is composed of hyperphosphorylated tau and other modifications of tau. These tangles form inside the neurons and block the transport system in the neurons [2-4]. Several hypotheses have been postulated to explain the primary triggers and drivers of AD. The notable ones are the prevailing amyloid cascade hypothesis, the well-recognized tau hypothesis, and the increasingly popular pathogen hypothesis.

Alzheimer's disease and the amyloid hypothesis

Amyloid plaques seen in the brain of Alzheimer disease patients are pathological aggregates of amyloid-β peptides (Aβ). Amyloid hypothesis postulates that extracellular amyloid plaques are the initiator of the downstream pathological events in AD.

There are two variants of amyloid beta: $A\beta_{40}$ (short Aβ) which has 40 amino acid residues and $A\beta_{42}$ (long Aβ) which has 42 - 43 amino acid residues. The short Aβ is found in the brain of healthy subjects as well as in the brain of AD patients; however long Aβ is abundant in AD brains. $A\beta_{40}$ is non-pathological whereas $A\beta_{42}$ is and plays a role in the formation of amyloid plaques seen in AD [5-7]. It is the metabolism of amyloid precursor protein (APP) that yields Aβ. APP is a transmembrane protein with a short half-life, it is metabolized rapidly down two pathways in all cells: the α and the β pathway. The α pathway does not yield $A\beta_{42}$, however the β pathway yields more of the $A\beta_{42}$ peptide which is amyloidogenic. The processing of APP through this pathway involves the cleavage of the precursor protein by β secretase enzyme followed by γ secretase cleavage. It has been proposed that the increase in the production of $A\beta_{42}$ in the β pathway is a result of the shift at the position of γ secretase cleavage, hence central to the production of more $A\beta_{42}$ is the position of γ secretase cleavage. If the cleavage is between residue 712 - 713, $A\beta_{40}$ is the result but if the cut is after 714 then $A\beta_{42}$ is the result [8,9]. The amyloid hypothesis has not been able to provide sufficient evidence to show that extracellular Aβ peptide accumulation is the primary trigger of AD because of poor correlation between amyloid plaque load and severity of cognitive impairment, as well as evidence against this hypothesis from clinical trials [10].

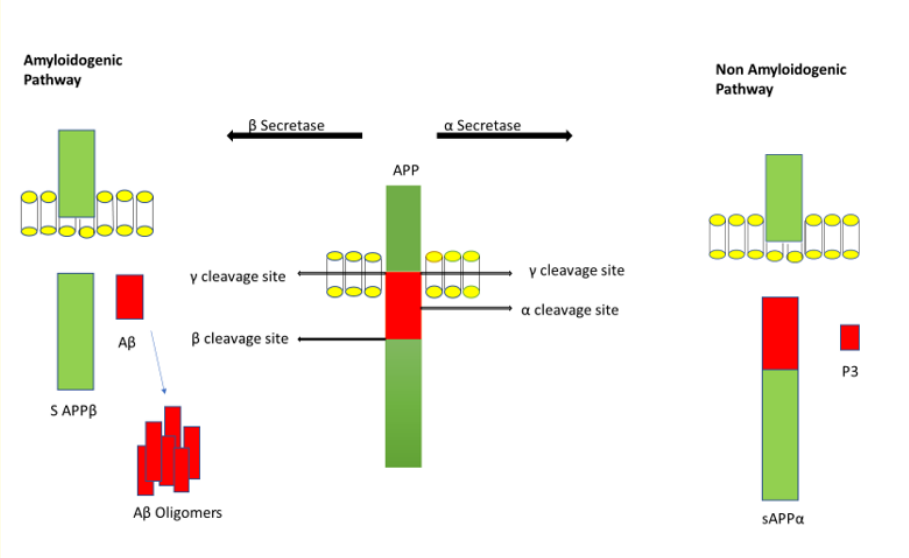

Figure 1: *Processing of APP through the α and β pathway.*

Alzheimer's disease and tau hypothesis

Intracellular neurofibrillary tangles (NFTs) are composed of hyperphosphorylated tau and some other post translational modifications of tau. Tau is a microtubule associated protein (MAP) that binds and stabilizes the microtubule (MT). Phosphorylation of tau is necessary for its physiological function; however, when tau is hyperphosphorylated it affects it function negatively and causes it to detach from the MT and self-aggregate into soluble oligomers. The soluble oligomers are toxic to the neurons, but as a defense mechanism the soluble oligomers polymerizes into Paired Helical Filament/Neurofibrillary Tangles (PHF/NFTs). The level of tau phosphorylation is a result of dynamic regulation between tau kinases and phosphatases. How tau becomes abnormally hyperphosphorylated in AD is not well understood; but this could be the result of imbalance between the activity of tau kinases and tau phosphatases [11-14].

Glycosylation is one of the most common co-/post translational modifications of protein, through which oligosaccharides are covalently attached to the sidechain of polypeptides. According to the nature of glycosidic bonds, two types of glycosylation are defined: O-linked and N-linked glycosylation [15]. There are three variants of tau seen in AD brain, they are: non-hyperphosphorylated functional tau (AD-tau), abnormally hyperphosphorylated tau (AD P-tau), which is readily soluble, and tau aggregated into PHFs (PHF-tau) which is insoluble. Studies have shown that PHF-tau from AD brain is glycosylated with several sugar moieties whereas tau purified from normal brain of controls is not. Enzymatic de-glycosylation of PHF tangles does not restore tau's biological activity, but it untwists PHFs into bundles of straight filament [16-18]. It appears that while hyperphosphorylation makes tau dysfunctional and polymerized; glycosylation probably helps maintain and stabilize NFT structure. Lectin staining technique have been used to demonstrate that AD-tau, which is active in stimulating MT assembly is not hyperphosphorylated; however, it is aberrantly glycosylated. On the other hand, tau from normal brain of controls is not glycosylated. This suggests that aberrant glycosylation may precede abnormal hyperphosphorylation of tau in AD brain. This raises the intriguing possibility that glycosylation might promote hyperphosphorylation. In addition to phosphorylation and glycosylation several other post-translational modifications of tau have been identified. These modifications include ubiquitination, glycation, polyamination, nitration, and proteolytic truncation [19-22].

Ubiquitination normally labels misfolded or damaged proteins for degradation through ubiquitin-proteosome system. Although PHF-tau is highly ubiquitinated, it is apparently not degraded rather it is deposited as NFTs in AD brain. It is not well understood why ubiquitinated PHF-tau fails to be degraded and cleared in AD. PHF-tau is also modified by glycation, and glycation refers to non-enzymatic linkage of glucose or other monosaccharide to amino acid side chain of the polypeptide, whereas glycosylation is an enzymatic linkage of polysaccharides or monosaccharide to amino acid side chain of the protein [23-25].

Tau protein undergoes several post-translational modifications during the pathological process seen in AD. Modifications such as abnormal hyperphosphorylation and glycosylation occur at early stage of tau pathology and appears to play a crucial role in the pathogenesis of neurofibrillary degeneration. Other modifications such as ubiquitination, glycation, and nitration are late events and probably represents failed mechanism of neurons to remove damaged and aggregated proteins [26,27].

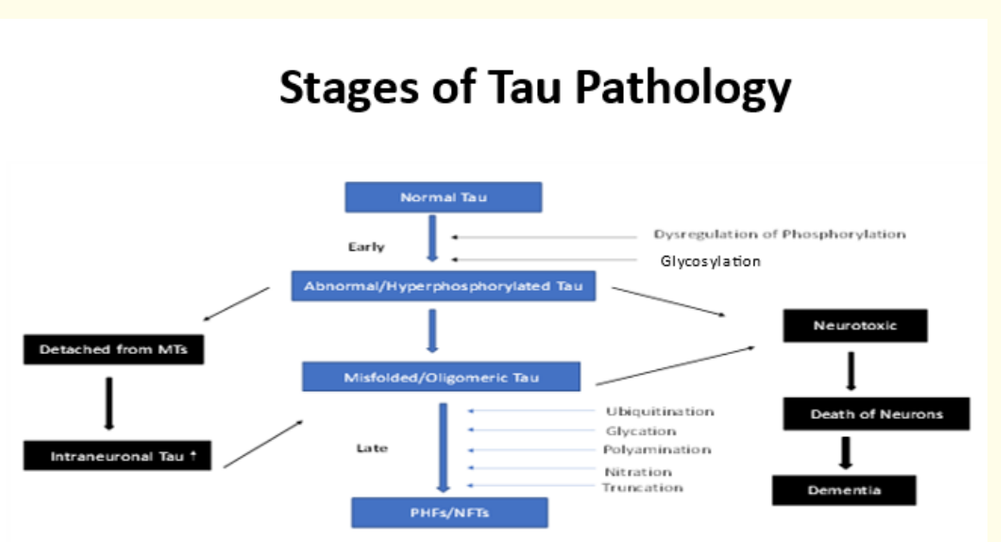

Figure 2: *Stages of Tau pathology and post-translational modifications [28].*

Alzheimer's disease and pathogen hypothesis

Evidence from human and animal studies suggest a strong correlation between the presence of Herpes Simplex Virus-1 (HSV-1) in the brain and the likelihood of Alzheimer's Disease [29]. Another study reported the presence of HSV DNA in amyloid plaques found in AD [30].

Denaro FJ., *et al*. and Morin I., *et al*. found Alzheimer's pathology in conjunction with herpes infection in the brain with abundant evidence tying markers of AD to identical markers of HSV-1 infection. One of such markers is up-regulation of heme oxygenase-1 (HO-1), which co-localizes to senile plaques and neurofibrillary tangles. Increase expression of HO-1 has also been observed in HSV-1 infected brains. Other coincident markers of AD and HSV infection includes phosphorylated eukaryotic initiation factor-2α (elF-2α), inducible prostaglandin synthase cyclooxygenase-2 [31]. HSV-1 life cycle follows two paths: lytic and latent cycle. During lytic phase, the virus replicates and viral particles are produced, but in latent cycle, one or another specific cell infected by the virus maintains the viral genome without productive virus infection. Reactivation of a latent infection of HSV-1 has been proposed as one of the possible causes of AD. Inflammatory responses and oxidative changes documented in AD brain are in support of an inflammatory cause; also, most people are latently infected by HSV-1, as there is a general agreement that a large percentage of human population has HSV-1, with a global prevalence of 65 - 90% [32]. Furthermore, the trigeminal ganglion located only a few millimeters from the entorhinal cortex is said to be the primary site of HSV-1 latency although other sites may be involved. In addition, novel data from Speljko T., *et al*. have shown that upon infection of cultured neuronal cells and mouse brain with HSV-1 there is accumulation of extracellular amyloid-beta protein as well as abnormal phosphorylation of tau protein [33].

Significance

An estimated 6.2 million Americans aged 65 years and older are living with AD today, and this number could grow to 13.8 million by 2060. Available data makes Alzheimer's the sixth leading cause of death in the United States and the fifth leading cause of death among American's aged 65 years and older [34]. Because there is yet no definitive treatment for AD, the patients suffering from the disease are on long term care and hospice services. More than 11 million family members and other unpaid care givers provided an estimated 15.3 billion hours of care to people with Alzheimer in 2020. The Medicaid payment for beneficiaries aged 65 years and older with AD or other dementias are more than three times as great as payment for beneficiaries without this condition [35,36]. Several studies have shown clinical correlation between various herpes viruses and neurodegeneration, others have demonstrated coincident markers for AD and HSV-1 infection, but there is yet no study investigating CMV and AD. Since CMV and HSV-1 belong to the same family of viruses; the herpes viridae and they share similar characteristics. CMV will therefore be used in this study to investigate AD [37]. Experiments using Human Cytomegalovirus (HCMV) in human will pose ethical challenges, also HCMV cannot effectively infect mice therefore Murine Cytomegalovirus (MCMV) which has similar kinetics with HCMV will be used to infect mice in this study [38].

Aim of the Study

The study will be carried out to determine the presence of neurodegenerative markers ($A\beta_{42}$ and high molecular weight tau) in IC-21 macrophage cell lines, and mice primary microglial cells following infection with Murine Cytomegalovirus (MCMV), as well as determine the modifications happening to tau.

Methods

IC-21 macrophage, 5 x 10^5 cells will be seeded unto 60mm dishes and infected 24 hours later with MCMV at a multiplicity of infection of 2 plaques forming units per cell (2PFU/cell). Cell lysates will be harvested at times (0, 6, 12, 24, 36, 48 and 72 hours) post infection in western lysis buffer (50 mM Tris, 1% SDS) and the total protein quantified. 20 µg protein will be loaded in each lane of 12% SDS-PAGE

and electrophoresis carried out. Separated proteins will be transferred to a blotting paper and membrane probed with rabbit polyclonal anti-Aβ, rabbit polyclonal anti-tau (sigma) and mouse monoclonal anti-β actin will be used as the loading control. Indirect immunofluorescence microscopy will be used to detect the presence of protein of interest. Primary microglial cells will be isolated from P2 brains of mice and homogenized in 2 ml Ts buffer (50 mM Tris-Hcl). MCMV infection will be done on day 15 and microglial cells will be disrupted with sonicator and centrifuged at 300,000g (450,000 rpm). Total protein will be quantified and 20 µg of protein loaded in each well of 12% SDS-PAGE. Electrophoresis will be carried out and separated proteins transferred to membrane and probed with antibodies.

Mass spectroscopy will also be used to investigate glycosylation on the full-length tau protein.

Limitation of the Study

Polyclonal antibodies will be used to detect our protein of interest, and this can cause background bands in the blot because of cross reactivity.

Conclusion

Presence of high molecular weight tau and $A\beta_{42}$ following infection of the cells with CMV shows that CMV which belongs to the same family (Herpesviridae) like HSV-1 also induces neurodegeneration.

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