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Anti-Viral Agents in Neurodegenerative Disorders: New Paradigm for Targeting Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease affecting geriatric populations for which several causes have been proposed. These include a relationship with known pathogens although the exact nature of such a relationship remains uncertain. Herpes simplex virus-1 has been proposed as potential cause of AD because of its ability to form ß amyloid(Aß) and neurofibrillary tangles due to tau hyperphosphorylation and action of beta & gamma secretase on amyloid precursor protein(APP) together with genetic association with apolipoprotein-E_4(ApoE-ε4), which points out to latent Herpes Simplex virus-1 as an agent causing AD. There are numerous studies that linked HSV-1 with AD like anti-HSV-1 IgM antibodies, nectin-2, heme oxygenase-1, phosphorylated eukaryotic initiation factor-2A, caspase-8 and nucleus-specific alteration of raphe neurons. Various possible mechanisms by which HSV-1 might lead to development of AD such as ApoE, ß-amyloid, tau phosphorylation, inflammation and oxidative stress are also discussed. Thus, this review discusses patent information and a strong relationship between latent HSV-1 and AD and also proposes antiviral therapy for AD.

Keywords: Alzheimer's disease, antiviral therapy, ß-amyloid plaques, biomarkers, herpes simplex virus-1 (HSV-1), inflammation.

INTRODUCTION

Herpes simplex is a common viral infection whose causative agent is Herpes Simplex Virus (HSV) also known as Human Herpes Simplex Virus. It is a contagious disease caused by HSV-1 causing cold sores and HSV-2 causing genital herpes. The usual treatment includes antivirals like Acyclovir, Valacyclovir and Famciclovir which shorten the outbreak. HSV usually migrates to the brain through branches of the trigeminal nerve to the meninges or via olfactory tract, and establishes a low-grade infection within brain. It can even remain latent in the brain which has been found in the post-mortem brain study. Alzheimer's disease is a neurodegenerative disease affecting the elderly population wherein there is decline in the memory power and cognitive behavior of the patients whose exact cause is unknown. It causes brain atrophy and usually affects after 5^{th} decade of life. The pathophysiology of Alzheimer's disease points to the formation of ß amyloid & neurofibrillary tangles due to destabilization of tau-protein responsible for microtubule stabilization, due to phosphorylation by kinases. Action of beta & gamma secretases cleaves APP causing production of ß amyloid. These events lead to degeneration of neurons which leads to loss of memory and dementia. As there is no certain cause, researchers keep trying and thus came up with new pointer towards latent HSV-1 causing AD.

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ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive decline in cognitive functions, which leads to memory loss and dementia. It involves degeneration in the temporal lobe of limbic and cortical brain structures. In 2010, estimate was that 35.5 million individuals were affected by dementia in the world with a prediction that this number will reach to 65.7 million by 2030, and 115.4 million by 2050 [1].

AD is classified by age of onset: early versus late-onset and familial versus sporadic. There is a clear genetic component in a small number of early-onset familial AD cases. While other causes of AD are unclear and the risk factors include increasing age, Down's syndrome, and head injury, there is accumulating evidence suggesting that infectious agents are important etiological factors in AD [2].

The characteristic feature of AD brain is pronounced cerebral cortex atrophy with loss of cortical and subcortical neurons. The pathological hallmarks of AD are senile plaques (i.e., spherical accumulations of the β-amyloid protein); accompanied by degenerating neuronal processes; and abundant neurofibrillary tangles, made up of paired helical filaments and other proteins. In advanced AD, hippocampus and associated regions of cortex witness abundant senile plaques and neurofibrillary tangles, while sparing areas such as the visual and motor cortices. This corresponds to the clinical features of marked impairment of memory and abstract reasoning, while preserving vision and movement [3].

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Loss of cholinergic neurons accounts for much of the learning and memory deficit in AD [4].

HERPES SIMPLEX VIRUS

Herpes simplex virus (HSV) belongs to *herpesviridae* family [5]. HSV is found in two variants i.e. HSV-1 and HSV-2. HSV-1 is responsible for diseases of mouth, face, skin, esophagus and brain. It causes encephalitis, corneal blindness, and several other disorders of the peripheral nervous system [6]; known for causing cold sores. HSV-2 causes infections of the genitals, rectum, skin, hands, or meninges, and is responsible for meningoencephalitis in neonates and meningitis in adults [6]; known as genital herpes [4, 7]. It is a contagious disease spreading through contact between person to person and the site of entry is skin and/or mucous membranes [7].

After viral inoculation, the incubation period is of 2 to 21 days, following which randomly distributed vesicles appear on skin. Tiny papules develop into vesicles, which produce ulceration. Soreness, itching, dysuria, and inguinal or femoral lymphadenopathy may accompany. Dysuria is common in women. Untreated eruptions of genital herpes last longer than the oral variety, with a primary episode lasting for 2 to 4 weeks. Recurrent genital herpes produces localized vesicles on an erythematous base, which without treatment exists for 7 to 12 days [8]. Herpes simplex virus types HSV-1 and HSV-2 establish latent infection in dorsal root ganglia for the entire life of the host from where they can reactivate to cause morbidity and mortality [6].

Internalization into the cells is by fusing the Herpes simplex virus (HSV) envelope with the host cell membrane through the glycoprotein interaction. The cell type decides whether the fusion occurs at the cell surface or, after endocytosis, with an endosomal membrane in low pH-dependent or independent manner [9]. HVEM (herpes virus entry mediator) is an immune modulator of the family Tumor Necrosis Factor Receptor (TNFR)- a host cell protein, which serves as a doorway for the wild type (wt) HSV-1 and HSV-2. The cell adhesion molecule nectin-1 (HveC, CD111) serves as a primary receptor for HSV-1 and HSV-2 on the keratinocytes, neurons and epithelial cells. Additionally, HSV-1 glycoprotein D (gD) binds to heparan sulfate (HS), which is modified by 3-O-sulfotransferases (3-OST). It has been shown that for directing the virus to the endocytic pathway in some cell types, the receptor interaction involving glycoprotein D (gD) is required [7, 10].

ROLE OF HSV-1 IN ALZHEIMER'S DISEASE

The first and foremost evidence of involvement of HSV-1 in AD was discovered by Ball, 1982 and Gannicliffe *et al.*, 1986 [11, 12] based on the observation that patients surviving Herpes simplex encephalis(HSE) showed clinical signs reminiscent of AD i.e., memory loss and cognitive impairment, and that brain regions primarily affected in HSE i.e. limbic system, frontal and temporal cortices were the same regions compromised in AD [1, 2]. The hypothesis suggested that in the later life, the immune system weakens, and HSV-1 spreads, either through branches of the trigeminal nerve to the meninges or via the olfactory tract, and establishes a low-grade infection within the brain which leads to inflammation and cellular changes, which promote, amyloid beta production and other changes leading to AD. It even establishes life-long latency in the brain which is supported by HSV-1 being found in brain tissue from patients with AD, specifically within the amyloid plaques [11]. Furthermore, HSV-1 infection of neural cells *in vitro* has been shown to induce AD-relevant cellular changes, i.e. amyloid beta production, tau hyperphosphorylation, and AD-type inflammatory signaling [12]. An association with ApoE genotype has also been found, which is consistent with facilitated HSV-1 spread in carriers of the ApoE ε4 allele [13].

Association of ApoE ε4 and brain

ApoE is a major cholesterol carrier responsible for lipid transport and injury repair in brain. It emerged as a major determinant in cognitive function and Alzheimer's disease, immunoregulation and infectious diseases. It has 3 major isoforms, differing by single amino acid substitutions and thus corresponding change in functions; with ApoE3 being the normal isoform. ApoE4 is associated with increased risk of Alzheimer's disease, impaired cognitive function and reduced neurite outgrowth [14]. Lipoproteins, including low density lipoproteins(LDL) and ApoE-containing lipoproteins have the ability to either inhibit or stimulate mitogen- & antigen- induced T-lymphocyte activation and proliferation. When these lipoproteins bind to immunosuppressive receptors, they inhibit lymphocyte activity [15]. Several evidences are consistent with the notion that ApoE binds to heparin and heparin- like macromolecules, which rescue the cellular response to mitogens which correlates with the removal of ApoE from the cell surface [3, 15]. Thus, viruses can bind to heparan sulphate proteoglycan(HSPG) on cell surfaces for primarily gaining entry. It has even been reported that the presence of HSV-1 increases the risk for AD and there is overrepresentation of the ε4 allele in the AD patients with HSV but not in those lacking HSV, which suggests an isoform specific effect of ApoE involving viruses that are connected directly to the risk of developing AD [18, 19] Fig. (**1**).

Many studies have used ApoE-transgenic mice for finding correlation between HSV-1 infection and ApoE isoform-specific effects on viral load, or on viral expression.

It has even been reported that ApoE-ε4 carriage led to potentially more damaging effects than ApoE-ε2 or ε3 carriage [16]. The brains of proliferative and latently infected ApoE-ε4 mice had a greater viral load [14,15]. Studies on cultured foetal neurons from ApoE-transgenic mouse brains showed that the infection increased the expression of HSV-1 immediate–early genes from e4 animals, and in ApoE-ε4 mice, and the latency was established later [17]. The trigeminal ganglia and brain of latently infected ApoE-ε4 mice evidenced greater viral load [18]. All these studies thus support the proposal that infection by HSV-1 is modulated by ApoE and majority of viral damage occurs in the brain of ApoE-ε4 carriers, resulting in AD [2].

HSV-1 and ß-Amyloid Plaque Formation

Genes for HSV-1 reactivation have been found to be associated with ß-amyloid deposits in the familial AD patients'

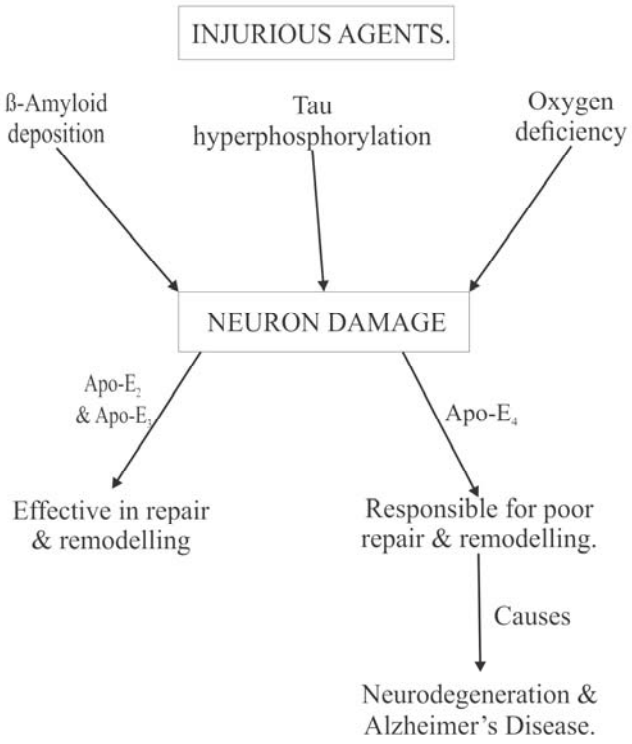

Fig. (1). Apolipoprotein E maybe involved in final common pathway mediating repair & remodelling of damaged neurons [15].

brains. In the AD patients, the amyloid plaques from the temporal and frontal cortices showed the presence of HSV-1 DNA [11].

The characteristic feature of AD is senile plaques composed of ß-amyloid, which is generated by enzymatic cleavage of amyloid precursor protein (695 amino acid long and is prevalent in neurons) [2] by enzymes ß and ϒ-secretases [2]. ß-amyloid clumps together to form the plaques. ϒ-secretase also processes nectin-1: a member of immunoglobin superfamily involved in synapse formation and serves as a chief receptor on neurons and epithelial cells for HSV-1 [15, 18]. ß-secretase is also involved with nectin-1 causing co-localization at synapses, and the interaction of virus and nectin-1 affects processing of the former [2]. The reason why HSV-1 causes Aß accumulation is unknown but there is a possibility that Aß fibrils may enhance the infection of several enveloped viruses including HSV-1 as it is required for synthesis of progeny viruses [19, 20]. Another possibility is that Aß might increase as part of their defense response as amyloid oligomers were evidenced as having bacteriostatic activity [21]. Therefore, oligomer formation prevents further damage to the host in response to infection and certain types of chemical change, but eventually through overproduction, results in toxicity.

Tau Hyperphosphorylation Evidences a Link between HSV-1 and Alzheimer's Disease

Tau is a microtubule associated protein, abundant in neurons. Its major function is regulation of microtubule dynamics, which is regulated by site-specific phosphorylation events; which if deregulated, as in diseased state, results in tau dysfunction and mislocation, followed by tau polymerization, neuronal dysfunction and death. Tau in phosphorylated and hyperphosphorylated forms comprising the neurofibrillary tangles [22].

It has been shown by immunochemistry, ELISA and western blotting techniques that cultured human neural cells infected with HSV-1 caused abnormal and hyperphosphorylation of tau, characteristic of AD brain. Together with it, there was increase in enzyme levels responsible for phosphorylation, i.e. glycogen synthase kinase 3ß and protein kinase A. There is evidence that HSV-1 encodes a protein that activates and is functionally homologous to protein kinase A. Zambrano and colleagues showed that cultures of cortical neurons from foetal mouse infected with HSV-1 displayed tau hyperphosphorylation, altered microtubule dynamics and neurite damage [2].

The reason why HSV-1 causes tau phosphorylation is unknown but it can be attributed to cellular defense mechanism or it would be required for viral replication and is supported by the fact that lithium, an inhibitor of glycogen synthase kinase 3ß, inhibits phosphorylation [23], also thereby inhibiting HSV-1 infection to be used to treat HSV-1 infection in brain [23, 24] Fig. (**2**).

Association of HSV-1, Inflammation & AD

Inflammation is a key response to many viral infections and even to HSV-1. There are many inflammatory mediators which are common to HSV-1 and Alzheimer's disease; and thus, these may prove to be the markers.

1. HSV-1 infection leads to production of tumor necrosis factor α(TNFα), interleukin 6(IL-6), interleukin 8(IL-8) and interferon inducible protein 10(IP-10) [25, 29], which are also found elevated in AD brains [25, 26].
2. Expression of RANTES(Regulated on activation normal t cell expressed and secreted) and MCP-1(Monocyte chemoattractant protein-1), is increased in case of AD as well as in HSV-1 infection [25].
3. In mice, interleukin 1ß is found to be protective against HSV-1 encephalitis [26]. It has been found elevated in AD which might explain that full-blown encephalitis is not found in the AD brain [27].
4. The peripheral inflammatory cytokines produced during systemic infection lead to immune cell stimulation in the central nervous system (CNS) and subsequent inflammation, which causes cognitive decline in elderly humans and memory loss in animals. Cognitive decline in humans may be a result of inflammation-induced reactivation of HSV-1 [28].
5. The innate immune cell reactivity increases with age and thus aging would lead to greater extent of inflammation in brain, contributing to neurological disease [29]. When older rodents were exposed to experimental peripheral infection or lipopolysaccharide injection, they showed memory defects as compared to younger animals [30, 31]. Several epidemiological studies on humans have shown association between the number or extent of infectious episodes and cognitive decline in elderly. Latent HSV-1 reactivates under stress and illness and thus repeated systemic infection causes brain inflammation which could cause AD [28].

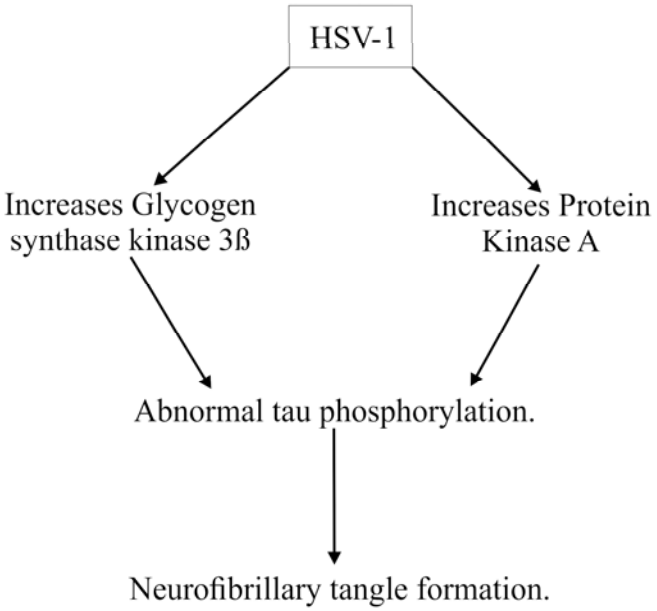

Fig. (2). Putative action of HSV-1 in the formation of neurofibrillary tangles [29].

Association of HSV-1, Oxidative Stress & AD

Oxidative damage is also common to HSV-1 and AD. It has been suggested that Aß and tau aggregation are compensatory responses to oxidative stress and culture studies showed that oxidative stress causes intracellular Aß accumulation and tau phosphorylation [28].

In AD brains and AD animal models, it has been observed that there is oxidation of major cell macromolecules nucleic acids, proteins and lipids in the early of AD [28]. Similarly, viral infection including HSV-1 and inflammatory response towards it can lead to increased formation of reactive oxygen(ROS) and nitrogen species (RNS), which can regulate host's inflammatory and immune responses and can cause oxidative damage to nucleic acids, proteins and lipid-containing structures to which nervous system is more susceptible [32]. On the basis of oxidative products detection in different cell types, it has been shown that not only latently infected neurons but uninfected neurons and glial cells are also damaged due to oxidative stress and persistent inflammation [32].

BIOLOGICAL MARKERS THAT LINK ALZHEIMER'S DISEASE AND HSV-1

Anti- HSV-1 IgM Antibodies

A large population based prospective study revealed that the elderly patients had increased risk of AD with positive titers of anti-HSV-1 IgM antibodies- markers for primary or reactivated HSV-1 infections. While it is not associated with anti-HSV-1 IgG antibodies- markers of a life-long infection [33].

Upregulation of Heme Oxygenase-1 (HO-1)

A report stated that immunoreactivity of HO-1 is greatly increased in astrocytes and neurons of hippocampus and cerebral cortex of AD subjects and colocalizes in senile plaques and neurofibrillary tangles. HSV-1 infected brains also observed increased HO-1 levels [34].

Phosphorylated Eukaryotic Initiation Factor-2α (eIF2α)

Protein kinases are part of cellular defense mechanism against virus and exist in various polymorphic forms, with one of them being protein kinase R (PKR). When PKR is activated, it phosphorylates the α-subunit of eIF2α, which causes inhibition of protein synthesis and replication as eIF2α is required for translation. It is observed that there is intense immunoreactivity against phosphorylated eIF2α in AD brain sections which is associated with neuronal degeneration in AD [34, 35].

Inducible Prostaglandin Synthase Cyclooxygenase-2(COX-2) and the Prostaglandin

Individuals with AD showing significant increase in inflammatory biomarkers suggest that inflammation might be a driving cause of the disease. COX-2 and prostaglandin-mediated signaling have been reported to be up-regulated in the brains with AD. Prostaglandin pathway actions may represent an intermediary step in HSV-1 reactivation, as treatment by celecoxib- selective inhibitor of COX-2, suppressed HSV-1 reactivation [36]. Some studies showed that non-steroidal anti-inflammatory drugs (NSAIDs) had protective effect against AD by preventing inflammation, which could cause reactivation of HSV-1 infection [34]. In addition, NSAIDs also inhibit generation of the pathogenic amyloid-β(1–42) peptide (Aβ42) independently of the inflammatory cyclooxygenase (COX) pathway.

Activation of Caspase-8

Caspase-8 activation has been observed in AD brain. Additionally, viral activation causes activation of caspase-8 leading to neuron degeneration due to apoptosis of host neurons [34]. It has been reported that Lithium inhibits caspase-3 activation and thereby possesses neuroprotective property [27].

Nucleus-Specific Alteration of raphe Nucleus

In neurodegenerative disorders, it has been noted that AD raphe and hippocampus evidence an alteration of serotonin transporter sites. It has been reported that there is a decrease in serotonin content in neocortex and cerebrospinal fluid due to degeneration of serotonergic nucleus raphe dorsalis in AD brains. Experiment on rabbits with HSV encephalitis showed decreased raphe serotonin, thus, suggesting HSV could reach limbic system via serotonergic projections from raphe neurons [34].

CAN ANTIVIRAL THERAPY BE PROPOSED FOR ALZHEIMER'S DISEASE?

Efficacy of antiviral treatment in AD is dependent on their ability to reduce HSV-1 induced hyperphosphorylated tau and Aß production. Most commonly used antivirals are acyclovir and valacyclovir- prodrug of acyclovir. Valacyclovir is used in HSE due to its greater oral bioavailability, efficiency and safety [37, 38].

Acyclovir is phosphorylated by viral thymidine kinase [30]. Acyclovir monophosphate form is phosphorylated to active triphosphate form by cellular kinases and has greater affinity for viral DNA polymerase. Triphosphorylated acyclovir gets incorporated into viral DNA, thus causing chain termination and acts by interfering with HSV-1 DNA replication [38].

Experiment on HSV-1 infected Vero cell cultures using acyclovir, penciclovir and foscarnet which inhibit viral DNA replication, showed a reduction in Aß and hyperphosphorylated tau accumulation [38]. Hyperphosphorylated tau accumulation was reducible to near zero values as its accumulation was dependent on HSV-1 DNA replication. Aß was reducible to only near-normal value as antiviral-induced decrease was due to reduction in the number of new viruses formed and this near-normal Aß as discussed previously has anti-viral activity through its overproduction which becomes toxic and thus treatment should focus on bringing Aß to near-normal values as done by acyclovir [37].

In another study on infected Vero cell culture, acyclovir treatment was compared with antiviral BAY 57-1293, which targets helicase-primase complex in the viral DNA replication. BAY 57-1293 was found to be more efficient in decreasing the accumulation of Aβ and hyperphosphorylation of tau, HSV-1 replication and the cell cluster size that form during infection [37].

Intravenous immunoglobulin (IVIG) was tested on infected Vero cell cultures and it was found to be effective in reducing accumulation of Aß and hyperphosphorylated tau, during infection stage before viral DNA replication and thus it might be effective in treating AD. It neutralizes extracellular virus, destroys HSV-1 infected cells in conjugation with lymphocytes and it has proved to have synergistic combination with acyclovir [2, 37].

In vivo studies of valacyclovir on rabbits latently infected with HSV-1 showed a possibility that viral levels could be monitored by assaying asymptomatic shedding of HSV-1 DNA in tears and saliva, which also occur in humans. The study compared DNA levels in tears, collected once a day for 6 days pre-infection and for 10 days post-infection. It was found that valacyclovir treatment in high doses reduced the number of copies of viral DNA [17, 39, 40].

Memantine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist is used alone or in combination with anticholinesterase for treating AD. Memantine also has strong antiviral activity by interfering with replication of virus and thus resembling other anti-HSV drugs such as amantidine and tromantadine. It reduces the effects of infection with human neurovirulent corona virus which caused encephalitis and a mutant which induces paralysis involving glutamate excitotoxicity in mice [37].

CURRENT & FUTURE DEVELOPMENTS

The review strongly supports that HSV-1 is the likely pathogen which gives rise to Alzheimer's disease in the long term by causing accumulation of ß-amyloid plaque and tau hyperphosphorylation. Thus, the anti-viral treatment could be used for treatment of AD and also suggests that it might prevent further decorations in patients. The great advantage of antiviral agent over current AD therapies is that it will provide a completely new approach in that they would inhibit a major cause of the disease rather than inhibiting the symptoms. Thus, repurposing the anti-viral drugs for Alzheimer's disease holds better future for virus-induced AD.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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