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Role of HSV-1 in Alzheimer's disease pathogenesis: A challenge for novel preventive/therapeutic strategies

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Abstract

Herpes simplex virus-1 (HSV-1) is a ubiquitous DNA virus able to establish a life-long latent infection in host sensory ganglia. Following periodic reactivations, the neovirions usually target the site of primary infection causing recurrent diseases in susceptible individuals. However, reactivated HSV-1 may also reach the brain resulting in severe herpetic encephalitis or in asymptomatic infections. These have been reportedly linked to neurodegenerative disorders, such as Alzheimer's disease (AD), suggesting antiviral preventive or/therapeutic treatments as possible strategies to counteract AD onset and progression. Here, we provide an overview of the AD-like mechanisms driven by HSV-1-infection in neurons and discuss the ongoing trials repurposing anti-herpetic drugs to treat AD as well as preventive strategies aimed at blocking virus infection.

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Introduction

Alzheimer's disease (AD) is the most common type of dementia in the elderly affecting millions of people worldwide [1]. Despite most of the underlying mechanisms have been unravelled, such as those involving the microtubule-associated protein-tau and amyloid-beta peptides (Aβs), very few successes have been gained in the field of therapeutic and/or preventive treatments for this devastating disease. During the last years, several evidence suggested infectious agents as risk factors for AD onset and progression, particularly herpes simplex virus-1 (HSV-1) infection/reactivations reaching the central nervous system (CNS) [2].

HSV-1 is a neurotropic double-stranded DNA virus that, following active replication in epithelial cells, establishes life-long latency mainly in the sensory neurons of trigeminal ganglia. In this site, the virus can periodically reactivate and neo-formed virions travel along axons to the site of primary infections, giving rise to cold sores and blister in symptomatic infected individuals. HSV-1 may also reach the CNS where it targets the brain regions most affected in AD [3–7] and triggers an acute inflammatory response resulting in the severe forms of herpetic encephalitis (HSE). Specifically, this response is driven by microglial cells, the brain-resident specialized population of macrophages that are enrolled as the first line of defense against viral agents. Upon activation, microglia release several inflammatory mediators to achieve a beneficial resolution of the viral infection. However, when this activation persists, microglia can lead to detrimental effects on neurons, contributing to HSE and its neuropathological sequelae [2,8].

Milder/subclinical infections may also occur eventually followed by latency [9]. Many studies linked repeated HSV-1 replications in the brain throughout life to the occurrence of an AD-like neurodegeneration in the elderly. First evidence came out from genetic studies correlating the presence of HSV-1 genome in the brain of few AD patients carrying the ε4 allele of apolipoprotein E

(*APOE*ε4), a genetic risk factor for AD and for HSV-1 recurrence [10]. Other subsequent population-based studies correlated the presence of specific anti-HSV-1 immunoglobulin M (IgM) or the increased avidity of anti-HSV-1 immunoglobulin G (IgG), both markers for HSV-1 reactivations, to a higher risk of developing AD pathology [11,12], particularly in *APOE*ε4 carriers [13]. In addition, Readhead et al. [14] recently published massive molecular and bioinformatics analyses of post-mortem brain samples from four independent cohorts of AD patients, reporting increased presence of herpes viruses in AD brains *versus* matched controls.

This review will provide a brief overview of recent scientific literature (up to 2021) describing the pathogenic mechanisms driven by HSV-1 in neurons and will discuss the most promising therapeutic strategies aimed at counteracting these viral effects.

HSV-1 and AD: experimental evidence

HSV-1 infection profoundly affects neuron physiology [2]. Ours *in vitro* studies indicate that in neuronal cells HSV-1 triggers intracellular Ca^{2+} signaling promoting amyloid precursor protein (APP) phosphorylation via GSK-3 activation and in turn the amyloidogenic APP processing leading to extra- and intra-cellular accumulation of Aβs and other APP cleavage products [15,16]. These possibly activate different pathways affecting the transcription of neurotoxic genes [17], DNA repair mechanisms [18], expression of synaptic proteins [19], and neurogenesis [20]. Consistently, other groups reported that the virus induces APP processing in human neuroblastoma cells via upregulation of cellular secretases [21], resulting in the production of a C-terminal APP fragment containing the Aβ domain [22]. Also, virus-induced defects in the autophagic machinery were reported to cause Aβ accumulation [23], and detection of Aβ persisted over 7 days post HSV-1 infection in primary adult hippocampal neurons [24]. Very recently, HSV-1 infection and its effects on Aβ production were also reproduced in 2D neuron cultures and 3D brain model derived from human-induced neuronal stem cells [25–27].

HSV-1-induced GSK-3 activation was also linked to tau phosphorylation in human neuroblastoma cells [28], and hyperphosphorylated tau was shown to accumulate into nuclei of infected cells [29]. Interestingly, a recent article showed transient tau phosphorylation in HSV-1-infected primary hippocampal neurons isolated from adult mice [24]. On the contrary, *in vivo* studies showed different tau phosphorylations lasting for days after virus inoculation [30] and significantly accumulating in the hippocampus of mice undergone several cycles of virus reactivations over life [7]. These animals particularly resembled an AD-like phenotype, showing also cerebral Aβ hyperproduction and deposition in plaques, signs of neuroinflammation, increased levels of oxidative stress damage and epigenetic alterations, paralleled by progressive and irreversible cognitive deficits [7,31,32].

Interestingly, other authors recently reported a protective antimicrobial property for Aβ against different pathogens, including HSV-1 [33–35]. Specifically, Eimer et al. proposed that Aβ oligomers entrap HSV-1 within the aggregation process leading to plaque deposition [33]. On the contrary, Ezzat et al. [36] showed that the virus itself catalyses Aβ aggregation *in vitro*, thus accelerating AD pathogenesis. However, both views converge to the same pathological event, i.e., the increased accumulation of Aβ peptides triggered by viral replication in the brain.

Anti-HSV-1 treatments and AD

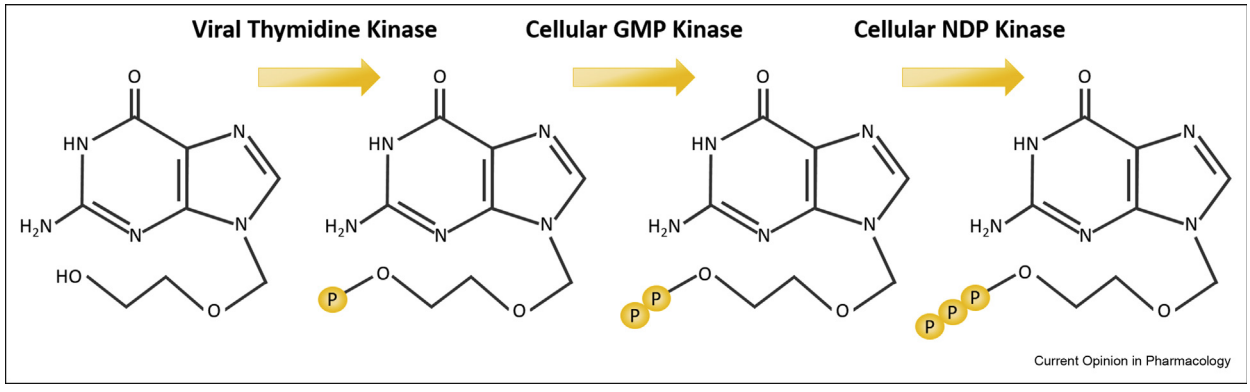
HSV-1 replication can be effectively inhibited by antiviral drugs such as acyclovir (ACV), penciclovir, foscarnet, and BAY 57–1293 [37]. These drugs, targeting viral DNA replication, in turn greatly reduced also HSV-1-driven tau phosphorylation and Aβ formation in *in vitro* models of infections [38,39]. These findings suggest specific anti-herpetic molecules as possible treatments to slow or stop AD progression, especially in those patients with a documented history of recurrent HSV-1 infection. An observational retrospective cohort study [40] on electronic health databases in Taiwan supported this chance. This study reported a 2.56-fold higher risk of developing dementia, including AD, over a 10-year follow-up period in Taiwanese individuals newly diagnosed with HSV infection in 2000. Notably, the authors evidenced a lower incidence of dementia in those HSV-infected people who had received an antiviral drug ($n = 7215$) compared to non-treated subjects ($n = 1147$), suggesting anti-herpetic drugs as a possible therapy in AD. However, this study has several possible biases due to the lack of in-depth clinical information of the analyzed population, including data on HSV-1 or HSV-2 seropositivity, duration of the treatment and the type of incident dementia. Other similar observational studies provided both concordant [41] and contrasting [42] results, being also affected by similar confounding biases. Recently, Loveheim et al. published results from a nested case-control study investigating if herpes antiviral drugs could prevent AD incidence in a very small, but well characterized cohort in terms of *APOE*ε4 and HSV-1 carriage status [43]. Their results, again, indicate an association between antiviral treatments (in this case even preventive) and a lower risk of AD, but further research should be performed both at basic and

clinical (e.g., well powered randomized clinical studies; identification of the correct timing, dosage and duration of the treatment) levels to support this observation.

Interestingly, two pilot clinical trials (ClinicalTrials.gov identifiers NCT02997982 and NCT03282916) are ongoing to evaluate the efficacy of valacyclovir (the most effective and bioavailable antiviral to treat HSV infections) in improving cognition and functions in HSV-seropositive AD patients. Upon oral administration, valacyclovir—L-valyl ester of ACV- is converted to the prodrug ACV, which in turn is transformed into the active compound only in virus-infected cells. Herein, ACV is phosphorylated to ACV monophosphate by the viral thymidine kinase and then by cellular kinases to its triphosphate form (Figure 1). The latter, acting as a DNA chain terminator, strongly inhibits viral DNA polymerase activity and thus viral replication [44]. L-valyl esterification of ACV, producing valacyclovir, improves oral bioavailability thus resulting in increased drug plasma levels compared with oral ACV [45]. This also results in higher ACV cerebrospinal fluid (CSF) concentrations, as they are dependent on drug levels in plasma (e.g., 20—50% of ACV plasma concentration) [45,46]. The NCT02997982 study is an open pilot phase-II trial enrolling 33 participants with AD or Mild Cognitive Impairment (MCI), HSV IgG positivity, and *APOE*ε4 carriage [47]. According to the study design, participants would have been orally administrated with valacyclovir (1.5—3.0 g daily) for 4 weeks and tested before and after the treatment period for cognitive functions, CSF AD biomarkers, and PET imaging to possibly detect active HSV infection within the brain. As properly attempted by this trial, this is a crucial point to address for checking the efficacy of a drug acting only on productive virus infection. This analysis, if successful and specific, would provide useful information to identify those AD patients bearing replicating HSV in their brains. This study was completed in April 2020, but its results have yet to be published. However, due to the few enrolled participants, the short treatment period and the absence of a placebo control administration, the expected results from this study would be only a starting point for future investigations. A previous open-label pilot study (not yet published) was performed at the University of Helsinki (EudraCt #2013-000101-24) investigating the effects of 12-week oral administration of valacyclovir in 30 HSV-seropositive AD participants that were assessed for changes in cognition and function.

A more complex and long-lasting study design underlies the NCT03282916 trial started in February 2018. This is a randomized, double-blinded, placebo-controlled phase II proof-of-concept trial enrolling 130 participants with mild AD and HSV seropositivity (IgG and IgM). Valacyclovir (2.0—4.0 g/day in 65 participants) or placebo (in 65 subjects) administration will last 78 weeks over which changes in cognitive parameters as well as PET imaging (to detected cerebral amyloid accumulation and tau phosphorylation) are assessed as primary and secondary outcomes, respectively. Moreover, structural MRI (to evaluate changes in cortical thinning), olfactory identification deficits and anti-HSV antibody titers in the blood are evaluated in exploratory analyses. Quantification of serum anti-HSV IgM titer would be a readout of viral reactivations within the treatment period. However, since it is planned only at the beginning and the end of the study, some viral reactivations might not be detected. Moreover, serum IgM titer does not actually reflect virus reactivation/replication in the

Figure 1



Schematic representation of the well-known mechanism of activation of the prodrug acyclovir (ACV). After the uptake in the HSV-infected cells, the nucleoside analogue ACV undergoes sequential phosphorylation steps. The first one is driven by the viral-encoded thymidine kinase that converts the prodrug in the monophosphate ACV. The latter is then phosphorylated by guanosine 5′ monophosphate (GMP) and nucleoside 5′ diphosphate (NDP) cellular kinases to yield the active ACV triphosphate. This inhibits viral DNA polymerase by competing with endogenous deoxyguanosine triphosphate for incorporation in the growing viral DNA strand. Here, it acts as a chain terminator blocking viral DNA replication. Hence, ACV impairs active viral replication but it is not able neither to affect latent virus infection nor to eradicate the virus from infected host.

brain. The study also plans to assay CSF AD biomarkers as well as plasma and CSF ACV concentration (to evaluate the degree of drug CNS penetration following oral valacyclovir) in those participants agreeing lumbar puncture. The latter is an interesting point to address since few studies reported that CNS ACV concentration may be affected by the loss of brain blood barrier integrity due to pathological conditions as well as by renal impairment [46,48,49]. However, additional detection of HSV genome would provide evidence of HSV replication in the brain. Overall, this study, expected to end in August 2022, attempts to evaluate the efficacy and safety of valacyclovir treatment in limiting AD progression in HSV-infected people, but it probably targets a too late stage of the disease. Authors themselves more recently (2021) proposed and started an additional trial (ClinicalTrials.gov identifiers NCT04710030) to evaluate the efficacy of valacyclovir in limiting the progression of the disease at the earlier stage MCI. This is a Phase II, placebo-controlled, 52-week trial using oral valacyclovir 4 g/day or placebo in 50 HSV-seropositive MCI patients exhibiting AD biomarkers. Participants will be assessed for ApoEε4 carriage, AD signature of MRI regional and whole brain cortical thinning, PET imaging of amyloid accumulation, plasma total tau, phospho-tau epitopes and neurofilament light protein, and plasma viral antibodies.

Despite the underlined limitations, if successful all these trials would add also important information supporting the view of HSV involvement in AD progression and pathogenesis. However, eventual negative results would not discredit it. Indeed, the limited duration of drug administration (4–78 week range) might not be enough to prevent HSV-dependent neurodegenerative events, especially if it does not strictly match active virus replication. On the other side, a longer treatment period might result in possible negative side effects linked to drug toxicity.

Anti-HSV preventive and innovative strategies and AD

To date, researchers' efforts to identify an effective anti-HSV vaccine in humans have been unsuccessful, although different vaccine technologies have been used, such as those based on killed or attenuated-live viruses, viral protein subunits, DNA and modified mRNA [50]. Many promising candidates successful in mouse, guinea pig or rabbit, failed to elicit effective immunity in human trials so far. This happens likely because animal models do not perfectly resemble neither HSV diseases in humans nor human immunity mechanisms. Thus, the development of effective anti-HSV vaccines, both preventive (i.e., able to prevent primary infection) or therapeutic (i.e., able to prevent outbreaks related to virus reactivations) represents a challenge for the ongoing research and several clinical trials are running. Since both types of vaccines would have the chance to avoid also the HSV-1-driven damages in the CNS, these researches deserve further efforts from benchside to bedside.

Interestingly, very recently a novel mechanism underlying alphaherpes virus neuroinvasiveness has been described [51]. According to the authors' results, HSV-1 captures cellular kinesin motor in epithelial cells within virion assembly, carries it between cells and engages the exogenous protein in neurons to travel toward nuclei. Authors referred to this process (i.e., repurposing of a cellular protein as an essential virion structural component) as assimilation, suggesting that it may offer new strategies to prevent virus infection in neurons.

Also some experimental data on the use of CRISPR/Cas9 endonuclease system to target the HSV genome in infected cells have been provided from both *in vitro* [52,53] and *in vivo* studies [54,55]. This two-component system is based on the endonuclease activity of the clustered regularly interspersed short palindromic repeat (CRISPR)-associated protein 9 (Cas9) and on a short guide RNA. The latter, by pairing to the target DNA sequence, recruits Cas9 to this DNA cleavage site, thus allowing a specific cut. The use of viral vectors to deliver this system in neurons evidenced how this approach could be potentially curative to treat latent HSV infection, eliminating viral DNA. However, safety issues should be carefully addressed before translating such approach in humans.

Conclusion

A growing body of evidence support recurrent HSV-1 infection as a potential risk factor for AD, and clinical trials repurposing anti-herpetic drugs to treat this disease and its earlier stages are ongoing. However, there is a huge need for additional research to unravel when and how the virus may reach the brain of susceptible individuals and which HSV-1-infected population suffers recurrent infection targeting the brain. Peripheral biomarkers of HSV-1 cerebral infections would strongly help in selecting the right population to treat with antivirals. However, whenever HSV-1 involvement in AD pathogenesis would be definitively proved, the development of preventive and/or therapeutic anti-HSV vaccines would be a challenging mandatory goal also for AD prevention in HSV susceptible people.

Conflict of interest statement

Nothing declared.

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