Abstract

Herpes simplex virus (HSV-1 and HSV-2) are one of the disease which is found all over the world which may lead to Alzheimer’s disease (AD). It causes cold sore or watery blisters on the skin and the mucous membrane of mouth or genitals, which are contagious and ubiquitous. It can spread with the contact of the saliva of the infected person. After the primary infection the virus undergoes in latent phase in the cell bodies of neurons which may reactivate if favorable condition is provided. There are many antiviral drugs present for the treatment or the disease but till now no vaccine has been made in case of HSV-1.

Introduction

Herpes Simplex Virus Type 1 belongs to the family of Herpesviridae (Arduino and Porter, 2008), subfamily is Alphaherpesvirinae and genera Simplexvirus (Whitley and Roizman, 2001). It is a linear double stranded DNA virus that can infect human host naturally (Arduino and Porter, 2008). The herpesvirus family can be divided into alpha, beta and gamma subgroups which consist of over 100 double stranded DNA viruses among which only eight herpesviruses are known to commonly infect humans (Shukla and Spear, 2001).

Viral structure

There are four components of herpes simplex viroin: double stranded DNA genome (152 kb) (Roizman and Sears, 1996) enclosed in an icosahedral nucleocapsid surrounded by a proteinaceous tegument and a lipoprotein envelope (Cardone et al., 2007). The DNA core which is enclosed in icosahedral capsid consist of 162 capsomeres, 7 different viral proteins (VPs), VP5 (UL19), VP19C (UL38), VP21 (UL26), VP22a (UL26.5), VP23 (UL18), VP24 (UL26), and VP26 (UL35) and gene product of UL6 and UL25. (Roizman and Sears, 1996; Zhou et al., 1998). VP5 is the major protein and is present on the surface together with VP19C, VP23 and VP26. The surrounding tegument protein consists of about 20 proteins which includes VP1/2 (UL36), VP11/12 (UL46), VP13/14 (UL47), VP16 (UL48), VP22 (UL49), ICP0, ICP4, and the virion host shutoff protein (UL41) plus the products of genes US2, US3, US10, US11, UL11, UL13, UL14, UL16, UL17, UL21, UL37, UL51, and UL56 (Homa et al., 1997; Mettenleiter, 2002) Mettenleiter, 2004). Out of 11 different glycoproteins (gB, gC, gD, gE, gG, gH, gL, gJ, gK, gL, and gM) at least 8 glycoproteins are involved in envelop synthesis (Roizman and Sears, 1996).

Host Shutoff protein

The Host Shutoff protein is made late in infection and packed into viroins and in newly infected cells. It degrades preexisting and newly transcribed mRNA by shutting off host protein synthesis (Karr et al., 1999; Kwong and Frenkel, 1987; Strom and Frenkel, 1987). It acts as an mRNA-specific RNase. It has been reported that (i) In the absence of other viral proteins vhs degrades mRNA as shown by inhibition of reporter gene expression in mammalian cells transiently co-transfected with a vhs expression vector (Jones et al., 1995; Pak et al., 1995); (ii) the vhs dependent nuclease activity of the detergent solubilized viroins can be specifically blocked by anti-vhs antisera (Zelus et al., 1996); (iii) vhs induces endonucleolytic cleavage of a number of RNA substrates (Elgadi et al., 1999) and (iv) vhs does not differentiate between cellular and viral mRNAs, but it shows a strong preference for mRNAs, as opposed to rRNA and tRNA, in all the systems tested (Krikorian et al., 1991; Oroskar et al., 1989; Zelus et al., 1996).

Viral Portal Protein

UL6 acts as a portal protein (Trus et al., 2004). The portal ring serves as a conduit for DNA entering and exiting the capsid (Cardone et al., 2007). The UL6 protein plays an effective role in many aspects of viral assembly and
encapsidation, including ring formation, incorporation into the growing procapsid shell, binding of the terminase, and acting as a conduit for the packaging of viral DNA into capsids.

**Figure 1**: Fitting of the UL6 portal protein into a vacant vertex in the HSV-1 capsid. (a) Interior view of a capsid model at 18-A° resolution.

**Mechanism of viral entry into the host cell**

**Formation of Hemifusion Intermediate**

There are three phases of Virus-induced membrane fusion (Chernomordik and Kozlov, 2005; Jardetzky and Lamb, 2004) In phase I opposing membranes are brought in close proximity through a viral glycoprotein binding a cellular receptor. In phase II the lipid mixing initiates between the two opposed membrane and is completed when the leaflets of outer membrane are mixed to form an intermediate called hemifusion and the phase III begins with the mixing of the inner membrane leaflets and pore formation and expansion. Once phase III gets over the fusion process completes. The functions of viral glycoproteins in the fusion process (close apposition, hemifusion, and complete fusion) are characterized by the three fusion process (Chernomordik and Kozlov, 2005; Blumenthal et al., 2003; Cohen and Melikyan, 1998). Four membrane glycoprotein (gD, gB and gHL complex) mediates the fusion process. Mutants lacking any one of the four glycoproteins are not infectious as their replication is blocked at membrane fusion (Spear, 2004). The current model for HSV-1 fusion is that the required glycoproteins form a fusion complex (Subramanian et al., 2005; Cocchi et al., 2004), where gD binds a cell surface receptor (herpesvirus entry mediator, nectin-1, or modified heparin sulfate), inducing a conformational change that leads to fusion mediated by gB and/or gHL (Cocchi et al., 2004; Zago et al., 2004). gB and gH are the most likely candidates to be involved in Phases II and III of the fusion process.

**Fig 2**: HSV Fig: virion and its two major modes of entry into cell
Structural components of a typical HSV virion are shown (box) above (fig. 2) (Akhtar and Shukla, 2007). HSV virions can enter into cells via a pH-independent fusion of viral envelope with the plasma membrane (I) or alternatively, via an endocytic pathway that may be phagocytosis-like (II) in terms of the viral uptake. In both pathways HSV particles may initially associate with filopodia-like membrane protrusions via heparin sulfate proteoglycan (HSPG). Unidirectional transport of extracellular particles bound to filopodia (HSV surfing) then brings the particles closer to the cell body for entry via interactions with the cellular receptors including gD receptor and possibly gB receptor. Fusion at the plasma membrane results in the release of the naked viral nucleocapsid in the cytoplasm for transport to the nucleus. Similarly, endocytosis also requires fusion of the enveloped particles with the vesicular membrane for the release of the viral nucleocapsid proximal to the nucleus (Akhtar and Shukla, 2007). Fusion at the plasma membrane is a pH-independent process (Spear, 2004).

**Figure 3**: Molecular interactions that facilitate HSV entry.

Initial attachment to cells is mediated by interaction between heparan sulfate proteoglycans (HSPGs) with HSV glycoproteins gC and/or gB as shown in (fig 3). Membrane fusion is required for the penetration of viral nucleocapsid and the tegument into the cytoplasm. Interaction between gD, gH-gL and a gD receptor may be sufficient to bring conformational changes within gD to trigger merging of viral and cellular membranes or lipid mixing. However, a fourth glycoprotein, gB, is also required for complete fusion and content mixing, which basically results in the release of the tegument and the nucleocapsid into the cytoplasm (Akhtar and Shukla, 2007).

**Uncoating the Herpes Simplex Virus Genome**

DNA was ejected as a linear single double helix with ejection occurring at one vertex assumed to be the portal. In the case of trypsin-treated capsids, DNA release was found to show a relationship with cleavage of a small proportion of the portal protein, UL6, suggesting UL6 cleavage may be involved in making the capsid permissive for DNA ejection (Newcomb and Brown., 2007). DNA ejection was observed only when capsids were warmed above 4°C in case of capsids bound to a solid surface. The proportion of capsids releasing their DNA increased as a function of incubation temperature. About all capsids eject their DNA when incubation was at 37°C. The results demonstrate heterogeneity among HSV-1 capsids with respect to their sensitivity to heat-induced DNA ejection (Newcomb and Brown., 2007).

**Figure 4**: Electron micrographs showing grid-associated HSV-1 C capsids in the process of releasing their DNA.
Capsids were warmed to 37°C for 30 min to promote DNA release, and prepared for electron microscopy by shadowing as described in Materials and Methods. White asterisks indicate DNA ends. Note that in each case the DNA originates at a capsid vertex (Newcomb and Brown, 2007).

Cellular receptors of gD
The main receptors gD exploit for cell entry are nectin-1, nectin-2, HVEM, and 3-O HS. HSV-1 and HSV-2 differ in their first choice of gD receptor types. HVEM and nectin-1 are used by both virus types, however, 3-O HS can only be used by HSV-1. Similarly, nectin-2 has not been shown to allow substantial wild-type HSV-1 entry and it may have a greater effect on HSV-2 entry (Spear, 2004).

Transmission
Symptomatic disease caused by HSV-1 is typically limited to cold sores of the mouth and keratitis in the eyes. HSV-2, in contrast, is mostly responsible for genital lesions. However, both viruses are capable of causing lesions on same body sites and both can cause life-threatening diseases in immunocompromised individuals including newborns, patients with human immunodeficiency virus (HIV) or patients undergoing immunosuppressive treatment (Whitley RJ et al, 2001). Transmission among humans involves physical contact and often occurs during kissing (HSV-1) or sexual intercourse (HSV-2). Herpes simplex viruses can affect areas of skin exposed to contact with an infected person. An example of this is herpetic whitlow which is a herpes infection on the fingers. This was a common disease of dental surgeons prior to the routine use of gloves when conducting treatment on patients. Both viruses may also be transmitted vertically during childbirth, although the real risk is very low (Corey and Wald, 2009). The risk of infection is minimal if the mother has no symptoms or exposed blisters during delivery. The risk is considerable when the mother gets the virus for the first time during late pregnancy (Kimberlin, 2007).

It needs to be acknowledged that genital HSV-1 infection has been common for a long time. For example, a Japanese study of women, published in 1976, documented 43% of genital herpes as caused by HSV-1 (Haddow LJ et al, 2006). In 1977, a university health clinic study showed that 37% of women with clinical diagnosis of genital herpes had HSV-1 isolated (Kalinyak et al, 1977). Among people with newly acquired genital herpes in Seattle in the mid to late 1980s, 32% had genital HSV-1 infection (Wald et al, 1994). Still, several well done studies have shown that the relative proportion of genital HSV-1 isolates has increased even more strikingly in the past two decades (Vyse et al, 2000). Two potential explanations that have been put forth include a decrease in HSV-1 acquisition among children, leaving them susceptible to HSV-1 in adolescence, and increase in oral-genital contact, or initiation of oral sex instead of genital-genital sex, among adolescents. Population based studies, although few have looked at secular trends in HSV-1 infection, do not suggest a prominent decrease in HSV-1 seroprevalence (Schillinger et al, 1976). Genital herpes is the most prevalent sexually transmitted disease (STD) in the United States. The most accurate estimates derived from seroprevalence surveys show that 1 person in 5 in the United States is infected with herpes simplex virus (HSV) type 2 (Fleming et al, 1976); these data are widely purported to estimate the impact of genital HSV. However, the estimates ignore the contribution of sexually acquired HSV-1 to the epidemic of genital herpes. Infection with HSV-1 usually causes cold sores (Lafferty et al, 1987).

HSV-1 virus is also unique in that the spread of infection is not dependent on a hematogenous or lymphatic route. Cell-to-cell contact is essential in HSV-1 and HSV-2 infection and revealing the mechanism of cell-to-cell spread is important to fully understand the overall viral infectious process. Viral spread from cell-to-cell depends on the same gD interaction with its receptor(s) as seen when free virions initially infect a host cell (Pertel et al, 2001).

Symptoms
Symptoms of herpes simplex virus infection include watery blisters in the skin or mucous membranes of the mouth, lips or genitals (Ryan et al, 2004). Lesions heal with a scab characteristic of herpetic disease. Sometimes, the viruses cause very mild or atypical symptoms during outbreaks. However, as neurotropic and neuroinvasive viruses, HSV-1 and -2 persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. After the initial or primary infection, some infected people experience sporadic episodes of viral reactivation or outbreaks. In an outbreak, the virus in a nerve cell becomes active and is transported via the neuron's axon to the skin, where virus replication and shedding occur and cause new sores (Ray et al, 2006).
HSV Latency in Murine Nervous System and Oxidative Damage to Neurons

HSV-1 latency was detected predominantly in the trigeminal ganglia, brainstem, olfactory bulbs, and temporal cortex. Latent HSV-1 infection was associated with focal chronic inflammation and consistently detectable evidence of oxidative damage involving primarily neurons. These results indicate that both acute and latent HSV-1 infections in the murine nervous system are associated with oxidative damage (Valyi et al., 2000).

![Figure 5 Neural Damage Associated with HSV Latency.](image)

HSV as a risk factor of Alzheimer’s disease (AD)

One of the causes of Herpes Simplex Encephalopathy (HSE) is a but very severe acute infection of the central nervous system (Whitley and Gnann, 2002). Although it has a very different course from Alzheimer’s disease (AD), it leads to the occurrence of bilateral hippocampal-inner temporal lesions resulting in profound verbal memory loss, characteristic of AD. HSV was proposed as a candidate environmental risk factor for AD on the basis of this hippocampal and temporal tropism of the virus (Pyles, 2001). HSV has been detected in the brain of many AD patients, both by direct detection of virus DNA by polymerase chain reaction (PCR) (Itzhaki, 2004) and by the detection of intrathecal antibodies (Wozniack et al., 2005). The risk of developing AD in subjects positive for HSV DNA presence in the brain who carried apolipoprotein E e4 allele (APOE e4) was several fold that of individuals possessing only one or neither of these factors (Itzhaki et al., 1997). However, this finding remains controversial as it has not been confirmed by another study (Beffert et al., 1998)

<table>
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<tr>
<th>HR</th>
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<th>p-value</th>
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<td>1.07-1.17</td>
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<td>APOE-4 allele</td>
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<tr>
<td>MMS score</td>
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Table 1: Hazard ratio of developing AD according to Anti-HSV IgM status.

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<tr>
<td>MMS score</td>
<td>0.83</td>
<td>0.75-0.92</td>
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Table 2: Hazard ratio of developing AD according to Anti-HSV IgG status.

IgM antibodies are present in the blood for a limited time period (the IgM response tends to drop within about one to two months (Knipe, 2001), but in HSE, IgM persisted at a range from 56 days to 328 days (Forsgren et al., 1989)), IgM positive status showed that HSV reactivation was recent. In addition, a high occurrence of positive IgM results among patients with established HSV infection (Morrow and Friedrich, 2006) has been observed. The risk of developing AD in anti-HSV immunoglobulin G (IgG) positive subjects (indicator of a lifelong infection to HSV) and IgM-positive subjects (indicator of primary infection or reactivation of the virus) (Letenneur et al., 2014).
Available medication

A. Acyclovir (Zovirax®) (gold standard) This was the first of a potent new class of antiviral agents, licensed in the 1982. It quickly replaced vidaribine for use in HSV infection. Available in oral, intravenous and topical (latter form generally not recommended because of minimal effectiveness) formulations.

B. Valacyclovir (Valtrex®) Parent compound (prodrug) of Acyclovir that is well absorbed and rapidly metabolized to the active form. It has the advantage of a 3-5x higher bioavailability, thus delivering higher levels of active drug with less frequent dosing.

C. Famciclovir (Famvir®) Parent compound (prodrug) of penciclovir, a newer nucleoside analog. Requires phosphorylation with viral thymidine kinase to become active (like acyclovir), so crossresistance occurs. Famciclovir inhibits the viral thymidine kinase less effectively than acyclovir, but has higher intracellular levels (good oral bioavailability) and a longer halflife (18-20h) than acyclovir, so it is efficacy is similar despite less frequent dosing intervals.

D. Foscarnet A phosphate analogue, it inhibits viral DNA polymerase at the pyrophosphate binding site and has little effect on cellular polymerases. Foscarnet does not require phosphorylation to become active, so it is effective against the TK-negative strains that are resistant to acyclovir, valacyclovir and famciclovir. It is currently licensed for the treatment of CMV infections, but is also used for therapy of acyclovir-resistant HSV and VZV as well.

Although acyclovir (a class C medication) is not FDA-approved for use in pregnancy, numerous studies have demonstrated its safety during pregnancy, and the CDC maintained acyclovir pregnancy registry has failed to show any increase in fetal anomalies in women who received acyclovir during the first trimester of pregnancy (Prober, 1992). The newer medications, valacyclovir and famciclovir, are both class B medications, but like acyclovir, neither is FDA-approved for use in pregnancy. Women should be carefully inspected for lesions immediately prior to delivery and Cesarian section should be performed for any woman with typical prodromal symptoms or lesions consistent with HSV. Cesarean section, if performed within 4 - 6 hours of membrane rupture, has been shown to reduce the risk of infection in neonates of mothers with primary HSV infection (Prober, 1992). Infants surviving neonatal HSV disease with CNS involvement had improved neurodevelopmental outcomes when they received suppressive therapy with oral acyclovir for 6 months (Kimberlin et al., 2012).

Blocking of Cytokine expression in Trigeminal Ganglia Latently infected Cell by Acyclovir

Cytokine and T-Cell marker gene expression (IFN-gamma, RANTES, TNF-alpha, CD4, and CD8) were compared in the TG of acyclovir-treated mice and untreated controls by RT-PCR. Consistent with previous observations HSV-1 latent infection induced a marked elevation of cytokine gene expression in the TG (Fig. 6) (Halford et al., 1997). While CD4 gene expression was only modestly elevated in the TG, CD8 gene expression was markedly elevated in HSV-1 latently infected TG relative to uninfected TG (Fig. 6) (Halford et al., 1997).

Figure 6: Cytokine transcription in latently infected TG of acyclovir- treated mice.
Antiviral Therapeutic Approach

Capsid assembly is essential and is highly conserved during the proliferation in all HSV-1 strains. It has been found that small interfering RNAs (siRNAs) can be used effectively against HSV-1 capsid protein. It was designed and chemically synthesized for capsid gene and showed their inhibitory effect on the expression of target mRNA and the total intracellular viral genome. It was confirmed by quantitative Real Time PCR, as well as on the replication of HSV-1 via plaque reduction assays and electron microscopy (Jin et al., 2014). siRNA is found to be an effective approach to inhibit the expression of capsid protein encoding genes including UL18, UL19, UL26, UL26.5, UL35 and UL38 in vitro. Interference of capsid proteins VP23 (UL18) and VP5 (UL19) individually or jointly greatly affected the replication of clinically isolated acyclovir-resistant HSV-1 as well as HSV-1/F and HSV-2/333 (Jin et al., 2014).

References