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Update On Emerging Antivirals For The Management Of Herpes Simplex Virus Infections: A Patenting Perspective

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Abstract

Herpes simplex virus (HSV) infections can be treated efficiently by the application of antiviral drugs. The herpes family of viruses is responsible for causing a wide variety of diseases in humans. The standard therapy for the management of such infections includes acyclovir (ACV) and penciclovir (PCV) with their respective prodrugs valaciclovir and famciclovir. Though effective, long term prophylaxis with the current drugs leads to development of drug-resistant viral isolates, particularly in immunocompromised patients. Moreover, some drugs are associated with dose-limiting toxicities which limit their further utility. Therefore, there is a need to develop new antiherpetic compounds with different mechanisms of action which will be safe and effective against emerging drug resistant viral isolates. Significant advances have been made towards the design and development of novel antiviral therapeutics during the last decade. As evident by their excellent antiviral activities, pharmaceutical companies are moving forward with several new compounds into various phases of clinical trials. This review provides an overview of structure and life cycle of HSV, progress in the development of new therapies, update on the advances in emerging therapeutics under clinical development and related recent patents for the treatment of Herpes simplex virus infections.

Keywords

Emerging therapeutics; infections life cycle; Herpes simplex virus; recent patents; treatment

Introduction

Herpes family of viruses is responsible for causing a wide variety of diseases in humans. Seroprevalence of many of these diseases is approaching 100% in the first years of life. This family of viruses has been known to establish lifelong latency causing recurrent episodes of the disease. While a few infections are typically mild and self-limiting, others are recurrent at any stage of life. A distinguishing feature shared by all the herpes viruses is their

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progression to severe form in immunocompromised individuals. Moreover, development of resistance is a major concern in such hosts since the viruses tend to replicate albeit treatment with first line antiviral drugs [1, 2]. Current treatment options for such resistant infections are challenging, because of a few alternate drugs which are potent. Furthermore the second line of therapies is associated with modest potencies, toxicity, and lack of bioavailability [3, 4].

A standard therapy for management of HSV infections includes acyclovir (ACV) and penciclovir (PCV) along with their respective prodrugs valaciclovir and famciclovir. Development of ACV revolutionized the treatment of HSV infections to a great extent. Since its introduction, this drug has become the standard line of therapy for all herpes simplex infections as well as herpes zoster infections. Although effective, delivery is limited by its hydrophilic nature and poor permeability across intestine and corneal tissues, leading to poor oral and ocular bioavailabilities. Moreover, long term prophylaxis with ACV may cause emergence of resistant viral strains. Such development is more prevalent in immunocompromised patients ranging from 3.5-10% [1-2, 5]. Though the exact mechanism of resistance development is not known, it appears to be mediated either by a mutation in thymidine Kinase (TK) (frequent) or mutation in viral DNA polymerase (less common) [5, 6]. Development of new therapies with different mechanism of actions and novel molecular targets should be designed to not only treat resistant infections but also to prevent their recurrence with single or a combination drug therapy [1,7]. Several novel compounds which can suppress viral replication and prevent reactivation in the target population have been identified and are progressing through various phases of clinical trials. Hence, the purpose of this review is to discuss the structure and life cycle of HSV, progress in the development of new therapies, emerging therapeutics under clinical development and finally related recent patents involving the treatment of herpes simplex virus infections.

Structure

Herpes simplex viruses type-1 and 2 (HSV-1 and HSV-2) are human neurotropic viruses belonging to the family *Herpesviridae*, subfamily *Alphaherpesvirinae* and the genus *Simplexvirus*. The term ‘herpes’ originates from Greek and implies to creep or crawl. HSV-1 and HSV-2 are frequent human pathogens causing infections of orofacial, ocular and genital mucosal surfaces. Although HSV-1 and HSV-2 show 70% genetic homology, they differ in terms of few antigenic and biological properties [8, 9]. HSV is a large enveloped DNA virus, approximately 150-200nm diameter. The basic components of a mature viral particle include a core containing linear double-stranded DNA (120 to 230 kbp); an icosadeltahedral capsid consisting of 162 capsomers; an amorphous proteinaceous tegument containing viral proteins and an external trilaminar lipid envelope studded with atleast 12 different glycoproteins Fig. (1) [10-15]. The 152-kbp length HSV genome encodes for 82 different proteins. The genome is composed of two regions, U_L (unique long) and U_S (unique short), covalently linked to each other and flanked by three segments. Each protein encoded by the genome is usually named by its location in U_L or U_S. HSV exhibits three origins of replication (ori); one copy of oriL and two copies of oriS [16-19]. These ori are palindromic sequences and any of the three regions suffice for the replication to initiate [20, 21].

HSV Lytic Cycle

HSV lytic infection involves the following steps: i) viral entry ii) viral replication and iii) viral assembly and egress. Each of these steps is described in detail below.

Viral Entry

HSV can infect a variety of host cells like lymphocytes, epithelial cells, fibroblasts and neurons. Hence, this virus is regarded as 'broad cell tropic'. The mechanism of entry varies depending on the cell type. Viral entry occurs in two different steps Fig. (2). In the first step, viral glycoproteins bind to the host cell receptors and in the second step, the viral envelope either fuses with the plasma membrane or undergoes endocytosis [22-25]. Viral fusion is a pH-independent process while endocytosis is a pH-dependent process. Although the viral envelope exhibits 12 different glycoproteins, only five of them-glycoprotein C (gC), gB, gD, gH and gL are essential for viral infection. gB functions as a homo-oligomer while gH and gL forms a functional hetero-oligomer [26-28]. The binding of gC to heparan sulfate (HS) initiates virus contact with the host cell [29]. Mutational analysis studies have demonstrated that two hydrophobic residues Ile (142) and Phe(146) play a vital role in maintaining this specific affinity [30, 31]. HSV binding can also occur even in the absence of gC but with reduced infectivity with the help of gB [32, 33]. gC and gB can also bind to C-type lectin dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), facilitating dendritic cell infection. Apart from HS and DC-SIGN, gB can bind to paired immunoglobulin-like type 2 receptor-α (PILRα), non-muscle myosin heavy chain IIA (NMMHCIIA) and myelin-associated glycoprotein (MAG) [34-37]. Initial tethering followed by viral fusion is facilitated by binding of gD to second receptors such as herpesvirus entry mediator (HVEM/HveA) (member of the tumor necrosis factor superfamily); nectin-1 (HveC) and nectin -2 (HveB) (cell adhesion molecules of the immunoglobulin superfamily); and 3-O-sulphated HS [38-43]. Finally, viral envelope fuses with host cell membrane facilitated by gB, gD and heterodimeric gH/gL [44-45]. Post fusion, viral nucleocapsid and tegument proteins are released into host cytoplasm, from where the proteins are transported into the nucleus by the dynein-dynactin protein complex. This process is aided by viral capsid protein VP26 and tegument protein U_L34. The capsids are propelled through the negative end of microtubules and released into nucleus through nuclear pore complexes (NPCs) [46-50].

Viral Replication

Post infection into nucleus, host RNA polymerase II initiates viral gene expression [51-53]. HSV genes are expressed in a temporal regulated manner, in three distinct classes: immediate early (IE/a), early (E/β) and late (L/y) genes Fig. (3). The virion protein VP16 in conjunction with cellular octamer DNA binding protein (Oct-1) induces the expression of five IE proteins (ICP0, ICP4, ICP22, ICP27 and ICP47). This protein synthesis usually occurs within 2-4 hours post infection. All the IE proteins except for ICP47 play a vital role in regulating the expression of E/ βgenes. ICP0 promotes transactivation of all the three classes of genes. Further, it acts as an E3 ubiquitin ligase and degrades several cellular proteins [54-57]. ICP4 helps in negatively regulating the expression of αand βgenes by binding to repressor sequence in its own promoter region, thus promoting their shutdown [58-61]. ICP22 plays an important role in regulating the expression of ICP0 while ICP27

regulates early and late gene transcription [62-64]. ICP47 plays a role in immune system evasion by preventing viral peptides from being presented to major histocompatibility complex (MHC) Class I molecules, evading the recognition by cytotoxic T-cells [65]. α proteins help in the transcription of β proteins, which usually proceeds 5-7 hours post infection. β proteins are mainly required for viral DNA replication [including origin-binding protein (U_L9), single-strand DNA-binding protein (SSB/ICP8/U_L29), DNA helicase-primase complex (U_L5/U_L8/U_L52), DNA polymerase (U_L30/U_L42)] and nucleotide metabolism [including thymidine kinase and ribonucleotide reductase] [66-68]. U_L9 binds to ori and U_L29 stimulates helicase-primase and polymerase activities. Further U_L29 negatively regulates the transcription of β proteins post viral DNA replication [69-72]. HSV DNA replicates *via* a theta mechanism initially and continues *via* sigma or rolling-circle mechanism [73]. Post DNA replication L/γ genes are transcribed, which mainly include viral structural components [74].

Viral Assembly and Egress

The late proteins are required for capsid assembly and are transported into nucleus *via* nuclear localization sequences (NLS). A procapsid contains 162 capsomers (150 hexons and 12 pentons) that lie on a capsid floor layer connected by 320 triplexes [75]. The hexons are composed of six molecules of major capsid protein (MCP/VP5) and VP26 joined together [76-78]. All the pentons, except for one (termed portal) show five molecules of VP5. The portal exhibits twelve-fold rotational symmetry with cylindrical shape and is composed of twelve molecules of U_L6 protein [79-81]. The portal facilitates DNA entry into capsid during viral assembly. The triplexes are small compact structures composed of one molecule of VP19 and two molecules of VP23 [75, 82]. These proteins hold the capsomers tight during capsid assembly. Another important capsid component includes the C capsid specific component (CCSC) having one molecule of each U_L17 and U_L25. This rod shaped structure appears at each capsid vertex, supporting the capsid against pressure gradient during DNA packing [83, 84]. The procapsid is assembled inside the nucleus and packaged with viral DNA to form a mature capsid. The re-envelopment model for viral egress proposes that a mature capsid initially fuses with inner nuclear membrane (primary envelopment) to form an enveloped particle and gain fuses with outer nuclear membrane (ONM) (de-envelopment) to release the capsids into cytoplasm. In the cytoplasm, capsids re-envelope (secondary envelopment), by budding into the Golgi compartment and are finally secreted from the infected cells Fig. (4) [85-87].

HSV Latent Cycle

A major hallmark of herpes viruses is their ability to undergo latentiation in the hosts for lifetime. HSV-1 can undergo latency in the trigeminal or cervical ganglia while the major site for HSV-2 is sacral ganglia. Following primary infection at oral or genital mucosal surfaces, the virion finds its entry into the innervating neuronal axon terminals Fig. (5). The capsid containing viral DNA undergoes retrograde transport along the axon via an active process occurring at an estimated rate of 0.5-3 (im/s [88]. Within the neuronal cell body, viral DNA is circularized and loaded with histone proteins to form nucleosomes and remains as extra unintegrated DNA. This arrangement facilitates latency for longer periods [89-90].

During latency, viral transcription is shutdown except for an 8.3kb latency associated transcript (LAT). This polyadenylated primary transcript is unstable and rapidly processed into two major stable introns (1.5kb and 2kb) with extended half-lives [91-94]. Although the exact function of these transcripts is unknown, it has been shown that they act as anti-apoptotic proteins protecting infected neurons [95-96]. Upon reactivation by proper stimuli including immunosupression, intercurrent illness, exposure to UV and/or stress, these viruses re-initiate the lytic cycle and cause various diseases [97].

New Anti-HSV Drugs

AIC316

AIC316, also known as BAY 57-1293 (N-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-N-methyl-2-[4-(2-pyridinyl) phenyl]acetamide) represents a new class of potent inhibitors of HSV which target the virus helicase primase complex Fig. (6). This novel non-nucleosidic inhibitor has shown excellent antiviral activity against HSV-1 and HSV-2, bovine herpesvirus and pseudorabies virus [98-100]. This compound possess the ability to inhibit the helicase primase complex encoded by HSV genes (UL5, UL52 and UL8) which results in the inhibition of viral DNA synthesis. BAY 57-1293 is effective against HSV both *in vitro* (Vero cells) and *in vivo* (mice model) [101]. AIC316 exposure confers resistance due to mutations in UL5 and UL52 genes [102]. A distinct mechanism of action enables it to be active against ACV-resistant HSV isolates suggesting potential utility of this agent in the treatment of resistant infections [103]. In addition, this compound may be used in combination with other drugs to overcome resistance in high-risk immunocompromised individuals [7]. Also, a recent Phase II clinical trial reported that this compound is well tolerated and highly effective.

CMX001

Alkoxyalkyl esters of cidofovir (CDV) such as hexadecy-loxypropyl CDV (CMX001) were developed to improve oral bioavailability of CDV Fig. (6) [104-105]. This compound was effective against clinical isolates of HSV as well as ACV, CDV, GCV and FOS-resistant isolates of HSV. More importantly, this lipid ester analog was more active than CDV itself against HSV, VZV, CMV, EBV, HHV-6, and HHV-8 *in vitro*, suggesting its potential utility for treatment of HSV infections [106-107]. In virus infected cells, CMX001 metabolizes to release CDV which inhibits viral DNA polymerase and in turn arrests viral replication [105]. CMX001 is 300-400 fold more active *in vitro* against HSV replication than ACV or CDV [108]. Furthermore, this compound is also efficacious in BALB/c mice inoculated with HSV-1 or 2 [109]. Also, CMX001 potentiates the efficacy of ACV and this combination synergistically inhibits the replication of HSV both *in vitro* and *in vivo*. Results from this study suggested that CMX001 may be effective in the treatment of ACV-resistant HSV infections and can be administered as an adjunct therapy in individuals with poor clinical response to ACV [110]. Phase I studies reported that this compound is well tolerated with significantly reduced kidney accumulation. CMX001 avoided the nephrotoxicity associated with CDV [109,111].

Valomaciclovir

Valomaciclovir (EPB-348) is the prodrug of H2G (R-9[4-hydroxy-2(hydroxymethyl) butyl] guanine), a guanosine analog with excellent activity against HSV-1, HSV-2, EBV and VZV Fig. (6) [112]. This compound was found to be less active or inactive against HHV-6A, HHV-6B, HHV-7, HHV-8 or HCMV [113]. EPB-348 is phosphorylated by viral TK homologs encoded by susceptible viruses. It forms triphosphate metabolite which acts as a competitive inhibitor of the viral DNA polymerase, thus interfering with viral DNA chain elongation. Although the *in vitro* potency of this compound is greater against VZV than ACV, development of resistance has been reported due to mutations in the TK gene [114,115]. Preclinical studies have shown that this compound is well tolerated. It is currently being evaluated for infectious mononucleosis and shingles in Phase II studies. A recent randomized, double-blinded, placebo-controlled non-inferiority study reported that once-daily valomaciclovir is safe and effective in immunocompetent patients when administered within 72 hr of onset of the acute rash of herpes zoster. However, further studies are warranted to optimize the doses and conditions necessary to develop this agent for acute herpes zoster therapy [116].

Entry and Fusion Peptidic Inhibitors

Entry of HSV-1 into host cells occurs via fusion of the viral envelope with the plasma membrane. However, this mechanism is very complex and the interactions between viral and cellular proteins are poorly understood. The most common approach is to employ specific inhibitors to dissect/hinder the function of gB, gH, and gD in the entry process [117]. Bultmann *et al.* have previously demonstrated that the EB peptide consisting of the RRKK tetramer attached to the 16-amino-acid FGF4 signal peptide, significantly inhibited HSV-1 entry and blocks viral infection during cell-to-cell spreading. Inhibition of HSV-1 entry and plaque formation was found to be dependent on virus concentrations and presence of serum, with 50% inhibitory concentrations typically ranging from 1-10 μM [117].

Also, a library of 138 overlapping peptides homologous to the 773-residue ectodomain of HSV-1 gB were synthesized and screened for the ability of the peptides to inhibit viral infection. Among these, seven 15-mer peptides significantly inhibited HSV-1 infection by more than 50% at a concentration of 100 μM [118]. The EC_{50} values of three peptides (gB94, gB122, and gB131) were below 20 μM. The gB131 peptide was a specific entry inhibitor with an EC_{50} value of approximately 12 μM. The gB122 peptide blocked viral entry (EC_{50}, ≈ 18 μM), protected cells from infection (EC_{50}, ≈ 72 μM), and inactivated virions in solution (EC_{50}, ≈ 138 μM) [118].

HSV membrane fusion appears to be an attractive target for anti-HSV therapy. Galdiero *et al.* have investigated different membranotropic domains of HSV-1 gH envelope glycoprotein to examine the structural basis of HSV membrane fusion and identify novel targets for inhibition. Five fusion peptides (gH220-262, gH381-420, gH493-537, gH493-512 and gH626-644) were synthesized and screened for their effect on inhibition of HSV infectivity [119]. Peptides gH493-537 and gH626-644 were found to inhibit the entry of HSV by 50-60% at 250 mM and 60–70% at 500 mM. However, peptides gH220-262 and gH381-420 did not exhibit antiviral activity up to 500 mM. Interestingly, the shorter peptide

gH493-512, corresponding only to the N-terminus of gH493-537, effectively inhibited HSV entry with approximately 60% inhibition observed at 250 mM and 90% inhibition at 500 mM. Peptides gH626–644 and gH493–512 demonstrated the strongest inhibitory effects of all peptides modeled on HSV-1 fusion glycoproteins till date. Their inhibitory effect could probably be due to their ability to partition into membranes and aggregate within them. Furthermore, the investigators hypothesized that these peptides could sterically hinder their relative domain, either in a pre-fusogenic or in an intermediate conformation. Such conformations could prevent the complete and functional interaction between gH and the membrane to fuse [120, 121].

This peptidic strategy could lead to the identification of functionally important regions of various glycoproteins or other membrane proteins and subsequently aid in the identification of novel inhibitors of HSV-1 entry and membrane fusion. Selected examples of antiviral peptides have been summarized in Table 1.

Other Novel Therapies with Promising Results

Shinji Nakama *et al.* recently reported anti-HSV activity of Bidens pilosa (*B. pilosa*), a tropical weed, in tissue culture cells and a cutaneous mouse HSV-1 infection model. *B. pilosa* extract demonstrated potent virucidal activity, inhibited plaque formation and suppressed virus yield in both Vero and RAW 264.7 cells infected with HSV-1 and HSV-2. This extract blocked the binding of virus to host cells and viral cell penetration. Interestingly, *B. pilosa* extract is effective against TK-deficient and phosphonoacetate-resistant HSV-1 strains. Furthermore, treatment with *B. pilosa* raised the survival rate of HSV-infected mice and stopped the development of skin lesions [122].

Antiviral activity of Bifidobacterium spp. against HSV-1 was recently studied. Efficacy of *B. adolescentis* SPM 0214 was tested using the plaque reduction and yield reduction assays against HSV-1. HSV-1 infected Vero cells treated with high concentration of B. adolescentis SPM 0214 allowed formation of very few plaques. However, at lower concentration of B. adolescentis SPM 0214, many plaques were formed. B. adolescentis SPM 0214 appears to be a potential new therapeutic tool against HSV-1, although the mechanism of the antiviral action of *Bifidobacterium* spp. is unknown [123].

Recently, non-nucleoside anti-HSV compounds have received significant attention. 1,6-Naphthyridines are a class of heterocyclic compounds exhibiting broad spectrum of biological activities such as inhibitor of HIV-1 integrase, HCMV, FGF receptor-1 tyrosine kinase, and enzyme acetyl-cholinesterase. A series of compounds were tested against HSV-1 and 3H-benzo[b]pyrazolo[3,4-h]-1,6-naphthyridines were found to be more effective inhibitors than their corresponding 3H-pyrido[2,3-b]pyrazolo[3,4-h]-1,6-naphthyridines. Among all the active compounds, 6-chloro-3-phenyl-9-fluoro-3H-benzo[b]pyrazolo[3,4-h]-1,6-naphthyridine reduced the virus yield by 91% at 50 μM and exhibited a low cyto-toxicity (CC_{50}=600 μM) [124].

Notoginsenoside ST-4 was investigated for its antiviral activity against HSV-1 and 2 *in vitro.* EC_{50} values determined by plaque reduction assay were 16.47 ± 0.67 and 19.44 ± 1.16 μM for HSV-1 and HSV-2, respectively. Antiviral activity of notoginsenoside ST-4 is

presumably due to penetration inhibition effects, which was further confirmed by fluorescence microscopy which demonstrated that notoginsenoside ST-4 blocked the viral penetration [125].

Ren *et al.* recently reported *in vitro* antiviral activity of total alkaloids extracted from Tripterygium hypoglaucum against HSV-1. A crude total alkaloids extract prepared from the roots of T. hypoglaucum was examined against HSV-1 infected Vero cells by plaque reduction assays. The alkaloids significantly reduced plaque formation at concentrations of 6.25-12.5 g/mL, the plaque reduction ratio reached 55-75% which was about 35% higher than that of ACV at the same concentration [126]. Monophosphorylated ACV prodrug derivatives (ACV ProTides) were developed and tested for their ability to suppress both HIV-1 and HSV-2. ACV ProTides exhibited efficacy in the sub-micromolar range in *ex vivo* lymphoid and cervicovaginal human tissues and EC_{50} between 3-12 μM in $CD4^+$ T cells. Also, ACV ProTides retained activity against ACV-resistant HSV-2 viral strains [127].

Palem *et al.* recently investigated the putative inhibitory effect of manzamine A on HSV-1 infection. This compound effectively inhibited viral replication and infection on SIRC, a corneal cell line at 1 μM; while ACV showed a comparable activity at 50 μM. Plaque reduction assays demonstrated that manzamine A reduced the release of infectious virus by approximately 10-fold. The investigators suggested that manzamines could reduce potent viral infections in corneal cells and prevent HSV-1 induced keratitis [128].

Recent Patents For Treatment Of Herpes Simplex Virus Infections

A US patent by Mitra discloses dipeptide and tripeptide ester derivatives of ACV and its analogs for the treatment of herpes virus infections of the eye [129]. Transporter targeted prodrug approach has gained significant attention in drug delivery [130, 131]. This patent provides methods for the synthesis of di- and tri-peptide mono- and di-esters of ACV and GCV and their derivatives. The esters described herein were reported to possess sufficient hydrophilic-lipophilic balance to be formulated into pharmacologically active compositions, such as aqueous eye drops. The compounds described in this patent were designed to target oligopeptide transporters for delivery into intraocular tissues. These prodrugs effectively reach the anterior segment and/or the vitreo-retinal region when administered either topically or systemically. Interestingly, these conjugates exhibited excellent antiviral activity against HSV as well as cytomegaloviruses. Although effective, dipeptide prodrugs are rapidly metabolized to parent drug resulting in limited bioavailability. For effective absorption at the blood ocular barrier (BOB), prolonged residence of intact prodrug is required. To overcome such limitation, specific stereoisomers of di-peptidyl esters of ACV and mono- and di-esters of GCV were designed.

These prodrugs were fairly stable with enhanced enzymatic stability and demonstrated efficient transporter translocation across ocular barriers. This patent disclosed prodrugs with excellent solution stability. The patents also disclose solubility facilitating formulation of stable eye drops [132].

Another recent patent by Mitra and Samanta discloses a novel prodrug strategy which is more lipophilic and at the same time site specific thereby providing a dual approach to

improve cellular absorption of ACV [133]. This patent describes conjugated compounds comprising a therapeutic or diagnostic agent linked to a substrate for a cell membrane transporter or receptor through a lipophilic linker. The inventors employed a lipid raft that is conjugated to ACV to impart lipophilicity and a targeting moiety (biotin) which can be recognized by a specific transporter/receptor (SMVT) on the other terminal of the lipid raft. It is well known that lipophilic prodrugs readily diffuse across the cell membrane by facilitated diffusion whereas transporter/receptor targeted prodrugs translocate compounds across the cell membrane via active transport via molecular recognition. Only a marginal improvement in cellular uptake was evident from each of the two approaches. However, this patent disclosed a novel approach which combines both lipid and transporter/receptor targeted delivery to generate synergistic effect. Compared to ACV, the uptake of targeted lipid prodrugs (B-R-ACV and B-12HS-ACV) increased by 10 and 8.3 times respectively, whereas the uptake of B-ACV, R-ACV and 12HS-ACV was higher by 3.5, 1.4 and 1.3 times respectively in Caco-2 cells. The targeted lipid prodrugs B-R-ACV and B-12HSACV exhibited much higher cellular accumulation than B-ACV, R-ACV and 12HS-ACV. Both the targeted lipid prodrugs B-R-ACV and B-12HS-ACV demonstrated higher affinity towards SMVT than B-ACV. Such enhanced affinity may be attributed to the presence of lipid raft which facilitates enhanced interaction of prodrug with membrane transporters/ receptors thereby assisting docking of the targeted ligand into the binding domain of transporter/receptor protein. The net effect observed was rapid translocation of the cargo across cell membrane. This novel prodrug design may also allow for enhanced plasma membrane uptake of hydrophilic therapeutic agents such as genes, siRNA, nucleosides, nucleotides, oligonucleotides or antisense oligonucleotides, peptides and proteins [134].

A recent US patent application by Hsu discusses compositions and methods for treating HSV infections [135]. This application reports therapeutic utility of green tea polyphenol compositions including (−)- epigallocatechin-3-gallate as well as green tea polyphenols with one on more ester-linked fatty acids. The green tea polyphenols of the present inventions include, but not limited to (-)-epigallocatechin-3-gallate, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin-3-gallate. Furthermore, proanthocyanidins, their enantiomers, isomers, pharmaceutically acceptable salts, and prodrugs of green tea polyphenols are included. Green tea is made from the plant named *“Camellia sinensis”* plant. It is rich in catechin polyphenols, in particular, epigallocatechin gallate, successfully inhibited HSV infection in a concentration dependent manner. Since epigallocatechin gallate is highly unstable and rapidly oxidized, it loses its antiviral abilities long before it is applied. Therefore, lipophilic tea polyphenols having an ester-linked C_1 to C_{30} hydrocarbon chain were prepared by catalytic esterification of green tea polyphenols to enhance stability and permeability of the green tea polyphenols. Antiviral screening studies indicated that epigallocatechin gallate inhibits HSV-1 by 95% and modified ester of epigallocatechin gallate inhibits HSV-1 by 99.46% [136, 137]. These compounds act by interfering with the virion envelope glycoproteins or by interfering with viral compounds for viral adsorption and cell entry. Though the modified ester of epigallocatechin gallate has demonstrated greater potency compared to epigallocatechin gallate against HSV-1 infections *in vitro*, further studies need to be conducted in order to completely delineate the exact mechanism of

action in humans. Nevertheless, natural products could improve the lives of many patients and offer a better health.

A US patent by Boyd discusses nucleoside analogs in combination for the treatment of herpes simplex infections [138]. This application discloses a pharmaceutical product comprising a nucleoside analogue active against HSV such as ACV/valaciclovir or peniclovir/famciclovir, and an im-munosuppressant such as cyclosporine A. These combined preparations are useful for simultaneous, separate or sequential therapy in the treatment and/or prevention of HSV infections. This patent reports that coadministration of penci-clovir/famciclovir with an immunosuppressant is particularly useful for the treatment of severe and/or prolonged HSV infections [139].

A recent US patent application by Whitten discusses therapeutic compositions for treating HSV infections. This therapeutic composition comprises a mixture of ACV, penci-clovir, and 2-Deoxy-D-Glucose. The inventor claims that these therapeutic compositions will meet a long felt need in the art of providing a treatment for lesions that result from HSV that significantly reduce the duration of a cold sore when vesicles have already emerged and a treatment that will prevent the outbreak of a lesion and vesicle formation when applied in the prodromal stage [140].

Burrell recently disclosed a therapeutic composition of glycyrrhizic acid, slippery elm, zinc oxide, allantoin, lysine monohydrochloride, L-carnitine, lipoic acid, salicylic Acid, citric Acid, vitamin E acetate, wheat germ oil, and shea butter for the treatment of HSV-1, HSV-2, canker sores, shingles, and other epidermal and oral ailments [141]. The present invention is directed to generate synergistic effects by combination of compounds designed to inhibit/ treat the virus itself as well as to alleviate the symptoms and triggers associated with HSV-1, HSV-2 and herpes zoster. The combination is prepared by mixing the ingredients until they are evenly distributed throughout the solution. The preferred composition of this combination include 1% Glycyrhizic acid, 0.05% Zinc Oxide, USP, 2% allantoin, 2% slippery elm, 2% Lysine (L) Monohydrochloride, USP, 2% Carnitine (L), 1% Lipoic Acid, DL-alpha (DL-thiotic acid, 0.025% Salicylic acid, USP crystalline poder, 0.5% citric acid, USP Hydrous powder, 16.25% wheat germ oil (cold pressed), 0.5% stevioside (90% extract), 4% Raspberry, 68.675% Shea butter.

A recent patent by Bornmann and Kalman disclosed the use of kinase inhibitors to inhibit kinases involved in pathogen-host cell interactions that are associated with or cause pathogenic infections including HSV [142]. This invention is directed towards development and identification of compounds that modulate/alter the way in which diverse viral pathogens interact with the host, so as to block or limit disease caused by these viruses and allow the host immune system to clear the viral pathogens. Several kinase inhibitors have been screened against herpes viruses. Some of the compounds tested such as ApCK103, Apck-43, LG2-55, and LG2-71 proved effective in treating infections caused by herpes virus. Also, the inventors claim that one or more of the kinase inhibitors can be selected in combination with antiviral agents such as CDV. Such combinations would lower the dosage requirements and thereby minimize toxic effects of this nucleoside analogue.

Novel hydroxybenzoic acid ester and analogues have been patented by Shenghua Guangzhou Pharmaceuticals [143]. Several compounds including 4-hydroxybenzoic acid ester; 2, 4-dihydroxybenzoic acid ester; 3, 4- dihydroxybenzoic acid ester; 2, 3, 4-trihydroxybenzoic acid ester; 3, 4, 5-trihydroxybenzoic acid ester or 3, 4, 6-trihydroxybenzoic acid ester have been synthesized. *In vitro* antiviral studies demonstrated that hydroxybenzoic acid esters and analogues are more potent than hydroxybenzoic acid and analogues. For example, propyl gallate was more stable than gallic acid, especially when it is in weak alkaline condition of the plasma and tissues (pH 7.4) or intestinal alkaline condition (pH 8.6). The antiviral activity of a hydroxybenzoic acid ester (propyl gallate) is higher than its corresponding acid (gallic acid).

A recent US patent application by Tkachuk describes the utility of RNA molecules because of their ability to adopt a wide variety of conformations thereby performing a range of cellular functions [144]. A new antiviral compound i.e, modified highly purified yeast RNA displayed pronounced multiple antiviral activities in a wide range of concentrations. This modified yeast RNA is capable of inhibiting the viral replication from several viruses including ortho-myxoviridae, paramyxovirus, hepatitis, herpesviridae families, enterovirus, adenovirus, influenza viruses, hepatitis C virus, genital herpes, human immunodeficiency virus and Coxsackie B virus. The antiherpetic activity of RNA-M was studied in a model of murine herpetic meningoencephalitis caused by HSV-1, as well as in a model of genital herpetic infection in guinea pigs infected by HSV-2. Results from this study demonstrated that this RNA-based drug has potent antiherpetic action and is effective in the treatment of herpetic diseases, especially genitals herpes. Since viral infections are frequently associated with several strains of viruses, antiviral agents with multiple mechanisms of action are warranted to ameliorate the rapidly escalating resistance to antiviral agents.

Cantin *et al*. in a recent US patent application discusses the use of compositions comprising pooled immunoglobulin for the treatment and/or prophylaxis of herpes infection and its associated disorders [145]. Human Immunoglobulin (IVIG) consisting of the polyclonal IgG fractions from thousands of donors was pooled. Consequently, poly reactive natural antibodies and antibodies specific for allotypic antigens must also be represented in the pool to suppress antibody-mediated autoimmune disease, chronic or acute inflammatory states in which damage is caused by activated leukocytes. The active component of IVIG includes sialylated IgG species which is prepared from human plasma or serum. In particular, the immunoglobulin comprised of sialylated IgG domains, is a monomeric Fc domain which is preferred for the treatment and prophylaxis of herpes infections including HSV-1 infection and its associated encephalitis and herpes stromal keratitis. The inhibitory activity of IVIG has been evaluated by studying HSV infection (B6- and 129-Rag/E strains) and studying suppression of inflammation and virus replication.

Current & Future Developments

The herpes family of viruses is responsible for causing a wide variety of infections in humans. With escalating sero-prevalence rates, treatment of herpes infections still remains a challenging task, regardless of the introduction of several therapeutics agents with excellent intrinsic antiviral activities. Current treatment options for the treatment of HSV infections

are reasonably safe and fairly effective. However, long term therapy with these agents is often associated with toxicities which limit their utility and ultimate druggability. Emergence of resistance and development of drug-resistant viral isolates have been observed especially in immunocom-promised patients, who are treated with antiviral drugs for longer periods of time. Hence, there is an apparent need to develop newer therapeutics with a novel mechanism of action, providing superior efficacy and diminished potential for adverse effects. All the new anti-herpetic compounds summarized in this review appear to be promising and have the potential to significantly enhance therapies for HSV infections. Development of novel compounds with enhanced efficacy and less potential for toxicity is obviously essential. Since many new compounds are currently in clinical development, it would be better if those compounds will be screened not only for their antiviral potency but also for their potential use in combination with other antivirals as multidrug regimens. Hopefully, some of these new molecules which are being developed would lead to block buster drugs in the near future and management of herpes infections would be less complicated.

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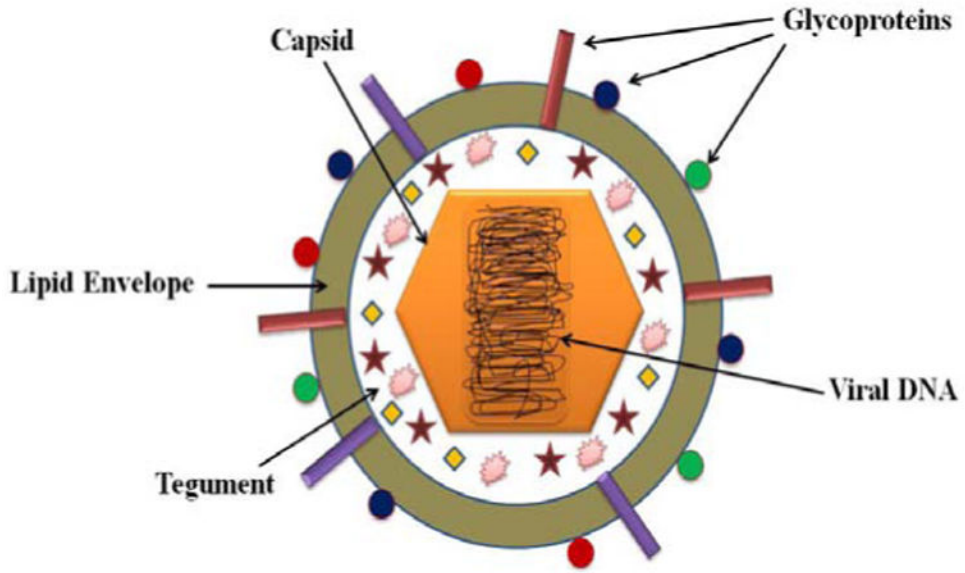

Fig. 1.
Structure of HSV.

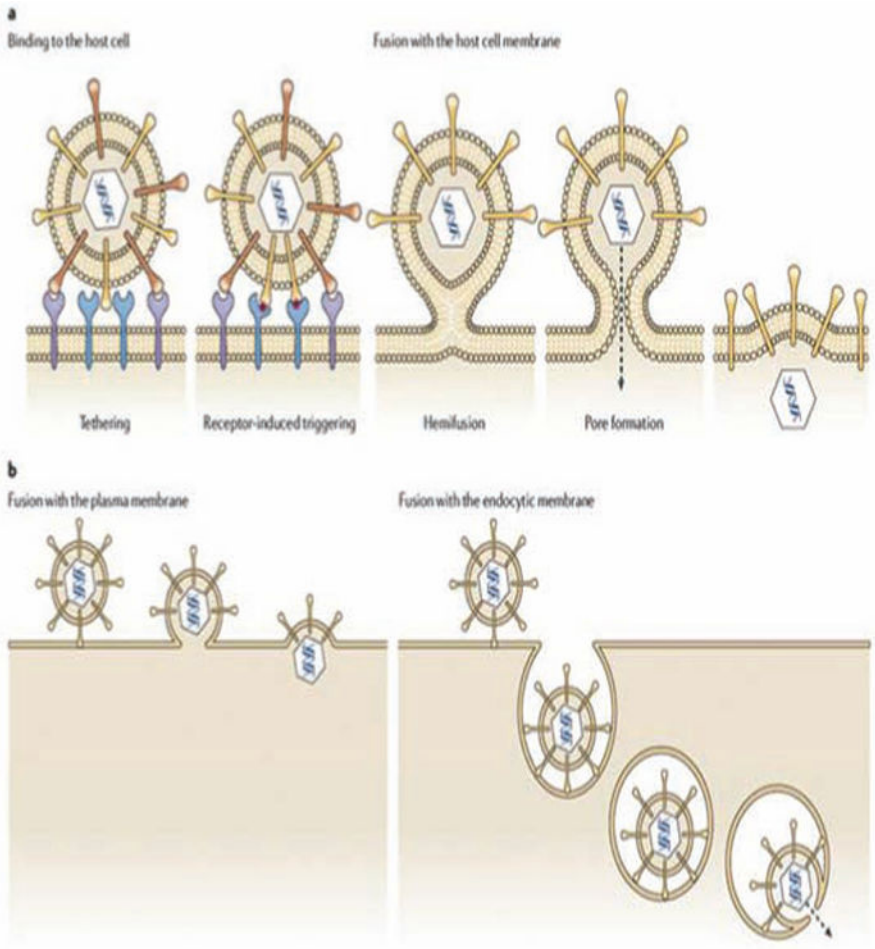

Fig. 2.
Mechanism of HSV entry into the host cells. Reproduced with permission from [25].

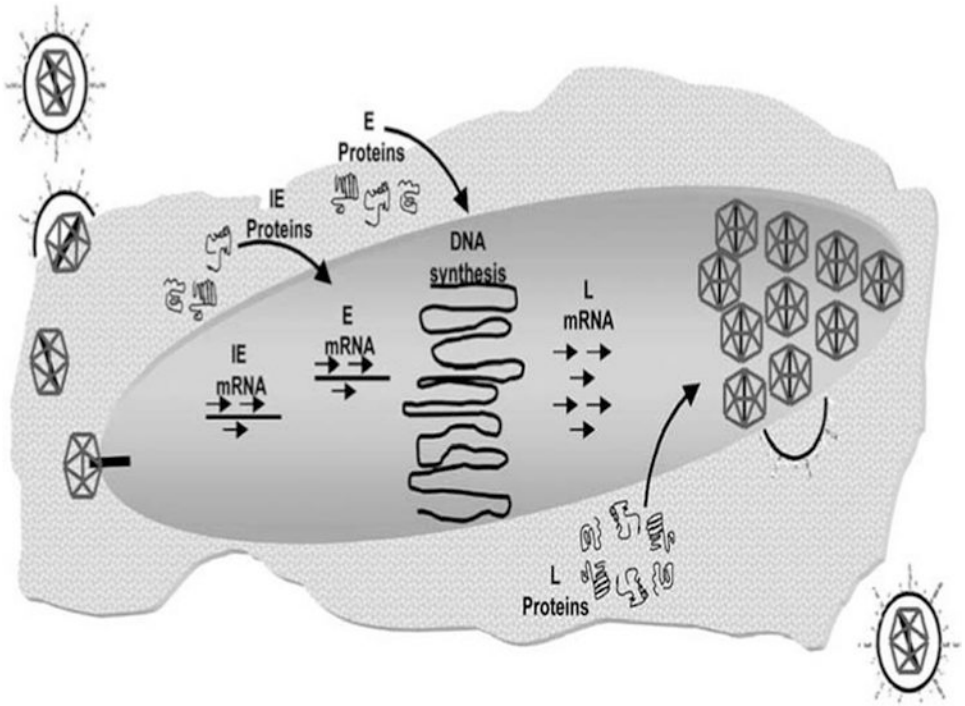

Fig. 3.
HSV viral replication. Reproduced with permission from [53].

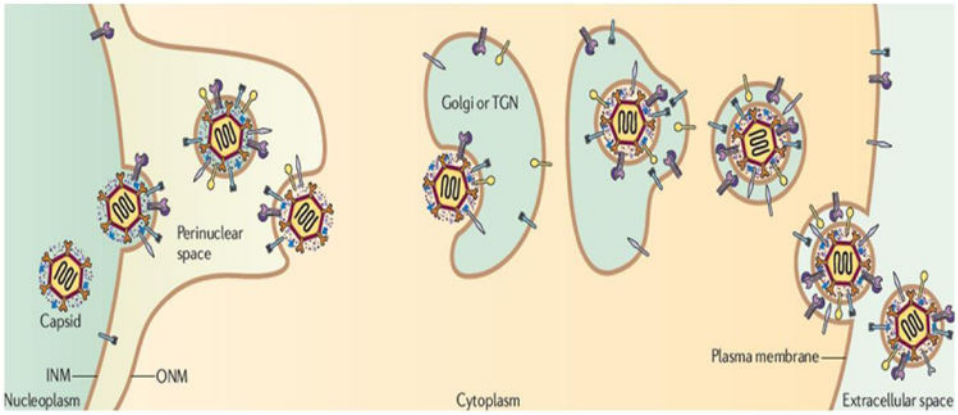

Fig. 4.
HSV viral egress. Reproduced with permission from [85].

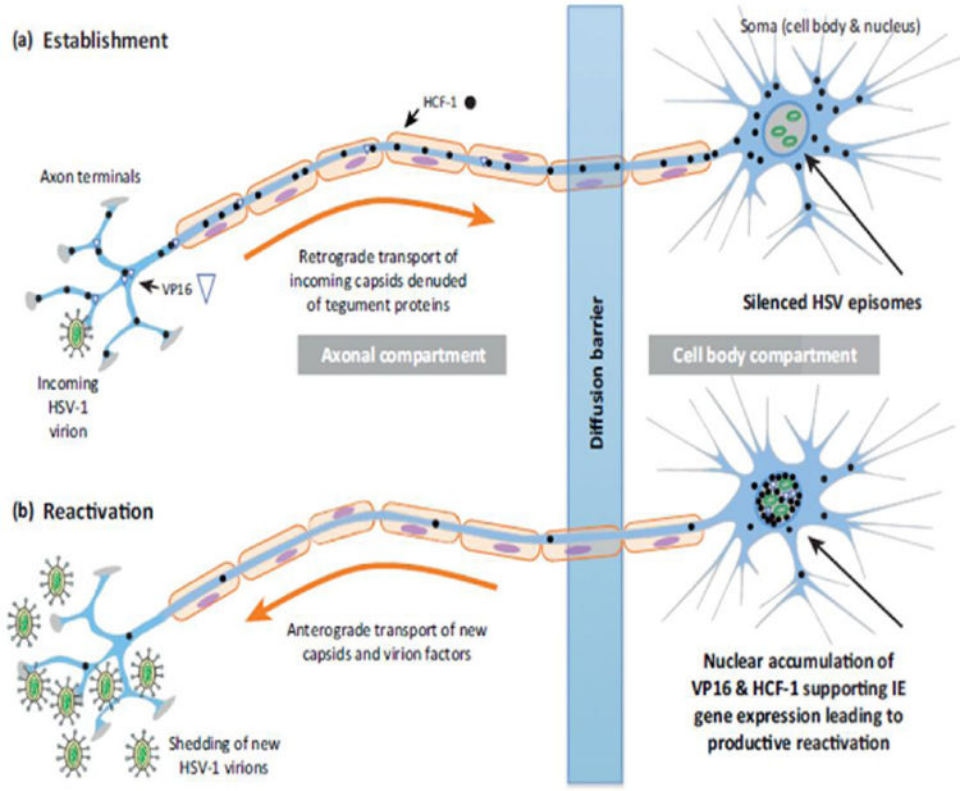

Fig. 5.
HSV latent cycle. Reproduced with permission from [89].

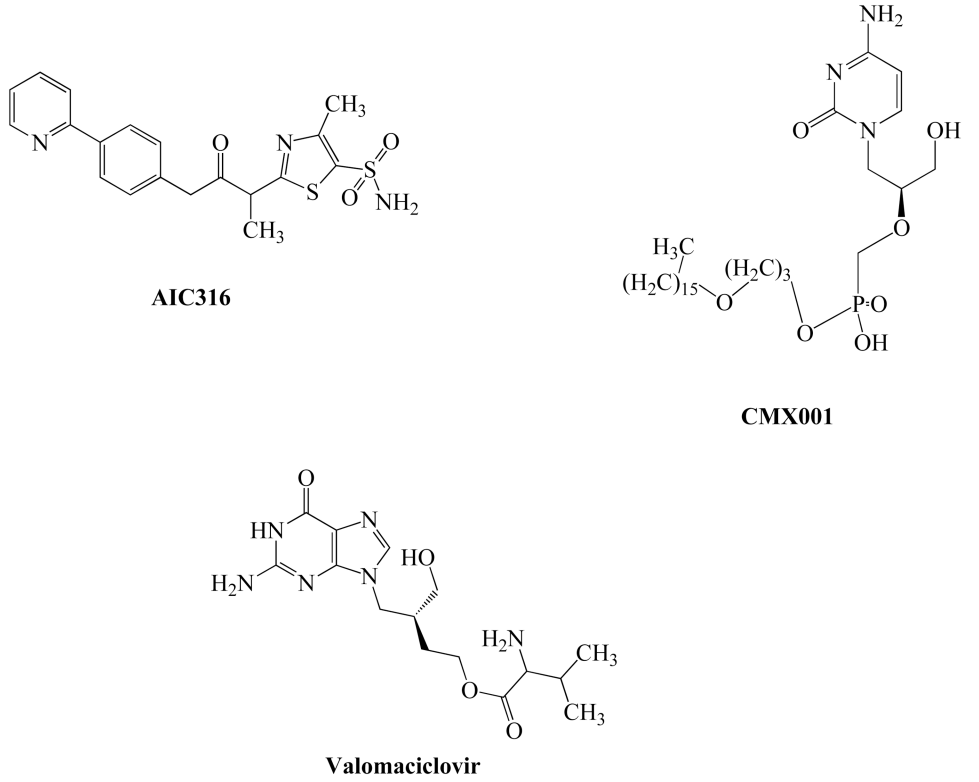

Fig. 6.
Inhibitors of HSV virus replication currently in clinical development.

Table 1
Selected examples of antiviral peptides effective towards HSV

Peptide	Sequence	Reference
EB	RRKKAAVALLPAVLLALLAP	[117]
gB94	KTTSSIEFARLQFTY	[118]
gB122	GHRRYFTFGGGYVYF	
gB131	HEVVPLEVYTRHEIK	
gH220–262	TWLATRGLLRSPGRYVYFSPSASTWPVGIWTTGELVLGCDAAL	[119]
gH381–420	RLTGLLATSGFAFVNAAHANGAVCLSDLLGFLAHSRALAG	
gH493–537	AAHLIDALYAEFLGGRVLTTPVVHRALFYASAVLRQPFLAGVPSA	
gH493–512	AAHLIDALYAEFLGGRVLTT	
gH626–644	GLASTLTRWAHYNALIRAF	
Defensin HNP-2	CYCRIPACIAGERRYGTCIYQGRLWAFCC	[146]
Brevinin-1	FLPVLAGIAAKVVPALFCKITKKC	
Tachyplesin	KWCFRVCYRGICYRRCR	[147]
Magainin-2	GIGKFLHSAKKFGKAFVGEIMNS	[148]
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	
Dermaseptin S4	ALWMTLLKKVLKAAAKAALNAVLVGANA	[149]
Bovine Lactoferricin	FKCRRWQWRMKKLGAPSITCVRRAF	[150]
Indolicidin	ILPWKWPWWPWRR	[151]