RESEARCH ARTICLE

Cytomegalovirus Replication Steps and the Actions of Antiviral Drugs

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Abstract: Human cytomegalovirus (HCMV) is a beta herpes virus that infects an active infection in the fetus and immunosuppressive patients. The virus encodes many proteins that work together with cellular fac-tors to achieve virus replication. In addition to vaccines, antiviral drugs can be deployed to manipulate how the virus replicates and minimize its pathogenicity. The five antiviral drugs approved by the Food and Drug Administration (FDA) have shown adverse reactions and the antiviral drug resistance were reported. Hence, this warrants the need for urgent development of a novel antiviral drug. Detailed understanding of the virus replication steps and how cellular signals interact with these steps will be key for pharmacological developments of for anti HCMV drugs. This review summarized all the drugs that target the virus proteins and cell signals that mediate CMV replication.

Keywords: Cytomegalovirus, immunosuppressive patients, Congenital Infection, Ganciclovir, Herpesvirus, Fomivirs.

1. INTRODUCTION

Cytomegalovirus (CMV) is classified as herpes virus [1]. It is an enveloped virus with dsDNA genome [2]. Upon entry, the virus establishes an active infection or initiate a state of latency [3]. Approximately, 36-90% of the population worldwide are seropositive to human cytomegalovirus (HCMV) [4], depending on their socioeconomic status [4-5]. In most cases, the immune system controls the virus replication which results in asymptomatic infection [6]. Nevertheless, symptomatic infection was linked to immunosuppressive individuals and newborns with CMV congenital infection [7]. For in utero infection, 10–15% of the infected fetus developed symptoms [8] such as hepatosplenomegaly, thrombocytopenia, microcephaly, psychomotor retardation, premature delivery and hearing loss that also developed in asymptomatically infected newborns. Both ganciclovir (GCV) and Val-ganciclovir (VGCV) are approved to treat CMV congenital infection [9] with fetal toxicities [10].

In human immunodeficiency virus (HIV) patients, HCMV infection is associated with retinitis [11]. In immunosuppressive patients, tissue-invasive HCMV disease is characterized by fever, anorexia, malaise, pneumonia, hepatitis, retinitis, ischemic colitis, transverse myelitis, atherosclerosis and gastro-intestinal ulcerations [12].

2. TREATMENT OF CYTOMEGALOVIRUS INFECTIONS

Five anti-HCMV drugs were approved to treat CMV [10b]. These drugs are used either systemically (GCV, cidovir (CDV), foscarnet (FOS) or locally (fomivirsen) [13]. The use of anti-CMV increases the survival of immunosuppressive patients [14]. In general, three antiviral drug strategies were applied for HCMV treatments: prophylactic therapy, pre-emptive therapy, and treatment of an established disease [12c, 15]. Using adaptive immunotherapy with CMV-specific T cell [16] successfully treat CMV infections which involve GCV resistant strains [17].

3. ANTIVIRAL DRUGS TOWARD HCMV

In general, antiviral drugs can be grouped into two based on their objectives. The first group targets the viral-specific structural proteins with less toxicity. The second group targets the cell signals and factors that mediate the virus replication. This group is associated with limited toxicity, has a high
barrier of resistance and inhibit a broad spectrum of viruses [18]. Since CMVs are species-specific, HCMV can be only studied in cell lines, whereas in vivo studies were done using CMV animal models [19]. In the following discussion, both in vitro and in vivo drugs against CMV in will be explained according to their interactions with virus replication steps.

4. REPLICATION STEPS OF CMV

CMV replication takes place in both nucleus and cytoplasm [20].

4.1. Virus Attachment

4.1.1. Cellular Receptors Mediate Virus Attachment

Virus attachment is mediated by its envelope which contains glycoproteins (gps) (gpB, gpH, gpL, gpO, gpN, gpM). These glycoproteins perform three complexes (gCI) (gpB homodimer), gCII (gpM/gpN), gCIII (gpH/gpL/gpO) and gH/gL/pUL (128, 130, 131A) [21]. Glycoproteins mediate the virus attachment to many cellular receptors such as Heparin sulphate proteoglycan (HSPGSR), cellular epidermal growth factors receptor (EGFR), integrin, (β1, β2 integrins) and platelet-derived growth factor receptor-α (PDGFRα) [22]. Glycoprotein B (gpB) is an essential protein which mediates CMV attachment and signaling transduction [21a, 23]. The protein has a structure similar to ADAMs integrin loop [24] and can be targeted to block CMV entry [25]. GpB forms a fusion core with gH/gL [21d, 26]. Targeting gH/gL complex by MSL-109 antibodies blocks CMV replication [27] (Fig. 1a), but high dosage is needed to achieve this therapeutic effect [28]. In addition, recombinant antibodies have been developed [29] to block the virus binding site (the gH/gL/UL128-131A complex)[30] but not gH/gL/gO. Valpromide (VPD) (homolog of Valproate, VPA) inhibits CMV infection by interfering with virus attachment [31].

4.1.1.1. Heparan Sulphate Proteoglycan Receptors (HSPGSR) and its Inhibitors

HSPGSRS are glycoproteins attached to heparan sulfate (HS) chains, a type of glycosaminoglycan (GAG) [32]. The receptor binding depends on the HS chains. Many polysaccharide compounds (sulfated polysaccharides; dextran sulfate, bacterial sulfated glycosaminoglycan, and chemically modified heparin) can bind to this receptor and block its function [33]. Binding to this receptor can disrupt the electrostatic interactions between sulfated/carboxyl groups of the HS chains toward cell surface (-ve charged) and the HCMV glycoproteins (+ve charged). This inhibits virus attachment (Fig. 1a) [34] and modifies membrane properties or induction of cellular signaling [35] involved in the virus replication.

Lactoferrin (LF) is a glycoprotein which works as an active carrier protein. It is excreted by the mammary glands and the mucosal epithelial cells [36]. The compound has antibacterial [37], antifungal [38] and antiviral activities (anti-HCMV) [39]. The positive charge N-terminal portions have an affinity to the negative amino acids group in the receptors [40]. This drug is used in combination with CMV approved drugs (Fig. 1a) [41].

In addition, both SB105 and SB105-A10 peptides have anti-CMV activities. They are classified as peptide-derivatized dendrimers (Fig. 1a) [42]. Their unique structure (lysine peptidyl branching core link with four functional peptide units) gives them a large space for interaction. The mechanism of reaction depends on the interaction between HCMV and the negative charge sulfate and carboxyl groups of HS.

4.1.1.2. Epithelial Growth Factor Receptor (EGFR) and its Inhibitors

EGFR is an intra-membrane protein which contains two domains: the extracellular domain for substrate binding and an
Cytomegalovirus Replication Steps and the Actions of Antiviral Drugs

Anti-Infective Agents, 2018, Vol. 16, No. 2

intracellular domain that initiate signals pathways. Binding of molecules to this receptor leads to autophosphorylation of EGFR [43] that mediate many cellular signals such as PIP2 to diacylglycerol (DG) and inositol triphosphate (IP3) by PLCc [43-44]. DG activates protein kinase C (PKC) whereas IP3 mediates calcium influx and stimulate actin fiber rearrangement [45]. Targeting this receptor by AG1478 prevent virus binding, entry, and replication (Fig. 1b) [46].

4.1.1.3. Platelets Derived Growth Factor Receptor (PDGFR)

PDGFR mediates HCMV attachment and induces signaling cascades in HCMV infected cell [48]. Imatinib was reported as anti-HCMV by its binding to the PDGFR (Fig. 1b) [47a, 48].

4.1.1.4. Integrin avb3 and its Inhibitors

Integrins are expressed as noncovalent heterodimer proteins with mediates many functions such as cellular adhesion and interaction with extracellular matrix (ECM) as well as immune cell circulation and functions [49]. HCMV binding to this receptor leads to phosphorylation of the b3 integrin subunit [45a], changes of Ca2 homeostasis [50]. It also activates phospholipases C and A2, arachidonic acid metabolites [51], mitogen-activated protein kinase (MAPK) [52], phosphatidyl-

4.1.1.5. Cell Signals Induced by Virus Attachment and Their Inhibitors

Attachment of HCMV to the cell in multiple of cellular signals involving cAMP and cGMP, calcium influx, cellular transcription factors NF-xB and Sp1, cellular kinases Akt and p70S6K in a PI3-K-dependent biphasic manner [22c, 56]. The virus regulates the expression cell cycle regulatory proteins such as cyclins A, B, D and E, and the tumor suppressor proteins p53 and Rb through its IE1 and IE2. This induces the macromolecules synthesis, moves the cell from (G0, G1) to late (G1/G1/M) [57]. This in turn stops the expression of proteins that mediates apoptosis and destroy the virally infected cell [58].

Leflunomide is rheumatoid arthritis treatment drug [59] which has an immunosuppressive activity [60]. The drug showed anti-HCMV activities involving CMV-resistant strains [61]. It affects the EGFR phosphorylation [60b], mediates the signals of tumor necrosis factor (TNF) and NF-xB activity [62]. The drug also interferes with CMV virion assembly (Fig. 2) [63]. A771726 is the active form of Leflunomide that interacts with pyrimidines biosynthesis through inhibition of dihydroorotate dehydrogenase (DHODH) [64]. The drug was not recommended for patients with MTX pneumonitis [65].

Another cell signals inhibitors are artesunate (an anti-malaria agent) [66]. It inhibits CMV replication with good bioavailability and safety. It interacts with transactivation activities of both NF-xB and Sp1[67]. The drug inhibits two effectors of the PI3K pathway (Akt, p70S6K). Sorafenib/Nexavar® is an anti-cancer drug with anti-HCMV activity [68]. Its action is linked to MIEP activity and MIE gene
expression in vitro [68b]. It affects the cellular kinases C and B Raf, mitogen-activated kinases (MEK), ERK/MAPK, and NF-κB activation pathway [69]. Another important compound is Geldanamycin (Hsp90 inhibitor), which showed anti HCMV [70] activity via the inactivation of Akt and PI3K and their mediated signals. Moreover, MG132 [71] blocks NF-κB by an accumulation of IκB (NF-κB inhibitor) which subsequently affect MIEP transcription.

Sirolimus and Everolimus reduce HCMV load in organ transplant recipients by working as rapamycin (mTOR) inhibitors [72]. The drug has been utilized to treat GCV-resistant HCMV strains [73]. Furthermore, PD 98059 inhibitors can inhibit the MEK/ERK pathway signals which very important for the virus replication [74]. COX-2 is a cellular enzyme essential for Prostaglandin E2 production [75] and accumulated in HCMV infected cells. Applying Non-cytotoxic COX-2 inhibitors block the induction of COX-2, and subsequently the prostaflaglin E2 production. It interacts with multiple HCMV replication steps, especially the expression of viral genome replication and immediate early (IE) 2, but not IE1.

4.2. Virus Entry Into Cells
4.2.1. Receptors Mediate Virus Entry and their Inhibitors

Cellular integrins (α2β1, α6β1, and αVβ3) work as attachment or post-attachment receptors for CMV [25]. In addition to mediating many cellular activities [49, 76], application of soluble integrin successfully blocks gpB attachment [22b, 25]. The motif with sequence Arg-Gly-Asp (RGD) mediates virus attachment through unique ECM protein family known as ADAMs [77]. A protein named RX5-7DLXXF/L is a member of this group that is well-identify to mediate HCMV entry [78].

HCMV entry link with its attachment. Binding of virions to cell receptor HSPGRs [79] through gpB [79b] and the gM/gN complex [80] resulted in a stable interaction. Subsequently, this interaction induces signal transduction mediated by post-binding to EGFR [46b, 81]. The binding of gpB or gpH to the integrins receptors induce rearrangement of actin cytoskeleton [25], phosphorylation of cytoplasmic domains of β1 and β3 integrin [25], focal adhesion kinase (FAK) [25], and Src [25, 82].

Polyphenols (flavonoids and resveratrol) are natural anti-inflammatory compounds available in many plants [83]. They have anti-inflammatory, antiproliferative, vasculo-protective, antimicrobial, and antiviral activities [84]. Baicalein (5, 6, 7-trihydroxyflavone) is one of the flavonoids that has been used as a treatment of the hepatic disorder, allergy, stroke induced by thrombin, and inflammation [85]. It blocks HCMV entry through EGFR tyrosine kinase activation [46], phosphatidylinositol 3-kinase (PI3K), Akt, and mitogen-activated protein kinases (Fig. 2). Moreover, Resveratrol (3, 5, 4’-trihydroxystilbene) [86] was used previously to treat the progression of inflammation, cardiovascular diseases, cancer [87] and viral infection [88]. In HCMV infection, resveratrol blocks EGFR phosphorylation and consequently affects all cellular signals mediated by HCMV IE genes expression (Fig. 2) [89]. The side effect of the drug is infant leukemia (MLL gene) [90], so it is not advisable to be used in pregnant patients.

CFI02 is another compound that interferes with [91] gpB fusion process and HCMV virus entry. The drug trial has stopped because of instability in vivo studies. The drug interacts with nuclear translocation of the virus, viral intermediate early and early gene expression. In addition, the previous compound Enfuvirtide,
with 36-amino acids, has the same structure of some viruses’ proteins such as HIV gp41 [92], HCMV gpB and gpH [93]. It has an excellent antiviral activity for both clinical and laboratory HCMV strains [93]. It affects viral fusion machinery [94]. In the same manner, Phosphorothioate-modified oligonucleotides, CpG oligodeoxynucleotides (ODNs) are commercial, single-stranded DNA with unmethylated cytosine-guanosine (CpG) dinucleotide [95]. This nucleotide interacts with toll receptor to induce immunomodulatory cascade, ending with stimulation of cytokines that activate the immune responses [96] with proper safety especially in vivo [95].

4.3. Uncoating

It is a post-entry event important to expel the virus nucleocapsid into the cytoplasm. Beyond entry, virus nucleocapsid and tegument proteins were released from the fusion with the endosome membrane [97].

4.4. Nuclear Translocation

Viral DNA replication occurs in the nucleus where the nucleocapsid is translocated into the nucleus through the virus teguments proteins pUL48, pUL47, and pp150/UL32 [98]. The virus protein pUL48 interacts with the microtubule motors for capsid translocation [99]. This step is a good target for future antiviral drugs. Using both nocodazole (anti-actin) and Podofilox (anti microtubules) resulting in the disruption of cellular microtubule network and block virus translocation and subsequently IE gene expression [100]. Cytochalasin B is an antiviral drug that targets the actin microfilaments assembly and disrupt viral nuclear translocation (Fig. 3) [101].

(3). Drug interact with virus translocation: the Fig. (3)

Cytochalasin B can affect the actin microfilaments structure and disrupt the virus translocation step. This step is very important for virus transcription and DNA replication.

4.5. Transcription and Translation Step

CMV gene expression starts directly as the virus nucleic acid release in the nucleus under control of the major intermediate-early promoter (MIEP). The viral genes are expressed in a cascade manner to produce three types of proteins immediate-early proteins (IE or α), early proteins (E or β) and late proteins (L or γ) [102]. The most critical IE proteins are major IE72 and IE86 gene products (Stenberg, 1996). They play an important role in controlling the virus and cellular expression to ensure a good environment for virus replication [103].

4.5.1. Expression of Early Proteins

In the nucleus, Daxx works as a transcriptional corepressor protein. It is an inactive protein which affects transcriptionally inactive heterochromatin. Viral tegument protein pp71 can start the gene expression by releasing Daxx from its inhibitor ATRX [104] by inducing its SUMOylation [105]. This induces proteasomal degradation through a ubiquitin-independent pathway [106]. In addition, it can target Rb family members and degrade it, which pushes the cell cycle to move to G0/G1 progression in infected cells [107]. The MIE region of HCMV consists of special MIE locus UL122–123, which expresses the essential IE1-72 and IE2-86 intermediate-early proteins.

IE2-86 can control the MIE expression through the block the Cell Specific Reference Signal (CRS) [108]. The interaction between both IE2-86 and CRS blocks the binding of RNA polymerase II, stop the assembly of replication complex and stop MIE gene expression. Also, IE2-86 can inhibit chromatin remodeling enzymes such as HDAC-1 and HDAC-2 [109] which lead to MIE gene silencing.

Moreover, IE2-86 promotes viral E genes with IE1-72 protein [110] and regulate cellular tumor suppressor Rb [111] to move cell cycle to phase G1/S that facilitates the virus DNA replication. The ability of IE2-86 to act as an immunomodulator, blocking of proinflammatory signaling expression in HCMV-infected cells by NF-κB inhibition [112] is significant to ensure that CMV escape from the immune system. Other IE proteins were expressed such as the HCMV UL36-38, TRS1/IRS1 and US3 loci [103b, 113]. They play a critical role in the virus replication and resemble good targets for the antiviral drug.

4.5.1.1. Target MIE Gene Expression and its Product

IE proteins are key proteins for CMV replication [114] and can be targeted to blocking the first replication step [115]. Fomivirsen is an approved drug that targets MIE gene expression. It is an antisense ODN composed of 21 phosphorothioate linked nucleotides (5′-GCGTTTGCTCTTCTTCTTGCG-3′) complementary to the mRNA of HCMV MIE transcriptional unit (Fig. 4) [116]. It is used to treat retinitis in AIDS patients [117]. The drug administrated intravitreal injection and many doses are needed to achieve the treatment [118]. Moreover, resistance to fomivirsen has been reported [119]. The drug can be used in combination with GCV with good effect [120]. Another MIE inhibitor is Amlohistatin A which targets histone deacetylases (HDACs) interact with MIEP activation step [121]. The function of IE2-86 depends mainly on its phosphorylation [122]. The ability to block the transactivation process by IE2-86 is a good target for the antiviral drug [123]. Another compound, 6-aminoquinoline (WC5). It so more effective toward CMV more than GCV [124]. In addition, this compound shows treatment efficacies towards HCMV resistant strains, GCV, FOS, and CDV. This drug opens a new hope to find a new treatment for HCMV infection. Moreover, A phosphorothioate oligonucleotide, GEM 132 (UL36 ANTI), interferes with CMV DNA replication. It has a complementary
structure to the intron-exon boundary of the IE genes UL36-UL37. It has shown a good efficacy compared with ganciclovir and fomiviren. It targets HCMV UL36 that mediates virus DNA origin of replication-dependent synthesis [125].

4.5.2. Expression of Early Genes (DNA Replication)

The DNA replication in the nucleus is mediated by the interaction between pp71 and Daxx [126]. These groups of proteins are important for viral DNA replication and work as immune response modulator proteins [20b]. The replication is associated with a special nuclear component (known as nuclear domain 10, ND10) [127].

4.5.2.1. UL54 DNA Polymerase (pol), pUL97 and their Inhibitors

DNA replication is mediated with UL54 DNA Polymerase (pol), the primary enzyme that mediates DNA replication. The replication complexes involve UL54 DNA Polymerase (pol), the polymerase accessory protein pUL44, the single-stranded DNA binding protein (UL57), and the primase-helicase complex (UL70, UL102, and UL105) [128]. All these proteins collectively form "replication fork proteins" [129]. These proteins have a highly conserved amino acid sequences among HCMV strains. The protein pUL44 forms homodimers and the interaction will be held between its N-terminal residues and the C-terminus of Pol [128b]. Together, all previous proteins with other four proteins encoded by UL112-113 gene and transactivators form the replication compartment [130]. The DNA genome will start its replication producing concatemer. The new genome is cut into particular lengths by concatemer terminase that is encapsulated inside the procapsid of new progeny viruses for assembly. Virus genome replication is a good target for the antiviral drug. Inhibition of DNA polymerase by incorporating the nucleotide analogs prevents HCMV replication. In the following discussion, the most common anti-CMV nucleotide analogs will be discussed.

4.5.2.1.1. Ganciclovir

Ganciclovir is the first drug of choice in treating systemic HCMV infections. It was administrated intravenously in 1989. The chemical structure of the drug is 9-(1, 3-dihydroxy-2-propoxyxymethyl) guanine, Cymevene, Cytovene. It needs three phosphorylation steps to work [10b]. The first activation step is mediated by the virus protein kinases enzyme (pUL97, serine/threonine kinase functional motifs) [131] while the other two are mediated by the cellular enzyme (cellular kinase). The drug involves in the newly synthesized strand of DNA and leads to the chain termination (Fig. 5) [132]. GCV is also used in the treatment of CMV congenital infection. The drug successfully improves children hearing ability with an adverse effect of delayed development [133]. Applying the produg (oral valganciclovir) either alone or following intravenous GCV reduces the virus load in the newborns who acquired congenital CMV infection [8, 134]. Unfortunately, the toxicity and teratogenicity from using both GCV and VGC are the barriers for using them in congenitally-infected infants.

4.5.2.1.2. Valganciclovir (VGC)

The poor bioavailability of GCV (5.6%) leads to the synthesis of its produrg, valganciclovir (the valyl ester of ganciclovir) (VGC) (Fig. 5). It has a better bioavailability (60%) than GCV, can be administrated orally [14a, 135] and used as the second line therapy drug after GCV to treat CMV-associated diseases [136]. The disadvantage of this drug rises from the development of granulocytopenia and thrombocytopenia due to the bone marrow suppression. The drug is metabolized to GCV by intestinal and liver cells. It was a recommended treatment for HCMV retinitis in HIV patients by FDA in 2000[137]. Mutations in HCMV DNA pol (54 genes) have been reported. It may be associated with the mutation in a UL97 mutation (stop ganciclovir phosphorylation (dGCV) or can be alone [138]. Currently, high resistance was reported in HCMV patients (D-/R+).
status) during solid organs transplantation [139] for both GCV and VGCV.

4.5.2.1.3. Foscarnet (FOS) (phosphonoformate sodium)

Foscarnet (FOS) is a pyrophosphate analog [140]. It was the second drug approved by FDA for HCMV infection treatment in 1991, especially for the treatment of retinitis in HIV patients, and for mucocutaneous-developed ACV-resistance (viral TK-deficient) HSV infections in immunocompromised patients. FOS resembles a pyrophosphate analog to the viral DNA polymerase. It blocks the release of pyrophosphate by DNA Pol that prevent nucleotide polymerization which results in chain termination (Fig. 5) [140]. The side effect is nephrotoxicity. The mutation was reported in UL54 gene. The combination between both ganciclovir and foscarnet was effective to treat life-threatening disease associated with CMV [141]. Another combination between DHPG and PFA showed a synergistic effect of suppressing the viral replication with lower concentrations, decreasing toxicity, lowering the rate of mutation even after a extended use [142].

4.5.2.1.4. Cidofovir (CDV)

Cidofovir (CDV) is a CMP analog [143], with the chemical structure of (CDV, (S)-1-(3-hydroxy-2-phosphonomethoxypropyl) cytosine, Vistide) and was approved in 1996. It is phosphorated by cellular kinases [144]. It is administrated intravenously. It is required to be treated by viral kinase for mono-phosphorylation and two steps activation by cellular kinases. It targets DNA polymerase for many DNA viruses [144-145]. It works as competitive nucleotide dCTP that binds to DNA polymerase [146] to block the chain elongation (Fig. 5). The side effect is kidney toxicity (electrolyte balance in kidney) [147]. The prodrug HDP cidofovir (hexadecyl oxy alkyl esters of CDV) was developed to improve the bioavailability when administrated orally. It also gives better inhibition in comparison with cidofovir [148]. Resistance to CDV was reported in the conserved regions of the viral DNA polymerase as a result of amino acid substitutions [150]. Interestingly, the mutant isolates also showed cross-resistance to other drugs involve ganciclovir, foscarnet, and cidofovir [151]. The drug was suggested to be used as second-line therapy.

Another prodrug is CMX001 (Fig. 5), which is the 1-O-hexadecyloxypropyl (HDP) prodrug of the acyclic nucleoside phosphonate cidofovir (CDV). It is administrated orally with less kidney toxicity than CDV [152]. The active form of HDP-CDV (CMX001) is cidofovir. It displays the same mechanism and targets the same viruses. It is more active than cidofovir against CMV infection in animal models [153]. Interestingly, alkoxy alkyl esters of CDV was developed, which is 1-O-octadecyl-2-β-benzyl-sn-glycero-3-CDV (ODBG-CDV), [83, 154] that showed a more potent result over HDP-CDV (CMX001). It treats CMV infection in hematopoietic-cell transplantation [155] with the development of resistance [156]. It showed an excellent result in treating congenital infection in GPCMV animal model compare with CDV with increased pups survival [157].

4.5.2.1.5. Acyclovir

It is a [9-(2-hydroxyethoxymethyl) guanine]. It is an analog of 2’-deoxyguanosine (Fig. 5) [158]. It needs an activation by both viral UL97 and cellular kinases as in GCV. The drug shows less effects compared to GCV and that is related to its shorter half time. The resistance for ACV has been reported in both UL54 or UL97 genes [159]. Valaciclovir (55%) (Valtrex®, GlaxoSmithKline, VACV) was synthesized to improve the oral bioavailability of ACV [160]. The drug is used as the prophylactic treatment for HCMV infection and established diseases in solid organ transplant recipients [159, 161].

†, (5). Drug interact with CMV DNA replication: The Fig. shows how the different analogous CMV inhibitors interact with DNA replication step and stop virus replication.
4.5.2.1.6. Cyclopropavir

Cyclopropavir is produced by replacing the acyclic side chain with methylenecyclopropane (Fig. 5). It shows antiviral activity [162] and requires phosphorylation similar to GCV [163]. Its diphosphate and triphosphate are held by the cellular enzyme, guanosine monophosphate kinase (GMPK) [164]. The drug can also undergo esterification step [165] to produce valcyclovir with 95% oral bioavailability in animals. It is more potent than GCV [166] with sensitivity to UL97 mutations. Moreover, phosphonate and cyclic phosphonate were developed and showed anti-CMV activity [167].

4.5.2.1.7. 4-hydroxyacridone-3-carboxamides

An example of these compounds is PNU-181465 (Fig. 5), which has antiviral activity against many herpes viruses involving HCMV [168]. Another drug that has been utilized for CMV inhibition is 1,6-naphthyridine and 7,8-dihydroisoquinoline derivatives [168a]. It works synergistically with GCV and is useful to treat CMV GCV-resistant strains.

4.5.2.1.8. Lobucavir

Lobucavir is a nucleoside analog in which a cyclobutyl ring replaces the sugar moiety. Guanosine derivative, lobucavir [RBHGC; LBV; (1R-1, 2, 3)-9-2, 3-bis hydroxymethyl cyclobutyl guanine] has an antiviral activity toward herpesviruses, including HCMV [169]. It targets the DNA synthesis and is used to treat ganciclovir-resistant infections (Fig. 5) [170].

4.5.2.2. Ribonucleoside Diphosphate Reductase and its Inhibitors

4.5.2.2.1. 10-Carboxymethylacridone (10-CMA)

Two compounds (Citrus pine-I, Compressive-I) of the 1-hydroxy acridone sub-class have shown antiviral activities toward HCMV. These agents target the virus ribonucleoside diphosphate reductase enzyme and affect the synthesis of the host of deoxyribonucleotides, which results in the inhibition of viral DNA replication [171].

4.5.2.3. Viral Helicase /Primase Complex and their Inhibitors

4.5.2.3.1. T-611

T-0902611 is a new drug that inhibits HCMV[172] (Fig. 6). It resembles a non-nucleoside HCMV inhibitor. T-661 resembles the imidazole-pyrimidine-based compound that targets the viral primase. The drug showed high anti-HCMV activity, less toxicity and a good pharmacological profile. The drug has passed phase I and II, but unfortunately, the trials stopped regarding formulation issues.

4.5.3. L protein Expression

The expression of the late gene was stimulated by E proteins (UL79, UL87, and UL95). In general, L proteins can be divided into two groups (γ1 and γ2). These proteins are structural proteins necessary for producing the new virus progeny [20].

4.6. Virus Assembly

Two main precursors mediate virus assembly: pAP, pUL80.5 complex and protease precursor pPR, pUL80a in the presence of serine protease (the UL80 gene product). Additionally, the protease is a member of an assembly scaffold that needs to undergo proteolytic and autocatalysis reactions to perform the capsid [173]. In herpes virus, a genome replication results in concatemer. Both viral proteins pUL56 (DNA terminase) and pUL89 (cleavage/packaging enzyme) mediate the cut and packaging of a new genome by binding to packaging (pac) sites in the concatemeric. The viral DNA is loaded into capsids at the portal protein (pUL104) [174]. The nucleocapsid will leave the nucleus through mechanisms known as envelopment, de-envelopment and re-envelopment processes (dual envelopment) [175]. The final envelope will acquire in Golgi apparatus from viral assembly compartment.

4.6.1. Benzimidazole Ribonucleoside Derivatives, BDCRB

Benzimidazoles (2-bromo-5,6-dichloro-1-β-D-ribofuranosyl benzimidazole [BDCRB]) [176] resembles one of anti-HCMV drugs [177]. The drug targets the DNA packaging step by interacting with the cutting of concatemeric to DNA.

![Diagram](image_url) (6). Drug interact with Ribonucleoside diphosphate reductase helicase /primase complex.
unit, and has less toxicity in immunosuppressed patients (Fig. 7) [178]. Moreover, TCRB (2, 5, 6-trichloro-1-β-D-ribofuranosyl benzimidazole) showed an antiviral activity against HCMV [179]. The mutations against the drug were found in both the UL89 and UL56 gene products [180], affecting the nuclease activity of the UL89 gene product and ATPase activity of UL56 gene product [181]. The use of BDCRB was discontinued as it is metabolically unstable [180, 182].

4.6.2. Letermovir or AIC246

Letermovir is the 3, 4- dihydro-quinazoline-4-yl-acetic acid derivatives. It has antiviral activity against many viruses [183] including HCMV with a safe concentration in phase I clinical trials [179]. The drug has moved to phase II trials. The drug interacts with capsid assembly, DNA processing/packing, or virus egress (Fig. 7) [184]. It shows good activity with multidrug-resistant HCMV strain as reported in lung transplant recipient [185]. Another drug, BAY 38-4766 interferes with HCMV terminase [186] with good effects toward AIC246-resistant strain. The mechanism of action of cleavage/packing inhibitors may be different on the same target [184b]. The mutations against this drug have been reported in both HCMV UL56 and UL89 genes [186a].

4.6.3. Maribavir

Maribavir has an anti-CMV activity in most CMV strains (isolates and laboratory-adapted strains resistant to GCV, PFA and BDCRB) with good bioavailability, less toxic, and with no side effects after long period administration [187]. The drug successfully inhibited the DNA synthesis without interacting with HCMV DNA polymerase and DNA processing. The drug has passed both Phase I/II clinical to treat HCMV infection in HIV-infected and stem cell transplantation patients with good concentration and activity [188]. However, the drug shows no response in the clinical trial III. Mutations have been isolated in UL97 that affects its phosphorylation and work as ATP-competitive kinase inhibitors [176, 189].

UL97 gene product plays a role in facilitating the nuclear egress of HCMV nucleocapsids, MBV may interferes with this protein [190] or block DNA encapsidation of viral genome without affecting the concatemer cleavage [191]. Another drug, indolocarbazoles [192] also affects the autophosphorylation of UL97 and GCV phosphorylation by UL97. The drug can affect both GCV-sensitive and resistant HCMV strains [193].

4.7. Virion Maturation and its Inhibitor

GpB mediates the virus attachment, fusion, and entry. It is synthesized as an N-glycosylated precursor polypeptide that is further modified by alteration of the glycosylation, phosphorylation at the C-terminus and cleavage by the proprotein convertase, furin. Targeting this cleavage step by using furin inhibitor (α1-PDX/hf) prevents the production of functional, mature gP8 that interferes with virus replication [194].

CONCLUSION

Targeting HCMV proteins such as DNA polymerase or IE effectively inhibits virus replication. The side effect and the development of drug-resistance are barriers against current clinical therapies for CMV. The development of new drugs is required urgently. CMV’s successful replication requires a multitude of cellular signals. Previous data mentions some inhibitors with an antiviral activity against CMV and with low toxicity. Cell signal inhibitors will be an important objective in future CMV treatment.

AUTHOR’S CONTRIBUTIONS

Ashwaq Ahmed Abdullah: Designed, conceptualized the write-up and drafted the manuscript and critically revised the manuscript.

Krishnan Nair Balakrishnan: Helped in drafting and final alignment of the Manuscript.
Jamila Abubakar Bala: Helped in drafting and final alignment of the manuscript.

Faez Firdaus Jesse Abdullah: Supervised the editing of the manuscript.

Zeenatul Allaudin Nazariah: Supervised the design and write-up of the manuscript.

Raseedee Abdullah: Supervised the design and write-up of the manuscript.

Noordin Mohamed Mustapha: Supervised the design and write-up of the manuscript.

Mohd-Azmi Mohd-Lila: Made considerable contributions to conception, supervised the design of the work and the write-up of the Manuscript.

All authors have read and approved the final manuscript.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

COMPLIANCE WITH ETHICAL STANDARDS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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[68] Cytomegalovirus Replication Steps and the Actions of Antiviral Drugs


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