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In Vitro Evaluation of the Activities of the Novel Anticytomegalovirus Compound AIC246 (Letermovir) against Herpesviruses and Other Human Pathogenic Viruses

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AIC246 (letermovir) is a potent anticytomegalovirus drug in clinical development. Here, we report a consistent antiviral efficacy of AIC246 against human cytomegalovirus laboratory strains, clinical isolates, and virus variants resistant to approved drugs. Furthermore, we describe a remarkable selectivity of AIC246 for human cytomegaloviruses compared to that of other alpha-, beta-, or gammaherpesviruses or nonrelated pathogenic viruses, including adeno-, hepadna-, retro-, orthomyxo-, and flaviviruses. Our data confirm and support an excellent and selective anticytomegaloviral activity of AIC246.

Human cytomegalovirus (HCMV) is one of eight human herpesviruses with worldwide distribution and a high clinical importance. Despite diagnostic and therapeutic advances, HCMV infection has remained a significant complication during pregnancy and in clinical situations associated with inefficient immunocompetence, such as organ or bone marrow transplantation, cancer, and AIDS. Currently approved anticytomegaloviral drugs include ganciclovir (GCV), its prodrug valganciclovir (VGCV), foscarnet (FOS), and cidofovir (CDV), which all uniformly target the viral DNA polymerase. Although efficacious, the use of these drugs is limited because of severe toxic side effects, low oral bioavailability (with the exception of VGCV), and the occurrence of drug resistance (10). Thus, there is an urgent need for novel safe and tolerable anticytomegalovirus drugs.

The 3,4-dihydroquinazoline AIC246 (letermovir) has shown a potent anticytomegaloviral activity *in vitro* and *in vivo*, a favorable safety and pharmacokinetic profile in phase I clinical trials, and proof of concept in a phase IIa trial (8, 9, 23). Recently, a clinical phase IIb dose-finding trial has been completed (clinicaltrials.gov). Analyses of the mode of action of AIC246 revealed that the drug interferes with DNA concatemer maturation and exerts its antiviral effect mainly through targeting the HCMV terminase complex, a heterodimeric enzyme which has no counterpart in mammalian cells (4). Based on this novel mode of action, AIC246 should provide new treatment options even for HCMV infections with virus variants that are resistant to current drugs. In fact, AIC246 recently proved to be highly efficacious in the treatment of a lung transplant recipient suffering from multidrug-resistant HCMV disease (6). Here, we extend our *in vitro* characterization of the AIC246 activities against (i) several cytomegalovirus variants and isolates; (ii) other alpha-, beta-, or gammaherpesviruses; and (iii) a panel of important human pathogenic viruses belonging to various families.

First we analyzed the efficacy of AIC246 against a panel of 17 different clinical HCMV isolates. Virus isolates WT1 to WT17 (Table 1) were collected from various sources, including reference laboratories and medical centers regularly conducting HCMV diagnostic assays and therapy. The susceptibilities of the virus isolates to AIC246 were determined by standard plaque reduction assays essentially as described by Pepin et al. (17). As summarized in Table 1, the data clearly demonstrate a potent antiviral activity of AIC246 against all 17 clinical isolates, with 50% effective concentrations (EC_{50}s) in the low nanomolar range. In general, AIC246 was consistently active and approximately 1,000-fold more potent than GCV, which was used as a reference compound in these experiments.

TABLE 1 Antiviral activity of AIC246 against a broad panel of different clinical HCMV isolates

Clinical isolate	$EC_{50} \pm SD$ (μM)[a]	
	AIC246	Ganciclovir
WT1	0.0015 ± 0.0003	3.0 ± 0.6
WT2	0.0015 ± 0.0008	1.8 ± 0.9
WT3	0.0015 ± 0.0001	0.9 ± 0.1
WT4	0.0020 ± 0.0006	1.4 ± 0.5
WT5	0.0020 ± 0.0004	1.8 ± 0.8
WT6[b]	0.0009 ± 0.0001	1.6 ± 0.3
WT7[b]	0.0010 ± 0.0003	1.5 ± 0.5
WT8[b]	0.0022 ± 0.0002	2.3 ± 1.7
WT9[b]	0.0008 ± 0.0001	1.5 ± 0.4
WT10[b]	0.0013 ± 0.0001	1.9 ± 0.6
WT11[b]	0.0011 ± 0.0004	1.5 ± 0.6
WT12[b]	0.0022 ± 0.0005	1.1 ± 0.3
WT13[b]	0.0023 ± 0.0003	1.2 ± 0.5
WT14[b]	0.0014 ± 0.0002	1.0 ± 0.2
WT15[b]	0.0029 ± 0.0003	1.6 ± 0.5
WT16	0.0031 ± 0.0010	0.9 ± 0.1
WT17	0.0009 ± 0.0003	1.4 ± 0.4

[a] EC_{50} values were determined by a plaque reduction assay. Data are means of results from at least three independent experiments and are expressed with standard deviations.
[b] Cell-associated virus (virus-infected cells) were used as viral inoculum.

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TABLE 2 Antiviral activity of AIC246 on HCMV variants resistant to approved drugs

HCMV variant	$EC_{50} \pm SD$ (μM)[a]	
	AIC246	Ganciclovir
Laboratory strain, AD169	0.0051 ± 0.0012	2.4 ± 2.5
AD169-derived drug-resistant variants[b]		
rGCV-1	0.0039 ± 0.0001	12 ± 0.7
RV-HG rGCV	0.0039 ± 0.0004	16 ± 5.2
GDGrXbaF4	0.0026 ± 0.0001	4.4 ± 1.9
rGCV-2	0.0016 ± 0.0005	32 ± 2.0
GDGrP53	0.0029 ± 0.0002	6.4 ± 0.7
759rD100	0.0024 ± 0.0010	>10

[a] EC_{50} values were determined by a CPE or plaque reduction assay; data are means of results from at least three independent experiments and are expressed with standard deviations.
[b] rGCV-1, GCV-resistant virus (UL97 M460I); RV-HG rGCV, GCV-resistant virus (UL97 A594V); GDGrXbaF4, GCV-resistant virus (UL97 del590-593); rGCV-2, GCV/CDV-resistant virus (UL54 K513E and UL97 M460I); GDGrP53, GCV/CDV-resistant virus (UL54 A987G); 759rD100, GCV/CDV-resistant virus (UL54 A987G and UL97 del590-593).

TABLE 3 Antiviral activity of AIC246 against alpha-, beta-, and gammaherpesviruses

Virus (strain)[b]	Drug	$EC_{50} \pm SD$ (μM)[a]
Alphaherpesviruses		
VZV (Oka)	GCV[c]	0.81 ± 0.05
	CDV[c]	0.28 ± 0.19
	AIC246	>10
HSV-1 (166v VP22-GFP)	ACV[c]	2.2 ± 0.2
	GCV[c]	0.70 ± 0.10
	AIC246	>10
HSV-2 (01-6332)	ACV[c]	2.8 ± 1.3
	GCV[c]	2.5 ± 0.6
	AIC246	>10
Betaherpesviruses		
HCMV (AD169-GFP)	GCV[c]	1.1 ± 0.0
	CDV[c]	0.10 ± 0.0002
	AIC246	0.0027 ± 0.0004
MCMV (Smith)	GCV[c]	4.3 ± 1.0
	CDV[c]	0.29 ± 0.04
	AIC246	4.5 ± 2.0
RCMV (Maastricht)	GCV[c]	0.85 ± 0.01
	CDV[c]	<0.12
	AIC246	>10
HHV-6 (typeA-GS)	CDV[c]	5.5 ± 2.4
	AIC246	>10
Gammaherpesvirus		
EBV (B95-8)	ART[c]	1.5 ± 0.4
	AIC246	>10

[a] EC_{50} values were determined by specific cell culture-based antiviral test systems. Data are means of results from at least three independent experiments and are expressed with standard deviations. Cytotoxicity studies (alamarBlue and/or microscopic evaluation) were performed with each drug in parallel to the antiviral assays. No toxicity was observed at the highest drug concentration used in these studies.
[b] VZV, varicella-zoster virus/primary human fibroblast cells (HFF); HSV-1 and -2, herpes simplex viruses 1 and 2/Vero cells; HCMV, human cytomegalovirus/primary human fibroblast cells (HFF); MCMV, murine cytomegalovirus/primary murine fibroblast cells (MEF); RCMV, rat cytomegalovirus/primary rat fibroblast cells (REF); HHV-6, human herpesvirus 6/HSB-2 cells; EBV, Epstein-Barr virus/293T cells.
[c] Reference compound.

The use of GCV or VGCV as the gold standard in the treatment of chronic HCMV infections may result in the selection of drug-resistant virus mutants (12). Therefore, we asked whether variants of HCMV showing resistance against approved antiviral drugs are comparably sensitive to AIC246. For this purpose, AD169-derived virus variants carrying confirmed GCV/VGCV resistance markers were analyzed in comparison to the parental laboratory strain AD169 by cytopathic effect (CPE)-based assays or plaque reduction assays as described previously (9, 15). The genotypic variations of the analyzed HCMVs in the UL97 (viral kinase) and/or UL54 (viral DNA polymerase) genes conferred either GCV resistance or GCV/CDV cross-resistance, as indicated in Table 2 (12). The degree of GCV resistance was monitored by a control setting (Table 2) indicating that the GCV phenotype ranged between full sensitivity (AD169), moderate resistance (GDGrXbaF4 and GDGrP53), and high-level resistance (all other variants). When analyzing the susceptibility toward AIC246, we observed that AIC246 was equally active against all these virus variants. In fact, none of the EC_{50}s obtained for the GCV-resistant AD169 variants exceeded the respective value obtained for the parental AD169 strain (Table 2). These results therefore confirmed and extended our previous finding that AIC246 is invariably active against HCMVs carrying clinically significant resistance mutations (9) and suggest that AIC246 might also be useful for treating patients infected with HCMV strains resistant to approved antiviral drugs (6).

As a next step in the profiling of this novel drug, we assessed the potential activity of AIC246 against a panel of different herpesviruses. Using cell culture-based replication assays, a selectivity profile of the antiviral spectrum of AIC246 was generated. The virus panel used included animal cytomegaloviruses (such as murine [MCMV] and rat [RCMV] cytomegalovirus) as well as human herpesviruses representing the three subfamilies, alpha-, beta-, and gammaherpesviruses (Table 3) (for virus replication assays, see references 1, 14, 15, 16, 18, and 20). The findings depicted in Table 3 indicate that AIC246 is remarkably specific for human cytomegaloviruses since no significant activity was noted against any other herpesvirus tested, including all analyzed human herpesviruses, i.e., varicella-zoster virus, herpes simplex viruses 1 and 2, human herpesvirus 6, and Epstein-Barr virus. Interestingly, the EC_{50}s obtained for RCMV and MCMC indicated that no (RCMV) or only a very low-level (MCMV, 4.51 μM) AIC246 sensitivity could be detected even for rodent cytomegaloviruses (Table 3). This is remarkable, as another drug targeting the HCMV terminase, BAY 38-4766, was shown to be highly active against MCMV (2). Therefore, in accordance with our previous findings which included AIC246 resistance selection, these data further support the hypothesis that AIC246 acts via a mode of interaction with the viral terminase that is distinct from that of other cleavage/packaging inhibitors (4).

To conclusively determine the therapeutic spectrum of AIC246, we addressed the putative antiviral activity of AIC246 against other nonrelated pathogenic viruses, such as human adeno-, hepadna-, retro-, orthomyxo- and flaviviruses. The respective antiviral assays were performed basically as described in references 5, 11, 14, 18, and 22, and the following reference drugs were used to control the assay validities: (i) roscovitine (19), (ii) lamivudine (3), (iii) efavirenz (13), (iv) Gö6976 (14), and (v) HCV-796 (7). The obtained EC_{50}s summarized in Table 4 further underline the high specificity of AIC246 for human cytomegalo-

TABLE 4 Effect of AIC246 on the replication of important human pathogenic viruses

Virus and strain/variant[b]	Drug	$EC_{50} \pm SD$ (μM)[a]
Adenovirus HAdV-2	Roscovitine[c]	1.4 ± 0.2
	AIC246	>10
Hepadnavirus HBV HepG2.2.15	Lamivudine[c]	0.21 ± 0.035
	AIC246	>30
Retrovirus HIV-1 LAI	Efavirenz[c]	0.0008 ± 0.0001
	AIC246	>11
Orthomyxovirus influenza A A/WSN/33	Gö6976[c]	1.7 ± 0.2
	AIC246	>10
Flavivirus HCV replicon	HCV-796[c]	0.001 ± 0.001
	AIC246	>32

[a] EC_{50} values were determined by specific cell culture-based antiviral test systems. Data are means of results from at least three independent experiments and are expressed with standard deviations. Cytotoxicity studies (alamarBlue and/or microscopic evaluation) were performed with each drug in parallel to the antiviral assays. No toxicity was observed at the highest drug concentration used in these studies.
[b] HAdV-2, human adenovirus/primary human fibroblast cells (HFF); HBV, hepatitis B virus/HepG2.2.15 cells; HIV-1, human immunodeficiency virus type 1/MT-4 cells; influenza A A/WSN/33, influenza A virus/293T cells; HCV, hepatitis C virus/Huh 5-2 cells.
[c] Reference compound.

viruses, as no specific inhibitory potential was detected for human adenovirus type 2 (HAdV-2), hepatitis B virus (HBV) (21), human immunodeficiency virus type 1 (HIV-1), human influenza A virus (subtype H1N1, strain A/WSN/33), and hepatitis C virus (HCV) (11).

Thus, in conclusion, our data underline a selective and potent antiviral efficacy of AIC246 against wild-type and drug-resistant HCMV variants and clinical HCMV isolates.

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