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Antiviral Research 56 (2002) 273–278

Antiviral Research

www.elsevier.com/locate/antiviral

Short communication

Z-isomers of 2-hydroxymethylcyclopropylidenemethyl adenine (synadenol) and guanine (synguanol) are active against ganciclovir- and foscarnet-resistant human cytomegalovirus UL97 mutants

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Received 7 May 2002; accepted 2 August 2002

Abstract

Emergence of drug-resistant human cytomegalovirus (HCMV) strains is a substantial problem during treatment of HCMV infections in immunocompromised patients. The Z-isomers of 2-hydroxymethylcyclopropylidenemethyl adenine (synadenol) and guanine (synguanol) were previously shown to be potent inhibitors of AD169 and Towne HCMV reference strains and postulated to share a common phosphorylation pathway with ganciclovir (GCV) possibly involving the UL97-encoded phosphotransferase. Analysis of synadenol and synguanol susceptibility of a series of HCMV isolates from immunocompromised untreated patients and from patients with treatment failure due to the emergence of GCV- and foscarnet (PFA)-resistant HCMV strains demonstrated that synadenol and synguanol are potent inhibitors of clinical HCMV isolates and are highly effective against both GCV- and PFA-resistant isolates. These results together with those showing resistance of a UL97 knock-out HCMV mutant to GCV as well as synadenol and synguanol suggest the involvement of UL97 phosphotransferase in synadenol and synguanol anabolism but with a substrate specificity different from that of GCV.

Keywords: Synadenol; Synguanol; Ganciclovir-resistance; Foscarnet-resistance; HCMV drug-susceptibility; HCMV UL97 phosphotransferase

Human cytomegalovirus (HCMV) is a major opportunistic pathogen in immunocompromised patients causing severe disseminated infections often associated with multiple organ localizations in untreated AIDS patients and in solid organ or

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0166-3542/02/$ - see front matter
PII: S0166-3542(02)00129-8

bone marrow transplant recipients (Jacobson and Mills, 1988). Antiviral drugs currently licensed for treatment of HCMV infections include ganciclovir (GCV), foscarnet (PFA) and cidofovir. However, the emergence of drug-resistant HCMV strains has been reported in AIDS patients (Baldanti et al., 1995, 1996, 1998a,c; Chou et al., 1995a,b, 1998, 2002; Drew et al., 1999; Erice et al., 1989; Gerna et al., 1992; Jabs et al., 2001 Smith et al., 1998) as well as transplant recipients (Baldanti et al., 1998b; Bienvenu et al., 2000; Eckle et al., 2000; Knox et al., 1991; Limaye et al., 2000; Lurain et al., 1996; Rosen et al., 1997). Indeed, after the introduction of highly active antiretroviral therapies (HAART), the emergence of HCMV drug-resistant strains has been more frequently reported in transplant recipients, while the emergence of drug-resistant HCMV strains in HAART-treated individuals is to date only anecdotal (Baldanti et al., 2002). Thus, development of new anti-HCMV compounds with different mechanisms of action is still warranted.

The Z-isomers of 2-hydroxymethylcyclopropylidenemethyl purines and pyrimidines were shown to be potent inhibitors of human and murine cytomegalovirus, Epstein–Barr virus and varicella zoster virus (Qiu et al., 1998; Rybak et al., 1999, 2000). In particular, synadenol and synguanol were active against HCMV reference strains Towne and AD169 with an IC_{50} value of 2 μM for both compounds in a plaque reduction assay (Drach et al., 1997). Addition of the drugs to cells at different times post-infection indicated that these compounds act at a similar time point as GCV, thus suggesting a mechanism of action similar to that of GCV. In addition, in vitro phosphorylation of GCV was inhibited by synadenol and synguanol, suggesting a common anabolism pathway for the three compounds (Drach et al., 1997).

In this study, the synadenol and synguanol susceptibilities of a series of HCMV isolates from immunocompromised untreated individuals and a series of GCV-resistant and double (GCV and PFA)-resistant HCMV isolates from AIDS patients carrying mutations in the UL97-encoded viral phosphotransferase and in both the UL97-encoded phosphotransferase and the UL54-encoded viral DNA polymerase, respectively, are reported. In parallel, an in vitro generated UL97 knock-out mutant was also analyzed.

HCMV isolates VR5611, VR6189 and VR6200 were recovered from blood of a heart transplant recipient and two patients with AIDS, respectively, who did not receive any previous treatment for HCMV disease. GCV-resistant HCMV isolates VR3480, VR4760, VR4990, VR4991, VR5120, VR5406 and VR6264 were recovered from blood of patients with AIDS and HCMV disease showing virologic failure on GCV treatment. The reported series of GCV-resistant clinical isolates was selected for this study because each of them showed the presence of a different mutation in UL97 (Table 1). Each mutation was proven by marker transfer experiments to confer a high degree of GCV-resistance (Baldanti et al., 1995, 1996, 1998c; Chou et al., 1995a, 2002; Lurain et al., 1994). In addition, isolates VR4760 and VR5120 were also shown to be PFA-resistant because of a mutation (V715M) in the viral DNA polymerase (Baldanti et al., 1996). Finally, the HCMV reference laboratory strain AD169 and an AD169-derived UL97 knock-out mutant lacking GCV-phosphorylating activity (Prichard et al., 1999) were included in the study.

GCV, synadenol and synguanol susceptibilities of all HCMV isolates were determined in triplicate using a reported immediate-early plaque reduction assay (Gerna et al., 1992). Drug resistance was defined as an increase in ID_{50} values $\geqslant$ threefold with respect to mean control values (Gerna et al., 1995).

Differences between synadenol and synguanol IC_{50} values of HCMV isolates from untreated patients and GCV-resistant isolates were analyzed using the t-test for unpaired data, and the relative potencies of the two analogs versus GCV-susceptible or GCV-resistant HCMV isolates were analyzed using the t-test for paired data.

Results of parallel GCV, synadenol and synguanol susceptibility testing of isolates from untreated patients or GCV-resistant isolates are reported in Table 1. In detail, synadenol and synguanol IC_{50} values for the three HCMV isolates from untreated patients were in the same range as GCV IC_{50} values ($P > 0.05$). Thus, these new analogs

Table 1
Synadenol and synguanol susceptibilities of HCMV isolates from untreated and GCV-treated immunocompromised patients

HCMV isolate[a]	UL97 mutation	Synadenol (μM)[b]		Synguanol (μM)[b]		GCV (μM)[b]	
		IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
GCV-susceptible							
VR5611	None	1.7	5.0	1.9	4.3	2.9	6.0
VR6189	None	1.8	4.7	2.0	4.9	1.8	3.5
VR6200	None	1.6	4.3	2.0	4.8	3.3	4.1
Mean value ± SD		1.7 ± 0.1	4.6 ± 0.2	2.0 ± 0.1	4.7 ± 0.3	2.7 ± 0.8	4.5 ± 1.3
GCV-resistant							
VR4760[c]	M460I	2.4	6.9	2.0	4.5	40.0	93.0
VR4991	C592G	1.7	4.9	1.1	4.7	25.0	92.5
VR6264	A594V	0.6	2.1	4.0	7.8	15.0	33.7
VR3480	L595del	4.3	8.4	1.8	6.8	22.0	75.0
VR5120[c]	L595S	2	4.2	2.2	4.8	25.0	72.5
VR5406	G598S	1.6	4.3	3.3	4.9	20.0	75.0
VR4990	C607Y	3.9	7.2	1.5	2.6	23.0	33.7
Mean value ± SD		2.4 ± 1.3	5.4 ± 2.2	2.3 ± 1.0	5.1 ± 1.7	24.2 ± 7.7	67.9 ± 24.8

[a] The three GCV-susceptible HCMV isolates originated from untreated immunocompromised patients (one heart transplant recipient and two patients with AIDS). The seven GCV-resistant HCMV isolates with different UL97 mutations were recovered from as many AIDS patients failing GCV therapy.

[b] IC_{50} and IC_{90} represent the 50 and 90% inhibitory concentrations, respectively.

[c] VR4760 and VR5120 were also shown to be PFA-resistant (IC_{50} 498.0 and 520.0 μM, respectively), because of the V715M change in the viral DNA polymerase (Baldanti et al., 1996).

showed a potency in inhibiting replication of HCMV clinical isolates in cell culture that was comparable to that of GCV. In addition, comparable IC_{90} values were found for the three drugs.

Moreover, as shown in Table 1, mean synadenol and synguanol IC_{50} and IC_{90} values for GCV-resistant HCMV isolates were not significantly different from those found for control HCMV isolates from untreated patients ($P > 0.05$). In addition, synadenol and synguanol showed a comparable potency in inhibiting replication of GCV-resistant isolates. Specifically, mean synadenol and synguanol IC_{50} values were 2.4 ± 1.3 and 2.3 ± 1.0 ($P > 0.05$), and the corresponding IC_{90} values were 5.4 ± 2.2 and 5.1 ± 1.7 ($P > 0.05$), respectively.

Table 2 shows the comparison among GCV, synadenol and synguanol susceptibilities of HCMV laboratory reference strain AD169 and an AD169-derived mutant (RCΔ97.19) with a large deletion in UL97 (Prichard et al., 1999).

Table 2
Activity of GCV, synadenol and synguanol on a HCMV laboratory reference strain and a UL97 knock-out mutant

HCMV strain[a]	IC_{50} (μM)[b]		
	GCV	Synadenol	Synguanol
AD169	3.8 ± 2.0	1.2 ± 0.4	2.4 ± 1.0
RCΔ97.19	19.6 ± 8.6	3.9 ± 0.9	9.6 ± 4.0
IC_{50} fold increase	5.2 ± 0.6	3.2 ± 0.7	4.0 ± 0.0

[a] HCMV AD169, laboratory reference strain; RCΔ97.19, AD169-derived UL97 knock-out mutant (kindly provided by Dr Mark Prichard, Aviron, Mountain View, CA).

[b] 50% Inhibitory concentration; data represent mean values (±SD) of three different experiments.

The observed fivefold increase in GCV IC_{50} value of RCΔ97.19 could account for a high level of GCV-resistance. In fact, as shown in Table 1, a

similar increase in GCV IC_{50} levels with respect to mean GCV IC_{50} control values was observed in clinical GCV-resistant isolates (mean = 8.9-fold, range = 5.5–14.8). This result is likely to be related to the reported lack of GCV phosphorylation in cells infected with RCΔ97.19 (Prichard et al., 1999). In parallel, a consistent increase in synadenol and synguanol IC_{50} levels (3.2 and 4.0 fold increase, respectively) was observed. These results support the involvement of the HCMV UL97 phosphotransferase in synadenol and synguanol anabolism. In fact, previous in vitro phosphorylation studies suggested a common anabolism pathway for synadenol, synguanol and GCV on the basis of competition experiments (Drach et al., 1997). In addition, uninfected cells did not show synadenol and synguanol phosphorylation activity (Drach et al., 1997).

It is interesting that UL97 phosphotransferase appears to be able to phosphorylate nucleoside analogs other than GCV. In fact, recent studies have shown that UL97 does not appear to be a nucleoside kinase (He et al., 1997; Michel et al., 1996, 1998), but it does contain functional regions homologous to conserved domains characteristic of protein kinases (Michel et al., 1998, 1999). However, the role of UL97 phosphotransferase appears to be different in the phosphorylation process of synadenol and synguanol compared to GCV. In fact, our data demonstrate that UL97 residues involved in GCV recognition and processing do not exert the same function for the new analogs, as shown by the susceptibility of all GCV-resistant UL97 mutants to both compounds. In addition, we observed that UL97 deletion had a great impact (fivefold increase) in GCV resistance of RCΔ97.19 with respect to its parental strain AD169.

In conclusion, synadenol and synguanol were shown to be potent inhibitors of replication of HCMV clinical isolates. In addition, these analogs were found to display full activity against a panel of GCV-resistant HCMV isolates carrying different mutations in the UL97-encoded phosphotransferase. Finally, although sharing a UL97-mediated phosphorylation process, synadenol and synguanol show a substrate specificity different from that of GCV. From the data presented here, synadenol and synguanol may seem promising for the treatment of GCV-resistant HCMV infections.

Acknowledgements

We thank Mark Prichard, Aviron, Mountain View, CA, for providing HCMV UL97 knock-out mutant RCΔ97.19 and Linda D'Arrigo for revision of the English. This work was partially supported by Ministero della Sanità, Istituto Superiore di Sanità, Programma Nazionale AIDS (grant no. 50D.12), Ricerca Finalizzata (grant no. 820RFM99/01), and by grant P01-AI46390 from N.I.H.

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