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Inhibition of Ganciclovir-Susceptible and -Resistant Human Cytomegalovirus Clinical Isolates by the Benzimidazole L-Riboside 1263W94

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The average 50% inhibitory concentration (IC_{50}) values for AD169 were 0.22 ± 0.09 μM 1263W94 and 5.36 ± 0.12 μM ganciclovir. For 35 human cytomegalovirus (HCMV) clinical isolates the average IC_{50} was 0.42 ± 0.09 μM 1263W94, and for 26 ganciclovir-susceptible HCMV clinical isolates the average IC_{50} was 3.78 ± 1.62 μM ganciclovir. Nine HCMV clinical isolates that were resistant to ganciclovir were completely susceptible to 1263W94.

Human cytomegalovirus (HCMV) causes considerable morbidity and mortality in the immunocompromised host (18, 19). Organ transplant recipients suffer from retinitis, gastrointestinal disease, hepatitis, and pneumonia caused by HCMV infections, whereas AIDS patients suffer from HCMV-induced retinitis and other complications (1). The current Food and Drug Administration-approved chemotherapies for HCMV infections consist of ganciclovir, foscarnet, cidofovir, and fomivirsen (5, 16, 17, 20). These antiviral drugs are effective against infections caused by HCMV; however, they are not ideal because of their toxicity and poor bioavailability. Furthermore, long-term treatment with these drugs often leads to the selection of drug-resistant mutants (6, 8, 9). Due to the problems associated with the currently used antiviral compounds for HCMV infection, there is an active search for more useful compounds to combat infections with HCMV.

The benzimidazole ribonucleosides represent a new class of antiviral compounds that inhibit HCMV replication by blocking the processing of progeny viral DNA (4, 10, 22). In an attempt to make a more stable derivative of benzimidazole riboside 2-bromo-5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (BDCRB), the L form of the compound, was synthesized (4). The L-riboside benzimidazole analogue of BDCRB, 1263W94, has potent activity against HCMV laboratory strains and clinical isolates as well as Epstein-Barr virus (4, 23). Preliminary studies suggest that 1263W94 inhibits HCMV replication by blocking viral DNA synthesis, but not by an effect on the viral DNA polymerase or the phosphotransferase encoded by the UL97 gene (4).

In this report, we show that 1263W94 inhibits the replication of the AD169 laboratory strain of HCMV and 35 HCMV clinical isolates at drug concentrations that are approximately 10-fold less than those required by ganciclovir. Nine of the 35 HCMV clinical isolates are resistant to ganciclovir, and several are also resistant to foscarnet and cidofovir (2, 3, 7, 8, 9, 11). All of these drug-resistant HCMV clinical isolates are susceptible to 1263W94. These results show that 1263W94 inhibits the replication of both ganciclovir-susceptible and single- and multiple-drug-resistant HCMV clinical isolates, confirming reports that 1263W94 has a mode of action different from that of ganciclovir, foscarnet, and cidofovir (4). These results also suggest that this drug is potentially useful for treating patients infected with HCMV clinical isolates that are resistant to the currently used antiviral drugs.

Determination of IC_{50} values of 1263W94 and ganciclovir for HCMV laboratory strains and clinical isolates by FACS analysis. Confluent human foreskin fibroblast cell monolayers (Clontech, San Diego, Calif.) were infected at low multiplicity of infection with the AD169 laboratory strain of HCMV, the 1263W94-resistant derivative of AD169, or 35 HCMV clinical isolates in the presence of various concentrations of 1263W94 or ganciclovir. The cells were harvested, permeabilized with methanol, and treated with fluorescein isothiocyanate-labeled monoclonal antibodies (MAbs) to the HCMV immediate-early (IE) or late antigens (direct fluorescent-antibody reagent 5090 or MAb 1G5.2; Chemicon International, Inc., Temecula, Calif.), and their drug susceptibilities were determined by the flow cytometry drug susceptibility (FACS) assay as described previously (12–14). Nine of the 35 HCMV clinical isolates were resistant to ganciclovir. The average 50% inhibitory concentration (IC_{50}) values for the AD169 laboratory strain were 0.22 ± 0.09 μM 1263W94 and 5.36 ± 0.12 μM ganciclovir on the basis of analysis of cells expressing the IE antigen and 0.31 ± 0.22 μM 1263W94 and 3.44 ± 1.01 μM ganciclovir on the basis of analysis of cells expressing the late antigen. The IC_{50} values for 2916rA were 57.08 ± 5.01 μM 1263W94 and 2.34 ± 0.92 μM ganciclovir using the IE antigen and >20 μM 1263W94 and 2.11 ± 0.98 μM ganciclovir using the late antigen. The IC_{50} values for the 35 clinical isolates are summarized in Table 1. The IC_{50} values ranged from 0.11 to 1.22 μM 1263W94, with an average IC_{50} value of 0.42 ± 0.22 μM 1263W94, using the IE antigen. The IC_{50} values ranged from 0.15 to 1.0 μM 1263W94, with an average IC_{50} value of 0.40 ± 0.17 μM 1263W94, using the late antigen. The IC_{50} values for 26 ganciclovir-susceptible HCMV clinical isolates ranged from

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TABLE 1. IC_{50} values of 1263W94 and ganciclovir for HCMV clinical isolates by FACS

Compound	No. of clinical samples	Avg[a] (range) IC_{50} (μM) with:	
		IE antigen	Late antigen
1263W94	35	0.42 ± 0.22 (0.11–1.22)	0.40 ± 0.17 (0.15–1.0)
Ganciclovir	26[b]	3.78 ± 1.62 (1.22–7.59)	3.76 ± 1.13 (2.63–7.79)
Ganciclovir	9[c]	33.41 ± 35.93 (9.21–>96)	33.41 ± 35.93 (9.21–>96)

[a] Values are means ± standard deviations.
[b] Susceptible.
[c] Resistant.

1.22 to 7.59 μM, with an average IC_{50} value of 3.78 ± 1.62 μM ganciclovir using the IE antigen and ranged from 2.63 to 7.79 μM, with an average IC_{50} value of 3.76 ± 1.13 μM ganciclovir, using the late antigen. Average IC_{50} values for drug-susceptible HCMV clinical isolates for either drug obtained by FACS analysis of HCMV-infected cells expressing either the IE or late antigens were statistically identical. The average IC_{50} values of 1263W94 for these HCMV clinical isolates were similar to the average IC_{50} values for the AD169 laboratory strain. Compound 1263W94 was 24 times more potent than ganciclovir against the AD169 laboratory strain of HCMV and approximately 10 times more effective than ganciclovir for inhibiting the replication of ganciclovir-susceptible HCMV clinical isolates in human foreskin fibroblast cell monolayers. The data in Table 1 show that nine of the HCMV clinical isolates are ganciclovir resistant (IC_{50} values of greater than 9 μM ganciclovir). Since all 35 HCMV clinical isolates were susceptible to 1263W96, the 9 ganciclovir-resistant HCMV clinical isolates were susceptible to 1263W94. These results show that 1263W94 inhibits the replication of ganciclovir-resistant HCMV clinical isolates and suggest that 1263W94 may be useful for the treatment of patients with ganciclovir-resistant HCMV disease.

Comparison of IC_{50} values of 1263W94 for HCMV clinical isolates determined by FACS assay, PRA, and the DNA hybridization assay. To determine if the FACS assay yields IC_{50} values for 1263W94 similar to those obtained with more-traditional methods such as the plaque reduction assay (PRA) (21) and the DNA hybridization assay (8, 9), the IC_{50} values for selected numbers of ganciclovir-susceptible and ganciclovir-resistant HCMV clinical isolates were determined by all three methods. The data are presented in Table 2. The average IC_{50} values of 1263W94 for 11 HCMV clinical isolates obtained with the FACS assay, the PRA, and the DNA hybridization assay were 0.38 ± 0.13, 0.35 ± 0.17, and 0.08 ± 0.04 μM, respectively. The average ganciclovir IC_{50} values for nine ganciclovir-susceptible HCMV clinical isolates obtained for the FACS assay, PRA, and the DNA hybridization assay were 3.54 ± 1.74, 3.46 ± 2.27, and 0.58 ± 0.34 μM, respectively. V917401-r is resistant to ganciclovir, and MR11979-r is resistant to ganciclovir, foscarnet, and cidofovir (14). For these isolates the IC_{50} values of ganciclovir were higher, but not those of 1263W94. The IC_{50} values of 1263W94 from the PRA are very similar to those obtained with the FACS assay for all of the HCMV clinical isolates. However, there is more variability between the PRA and the FACS assay for ganciclovir. The DNA hybridization assays gave substantially lower IC_{50} values than either the FACS assay or the PRA for both compounds. This comparison between the FACS assay and the PRA confirms the utility of the FACS assay for determining IC_{50} values and extends its use to antiviral compounds that inhibit HCMV replication by a mechanism different from that of ganciclovir. An analysis of bias and precision of the IC_{50} values obtained by the FACS assay and the PRA for the ganciclovir-susceptible clinical isolates showed a less than twofold difference.

TABLE 2. 1263W94 and ganciclovir IC_{50} values for HCMV clinical isolates: comparison of phenotypic assays

Clinical isolate	IC_{50} (μM) of indicated drug by:					
	FACS assay with IE antigen		PRA		DNA hybridization	
	GCV	1263W94	GCV	1263W94	GCV	1263W94
C8301	3.63	0.34	1.1	0.12	0.25	0.04
C8302	7.52	0.15	2.8	0.16	0.3	0.1
C8303	4.47	0.56	4.5	0.54	0.8	0.09
C8501	3.95	0.59	4.0	0.34	1.0	0.1
C8910	3.10	0.42	1.4	0.19	0.7	0.12
C8912	2.43	0.31	2.6	0.21	0.5	0.04
C9003	2.85	0.29	1.0	0.56	1.1	0.12
C9207	2.55	0.38	6.7	0.44	0.15	0.03
C9213	1.40	0.46	7.0	0.40	0.4	0.04
MR11979-r	>96	0.31	>96	0.61	ND[a]	ND
V917401-r	>96	0.33	>96	0.32	ND	ND

[a] ND, not determined.

This report shows that 1263W94 inhibits the replication of the AD169 laboratory strain of HCMV and 35 HCMV clinical isolates at concentrations of the compound below 1 μM. Ganciclovir-resistant HCMV clinical isolates were susceptible to 1263W94 as was one HCMV clinical isolate that was ganciclovir, foscarnet, and cidofovir resistant (14). These results suggest that 1263W94 could be used for the treatment of patients with diseases caused by HCMV that is resistant to the currently licensed antiviral drugs. Compound 1263W94 joins other compounds such as BAY38-4766 and BAY43-9695 that inhibit ganciclovir-susceptible and ganciclovir-resistant HCMV clinical isolates at concentrations below 1 μM (15). These compounds have the potential to be used in the treatment of patients infected with ganciclovir-resistant HCMV.

Three phenotypic drug susceptibility assays, a FACS assay, the PRA, and the DNA hybridization assay, were used to determine IC_{50} values of 1263W94 and ganciclovir for HCMV laboratory strains and clinical isolates. The FACS assay yielded statistically equivalent IC_{50} values when monoclonal antibodies to the IE or late antigens were used to identify HCMV-infected cells, suggesting that the assay does not depend on the mode of action of the compound to yield meaningful IC_{50} values. FACS analysis of cells synthesizing the IE antigen for determining the effect of drugs that interfere with viral DNA synthesis is possible because of the low multiplicity of infection used in these experiments. Under these experimental conditions, the assay measures the effect of antiviral drugs on the spread of virus from a few initially infected cells to the surrounding cells in the monolayer. Furthermore, the FACS assay and the PRA gave equivalent results for drug susceptibility, indicating that the rapid FACS assay is at least as good as the

PRA. However, the DNA hybridization assay gave lower IC_{50} values than either the FACS assay or the PRA. We have previously established that the FACS assay can be used to determine the IC_{50} values of ganciclovir, foscarnet, and cidofovir for a wide variety of HCMV laboratory strains and clinical isolates (12–14). These drugs inhibit HCMV replication by interfering with the activities of the UL97 and Pol gene products leading to the prevention of viral DNA synthesis (5, 6). The inhibition of the replication of ganciclovir-resistant HCMV clinical isolates by 1263W94 suggests that this novel compound had a mode of action different from that of ganciclovir. Therefore, this report expands the use of FACS assays to include antiviral compounds with in vitro effects against HCMV that have modes of action different from those of ganciclovir, foscarnet, and cidofovir. Since the FACS assay yields IC_{50} values equivalent to those from the PRA for these compounds for HCMV clinical isolates, the FACS assay should be used for drug susceptibility testing of HCMV clinical isolates.

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