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Sensitivities of Human Cytomegalovirus (HCMV) Clinical Isolates to Cidofovir. J.M. Cherrington[1], S.J.W. Allen[1], A.S. Mulato[1], R. Miner[2], W.L. Drew[2], and M.S. Chen[1]. [1]Gilead Sciences, Inc., Foster City, CA. 94404; [2] Mt. Zion Medical Center, UCSF, San Francisco, CA. 94115.

Cidofovir [CDV, (S)-(1)-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC] sensitivities among HCMV clinical isolates from patients enrolled in the GS-101 clinical trial were determined. GS-101 was a Phase I/II trial addressing safety and antiviral effects of CDV in patients shedding HCMV but without HCMV disease. Patients had semen HCMV titers ranging from 2.5 X 10^1 to 5 X 10^7 pfu/ml. They received a variety of CDV dosing regimens and durations of therapy. CDV susceptibilities of paired pre- and post-CDV therapy semen isolates from 18 patients have been analyzed. The IC_{50} values for pre-therapy isolates ranged from 0.32 μM to 1.85 μM and for post-therapy isolates from 0.55 μM to 2 μM. No significant changes in CDV susceptibilities have been noted for HCMV during various regimens of CDV administration in the clinic. In addition to these findings, we also obtained a clinical isolate from the retina of a patient with CMV retinitis who had received both Ganciclovir (GCV) and Foscarnet (PFA) therapy (but not CDV). We found that this isolate was 40-fold resistant to GCV, 5-fold resistant to PFA and 40-fold resistant to CDV, relative to average IC50 values for wild type clinical isolates. DNA sequence analysis of the DNA polymerase gene from this drug resistant mutant identified 6 amino acid substitutions and 1 amino acid insertion when compared to the wild type laboratory strain, AD169. None of these substitutions/ insertions has been demonstrated previously to be responsible for HCMV resistance to GCV, PFA or CDV. Construction of recombinant viruses carrying defined single and multiple DNA polymerase mutations are in progress. We are also investigating the kinetic properties of the HCMV DNA polymerases purified from the wild type virus and the clinical isolate.

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Selection and Characterization of HCMV Resistant to the Benzimidazole Ribonucleoside TCRB. P.M. Bush, S.R. Turk, R.G. Ptak, F.P. Albayya, A.C. Westerman, L. B. Townsend and J. C. Drach, University of Michigan, Ann Arbor, MI 48109; M.R. Underwood and K. K. Biron, Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA.

We have previously described the activity against human cytomegalovirus (HCMV) of a new class of benzimidazole ribonucleosides (Townsend *et al.*, Drach *et al.*; 5th and 6th ICAR; 1992, 1993). Substitution with halogen at the 2-position was critical for anti-HCMV activity. 2,5,6-Trichloro-1-(ß-D-ribofuranosyl)benzimidazole (TCRB) was the lead compound in this series and has been used to select drug-resistant HCMV. Human foreskin fibroblasts infected with a low m.o.i. of the Towne strain of HCMV were incubated in the presence and absence of TCRB in the following sequence: 10 μM TCRB for 45 days, no drug for 10 days, 10 μM drug for 7 days, and 30 μM TCRB for 13 days. Separate plaques were isolated by limiting dilution in 96-well plates; eight variants were obtained whose IC_{50}'s against TCRB were 10 to 15-fold greater than wild-type virus. Two of these strains (B-11 and D-10) were passaged two times in the absence of drug and characterized more extensively. The strains were an average of 8 and 9-fold resistant, respectively, to TCRB in several experiments. Both strains were cross resistant to the 2,4,5,6-tetrachloro analog of TCRB; its 2'-deoxy and 5'-deoxy analogs; and to its 2-Br analog, BDCRB. Neither strain was resistant to ganciclovir nor to the acyclic pyrrolopyrimidine analogs UMJD 102 and 183. Based upon the discovery that resistance to BDCRB of an AD169 strain of HCMV mapped to exon 2 of the UL89 gene (Biron, Herpesvirus Workshop, 1994; Underwood *et al.*, ICAR, 1995) the UL89 gene of the Towne strains resistant to TCRB were sequenced. Strains B-11 and D-10 were again purified by limiting dilution, resistance to TCRB confirmed, and exon 2 amplified by PCR. The PCR DNA's were sequenced and the same mutation first found in the AD169 virus resistant to BDCRB (Asp 344 Glu) was found in the Towne strains resistant to TCRB. These results demonstrate the importance of UL89 in the action of benzimidazole ribonucleosides and strongly suggest that the UL89 gene product is the drug target. The studies were supported by grant U01-AI31718 from the N.I.A.I.D. and by Burroughs Wellcome Co.