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Characterization of Drug Resistance-Associated Mutations in the Human Cytomegalovirus DNA Polymerase.

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To determine the role of human cytomegalovirus (HCMV) DNA polymerase (UL54) mutations in HCMV drug resistance, recombinant UL54 mutant viruses were generated. Specific mutations or a segment of the UL54 sequence from the clinical isolates were transferred to laboratory strains AD169 and Towne by homologous recombination. The recombinant virus containing V781I in UL54 showed a 5-fold reduced sensitivity to foscarnet and a 2-fold reduced sensitivity to ganciclovir. Recently it has been shown that a recombinant UL54 mutant virus (V781I) generated by use of cotransfection of overlapping HCMV DNA, caused foscarnet resistance only (Cihlar et al, 1998; J Virol). We are now investigating whether the alterations D588N, E756K, and V787L in HCMV DNA polymerase are correlated to foscarnet resistance.

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Phenotypic and genotypic resistance pattern of human cytomegalovirus isolates obtained from vitreous and urine samples of AIDS patients with CMV-retinitis

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Human cytomegalovirus (CMV) retinitis is one of the most common opportunistic infections in patients with AIDS leading untreated to blindness. Although, rates of mortality and disease progression are improved by highly active antiretroviral therapy (HAART), current clinical anti-CMV drugs like ganciclovir (GCV), foscarnet (PFA) and cidofovir (HPMPC) still have the disadvantage of therapy failure due to mutations in the viral polymerase-gene (UL54) and/or viral kinase-gene (UL97) after maintenance therapy. To study the effects of continuous anti-CMV-treatment we compared 10 vitreous and urine isolates from AIDS patients with clinically proven CMV retinitis after HAART in terms of their (1) drug sensitivity; (2) UL54 and UL97 mutation pattern and (3) growth specificity in different cell types like human foreskin fibroblasts (HFF) and retinal pigmental endothelial (RPE) cells. Phenotypic resistance to GCV was shown in 6 isolates; one virus strain was resistant to GCV and PFA, whereas one isolate yielded multiresistance to GCV, PFA and HPMPC. Phenotypic resistance correlated with specific mutations within the UL54- and UL97-gene. All strains showed different growth properties in RPE cells and HFF. We showed that anti-CMV treatment under HAART yields CMV resistance patterns similar to those reported previously for patients without HAART. We also demonstrated establishment of organ specific CMV strains in terms of specific gene mutations, cell tropism and growth properties.

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Phenotypic and Genotypic Characterization of Antiviral Drug-Resistant Cytomegalovirus in Transplant Recipients.

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A total of 160 cytomegalovirus isolates from 110 bone marrow and solid organ transplant recipients, who had received ganciclovir (GCV) prophylaxis or pre-emptive therapy, were phenotypically tested for GCV resistance by the plaque reduction assay. Isolates from 8 patients were found to be resistant to GCV (IC_{50} >10 μM). Seven of these patients were lung transplant recipients and 1 was a kidney transplant recipient. All 8 recipients were CMV seronegative (R-) and received organs from CMV seropositive donors (D+). Serial isolates have been collected from 7 of these patients for a total of 24 isolates. Nine of the 24 isolates are GCV sensitive (IC_{50} < 6 μM) and were obtained either early post-transplant or after a change in antiviral treatment regimen. Genotypic changes in the UL97 coding sequence at amino acid residues known to confer GCV resistance have been identified in 12 of the resistant isolates. These include 460 M→I (1), 520 H→Q (5), 594 A→V (3) and 595 L→S (2). One isolate had a 594 A→P change, which has not yet been marker transferred to confirm linkage to the resistant phenotype. One isolate is a double mutant with a 987 A→G substitution in the DNA polymerase. Although a large number of CMV isolates were obtained from this group of patients, GCV resistance only occurred under D+/R- serological conditions. This supports the hypothesis that the development of antiviral drug resistance is a significant risk factor associated with primary CMV infection in transplant recipients.

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Structure-Activity Relationships of (*E*)-5-(2-Bromovinyl) uracil and Related Pyrimidine L-Nucleosides as Antiviral Agents for Varicella Zoster Virus

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The current treatment for VZV infections is based on acyclovir (ACV) and famciclovir. However, low efficacy and/or low oral bioavailability prompt the development of new antiviral agents for the treatment of VZV infections. As a result of our continuing efforts to discover novel antiviral nucleosides, we have recently synthesized a series of (*E*)-5-(2-bromovinyl)uracil analogues as potential anti-VZV agents in the hope to improve the current anti-VZV agents. (*E*)-5-(2-Bromovinyl)-2'-deoxy-L-uridine (L-BVDU), (*E*)-5-(2-bromovinyl)-1-β-L-arabinofuranosyl uracil (L-BVAU), (*E*)-5-(2-bromovinyl)-1-2'-deoxy-2'-fluoro-β-L-ribofuranosyl uracil (L-FBVRU), and (*E*)-5-(2-bromovinyl)-1-2'-deoxy-2'-fluoro-β-L-arabino-furanosyl uracil (L-FBVAU) were synthesized *via* appropriate 5-iodouracil analogues from L-arabinose. D- and L-Oxathiolane and -dioxolane nucleo-sides bearing (*E*)-5-(2-bromovinyl)uracil were prepared by glycosylation reaction of oxathiolane and dioxolane intermediates with silylated uracil analogues using TMSI as the coupling agent. L-Dioxolane nucleosides with 5-vinyl and 5-acetylene, and 5-ethyluracil were obtained from β-L-I-OddU by palladium-catalyzed coupling reaction. The synthesized compounds were evaluated *in vitro* against varicella zoster virus (VZV), Epstein-Barr virus (EBV), and herpes simplex virus 1 and 2 (HSV-1 and HSV-2). Among the tested compounds, β-L-CV-OddU, β-L-BV-OddU, and β-L-IV-OddU exhibited potent *in vitro* antiviral activity against VZV with EC_{50} values of 0.15, 0.07, and 0.035 μM, respectively. (This research was supported by NIH grant AI 33655).