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Contrasting drug resistance phenotypes resulting from cytomegalovirus DNA polymerase mutations at the same exonuclease locus

Sunwen Chou^{*,a,b}, Gail Marousek^b, Shaobing Li^c, and Adriana Weinberg^{c,d}

^aDivision of Infectious Diseases, Oregon Health and Science University, Portland, Oregon

^bVA Medical Center, Portland, Oregon

^cDivision of Infectious Diseases, University of Colorado Health Sciences Center, Denver, Colorado

^dPediatric Infectious Diseases, University of Colorado Health Sciences Center, Denver, Colorado

Abstract

Background—Diverse mutations in the cytomegalovirus (CMV) DNA polymerase (*pol*) gene confer resistance to one or more of the antiviral drugs ganciclovir, foscarnet or cidofovir. The levels of resistance conferred by specific mutations are variable, ranging from insignificant resistance to triple-drug resistance.

Objectives—Three *pol* mutations, I521T, P522A and P522L, detected in patients who received antiviral therapy for CMV infection, were studied by recombinant phenotyping to characterize their associated drug resistance.

Study Design—The individual mutations were transferred by homologous recombination into a reference CMV strain modified with a reporter gene and the drug concentrations required to reduce the reporter signal by 50% (IC50) were determined.

Results—The mutations I521T and P522A each conferred 3- to 4-fold increases in IC50 to both ganciclovir and cidofovir, while mutation P522L conferred no significant resistance to either drug. None of these mutations conferred foscarnet resistance.

Conclusions—The resistance phenotypes of mutations I521T and P522A are as predicted from the known mutation P522S, but divergent results with P522L indicate that different amino acid substitutions at the same position may not have the same effect on drug resistance. New mutations must be individually validated for proper interpretation of genotypic resistance testing.

Introduction

The human cytomegalovirus (CMV) DNA polymerase, encoded by the UL54 (*pol*) gene, is the target for all current systemic CMV drugs, including ganciclovir (GCV), foscarnet (FOS) and cidofovir (CDV). A considerable diversity of mutations in *pol* can confer resistance to any or all of these drugs, although GCV resistance usually begins with mutations in the viral UL97

*Corresponding author. Mailing address: VA Medical Center P31D, 3710 SW U.S. Veterans Hospital Road, Portland OR 97239, Phone 503-273-5115, FAX 503-273-5116, E-mail: chous@ohsu.edu.

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kinase gene¹. Because they confer different levels of resistance and cross-resistance, over 30 individual *pol* mutations have been characterized by recombinant phenotyping (marker transfer), a process in which they are transferred in isolation to reference drug sensitive CMV strains to determine the resulting level of resistance to each drug^{2–4}. The results are used for genotypic CMV resistance testing, where the UL97 and *pol* DNA sequences amplified from clinical specimens are checked for mutations known to confer drug resistance. Yet new mutations continue to be identified, and some published ones remain uncharacterized, resulting in less reliable interpretations of drug resistance based on similarities with known mutations. The traditional phenotypic approach of testing clinical isolates directly for drug sensitivity in cell culture is limited by current clinical laboratory practices that do not provide a live viral isolate, and the technical complexity of such assays. Continuing our practice of providing recombinant phenotyping data where they are lacking, we report here on 3 mutations at an exonuclease domain of *pol* that illustrate the divergent phenotypes that can result from different mutations at the same locus.

Methods

Strains and procedures for performing recombinant phenotyping have been published⁵. Briefly, reference strain T2211 was derived from standard strain AD169 by introducing unique restriction sites in *pol* and UL97, along with a secreted alkaline phosphatase (SEAP) expression cassette at US6, which enabled viral quantitation by assay of supernatant SEAP activity using a chemiluminescent substrate. PCR products containing the desired mutations were created by using specific mutagenic primers and then cloned into plasmid transfer vectors that contained a 6.6 kb *pol* region of the CMV genome. Mutations were transferred by digesting strain T2211 genomic viral DNA with restriction enzyme PmeI, and cotransfecting with the linearized transfer vector into human foreskin fibroblast cultures. The resulting recombinant viruses were checked throughout their *pol* sequence for presence of the desired mutation and absence of extraneous ones, and plaque-purified twice before determining the drug concentration required to reduce supernatant SEAP activity by 50% (IC50) 5–6 days after inoculation of calibrated input virus at a multiplicity of ~0.02. Each run included the sensitive parent strain T2211 as a control. Multiple assays were done on different dates and the mean and standard error IC50 values for each drug (GCV, FOS, CDV) calculated for each strain and compared to values for the parental T2211.

Results

CMV *pol* mutations at codons I521T, P522A and P522L in the exonuclease III domain were studied. Mutation I521T was published as part of a genotype of multiple UL97 and *pol* mutations in a pediatric stem cell recipient who developed multi-drug resistant CMV and fatal CMV encephalitis⁶. More recently, another pediatric stem cell recipient in Denver received 7 months of GCV treatment and had recurrent low-level CMV DNA in the peripheral blood, which contained *pol* mutation I521T and UL97 mutation C603W, the latter as a minority subpopulation mixed with wild type sequence (unpublished). The CMV DNA cleared from the blood after switching therapy to FOS, and was not associated with symptomatic disease. Mutation P522A was reported along with other mutations in UL97 and *pol* in a CMV isolate from a leukemia patient who was viremic after extended GCV and FOS therapy⁷. Mutation P522L was reported in conjunction with UL97 mutation E596G (the latter as ~70% of the sequence population) in a lung transplant recipient after >1 year of GCV therapy but without associated disease⁸.

Recombinant viruses containing each of the *pol* mutations I521T, P522A and P522L were constructed and plaque purified, and showed a normal growth phenotype in cell culture. Their drug resistance phenotypes are shown in Table 1. Mutations I521T and P522A showed

moderate 3- to 4-fold increases in IC₅₀ to both GCV and CDV, which is identical to the phenotype published for *pol* mutation P522S², despite significant differences in the techniques used for recombinant phenotyping. In contrast, mutation P522L conferred barely measurable increases in IC₅₀ values for GCV and CDV (less than 1.4-fold) and would not be considered to confer resistance to these drugs. None of the 3 mutations significantly affected FOS sensitivity.

Discussion

The interpretation of CMV DNA polymerase drug resistance mutations is complicated by their diversity and a track record of suspected resistance phenotypes that were not subsequently confirmed by recombinant phenotyping, including mutations at codons 515, 737, 845 and 971^{3,4,9}. The three mutations studied here are located in the exonuclease III domain, as recognized by sequence homology to the herpes simplex DNA polymerase structure¹⁰. Based on several established resistance mutations in this domain, including P522S^{2,3}, the general expectation is that exonuclease III mutations confer dual GCV-CDV resistance¹. Recombinant phenotyping proved this prediction to be correct for the mutations I521T and P522A, which had the same level of GCV-CDV resistance as P522S, but did not validate the published hypothesis that P522L similarly conferred GCV and CDV resistance⁸. The latter case did not have symptomatic CMV disease and was treated with a combination of GCV, FOS and CMV immunoglobulin with a reduction in viral load.

All of the cases involved in this study showed evidence of UL97 mutation accompanying the *pol* mutations, and 2 cases had multiple *pol* mutations that contributed to multi-drug resistance. Because the direct phenotypic testing of clinical isolates for antiviral drug resistance is impractical and rarely available, accurate interpretation of the UL97 and *pol* mutation profiles is important for guiding the selection of alternate therapy. When new mutations are detected, their resistance phenotypes need to be experimentally determined, so that the genotype-phenotype information database can be improved for the benefit of subsequent genotypic resistance testing. Interpretations of phenotype based on analogies to known mutations must always remain tentative.

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Table 1

Genotypes and phenotypes of recombinant viruses

Strain	UL54- <i>pol</i> mutation	SEAP IC ₅₀ (μM) ^I		
		GCV	FOS	CDV
T2211	-	1.1 ± 0.1	36 ± 3	0.22 ± 0.01
T3003	I521T	3.4 ± 0.5	33 ± 2	0.85 ± 0.1
T3005	P522A	3.3 ± 0.4	35 ± 2	0.90 ± 0.08
T3001	P522L	1.4 ± 0.07	43 ± 3	0.30 ± 0.01

^I mean ± SEM of at least 8 determinations from 3 or more setup dates