ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1995, p. 1204–1205 Vol. 39, No. 5
0066-4804/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Novel Mutation in the UL97 Gene of a Clinical Cytomegalovirus Strain Conferring Resistance to Ganciclovir

MICHELLE N. HANSON,[1] LAUREL C. PREHEIM,[2] SUNWEN CHOU,[3] CHRISTINE L. TALARICO,[4] KAREN K. BIRON,[4] AND ALEJO ERICE[1,5]*

Departments of Laboratory Medicine and Pathology[1] and Medicine,[5] University of Minnesota Medical School, Minneapolis, Minnesota; Department of Medicine, Veterans Administration Medical Center, Omaha, Nebraska[2]; Medical and Research Services, Veterans Affairs Medical Center, Portland, Oregon[3]; and Division of Virology, Burroughs Wellcome Company, Research Triangle Park, North Carolina[4]

Received 24 October 1994/Returned for modification 15 December 1994/Accepted 9 March 1995

Characterization of a ganciclovir-resistant cytomegalovirus strain from a patient with AIDS showed a histidine-to-glutamine change at residue 520 of UL97 (Q520 mutation). In anabolism studies, Q520 was associated with impaired phosphorylation of ganciclovir. Transfer of Q520 to a recombinant virus resulted in a ganciclovir-resistant phenotype.

Resistance of clinical and laboratory cytomegalovirus (CMV) strains to ganciclovir has been associated with impaired phosphorylation of this compound in virus-infected cells (11). Characterization of ganciclovir-resistant CMV laboratory strains has demonstrated the presence of amino acid deletions (residues 590 to 593) or substitutions (residue 460) in conserved regions of the UL97 protein and/or point mutations in the DNA polymerase of the virus (2, 9, 10, 12, 13). In clinical CMV strains, resistance to ganciclovir has been associated with substitutions (residues 460, 594, and 595) or deletions (residue 595) in UL97 (1, 4, 14). We have identified a novel mutation in the UL97 protein of a ganciclovir-resistant CMV strain and demonstrated that this mutation is responsible for resistance to ganciclovir.

(This work was presented in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., 6 October 1994.)

Viruses studied were from an AIDS patient with progressive CMV disease (retinitis and gastrointestinal disease) and included an early isolate obtained prior to ganciclovir therapy and a late isolate (C-9330) from a sample of rectal tissue obtained after 421 days of ganciclovir therapy. Ganciclovir susceptibilities were determined by a DNA hybridization method (3). A fragment of the catalytic domain of UL97 was amplified by PCR using viral DNA purified from fibroblasts infected with the patient's CMV isolates and primers designed on the basis of published data on strain AD169. Primer sequences were 5′-CATCGACGTTTCCACACAGAC-3′ (Z2671, forward) and 5′-TTGCGCCGCCAGAATGAGCAG-3′ (Z2672, reverse). PCRs were performed with *Taq* polymerase and consisted of 35 cycles at 95°C for 1 min, 55°C for 2 min, and 72°C for 1 min. Amplified products were filter purified and sequenced with a commercial kit (Prism Ready Reaction Dyedeoxy Terminator Cycle sequencing kit; Applied Biosystems) and sequencing primers Z2671 (forward), Z2828 (5′-ATCCGGATTACAACGAGCGCT-3′, forward), Z2672 (reverse), and Z2829 (5′-TAACATTCGCGCAGACGGTGC-3′, reverse). UL97 sequences were aligned with that of strain AD169 to determine whether mutations were present in the region analyzed. A recombinant virus (C-9330-5) was obtained in marker transfer experiments after cotransfection of MRC5 cells with full-length AD169 DNA and an 858-bp UL97 DNA fragment (encompassing codon 520) amplified by PCR from strain C-9330 using primers VS9714 (5′-ATGTTCTTGCGC CTTACGCA-3′, forward) and CT9729 (5′-CCATGCGCAC CTCGTCC-3′, reverse) (12). For ganciclovir anabolism studies, MRC5 cells infected with isolate C-9330 or the recombinant strain C-9330-5 were pulse-labeled with purified 8-^{14}C-ganciclovir, and anabolites of the drug were measured with a cation-exchange column (11).

Ganciclovir susceptibilities were determined at least on two occasions for each virus. The early CMV isolate was sensitive, with a mean 50% inhibitory concentration of ganciclovir of 1.2 ± 0.04 μM (standard error). C-9330 and the recombinant strain C-9330-5 were resistant, with mean 50% inhibitory concentrations of ganciclovir of >200 and 12.7 ± 1.25 μM, respectively. The mean 50% inhibitory concentration for control strain AD169 was 1.27 ± 0.83 μM. C-9330 contained a single nucleotide change (CAC to CAG) at nucleotide 1560 which resulted in a histidine-to-glutamine (H to Q) substitution at residue 520 of UL97 (Q520 mutation). This mutation was not found in the patient's early isolate or the control strain AD169. Sequence analysis of UL97 codons 300 to 700 of the recombinant strain C-9330-5 confirmed the presence of Q520 and the absence of other mutations. Cells infected with C-9330 or the recombinant C-9330-5 showed a marked reduction in total phosphorylation of ganciclovir (34 and 21% of that for control strain AD169, respectively). These reduced phosphorylation levels were similar to those in cells infected with the ganciclovir-resistant control strain 759^{r}D100.

Screening for Q520 was performed by digesting with *Alu*I a PCR product obtained with primers CPT1088 (5′-ACGGTG CTCACGGTCTGGAT-3′) and CPT1587M (5′-CTGCAG CGGCATGGGTCGGAAAGCAAG-3′). In the presence of Q520, primer CPT1587M creates an additional *Alu*I site. Therefore, digestion of DNA from Q520 mutants resulted in three fragments (285, 188, and 27 bp) instead of the two fragments (312 and 188 bp) obtained with digestion of wild-type CMV strains. This distinctive change in the restriction enzyme pattern was readily visualized by gel electrophoresis (Fig. 1).

That Q520 is responsible for resistance to ganciclovir is supported by (i) its absence in the ganciclovir-sensitive virus isolated prior to therapy, (ii) the demonstration that transfer of

* Corresponding author. Mailing address: University of Minnesota, Box 609 UMHC, 420 Delaware St. SE, Minneapolis, MN 55455-0392. Phone: (612) 626-5670 or 0920. Fax: (612) 625-5468. Electronic mail address: erice001@maroon.tc.umn.edu.

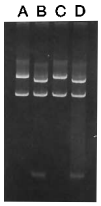

FIG. 1. Detection of the Q520 mutation by restriction analysis. Fragments of the UL97 region amplified by PCR using primers CPT1088 and CPT1587M (see the text) were digested with *Alu*I and analyzed on a 15% polyacrylamide gel. Lane A, pretherapy isolate; lane B, isolate C-9330; lane C, strain AD169; lane D, recombinant strain C-9330-5. Viruses with the Q520 mutation have an additional *Alu*I restriction site which reduces the size of the higher-molecular-weight band and yields an additional smaller fragment.

Q520 to a recombinant virus conferred resistance to ganciclovir, and (iii) the marked reduction in the ability of cells infected with Q520 mutants to phosphorylate ganciclovir. In a recent study, characterization of 10 ganciclovir-resistant clinical strains demonstrated that all contained mutations at residue 460, 594, or 595 of UL97 (4). None of those strains contained a mutation at residue 520, suggesting that the occurrence of this mutation is low. Nevertheless, Q520 may have functional importance because it occurs at a conserved region of UL97 (subdomain VIII) which appears to be important in the catalytic activity of the protein (8).

The 50% inhibitory concentration of ganciclovir for C-9330 was higher than that for the recombinant virus C-9330-5. One possibility is that C-9330 could also contain a mutation in the DNA polymerase gene that would make it more resistant to ganciclovir than the single-mutation recombinant virus. Point mutations located within conserved regions of the DNA polymerase gene of CMV laboratory strains have been associated with resistance to ganciclovir (10, 12). Sequencing studies of the DNA polymerase gene of C-9330 are in progress in our laboratory.

The clinical course of the patient from whom the Q520 mutant CMV was isolated was similar to those of three patients described earlier by our group, who were also infected with ganciclovir-resistant CMV strains (7). In one prospective study, the isolation of ganciclovir-resistant CMV from patients with AIDS was associated with progression of retinitis (5). Therefore, infections with ganciclovir-resistant CMV can be severe and cause progressive end organ disease. Rapid detection of ganciclovir-resistant viruses may facilitate clinical decisions to switch to alternative therapies. Characterization of mutations conferring resistance to ganciclovir may provide the basis for the development of molecular assays for rapid detection of resistant CMV strains.

This work was supported by National Institutes of Health grants AM13083 and A127761 and grants from the Minnesota Medical Foundation.

We are indebted to Sharon Henry for technical assistance and to Julian Verheyden (Syntex Corporation, Palo Alto, Calif.) for providing ganciclovir.

REFERENCES

1. **Baldanti, F., E. Silini, A. Sarasini, C. L. Talarico, S. C. Stanat, K. K. Biron, M. Furione, F. Bono, G. Palú, and G. Gerna.** 1995. A three-nucleotide deletion in the UL97 open reading frame is responsible for the ganciclovir resistance of a human cytomegalovirus isolate. J. Virol. **69:**796–800.
2. **Biron, K. K., J. A. Fyfe, S. C. Stanat, L. K. Leslie, J. B. Sorrell, C. U. Lambe, and D. M. Coen.** 1986. A human cytomegalovirus mutant resistant to the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl} guanine (BW B759U) induces reduced levels of BW B759U triphosphate. Proc. Natl. Acad. Sci. USA **83:**8769–8773.
3. **Boivin, G., A. Erice, D. D. Crane, D. L. Dunn, and H. H. Balfour, Jr.** 1993. Ganciclovir susceptibilities of cytomegalovirus (CMV) isolates from solid organ transplant recipients with CMV viremia after antiviral prophylaxis. J. Infect. Dis. **168:**332–335.
4. **Chou, S., A. Erice, M. C. Jordan, G. M. Vercellotti, K. R. Michels, C. L. Talarico, S. C. Stanat, and K. K. Biron.** 1995. Analysis of the UL97 phosphotransferase coding sequence in clinical cytomegalovirus isolates and identification of mutations conferring ganciclovir resistance. J. Infect. Dis. **171:**576–583.
5. **Drew, W. L., R. C. Miner, D. F. Busch, S. E. Follansbee, J. Gullett, S. G. Mehalko, S. M. Gordon, W. F. Owen, Jr., T. R. Matthews, W. C. Buhles, and B. DeArmond.** 1991. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J. Infect. Dis. **163:**716–719.
6. **Drobyski, W. R., K. K. Knox, D. R. Carrigan, and R. C. Ash.** 1991. Foscarnet therapy of ganciclovir-resistant cytomegalovirus in marrow transplantation. Transplantation **52:**155–157.
7. **Erice, A., S. Chou, K. K. Biron, S. C. Stanat, H. H. Balfour, Jr., and M. C. Jordan.** 1989. Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. N. Engl. J. Med. **320:**289–293.
8. **Hanks, S. K., A. M. Quinn, and T. Hunter.** 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domain. Science **214:**42–52.
9. **Lurain, N. S., L. E. Spafford, and K. D. Thompson.** 1994. Mutation in the UL97 open reading frame of human cytomegalovirus strains resistant to ganciclovir. J. Virol. **68:**4427–4431.
10. **Lurain, N. S., K. D. Thompson, E. W. Holmes, and G. S. Read.** 1992. Point mutations in the DNA polymerase gene of human cytomegalovirus that result in resistance to antiviral agents. J. Virol. **66:**7146–7152.
11. **Stanat, S. C., J. E. Reardon, A. Erice, M. C. Jordan, W. L. Drew, and K. K. Biron.** 1991. Ganciclovir-resistant cytomegalovirus clinical isolates: mode of resistance to ganciclovir. Antimicrob. Agents Chemother. **35:** 2191–2197.
12. **Sullivan, V., K. K. Biron, C. Talarico, S. C. Stanat, M. Davis, L. M. Pozzi, and D. M. Coen.** 1993. A point mutation in the human cytomegalovirus DNA polymerase gene confers resistance to ganciclovir and phosphonylmethoxyalkyl derivatives. Antimicrob. Agents Chemother. **37:**19–25.
13. **Sullivan, V., C. L. Talarico, S. C. Stanat, M. Davis, D. M. Coen, and K. K. Biron.** 1992. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature (London) **358:**162–164.
14. **Wolf, D. G., I. L. Smith, J. L. Delphine, W. R. Freeman, M. Flores-Aguilar, and S. S. Spector.** 1995. Mutations in human cytomegalovirus UL97 confer clinical resistance to ganciclovir and can be detected directly in patient plasma. J. Clin. Invest. **95:**257–263.