Single Amino Acid Change in DNA Polymerase Is Associated with Foscarnet Resistance in a Varicella-Zoster Virus Strain Recovered from a Patient with AIDS

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The genetic characterization of a foscarnet-resistant strain of varicella-zoster virus (VZV) that was isolated from a patient with AIDS is reported. Compared with the sequence of the Dumas reference strain, this strain, which was isolated from a patient in whom foscarnet treatment failed, had two point mutations. The emergence of one of the mutations, which includes a change from a glutamic acid to a lysine at position 512 in the DNA polymerase, suggests that this mutation is implicated in the VZV foscarnet resistance. The other mutation, which replaces serine 863 by a glycine, is also present in 2 susceptible strains—Oka and a wild-type isolate.

Varicella-zoster virus (VZV) can cause persistent and occasionally disseminated herpes zoster in patients with AIDS. Acyclovir is the first line of treatment for VZV infection in immunodeficient patients [1]. Since 1988, cases of herpes zoster and meningoencephalitis resistant to acyclovir have been described [2, 3]. Acyclovir-resistant VZV infection usually responds to foscarnet therapy [4].

Most studies of VZV sensitivity to foscarnet have yielded conflicting results between clinical and in vitro resistance. Safrin et al. [4] described a case of herpes zoster in a patient with AIDS, which responded to foscarnet despite altered in vitro sensitivity. In contrast, 2 patients with in vitro–susceptible strains failed to respond to foscarnet: 1 had AIDS [4] and 1 had received a bone-marrow transplant [5].

One case of clinical resistance to foscarnet with corresponding in vitro resistance has been described in our laboratory [6]. In brief, a 28-year-old AIDS patient, who received intravenous foscarnet (6 g daily) from October 1992 as maintenance therapy for cytomegalovirus (CMV) retinitis, had a first episode of left brachial zoster in October 1993 followed by a recurrence in February 1994; both episodes were treated with acyclovir. Five weeks later, the patient had a second recurrence of brachial zoster, which was treated with intravenous acyclovir for 14 days, and foscarnet was discontinued. Three days after the beginning of acyclovir treatment, we isolated a VZV strain from a skin lesion on the patient’s left arm by culture of a skin biopsy. Since treatment with acyclovir failed, the patient's therapy was changed to foscarnet (12 g daily) for 15 days; however, the lesions did not respond. The isolate was found to be resistant to foscarnet in vitro. The average IC₅₀ of foscarnet was 350 μM for the isolate compared with 88 μM for the reference Oka strain that was evaluated in parallel. The mean IC₅₀ of foscarnet for the isolate was higher than that for 2 susceptible strains recovered from human immunodeficiency virus–infected patients who did not receive foscarnet [6].

Herein, we report the genetic characterization of this foscarnet resistance. Foscarnet is a pyrophosphate analogue that inhibits VZV DNA polymerase. To detect the emergence of mutations during treatment, we compared the sequence of the DNA polymerase gene of the strain isolated after foscarnet failure to that of the reference strain Dumas.

Materials and Methods

Amplification of the VZV DNA polymerase gene. VZV DNA was extracted from an MRC5 cell culture of a skin biopsy from the patient. Cells were treated with trypsin, recovered, and centrifuged. Cell pellets were lysed and extracted with phenol-chloroform before precipitation with alcohol. The primer sequences used to amplify a fragment containing the DNA polymerase gene (open-reading frame 28 position 50636–47055) were determined from the sequence of the Dumas strain [7]. The sequences of the two primers were (Pol1) 5'-CGGGATCCGTCATATTAATAGT-CGCAC and (Pol2) 5'-CGGGATCTAAACGTAACCAGGG-GCCC, with addition of the BamHI site at the 5' end. Amplification involved a hot-start procedure with a polymerase chain reaction (PCR) kit (Taq Extender PCR Additive kit; Stratagene, La Jolla, CA). The first-round cycle was 95°C for 7 min, followed by 38 rounds at 95°C for 1 min, 58°C for 1 min, and 72°C for 4 min. The final cycle was 72°C for 12 min.

Cloning and sequencing. After being analyzed in 0.8% agarose gel, the amplification products were purified by use of the Wizard PCR Prep kit (Promega, Madison, WI). Amplified products of the VZV isolate were digested and cloned to the BamHI site of the Bluescript II SK(+) plasmid (Stratagene). Clones were sequenced as described by Mabillat et al. [8], with the procedure being adapted to a sequencing kit (T7; Pharmacia, Milwaukee).
The sample was sequenced on both strands. Mutations were confirmed on 2 other clones of the same strain.

**Sequence analysis.** The full VZV DNA polymerase gene sequence was determined by use of computer software (GeneWorks 2.45; IntelliGenetics, Mountain View, CA), and the sequence was compared with that of the Dumas strain [7] obtained from GenBank.

**Results**

Our PCR method allowed us to amplify a fragment of the 3990 bp that contained the VZV DNA polymerase gene. Compared with the sequence of the Dumas strain [7], the strain from the VZV isolate had three mutations: G to A at position 49103 of the complete genome and position 1534 of the DNA polymerase gene, which entails a nonconservative change from an acidic amino acid (glutamic acid) to a basic amino acid (lysine) at position 512; A to G at position 48050 of the complete genome and position 2587 of the DNA polymerase gene, which changed the serine at position 863 into a glycine; and a silent mutation, T to C at position 49004 of the complete genome and position 1632 of the DNA polymerase gene.

**Discussion**

We have observed the genetic basis for a case of VZV resistance to foscarnet. Compared with the sequence of the Dumas reference strain, the sequence of the strain isolated after foscarnet failure had two point mutations.

The mutation replacing serine 863 by a glycine in the VZV strain we characterized as resistant was also present in 2 susceptible strains (data not shown), an Oka strain (a reference strain), and a wild-type isolate recovered from a patient with varicella who never received any antiviral drugs. This finding shows that this mutation is not responsible for foscarnet resistance.

We detected a mutation in the sequence coding for the glutamic acid at position 512 leading to a lysine substitution in the VZV strain resistant to foscarnet. The emergence of this mutation in the DNA polymerase gene suggests that this mutation is implicated in resistance. One drug-resistant VZV mutant exhibiting the same mutation has been mentioned [9].

By analogy with the DNA polymerase gene of herpes simplex virus, we situated the mutation at position 512 between the conserved regions IV and A described elsewhere [10–12]. Although outside a conserved region, this nonconservative mutation was located in a region common to all herpesviruses (figure 1). It has been demonstrated that the mutation causing resistance of CMV to ganciclovir may be situated outside a conserved region of the DNA polymerase gene: The conservative substitution of a leucine by an isoleucine was also found between conserved region IV and A and accounted for resistance of mutant CMV strains to ganciclovir [13]. In other herpesviruses, substitution of a single amino acid in a conserved region of the DNA polymerase gene can cause resistance. Indeed, a single conservative or nonconservative mutation can lead to resistance of herpes simplex virus to foscarnet or phosphonoacetic acid [9–11]. The same phenomenon has been observed in ganciclovir-resistant CMV mutants derived from CMV strain AD169 [13, 14], and in isolates from patients resistant to ganciclovir and foscarnet [15].

We conclude that a single amino acid substitution at position 512 in the VZV DNA polymerase gene may confer resistance to foscarnet. However, a study of a larger number of strains (foscarnet-resistant clinical isolates or laboratory mutants) will be necessary to obtain a fuller picture of the genetic bases of resistance.

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**Figure 1.** Structure of VZV DNA polymerase gene. Upper part of figure shows positions of polymerase chain reaction primers (Pol1–Pol2) relative to VZV DNA polymerase gene. Black boxes on representation of corresponding protein correspond to conserved domains. Amino acid (aa) sequence of fragment of isolate (between conserved regions IV and A, residues 500–533) contains mutation associated with foscarnet-resistant phenotype (bold type). Sequences of same region of VZV (Dumas strain), herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus (HHV) 6 and 7 DNA polymerases are shown for comparison. Conserved aa among HSV and other herpesviruses are shaded in gray.
VZV DNA polymerase gene–mediated resistance to foscarnet, and such a study may open the way to a genetic test for resistance that does not require VZV isolation by cell culture.

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References