Characterization of the DNA polymerase gene of varicella-zoster viruses resistant to acyclovir

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The nucleotide changes of the DNA polymerase gene and the susceptibility of acyclovir (ACV)-resistant varicella-zoster virus (VZV) mutants to anti-herpetic drugs were determined and compared to those of herpes simplex virus type 1 (HSV-1) mutants. The seven ACV-resistant VZV mutants were classified into three groups, N779S, G805C and V892M, according to the sequences of their DNA polymerase genes. The amino acid substitutions N779S and G805C were identical in position to the N815S and G814C mutations in the HSV-1 DNA polymerase mutants, respectively, and the V892M amino acid substitution was similar to the HSV-1 V893M mutation. All three groups of VZV mutants were susceptible to ACV, phosphonoacetic acid, vidarabine and apheridicolin, at levels similar to those seen with the respective HSV-1 mutants, except for subtle differences that were due possibly to the non-conserved regions in their sequences. Although both the HSV-1 and the VZV DNA polymerase genes show 53% sequence similarity, both viruses essentially show a similar biochemical behaviour.

Varicella-zoster virus (VZV) infection has been treated mainly with acyclovir (ACV) and penciclovir, both of which are anti-viral nucleoside analogues that exhibit anti-VZV activity through phosphorylation by thymidine kinase (TK) and inhibition of viral DNA synthesis (Elion et al., 1977; Biron & Elion, 1980; Elion, 1993; Boyd et al., 1987). In immunocompetent hosts and in clinical practice, conventional ACV treatments rarely generate ACV-resistant viruses. However, in immunocompromised hosts, such as patients in the late phase of human immunodeficiency virus infection, VZV infection tends to be severe and prolonged; ACV-resistant VZV mutants have emerged after long-term treatment with ACV (Pahwa et al., 1988; Hoppenjans et al., 1990; Jacobson et al., 1990; Talarico et al., 1993; Boivin et al., 1994; Snoeck et al., 1994). Most of the ACV-resistant mutants that have been isolated either from cell culture or from patients have mutations within the TK gene. However, the DNA polymerase of foscamet-resistant VZV isolated from a patient with AIDS has been characterized and the mutation responsible for the foscamet resistance was located within the non-conserved region of the DNA polymerase gene (Visse et al., 1998).

In this study, we determined the nucleotide changes in the DNA polymerase gene of ACV-resistant VZV mutants. These mutants were analysed for their susceptibility to anti-herpetic drugs. In addition, we have demonstrated identical amino acid substitutions and a discrete discrepancy in the drug susceptibility between VZV and herpes simplex virus type 1 (HSV-1) DNA polymerase mutants.

Human embryonic lung (HEL) cells were grown and maintained in Eagle’s minimum essential medium supplemented with 10% foetal bovine serum (FBS) for growth or 2% FBS for maintenance. We used the VZV parent Kawaguchi strain which was serially plaque-purified six times using cell-free virus (Shiraki et al., 1983, 1985, 1992). The ACV-resistant virus strains A1–A6 and A8, each with mutations in the DNA polymerase gene, were isolated in the presence of increasing concentrations (4.5, 11.3 and 22.5 µg/ml) of ACV (100 µM). Mutants were passaged three times at each concentration until the appearance of typical cytopathology. After two plaque purification steps in the presence of 100 µM ACV, the plaque-purified viruses were used as ACV-resistant mutants (Shiraki et al., 1983, 1990).

VZV DNA was prepared from cell cultures infected with the parent Kawaguchi strain or from the various mutants (Shiraki et al., 1991a, b). The 3.6 kb fragment encoding the VZV DNA polymerase gene was amplified by PCR and sequenced using the Auto Sequencer Core kit (Toyobo) with Cy5-labelled primers designed according to the sequence of VZV Dumas strain (Davison & Scott, 1986). The cycle
Table 1. Genotypic and phenotypic characterization of ACV-resistant VZV

<table>
<thead>
<tr>
<th>Clone</th>
<th>Mutation</th>
<th>EC₅₀ (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nucleotide</td>
<td>Amino acid</td>
</tr>
<tr>
<td>A1</td>
<td>48224 GGC → TGC</td>
<td>G⁶⁸⁵C</td>
</tr>
<tr>
<td>A2</td>
<td>48074 GTG → ATG</td>
<td>V⁶⁸⁵M</td>
</tr>
<tr>
<td>A3</td>
<td>48224 GGC → TGC</td>
<td>G⁶⁸⁵C</td>
</tr>
<tr>
<td>A4</td>
<td>48224 GGC → TGC</td>
<td>G⁶⁸⁵C</td>
</tr>
<tr>
<td>A5</td>
<td>48074 GTG → ATG</td>
<td>V⁶⁸⁵M</td>
</tr>
<tr>
<td>A6</td>
<td>48301 AΔT → AGT</td>
<td>N⁷⁷⁹S</td>
</tr>
<tr>
<td>A8</td>
<td>48301 AΔT → AGT</td>
<td>N⁷⁷⁹S</td>
</tr>
<tr>
<td>Kawaguchi</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* EC₅₀ is expressed as the mean ± SE of more than three independent experiments.

Fig. 1. Mutations in the HSV-1 and VZV DNA polymerase gene. Filled boxes show the conserved regions I–VII of the HSV-1 DNA polymerase gene. Region A, which shows a lesser degree of conservation, is also shown. HSV-1 DNA polymerase mutants reported previously are shown above. The amino acid sequence of conserved regions III and I of HSV-1 and VZV are shown below; mutation sites are boxed. VZV mutants N⁷⁷⁹S, G⁸⁰⁵S and V⁸⁵⁵M identified in this study are shown in the open boxes. Dotted arrows indicate the different amino acids between the parent Kawaguchi strain and the Dumas strain in the EMBL database. The numbers in parentheses indicate the sources from where other mutants were reported. 1, Larder et al. (1987); 2, Gibbs et al. (1988); 3, Tsurumi et al. (1987); 4, Knopf & Weisshart (1988); 5, Hwang et al. (1992); 6, Marcy et al. (1990); 7, Collins et al. (1989); 8, Chiou et al. (1995); 9, Visse et al. (1998); 10, Collins & Darby (1991); 11, Visse et al. (1999).
sequencing reaction products were run on the ALF DNA Sequencer (Pharmacia).

The susceptibility of each ACV-resistant VZV mutant to ACV (Sigma), phosphonoacetic acid (PAA) (Sigma), 9-β-d-arabinofuranosyladenine (Ara-A) (ICN) and aphidicolin (Aph) (Wako Pure Chemical Industries) was determined by plaque reduction assays in HEL cells (Ida et al., 1999; Shiraki et al., 1983, 1992). Briefly, confluent monolayers of HEL cells in 60 mm plastic Petri dishes (in duplicate) were inoculated with 100 p.f.u. per dish of cell-free virus in 0.2 ml SPGC medium (PBS supplemented with 5% sucrose, 0.1% sodium glutamate and 10% FBS). After incubation for 1 h to permit adsorption, 5 ml of maintenance medium and the various concentrations of drug were added. After 5 days of incubation, cells were fixed and stained, after which plaques were counted. The 50% effective concentration (EC50) was defined as the concentration that reduced plaque formation by 50%.

Genotypic and phenotypic characterizations of ACV-resistant mutants are summarized in Table 1. Sequence analysis of the DNA polymerase gene of ACV-resistant mutants showed that each mutant had a single nucleotide substitution. All seven ACV-resistant mutants showed only a single amino acid change in the whole protein. The ACV-resistant mutants A1, A3 and A4 showed a G → T change at nucleotide position 48224, which resulted in the amino acid substitution G866C. These three mutants were resistant to ACV and PAA, sensitive to Ara-A and hypersensitive to Aph. The ACV-resistant mutants A2 and A5 showed a G → A change at nucleotide position 48074, which resulted in the amino acid substitution V854M. These two mutants were resistant to ACV, PAA and Ara-A and hypersensitive to Aph. The ACV-resistant mutants A6 and A8 showed an A → G change at nucleotide position 48301, which resulted in the amino acid substitution N779S. These two mutants were resistant to ACV, sensitive to Aph and hypersensitive to PAA and Ara-A. Thus, seven ACV-resistant mutants were classified into three groups according to their genotypic and phenotypic characterizations. Mutations in the TK gene of ACV-resistant mutants were observed near the guanosine homopolymer in HSV (Sasadeusz et al., 1997) or downstream from two sequential guanosine nucleotides in VZV (Ida et al., 1999), but the nucleotide changes in the VZV DNA polymerase gene were not related to these sequences, thus indicating that these mutations were probably not induced by ACV.

The vast majority of drug-resistant mutants in the HSV-1 DNA polymerase gene reported previously have been mapped within the conserved regions I–III and A (Larder et al., 1987; Marcy et al., 1990; Chiou et al., 1995; Andrei et al., 2000). Many drug-resistant mutations were reported to be located especially within regions II and III. We compared the DNA polymerase gene sequence of the parent Kawaguchi strain used in this study with that of the Dumas strain in the EMBL database. The parent Kawaguchi strain had six nucleotide differences compared with the Dumas strain; T → C at nucleotide position 47162, G → A at nucleotide position 47939, A → G at nucleotide position 48050, C → T at nucleotide positions 48825 and 49535 and G → T at nucleotide position 50081, resulting in three amino acid substitutions, G196S, S862G and C1140R (Fig. 1). The amino acid changes between the Kawaguchi and the Dumas strains were located in non-conserved regions within the DNA polymerase gene. These data suggest that the difference in DNA sequence between the two strains may not effect the enzyme activity of the DNA polymerase.

Visse et al. (1998) have reported a foscarnet-resistant VZV strain from a patient with AIDS; its amino acid changes compared with the Dumas strain are E512K and S863G (Fig. 1). The amino acid substitution at residue 863 is identical to that in the Kawaguchi strain. Visse et al. (1998) reported that the E512K substitution in the non-conserved region implicated foscarnet resistance. Collins & Darby (1991) have reported two ACV-resistant VZV mutants with A684T and N775S mutations in the VZV DNA polymerase gene. The latter mutation is identical to the N775S mutation in our study.

We compared the mutations of the VZV DNA polymerase gene with those of the HSV-1 DNA polymerase gene, as shown in Fig. 1. The positions of the three mutations at amino acid residues 779, 805 and 855 found in the ACV-resistant VZV corresponded to amino acid residues 815, 841 and 890 of HSV-1, respectively, which were within the conserved regions III and I of the HSV-1 DNA polymerase gene. The mutations found at residues 779 and 805 of VZV were identical to residues 815 and 841 in HSV-1, respectively, as reported previously (Larder et al., 1987; Chiou et al., 1995). Region III of the VZV DNA polymerase gene may interact directly with drug–substrate binding, in a manner similar to that of the conserved region III of the HSV-1 DNA polymerase gene (Larder et al., 1987; Gibbs et al., 1988). Marcy et al. (1990) have reported that one of the HSV-1 DNA polymerase mutations has a V882M change. Although V855 of VZV corresponds to V880 of HSV-1, as shown in Fig. 1, the V855M mutation in VZV may correspond to the V880M mutation in HSV-1 by way of biochemical behaviour.

We compared the drug susceptibility of the VZV DNA polymerase mutants with those reported for the HSV-1 DNA polymerase mutants (Table 2) containing identical amino acid substitutions (Larder et al., 1987; Chiou et al., 1995; Marcy et al., 1990). This indicates that the role of the conserved regions is identical between the two DNA polymerases. The HSV-1 mutants with the N815S mutation were susceptible to PAA and Ara-A and more resistant to ACV, similar to the VZV mutants with the N775S mutation. Although HSV-1 N815S mutants were resistant to Aph, VZV N775S mutants were as sensitive to Aph as wild-type VZV. The mutants with a G to C change at amino acids 841 and 805 of HSV-1 and VZV, respectively, showed similar susceptibility to Ara-A and were resistant to both ACV and PAA. The mutants with a V to M change at 892 of HSV-1 and 855 of VZV showed similar susceptibility to
Aph and similar resistance to PAA. Among the four anti-herpetic drugs, the VZV V855M mutant was more resistant to ACV than the HSV-1 V892M mutant. In spite of both the identical and similar amino acid substitutions, ACV-resistant mutants of VZV and HSV-1 showed a different susceptibility to ACV and Aph. It was suggested that the discrepancy in drug susceptibility between HSV-1 and VZV might be reflected from the non-conserved regions, which may have caused a minute difference in the affinity of the DNA polymerase to ACV and Aph.

In this study, the seven ACV-resistant VZV mutants were classified into three groups, N779S, G805C and V855M, according to the sequences of their DNA polymerase genes. The amino acid substitutions N779S and G805C were identical in position to N815S and G814C, respectively, and V855M was similar in position to V892M, as reported for the HSV-1 DNA polymerase mutants (Fig. 1). The sequence similarity between the HSV-1 and the VZV DNA polymerase genes is only 53%, but both DNA polymerases are biochemically similar, due possibly to the highly conserved regions that are essential for enzyme activity. The N779S and V855M mutants exhibited a discrepancy in the ratio of sensitivity to ACV or Aph in comparison to the corresponding HSV-1 mutants. It was suggested that the different conformations created by the differences in the non-conserved regions of VZV and HSV-1 may have caused the discrepancy in drug susceptibility of the ACV-resistant mutants with identical and similar amino acid substitutions.

We thank Ms Jacqueline Brown for her editorial assistance.

References


Table 2. Comparison of the sensitivity of ACV-resistant HSV-1 to anti-herpetic drugs with ACV-resistant VZV

The ratio of sensitivity was determined as the EC_{50} ratio of the mutant over the parent. The substitutions N779S, G805C and V855M of the HSV-1 mutants were referenced from Larder et al. (1987), Coen et al. (1985) and Marcy et al. (1990), respectively.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Amino acid substitution</th>
<th>Clone</th>
<th>ACV</th>
<th>PAA</th>
<th>Ara-A</th>
<th>Aph</th>
</tr>
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<tbody>
<tr>
<td>HSV-1</td>
<td>N779S</td>
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<td>59</td>
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<td>0.10</td>
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<td></td>
<td>N779S</td>
<td>TP4.1</td>
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<td>0.12</td>
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<tr>
<td></td>
<td>G814C</td>
<td>AraA7</td>
<td>15</td>
<td>&gt; 3</td>
<td>3</td>
<td>&lt; 0.33</td>
</tr>
<tr>
<td>VZV</td>
<td>V892M</td>
<td>–</td>
<td>2.8*</td>
<td>4.9*</td>
<td>ND</td>
<td>0.54*</td>
</tr>
<tr>
<td></td>
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<td>A6</td>
<td>38</td>
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<td>0.05</td>
<td>1.3</td>
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<tr>
<td></td>
<td>N779S</td>
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<td>31</td>
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<tr>
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<td>6.0</td>
<td>2.1</td>
<td>0.33</td>
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* These data were obtained from Marcy et al. (1990).

ND, No data.


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