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Short Communication

Analysis of mutations in the thymidine kinase gene of varicella zoster virus associated with resistance to 5-iodo-2′-deoxyuridine and 5-bromo-2′-deoxyuridine

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Abstract

We have analyzed mutations in the thymidine kinase (TK) gene of varicella zoster virus (VZV) which showed resistance to 5-iodo-2′-deoxyuridine (IDU) and 5-bromo-2′-deoxyuridine (BrDU). Through sequencing of the TK gene, we found three amino acids were exchanged (41 Asn → Ser, 266 Cys → Ile, 288 Ser → Leu). These mutations were not located at either the nucleoside- or the ATP-binding site. This result suggests that the resistance to IDU and BrDU in this particular strain is due to the change in conformation of TK rather than the replacement of amino acids in the binding sites.

Keywords: Varicella zoster virus; Thymidine kinase; Sequence analysis

Varicella zoster virus (VZV) is the causative agent of chickenpox and shingles. This virus encodes a 35-kDa protein which has thymidine kinase (TK) activity (Dobersen et al., 1976; Lopetegui et al., 1983). The VZV-TK activates anti-VZV agents such as 9-(2-hydroxyethoxymethyl)guanosine, acyclovir (ACV), 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BVaraU), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), currently the most effective compounds in the clinical treatment of VZV infection (Biron and Elion, 1980; Machida et al., 1982; De Clercq, 1988).

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Table 1
Primers used in PCR of the VZV TK gene

Primer	Position	Sequence
VZ-TKF	64721-64745	5′-AAAACACTTGGCCCGAATTCGACTA-3′ (*EcoRI*)
VZ-TKR	65877-65901	5′-AACACGTACAC*TGAGTATGACAAT-3′ [a] (*XhoI*)

Note: The primers were designed to be complementary to the VZV sequence (Davison and Scott, 1986; Lacey et al., 1991).
[a] The presence of a mismatch between the primer and the target DNA designed to create a *XhoI* site is indicated by an asterisk.

Several investigators have reported the resistant VZV strain against these anti-VZV compounds (Sakuma, 1984; Pahwa et al., 1988; Jacobson et al., 1990; Lacey et al., 1991; Talarico et al., 1993).

In this study, we analyzed the TK peptide sequence of VZV which has only shown resistance to 5-iodo-2′-deoxyuridine (IDU) and 5-bromo-2′-deoxyuridine (BrDU), whereas it is susceptible to the other nucleoside analogs such as ACV, BVaraU, and BVDU. This virus strain, termed Ito strain, was isolated from a patient with chicken pox, and proved to be lowered in TK affinity to IDU and BrDU as substrate (Shigeta et al., 1986). The CaQu strain which was kindly provided by N.J. Schmidt, California Department of Health, Berkley, was used as standard TK-positive strain. The Ito and CaQu strains were prepared as cell-free virus and stored in −80°C until use (Baba and Shigeta, 1983).

To analyze the TK gene mutations, we carried out polymerase chain reaction (PCR) amplification and the whole TK gene was analyzed for the nucleotide sequence. The DNA was prepared from 100 μl of the cell-free virus stock solution. Briefly, SDS (final 0.1%) was added to the virus stock solution and the mixture was heated at 65°C for 5 min. After heating, samples were phenolized and precipitated with ethanol and finally precipitated samples were dissolved in 100 μl of distilled water.

A-10 μl of sample DNA was added to 90 μl PCR reaction mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM each of dATP, dTTP, dCTP, dGTP, 1 μM pair of oligonucleotides primer (Table 1) and 2.5 U of *Taq* polymerase (Perkin–Elmer Cetus; AmpliTaq). The reaction mixture was overlaid with 100 μl of mineral oil. PCR reaction was carried out in a DNA Thermal Cycler (Perkin–Elmer Cetus) for 30 cycles (1 min at 93°C, 1 min at 55°C, and 2 min at 72°C). After PCR reaction, the products were analyzed by electrophoresis in a 1% SeaKem agarose (FMC BioProducts, MD) containing 0.5 μg/ml ethidium bromide. The expected size of the products from DNA of two virus strains was 1181 bp. As shown in Fig. 1A, the TK gene was clearly amplified from both strains' DNA, but was negative in the control, in which distilled water was added instead of DNA sample.

To determine whether a difference in TK gene sequences exists between two strains, we performed single-strand conformation polymorphisms-PCR (SSCP-PCR) (Orita et al., 1989) combined with the internal labeling method with [α-^{32}P]dCTP in the PCR

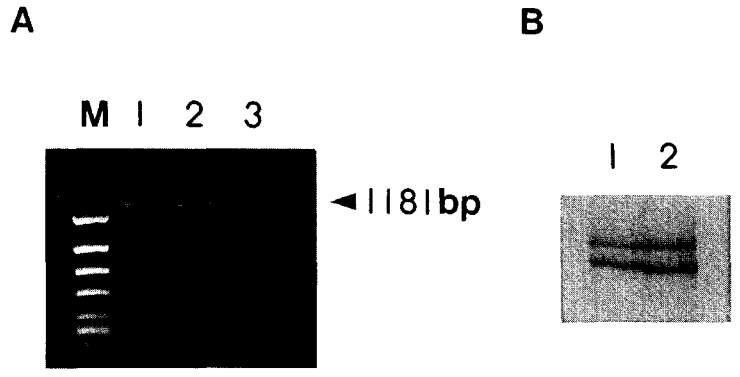

Fig. 1. (A) Ethidium bromide staining of amplified PCR products. Lane M, DNA size marker (ϕX174 Hinc II digest); lane 1, CaQu strain; lane 2, Ito strain, lane 3, negative control (distilled water instead of sample DNA was added to the PCR reaction mixture). Lanes 1 and 2 were clearly positive for 1181 bp PCR products (B) Autoradiogram of single-strand conformation polymorphism-PCR (SSCP-PCR) analysis. Lane 1, CaQu strain; lane 2, Ito strain. Samples were amplified with internal labeling using [^{32}P]dCTP and developed on X-ray film.

reaction mixture as described above. After SSCP-PCR, the products were electrophoresed in a 6% acrylamide gel. After the loading, the gel was dried and exposed to X-ray film at −70°C for autoradiography. As shown in Fig. 1B, single-stranded DNA fragments of these strains showed different mobility shift. This result suggested the presence of some mutations in TK gene of Ito strain compared with that of CaQu strain.

In order to analyze the mutating point in the TK gene of the Ito strain, the 1181 bp PCR products were cleaved with *Eco*RI and *Xho*I, and ligated into pBluescript plasmid which had been treated with *Eco*RI and *Xho*I, and plasmids were amplified in *Escherichia coli* (JM-109 strain). Four clone samples were obtained and analyzed for nucleotide sequence to avoid the misincorporation of *Taq* polymerase. The sequencing was carried out using the dideoxynucleotide chain termination method (Sanger et al., 1979).

Compared with the TK gene sequence of the Dumas strain (Davison and Scott, 1986), the point mutations were observed at 4 position (64928 A : T to G : C, 64979 G : C to T : A, 65605 G : C to A : T, 65669 C : G to T : A) in the TK gene of the Ito strain (Fig. 2). As a result of these mutations in nucleotides, it was disclosed that the translate amino acids were exchanged at three points (41 Asn → Ser, 266 Cys → Ile, 288 Ser → Leu). Among them, the 288 Ser → Leu amino acid difference from the Dumas strain has also been reported in the wild-type strain Ellen (Sawyer et al., 1988). This fact indicates the resistance to IDU and BrDU is due to the exchanges of the two other amino acids (41 Asn → Ser and 266 Cys → Ile).

Lacey et al. (1991) previously reported on the TK sequence of the drug resistant VZV strains which showed resistance to ACV, PYaraU, and BVaraU. As shown in Fig. 2, the mutation points of the Ito strain are quite different from those of previously reported resistant strains.

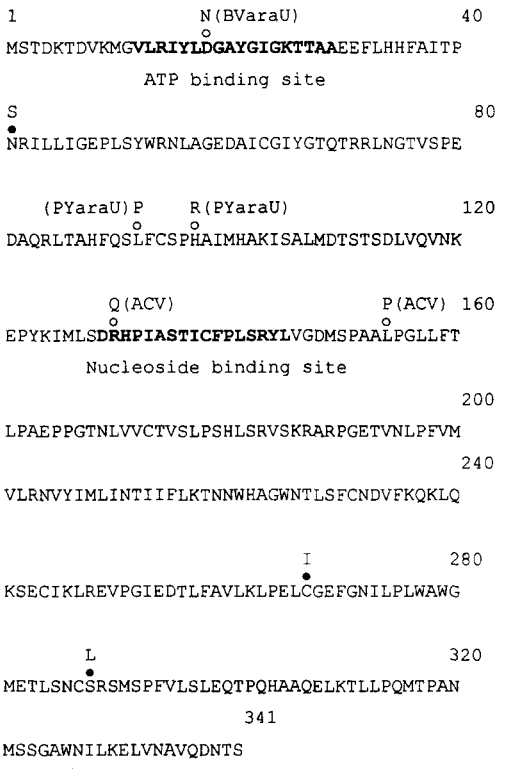

Fig. 2. Location of mutations observed in several drug-resistant VZV TK genes, which were previously reported (Lacey et al., 1991) and reported in this study. The amino acid sequence of the VZV TK gene is given in the single-letter code from amino acid 1 to 341. The positions at which amino acids of resistant strains by Lacey et al. are replaced are marked with an open circle, and those of the Ito strain are marked with a filled circle (41 Asn → Ser, 266 Cys → Ile, 288 Ser → Leu). The bold letter codes, amino acids 12–29 and 129–145, indicate the proposed ATP- and the nucleoside-binding site, respectively.

In the present report, we described a sequence analysis of VZV mutant strain (Ito strain) which is TK-positive, but resistant to IDU and BrUD. The characteristics of the Ito strain and its TK were reported previously and its susceptibility to nucleoside analogs was summarized in Table 2. The Ito-TK protein has mutations at three amino

Table 2
Inhibitory effect of antiviral compounds on focus formation

Virus strain	ID_{50} (μM)				ID_{90} (μM)			
	IDU	BrDU	BVDU	ACV	IDU	BrDU	BVDU	ACV
CaQu	0.84	1.2	0.026	8.0	1.9	2.4	0.075	17.3
Ito	25.0	30.0	0.012	17.3	50.0	60.0	0.024	54.4

Note: From Shigeta et al. (1986).

acids compared with standard VZV strains (Dumas strain), but, surprisingly, these mutation points are neither at the nucleoside- nor the ATP-binding site. Talarico et al. (1993) also reported that several acyclovir-resistant strains isolated from AIDS patients showed amino acid mutations out of the two binding sites. These results suggest that the resistance to IDU and BrDU of the Ito strain and altered affinity of TK to IDU and BrDU may be due to change of the protein structure.

In conclusion, we analyzed the TK gene of the Ito strain which is resistant to IDU and BrDU, but susceptible to BVDU and ACV. Through sequencing, exchanges of amino acids in three points of TK peptide were observed. These amino acids exchanges were not located at either the nucleoside- or the ATP-binding site. These data suggest that the resistance to IDU and BrDU of the Ito strain is due to change of conformation of TK protein rather than the replacement of amino acids at the nucleoside- or the ATP-binding site.

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