A neonate with herpes simplex virus 1 encephalitis was treated with intravenous acyclovir. During the course of therapy, the infection became intractable to the treatment and a mutation in the viral thymidine kinase gene (nucleotide G375T, amino acid Q125H) developed. This mutation was demonstrated in vitro to confer acyclovir resistance.
and designated vTK-375G. Subsequently, a G375T-mutant vTK expression plasmid was constructed by site-directed mutagenesis using the following primers, reagents, and PCR conditions and designated vTK-375T. Primers 5’-ATATAACATGGGCATGCC TTATGCcand 5’-GGGCGCTTGTCATTACCAC were designed for the inverse PCR (the underlined T is the targeted nucleotide), and vTK-375G was used as the template. The reaction was performed using a PrimeSTAR GXL DNA polymerase kit (TaKaRa Bio, Otsu, Japan), and the amplification conditions included an initial denaturation step of 2 min at 94°C, followed by 10 cycles of 10 s at 98°C, 15 s at 55°C, and 7 min at 68°C. Digestion and self-ligation were performed with a KOD mutagenesis kit (Toyobo, Osaka, Japan). G375T substitution without other nucleotide changes was confirmed by sequencing analysis. The expression plasmid for the ACV-sensitive HSV-1 vTK-TAS strain (5) was constructed as a positive control and designated vTK-TAS. Empty pTARGET served as a negative control. In the final stage of the assay, the titers of the replicated TAR were determined by the standard plaque assays. Then, \( \Delta \log_{10} \) PFU values were calculated as follows: \( \Delta \log_{10} \) PFU = log_{10} (PFU per milliliter of the replicated TAR at each ACV concentration) – log_{10} (PFU per milliliter of the replicated TAR at the ACV concentration of 0 µg/ml). This value represents the inhibitory effect of ACV on TAR replication, which is brought about by the transfection. Thus, the higher the value is, the lower the activity of the expressed vTK. In this way, the vTK-related resistance of HSV-1 can be judged from the \( \Delta \log_{10} \) PFU values. The sensitivities to ganciclovir (GCV; Sigma-Aldrich Chemical Company, St. Louis, MO), penciclovir (PCV; Wako), and brivudine (BVDU; Sigma-Aldrich) were also tested in the same way.

TAR replication in 293T cells transfected with a negative control was not affected by any concentrations of any antiviral compounds (Fig. 2). When ACV was used, \( \Delta \log_{10} \) PFU values elicited by vTK-375G transfection were at almost the same level as those elicited by vTK-TAS transfection, indicating that HSV-1 in sample 1 and TAS had nearly equal levels of sensitivity to ACV. However, \( \Delta \log_{10} \) PFU values elicited by vTK-375T transfection were significantly higher than those elicited by vTK-375G transfection (Welch’s t test; \( P = 0.004, <0.001 \), and \( 0.045 \), at ACV concentrations of 0.4, 4, and 40 µg/ml, respectively), indicating that the HSV-1 with the G375T mutation in the vTK gene had acquired ACV resistance (Fig. 2A). When GCV, PCV, and BVDU were used, transfection of vTK-375G, vTK-375T, and vTK-TAS resulted in almost the same level of \( \Delta \log_{10} \) PFU values (Fig. 2B to D). The HSV-1 G375T mutant was therefore considered to be sensitive to these drugs.

To our knowledge, this is the first report of a patient with ACV-resistant neonatal HSV-1 disease. Neonatal HSV infection is estimated to occur in 1 in every 3,500 to 5,000 deliveries (6). Approximately 30% of the patients are diagnosed as having NHE (7). Although the introduction of ACV has significantly improved the prognosis, NHE is still a severe disease with a mortality rate of 6%, and 70% of the survivors suffer from moderate-to-severe neurological abnormalities (7, 8). ACV-resistant HSV mainly threatens immunocompromised patients, and the prevalence among them is reported to range from 3.5% to 10%. In immunocompetent individuals, the prevalence of ACV-resistant HSV is far lower, ranging from 0.1% to 0.7% (2). Neonatal ACV-resistant HSV infections are quite rare, and all the cases previously described have been caused by ACV-resistant HSV-2 (9–11).

The present study also showed for the first time that a Q125H amino acid substitution in the vTK polypeptide induces ACV resistance. Using a method previously described (4), it was confirmed that the Q125H mutation was not a part of natural polymorphism. Q125 of HSV-1 TK has been shown to be located above the nucleotide binding pocket in the three-dimensional (3D) structure of the vTK protein (12). Several studies have shown that substitution of Q125 to other amino acids changes vTK activity; Q125E and Q125L are associated with resistance to ACV, and Q125N leads to hypersensitivity to ACV (13, 14). Interestingly, the Q125H mutation did not induce cross-resistance to GCV, PCV, and BVDU, suggesting that these drugs may be effective with respect to this specific mutant.

This study showed also for the first time a central nervous system infection caused by a virologically confirmed ACV-resistant HSV-1 strain. There is one report of a possibly ACV-resistant HSV-1 encephalitis adult patient (15). In that report, virus isolation from the CSF failed, but an amino acid substitution of R41H found in the vTK polypeptide was suspected to be responsible for the ACV resistance, although it has not been virologically confirmed whether the mutation confers ACV resistance. The method
used in the present study may be suitable for such a condition. However, it should be kept in mind that the method can be applied only for vTK-related ACV resistance and not for DNA polymerase-related resistance.

ACV-resistant HSV is usually seen in patients with a history of ACV treatment (16). In the present case, administration of ACV may possibly have induced the appearance of the ACV-resistant HSV-1 strain. Although a recent study showed a benefit of oral ACV suppressive therapy for survivors of NHE (17), emergence of ACV-resistant HSV during the suppressive therapy was also reported (18). Thus, sensitivity of the causative HSV to antiviral drugs should be carefully monitored. This patient did not receive the suppressive therapy because he suffered from NHE before the beneficial effect of the therapy was reported.

It is recommended to repeat the lumbar puncture after 21 days from the initiation of ACV administration in the treatment of NHE (19). On the other hand, persistence of CSF HSV DNA is reported to be associated with poor neurodevelopmental outcomes of NHE patients (20). CSF HSV DNA quantification was conducted weekly in this patient to monitor the HSV-1 genome level in a real-time manner. We considered that the practice was beneficial, although further discussion is needed. In fact, the frequent monitoring enabled us to treat NHE with an appropriate choice of antiviral drugs.

In conclusion, ACV-resistant HSV-1 was virologically confirmed for the first time in a NHE patient. A nucleotide mutation, G375T in the HSV-1 TK gene, leading to a Q125H amino acid substitution, conferred ACV resistance.

Nucleotide sequence accession numbers. The vTK DNA sequence data have been deposited in the DNA Data Bank of Japan (DDBJ) under accession no. AB713519 (CSF sample 1) and AB713520 (CSF sample 2).

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REFERENCES

7. Kimberlin DW, Lin CY, Jacobs RF, Powell DA, Frenkel LM, Gruber WC, Rathore M, Bradley JS, Diaz PS, Kumar M, Arvin AM, Gutierrez K, Shelton M, Weiner LB, Slesman JW, de Sierra TM, Soong SJ, Kiell FIG 2 The inhibitory effects of antiviral compounds on replication of TAR in 293T cells transfected with each of the plasmids vTK-375G (○), vTK-375T (□), vTK-TAS (×), and empty pTARGET (Δ). Each experiment was performed in triplicate, and the error bars indicate standard deviations.


