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Genotypic and phenotypic characterization of the thymidine kinase of ACV-resistant HSV-1 derived from an acyclovir-sensitive herpes simplex virus type 1 strain☆

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Abstract

Twenty-four strains of acyclovir (ACV)-resistant (ACV^r) herpes simplex virus type 1 (HSV-1) were generated from the HSV-1 TAS strain by exposure to ACV, and the genotype and phenotype of the thymidine kinase (TK) from these mutants were analyzed. The TK polypeptide of the ACV^r HSV-1 strains was examined by Western blot using an anti-HSV-1 TK rabbit serum. The sensitivity of each strain to ACV, foscarnet and cidofovir (CDV) was also determined. A single guanine (G) insertion or a single cytosine (C) deletion was detected in 12 of the 24 ACV^r strains at the G or C homopolymer stretches within the TK gene. Genotypic analysis predicted that two thirds of the ACV^r HSV-1 strains expressed truncated TK polypeptides, while one third expressed viral TK polypeptide with a single amino acid substitution at various sites. Western blot abnormalities in the viral TK polypeptides were identified in 21 ACV^r strains. There was an inverse correlation between the susceptibility of the HSV-1 mutant strains to ACV and that to CDV. Nucleotide sequencing of the TK gene and Western blot analysis of the viral TK polypeptides are considered to be one of the methods for predicting virus sensitivity to ACV and CDV.

Keywords: Herpes simplex virus type 1; Acyclovir; Cidofovir; Thymidine kinase; Mutation

1. Introduction

As acyclovir (ACV) has become widely used for the treatment of herpes virus infections, infections by ACV-resistant (ACV^r) herpes simplex virus types 1 and 2 (HSV-1 and HSV-2, respectively) and varicella-zoster virus (VZV) have raised con-

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siderable concern (Chatis and Crumpacker, 1991; Crumpacker et al., 1982; Englund et al., 1990; Erlich et al., 1989; Jacobson et al., 1990; Ljungman et al., 1990; Modiano et al., 1995; Pahwa et al., 1988; Saijo et al., 1998; Sibrack et al., 1982). ACV is phosphorylated by virus-induced thymidine kinase (TK) to the monophosphate, ACV monophosphate (ACVMP), whereupon ACVMP is converted to ACV diphosphate (ACVDP) and ACV triphosphate (ACVTP) by cellular enzymes. ACVTP is incorporated into viral DNA, resulting in termination of viral DNA chain elongation and inhibition of virus replication (Elion et al., 1977). Thus, ACV^r herpes viruses are either TK mutants and/or DNA polymerase mutants, although the former are far more frequent than the latter.

Phosphonoformic acid (foscarnet, PFA) and (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (cidofovir, CDV) are the preferred choices for the treatment of infections by ACV^r HSV or ACV^r VZV (Chatis et al., 1989; Erlich et al., 1989; Lalezari et al., 1997; Safrin, 1992; Safrin et al., 1990, 1991; Snoeck et al., 1994; Youle et al., 1988).

There have been many reports on mutations in the TK gene of herpes viruses, whether laboratory-derived or isolated from patients with ACV^r herpes infections (Chatis and Crumpacker, 1991; Hwang and Chen, 1995; Kit et al., 1987; Kost et al., 1993; Michael et al., 1995; Nugier et al., 1991; Palù et al., 1992; Saijo et al., 1999; Sasadeusz et al., 1997). According to these reports, several types of nucleotide mutations can occur in the TK gene of ACV^r HSV-1 isolates; i.e. frameshift mutations and nucleotide substitutions resulting in the production of a truncated TK polypeptide or a full-length TK polypeptide with an amino acid substitution. Recently, it was reported that 14 of the 15 ACV^r HSV-1 mutants selected after the passage in the presence of ACV at the concentration of 10 μg/ml expressed the frameshift mutant TK polypeptides (Sarisky et al., 2001). We also independently established 24 ACV^r HSV-1 strains from ACV-sensitive (ACV^s) HSV-1 and obtained basic and detailed information on the ACV^r HSV-1 strains by genotypic and phenotypic characterization of the TK proteins expressed by these strains. Furthermore, the sensitivities of the ACV^r HSV-1 strains to ACV and CDV were also determined and compared with the TK polypeptide characteristics.

2. Materials and methods

2.1. Compounds

ACV (Sigma Chemical Company, St. Louis, MO), PFA (Sigma) and CDV (Gilead Sciences, Foster City, CA) were used. [*Methyl*-^{3}H]thymidine was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). ^{3}H-labelled ACV (Sigma) was also used.

2.2. Cells and viruses

Green monkey kidney (Vero) cells and TK-deficient 143B/TK$^-$ neo R cell line (Riken Cell Bank, Tsukuba, Japan) were used. HSV-1 VR-3 and HSV-1 TAS were used as the laboratory and wild ACV^s strains, respectively (Saijo et al., 1998; Suzutani et al., 1988). Twenty-four ACV^r HSV-1 strains were generated from plaque-purified HSV-1 TAS, as follows. Vero cells were infected with the plaque-purified HSV-1 TAS at a multiplicity of infection (m.o.i.) of one plaque forming unit (p.f.u.)/cell, and cultivated for 2 days in Eagle's minimum essential medium containing 2% fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin (MEM-2FBS), supplemented with 0.1 μg/ml of ACV. The harvested cells were treated by sonication and then centrifuged. The supernatant fraction was inoculated into Vero cells and cultivated for 2 days in MEM-2FBS supplemented with 0.3 μg/ml of ACV. The concentration of ACV in the culture medium was gradually increased from 0.1 to 3.0 μg/ml. The HSV-1 strains, which grew at 3.0 μg/ml, were plaque-purified and used for further analysis. We selected strains at an ACV concentration of 3.0 μg/ml because the serum concentration of ACV during high-dose ACV therapy was 3.0 μg/ml in a patient with a mucocutaneous ACV^r HSV-1 infection (Saijo et al., 1998).

2.3. Plaque reduction assay

The susceptibility of HSV-1 strains to ACV, PFA and CDV was assessed by a plaque reduction method in Vero cells, as described previously (Saijo et al., 1992). All strains were tested for sensitivity to antiviral agents in the same experiment and at the same time in order to determine the sensitivities in the completely same conditions.

2.4. Measurement of TK activity

TK (EC 2. 7. 1.21) activity in virus-infected 143B/TK$^-$ neo R cell extracts was evaluated according to the method of Jamieson (Jamieson et al., 1974; Suzutani et al., 1988).

2.5. Measurement of ACV-phosphorylation activity

ACV-phospharylation activity in virus-infected 143B/TK$^-$ neo R cells was evaluated as reported previously (Saijo et al., 1998, 2002a).

2.6. Nucleotide sequencing

DNA sequencing of the TK gene was performed by cycle sequencing of PCR-generated products amplified from purified viral DNA of Vero cells infected with each strain, as described previously (Saijo et al., 1999).

2.7. Western blotting

The size of the TK polypeptides expressed in HSV-1 TAS-, VR-3- and ACV^r strain-infected Vero cells was examined by Western blotting. Vero cells infected with each virus at an m.o.i. of 1 p.f.u./cell were suspended in an appropriate amount of PBS at 24 h post-infection. The suspensions of infected Vero cells were sonicated at full power on ice for 1 min. Then, the sonicated materials were centrifuged at 12 000 rpm for 10 min at 4 °C. The supernatant and pellet fractions were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Western blotting was performed as described previously (Saijo et al., 1999).

2.8. Statistics

The relationship between the susceptibilities of the tested HSV-1 strains to ACV and those to CDV was assessed by Spearman's correlation coefficient by rank.

3. Results

3.1. Nucleotide sequence of TK genes

Genotypic characteristics of the TK genes of the 24 ACV^r strains are summarized in Table 1. A single guanine (G) insertion within the seven-G residues from nucleotide positions 430–436 was detected in eight ACV^r strains (CL1-8). A single cytosine (C) deletion within the homopolymer C residues in the TK gene was detected in four strains (CL9–12). Of these four strains, the mutation was detected within the six-C residues (positions 548–553) in two strains (CL11 and 12) and within the four-C residues (positions 1061–1064) in two strains (CL9 and 10). Interestingly, both six- and four-C residues lay between adenosine nucleotides. There are 11 C-homopolymer stretches, which consist of four or more C residues, within the TK gene and three of them have the sequence 'ACCCCCCA' or 'ACCCCA' (Saijo et al., 1999). The sequence 'CCCC' with adenosine on both ends is thus a hot spot for the C deletion mutation.

The deletion of 496 nucleotide residues from positions 340–835 in the TK gene was found in one strain (CL13).

A single nucleotide substitution in the TK gene was found in 11 of the 24 ACV^r strains. Six of the 11 substitutions were 'C to T'. Seven of the 11 substitutions occurred at AC-rich residues such as 'AC', 'ACC' and 'ACCC'. Three (CL14-16) of these 11 ACV^r strains were expected to express truncated TK polypeptides because of the early appearance of a stop codon. The other eight strains were expected to express full-length TK

Table 1
Characteristics of TK polypeptides, susceptibility to ACV and CDV, nucleotide mutations in the TK gene, number of amino acid residues in the TK polypeptides, viral TK activity, staining patterns by Western blotting, and accession number, for the different HSV-1 strains

Strains	IC_{50} (μg/ml)		Mutations[a]	Size of TK polypeptide	Categories	Accession number.
	ACV	CDV				
TAS	0.60	1.3	No mutations	376	Authentic	AB047358
VR-3	0.70	0.80	No mutations	376	Authentic	AB009254
CL1	>100	0.20	G added within 7-Gs (430–436)	227	Not stained	AB047359
CL9	>100	0.0086	C deleted within 4-Cs (1061–1064)	407	Elongated	AB047365
CL11	>100	0.14	C deleted within 6-Cs (548–553)	407	Not stained	AB047366
CL13	>100	NT[b]	Deletion of nucleotide residues (340–835)	262	Not stained	AB047367
CL14	>100	0.13	C310T	103	Not stained	AB047368
CL15	>100	NT	C325T	108	Not stained	AB047369
CL16	>100	NT	C841T	280	Truncated	AB047370
CL17	70	0.14	G163A (Asp 55 Asn)	376	Insoluble	AB047371
CL18	4.8	0.54	C194A (Thr 65 Asn)	376	Authentic	AB047372
CL19	16	0.44	C25T (Pro 84 Ser)	376	Authentic	AB047373
CL20	>100	0.062	C518G (Pro 173 Arg)	376	Insoluble	AB047374
CL21	10	1.3	T598G (Gly 200 Cys)	376	Insoluble	AB047375
CL22	80	0.064	C734T (Thr 245 Met)	376	Insoluble	AB047376
CL23	40	0.042	C860T (Thr 287 Met)	376	Insoluble	AB047377
CL24	72	0.20	G1006A (Cys 336 Tyr)	376	Authentic	AB047378
Mock-	–	–	–	–	Not stained	–

[a] 'C310T' represents the nucleotide substitution of thymine (T) for cytosine (C) at position 310. 'Asp 55 Asn' represents the amino acid substitution of Asn for Asp at position 55.
[b] 'NT' indicates 'not tested'.

polypeptides with a single amino acid substitution at various sites (Table 1).

3.2. Phenotypic characteristics of TK polypeptides

ACV^r HSV-1 strains, which expressed truncated TK proteins, did not express any viral TK activity. The viral TK activity of HSV-1 CL18, CL19, CL22 and CL24 was 93.7, 1.4, 10.3 and 23.8% of that of HSV-1 TAS, respectively. The TK activity levels of the other ACV^r HSV-1 strains were found to be less than 1%. In order to clarify whether these four strains, which expressed viral TK activity, became ACV-resistant due to decrease in ACV-phosphorylation activity, the ACV-phosphorylation activity in viral strain-infected cells was measured in comparison with the control. As shown Fig. 1, the ACV-phosphorylation activity of CL18 retained 6% of that of HSV-1 TAS, while

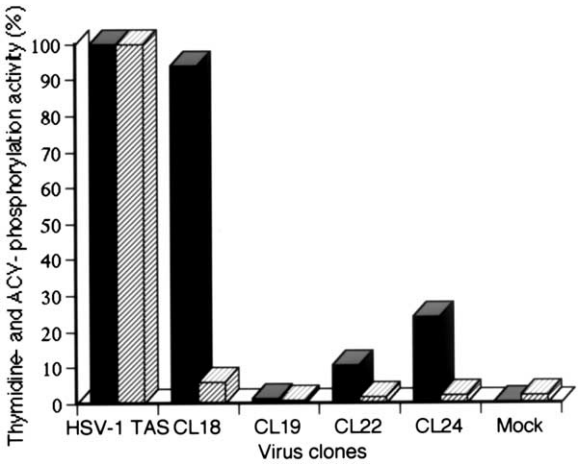

Fig. 1. Thymidine-phosphorylation (black bar) and ACV-phosphorylation (diagonal bar) activities of HSV-1 TAS-, CL18-, CL19, CL22, CL24 and mock-infected 143B/TK$^-$ neo R cells. Both thymidine-phosphorylation and ACV-phosphorylation activities of HSV-1 TAS-infected cells were defined as 100%.

those of the other three strains were the same as that of mock-infected cells.

3.3. Susceptibility to antiviral agents

As mentioned above, eight strains coded a single G insertion within the seven-G residues in the TK gene (CL1–8) and four strains did a single C deletion within the four and six-C residues, respectively (CL9–10 and CL11–12, respectively). Furthermore, four strains (CL13–16) were expected to express truncated TK polypeptide due to the long nucleotide deletion or due to the early appearance of stop codon at the various sites (Table 1). Therefore, HSV-1 CL1, CL9, CL11, and CL14 were selected and regarded as the representatives of these four mutant groups, HSV-1 CL1–8, CL9–10, CL11–12, and CL13–16, respectively. These four representative strains, the other ACV^r strains (CL17–24), totally 12 ACV^r strains, and the ACV^s HSV-1 TAS and VR-3 strains were tested for sensitivity to antiviral agents by the plaque reduction assay. The ACV IC_{50} values for HSV-1 TAS, HSV-1 VR-3 and all the tested 15 ACV^r mutant strains (including HSV-1 strains CL13 and CL15–16) are shown in Table 1. The IC_{50} of ACV for the tested 15 ACV^r strains was from 4.8 to >100 μg/ml, although for most of the strains it was >100 μg/ml. All the tested 12 ACV^r strains as well as two ACV^s strains were equally susceptible to PFA, showing IC_{50} values around 10 μg/ml. The IC_{50} values of CDV for the tested 12 ACV^r strains and two ACV^s strains ranged from 0.0086 to 1.3 μg/ml (Table 1).

3.4. Western blotting of TK polypeptides

The TK polypeptide in HSV-1 CL9 was detected in the pellet fractions from the virus-infected cells by Western blotting at a relatively higher position on the gel than the intact viral TK polypeptide, as reported previously (Saijo et al., 1999) (Fig. 2c, right). This type of the mutant TK polypeptide with the delayed mobility was reported among the ACV^r HSV-2 clinical isolates by Chatis and Crumpacker (1991). This type of staining pattern is designated 'elongated'. A truncated TK polypeptide was detected in HSV-1 CL16-infected cells (Fig. 2c, right) at an expected position and this type is designated 'truncated'. Data not shown here because of the weak visualization, the elongated TK and the truncated TK were also detected in the unfractionated virus-infected cells. On the other hand, truncated TK polypeptides were not detected by Western blotting in the supernatant or pellet fractions from cells infected with ACV^r HSV-1 CL1–8 and CL11–15 (data not shown). Based on these results, the staining patterns of the TK polypeptides with the antibody can be categorized as 'elongated', 'truncated' and 'not stained', respectively.

The TK polypeptides of HSV-1 CL17–24 were expected to be stained with the anti-TK rabbit serum in the same way as those of HSV-1 TAS. However, only the TK polypeptides of strains CL18, CL19 and CL24 showed this staining pattern, described as 'authentic' (Fig. 2a). The TK polypeptides were detected in both soluble and insoluble fractions from virus-infected Vero cells. On the other hand, the staining patterns of the TK polypeptides of strains CL17 and CL20–23 were different from that of the 'authentic' type (Fig. 2a). The TK polypeptides expressed by strains CL17 and CL20–23 were detected only in the insoluble fractions, indicating that these five TK polypeptides could not form exact secondary or tertiary structures and assumed an aggregated format (Fig. 2b and c, left). We denoted this type of staining as 'insoluble'. In summary, the staining pattern of these TK polypeptides were categorized into 'authentic', 'insoluble', 'elongated', 'truncated' and 'not stained'.

3.5. Western blotting of TK polypeptides and TK activities

Viral TK activity of ACV^r strains was classified into three categories, 'TK-altered ($>15\%$ of intact TK activity)', 'low TK-producer (1–15% of intact TK activity)' and 'TK-deficient ($<1\%$ of intact TK activity)' (Hill et al., 1991). Of the three strains categorized as 'authentic', two (CL18, CL24) were classified as TK-altered and one (CL19) as low TK-producer, while of the five strains categorized as 'insoluble', four (CL17, CL20, CL21, CL23) were classified as TK-deficient and one (CL22) as

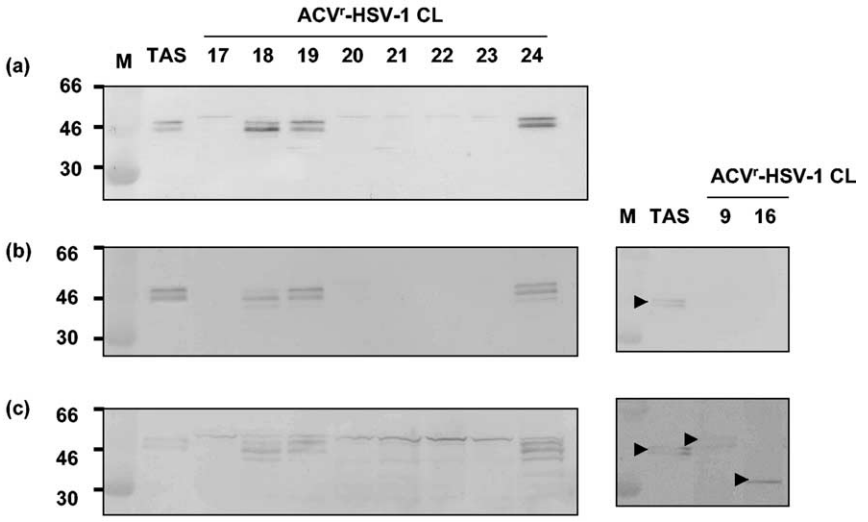

Fig. 2. Western blotting analysis of the size of TK polypeptides in virus-infected Vero cells. 'M' represents 'Marker'. The Western blotting pattern of the parent strain HSV-1 TAS is shown in lane 2 of both left and right figures. The staining patterns of viral TK polypeptides expressed by strains CL17-24 and by strains CL9 and CL16 are shown in Lanes 3–10 of left figure and in Lanes 3 and 4 of right figure, respectively. Western blotting was performed with samples from unfractionated virus-infected cells (a), from supernatant fractions (b) and from pellet fractions (c).

low TK-producer (Table 1, Fig. 1). All other ACV^r strains, whose TK polypeptides were classified as 'elongated', 'not stained' and 'truncated', were classified as TK-deficient.

3.6. Staining patterns of TK polypeptides and susceptibility to CDV

We further analyzed the relationship between the Western blot staining patterns of the TK polypeptides and susceptibility to CDV. The HSV-1 TAS and VR-3 strains were confirmed to be sensitive to CDV, but their CDV sensitivities were lower than those of most ACV^r strains (Table 1, Fig. 3). Of the tested 12 ACV^r strains (CL1, CL9, CL11, CL13, CL17–24), HSV-1 strain CL9, which had an 'elongated' staining pattern, was the most sensitive to CDV (IC_{50}: 0.0086 μg/ml). The ACV^r HSV-1 strains in which the staining patterns were 'authentic' or 'not stained' had moderate sensitivity to CDV, their IC_{50} values being between 0.08 and 0.8 μg/ml. Three of the five ACV^r strains, for which the staining pattern was 'insoluble', were very sensitive to CDV, with an IC_{50} of less than 0.08 μg/ml, while the other two strains were moderately sensitive to CDV.

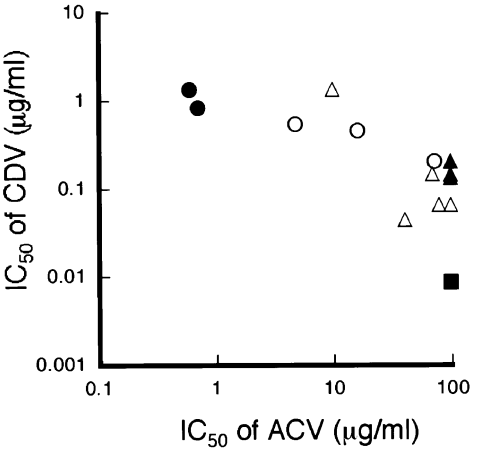

Fig. 3. Correlation of the susceptibility of HSV-1 strains to ACV with that to CDV. The IC_{50} value '>100 μg/ml' was regarded as '100 μg/ml'. The figures '●', '○', '▲', '△' and '■' represent 'wild type', 'authentic', 'not stained', 'insoluble' and 'elongated' strains, respectively.

To analyze the relationship between the sensitivities to ACV and CDV for the different ACV^r

HSV-1 strains (CL1, CL9, CL11, CL13, CL17–24) together with HSV-1 TAS and VR-3 wild strains, the IC_{50} values of ACV and CDV for all these strains were plotted (Fig. 3). There was a significant inverse correlation between the virus' sensitivities to ACV and CDV ($P < 0.05$) (Fig. 3).

4. Discussion

All the ACV^r strains (CL18, CL19, CL22, CL24), which expressed some degree of viral TK activity, expressed decreased ACV-phosphorylation activities (Fig. 1), indicating that the mutations detected in the TK genes of these ACV^r strains were responsible for the resistance to ACV.

In the present study, we explored, the relative frequencies of the mutational types in the TK genes of ACV^r strains. Frameshift mutations of a single G insertion within the seven-G stretch and of a single C deletion within two of the C homopolymer stretches were identified in one third and one sixth of the strains, respectively. On the other hand, a nucleotide substitution was detected in approximately half of the ACV^r mutants. Approximately half of the ACV^r HSV clinical isolates had insertion or deletion mutations within the G or C homopolymer stretches in the TK gene in the present and the previous studies (Gaudreau et al., 1998; Morfin et al., 2000; Sasadeusz et al., 1997). There were no differences in the relative frequencies of the mutational types in the TK genes between the laboratory derived ACV^r strains and ACV^r clinical isolates.

A single amino acid substitution in the TK polypeptide was demonstrated in eight of the 24 ACV^r strains. The positions of the amino acid substitutions in the TK polypeptides are shown in Fig. 4, together with those described in the previous reports (Gaudreau et al., 1998; Morfin et al., 2000). Amino acid substitution-mutations responsible for the resistance to ACV were identified throughout the TK protein, indicating that the accumulation of data on amino acid substitutions in TK polypeptides from ACV^r viruses is considered to be important for the assessment of virus sensitivity to ACV.

The TK polypeptides, in which a single amino acid substitution was detected, of five ACV^r strains were detected only in the insoluble fractions prepared from virus-infected cells (Fig. 2). Although the mechanism by which a single amino acid change made the TK polypeptide insoluble is unknown, there are two possible explanations. The first is that the TK polypeptides of these ACV^r strains may be processed in an unknown manner by cellular factors in virus-infected cells and the processing may make the TK polypeptides insoluble. The other explanation is that the single amino acid substitution itself induces a structural change in the TK protein, resulting in an aggregation of the TK polypeptides.

The staining patterns of TK polypeptides in the Western blotting with our anti-HSV-1 TK rabbit serum were classified into the five categories as described above. Possible explanation for 'not stained' is as follows: (1) TK polypeptides were not expressed, (2) TK polypeptides were expressed but were rapidly destroyed by proteases, and (3) the antigenic regions were lost by truncation. The last explanation is the most plausible, as the antigenic regions are thought to be located on the C-terminal half of the TK polypeptide. Although the Western blotting was just performed by using the specific antibodies raised against HSV-1 TK polypeptide in the study, the procedure

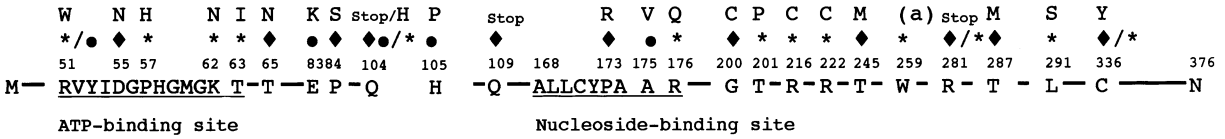

Fig. 4. Diagram of HSV-1 TK-mutations conferring ACV resistance. Amino acid substitutions defined in the present study (♦) and in the reports by Gaudreau et al. (1998) (*) and by Morfin et al. (2000) (●). ATP- and nucleoside-binding sites are shown according to the report by Darby et al. (1986). Frameshift mutations such as a single nucleotide insertion and deletion within the homopolymer stretch are not illustrated in this Figure.

for the production of this antibody was reported in detail (Saijo et al., 1999). Therefore, the Western blotting in the study is available not only in our laboratory but also in other laboratories.

The characterization of TK polypeptides of ACV^r HSV-2 clinical isolates was performed with an immunoprecipitation using the monoclonal antibody to HSV-2 TK polypeptide (Chatis and Crumpacker, 1991). The TK polypeptides of ten ACVr HSV-2 strains were classified into 'authentic or insoluble (not determined in their report) ', 'not stained', 'elongated' and 'truncated' according to our classifications. We believe that our study extended the knowledge on TK mutants of ACV^r HSV-1 and HSV-2 strains.

Our results showed, for the first time, that there was statistically an inverse correlation between sensitivity to ACV and sensitivity to CDV (Fig. 3, $P < 0.05$). Furthermore, the staining patterns of TK polypeptides in Western blotting were related to the sensitivities to ACV and CDV (Fig. 3). For example, the ACV^r strains with 'elongated' TK polypeptides were highly sensitive to CDV and three of the five ACV^r strains with 'insoluble' TK polypeptides were also highly sensitive to CDV. The ACV^r HSV-1 R98-0 and ACV^r and PFA-resistant R98-3 strains, which were isolated from the patient with Wiskott–Aldrich syndrome and whose TK polypeptides were completely the same as that of HSV-1 CL9, were also highly sensitive to CDV, suggesting that the mutation in the TK polypeptide was responsible for the high sensitivity to CDV (Saijo et al., 2002b). Although data are not shown here, there were no mutations in the DNA polymerase genes of the highly sensitive strains compared with the parent HSV-1 TAS strain, excluding the possibility that the DNA polymerase in these strains were responsible for the high sensitivity to CDV. The ACV^r strains with 'not stained', 'truncated' or 'elongated' TK polypeptides were highly resistant to ACV. Thus, Western blotting is considered to be one of the rapid and effective methods for predicting the sensitivity of HSV to ACV and CDV. The phenomenon that ACV^r HSV strains that were TK-altered or deficient were more sensitive to CDV than the TK-intact strains were reported (Chakrabarti et al., 2000; Mendel et al., 1995). This increase in sensitivity to CDV may be related to the smaller dCTP pool, which competes with CDV diphosphate for the viral DNA polymerase, in the cells infected with ACV^r TK-associated HSV (Mendel et al., 1995). Although, the increased sensitivity of ACV^r strains to CDV was explained as described above, further study is needed to elucidate the mechanism for the inverse correlation in the sensitivity between to ACV and to CDV among the ACV^r strains.

Finally, it must be noted that approximately half of the ACV^r HSV-1 strains were due to the frameshift mutations in the present study, while 14 of the 15 ACV^r HSV-1 were due to the same mutations in the report by Sarisky et al. (2001). The difference might be due to the differences in conditions for the selection of ACVr mutants such as cell types (Vero cell vs. MRC-5 cells), the ACV concentration for selection (3 vs. 10 μg/ml) and other unspecified conditions.

In summary, we determined the basic genotypic and phenotypic characteristics of the TK polypeptides expressed by ACV^r HSV-1 strains. Nucleotide sequencing of the TK genes and Western blot analysis using the anti-HSV-1 TK antibodies to determine the size and staining pattern of the TK polypeptides can be considered as rapid and effective means for estimating virus sensitivity to ACV and CDV.

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