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Short communication

Susceptibility of protein kinase (ORF47)-deficient varicella-zoster virus strains to anti-herpesvirus nucleosides

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Abstract

To clarify whether varicella-zoster virus (VZV) protein kinase (PK; ORF47) takes part in phosphorylation of anti-herpesvirus nucleosides, thymidine kinase (TK) deficient, and PK/TK double deficient recombinant VZV strains were isolated and their susceptibility, and that of wild type and PK-deficient strains to various nucleoside analogs was evaluated. The PK-deficient VZV strains showed a sensitivity equal to that of the wild type strain against all compounds tested, including ganciclovir. This indicates that PK is not involved in phosphorylation of the tested nucleosides in VZV-infected cells. Published by Elsevier Science B.V.

Keywords: Varicella-zoster virus; Protein kinase; Antiviral nucleosides

Herpes simplex virus (HSV) UL13, varicella-zoster virus (VZV) ORF47, human cytomegalovirus (HCMV) UL97 and Epstein–Barr virus (EBV) BGLF4 genes have been identified as conserved genes in every herpesvirus subfamily (Chee et al., 1989; Smith and Smith 1989). Highly conserved motifs of cellular protein kinases (PK) were found in these herpesvirus genes and the serine/threonine kinase activity of HSV-1 UL13, VZV ORF47 and HCMV UL97 gene products was demonstrated (Cunningham et al., 1992; Ng and Grose 1992; He et al., 1997). Two independent studies showed that HCMV UL97 PK is involved in ganciclovir (GCV)-phosphorylation (Littler et al., 1992; Sullivan et al., 1992). This finding was supported by the identification of mutations in UL97 genes from clinical GCV-resistant (GCV^r)-HCMV isolates, along with the GCV-sensitive phenotype of the recombinant vaccinia virus and HSV-1 strains containing the UL97 gene (Metzger et al., 1994; Chou et al., 1995; Wolf et al., 1995; Ng et al., 1996).

The homology of herpesvirus PKs and the evidence that UL97 can control GCV phosphorylation, led to the hypothesis that HSV UL13 and

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VZV ORF47 can phosphorylate GCV. We previously analyzed the susceptibility of COS cells expressing VZV ORF47 PK to anti-herpesvirus nucleosides. The results indicated that VZV PK can phosphorylate GCV and two other deoxyguanosine analogs, 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine (OXT-G) and (+)-9-[(1R,2R,3S)-2,3-bis(hydroxymethyl)-cyclobutyl]guanine (cOXT-G) (Koyano et al., 1996). In this study, to clarify whether ORF47 PK is involved in the phosphorylation of anti-herpesvirus nucleosides in VZV-infected cells, a set of recombinant VZV strains with either or both of thymidine kinase (TK) and PK deleted were isolated, and the susceptibilities of these recombinant strains to anti-herpesvirus nucleosides were determined.

1. Isolation of recombinant VZV strains

We used the ROka (wild type) and ROka 47S (PK-deficient) strains, which are recombinant strains produced by transfection of MeWo cells with four overlapping VZV cosmids (Heineman and Cohen 1995). The mutant TK gene from the TK-deficient VZV strain (YSR strain) was introduced into both the ROka and ROka 47S strains, as follows: the mutant TK gene, which has a single base deletion (loss of A:T at position 65298), was prepared with PCR using primers 4 and 8′ (Lacey et al., 1991), and was co-transfected with the genomic DNAs of ROka or ROka 47S strains into human embryo lung fibroblast cells. Recombinant TK-deficient strains were selected and cloned three times in a medium containing 1 μg/ml of 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU). TK-deficient characteristics of the isolated strains, ROka-YSR and ROka-47S/YSR, were confirmed by the plaque autoradiography method (Suzutani et al., 1995). The nucleotide sequences of PK genes from four VZV strains were determined. The ROka and ROka-YSR coded wild type PK gene and the ROka 47S and ROka-47S/YSR strains were found to contain a mutant PK gene with two stop codons at positions 166 and 167, as expected (Heineman and Cohen 1995).

2. Plaque reduction assay

The 50% inhibitory concentration (IC_{50}) of nucleosides against VZV strains was examined in a plaque reduction assay (Koyano et al., 1996). Strains ROka-YSR and ROka-47S/YSR were resistant to acyclovir (ACV) and brovavir (BV-araU), but these strains were as susceptible to phosphonoacetic acid (PAA) and vidarabine (araA) as the ROka and ROka 47S strains (Table 1). This indicates that ROka-YSR and ROka-47S/YSR have TK-deficient phenotypes and that none of the strains have mutations in their DNA polymerase. GCV and cOXT-G were effective against

Table 1
Inhibitory effects of different compounds on the plaque formation of VZV strains[a]

Compounds	IC_{50} (μg/ml)[b]			
	ROka	ROka-YSR	ROka 47S	ROka-47S/YSR
PAA	4.8 ± 0.25	2.7 ± 0.07	2.9 ± 0.50	2.4 ± 0.67
AraA	2.0 ± 0.01	1.3 ± 0.38	3.1 ± 0.04	3.7 ± 1.4
ACV	2.1 ± 0.11	17.6 ± 0.21	1.6 ± 0.28	20.6 ± 1.3
BV-araU	0.0019 ± 0.0002	>100	0.00075 ± 0.00003	>100
GCV	1.7 ± 0.24	3.4 ± 0.90	0.87 ± 0.02	4.8 ± 0.57
COXT-G	0.23 ± 0.03	0.71 ± 0.06	0.16 ± 0.03	0.64 ± 0.08
OXT-G	2.9 ± 0.21	1.1 ± 0.11	0.72 ± 0.06	0.72 ± 0.37
S2242	0.21 ± 0.004	0.19 ± 0.007	0.080 ± 0.005	0.17 ± 0.025

[a] Results are the averages for two to four different experiments.
[b] IC_{50}, 50% Inhibitory concentration for VZV plaque formation.

all strains, although TK-deficient strains showed slightly reduced susceptibility. This result is in contrast to the important effect of HSV TK on phosphorylation of GCV and cOXT-G as previously reported (Smee et al., 1985; Koyano et al. 1996). Part of the difference between HSV and VZV would be caused by differences in the degree of dependence on cellular enzymes for the metabolism of nucleosides and nucleotides. HSV shuts off host protein synthesis soon after virus entry by the virion protein encoded by the UL41 gene, resulting in suppression of cellular enzyme activities (Kwong et al., 1988; Suzutani et al., 1988), but VZV does not induce such shut-off of host proein synthesis (unpublished results). There were no differences in the susceptibility of individual strains to OXT-G or 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]adenine (S2242) (Neyts et al., 1994), suggesting that neither viral TK nor PK are related to the phosphorylation of OXT-G or S2242 in VZV-infected cells. Contradictory observations in the phosphorylation pathway of OXT-G were reported; some studies indicated that VZV TK participates in the phosphorylation of OXT-G (Andrei et al., 1995; Koyano et al., 1996), while others did not support this (Sakuma et al., 1991; this paper). These conflicting results could be brought about by differences in cellular conditions, because OXT-G may be phosphorylated by both viral and cellular enzyme(s), and higher activity of cellular enzymes may mask the contribution of viral TK.

Our results indicated VZV ORF47 PK is not involved in the phosphorylation of the tested nucleosides in VZV-infected cells, as is also the case for the HSV-1 counterpart, UL13 PK (Ng and Grose 1992). Another enzyme, TK, also participates in the phosphorylation of GCV and cOXT-G, although its contribution is much lower as compared to HSV TK (Smee et al., 1985). To elucidate the nature of the enzyme(s) taking part in the phosphorylation of nucleoside analogs, including GCV in VZV-infected cells, further studies should be carried out using different approaches such as the isolation of large numbers of GCV^r-VZV strains and identification of mutation sites in these strains.

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