ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1986, p. 894–898
0066-4804/86/050894-05$02.00/0

Vol. 29, No. 5

# Susceptibility to Other Antiherpes Drugs of Pathogenic Variants of Herpes Simplex Virus Selected for Resistance to Acyclovir

BRENDAN A. LARDER† AND GRAHAM DARBY†*

*Division of Virology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom*

Received 16 October 1985/Accepted 31 January 1986

**Cross-resistance data for a group of nine acyclovir-resistant variants of herpes simplex virus type 1 are reported. These mutants, which express either altered thymidine kinase (TK) or DNA polymerase, were all derived from the same wild-type (wt) strain after exposure to acyclovir in tissue culture. Furthermore, all variants have pathogenic properties similar to the wt parental strain as assessed using mouse model systems (G. Darby, H. J. Field, and S. A. Salisbury, Nature (London) 289:81–83, 1981; B. A. Larder and G. Darby, Virology 146:262–271, 1985). Two groups of antiherpes compounds were used: those requiring activation by TK and those whose action is independent of that enzyme. The TK substrate-specificity mutants were generally resistant to the TK-activated drugs but showed wt susceptibility to phosphonoacetic acid, 9-β-D-arabinofuranosyladenine, and aphidicolin. The DNA polymerase mutants were relatively susceptible to most TK-activated drugs, although two were resistant to 5-(trifluoromethyl)-2′-deoxyuridine. The polymerase mutants showed a more complex pattern of susceptibility, however, to those compounds whose mode of action is independent of TK. In general, these variants showed similar responses to phosphonoacetic acid, phosphonoformate, and 9-β-D-arabinofuranosyladenine, a particular variant being either resistant, susceptible, or hypersensitive to all three. The response of each variant to aphidicolin, however, appeared to be the inverse of its response to the other three drugs. The cross-resistance patterns are discussed, and their implications for combined or successive therapies are considered.**

The newly developed antiviral agent acyclovir (ACV; 9-[(2-hydroxyethoxy)methyl]guanine; 9) is now in extensive clinical use for the treatment of herpes simplex virus (HSV) infections. However, recent reports have warned that resistant strains may emerge during therapy and thus pose a potential threat to future treatment (2, 25, 28, 31). In view of these observations, it is important to assess the susceptibility of ACV-resistant mutants to other antiherpes drugs. Any patterns that are recognized may then be exploited both in therapy and in the future design of antiherpes compounds.

Selection for ACV resistance in tissue culture most frequently results in the isolation of variants defective in the expression of thymidine kinase (TK) activity (5, 11, 27), a virus-coded enzyme required to initiate activation of ACV by phosphorylation (19). Although $TK^-$ mutants replicate in culture under normal conditions, they are considerably attenuated in animal model systems (10, 14, 30), suggesting they are unlikely to be of major clinical importance in normal individuals. In contrast, some HSV variants selected with ACV retain the ability to induce TK activity, and these often show little change in pathogenic properties compared with wild-type (wt) virus (7, 10, 13). Variants of this latter type, although less common than $TK^-$ mutants, cause greater concern. They fall into two groups: firstly those which induce TK with altered substrate specificity such that the variant enzyme phosphorylates thymidine but not ACV (24) and, secondly, mutants which induce altered DNA polymerase with reduced sensitivity to inhibition of ACV-triphosphate (6, 16), the active form of the drug (8, 17, 18). Because both groups of $TK^+$ variants have proved more difficult to isolate in culture than $TK^-$ mutants, little information is available regarding the spectrum of susceptibility of such ACV-resistant strains to other drugs.

We report here cross-resistance data for a group of DNA polymerase and TK mutants, all of which were derived from the same HSV type 1 (HSV-1) wt strain after exposure to ACV in culture. A number of interesting patterns of cross resistance and susceptibility were observed. The implications of these findings with regard to treatment of ACV-resistant strains and to the possibility of preventing their emergence are discussed.

## MATERIALS AND METHODS

**Cells and tissue culture.** Baby hamster kidney-21 (BHK) cells and 5-bromo-2′-deoxyuridine-resistant BHK (BU-BHK) cells were the cell lines used in this study. BU-BHK cells express very low levels of cellular TK activity. Both cell lines were free of mycoplasma contamination.

Cells were maintained in Glasgow modified Eagle medium supplemented with 10% newborn calf serum and 10% tryptose phosphate broth. In plaque reduction assays, tryptose phosphate broth was omitted from the medium and 1% carboxymethyl cellulose (CMC) was included in the overlay ($EC_{10}$-CMC medium).

**Viruses.** The wt HSV-1 strain SC16 (20) and ACV-resistant mutants derived from it were used. The isolation and properties of the TK substrate-specificity mutants SC16 S1 (S1) and SC16 Tr7 (Tr7) were described previously (7, 24). S1 induces about 30% TK activity, and Tr7 induces about 100% TK activity compared with the wt (7, 24). The DNA polymerase mutants (transformed cell passage series) were isolated recently following passage of SC16 in TK-transformed BU-BHK cells (BHK TK1) in the presence of increasing concentrations of ACV (23). All of these mutants induce TK activity levels similar to that of the wt SC16 (23).

Virus stocks, derived from single-plaque isolates of each

* Corresponding author.

† Present address: Department of Biochemical Virology, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, United Kingdom.

TABLE 1. Susceptibility of HSV polymerase mutants to TK-independent drugs

| Virus | Phenotype | $ED_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | ACV | PAA | PFA | araA | Aphidicolin |
| SC16 | wt | 0.15 | 55 | 26 | 6.7 | 0.4 |
| TP2.4 | $PAA^r$ | 2.9 | 577 | 133 | 67 | 0.22 |
| TP2.5 | | 7.1 | 1,095 | 443 | 94 | 0.12 |
| TP1.3 | $PAA^s$ | 0.5 | 73 | 30 | 9.4 | 0.49 |
| TP2.7 | | 4.4 | 146 | 53 | 7.5 | 0.28 |
| TP4.4 | | 35 | 33 | 19 | 1.4 | 1.73 |
| TP3.2 | $PAA^{hs}$ | 8.8 | 10 | 5 | 0.7 | 1.0 |
| TP4.1 | | 28 | 13 | 6 | 0.8 | 1.23 |

strain, were made by infection of BHK cells at low multiplicity.

**Plaque reduction assays.** The susceptibility of viruses to antiherpes compounds was assessed by plaque reduction assay using monolayers of BHK or BU-BHK cells as described previously (11). Briefly, dishes (5 cm) of cells were inoculated with 100 to 250 PFU of virus per dish in 0.2 ml of $EC_{10}$. The dishes were incubated at 37°C for 30 to 60 min to allow virus adsorption, after which the inoculum was removed and 5 ml of $EC_{10}$-CMC containing the required drug concentration was added. Plaques were allowed to develop for 2 days, and the cells were then fixed and stained. The $ED_{50}$, defined as the dose required for 50% plaque reduction, was obtained from a plot of the reduction in plaque number (relative to the no-drug control) against drug concentration. Plaque reduction assays with each drug were performed at least twice.

**Antiviral drugs.** The following compounds were gifts: ACV and 9[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]guanine (alternatively known as BIOLF-62, 2′-NDG, BW759, or DHPG) from P. Collins, The Wellcome Research Laboratories, Beckenham, Kent, United Kingdom; (*E*)-5-2-bromovinyl-2′-deoxyuridine (BVdU) from E. De Clercq, Rega Institute, Louvain, Belgium; phosphonoacetic acid (PAA) and aphidicolin from R. W. Honess, National Institute for Medical Research, Mill Hill, London, United Kingdom; and phosphonoformate (PFA) from B. Oberg, Astra Lakemedel, Sonderstalje, Sweden. 5-Iodo-2′-deoxyuridine (IUdR), 5-trifluoromethyl-2′-deoxyuridine (TFT), and 9-β-D-arabinofuranosyladenine (araA) were purchased from the Sigma Chemical Co., Poole, Dorset, United Kingdom.

## RESULTS

The wt strain (SC16) and nine ACV-resistant $TK^+$ mutants were tested for susceptibility to a number of different antiviral compounds by plaque reduction assay. Two of the variants used were TK substrate-specificity mutants (Tr7 and S1), and the remainder were resistant at the level of DNA replication (transformed cell passage series).

The compounds we used can be broadly divided into two groups, those which require activation by TK (DHPG, BVdU, IUdR, and TFT) and those whose action is independent of TK (PAA, PFA, araA, and aphidicolin). It is believed that the inhibitory effects of these compounds or their active derivatives result from their interaction with viral DNA polymerase, causing inhibition of the enzyme or producing deleterious effects after incorporation into viral DNA (3, 15, 29; reviewed in reference 22).

$ED_{50}$s were obtained directly from the inhibition curves derived from plaque reduction data.

**Susceptibility of DNA polymerase mutants to PAA, PFA, araA, and aphidicolin.** The seven ACV-resistant DNA polymerase mutants have been classified in three groups according to their susceptibility to PAA (23). These groups are defined as follows: PAA resistant ($PAA^r$) with an $ED_{50}$ greater than threefold that of the wt, PAA hypersensitive ($PAA^{hs}$) with an $ED_{50}$ less than one-third-fold that of the wt, and PAA susceptible ($PAA^s$), the remainder. The resistance of these variants to drugs whose action is independent of TK is shown in Table 1. Several points emerge. Firstly, no correlation was seen between the degree of ACV resistance and resistance or susceptibility to PAA. For example, TP2.5 and TP3.2 showed a similar reduction in susceptibility to ACV ($ED_{50}$s 40- and 50-fold higher, respectively, than wt values), although TP2.5 was $PAA^r$ and TP3.2 was hypersensitive to the drug. Secondly, all mutants showed very similar patterns of susceptibility to PAA, PFA, and araA. Hence, the $PAA^r$ mutants (TP2.4 and TP2.5) were both $PFA^r$ and $araA^r$. Thirdly, an inverse relationship was found between PAA and aphidicolin susceptibility. Thus, the $PAA^r$ variants showed increased susceptibility to aphidicolin compared with the wt, and the $PAA^{hs}$ strains were slightly resistant. Interestingly, the $PAA^s$ variant, TP4.4, had some characteristics of the $PAA^{hs}$ strains, because it was significantly resistant to aphidicolin (with an $ED_{50}$ fourfold that of the wt) and showed hypersensitivity to araA (giving an $ED_{50}$ fivefold lower than that of the wt).

**Susceptibility of DNA polymerase mutants to DHPG, BVdU, TFT, and IUdR.** The polymerase mutants were also tested for susceptibility to a number of TK-activated nucleoside analogs; the $ED_{50}$ data are shown in Table 2. The mutants showed the least change in susceptibility to BVdU, since the greatest increase in $ED_{50}$ (seen with TP4.1) was less than twofold that of the wt. The situation with DHPG and IUdR was different in that most of the variants showed marginal but significant resistance to these compounds. For example, the increases in $ED_{50}$s for DHPG were between two- and fivefold compared to that of the wt, and for IUdR they were up to sixfold. Most of the mutants were significantly less susceptible to TFT, the most resistant being the $PAA^r$ mutants (TP2.4 and TP2.5), with $ED_{50}$s nearly 50-fold that of the wt.

**Susceptibility of TK substrate-specificity mutants to antiviral agents.** The TK substrate-specificity mutants S1 and Tr7 were also tested for their susceptibility to the drugs used as

TABLE 2. Susceptibility of HSV polymerase mutants to TK-activated drugs

| Virus | Phenotype | $ED_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | ACV | BVdU | DHPG | IUdR | TFT[a] |
| SC16 | wt | 0.15 | 0.02 | 0.17 | 0.45 | 0.57 |
| TP2.4 | $PAA^r$ | 2.9 | 0.02 | 0.74 | 0.79 | 28 |
| TP2.5 | | 7.1 | 0.03 | 0.74 | 2.5 | 28 |
| TP1.3 | $PAA^s$ | 0.5 | 0.02 | 0.4 | 0.9 | 1.5 |
| TP2.7 | | 4.4 | 0.025 | 0.46 | 0.6 | 8.1 |
| TP4.4 | | 35 | 0.02 | 0.67 | 1.8 | 5.4 |
| TP3.2 | $PAA^{hs}$ | 8.8 | 0.025 | 0.55 | 0.5 | 2.4 |
| TP4.1 | | 28 | 0.033 | 0.7 | 0.7 | 4.4 |

[a] Plaque reduction assays were performed in BU-BHK cells.

TABLE 3. Susceptibility of TK substrate-specificity HSV mutants to other antiherpes drugs

| Virus | $ED_{50}$ (μM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ACV | PAA | araA | Aphidicolin | BVdU | DHPG | IUdR | TFT[a] |
| SC16 | 0.15 | 55 | 6.7 | 0.4 | 0.02 | 0.17 | 0.45 | 0.57 |
| S1 | 11.1 | 65 | 15 | 0.34 | 0.54 | 1.0 | 1.3 | 20 |
| Tr7 | 22.2 | 65 | 13 | 0.55 | 0.36 | 21 | 0.7 | 2.0 |

[a] Plaque reduction assays were performed in BU-BHK cells.

described above with the polymerase mutants (Table 3). Predictably, these variants showed normal susceptibility to inhibitors that do not require prior activation by TK. Thus, S1 and Tr7 showed no significant changes in susceptibility to PAA, araA, or aphidicolin.

The picture with TK-activated compounds, however, was different, because both mutants were less susceptible to these inhibitors. The degrees of resistance observed varied considerably. For example, Tr7 was significantly resistant to

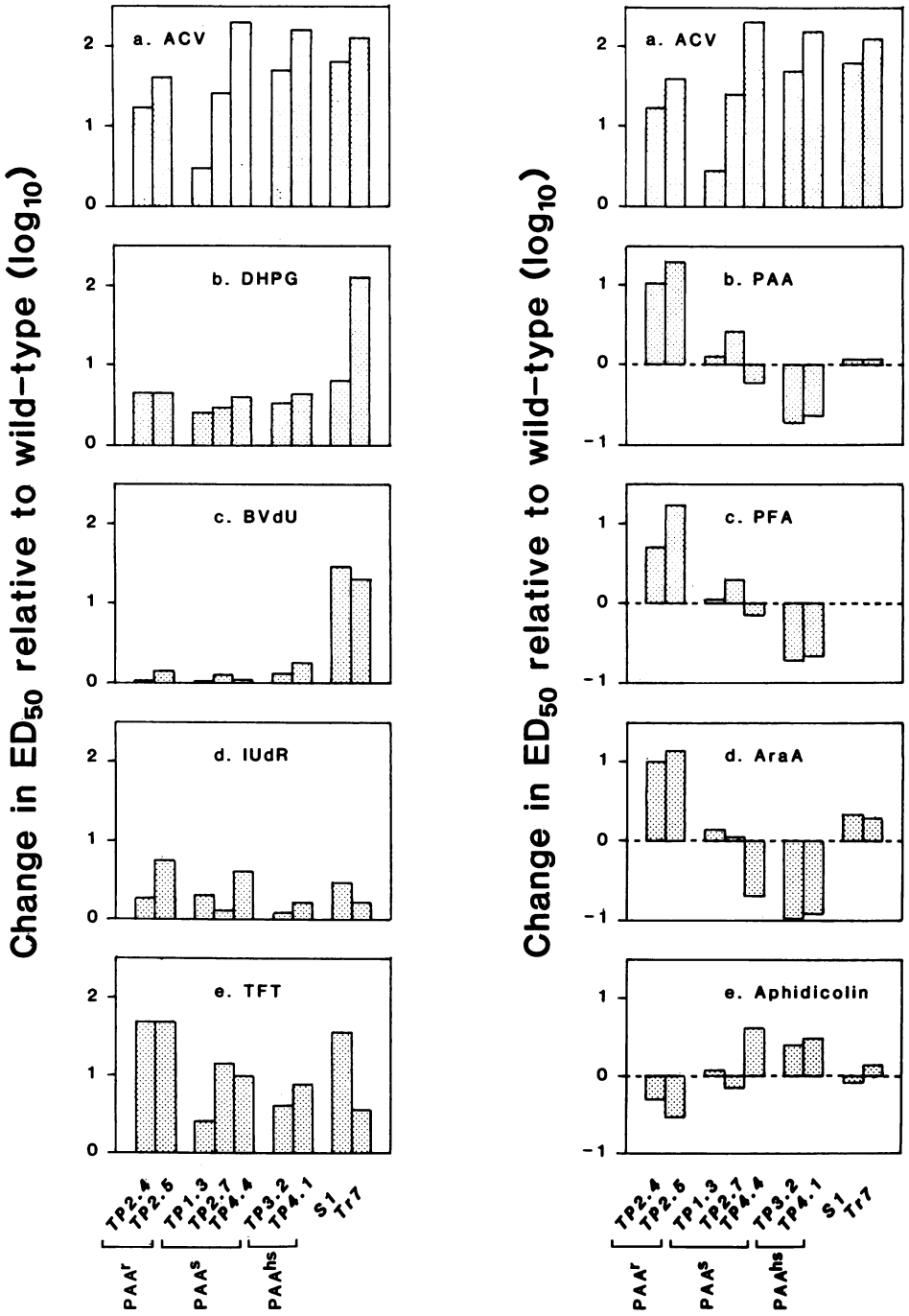


FIG. 1. Summary of cross-resistance data for all mutants and drugs used in this study. The values shown are the $ED_{50}$s for the mutants calculated relative to that of the wt virus using the formula: change in $ED_{50}$ = $ED_{50}$ of mutant/$ED_{50}$ of wt. The values are plotted on a log scale.

DHPG ($ED_{50}$, 124-fold that of the wt) and BVdU ($ED_{50}$, 20-fold that of the wt) but showed only slightly decreased susceptibility to TFT and IUdR. The mutant S1 gave a different pattern, as it was considerably less resistant to DHPG ($ED_{50}$, 6-fold that of the wt) but more resistant to BVdU and TFT ($ED_{50}$s, about 30- and 35-fold, respectively, that of the wt). Like Tr7, this variant showed only a marginal decrease in susceptibility to IUdR.

The data for all drugs and mutants are summarized in Fig. 1.

## DISCUSSION

The major interest in cross resistance is that it may provide information which will aid the design of suitable therapy for infections caused by drug-resistant variants of HSV. It may also suggest drug combinations which would allow us to minimize the frequency of appearance of resistant isolates. We chose ACV-resistant isolates for this study as ACV is now the most widely used and successful of the antiherpes drugs. Furthermore, ACV-resistant strains have already been isolated from patients undergoing therapy (2, 25, 28, 31). We specifically excluded viruses deficient in TK expression, because variants of this type exhibit markedly reduced virulence in vivo (10, 14, 30). Although we are clearly not yet in the situation in which the clinician has at his disposal a selection of antiherpes drugs with different modes of action, there are many compounds with considerable clinical potential, and it is appropriate that these compounds be examined in relation to their effects on ACV-resistant mutants. Such studies may provide guidance for the future design and use of antiherpes drugs.

The picture which emerged with the TK substrate-specificity mutants (S1 and Tr7) was fairly clear. As expected, these viruses were as susceptible as the wt to drugs whose action is independent of the virus-induced TK. Thus, replication of these strains was inhibited by PAA, araA, and aphidicolin, and we expect that in vivo the response of the TK substrate-specificity mutants to these drugs would resemble the response of the wt virus. Furthermore, combined therapy with ACV and any drug in this group would be expected to prevent the appearance of TK substrate-specificity mutants, because a virus would require multiple lesions if it is to exhibit resistance to such a combination. The TK substrate-specificity mutants were in general resistant to other nucleoside analogs, such as DHPG, BVdU, and TFT, although they showed only marginal resistance to IUdR.

The situation with DNA polymerase mutants is more complex. All drugs used in this survey are believed to interact ultimately with the virus-coded DNA polymerase, and so we might expect altered susceptibility to any of these compounds. Surprisingly, all seven polymerase mutants were susceptible to BVdU and relatively susceptible to DHPG and IUdR, showing smaller than 10-fold changes in the $ED_{50}$ relative to that of the wt virus. Thus, alterations at the DNA polymerase locus which conferred ACV resistance resulted in relatively little change in susceptibility to the other nucleoside analogs. TFT was exceptional in that several polymerase mutants (TP2.4, TP2.5, TP2.7, and TP4.4) showed changes of 10-fold or greater in their response to this compound. Again, these results indicate that there may be advantages in using drugs in combination rather than individually in the treatment of herpes infections.

A more intriguing observation was the response of polymerase mutants to those compounds whose action is independent of TK (PAA, PFA, araA, and aphidicolin). With these compounds, we observed not only increases in resistance but also, with almost equal frequency, significant decreases. The observation that polymerase mutants isolated by using one compound may be hypersensitive to others is not new (1, 4, 12, 21, 26); however, the pattern was striking. Each mutant appeared to respond similarly to PAA, PFA, and araA. In general, a variant was either resistant to all three, hypersensitive to all three, or susceptible to all three. This suggests that the interactions of these compounds with the polymerase might be analogous, not a surprising conclusion for PFA and PAA, which are both pyrophosphate analogs, but certainly surprising for the nucleoside analog araA. In contrast, the response of these variants to aphidicolin appeared to be the inverse of their responses to PAA, PFA, and araA. Viruses resistant to the latter were hypersensitive to aphidicolin, and those hypersensitive to PAA, PFA, and araA were resistant to aphidicolin. Those viruses similar to the wt in susceptibility to PAA, PFA, and araA remained susceptible to aphidicolin.

It is inevitable that reliance on a single compound, however effective, for the treatment of HSV infections will lead to the establishment of resistant virus strains in the population. Alternatives to ACV will be required in the future, and it appears from the present study that none of the drugs whose mode of action involves TK or DNA polymerase will provide the ideal alternative. Although it may be possible to develop cocktails of drugs able to deal with ACV-resistant variants (e.g., PFA plus aphidicolin), there is clearly a need to direct our efforts toward developing drugs targetted to other virus-specific functions.

## ACKNOWLEDGMENTS

This work was supported by the Medical Research Council, United Kingdom. B.A.L. was in receipt of a Junior Research Fellowship at Churchill College, Cambridge University.

We thank Elizabeth Lay for technical assistance.

## LITERATURE CITED

1. **Bastow, K. F., D. D. Derse, and Y.-C. Cheng.** 1983. Susceptibility of phosphonoformic acid-resistant herpes simplex virus variants to arabinosylnucleosides and aphidicolin. Antimicrob. Agents Chemother. **23**:914–917.
2. **Burns, W. H., R. Saral, G. W. Santos, O. L. Laskin, P. S. Leithan, C. McLaren, and D. W. Barry.** 1982. Isolation and characterisation of resistant herpes simplex virus after acyclovir therapy. Lancet **i**:421–423.
3. **Cheng, Y.-C., S. P. Grill, G. E. Dutschman, K. Nakayama, and K. F. Bastow.** 1983. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl)guanine, a new anti-herpes virus compound, in herpes simplex virus-infected cells. J. Biol. Chem. **258**:12460–12464.
4. **Coen, D. M., H. E. Fleming, Jr., L. K. Leslie, and M. J. Retondo.** 1985. Sensitivity of arabinosyladenine-resistant mutants of herpes simplex virus to other antiviral drugs and mapping of drug hypersensitivity mutations to the DNA polymerase locus. J. Virol. **53**:477–488.
5. **Coen, D. M., and P. A. Schaffer.** 1980. Two distinct loci confer resistance to acycloguanosine in herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA **77**:2265–2269.
6. **Darby, G., M. J. Churcher, and B. A. Larder.** 1984. Cooperative effects between two acyclovir resistance loci in herpes simplex virus. J. Virol. **50**:838–846.
7. **Darby, G., H. J. Field, and S. A. Salisbury.** 1981. Altered substrate-specificity of herpes simplex virus thymidine kinase confers acyclovir resistance. Nature (London) **289**:81–83.
8. **Derse, D., Y.-C. Cheng, P. A. Furman, M. H. St. Clair, and G. B. Elion.** 1981. Inhibition of purified human and herpes simplex virus-induced DNA polymerases by 9-(2-hydroxyeth-

oxymethyl)guanine triphosphate. Effects on primer-template function. J. Biol. Chem. **256**:11447–11451.
9. **Elion, G. B., P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, and H. J. Schaeffer.** 1977. Selectivity of action of an antiherpetic agent 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. USA **74**:5716–5720.
10. **Field, H. J., and G. Darby.** 1980. Pathogenicity in mice of strains of herpes simplex virus which are resistant to acyclovir in vitro and in vivo. Antimicrob. Agents Chemother. **17**:209–216.
11. **Field, H. J., G. Darby, and P. Wildy.** 1980. Isolation and characterisation of acyclovir-resistant mutants of herpes simplex virus. J. Gen. Virol. **49**:115–124.
12. **Field, H. J., A. McMillan, and G. Darby.** 1981. The sensitivity of acyclovir-resistant mutants of herpes simplex virus to other antiviral drugs. J. Infect. Dis. **143**:281–285.
13. **Field, H. J., and J. Neden.** 1982. Isolation of bromovinyldeoxyuridine-resistant strains of herpes simplex virus and successful chemotherapy of mice infected by one such strain by acyclovir. Antiviral Res. **2**:243–254.
14. **Field, H. J., and P. Wildy.** 1978. The pathogenicity of thymidine kinase deficient mutants of herpes simplex virus in mice. J. Hyg. **81**:267–277.
15. **Frank, K. B., D. Derse, K. F. Bastow, and Y.-C. Cheng.** 1984. Novel interaction of aphidicolin with herpes simplex virus DNA polymerase and polymerase-associated exonuclease. J. Biol. Chem. **259**:13282–13286.
16. **Furman, P. A., D. M. Coen, M. H. St. Clair, and P. A. Schaffer.** 1981. Acyclovir-resistant mutants of herpes simplex virus type 1 express altered DNA polymerase or reduced acyclovir phosphorylating activities. J. Virol. **40**:936–941.
17. **Furman, P. A., M. H. St. Clair, J. A. Fyfe, J. L. Rideout, P. M. Keller, and G. B. Elion.** 1979. Inhibition of herpes simplex virus-induced DNA polymerase activity and viral DNA replication by 9-(2-hydroxyethoxymethyl)guanine and its triphosphate. J. Virol. **32**:72–77.
18. **Furman, P. A., M. H. St. Clair, and T. Spector.** 1984. Acyclovir triphosphate is a suicide inactivator of the herpes simplex virus DNA polymerase. J. Biol. Chem. **259**:9575–9579.
19. **Fyfe, J. A., P. M. Keller, P. A. Furman, R. L. Miller, and G. B. Elion.** 1978. Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound 9-(2-hydroxyethoxymethyl)guanine. J. Biol. Chem. **253**:8721–8727.
20. **Hill, T. J., H. J. Field, and W. A. Blythe.** 1975. Acute and recurrent infection with herpes simplex virus in the mouse: a model for studying latency and recurrent disease. J. Gen. Virol. **28**:341–353.
21. **Honess, R. W., D. J. M. Purifoy, D. Young, R. Gopal, N. Cammack, and P. O'Hare.** 1984. Single mutations at many sites within the DNA polymerase locus of herpes simplex viruses can confer hypersensitivity to aphidicolin and resistance to phosphonoacetic acid. J. Gen. Virol. **65**:1–17.
22. **Larder, B. A., and G. Darby.** 1984. Virus drug-resistance: mechanisms and consequences. Antiviral Res. **4**:1–42.
23. **Larder, B. A., and G. Darby.** 1985. Selection and characterisation of acyclovir-resistant herpes simplex virus type 1 mutants inducing altered DNA polymerase activities. Virology **146**: 262–271.
24. **Larder, B. A., D. Derse, Y.-C. Cheng, and G. Darby.** 1983. Properties of purified enzymes induced by pathogenic drug-resistant mutants of herpes simplex virus. Evidence for virus variants expressing normal DNA polymerases and altered thymidine kinases. J. Biol. Chem. **285**:2027–2033.
25. **McLaren, C., L. Corey, C. Dekker, and D. W. Barry.** 1983. In vitro sensitivity to acyclovir in genital herpes simplex viruses from acyclovir-treated patients. J. Infect. Dis. **148**:868–875.
26. **Nishiyama, Y., S. Suzuki, M. Yamauchi, K. Maeno, and S. Yoshida.** 1984. Characterisation of an aphidicolin-resistant mutant of herpes simplex virus type 2 which induces an altered viral DNA polymerase. Virology **135**:87–96.
27. **Schnipper, L. E., and C. S. Crumpacker.** 1980. Resistance of herpes simplex virus to acycloguanosine: the role of the viral thymidine kinase and DNA polymerase loci. Proc. Natl. Acad. Sci. USA **77**:2270–2273.
28. **Sibrak, C. D., L. D. Gutman, C. M. Wiffert, C. McLaren, M. H. St. Clair, P. M. Keller, and D. W. Barry.** 1982. Pathogenicity of acyclovir-resistant herpes simplex virus type 1 from an immunodeficient child. J. Infect. Dis. **146**:673–682.
29. **St. Clair, M. H., W. H. Miller, R. L. Miller, C. U. Lambe, and P. A. Furman.** 1984. Inhibition of cellular α DNA polymerase and herpes simplex virus-induced DNA polymerases by the triphosphate of BW759U. Antimicrob. Agents Chemother. **25**:191–194.
30. **Tenser, R. B., S. Ressel, and M. E. Dunstan.** 1981. Herpes simplex virus thymidine kinase expression in trigeminal ganglion infection: correlation of enzyme activity with ganglion virus titre and evidence of *in vivo* complementation. Virology **112**:328–341.
31. **Wade, J. C., C. McLaren, and J. D. Meyers.** 1983. Frequency and significance of acyclovir-resistant herpes simplex virus isolated from marrow transplant patients receiving multiple courses of treatment with acyclovir. J. Infect. Dis. **148**: 1077–1082.