Sensitivity of Viruses to Phosphorylated 9-(2-hydroxyethoxymethyl)guanine Revealed in TK-transformed Cells

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SUMMARY

Vaccinia and pseudorabies viruses are resistant to ACV [Acyclovir or 9-(2-hydroxyethoxymethyl)guanine] in normal cells. However, both viruses are sensitive in thymidine kinase (TK)-transformed cells in which the resident HSV-specific TK is able to phosphorylate the drug. This demonstrates the sensitivity of these viruses to phosphorylated ACV and suggests a wider antiviral activity for the phosphorylated drug.

Acyclovir [ACV or 9-(2-hydroxyethoxymethyl)guanine] is a potent inhibitor of herpes simplex virus replication (Elion et al. 1977; Schaeffer et al. 1978). Its activity against the virus is coupled with low toxicity for uninfected human cells and this provides hope that the drug will prove useful for the treatment of herpes infections in man. Experiments in a number of animal model systems have supported this conclusion (Schaeffer et al. 1978; Field et al. 1979; Klein et al. 1979; Park et al. 1979; Pavan-Langston et al. 1979). However, one consequence of the highly specific mode of action of ACV is a narrow spectrum of activity. The drug is only inhibitory to certain members of the herpes virus group (Schaeffer et al. 1978). This is illustrated in Fig. 1 where we have examined the ACV-sensitivities of two HSV type I strains (HFEM and SC16) and vaccinia and pseudorabies viruses. The test system was a plaque reduction assay in BHK cells. The HSV strains were extremely sensitive to the drug with ED50 doses of ACV in the range 0.01 to 0.02 μg/ml. In contrast, the ED50 doses for vaccinia and pseudorabies (a member of the herpes group) were more than 1000-fold higher (32 μg/ml and 25 μg/ml respectively). The result with vaccinia virus was consistent with the earlier observations of Fyfe et al. (1978).

Studies on the mode of action of ACV have shown that it is phosphorylated in HSV-infected cells by the virus-specified enzyme, thymidine kinase (TK; Fyfe et al. 1978). It is the phosphorylated drug which inhibits replication by interference with virus DNA synthesis (Elion et al. 1977). It has been shown that failure to convert the drug to the ‘active’ phosphorylated form results in resistance. We have two lines of evidence which support this. Firstly, viruses with a TK− phenotype have been shown to have natural resistance to ACV. Secondly, it has been shown that the majority of ACV-resistant mutants isolated following serial passages of herpes simplex in the presence of the drug have defects in TK expression (Field et al. 1980). D. M. Coen and P. A. Schaeffer have obtained similar data with ACV-resistant mutants (personal communication).

The availability of ‘biochemically transformed’ cells which carry and express the HSV TK gene (TK-transformed cells; Munyon et al. 1971; Wigler et al. 1977; Minson et al. 1978) provides a system in which the drug can be ‘activated’ to its phosphorylated form by the resident virus-specified enzyme. The TK-transformed cells can therefore be used to test directly the sensitivity of viruses to the phosphorylated compound. One prediction based on the arguments above is that virus which has acquired resistance to ACV by loss of ability to express TK would become sensitive when tested in a TK-transformed cell. This is the case...
Fig. 1. Virus sensitivities to ACV measured in BHK cells. Monolayers of cells in 5 cm plastic dishes were inoculated with 100 to 250 p.f.u./dish of virus in 0.2 ml Glasgow modified Eagle’s medium containing 10% calf serum (ECm). After 1 h to permit adsorption, 5 ml of ECm containing 10% carboxymethylcellulose and the required concentration of ACV were added. Plaques were allowed to develop for 2 days and the cells were then fixed and stained. To obtain the ED₅₀, a plot was constructed of the reduction in plaque number (relative to the no-drug control) against drug concentration (on a log scale). The dose required for 50% plaque reduction was obtained from the graph. The sensitivities of two HSV-1 strains, HFEM (■) and SCI6 (□) were compared with those of vaccinia virus (○) and pseudorabies virus (○).

with the BrdUrd-derived TK⁻ virus, C1 (101) TK⁻. In six different cell types tested, C1 (101) TK⁻ was at least 10-fold more resistant to ACV than its parent, C1 (101). However, when these viruses were tested in the TK transformed line, D₂₁ (Minson et al. 1978), the TK⁻ virus was as sensitive as its parent. The ED₅₀ of ACV in both cell types was 0.01 µg/ml (Field et al. 1980).

If ACV is phosphorylated efficiently by the virus-induced TK in HSV-infected TK-transformed cells, then we would expect the resident kinase gene to have only a minor role in determining the sensitivity of the virus to the drug. We would then predict that the virus would show similar sensitivity in TK-transformed cells and the parent non-transformed line. This has been confirmed with the HSV-1 strain, SCI6, whose sensitivity measured by plaque reduction assay in D₂₁ cells (ED₅₀ dose 0.02 µg/ml) is very similar to its sensitivity in the parent LMTK⁻ line (ED₅₀ dose 0.03 µg/ml).

It was of interest to examine viruses which were insensitive to ACV in standard cell systems. We therefore compared vaccinia and pseudorabies viruses in LMTK⁻ and D₂₁ cells (Fig. 2). The ED₅₀ values obtained in LMTK⁻ cells were of the same order as those in BHK cells (vaccinia 32 µg/ml and pseudorabies 11 µg/ml). However, in D₂₁ cells both viruses showed a dramatic decrease in resistance. The ED₅₀ for pseudorabies virus was 0.03 µg/ml, which was similar to values obtained with herpes simplex strains and implies that pseudorabies and HSV have similar sensitivities to the phosphorylated drug. Vaccinia virus was more resistant (ED₅₀ 0.11 µg/ml) but it was, nevertheless, 300-fold more sensitive in D₂₁ than in LMTK⁻.

We considered the possibility that failure of the viruses to plaque in TK-transformed cells at low concentrations of ACV was reflecting the toxicity of the phosphorylated compound for the cells (Nishiyama & Rapp, 1979). However, we measured the effect of ACV on LMTK⁻ and D₂₁ cells using a growth inhibition system and showed that the incorporation
Fig. 2. Virus sensitivities to ACV measured in D21 and LMTK− cells. Monolayers of cells in 5 cm plastic dishes were inoculated with 100 to 250 p.f.u./dish of virus (titrated in the cell type used for the assay). The virus was in 0.2 ml EC10. After 1 h adsorption 5 ml EC10 containing 10% carboxymethylcellulose and the required concentration of ACV was added. Plaques were allowed to develop for 3 days. The EDso was obtained as described in Fig. 1. (a) Pseudorabies virus was assessed in D21 (●) and LMTK− (○) cells. (b) Vaccinia virus was assessed in D21 (●) and LMTK− (○) cells.

of the HSV TK gene into LMTK− cells resulted in this case in only a 10-fold increase in sensitivity to the drug (EDso for LMTK− 10 μg/ml, for D21 1 μg/ml). This compares with a 300-fold increase in sensitivity of vaccinia and pseudorabies viruses in D21 cells relative to LMTK−, and implies that the virus DNA polymerase systems are more sensitive to phosphorylated ACV than the DNA synthesis machinery of the cells.

These experiments demonstrate the value of biochemically transformed cells in revealing the sensitivity of viruses to phosphorylated ACV. It is possible that other viruses will be found to have polymerase systems which are sensitive, and we are currently investigating several other DNA and RNA viruses. The system is also useful for the study of ACV-resistant mutants of HSV as it allows us to detect subtle changes at the polymerase locus in viruses which are defective in expression of TK (Field et al. 1980). It may also be useful for studying the role of HSV TK in the mode of action of other drugs which inhibit the replication of herpes viruses.

Finally, we conclude from these data that a major factor contributing to the resistance to vaccinia and pseudorabies viruses to ACV is their failure to phosphorylate the drug in infected cells. On this basis we suggest that phosphorylated ACV will have a wider spectrum of antiviral activity than the unphosphorylated compound, although it is likely to be coupled with increased toxicity.
Short communications

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Division of Virology
Department of Pathology
Cambridge University
Cambridge, U.K.

Graham Darby
Brendan A. Larder
Kenneth F. Bastow
Hugh J. Field

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