ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1983, p. 332-334
0066-4804/83/020332-03$02.00/0

Vol. 23, No. 2

Effect of Acyclovir on Viral Protein Synthesis in Cells Infected with Herpes Simplex Virus Type 1

PHILLIP A. FURMAN* AND PAUL V. McGUIRT

Wellcome Research Laboratories, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709

Received 16 August 1982/Accepted 9 December 1982

The effect of the antiviral agent 9-(2-hydroxyethoxymethyl)guanine (acyclovir) on herpes simplex virus type 1 protein synthesis during virus replication was examined. Treatment of infected cells with acyclovir markedly affected the amounts of the four major glycosylated and certain non-glycosylated viral polypeptides synthesized; other viral polypeptides were made in normal amounts. The reduced amount of late protein synthesis was most likely due to the inhibition of progeny viral DNA synthesis by acyclovir.

The nucleoside analog 9-(2-hydroxyethoxymethyl)guanine (acyclovir; Zovirax, Burroughs Wellcome Co.) is a potent and selective inhibitor of herpes simplex virus (HSV) replication (2, 10). Acyclovir exerts its antiviral effect after being converted to acyclovir triphosphate, which inhibits the viral nucleotidyl transferase (DNA polymerase) more effectively than it does the host cell α DNA polymerase (2, 3). It has been reported that when HSV DNA synthesis is blocked, alterations in the production of certain classes of HSV-specific polypeptides can be detected (7, 12). These reports prompted us to investigate the effect of acyclovir on HSV type 1 (HSV-1) protein synthesis. In this study the effect of acyclovir on the synthesis of viral polypeptides from the α, β, and γ classes (4, 5) was examined, as well as its effect on viral glycoprotein synthesis.

A polypeptide was selected from each class, and its synthesis was followed in the presence and absence of acyclovir as a function of time. As a representative of the α class (immediate early class; first polypeptides to appear after release from a cycloheximide block), the high-molecular-weight nonstructural polypeptide ICP4 was chosen. ICP6 was selected to represent the β class (early class, but not made immediately after cycloheximide withdrawal). ICP5 was selected to represent the γ or late class, and the recently designated γ_2 class (7) was represented by ICP17. To determine the effect of acyclovir (100 μM, a 10-fold-greater concentration than is needed to inhibit HSV-1 DNA synthesis by >90% [3]) on the kinetics of synthesis of each of the four classes of polypeptides, we infected HEp-2 cells with the KOS strain of HSV-1 (20 PFU/cell) and pulse-labeled them with ^{14}C-amino acid mixture (5 μCi/ml; Amersham Corp.). Sodium dodecyl sulfate (SDS) extracts of the cells containing 30 μg of protein were analyzed by polyacrylamide gel electrophoresis (PAGE) (8). The synthesis of α polypeptides (Fig. 1A) appeared to be stimulated when cells were treated with acyclovir, but β polypeptide synthesis did not appear to be altered significantly (Fig. 1B). The synthesis of γ polypeptides was reduced by about 40 to 50% (Fig. 1C) and γ_2 polypeptide synthesis was reduced 90 to 95% (Fig. 1D) by acyclovir treatment. Late polypeptide synthesis was inhibited in a dose-dependent manner (data not shown).

It is apparent from the work of several investigators that the glycoproteins of the virion envelope are involved in the process of adsorption and penetration (6, 9). In particular, the glycoprotein gB appears to affect infectivity at the level of penetration (6, 9). A decrease in synthesis of glycoprotein gB has been reported to result in a reduction of virus infectivity (6, 9). Therefore, the effect of acyclovir on glycoprotein synthesis was examined to determine whether drug treatment caused a reduction of viral glycoprotein synthesis. HEp-2 cells infected with 20 PFU of the KOS strain of HSV-1 per cell were labeled with [^{14}C]glucosamine (2 μCi/ml; Amersham Corp.) from 4 to 24 h postinfection in the presence of various concentrations of drug. Samples were processed and electrophoresed as described in the legend to Fig. 2. The synthesis of all five major glycoproteins, gA, gB, gC, gD, and gE (1, 11), was reduced in a dose-dependent manner by acyclovir. The synthesis of other [^{14}C]glucosamine-labeled polypeptides, presumably corresponding to partially glycosylated intermediates of the major HSV-1

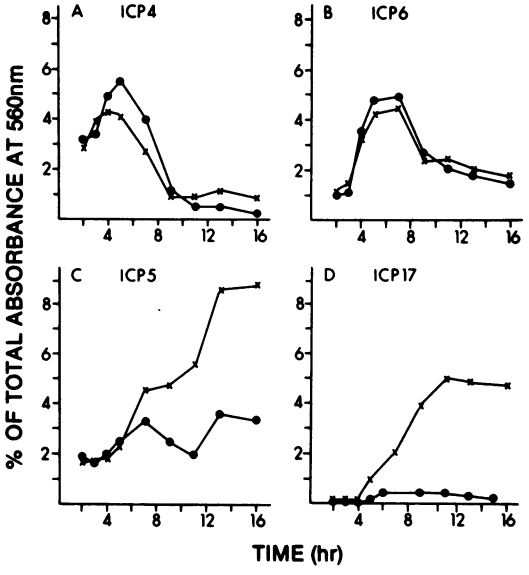

FIG. 1. Relative rates of representative polypeptide synthesis. HEp-2 cells were infected with the KOS strain of HSV-1 at a multiplicity of 20 PFU/cell. At various times postinfection, cells were labeled for 1 h with ^{14}C-amino acid mixture (5 μCi/ml; specific activity, 54 mCi/milligramatom of carbon) in amino acid-free medium supplemented with normal amounts of arginine and glutamine, 1/10 the concentration of essential amino acids, and 2% dialyzed fetal calf serum (labeling medium). At the end of the labeling period, cells were harvested and extracted with SDS, and the extracts were analyzed by PAGE (8). The amount of representative α (ICP4), β (ICP6), γ (ICP5), and γ_2 (ICP17) polypeptides synthesized in the presence (●) or absence (X) of acyclovir was calculated from an autoradiograph of a polyacrylamide gel. The autoradiogram was scanned with a densitometer, and each peak was converted to a percentage of the total absorbance.

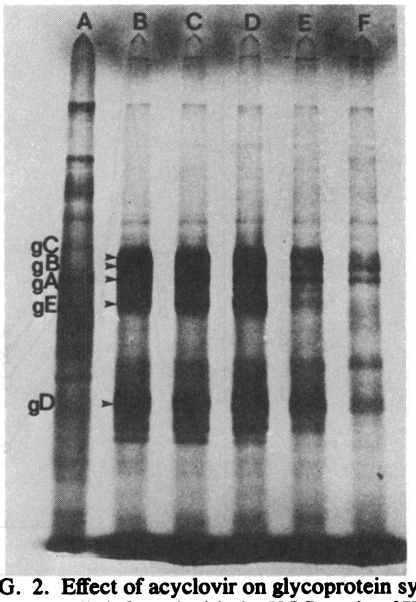

FIG. 2. Effect of acyclovir on glycoprotein synthesis. HEp-2 cells infected with the KOS strain of HSV-1 at a multiplicity of 10 PFU/cell were incubated in Eagle minimal essential medium containing acyclovir at the following concentrations: B, 0 μM; C, 0.1 μM; D, 1.0 μM; E, 10 μM; or F, 100 μM. Lane A, Uninfected cells (control). At 4 h postinfection, [^{14}C]glucosamine (2 μCi/ml, 61 mCi/mmol) was added to each plate of cells. Infected cells were harvested at 24 h postinfection, extracted with SDS, and analyzed by PAGE (9).

glycoproteins, was also reduced by acyclovir treatment.

The overall effect of acyclovir on HSV-1 protein synthesis was examined by following the incorporation of ^{3}H-amino acids (2 μCi/ml; Amersham Corp.) into acid-precipitable material (Fig. 3). Inhibition of total protein synthesis was not obvious at low drug concentrations. However, at acyclovir concentrations of 10 μM and above, a reduction in total protein synthesis reflected the patterns seen with individual polypeptides. The predominant polypeptides being synthesized by 8 h after infection were of the γ class. A large reduction in the synthesis of γ polypeptides could, therefore, result in the reduction in overall protein synthesis shown in Fig. 2.

This study shows that the production of certain classes of HSV-1 polypeptides can be altered by acyclovir treatment. As is the case for arabinofuranosylcytosine (7, 12), this drug effect is most likely due to inhibition of HSV-1 DNA synthesis. It has been suggested that since parental DNA can serve as a template for mRNAs for α, β, and γ polypeptides, the apparent stimulation of α and the possible stimulation of β polypeptides may be a result of more parental DNA being available for transcription (7). The inhibition of virus progeny DNA synthesis by acyclovir may be responsible for the decrease in γ polypeptide synthesis, since a greater proportion of the γ polypeptides are made from progeny DNA. The reduction in γ polypeptide synthesis, although a secondary effect of acyclovir, could play a role in the inhibition of virus replication. Furthermore, the reduction of viral glycoprotein synthesis by acyclovir could affect the infectivity of newly made virus (7, 12).

We thank M. Lyon for excellent technical assistance, G. B. Elion for critical reading of the manuscript and continued support, and K. K. Biron for critical reading of the manuscript. For the use of the gel scanner and PDP-11 computer, we thank W. K. Joklik of the Department of Microbiology, Duke University.

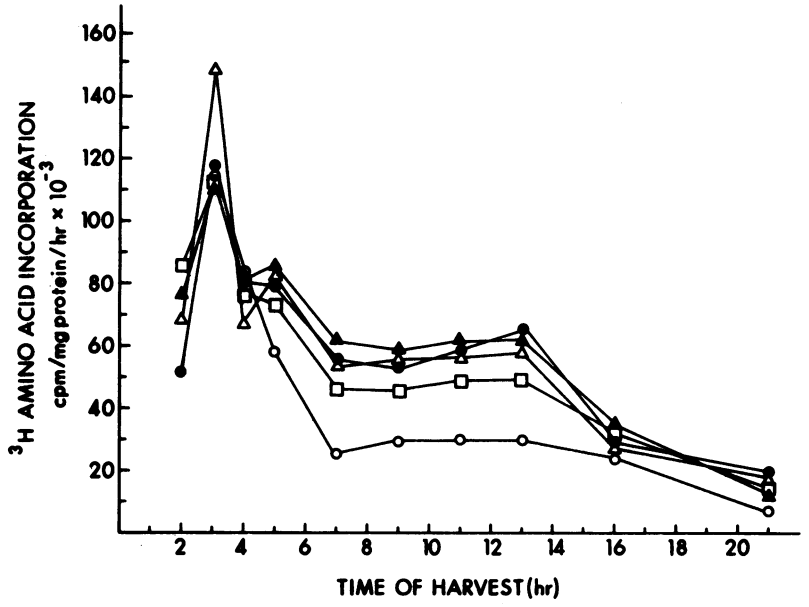

FIG. 3. Effect of acyclovir on total infected cell protein synthesis. HEp-2 cells infected with the KOS strain of HSV-1 at a multiplicity of 20 PFU/cell were incubated with acyclovir at the following concentrations: (●) 0 μM, (▲) 0.1 μM, (△) 1.0 μM, (□) 10 μM, and (○) 100 μM. At the indicated times after drug addition, the cells were pulse-labeled for 1 h with ^{3}H-amino acid mixture (2 μCi/ml) in labeling medium (see the legend to Fig. 1). After being labeled, the cells were washed three times with phosphate-buffered saline, and samples were assayed for total acid-precipitable radioactivity.

LITERATURE CITED

1. **Bauke, R. B., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an F_c-binding glycoprotein. J. Virol. 32:779–789.
2. **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. U.S.A. 74:5716–5720.
3. **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 replication by 9-(2-hydroxy-ethoxymethyl)guanine and its triphosphate. J. Virol. 32:72–77.
4. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpesvirus polypeptides in the infected cell. J. Virol. 12:1347–1365.
5. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14:8–19.
6. **Little, S. P., J. T. Jofre, R. J. Courtney, and P. A. Schaffer.** 1981. A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. J. Virol. 115:149–160.
7. **Powell, K. L., D. J. M. Purifoy, and R. J. Courtney.** 1975. The synthesis of herpes simplex virus proteins in the absence of virus DNA synthesis. Biochem. Biophys. Res. Commun. 66:262–271.
8. **Samuel, C. E., and W. K. Joklik.** 1974. A protein synthesizing system from interferon-treated cells that discriminates between cellular and viral messenger RNAs. Virology 58:476–491.
9. **Sarmiento, M., M. Haffey, and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7 (B_2) in virion infectivity. J. Virol. 29:1149–1158.
10. **Schaeffer, H. J., L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, and P. Collins.** 1978. 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature (London) 272:583–585.
11. **Spear, P. G.** 1976. Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. 17:991–1008.
12. **Ward, R. L., and J. G. Stevens.** 1975. Effect of cytosine arabinoside on virus-specific protein synthesis in cells infected with herpes simplex virus. J. Virol. 15:71–80.