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Gene Expression of Herpes Simplex Virus
III. Effect of Arabinosyladenine on Viral Polypeptide Synthesis

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Arabinosyladenine, an established antiherpetic drug, was used to block herpes simplex virus type 1 DNA synthesis quantitatively in infected xeroderma pigmentosum cells. Kinetic analyses of viral polypeptides synthesized in the presence and absence of this drug revealed that there were at least six distinct kinetic classes of polypeptides. These differed in time of appearance after infection, time of maximum rate of synthesis, kinetics of turnoff, and sensitivity to arabinosyladenine. This study showed that arabinosyladenine had the following three main effects on herpes simplex virus type 1 gene expression. (i) The turnon of immediate early and delayed early polypeptides (kinetic classes 1 and 2) was retarded. (ii) The turnoff of early (immediate early and delayed early) polypeptides (classes 1 through 3) was delayed. (iii) The synthesis of late polypeptides (classes 4 through 6) was inhibited by arabinosyladenine, with class 6 severely (80 to 90%) inhibited. The kinetic data presented here, along with the findings of other workers on the effects of inhibition of viral DNA synthesis, suggest that viral DNA replication is required for optimum synthesis of late viral polypeptides.

As a potent and effective antiherpetic drug, the nucleoside analog 9-β-D-arabinofuranosyladenine (ara-A) has received considerable attention over the past several years. Studies have shown a strong correlation between its antiviral activity and its preferential inhibition of viral DNA synthesis (12, 13). This inhibition appears to be mediated through the intracellular conversion of ara-A to arabinofuranosyladenine triphosphate and the subsequent inhibition of viral DNA polymerase activity (10).

Recently, we utilized this property of ara-A to block viral DNA replication so that UV mapping studies could be carried out on the prereplicative genes of herpes simplex virus type 1 (HSV-1) (8). In these studies, repair of the UV-irradiated viral genome was prevented by using DNA repair-negative xeroderma pigmentosum (XP) fibroblasts as host cells.

Before ara-A was used to inhibit HSV-1 DNA synthesis in studies on viral gene expression, it was necessary to characterize the kinetics of virus multiplication and the syntheses of DNA and polypeptides in infected XP cells in the presence and absence of the drug. The experiments described here established the parameters of HSV-1 multiplication in XP cells and showed that ara-A quantitatively blocked HSV-1 DNA replication in these cells. More importantly, we found that ara-A inhibition had some profound effects on the normal temporal control of viral gene expression. These effects included a delayed turnoff of synthesis of early viral proteins and a severe inhibition of synthesis of late, postreplicative viral proteins.

MATERIALS AND METHODS

Virus and cells. HSV-1 strain F (4) was propagated in HEp-2 (human epidermoid carcinoma) cells (both obtained from B. Roizman, University of Chicago, Chicago, Ill.). XP fibroblasts of the A complementation group (CRL 1223, XP-12BE) were obtained from the American Type Culture Collection, Rockville, Md. The virus was purified by sucrose gradient centrifugation, and the titers were determined by a plaque assay in Vero and XP cells. All methods used were as previously described (8).

Infection of cells. XP cell monolayers (85 to 96% confluent) were infected at a multiplicity of 50 PFU/cell for 1 h at 37°C with virus in phosphate-buffered saline (PBS) containing 0.1% glucose and 1% inactivated calf serum. Virus was removed, and the cells were rinsed and then overlaid with Dulbecco modified Eagle medium containing 1% inactivated calf serum. Time after infection was defined as time after addition of virus. When ara-A was added from the time of infection, the adenosine deaminase inhibitor covidarabine (1 μg/ml) and ara-A (0 to 250 μM) were present in both the infecting medium and the overlay medium.

Kinetics of virus multiplication and DNA synthesis. To measure the kinetics of DNA synthesis in HSV-1-infected XP cells, 25-cm^2 cultures were infected, overlaid with Dulbecco modified Eagle medium containing 1% inactivated calf serum, and pulse-la-

beled for 30 min with 2 μCi of [*methyl*-^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.) per ml at different times postinfection. The labeling medium was removed and saved for determining the titers of extracellular virus. The cells were rinsed three times with cold PBS, scraped into 2 ml of PBS, and mixed with a Vortex mixer; 1 ml of cell suspension was treated with 0.2% sodium dodecyl sulfate, and the DNA from a 50-μl sample was precipitated with 5% cold trichloroacetic acid. The precipitate was collected on GF/C glass fiber filters (Whatman), washed, and dried, and the [^{3}H]thymidine incorporation was determined by liquid scintillation spectroscopy. The other 1 ml of cell suspension was sonicated for 2 min to disrupt the cells, serial dilutions were made in PBS containing 0.1% glucose and 1% inactivated calf serum, and the intracellular virus titers were determined by a plaque assay on Vero cells.

Inhibition of viral and host DNA synthesis by ara-A. To measure the inhibition of viral DNA synthesis, XP cells were infected as described above, washed with PBS, and then overlaid with Dulbecco modified Eagle medium containing 1% inactivated calf serum, 14 μCi of [*methyl*-^{3}H]thymidine (New England Nuclear Corp.) per ml, 1 μg of covidarabine (2-deoxycoformycin) per ml, and 0 to 250 μM ara-A. These compounds were kindly supplied by H. Machamer, Parke, Davis & Co., Detroit, Mich., or were obtained from Sigma Chemical Co., St. Louis, Mo. At either 8 or 18 h postinfection, the infected cells were rinsed with ice-cold PBS, suspended in cold PBS, centrifuged at 3,000 rpm for 10 min, and suspended in NET buffer (120 mM NaCl, 1 mM EDTA, 10 mM Tris-hydrochloride, pH 7.4). The EDTA concentration was increased to 5 mM, sodium dodecyl sulfate was added to 0.5%, and a 10-μl sample was removed for determination of total thymidine incorporation into DNA. The DNA was extracted three times with phenol (saturated with NET buffer and containing 0.08% 8-hydroxyquinoline) and then three times with $CHCl_3$-isoamyl alcohol (50:1). CsCl was added to the DNA solutions to a density of 1.712 g/cm^3 (7.177 molal). The samples were centrifuged at 35,000 rpm for 90 h at 20°C in a Spinco SW50.1 rotor. Fractions were collected dropwise and assayed for density by refractometry and trichloroacetic acid-insoluble [^{3}H]thymidine incorporation. The viral DNA banded at 1.725 g/cm^3, and the host DNA banded at 1.698 g/cm^3.

Analysis of viral proteins. Proteins were labeled by incubating infected cell cultures for 30 or 60 min at 37°C in minimal essential medium containing 1/100 the normal concentration of methionine, 10 to 12 μCi of [^{35}S]methionine (Amersham) per ml, and, when indicated, covidarabine (1 μg/ml) and ara-A. The cells were lysed, and the viral polypeptides were quantitated by polyacrylamide gel electrophoresis, autoradiography, and film densitometry as previously described (8). To compare the rates of polypeptide synthesis in the absence and presence of ara-A, experiments were performed in parallel under identical conditions with the exception of the added drug.

RESULTS

Kinetics of virus multiplication and DNA synthesis in infected XP cells. We chose XP cells as host cells to study HSV-1 gene expression for the following two reasons. First, these cells provide a DNA repair-negative system in which to perform UV mapping studies of viral gene expression (8). Second, when infected with HSV-1, they exhibit a more rapid and thorough turnoff of host RNA synthesis than the other cell lines which we have used. This has been advantageous for analyzing HSV-1 mRNA's (16).

It was necessary to establish virus growth parameters in these cells in order to correlate the effects of ara-A with the kinetics of viral DNA synthesis. Figure 1 shows the kinetics of intra- and extracellular virus production and [^{3}H]thymidine incorporation. The rate of thymidine incorporation decreased during the first 2.5 h, increased, reached a peak at about 4.5 h, and then decreased with increasing time postinfection. This paralleled the pattern of thymidine incorporation of HSV-1-infected HEp-2 cells, in which the peak rate of thymidine incorporation coincided with the peak of incorporation into viral DNA (11). Intracellular virus production began at the peak of thymidine incorporation (4 to 5 h postinfection) and reached a maximum level at 10 to 12 h postinfection. Subsequently, virus began to be released from the cells and increased in the extracellular medium.

Inhibition of viral DNA synthesis by ara-A. To determine the effectiveness of ara-A in inhibiting viral DNA synthesis in infected XP cells, two sets of cell cultures were infected. At 1 h after infection, [^{3}H]thymidine, covidarabine (to inhibit adenosine deaminase), and varying concentrations of ara-A were added to the medium. One set of cultures was harvested at 8 h postinfection, and the other set was harvested at

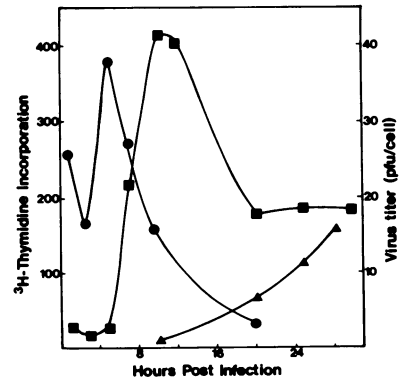

FIG. 1. *Time course of virus production and DNA synthesis in infected XP cells. The experimental procedures used are described in the text. Symbols: ●, [^{3}H]thymidine incorporation into total DNA; ■, intracellular virus titer; ▲, extracellular virus titer.*

18 h postinfection. The DNA was extracted and separated into viral and host components by buoyant density centrifugation. Table 1 shows the incorporation of thymidine into viral and host DNAs. As reported previously for infected KB cells (12, 13), viral DNA synthesis was much more sensitive to ara-A inhibition than host DNA synthesis. At a concentration of 100 μM, ara-A reduced viral DNA synthesis to less than 0.19% of the synthesis in the ara-A-free control, whereas host synthesis was decreased to only 15 to 16% of the control value. At 170 μM ara-A, viral DNA synthesis was less than 0.08% of the control value by 8 h postinfection. Because of tailing of host DNA into the region of viral DNA in the gradients, the values shown for viral DNA synthesis represent maximum levels. These results show that ara-A plus covidarabine almost quantitatively blocked viral DNA synthesis in HSV-1-infected XP cells.

Effect of ara-A concentration on viral polypeptide synthesis. To determine the effects of ara-A inhibition on viral gene expression, we examined the viral polypeptides that were labeled at 8.5 h postinfection in the presence of different concentrations of ara-A. Although most viral proteins appeared to be synthesized in the presence of ara-A, a number of quantitative changes in the viral polypeptide pattern became apparent as we increased the ara-A concentration (Fig. 2). The most striking change was the severe inhibition of synthesis of a number of late proteins, including viral polypeptides 240, 154, 103, 77, 53, 50, and 48 (designations correspond to molecular weights [$\times 10^3$]). As discussed below, these polypeptides belong to the two latest-appearing kinetic classes of viral proteins, classes 5 and 6. These apparently correspond in part to the γ polypeptides (e.g., ICP 1-2, 5, 15, 21, 31, 32, and 35) described by Honess and Roizman (5) and to the γ_2 class reported by Powell et al. (9), which was inhibited severely by ara-C.

Figure 3 shows the relative amounts of some of these polypeptides synthesized at different ara-A concentrations. This figure shows that the inhibition of polypeptide synthesis roughly paralleled the inhibition of viral DNA synthesis by ara-A.

The second observation to come from these studies was that with increasing ara-A concentrations, the normal turnoff of a number of early proteins (viral polypeptides 165, 145, 126, 92, 86, 55, and 41) was delayed (Fig. 2). This caused an apparent stimulation of labeling of early proteins at 8.5 h postinfection with increasing ara-A concentrations (Fig. 3). As shown below, these proteins belong to the three earliest classes of viral proteins. These classes consist of the immediate early and delayed early proteins (8) and correspond in part to the α and β classes described by Honess and Roizman (5).

Effect of time of ara-A addition on viral gene expression. In another experiment, cultures of XP cells were infected with HSV-1, and ara-A was added at different times postinfection. All cultures were pulse-labeled with [^{35}S]methionine at 11 h, and the proteins were analyzed by gel electrophoresis (Fig. 4). To achieve full inhibition of synthesis of the late viral proteins (e.g., polypeptides 240, 154, 103, 77, 61, 53, and 50) ara-A had to be added by 2 h postinfection. This is more clearly shown in Fig. 5, where the relative amounts of methionine incorporation into these proteins are plotted against the time

TABLE 1. *Effect of ara-A on [^{3}H]thymidine incorporation into viral and host cell DNAs*

Concn of ara-A (μM)[a]	^{3}H incorporation at 8 h postinfection[b]				^{3}H incorporation at 18 h postinfection			
	HSV DNA		Host DNA		HSV DNA		Host DNA	
	Amt (cpm, $\times 10^3$)	% of control	Amt (cpm, $\times 10^3$)	% of control	Amt (cpm, $\times 10^3$)	% of control	Amt (cpm, $\times 10^3$)	% of control
0	107.7	100	22.9	100	435	100	48.8	100
4					136.2	31.3	66.8	137
10					53.9	12.4	48.0	98.3
20	5.25	4.87	19.6	85.4	23.1	5.03	44.4	90.9
50	0.53	0.49	5.91	25.8				
60					2.74	0.63	9.87	22.7
100	0.16	0.153	3.73	16.3	0.84	0.19	7.47	15.3
170	0.09	0.08	2.80	12.2				
250	0.09	0.08	2.34	10.2				

[a] Final concentration of ara-A, added along with covidarabine (1 μg/ml) after removal of virus at 1 h postinfection.

[b] Values are the amounts of [^{3}H]thymidine incorporated during labeling periods of 1 to 8 h or 1 to 18 h postinfection into viral DNA (1.725 g/cm^3) and host DNA (1.695 g/cm^3) after buoyant density separation in CsCl gradients.

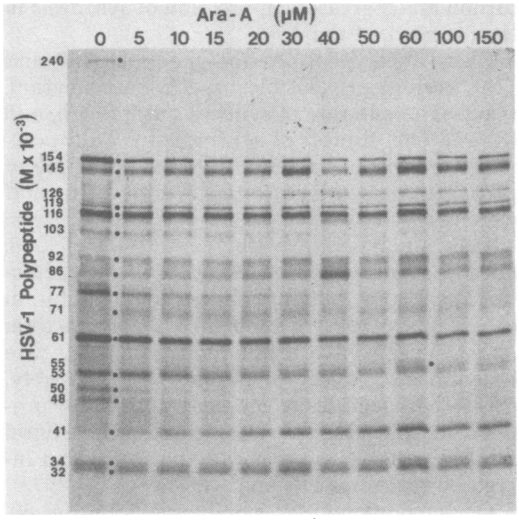

FIG. 2. *Effect of ara-A concentration on viral polypeptide synthesis. Monolayer cultures of XP fibroblasts (25 cm²) were infected with HSV-1 at a multiplicity of 50 PFU/cell. Covidarabine (1 μg/ml) and ara-A at the indicated concentrations were present from the time of virus addition until termination of the labeling period. At 8.5 h after infection, the cells were labeled with [³⁵S]methionine (10 μCi/ml) for 1 h. The cells were lysed, and the polypeptides were analyzed by polyacrylamide gel electrophoresis. M, Molecular weight.*

of addition of ara-A. A comparison of these data with the data in Fig. 1 shows that ara-A had to be present from before the onset of viral DNA synthesis to achieve maximum inhibition of late viral protein synthesis.

Kinetics of viral polypeptide synthesis in the presence and absence of ara-A. To determine the effects of ara-A on the kinetics of synthesis of the different viral polypeptide classes, duplicate sets of cell cultures were infected with HSV-1. One set contained ara-A and covidarabine from the time of virus addition, and the control set contained no drugs. At different times after infection, the cultures were pulse-labeled with [³⁵S]methionine, and the proteins were analyzed by gel electrophoresis and autoradiography. In the control experiment with no added drugs (Fig. 6A), a number of different temporal classes of viral polypeptides were found, as first reported by Honess and Roizman (5). Figure 6B shows the changes in the normal time course of viral polypeptide expression when viral DNA synthesis was blocked by ara-A.

These data show that ara-A produced the following two major changes in the kinetics of HSV-1 polypeptide synthesis: (i) a delayed turnoff of synthesis of the immediate and delayed early proteins (e.g., viral proteins 145, 126, 92, and 55) and (ii) a severe reduction in the amount of synthesis of a number of late proteins (e.g., viral polypeptides 240, 154, 103, 77, and 53).

The kinetics of polypeptide synthesis were analyzed by measuring the relative amount of methionine incorporated into each protein band by densitometry of the autoradiograms, as described previously (8). These analyses (Fig. 7) showed that the viral proteins fell into at least six classes. These classes differed from each other in the time postinfection of turnon, in the time of maximum rate of synthesis, in the kinetics of turnoff of synthesis, and in the manner in which they were affected by ara-A.

Class 1 consists of the earliest-appearing viral polypeptides. These were present by 1 h, reached a maximum rate of synthesis at about 2 h, and were no longer observed at 7 h postinfection. This class consists of two immediate

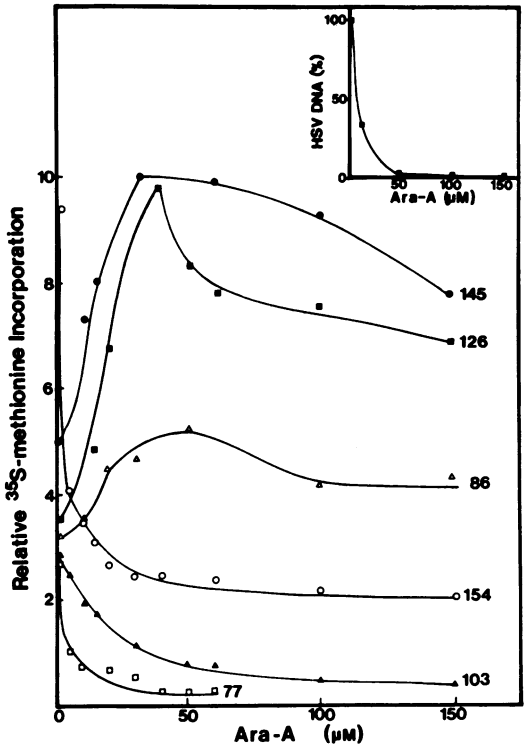

FIG. 3. *Effect of ara-A concentration on the relative amounts of viral polypeptides synthesized. The relative amounts of viral polypeptide synthesis were determined by densitometry of the gel autoradiogram of Fig. 2. The ordinate values represent arbitrary densitometry units. The relative [³H]thymidine incorporation into HSV-1 DNA (insert) was taken from Table 1. Viral polypeptide designations (molecular weight ×10³) are at the right.*

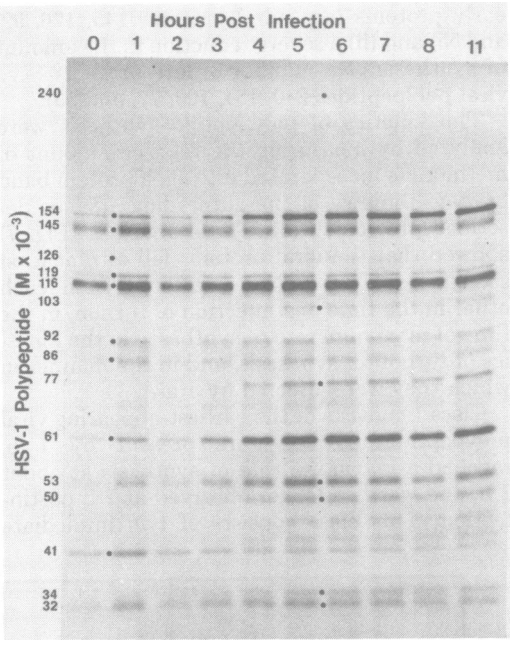

FIG. 4. *Effect of time of addition of ara-A on viral polypeptide synthesis. Cultures of XP cells were infected and the radiolabeled polypeptides were analyzed as described in the text and in the legend to Fig. 3. Ara-A (100 μM) and covidarabine (1 μg/ml) were added at the times indicated and were present throughout the labeling period with [^{35}S]methionine from 11 to 12 h postinfection. M, Molecular weight.*

early polypeptides, 165 and 55 (i.e., the polypeptides whose mRNA's are synthesized in the presence of cycloheximide). These appeared to correspond to polypeptides ICP 4 and 27 of Honess and Roizman (5). Immediate early protein 123 (ICP 0) also may belong to this class, but it was not clearly resolved in these experiments. When ara-A was present, these proteins reached the peak rate of synthesis later (at 4 h postinfection) and were turned off more slowly than normal.

Class 2 viral polypeptides (e.g., polypeptides 130, 126, and 86) were first apparent by 2 h postinfection, reached a peak rate of synthesis at 3 h, showed 50% turnoff by 5.5 h, and were almost completely shut off by 10 h postinfection. These polypeptides, as well as some class 3 polypeptides (e.g., polypeptide 119), behaved as delayed early gene products in that they required a prior period of viral protein synthesis, were made in the absence of viral DNA synthesis, and appeared to belong mainly to the β class (5). Because of different sensitivities to ara-A, class 2 polypeptides were divided into two subclasses (Fig. 7, broken lines). Class 2B polypeptides (proteins 34 and 32) showed a greater delay in turnon and a greater suppression of synthesis in the presence of ara-A.

Class 3 polypeptides (polypeptides 145 and 119) were clearly visible by 2 h postinfection, reached a peak rate of synthesis at 3 to 4 h, and showed 50% shutoff of synthesis by 7.5 h postinfection. In the presence of ara-A, the time of peak rate of synthesis and the time of turnoff were delayed by 2 to 3 h.

Class 4 polypeptides (polypeptides 116, 71, 66, and 61) were turned on at about 1 h postinfection, peaked at 5 h, and showed 50% inhibition of rate of synthesis at about 10.5 h postinfection. Ara-A caused only a slight depression in the rate of synthesis. These polypeptides and, possibly, class 5 polypeptides resembled "quasi-late" proteins in that they first appeared before viral DNA synthesis, but were synthesized at an increased rate after DNA synthesis.

Class 5 polypeptides (polypeptides 154, 55, 50, and 48) were turned on at 2 h, reached a maximum rate of synthesis at about 7 h, and showed 50% reduction in rate of synthesis by about 12 h

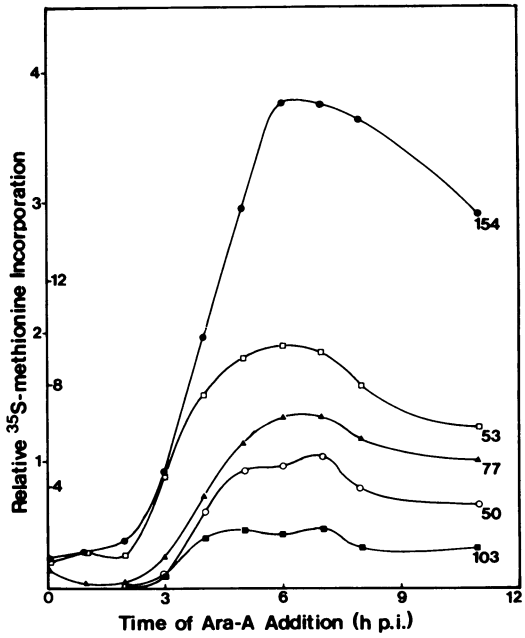

FIG. 5. *Effect of time of ara-A addition on the synthesis of late viral polypeptides. The data were determined from densitometer scans of the labeled polypeptides in the gel autoradiogram of Fig. 4. The ordinate values represent arbitrary densitometer units. The outer numbers are values (×10³) for polypeptide 154; the inner numbers are values (×10²) for the other polypeptides. Viral polypeptide designations (molecular weight, ×10³) are at the right. p.i., Postinfection.*

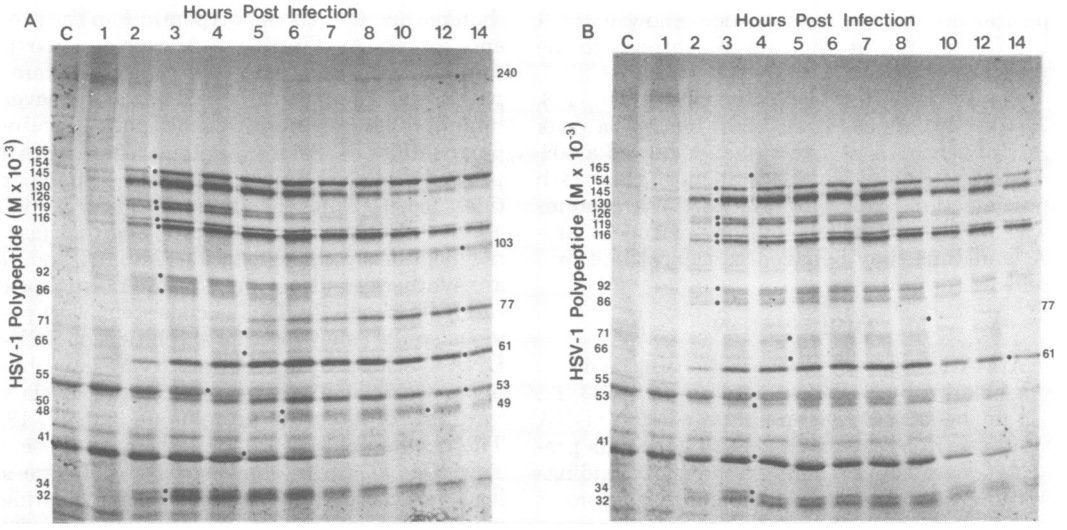

FIG. 6. *Time course of HSV-1 polypeptide synthesis in the absence and presence of ara-A. The experimental procedures used are described in the text. (A) Polypeptides synthesized without added drugs. (B) Polypeptides synthesized in the presence of ara-A (150 μM) and covidarabine (1 μg/ml). Times represent the midpoints of 30-min labeling periods with [^{35}S]methionine (12 μCi/ml). C, Uninfected control; M, Molecular weight.*

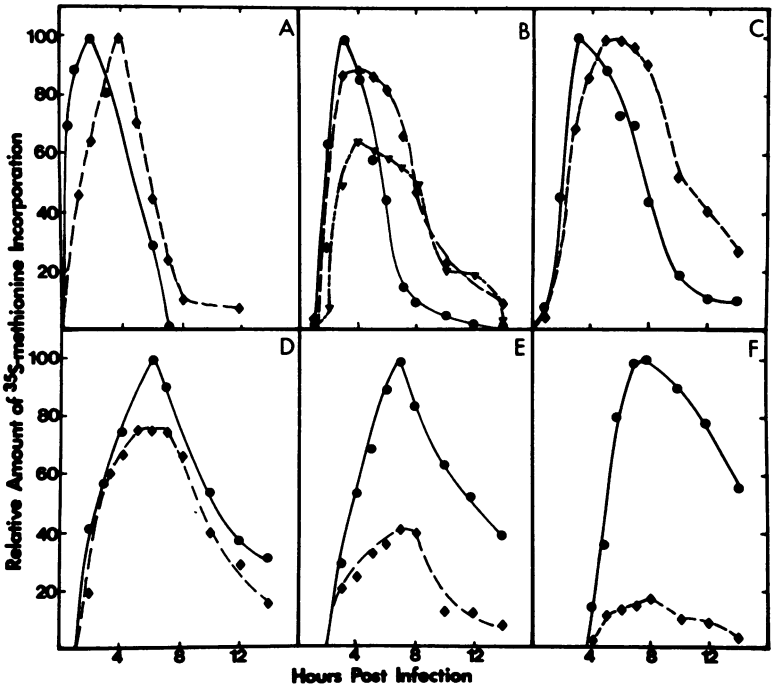

FIG. 7. *Kinetics of viral polypeptide synthesis in the absence (solid lines) and presence (broken lines) of ara-A. The data were determined from densitometer scans of the autoradiograms shown in Fig. 6. The points for each curve represent averages of all of the viral polypeptides that were analyzed in each kinetic class. Each point represents the percentage of incorporation relative to the maximum (or peak) incorporation without drug. Class 2 polypeptides were subdivided into two groups according to their inhibition by ara-A. (A) Class 1. (B) Class 2. (C) Class 3. (D) Class 4. (E) Class 5. (F) Class 6.*

postinfection. These polypeptides showed about a 60% reduction in the rate of synthesis in the presence of ara-A.

Class 6 polypeptides (polypeptides 240, 103, 77, and 49) appeared at 3.5 h, reached a peak rate of synthesis at 7 to 8 h, and showed a 50% turnoff of rate of synthesis at more than 14 h postinfection. The synthesis of these proteins was inhibited 80 to 90% by the presence of ara-A, and thus they behaved as typical late proteins.

DISCUSSION

Ara-A is a potent antiherpetic drug and has been shown by Shipman and co-workers (3, 12, 13) to be a selective inhibitor of HSV DNA synthesis in infected KB cells. The results presented here support and extend these findings and show that the appropriate concentrations of ara-A, along with the adenosine deaminase inhibitor covidarabine, effectively inhibit HSV-1 DNA synthesis in infected XP cells. With 170 μM ara-A, thymidine incorporation into viral DNA was less than 0.08% of the control levels.

We also showed that ara-A has a number of striking effects on the expression of viral polypeptides. Kinetic analyses of the viral polypeptides produced in HSV-1-infected cells in the absence and presence of ara-A (Fig. 6 and 7) revealed that there are at least six distinct kinetic classes of viral polypeptides. These differ in the time of initial appearance after infection, time of maximum rate of synthesis, kinetics of turnoff, and sensitivity to ara-A.

It is of interest that the immediate early polypeptides (polypeptides 165, 145, 123, 86, 71, and 55) (8, 16), i.e., those synthesized immediately after removal of a cycloheximide block, belong not only to the earliest kinetic class, but also to classes 1 through 3. Thus, even though the mRNA's for these proteins are synthesized in the absence of protein synthesis, not all immediate early proteins are expressed with the same kinetics during normal infection. This could be due to different transcriptional or translational rates.

In addition to its well-established action on DNA synthesis, ara-A appears to have three main effects on the normal temporal controls of viral gene expression. First, it causes the immediate early and delayed early polypeptides of classes 1 through 3 to be delayed in reaching the maximum rate of synthesis. As Fig. 7 shows, this maximum rate occurs 1 to 3 h later than normal in the presence of the drug. Second, ara-A causes a delay in the turnoff of synthesis of the polypeptides of classes 1 through 3. Thus, the time of 50% turnoff is 1 h later than normal for class 1, about 2.5 h later for class 2, and up to 3 h later than normal for class 3 polypeptides in the presence of ara-A. Third, ara-A causes a partial suppression of the rates of synthesis of class 5 proteins and some class 3 proteins and a severe inhibition of synthesis of the postreplicative polypeptides of classes 5 and 6. When ara-A is present, the class 5 polypeptides (e.g., polypeptides 154, 50, and 48) are synthesized at only about 40% of the maximum rate, and the class 6 polypeptides (e.g., polypeptides 240, 103, and 77) are synthesized at only 15% of the normal peak rate (Fig. 6 and 7). This is consistent with previous studies showing that inhibition of HSV-1 DNA synthesis by ara-C, hydroxyurea, or phosphonoacetic acid greatly reduces the levels of synthesis of late viral proteins (5, 6, 9, 17, 18). The results presented here agree with those of Powell et al. (9) in showing that there are at least two classes of postreplicative polypeptides which differ with respect to dependence on viral DNA synthesis, one of which is severely inhibited.

The effects of ara-A on late gene expression are probably the result of inhibition of viral DNA synthesis by this drug. The inhibition of synthesis of class 5 and 6 polypeptides with increasing ara-A concentrations parallels the inhibition of HSV-1 DNA synthesis (Fig. 3), and the escape from this inhibition by delaying the time of ara-A addition postinfection roughly parallels the initial kinetics of viral DNA synthesis (Fig. 5).

The requirement of DNA replication for late gene expression may be related in part to the greater instability of the late mRNA's (18). However, a number of studies have shown that late viral transcription is dependent on viral DNA replication. Several studies by Swanstrom et al. (14, 15) have shown that blockage of HSV-1 DNA synthesis by hydroxyurea or mitomycin C results in restricted transcription. More recent experiments involving hybridization to restriction fragments of HSV-1 DNA have shown that late transcription is inhibited by ara-C and phosphonoacetate (1, 7), as well as by ara-A (4a).

A new finding that was not apparent from previous studies in which hydroxyurea, ara-C, or phosphonoacetic acid was used to block HSV DNA synthesis is the effect of ara-A on early viral gene expression. Both immediate early and delayed early gene products (polypeptides of classes 1 through 3) are expressed in the presence of ara-A, but there is both a 1- to 3-h delay in their reaching the maximum rate of synthesis and a 2- to 3-h delay in their kinetics of turnoff (Fig. 7). Additional evidence for the latter effect comes from the apparent stimulation of synthesis of these polypeptides (e.g., polypeptides 145 and 126) by ara-A at late times (8.5 h) after

infection (Fig. 3). Since it is known that arabinosyl nucleotides can inhibit a variety of enzymes (2), it is not clear whether the effects of ara-A on early proteins are a direct result of inhibition of viral DNA synthesis or the result of ara-A action on other enzyme systems.

ACKNOWLEDGMENTS

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