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Antiviral Activity of Arabinosyladenine and Arabinosylhypoxanthine in Herpes Simplex Virus-Infected KB Cells: Selective Inhibition of Viral Deoxyribonucleic Acid Synthesis in the Presence of an Adenosine Deaminase Inhibitor

PAULINE M. SCHWARTZ,[1] CHARLES SHIPMAN, JR., and JOHN C. DRACH*

The Dental Research Institute, Department of Oral Biology, School of Dentistry; Department of Microbiology, School of Medicine; and Interdepartmental Graduate Program in Medicinal Chemistry, The University of Michigan, Ann Arbor, Michigan 48109*

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The antiviral activity of the fraudulent nucleoside arabinosyladenine (ara-A) against herpes simplex virus (HSV) type 1 was increased nearly 20-fold by the adenosine deaminase inhibitor, coformycin. The combination of ara-A plus coformycin was 90 times more potent in blocking HSV replication than was arabinosylhypoxanthine (ara-H). In suspension culture both drugs were more active than they were in monolayer culture. Deoxyribonucleic acid (DNA) synthesis also was inhibited by the nucleosides. Depending upon the species of DNA examined, ara-A was 8 to 15 times more active in the presence of coformycin, and the combination was 35 to 70 times more potent than ara-H. Both drugs inhibited total DNA synthesis to the same extent in uninfected and HSV-infected KB cells. In contrast, viral DNA synthesis was three to six times more susceptible to inhibition than was cellular DNA synthesis. Inhibition of viral DNA synthesis was more pronounced in suspension culture than in monolayer culture. However, the method of cell propagation did not alter the degree to which the drugs inhibited DNA synthesis in uninfected KB cells. An index has been derived to quantitate the extent of the selective inhibition of viral or cellular DNA synthesis. Fifty percent inhibitory concentrations of a drug were calculated for uninfected KB DNA synthesis and viral DNA synthesis and expressed as a ratio. The logarithm of this ratio was termed the selective index and was positive if viral DNA synthesis was inhibited preferentially or negative if uninfected KB DNA synthesis was more strongly inhibited. Data from experiments performed in monolayer culture gave positive selective index values of 0.3, 0.5, and 0.4 for ara-A plus coformycin, ara-A, and ara-H, respectively. Values of 0.7 and 0.6 were obtained from suspension culture data for ara-A plus coformycin and ara-H, respectively. Considered collectively, the data presented in this communication establish that coformycin increased the potency of ara-A but did not increase its selectivity.

The fraudulent nucleoside 9-β-D-arabinofuranosyladenine (ara-A) is active against a broad spectrum of deoxyribonucleic acid (DNA) viruses, including herpes simplex virus (HSV) type 1 (4, 21, 25, 28). In cell culture ara-A significantly reduced viral replication at drug concentrations that had no cytotoxic effect in uninfected cultures (13, 25, 27). In experimental animals, ara-A has been shown to be effective against several DNA virus infections without significant host toxicity (9, 21, 28). Early clinical studies in humans have indicated its usefulness against herpetic keratitis and cases of disseminated herpes zoster infection without significant toxicity (18).

Deamination of ara-A by adenosine deaminase from many sources yields 9-β-D-arabinofuranosylhypoxanthine (ara-H) (4, 10). Although ara-H also is active against DNA viruses, it is considerably less active than ara-A (21, 25, 28). Consequently, inhibitors of adenosine deaminase have been developed to potentiate the activity of ara-A. Investigators at Parke, Davis and Co. first noted in 1967 that such an inhibitor (covidarabine [31]) increased

[1] Present address: LAC/USC Cancer Center, 1720 Zonal Ave., Los Angeles, Calif. 90033.

the antiviral potency of ara-A (25). This observation recently was confirmed by us (P. M. Schwartz, C. Shipman, Jr., and J. C. Drach, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Abstr. 33, 1974) using coformycin (15) and by Bryson and Conner (2) and Conner et al. (5) using covidarabine. Plunkett and Cohen (19) have reported that the antiproliferative activity of ara-A also could be increased through the use of erythro-9-(2-hydroxy-3-nonyl)adenine, another potent inhibitor of adenosine deaminase (22).

Based upon experience in both cell culture and whole animals, the activity of ara-A and ara-H seems to be selective. In animal models the drugs exhibit antiviral activity at nontoxic doses, and in cell culture the nucleosides appear to affect virus-infected cells to a greater extent than uninfected cells. For example, Sidwell and Huffman (27) found that ara-A inhibited HSV-induced cytopathic effects at drug levels that did not produce cytotoxic effects in uninfected cultures. Shannon et al. (24) reported significant inhibition of Gross murine leukemia virus-induced plaque formation by ara-A at concentrations that produced no cytotoxicity in uninfected cells. In part I of this series, Shipman et al. (26) established that the selective activity of ara-A and ara-H could be observed at the level of DNA synthesis. Specifically, HSV DNA synthesis was inhibited to a greater extent by ara-A and ara-H than was cellular DNA synthesis in synchronous cultures of KB cells.

As a concurrent approach to a more detailed understanding of the biochemical action of ara-A, we addressed two points not considered in the first part of this series (26): namely, (i) was the selective inhibition of viral DNA synthesis dependent upon the use of synchronized cell cultures and (ii) did ara-A act selectively if it was used in concert with an adenosine deaminase inhibitor? We now have examined the activity of ara-A and ara-H in asynchronous cultures of KB cells using the same strain of HSV as studied previously (26). Here we present evidence that ara-A and ara-H are selective inhibitors of viral DNA synthesis regardless of the method used for cell propagtion and that coformycin did not alter the selectivity of ara-A.

(This investigation is based on portions of a dissertation submitted by P. M. Schwartz in partial fullfillment of the requirements for the Ph.D. degree in Medicinal Chemistry.)

MATERIALS AND METHODS

Materials. Coformycin was provided through the courtesy of H. Umezawa, Institute of Microbial Chemistry, Tokyo, Japan. All other chemicals, radioisotopes, and sera were obtained as noted in the preceding communication (26).

Cell culture techniques. The source of cells and HSV; the routine growth and passage of BHK-21/4 cells and KB cells; the propagation and titration of HSV; and techniques used for the enumeration of cells, the determination of cell viability, and the detection of mycoplasma contamination have been described previously (26).

Statistical analyses. Dose-response relationships were constructed by linearly regressing probit values of the percentage of inhibition of DNA synthesis or plaque formation against log drug concentrations. The 50% inhibitory (I_{50}) concentrations and the 95% confidence intervals were calculated from the regression lines using the methods described by Goldstein (8). Identity of any two dose-response curves was tested using one-way covariance analysis (7) by calculating P values for the equality of the regressions and slopes. The slopes of all the pairs of dose-response curves compared in this study were similar ($P > 0.350$), consequently P values given in the text refer only to the comparison of regressions.

Protocol for studies in monolayer cultures. KB cells were planted in plastic tissue culture flasks (75-cm^2 surface area) (Falcon Plastics, Oxnard, Calif.) 18 to 24 h before infection with HSV. At the onset of experiments, logarithmically growing, replicate, monolayer cultures were 60 to 70% confluent and contained 4×10^6 to 6×10^6 cells per flask. The medium was decanted, and the cultures were infected with 10 plaque-forming units (PFU) of HSV per cell. The HSV was contained in 1.0 ml of minimal essential medium (MEM) with Hanks salts supplemented with 10% unheated calf serum. Mock-infected monolayer cultures received 1.0 ml of medium alone. After a 1-h adsorption period at 37 C, the cell sheet was rinsed twice with 5 ml of MEM with Hanks salts to remove unabsorbed virus, and 10.0 ml of medium containing [^{3}H]thymidine (dThd) (5 μM, 2 or 3 μCi/ml), 2× arginine and 10% heated calf serum (23) was added. Medium also contained ara-A, with or without 3.5 μM coformycin, or ara-H, as illustrated in Fig. 1. Drugs were added to the medium at the selected concentrations immediately before use. After a 12-h incubation at 37 C, 0.5 ml of 0.25 mM ethylenediaminetetraacetate was added to uninfected monolayer cultures, and after several minutes at 37 C flasks were shaken to suspend the cells. Infected monolayers were shaken to suspend cells, and 1.0-ml samples were removed and stored at −76 C for subsequent virus assay. Ethylenediaminetetraacete (0.45 ml) then was added to the remaining medium. Samples of the cells were utilized to determine cell number and viability. One hundred-microliter portions of the suspensions also were spotted in triplicate on filter paper circles which were processed for the determination of incorporated label as described previously (26). Data were tabulated on the basis of tritium incorporated into acid-insoluble material per 10^3 cells. Inhibition of DNA synthesis was determined at the various drug levels as a percentage of a control without drugs. Five-milliliter portions of infected cell suspensions were centrifuged at $600 \times g$ for 5 min, and the pellets were stored at −76 C for subsequent sepa-

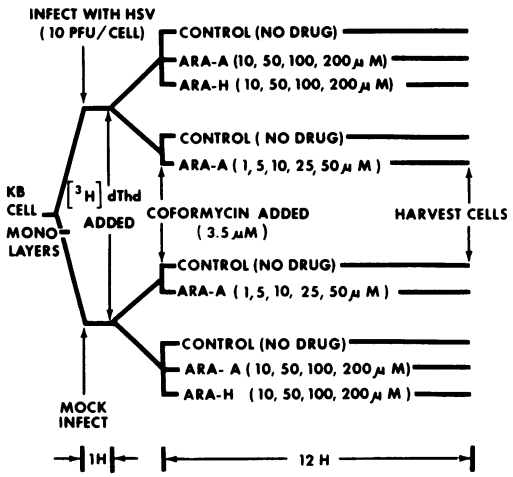

FIG. 1. *Experimental protocol for studies performed in monolayer cultures. In all experiments involving ara-A plus coformycin, the inhibitor also was present in the corresponding control flasks.*

ration of viral and cellular DNA. After isopycnic centrifugation in CsCl gradients (see reference 26), the amount of label was proportioned between viral and cellular DNA on the basis of peak areas. Areas were determined by integration after the peaks were fully separated using a Dupont 310 curve resolver set to fit Lorentzian distribution curves.

Protocol for studies in suspension cultures. Suspension cultures of KB cells were grown and maintained as described previously (26). Logarithmically growing cultures were used in experiments 5 to 14 days after their initiation from a monolayer culture. The general protocol for experiments in suspension cultures was that depicted in Fig. 1 with the following differences: (i) no studies were performed with ara-A alone, and (ii) all experiments were modified to take advantage of multiple sampling times. Thus, 400 ml of cell suspension from a single spinner flask was centrifued at 300 × *g* for 10 min at 4 C. All but 5 ml of the supernatant was removed and the loose pellet was suspended by gentle swirling. Suspended cells were infected with HSV to give 2 or 3 PFU/cell in a total volume of approximately 5 ml. Infective center assays established that 82% of the cells were infected resulting in an actual multiplicity of infection (MOI) of 0.9 or 1.4. Both virus-infected and mock-infected suspensions were kept at room temperature for 1 h and swirled periodically. To remove unadsorbed virus, cells were resuspended in 30 to 50 ml of MEM with spinner salts, and centrifuged again. After removal of the supernatant medium, the virus- and mock-infected cells were resuspended at a final density of 2×10^5 to 3×10^5 cells/ml and split into four separate spinner flasks. Each flask contained 100 ml of MEM with spinner salts buffered with 10 mM HEPES [*N*-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid] (pH 7.2 at 37 C), 2× arginine, 10% extensively heated calf serum (23), [^{3}H]dThd (5 μM, 3 μCi/ml), and selected concentrations of ara-A plus 3.5 μM coformycin or ara-H. The flasks were incubated at 37 C, and portions of the suspension were removed at selected times for the subsequent determination of cell number, cell viability, incorporated label, virus titer, and the separation of cellular and viral DNA species. Portion volumes and methods of determination were similar to those described in the preceding section.

RESULTS

The effects of ara-A and ara-H on virion replication and DNA synthesis were examined in asynchronous populations of KB cells so that a direct comparison could be made with the data obtained in synchronized cultures (26). Coformycin (3.5 μM) also was included in the experiments with ara-A to determine whether the selectivity observed previously might be a consequence of deamination of ara-A to ara-H.

Studies in suspension culture. Virion production was examined after HSV-infected KB cells were exposed to selected concentrations of the drugs for 12 h. During this time there was no decrease in viability of uninfected cells as measured by trypan blue dye exclusion. Ara-A in the presence of coformycin reduced HSV replication by 50% at a concentration of approximately 0.5 μM, whereas 68 μM ara-H was required to produce the same effect (Table 1, Fig. 2). Coformycin alone did not affect HSV replication at a concentration that completely blocked the deamination of ara-A (3.5 μM).

Incorporation of labeled dThd into acid-insoluble material was examined in these cultures and in corresponding mock-infected cultures. In the absence of drugs uninfected KB cells incorporated label at a constant rate during a 12-h period. HSV-infected cultures also incorporated label at this rate for 3 h, after which the rate increased owing to the synthesis of viral DNA (Fig. 3, inset). In the presence of drug, label incorporation was reduced at all times examined. On the basis of the percentage of label incorporated in the absence of drugs, maximal inhibition was observed from 6 to 12 h. Data obtained after a 12-h exposure to [^{3}H]dThd and drug is presented in Fig. 3. Comparisons of dose-response relationships as described in Materials and Methods revealed that total DNA synthesis was inhibited to the same extent in mock-infected and HSV-infected cultures by both drugs ($P = 0.383$ for ara-A plus coformycin and $P = 0.829$ for ara-H).

Lysates also were prepared from the HSV-infected cells and were subjected to isopycnic centrifugation in CsCl gradients to separate cellular and viral DNA. The amount of label incorporated into each DNA species increased for 12 h after virus adsorption both in the absence of drug and in the presence of 10 μM ara-

TABLE 1. *Inhibition of HSV replication and DNA synthesis by ara-A and ara-H*

Method of culture propagation	Parameter measured	50% inhibitory concentrations (μM) and corresponding 95% confidence interval[a]		
		Ara-A plus 3.5 μM coformycin	Ara-A	Ara-H
Monolayer	HSV replication	2.1 (1.0–4.6)	43 (29–65)	191 (137–265)
	Viral DNA synthesis	2.6 (2.0–3.3)	20 (13–30)	126 (88–182)
	Cellular DNA synthesis[b]	8.0 (6.1–10.5)	67 (34–129)	1790 (710–4,490)[c]
	Uninfected KB DNA synthesis	4.9 (4.4–5.4)	62 (45–85)	309 (165–578)
Suspension	HSV replication	0.5[d]		69[d]
	Viral DNA synthesis	0.9[d]		60[d]
	Cellular DNA synthesis[b]	5.1[d]		179[d]
	Uninfected KB DNA synthesis	5.0[d]		226[d]
Synchronized suspension[e]	HSV replication		15 (9.8–25)	33 (15–75)
	Viral DNA synthesis		7 (3.4–14)	18 (12–28)
	Cellular DNA synthesis[b]		37 (14–97)	45 (10–206)
	Uninfected KB DNA synthesis		86 (59–126)	253 (153–416)

[a] That range of values which, at the 95% confidence level, contains the true value.
[b] In HSV-infected KB cells.
[c] $r^2 < 0.2$ for fit of data points to dose-response curve.
[d] Not determined owing to insufficient data.
[e] Calculated from the data of Shipman et al. (26).

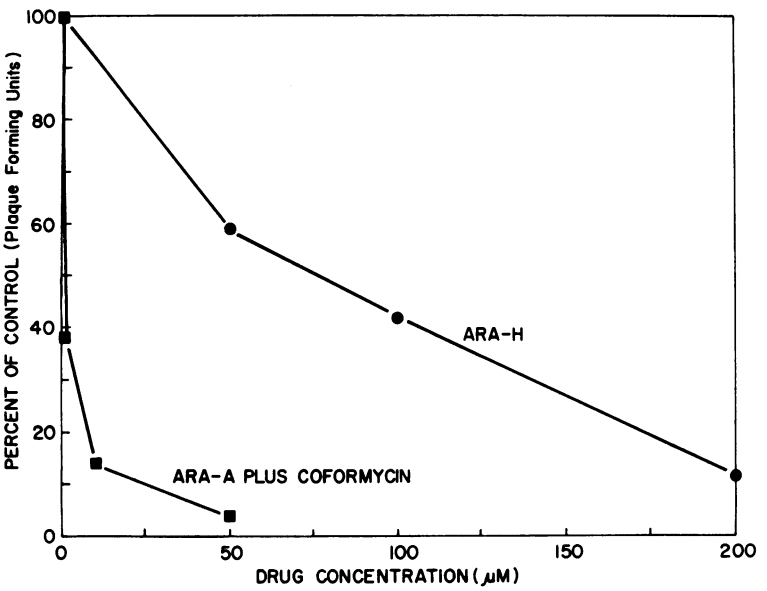

FIG. 2. *Effect of drugs on HSV replication in suspension cultures of KB cells. HSV-infected cultures contained no drug or selected concentrations of ara-A plus 3.5 μM coformycin or ara-H. Samples of virus-infected cells were disrupted by freeze-thawing and were assayed for plaque-forming ability on BHK-21/4 cells. Each assay was performed in triplicate, and the mean is expressed as a percentage of the number of plaques formed by samples from an infected culture without drug.*

A plus coformycin. No additional labeling occurred for up to 25 h. Figure 4 illustrates the separation of the two DNA species and shows the effects of ara-A plus 3.5 μM coformycin (Fig. 4A) and ara-H (Fig. 4B) on the amount of [^{3}H]dThd incorporated during 12 h of labeling. As is evident from the figure, viral DNA synthesis was inhibited to a significantly greater extent than was cellular DNA synthesis within the infected cell ($P = 0.022$ for both drugs). A

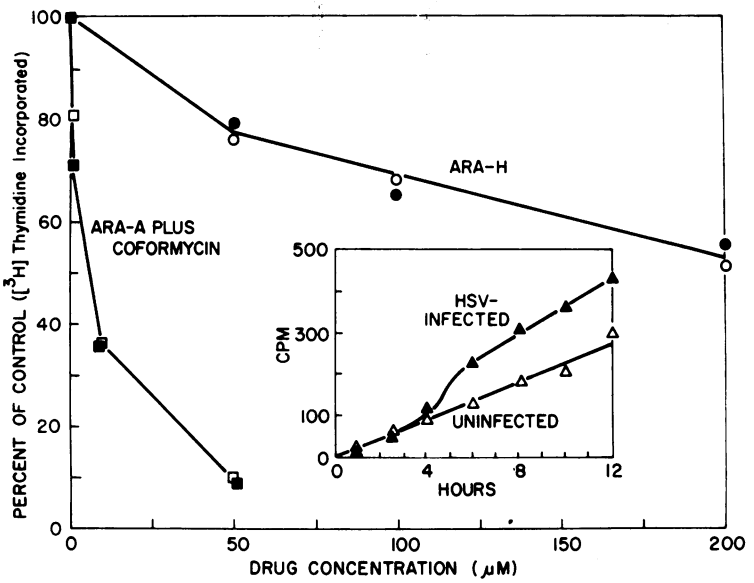

FIG. 3. *Effect of drugs on incorporation of [^{3}H]dThd into DNA of KB cells grown in suspension culture. HSV-infected cultures described in Fig. 2 (closed symbols) and corresponding mock-infected cultures (open symbols) were exposed to [^{3}H]dThd and drugs. Assays were performed in triplicate and expressed as a percentage of the amount of label incorporated by cultures that contained no drug. The amount of label incorporated per 10^3 cells in the absence of drug (the control cultures in the ara-A experiment) is shown in the inset.*

similar relationship existed between inhibition of viral DNA synthesis and inhibition of DNA synthesis in uninfected KB cells ($P = 0.025$ for ara-A plus coformycin, $P = 0.037$ for ara-H). I_{50} concentrations were calculated from dose-response relationships (see Materials and Methods) to quantitate the inhibition of DNA synthesis by the two drugs. These results, along with those for inhibition of HSV replication, are summarized in Table 1. Cellular DNA synthesis in HSV-infected cells required I_{50} concentrations approximately the same as those required in uninfected cells. In contrast, significantly lower concentrations of the two nucleosides were required for 50% inhibition of viral DNA synthesis.

There was close correspondence between the I_{50} concentration of ara-A plus coformycin required for inhibition of viral DNA synthesis and the I_{50} concentration required for inhibition of virion production. A similar relationship existed for ara-H (Table 1). Furthermore, virtually identical dose-response relationships existed for inhibition of viral DNA synthesis and inhibition of virion production by both drugs ($P = 0.776$ for ara-A plus coformycin, $P = 0.842$ for ara-H).

Studies in monolayer culture. Monolayer cultures of KB cells mock-infected or infected with HSV were used for more extensive comparisons of ara-A, with or without coformycin, and ara-H. The effect of a 12-h exposure to these drugs on HSV replication is presented in Fig. 5. Calculation of I_{50} concentrations from these data showed that in the presence of 3.5 μM coformycin, 2.1 μM ara-A was required to inhibit HSV production by 50%, whereas in the absence of the adenosine deaminase inhibitor 43 μM ara-A or 190 μM ara-H was required (Table 1).

Labeled dThd incorporation was measured in these and corresponding mock-infected cultures after the 12-h exposure to [^{3}H]dThd and drugs. Figure 6 illustrates the inhibition of label incorporation by each nucleoside. Comparison of dose-response curves shows that total DNA synthesis was inhibited to the same extent in mock-infected and HSV-infected cultures by ara-A plus coformycin ($P = 0.525$) or ara-H ($P = 0.692$). In contrast, ara-A inhibited total DNA synthesis in HSV-infected cultures to a greater extent than it inhibited DNA synthesis in uninfected cultures ($P = 0.015$). An I_{50} concentration of 31 μM was required in the former case, and a 62 μM concentration was required in the latter.

Viral and cellular DNA from the HSV-infected monolayer cultures was separated as detailed above, and drug effects were noted. In all cases examined, except in one experiment at 10 μM ara-H, viral DNA synthesis was inhibited more strongly than was cellular DNA synthesis (Fig. 7). All dose-response curves representing

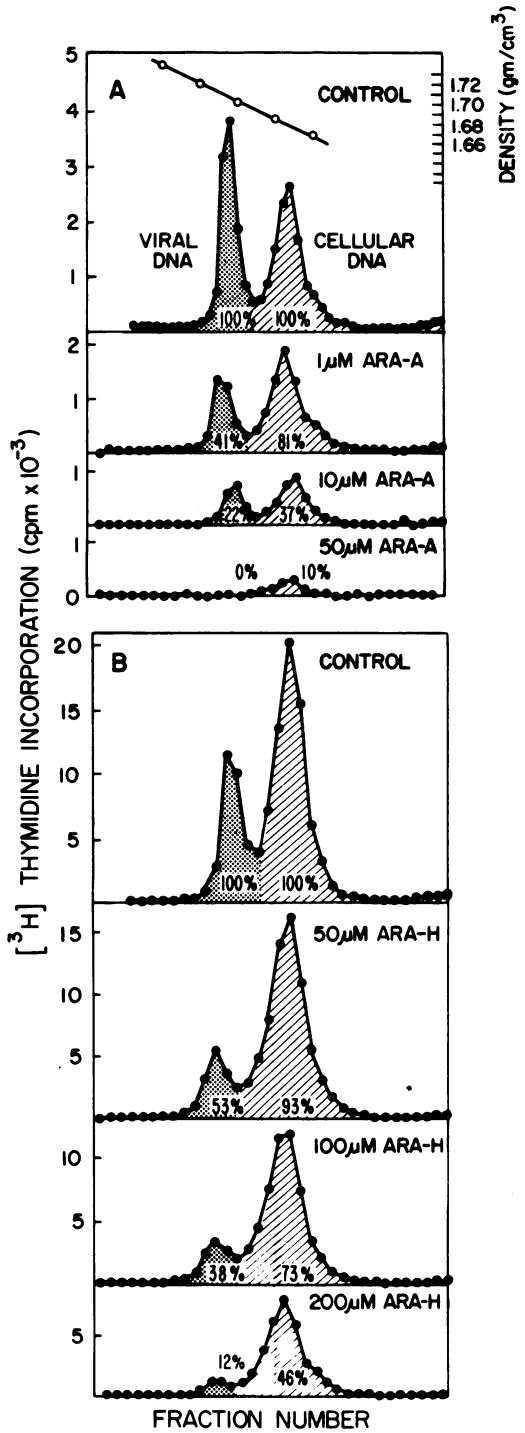

FIG. 4. *Influence of drugs on DNA synthesis in HSV-infected KB cells grown in suspension culture. DNA was labeled during the experiments presented in Fig. 2 and 3. Cells were lysed and DNA species were separated by isopycnic centrifugation in CsCl gradients. Amount of label incorporated into cellular and viral DNA in the presence of selected concentrations of ara-A plus 3.5 μM coformycin (A) or ara-H (B) is expressed as a percentage of the amount incorporated into the respective DNA species in cell cultures without drugs.*

inhibition of viral DNA synthesis differed significantly ($P \leq 0.014$) from the corresponding curves representing inhibition of cellular DNA synthesis (Fig. 7). Similar significant differences ($P \leq 0.023$) existed between inhibition of viral DNA synthesis and inhibition of DNA synthesis in uninfected KB cells (Fig. 6). As in suspension cultures, significantly lower concentrations of ara-A plus coformycin, ara-A, or ara-H were required for inhibition of viral DNA synthesis than were required for the same inhibition of cellular DNA synthesis in either uninfected or HSV-infected cells (Table 1).

Correspondence between inhibition of viral DNA synthesis and inhibition of HSV replication production was not as good as that observed in suspension cultures. I_{50} concentrations differed more widely (see Table 1), and dose-response curves were dissimilar ($P = 0.086$ and 0.010 for ara-A and ara-H, respectively). On the other hand there was agreement in I_{50} concentrations and dose-response curves ($P = 0.273$) for ara-A in the presence of coformycin.

DISCUSSION

Ara-A and ara-H inhibited the replication of HSV type 1 in KB cells. Experiments in monolayer culture demonstrated that the antiviral activity of ara-A was approximately four times greater than that of ara-H. The potency of ara-A, however, depends directly upon the rate of deamination to ara-H. This, in turn, depends upon the type of cells studied, the number of cells present, and the length of the incubation (29; C. Shipman, J. N. Sandberg, P. M. Schwartz, and J. C. Drach, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 358, 1975). To eliminate these variables, the activity of ara-A was measured in the presence of coformycin, an adenosine deaminase inhibitor. Coformycin itself was devoid of antiviral activity at the concentration used in this study and in a study by Williams and Lerner (30). In the presence of coformycin, 2.1 μM ara-A inhibited viral replication by 50% compared with 43 μM ara-A required in its absence. Thus, ara-A plus coformycin was 90 times more effective as an antiviral agent than ara-H when assayed in monolayer cultures. In comparison, Bryson and Conner (2) reported that 1.8 μM ara-A plus covidarabine inhibited varicella zoster virus replication by 50% and the combination was 40- to 50-fold

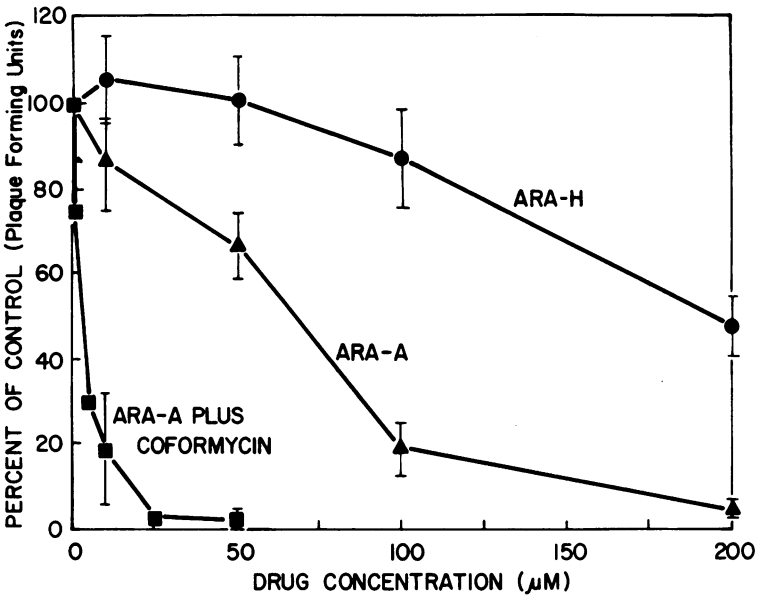

FIG. 5. *Effect of drugs on HSV replication in monolayer cultures of KB cells. HSV-infected cultures contained no drug or selected concentrations of ara-A plus 3.5 μM coformycin, ara-A alone, or ara-H. Portions from cultures were assayed for plaque-forming ability as described in the legend to Fig. 2. Data points with vertical bars represent the mean ± standard error of six (ara-A plus coformycin), three (ara-A), or four (ara-H) separate determinations. Points without bars are the means of two determinations. Each determination was performed in triplicate.*

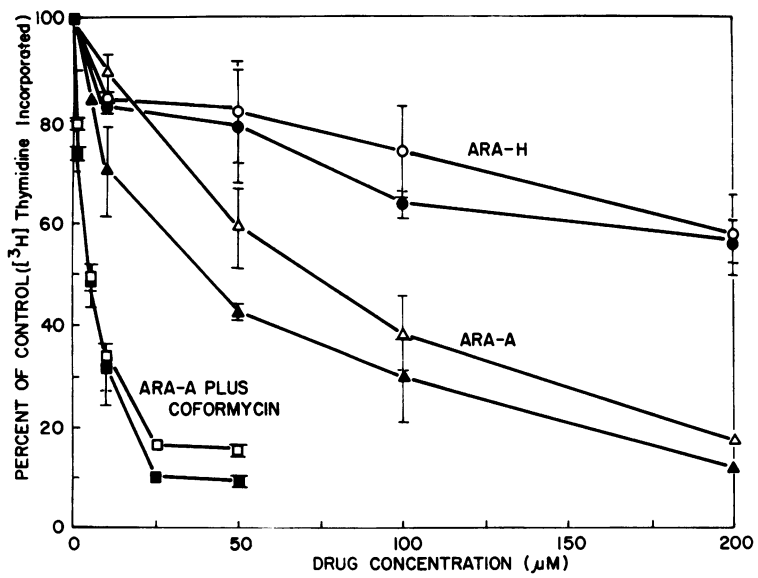

FIG. 6. *Influence of drugs on incorporaton of [^{3}H]dThd into DNA of KB cells grown in monolayer culture. HSV-infected cultures described in Fig. 5 (closed symbols) and corresponding mock-infected cultures (open symbols) were exposed to [^{3}H]dThd and drugs. Assays were performed in triplicate and expressed as a percentage of the amount of label incorporated in the absence of drugs. (Control cultures incorporated approximately 200 and 400 counts/min per 10^3 cells in mock-infected and HSV-infected cultures, respectively.) The means and deviations of the data are expressed as in Fig. 5.*

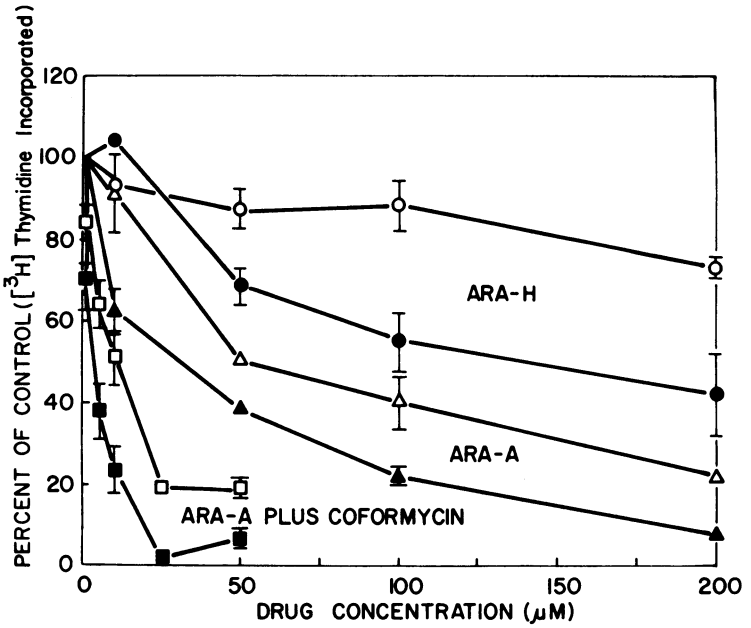

FIG. 7. *Effect of drugs on DNA synthesis in HSV-infected KB cells grown in monolayer culture. DNA was labeled in the experiments depicted in Fig. 5 and 6, and viral DNA was separated from cellular DNA by isopycnic centrifugation. The amount of label incorporated into viral DNA (closed symbols) and cellular DNA (open symbols) is expressed as a percentage of the amount incorporated into the respective DNA species in cell cultures without drugs. The means and deviations of the data are expressed as in Fig. 5.*

more potent than ara-H. Both ara-A and ara-H were more active in suspension cultures than they were in monolayer cultures (Table 1). Furthermore, comparisons with data obtained in the companion study (26) also showed that ara-A and ara-H had more antiviral activity in synchronized suspension cultures than in monolayer cultures. For example, the I_{50} concentration for ara-H in monolayer cultures was 191 μM compared with 33 μM in synchronized suspension cultures (without overlap of confidence intervals). The I_{50} concentration for ara-H in asynchronous suspension cultures (69 μM) also appeared to be lower than in monolayer cultures. No conclusions can be drawn, however, about similarities or differences in activity between synchronous and asynchronous suspension cultures without more data from the latter system. The reasons for the differences in drug potency between monolayer and suspension cultures are unknown but may reflect differences in the multiplicities of HSV infection. Monolayer cell sheets were infected at an MOI of 10 for 1 h at 37 C, whereas suspension cultures were infected at an MOI of 0.9 or 1.4 in a slurry for 1 h at room temperature. The former cultures produced 40 to 80 PFU/cell compared with a yield of 2 to 5 PFU/cell from the latter. Hence, the drugs were more active in cells infected at the lower multiplicity. Miller et al. (13) and Marks (12) also observed that ara-A was more effective in blocking replication of HSV or cytomegalovirus at lower MOIs.

Ara-A was a more potent inhibitor of DNA synthesis than was ara-H and was most potent in the presence of coformycin. Although there was some variation depending on the type of DNA synthesis examined or the culture conditions utilized, ara-A was two to six times more potent than ara-H and, in the presence of coformycin, was 35 to 70 times more potent. Differences in drug activities from monolayer to suspension cultures are most informative. As with inhibition of HSV replication, inhibition of viral DNA synthesis was more pronounced in suspension cultures than in monolayers. In contrast, the method of cell propagation did not alter the effectiveness of drugs on DNA synthesis in uninfected KB cells. For example, 126, 60, and 18 μM ara-H were required to inhibit viral DNA synthesis by 50% in monolayer, asynchronous, and synchronous suspension cultures, respectively (Table 1). The first and last values clearly are different, with no overlap of confidence intervals. However, 309, 226, and 253 μM ara-H were required to inhibit DNA synthesis

in uninfected KB cells by 50%. Based upon the coincidence of confidence intervals, these values are virtually the same. Although our data are not as complete, a similar situation existed for the activity of ara-A, with or without coformycin. The relationship between the method of cell propagation and the effect of drugs on cellular DNA synthesis in HSV-infected cells is less clear. Cellular DNA synthesis appeared to be more susceptible to inhibition by ara-A and ara-H in synchronized cell cultures than it was in either asynchronous suspension or monolayer cultures. We are currently unable to explain this phenomenon.

In all experiments viral DNA synthesis was inhibited to a greater extent than was DNA synthesis in uninfected KB cells. To more clearly define this relationship, we have derived an index that quantitates the preferential inhibition of viral or cellular DNA synthesis. The I_{50} concentration of a drug for DNA synthesis in uninfected cells is divided by the I_{50} concentration for viral DNA synthesis. We have termed the logarithm of this ratio the selective index (SI) (additional details may be found in Drach and Shipman, Third Conf. on Antiviral Substances, New York Academy of Sciences, 1976). It is positive if viral DNA synthesis is inhibited preferentially and negative if uninfected cellular DNA synthesis is more strongly inhibited. Owing to the range of values defined by the 95% confidence intervals of the component I_{50} concentrations, differences of 0.1 to 0.2 units probably are not meaningful. Table 2 gives SI values for the cases presented in this and the prior communication (26). All values were positive and were similar for each nucleoside tested under the same culture conditions. Growth of cells in suspension cultures resulted in the greatest selectivity because of the increased drug sensitivity of viral DNA synthesis. On the other hand, inhibition of adenosine deaminase by coformycin did not affect the selectivity of ara-A even though it greatly increased the potency of the drug.

Bennett et al. (1) also have reported that ara-A acts selectively. Their observations were based, however, on a comparison between inhibition of DNA synthesis in uninfected HEp-2 cells and inhibition of total, not viral alone, DNA synthesis in HSV-infected cells. In this system ara-A inhibited dThd incorporation only in HSV-infected cells. The nearly complete lack of inhibition by 375 μM ara-A in uninfected HEp-2 cells is quite surprising and contrasts with our observation that 62 μM ara-A inhibited DNA synthesis in uninfected KB cells by 50%. The apparent lack of inhibition in HEp-2 cells may be explained on the basis of a threefold increase in the labeling of soluble dThd nucleotides (1). Our results also showed selectivity based on total DNA synthesis, but only with ara-A in the absence of coformycin. We speculate that the differential effect on total DNA synthesis was not the result of a selective action of the drug on a biochemical system, but rather was the result of a more extensive deamination of ara-A by the less perturbed uninfected cells.

The possibility that ara-A did not act selectively against viral DNA synthesis must be considered. In this and the prior (26) article the incorporation of [^{3}H]dThd into acid-precipitable material has been equated with DNA synthesis. We have not ruled out the possibility that the selective effect was the result of differential changes in the specific activity of [^{3}H]dThd that might accompany drug treatment or HSV infection. HSV infection does increase dThd nucleotide pools in mammalian cells (3, 16, 20), but for changes in pool sizes to explain the selective effect, it would be necessary for cellular and viral DNA to be synthesized from separate nuclear dThd nucleotide pools in HSV-infected cells which changed independently with changes in drug concentration. In a separate study we (Drach et al., unpublished observations) have found that 120 μM ara-A did not alter the labeling of dThd nucleotides in B-mix K-44/6 cells. The recent data of Müller et al. (W. E. G. Müller, R. K. Zahn, K. Bittlingmaier, and D. Falke, Ann. N.Y. Acad. Sci., in press) also support the conclusion that we measured a selective inhibition of DNA synthesis and not just of [^{3}H]dThd incorporation. The 5'-triphosphate of ara-A was found to be a more potent inhibitor of HSV DNA polymerase than of cellular DNA polymerases (K_i = 0.14, 7.4, and 5.6 μM, respectively, for HSV and α- and β-DNA polymerases). Such differences are adequate to account for the selectivity we have noted. Nonetheless, experimental determination of the specific activity of [^{3}H]dThd incorporated into DNA will be required to totally resolve the issue.

TABLE 2. *Selective indexes for ara-A and ara-H*

Method of culture propagation	SI value		
	Ara-A plus 3.5 μM coformycin	Ara-A	Ara-H
Monolayer	0.3	0.5	0.4
Suspension	0.7		0.6
Synchronized suspension[a]		1.1	1.1

[a] Calculated from the data of Shipman et al. (26).

If ara-A acted selectively against viral DNA synthesis, the additional question arises of whether or not this action was responsible for the differential effects on virion and cell viability. Several studies (see reference 25) have demonstrated antiviral activity for ara-A at dose levels that were not cytotoxic to uninfected cells. As discussed previously (26), this differential sensitivity could arise because HSV-induced enzymes involved in DNA metabolism were preferentially susceptible to ara-A or its nucleotides, thereby resulting in selective toxicity to HSV-infected cells. Nonetheless, other mechanisms also may be operative. The greatest antiviral effects occurred when ara-A was added from 2 h before to 3 h after virus adsorption (1, 26), indicating that if ara-A was present during viral DNA synthesis then antiviral activity was expressed. When ara-A is used without adenosine deaminase inhibitors, it is metabolized, and therefore is present only for a limited time. This restricted exposure of HSV-infected cells to ara-A apparently is adequate to decisively inhibit viral DNA synthesis and hence virion replication. Uninfected cells, on the other hand, recover from a limited exposure to ara-A. We (C. Shipman, J. Novack, and J. C. Drach, Fed. Proc. 32:699, 1973) have observed that B-mix K-44/6 cells inhibited by 600 μM ara-A resumed growth after the removal of the drug. Similarly Cohen et al. (6, 17) found that L cells resumed multiplication after a 9- or 10-h exposure to 200 μM ara-A. Müller et al. (14) also noted that the inhibitory effect of ara-A was completely reversible over a limited concentration range. Furthermore, LePage (11) found the Ehrlich carcinoma ascites cells recovered their ability to synthesize DNA as ara-A levels declined in host animals. An entirely different situation may exist if the metabolism of ara-A is blocked. Even though we observed no decrease in cell viability (as measured by trypan blue dye exclusion) during the 12-h exposure of KB cells to ara-A plus coformycin, Plunkett and Cohen (19) have reported that cytostatic concentrations of ara-A become lethal to 99.9% of L cells in the presence of an adenosine deaminase inhibitor. Viability did not decline, however, until 24 to 36 h after the addition of up to 20 μM ara-A. It is conceivable, therefore, that, although KB cells appeared to be viable after the 12-h exposure to ara-A plus coformycin, irreversible damage had been done but would not be expressed until later in the replication cycle. If this were the case, then the selective inhibition of viral DNA synthesis in the presence of coformycin would not translate into selective action at the cellular level. Consequently, the selectivity of ara-A at the cellular level may depend not only upon selective inhibition of viral DNA synthesis, but also upon its subsequent metabolism to ara-H.

Selectivity of drug action in vivo, defined as the ability of ara-A to inhibit viral replication at nontoxic doses, probably is more complex. In addition to the two factors mentioned above, the total cellular and physiological disposition of the drug also would be involved. Therefore, it is of considerable interest to learn what contribution the selective inhibition of viral DNA synthesis makes to the overall selectivity of drug action in vivo. Our in vitro studies predict that in vivo ara-A plus coformycin should be more potent than ara-A, but no more selective (see Tables 1 and 2). The antiviral and toxicological evaluation of ara-A plus an adenosine deaminase inhibitor in experimental animals should provide an interesting test of this prediction.

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