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Combined effects of interferon-α and acyclovir on herpes simplex virus type 1 DNA polymerase and alkaline DNase

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Abstract

Treatment of cells with combinations of human interferon-α (IFN-α) and the nucleoside analog, acyclovir (ACV), leads to the synergistic inhibition of herpes simplex virus type 1 (HSV-1) replication. We have examined the effect of these agents on the replication of HSV-1 DNA and the synthesis of early viral enzymes to understand the mechanism(s) responsible for this synergistic activity. Combination treatment with 100 IU/ml IFN-α and 5 μM ACV led to HSV-1 DNA levels more than 8-fold lower than in cells treated with ACV alone, while IFN-α treatment alone had no detectable effect on viral DNA synthesis. Steady state levels of DNA polymerase were reduced approximately 50% by IFN-α and 25% by ACV, but combination treatment did not decrease enzyme levels to an extent greater than the sum of these effects. In contrast, the activity of another early viral enzyme, alkaline DNase, was reduced less than 20% by IFN-α alone or combination treatment and was unaffected by ACV treatment. No decrease in the level of mRNA encoding either enzyme was detected in IFN-α-treated cells although ACV treatment reduced polymerase mRNA levels. These studies suggest that the synergistic anti-HSV activities of IFN-α with ACV could be mediated, in part, through some post-transcriptional mechanism induced by IFN-α treatment, leading to the reduction in production of viral early enzymes, especially DNA polymerase.

Keywords: Interferon; Herpes simplex virus; Acyclovir; Synergy

1. Introduction

Interferons (IFNs) have been shown to have synergistic anti-herpes simplex virus (HSV) activity when combined with nucleoside analogs in cell cultures (Levin and Leary, 1981; Hammer et al., 1982; Janz and Wigand, 1982; Crane et al., 1984; Czarniecki et al., 1984; Eppstein and Marsh, 1984; Moran et al., 1985; Hartshorn et al., 1986; Kawaguchi et al., 1986; Scheck et al., 1986; Hall and Duncan, 1988; Hanada et al., 1989; Taylor et

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al., 1989, 1991), in animal models (Janz and Wigand, 1982; Crane et al., 1984; Fraser-Smith et al., 1984; Trousdale and Nesburn, 1984; Connell et al., 1985), and in humans (Colin et al., 1983; De Koning et al., 1983; Sundmacher et al., 1984; Van Bijsterveld et al., 1989). This synergistic activity has been shown to extend to other herpesviruses, including cytomegalovirus and varicella-zoster virus (Levin and Leary, 1981; Rasmussen et al., 1984; Rose et al., 1983; Smith et al., 1983; Wade et al., 1983; Baba et al., 1984), as well as human retroviruses (Hartshorn et al., 1986, 1987; Davey et al., 1991; Ito et al., 1991). The mechanism(s) by which IFNs act to participate in this synergism with nucleoside analogs is not known. Our previous studies indicate that IFN-α treatment alters the metabolism of nucleosides in HSV-1-infected cells and suggests that these alterations may contribute to the synergism seen between IFNs and acyclovir (ACV) or other acyclic nucleoside analogs (O'Brien et al., 1990; Taylor et al., 1991). These detected alterations in nucleoside metabolism include a decrease in the uptake and phosphorylation of thymidine and a decrease in the size of deoxyribonucleoside triphosphate pools. The decrease in thymidine uptake and phosphorylation detected in IFN-α-treated Vero cells and human cornea stromal cells appears to be due to a reduction in the level of the early viral enzyme, thymidine kinase (O'Brien et al., 1990; Taylor et al., 1994). In the studies reported here we examined the effects of IFN-α and ACV on two other early viral enzymes to determine if the inhibitory effects of IFN-α treatment on early protein synthesis is a generalized effect and if an IFN-induced reduction in DNA polymerase could be a contributing factor in the synergism with ACV. In addition to DNA polymerase, which is the target enzyme for inhibition by ACV-triphosphate (ACV-TP) (Elion et al., 1982), we examined DNase, an enzyme not known to be affected by ACV.

We have found that DNA polymerase activity was decreased in cells treated with IFN-α, ACV, or the combination when compared to untreated, infected cells. The inhibition of expression of DNA polymerase in cells treated with ACV appears to be through a decrease in mRNA levels, whereas in IFN-α-treated cells the inhibition appears to occur after transcription. In cells treated with a combination of IFN-α and ACV the decrease in enzyme activity appeared to be due to a combination of both mechanisms. DNase activity was also decreased by IFN-α-treatment, but to a lesser extent than polymerase, while levels of DNase activity were unaffected by ACV treatment. No consistent change in the levels of mRNA for DNase was detected in either Vero cells or cornea stromal fibroblasts treated with IFN-α, ACV or combination treatment. The observed decrease in HSV-1 DNA polymerase activity is likely an important contributor to the mechanism of synergism between IFN-α and ACV.

2. Methods

2.1. Cells and virus

Human cornea stromal cells, derived from donor corneas (Taylor et al., 1991), were grown in DMEM with 5% fetal bovine serum and Mito-Plus serum extender (Collaborative Biomedical Products, Bedford, MA). Vero cells, a continuous monkey kidney cell line (obtained from American Type Culture Collection, ATCC CCL81), were grown in DMEM with 10% newborn calf serum. The McKrae strain of HSV-1 was used throughout the studies.

2.2. Treatment of cells

Confluent cultures were treated with 100 IU/ml or 1000 IU/ml of recombinant human IFN-α2a (Hoffmann LaRoche, Nutley, NJ) in medium containing 2% serum for 16–24 h. IFN-α-containing medium was removed and cells were infected at a multiplicity of infection (MOI) of 5 pfu/cell. After 1 h, the inoculum was removed and cells were treated with medium containing 5 μM ACV (Glaxo-Wellcome, Research Triangle Park, NC). At 6 or 16 h after infection, cells were harvested and extracts prepared for immunoblots or enzyme assay, DNA was extracted for quantitation of viral DNA by dot blot hybridization, or cytoplas-

mic RNA was extracted for detection of viral mRNA by Northern blots. The concentrations of IFN-α and ACV used in these studies have been shown previously to produce synergistic inhibition of virus replication (Taylor et al., 1989).

2.3. Detection of viral DNA

At 6 and 16 h after virus infection cells were harvested by trypsin treatment and DNA extracted by the method of Nicolaides and Stoeckert (1990). DNA was diluted to 2 μg/100 μl in water, heated to 100°C for 10 min, and chilled on ice. Serial 2-fold dilutions of DNA were mixed with a equal volumes of denaturation buffer (0.8 M NaOH, 20 mM EDTA). Mixtures were held for 10 min and then neutralized with an equal volume of ice cold 2 M ammonium acetate, pH 7.0. Samples were blotted to nitrocellulose, rinsed in 2 × SSC, then 6 × SSC, and dried in vacuo for 2 h at 80°C. Blots were hybridized with pXK19, a plasmid containing a 1.3-kb *Bam*HI-*Stu*I fragment of HSV-1 DNA including the first intron and part of the first exon of the ICP0 gene. This probe was ^{32}P-labeled by nick-translation and hybridized to the blots overnight at 42°C in 50% formamide, 5 × SSC, 20 mM sodium phosphate buffer, pH 6.5, 0.05 mg/ml salmon sperm DNA, 5 mM EDTA, 1 × Denhardt's, and 10% dextran sulphate. Blots were then washed in 2 × SSC at 65°C twice for 15 min each, once in 2 × SSC with 0.1% SDS at 65°C for 30 min, and 2 h at 65°C in 0.1 × SSC. Hybridization was detected by autoradiography.

2.4. Preparation for enzyme assay of cell extracts

Extracts were prepared by the method of Elias et al. (1986). Briefly, cells were suspended by trypsin/EDTA treatment and washed once with buffer containing 20 mM HEPES, pH7.6, 150 mM NaCl, and 0.5 mM dithiothreitol (DTT). Cells were suspended in 2 ml of lysis buffer containing 20 mM HEPES, 0.5 mM DTT, 10 mM $NaHSO_3$ pH 7.0, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 2 μg/ml leupeptin and lysed by Dounce homogenization. Nuclei were collected by centrifugation at 1500 rpm for 10 min and washed in lysis buffer. The nuclear pellet was suspended in lysis buffer containing 10% (w/v) sucrose and extracts were stored at −80°C until enzyme activity and protein content could be measured.

2.5. Immunoblots

Cell extracts were prepared and immunoblots were performed as previously described (Taylor et al., 1994) using rabbit polyclonal antibody specific for HSV-1 DNA polymerase (supplied by Donald Coen, Harvard University) and detected using the ECL chemiluminescence detection system (Amersham, Arlington Heights, IL). The relative amounts of protein in the detected bands were quantitated with the Ambis Image Analysis System (Ambis, San Diego, CA).

2.6. Enzyme assays

DNase activity was measured by the method of Williams et al. (1989) using ^{32}P-nick-translated calf thymus DNA prepared by the method of Knopf and Weisshart (1990) as a substrate. Reaction mixtures totaling 100 μl contained 50 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, 10 mM DTT, 20 μg bovine serum albumin (BSA), 6 μg ^{32}P-labeled double-stranded calf thymus DNA (specific activity determined for each experiment, range 12000–65000 cpm/μg), and cell extract containing 20 μg protein. Reactions were incubated at 37°C in shaking water bath for 15 min and then terminated by the addition of 25 μl (2 mg/ml) of sheared, double-stranded calf thymus DNA followed by 25 μl of cold 50% trichloroacetic acid (TCA). Reactions were held on ice for 10 min and then centrifuged at 1200 × g for 10 min. A 0.1-ml sample of the supernatant was neutralized with 10 μl of 1.2 M KOH and solubilized radioactivity determined in a scintillation counter. One unit of enzyme activity was defined as the amount that converted 1 μg of DNA to acid soluble material per h.

DNA polymerase activity was measured by the method of Williams et al. (1989). Reactions mixtures contained 70 mM Tris–HCl, pH. 8.0, 0.2 M KCl, 4 mM $MgCl_2$, 70 μg BSA, 0.7 mM DTT, 2% (v/v) glycerol, 100 μM each of dATP, dGTP, and dCTP, 1 μM [^{3}H]TTP (500 μCi/μmol, DuPont, Boston, MA), 13 μg of activated calf thymus DNA, and cell extract containing 5 μg of protein. Reactions were incubated at 37°C for 15 min, then terminated and incorporated radioactivity determined by the method of Ruth and Cheng (1981). One unit of polymerase activity was defined as that amount of enzyme that incorporated 1 pmol of TTP per h. Enzyme assays were done in triplicate and carried out at conditions that demonstrated a linear increase in activity with respect to time and enzyme concentration. Statistical comparisons of enzyme activities among treatment groups were done by one-way analysis of variance and comparison of means by the Bonferroni *t*-test.

2.7. Extraction of RNA and Northern blots

Total cytoplasmic RNA was extracted by the method of Sambrook et al. (1989). Northern blots were done as previously described (Taylor et al., 1994). Probes for detection of HSV-1 mRNAs were cloned genomic DNA, ^{32}P-labeled by nick translation. The probe for DNA polymerase was a 2.9-kb *Eco*RI-*Pst*I fragment which hybridized to the 3.9-kb mRNA for UL30 (DNA polymerase) as well as the 1.0-kb mRNA for UL 31 and a 2.8-kb mRNA for UL32. The alkaline DNase probe was a 586-bp *Nco*I-*Xho*I fragment which detected the 2.2-kb UL12 (DNase) mRNA as well as a 1.9-kb colinear mRNA expressed as a γ1 class gene (Draper et al., 1986). Probes for HSV-1 genes were prepared with the assistance of Kent Wilcox (Medical College of Wisconsin) by subcloning of restriction enzyme fragments from an *Eco*RI library of the KOS strain of HSV-1 (Goldin et al., 1981). Levels of mRNAs for individual genes were quantitated by densitometric scanning of autoradiograms of Northern blots using the Ambis Image Analysis System (Ambis, San Diego, CA).

3. Results

3.1. Effect of IFN-α and ACV treatment on HSV-1 DNA synthesis

We had demonstrated earlier that synergistic inhibition of replication of HSV-1 occurred when cells were treated with a wide range of doses of both IFN-α and ACV. Pretreatment with 100 IU/ml of IFN-α for 24 h, followed by infection with HSV-1 at a MOI of 5 and treatment with 5 μM ACV (Taylor et al., 1989, 1991) were conditions that achieved maximal measurable synergistic inhibition of virus replication, resulting in nearly complete inhibition of viral cytopathic effect. Therefore, these treatment conditions were chosen for the studies reported here. DNA was extracted at 6 and 16 h after infection from cells treated according to this schedule as well as from cells treated with IFN-α or ACV alone, or cells not treated with either antiviral. At 6 h after infection, IFN-α treatment alone resulted in no detectable inhibition of virus DNA synthesis (Fig. 1). ACV treatment inhibited viral DNA synthesis approximately 4–8-fold. Cells treated with both IFN-α and ACV showed a much greater ($\geq$64-fold) inhibition of viral DNA synthesis, consistent with the synergistic anti-HSV-1 activity of this combination. Similar inhibition of viral DNA synthesis persisted in samples prepared from cells at 16 h after infection. These results indicated that antiviral synergism between IFN-α and ACV occurred early during virus replication, prior to or during the synthesis of viral DNA.

3.2. Effect of IFN-α and ACV treatment on DNA polymerase

To determine whether the detected inhibition of DNA synthesis occurred because of a comparable decrease in the levels of HSV-1 DNA polymerase activity present in treated cells, enzyme activity was measured. At 6 h after infection of Vero cells, DNA polymerase activity was decreased from 58.2 ± 1.5 pmol TTP incorporated/min per mg protein in untreated cells to 30.4 ± 5.7 pmol/min per mg protein in cells treated with 100 IU/ml of IFN-α, a decrease of 48% (see Table 1 for repre-

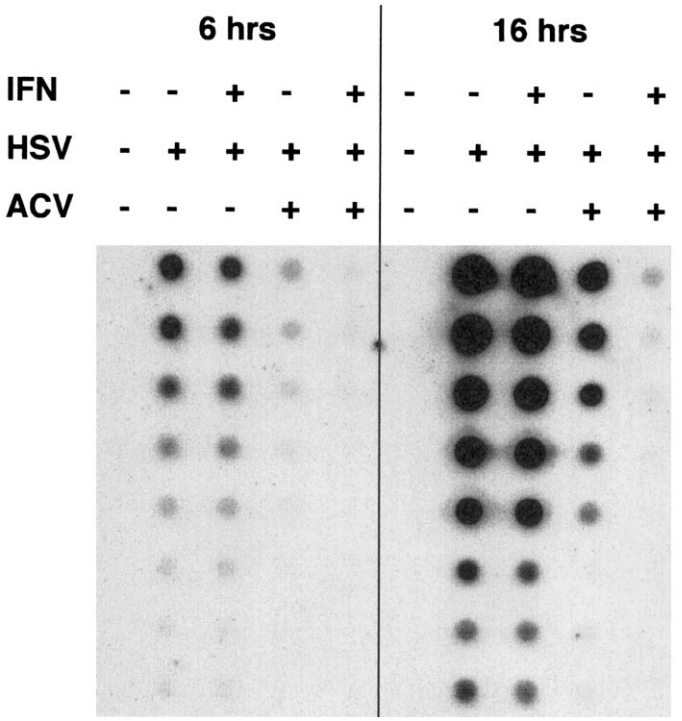

Fig. 1. Effects of IFN-α and ACV on HSV-1 DNA content. Vero cells were treated with or without 100 IU/ml IFN-α for 24 h. Cells were infected with HSV-1 at an MOI of 5 and medium with or without 5 μM ACV was added. At 6 or 16 h after infection, DNA was extracted. One μg and serial 2-fold dilutions of DNA were blotted and hybridized with a ^{32}P-labeled probe from the region of the viral genome encoding ICP0. Hybridized probe was detected by autoradiography.

sentative experiments). In human cornea stromal cells a similar decrease was observed (Table 1). Treatment of Vero cells with IFN-α alone at 1000 IU/ml resulted in a greater decrease in the amount of enzyme activity than treatment with 100 IU/ml, a 73% decrease in Vero cells and a 76% decrease in stromal cells. Under the conditions used, viral yields were reduced by 36% after treatment with 100 IU/ml and 68% after treatment with 1000 IU/ml of IFN-α.

ACV treatment of the two cell types also consistently reduced polymerase activity, although to an extent less than IFN-α. The measurement of enzyme activity in extracts from cells treated with ACV could be complicated by the presence of residual ACV-TP in the extract. However, based upon measurements of ACV-TP pools in cell extracts, the concentration of ACV-TP present in reaction mixtures would be less than 5 nM, a concentration 16-fold lower than the K_i for the viral polymerase (Elion et al., 1982). The concentration of dGTP competing with this ACV-TP in the reaction mixtures was 100 μM, so the ACV-TP present in extracts prepared from ACV-treated cells should not interfere with the assay. Combined treatment of cells with ACV and IFN-α resulted in an inhibition similar to or greater than that seen with 100 IU/ml of IFN-α alone.

At 16 h after infection, levels of enzyme activity in HSV-1-infected, untreated Vero cells had increased approximately three-fold compared to those at 6 h after infection (compare enzyme activities in HSV-1 cultures in Table 1, 6 and 16 h in Vero cells). The decrease in enzyme activity as a result of treatments persisted at 16 hours after infection indicating that the reduced steady state level of DNA polymerase was not due simply to a delay in the synthesis of enzyme.

Western blots using antibody specific for HSV DNA polymerase showed that, consistent with the

Table 1
The effect of IFN-α and ACV treatment on HSV DNA polymerase activity

Cell type	Hours PI	Treatment	Polymerase activity[a] (pmol TTP incorporated min/mg protein)	% of HSV-infected
Vero	6	None	8.7 ± 8.5[b]	14.9
		HSV	58.2 ± 1.5	100.0
		HSV+IFN (100 IU/ml)	30.4 ± 5.7[b,c]	52.2
		HSV+IFN (1000 IU/ml)	19.4 ± 3.8[b]	33.4
		HSV+5 μM ACV	38.4 ± 5.7[b,c]	66.7
		HSV+IFN (100 IU/ml)+5 μM ACV	13.6 ± 2.5[b]	23.4
HCS	6	None	1.1 ± 1.0[b]	1.6
		HSV	70.9 ± 25.1	100.0
		HSV+IFN (100 IU/ml)	34.1 ± 11.8	48.1
		HSV+IFN (1000 IU/ml)	8.2 ± 2.5b[b]	11.7
		HSV+5 μM ACV	52.9 ± 9.7	74.6
		HSV+IFN (100 IU/ml)+5 μM ACV	45.6 ± 9.0	64.4
		HSV+IFN (1000 IU/ml)+5 μM ACV	14.5 ± 1.4[b]	20.5
Vero	16	None	1.4 ± 0.1[b]	0.6
		HSV	240.5 ± 48.4	100.0
		HSV+IFN (100 IU/ml)	139.4 ± 11.9	58.0
		HSV+IFN (1000 IU/ml)	112.3 ± 26.6[b]	46.7
		HSV+5 μM ACV	219.0 ± 54.0	91.1
		HSV+ IFN (100 IU/ml) + 5 μM ACV	90.4 ± 41.6[b]	37.6

[a] All assays performed in triplicate; data presented as the mean ± S.D.
[b] Statistically different ($P<0.05$) from HSV-infected, untreated as determined by a one-way analysis of variance and comparison of multiple means by the Bonferroni t-test.
[c] Statistically different ($P<0.05$) from combination (IFN+ACV) treated.

enzyme activity measurements, IFN-α decreased the amount of polymerase polypeptide, while ACV treatment did not detectably reduce polymerase levels (Fig. 2). Combination treatment led to decreases in polypeptide equivalent to those seen in cell treated with IFN-α alone.

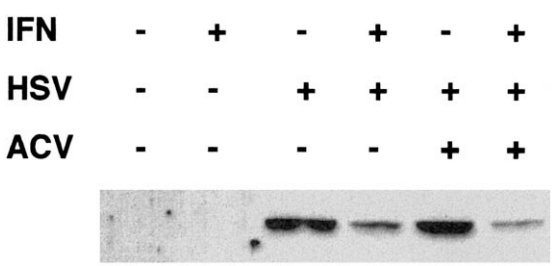

Fig. 2. Effect of IFN-α and ACV on DNA polymerase. Human cornea stromal cells were treated as described in Fig. 1 and at 6 h after infection cells were harvested, lysed, and electrophoresed on SDS-PAGE. Immunoblots were performed using antibody specific for HSV-1 DNA polymerase.

3.3. Effect of IFN-α and ACV treatment on DNA polymerase mRNA

It has been reported that IFN-α treatment can cause an inhibition of transcription of HSV-1 genes, including immediate early and early genes in other cell types (Gloger and Panet, 1984; Mittnacht et al., 1988; Oberman and Panet, 1988; Destasio and Taylor, 1990). To determine if the effects of IFN-α treatment on DNA polymerase activity occur at the level of mRNA expression we prepared Northern blots of total cytoplasmic RNA from Vero and human cornea stromal cells which had been treated as described above. Although IFN-α treatment reduced steady-state lev-

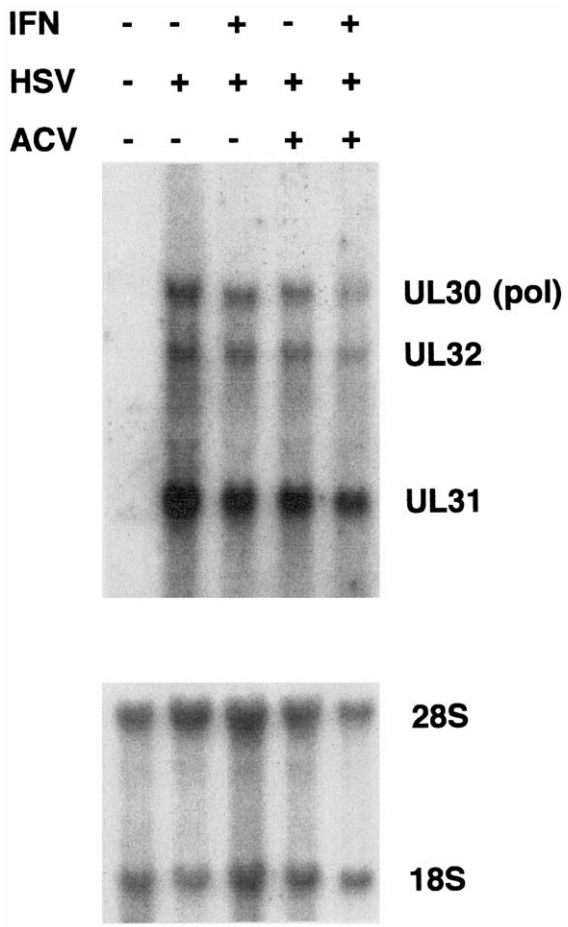

Fig. 3. Effect of IFN-α and ACV on DNA polymerase mRNA. Human cornea stromal cells were treated as described in Fig. 1 and at 6 h after infection total cytoplasmic RNA extracted. Northern blots were probed with a ^{32}P-labeled probe from the region of the HSV-1 genome encoding UL30 (DNA polymerase), UL31, and UL32. Hybridized probe was detected by autoradiography. Blot was stripped and reprobed with ^{32}P-labeled probe for ribosomal RNA to evaluate relative RNA load.

els of HSV-1 DNA polymerase as measured by enzyme assay and Western blot, there was no measurable reduction of polymerase mRNA in either cell type (Fig. 3, Table 2). Therefore, the reduced amount of HSV-1 DNA polymerase detected in IFN-α-treated cells did not result from a decrease in mRNA. Treatment of cells with ACV did decrease the level of DNA polymerase mRNA in both cell types (Fig. 3, Table 2). Combination treatment resulted in a decrease in mRNA levels that was not different from that seen with ACV alone.

3.4. Effect of IFN-α and ACV treatment on DNase

To determine if the effects of IFN-α and ACV on DNA polymerase were specific for that enzyme or were also seen with another early viral enzyme we examined the levels of viral alkaline DNase and its mRNA. DNase levels present in Vero or stromal cells treated with IFN-α at 100 IU/ml were consistently less than levels in untreated, infected cells (Table 3), but the extent of reduction was not as great as for polymerase. Combination treatment reduced activity to the same extent as IFN-α alone. No reduction was detected with ACV treatment. DNase levels at 16 h after infection in Vero cells treated with IFN-α or ACV were not different than those in untreated cells suggesting that DNase production was only delayed by IFN treatment alone. Cells treated with the combination of IFN-α and ACV had enzyme levels reduced compared to those of untreated cells at 16 h after infection (Table 3). Levels of mRNA for DNase in Vero cells and stromal cells at 6 h after infection following any of the treatments were not different than in untreated cells (data not shown).

4. Discussion

IFN-α and ACV act synergistically to inhibit the replication of HSV-1. The mechanism(s) responsible for the synergy have not been determined. Earlier studies indicated that IFN-α treatment decreased production of early viral proteins, possibly through the inhibition of the production of the immediate early transcriptional activator, ICP4 (Gloger and Panet, 1984; Mittnacht et al., 1988; Oberman and Panet, 1988; Destasio and Taylor, 1990). We have detected no reduction in ICP4 levels in our cell culture systems with the conditions of IFN-α treatment used for these studies described here, but have detected a decrease in the levels of some, but not all early

Table 2
Effects of IFN and ACV on levels of DNA polymerase mRNA and other mRNAs encoded in adjacent regions of the HSV genome

Amount of indicated mRNA present[a] (relative to HSV-infected, untreated cells) in cells treated with:									
	UL 30 (DNA POL)			UL 31			UL32		
Cell	IFN	ACV	IFN/ACV	IFN	ACV	IFN/ACV	IFN	ACV	IFN/ACV
Vero	1.08 ± 0.23[b]	0.75 ± 0.13[b]	0.35 ± 0.12	0.77 ± 0.08[b]	0.82 ± 0.21[b]	0.52 ± 0.10	0.66 ± 0.34[b]	0.85 ± 0.10[b]	0.34 ± 0.13
HCS	1.09 ± 0.07[b]	0.60 ± 0.18	0.59 ± 0.19	1.14 ± 0.12[b]	0.72 ± 0.19	0.41 ± 0.33	nd[c]	nd	nd

[a] Amounts of mRNA present on a Northern blot were normalized based upon densitometric scans of blots hybridized with a probe for rRNA. These normalized values were used to determine the amount of mRNA present in cells treated as indicated relative to the amount of mRNA detected in infected cells not treated. Values represent the mean $\pm$ S.D. of three determinations for Vero cells and five determinations for corneal cells.
[b] Normalized mean values are greater than two standard deviations different from the mean levels of combination treated.
[c] nd, None detected.

proteins examined (Taylor et al., 1994, 1998). To determine if a generalized inhibition of early protein production occurred that could lead to a synergistic inhibition of HSV-1 replication with ACV we examined the production of DNA polymerase and DNase in the presence of IFN-α and combinations of IFN-α with ACV.

In contrast to the reports of others (Klotzbücher et al., 1990), IFN-α treatment of Vero or corneal cells did not detectably decrease the levels of mRNA for the HSV-1 DNA polymerase. The reasons for this difference in effect from that previously reported is not known, although differences in cell type, IFN-type, IFN-α dosage or other culture conditions may play a role. We have shown that the two cell types used for this study do differ in their response to the anti-HSV activity of several IFN types and that the extent of the antiviral effect is influenced by the MOI (Taylor et al., 1998).

ACV treatment decreased the levels of HSV DNA polymerase mRNA in Vero cells by 25% and in stromal cells by 40%. This reduction in polymerase mRNA by ACV is consistent with the report by Wobbe et al. (1993) who demonstrated that another inhibitor of DNA synthesis, phosphonoacetic acid, reduced the accumulation of HSV-1 DNA polymerase mRNA. In cells treated with the combination of IFN-α and ACV, the inhibition of RNA synthesis was equivalent to that with ACV alone.

Although mRNA levels were not affected by IFN-α treatment, detectable DNA polymerase polypeptide, measured by Western blot, or enzyme activity were reduced to a similar extent. This effect at some post-transcriptional/translational level was similar to that which we have reported for IFN-α on the expression of HSV-1 thymidine kinase (Taylor et al., 1994).

The extent of inhibition by ACV on polymerase activity was similar to the effect of ACV on mRNA levels, suggesting that regulation occurs at the level of transcription or mRNA stability. In general, combination treatment reduced polymerase activity more than IFN-α or ACV alone, suggesting that regulation of RNA levels by ACV combined with the post-transcriptional/translational regulation of IFN-α to give a greater decrease in active enzyme.

There was not a direct correlation between the extent of inhibition of polymerase and the decrease in the amount of viral DNA in treatment groups. IFN-α decreased DNA polymerase activity by approximately 50%, but had no measurable effect on the amount of DNA synthesized. This finding may indicate that under normal conditions in the infected cell there is an excess of DNA polymerase and that some decrease in enzyme can be tolerated without causing decreased DNA synthesis. In the cells treated with ACV, there was a smaller decrease in polymerase activity than with IFN-α treatment, but a detectable decrease in DNA synthesis occurred. This decrease was prob-

Table 3
Effects of IFN-α and ACV treatment on HSV DNase activity

Cell type	Hours PI	Treatment	DNase activity[a] (units/mg protein)	% of HSV-infected
Vero	6	None	92.1 ± 12.5[b]	36.3
		HSV	253.4 ± 31.7	100.0
		HSV+IFN (100 IU/ml)	153.8 ± 13.4[b]	60.7
		HSV+IFN (1000 IU/ml)	150.3 ± 13.7[b]	59.3
		HSV+5 μM ACV	217.2 ± 46.3	85.7
		HSV+IFN (100 IU/ml)+5 μM ACV	152.5 ± 7.2[b]	60.2
HCS	6	None	72.6 ± 16.1[b]	32.3
		HSV	224.6 ± 42.0	100.0
		HSV+IFN (100 IU/ml)	197.4 ± 27.1	87.9
		HSV+5 μM ACV	211.4 ± 6.2[c]	94.1
		HSV+IFN (100 IU/ml)+5 μM ACV	160.6 ± 22.4	71.5
Vero	16	None	134.4 ± 65.4[b]	17.5
		HSV	767.7 ± 93.1	100.0
		HSV+IFN (100 IU/ml)	736.1 ± 156.0	95.9
		HSV+IFN (1000 IU/ml)	443.8 ± 278.6	57.8
		HSV+5 μM ACV	697.4 ± 96.5	90.8
		HSV+IFN (100 IU/ml)+5 μM ACV	576.8 ± 28.0	75.1

[a] All assays performed in triplicate, data presented as mean $\pm$ S.D.
[b] Statistically different ($P<0.05$) from HSV-infected, untreated as determined by a one-way analysis of variance and comparison of multiple means by the Bonferroni t-test.
[c] Statistically different ($P<0.05$) from combination (IFN+ACV) treated.

ably due to the direct effects of ACV-TP on DNA synthesis, i.e. by causing DNA chain termination (Furman et al., 1984; Reardon and Spector, 1989). The effect of the combination of ACV and IFN-α on DNA synthesis was much greater than would be predicted from additive effects of each treatment alone. The mechanisms for this synergy appear to be complex. IFN-α and ACV treatment both contribute to the decrease in DNA polymerase. Coupled with this decrease in enzyme, ACV is phosphorylated to ACV-TP which then inhibits the action of the enzyme that is produced. In addition, we have shown previously that IFN-α-treatment of HSV-1-infected cells alters nucleotide metabolism leading to a decrease in the pool of dGTP present (O'Brien et al., 1990). Therefore, in the IFN-α/ACV combination-treated cell there is a reduced amount of polymerase present, there is an inhibitor of that enzyme, and there is a reduction in the amount of dGTP which competes with ACV-TP for incorporation into viral DNA, all contributing to enhanced antiviral activity.

The decrease in DNase activity detected in IFN-α-treated cells was small compared to that of DNA polymerase or the effect we have previously demonstrated on thymidine kinase (Taylor et al., 1994). This suggests that IFN-α effects on early protein synthesis are somewhat selective. Although the extent of the reduction of the early enzymes we have measured varies, our results suggest that some level of inhibition by IFN-α may also exist for other early proteins that participate in efficient DNA synthesis, e.g. the major DNA binding protein, helicase-primase, etc. We have demonstrated some inhibition of ribonucleotide reductase and ICP8 as well (O'Brien et al., 1998; Taylor et al., 1998). An early study suggested that HSV-1 alkaline DNase activity may contribute to the nucleoside pool by degrading cellular DNA (Nutter et al., 1985). Inhibition of such activity leading to further reductions in nucleotide pools sizes could further augment ACV's activity. More recent studies suggest that DNase may play a role later in virus replication in the processing of viral DNA replication intermediates into unit length genome and viral capsid assembly and release from the nucleus (Martinez et al., 1996a,b).

Alone, the modest changes in the production of early proteins following IFN-α treatment may not be sufficient to cause a detectable decrease in the synthesis of viral DNA, but when combined with the effects of ACV on production and action of the polymerase, the result could contribute to the synergistic inhibition of DNA synthesis and virus replication. Mechanisms responsible for the apparent post-transcriptional/translational effects of IFN-α are unknown, but recent reports suggest that the double-stranded RNA-activated IFN-inducible protein kinase (PKR) which inhibits translation initiation may participate in inhibition of HSV-1 replication (Chou et al., 1995). Therefore, translational regulation of HSV-1 early proteins may be one mechanism by which IFN-α regulates virus replication and contributes to the synergistic inhibition of HSV-1 replication with nucleoside analogs.

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