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The effects of interferon-α and acyclovir on herpes simplex virus type-1 ribonucleotide reductase

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Abstract

Herpes simplex virus-type 1 (HSV-1) encodes both the small (UL40) and large (UL39) subunits of the enzyme, ribonucleotide reductase. Treatment of HSV-1-infected cells with interferon-α (IFN-α) reduced the levels of both enzyme subunits. Reduced steady state levels of the large subunit were demonstrated by immunoblot using polyclonal antibody specific for the viral enzyme. Reduction in the amount of small subunit was shown by a reduction in the electron spin resonance signal derived from the iron-containing tyrosyl free-radical present in this subunit. Treatment of cells with 100 IU/ml of IFN-α decreased levels of both subunits resulting in a reduction in enzyme activity as measured by conversion of CDP to dCDP. The decrease in the amount of the large subunit was not due to a reduction in the level of its mRNA. The combination of IFN-α and ACV treatment of human cornea stromal cells did not result in a further reduction in amounts of ribonucleotide reductase relative to that detected with IFN-α alone. The IFN-α-induced reduction in ribonucleotide reductase activity is the likely cause of decreased levels of dGTP which we have previously demonstrated in IFN-α-treated, infected cells.

Keywords: Herpes simplex virus; Acyclovir; Interferon; Ribonucleotide reductase; Synergy

1. Introduction

Interferons (IFNs) combined with acyclic nucleoside analogs such as acyclovir (ACV) or ganciclovir synergistically inhibit herpes simplex virus type 1 (HSV-1) replication (Hall and Duncan, 1988; Hanada et al., 1989; Lazdins et al., 1990; O'Brien et al., 1990; Taylor et al., 1989, 1991). The mechanism responsible for this synergy is not clearly defined. The effects of IFNs on nucleoside metabolism is one area of focus which has contributed to our understanding of IFNs, influence on the antiviral activity of IFN–nucleoside

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analog combinations. For example, among combinations tested, those which combine IFN with acyclic guanine analogs were determined to be the most highly synergistic (Taylor et al., 1991). Combinations of IFN and analogs such as trifluorothymidine and bromovinyl deoxyuridine produced additive effects. Combinations of IFN and DNA polymerase inhibitors such as phophonoformic acid (PFA) resulted in 'moderate' synergy relative to IFN and ACV (Hall and Duncan, 1988; Janz and Wigand, 1982).

In HSV-1-infected cells treatment with recombinant human IFN-α2a (IFN-α) treatment leads to a decrease in the size of deoxyribonucleotide pools with the greatest affect being on the dGTP pool (O'Brien et al., 1990). The effects of IFN-α treatment appear to be selective for deoxynucleoside triphosphate (dNTP) synthesis since there was no detectable effect on the size of the nucleoside triphosphate pools in infected cells (O'Brien et al., 1995). It has been observed by others that combinations of ACV with ribonucleotide reductase inhibitors result in synergistic antiviral activity (Spector et al., 1985; 1989) The hypothesis offered to account for synergy is based on the reduction of dNTP pools. It is believed that because of reduced amounts of dGTP in the cell, ACV-5′-triphosphate (ACV-TP) more effectively inhibits the activity of the viral DNA polymerase. Thus, we sought to determine whether the decreased nucleotide pools observed in IFN-treated, HSV-infected cells were possibly due to reduced ribonucleotide reductase activity.

In HSV-1 infected cells the pools of all four dNTPs are increased about 2–15-fold relative to uninfected cells (Furman et al., 1982). In order to achieve this pool expansion two events must occur. First, a virus encoded thymidine kinase is produced which greatly expands TTP pools (Taylor et al., 1994; Elion et al., 1977). Second, a viral encoded ribonucleotide reductase is produced which is insensitive to feedback inhibition by dNTPs resulting in the greatly expanded pools of dCTP, dATP, and dGTP. The viral ribonucleotide reductase is encoded by viral genes UL39, the gene for the 140 kDa large subunit, and UL40, the gene for the small subunit, which possesses the iron-dependent tyrosyl radical (Averett et al., 1983; Frame et al., 1985; Bacchetti et al., 1986; Swain and Galloway, 1986; Ingemarson and Lankinen, 1987). The enzyme is similar in structure to the ribonucleotide reductases found in bacterial and mammalian cells, existing as a heterotetramer containing two copies of each of the two subunits (Sjoberg et al., 1985; Reichard, 1987, 1993). Chemical inhibitors of ribonucleotide reductase activity can effectively inhibit the replication of HSV-1 in vitro (Paradis et al., 1988; Spector, 1985; Prichard and Shipman, 1995) and in vivo (Lobe et al., 1991; Safrin et al., 1993). The viral ribonucleotide reductase is not absolutely required for virus replication (Goldstein and Weller, 1988a). Virus possessing defects in ribonucleotide reductase genes replicate poorly in most cell types, however, some cells such as *S* phase cells, which contain elevated dNTP pools are capable of supporting replication of ribonucleotide reductase deficient virus. Cellular nucleotide pools, however, appear incapable of restoring replication to wild type levels (Prichard and Shipman, 1995). Our studies have documented that IFN-α treatment reduces the ability of HSV-1 to increase the dNTP pools in corneal stromal cells and Vero cells (O'Brien et al., 1990). It is unlikely that IFN-α would result in the direct inhibition of the viral enzyme. The effects of IFN-α are more likely to be mediated through its affects on HSV-1 enzyme production at the transcriptional or translational level (Mittnacht et al., 1988; Destasio and Taylor, 1990; Taylor et al., 1994; Nicholl and Preston, 1996). In this manuscript we report the effects of IFN-α on the levels of ribonucleotide reductase RNA and protein.

2. Materials and methods

2.1. Cells and virus

Human stromal fibroblast cells derived from donor corneas provided by the Wisconsin Lions Eye Bank (Milwaukee, WI) (Taylor et al., 1991) were grown in DMEM with 5% fetal bovine serum and Mito-Plus serum extender (Collaborative Res. Prod., Bedford, MA). Vero cells, a continuous monkey kidney cell line, were grown in

DMEM with 10% newborn calf serum. The McKrae strain of HSV-1 propagated in Vero cells was used throughout the studies.

2.2. Treatment of cells

Confluent cultures were treated with 100 IU/ml of recombinant human IFN-α2a (Hoffmann LaRoche, Nutley, NJ) in medium with 2% serum for 16–24 h. IFN-α-containing medium was removed and cells were infected at an MOI of 5 PFU per cell. After 1 h for virus adsorption, inoculum was removed and cells were treated with 5 μM ACV (Burroughs Wellcome, Research Triangle Park, NC). These treatment conditions have been demonstrated to lead to synergistic inhibition of HSV replication (Taylor et al., 1989). At various times after infection cells were harvested by scraping and extracts prepared for enzyme assay, immunoblot, or total cytoplasmic RNA.

2.3. Preparation of cell extracts

For measurement of ribonucleotide reductase activity cells were treated and harvested as described above. Cell pellets were suspended in 100 mM HEPES, pH 7.6, 2 mM DTT, and 0.2 mg/ml aprotinin and Dounce homogenized to lyse. Nuclei and cell debris were pelleted by centrifugation at $10000 \times g$ for 20 min at 4°C. Both the large and small subunits are localized to the cytoplasm of cells (Conner et al., 1995). The supernatant was centrifuged at $100000 \times g$ for 90 min. The resulting supernatant from this high speed spin was treated with $(NH_4)_2SO_4$ to 45% saturation and after 30 min precipitated material was collected by centrifugation at $10000 \times g$ at 4°C for 20 min. Pelleted protein was dissolved in homogenization buffer and chromatographed on a Sephadex G-25 column, eluting in homogenization buffer. Fractions containing peak OD_{280} readings were pooled and frozen at -80°C until protein determinations could be performed and enzyme activity could be assayed. Protein content of cell extracts was determined using the BCA protein assay (Pierce Chem., Rockford, IL). Western immunoblots to detect the large subunit of the viral ribonucleotide reductase were performed on extracts prepared for enzyme activity analysis and on cells lysates prepared by direct lysis of cells in SDS gel loading buffer containing 0.2 mg/ml aprotinin and 0.1 mM N^{α}-ρ-tosyl-L-lysine chloromethyl ketone (TLCK) to inhibit proteolysis (Ingemarson and Lankinen, 1987). Primary antibody (supplied by Sylvia Bacchetti, McMaster University) was polyclonal rabbit antibody prepared against the large subunit of HSV ribonucleotide reductase (Bacchetti et al., 1984). Immunoreactive material was detected using staphylococcal protein A linked to horseradish peroxidase and the ECL chemiluminescence detection system (Amersham, Arlington Heights, IL). Data were quantitated by densitometric scanning of blots using the Ambis Image Acquisition and Analysis System (Ambis, San Diego, CA) as previously described (Taylor et al., 1994).

2.4. Enzyme assays

Ribonucleotide reductase activity was measured by conversion of ^{14}C-labeled CDP to dCDP. Reaction mixtures consisted of 200 mM HEPES, pH 8.1, 10 mM DTT, 10 μM CDP, 100 μg/ml bovine serum albumin, 0.4 μl [^{14}C]CDP (0.04 μCi) and 25–50 μg of cell protein in a total of 50 μl. Reactions were incubated at 37°C for 15 min and stopped by addition of hydroxyurea to 20 mM and EDTA to 10 μM. Samples were boiled for 3 min and then 1 ml of crude snake venom phosphodiesterase at 0.5 mg/ml in 12 mM Tris, pH 9.0, 4 mM $MgCl_2$, and 1 mM deoxycytidine was added. Reactions were incubate for 30 min and then boiled for 3 min. The entire sample was applied to a 0.5 ml Dowex-1 borate column and eluted with 1.5 ml water. Radioactivity present in 1 ml was measured in a scintillation counter. All enzyme assays were carried out in triplicate and under conditions which were determined to be in the range of protein concentration and substrate concentration that demonstrated a linear increase in activity with respect to time and enzyme concentration. Comparisons between enzyme activities in infected cells of each treatment group and in infected, untreated cells were made using a one-way analysis of variance (ANOVA) and the Bonnferroni t-test for multiple comparisons.

2.5. Extraction of RNA and Northern blots

Total cytoplasmic RNA was extracted (Sambrook et al., 1989) and Northern blot were done as previously described (Taylor et al., 1994). Probes for detection of mRNAs were cloned genomic HSV DNA, ^{32}P-labeled by nick translation. The ribonucleotide reductase probe was an *Xho*I fragment (nucleotide residues 86412–88657) of viral DNA prepared with the assistance of Kent Wilcox (Medical College of Wisconsin) by subcloning of a restriction enzyme fragment from an *Eco*RI library of the KOS strain of HSV-1 (Goldin et al., 1981). The probe hybridized to a 4.8-kb mRNA for UL 39, encoding the large subunit of the enzyme. Data were quantitated by densitometric scanning of blots using the Ambis Image Acquisition and Analysis System (Ambis, San Diego, CA) as previously described (Taylor et al., 1994).

2.6. Electron spin resonance detection of enyzme

Cells were treated as described above and harvested at 6 and 16 h after infection, treated with trypsin and EDTA, and collected by centrifugation at $200 \times g$ for 5 min. Cells were washed in phosphate buffered saline, counted, and collected by centrifugation. The packed cell pellet was pipetted gently to form a slurry and cells were transferred into a glass capillary tube sealed at one end with Parafilm (American National Can, Greenwich, CT). Tubes were sealed with Parafilm and slowly placed in liquid nitrogen. The cells were held in liquid nitrogen for 60 s, withdrawn and the Parafilm removed. Tubes were warmed slightly and the frozen rod of cells expelled into liquid nitrogen. The cell rods were chilled for 30 s in liquid nitrogen prior to transfer into prechilled tubes and then stored at −80°C. Because of the limitations in the number of cornea stromal cells that could be obtained from a single pair of corneas comparisons could not be made between all treatment groups on the same batch of cells. Therefore, measurements were made comparing the signal present in ACV-treated cells to those present in IFN-α/ACV combination treated cells only.

The tyrosyl free radical of ribonucleotide reductase was detected using ESR spectroscopy. The ESR measurements were carried out at the National Biomedical ESR Center of the Medical College of Wisconsin. X-band ESR spectra were obtained using a standard Century Series Varian E-109 spectrometer operating at X-band (9–9.5 GHz) with 100 kHz field modulation. ESR measurements were made on frozen cell rods at −196°C in quartz finger dewars. Each spectra was recorded 25 times and averaged using the Viking computer program (National Biomedical ESR Center, MCW). The baseline background spectrum was obtained for water–buffer frozen rod under the same spectrometer conditions and subtracted from the sample spectrum using a computer program called Sumspec (National Biomedical ESR Center, MCW).

3. Results

3.1. Effects of IFN-α and ACV on ribonucleotide reductase activity

In four independent experiments treatment of human cornea stromal cells with 100 IU/ml of IFN-α reduced enzyme activity in the range of 21–68% relative to infected, untreated cells at 6 h after infection. The results of two such experiments is shown in Table 1. IFN-α pretreatment significantly reduced the amount of ribonucleotide reductase activity induced in stromal cells. ACV treatment alone had no reproducible effect on virus enzyme activity levels. Combined IFN-α and ACV treatment produced no additional significant reductions in enzyme levels beyond those seen with IFN-α alone. In a similar set of experiments, treatment of Vero cells with 100 IU/ml of IFN-α reduced the amount of viral ribonucleotide reductase induced by about 6–20% (Table 1). As in stromal cells, treatment with ACV alone did not significantly change the amount of enzyme activity present. Combined IFN-α and ACV treatment also reduced the amount of enzyme activity in infected cells. Although these effects of IFN-α treatment on infected Vero cells were reproducible, the differences in enzyme activity with

and without IFN-α were not statistically significant. Thus, the effects of IFN-α in the two cell types were similar, but statistically significant effects of IFN-α were observed only in low passage human cornea stromal cells.

3.2. Effects of IFN-α and ACV on the amount of the large subunit of ribonucleotide reductase

Cell extracts were analyzed using Western blots to determine whether the amount of the large subunit/mg of total cytoplasmic protein was changed in HSV-1-infected, IFN-α-treated cells relative to that in infected, untreated cells. The large subunit of ribonucleotide reductase is encoded by the HSV-1 UL39 gene which is regulated with some of the characteristics of an immediate early class of HSV-1 gene (Nicholl and Preston, 1996). The 140-kDa full-length gene product (ICP6) is subject to proteolytic cleavage resulting in a 110-kDa product which retains enzyme activity (Ingemarson and Lankinen, 1987). Both of these products are recognized by the antibody used in this study. Treatment with 100 IU/ml of IFN-α reduced the total detectable amount of large subunit to 16% (range 7–25%) of that detected in HSV-1-infected, untreated human cornea stromal cells at 6 h after infection (Fig. 1). ACV treatment alone did not result in a detectable change in the level of large subunit relative to that in infected, untreated cells, while treatment with both IFN-α and ACV reduced the amount of large subunit to a level equal to that in cells treated with IFN-α alone. IFN-α treatment of Vero cells reduced the level of the large subunit to about 63% (range 50–84%) of that detected in infected, untreated cells. The extent of the reduction in Vero cells treated with combinations of IFN-α and ACV was similar to that in infected cells treated with IFN-α alone while ACV treatment alone had no effect on the levels of large subunit.

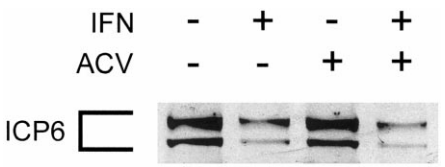

Fig. 1. Western blot of cytoplasmic extracts from HSV-1-infected human cornea stromal cells prepared 6 h after infection. Cells were mock-treated or treated with 100 IU/ml IFN-α 24 h prior to infection. Following infection at an MOI of 5 PFU/cell, cells were treated with 5 μM ACV as indicated. The HSV-1 ribonucleotide reductase-specific antiserum used to probe the blot detected both the full length 140-kDa ICP6 protein (upper band) and its 110-kDa breakdown product (lower band) of the viral enzyme.

Table 1
The effect of IFN-α and ACV on HSV-1 ribonucleotide reductase in human cornea stromal cells and Vero cells

Cell type	Treatment[a]	Enzyme activity[b]	Percent decrease[c]
Cornea	None	965 ± 225	—
	IFN	308 ± 64[d]	68
	ACV	960 ± 37	0
	IFN+ACV	274 ± 48[d]	72
Cornea	None	1016 ± 86	—
	IFN	745 ± 119[d]	27
	ACV	1038 ± 25	0
	IFN+ACV	410 ± 83[d]	60
Vero	None	4775 ± 681	—
	IFN	3975 ± 413	17
	ACV	4746 ± 318	1
	IFN+ACV	3323 ± 151[d]	30
Vero	None	3266 ± 250	—
	IFN	3053 ± 771	6
	ACV	3714 ± 912	0
	IFN+ACV	2217 ± 407	31

[a] Cells were plated at 5×10^4 cells/cm^2 and the next day mock-treated or treated with 100 IU/ml IFN-α. After 24 h, cells were infected at MOI = 5 with HSV-1 and mock-treated or treated with 5 μM ACV. After 6 h, cell lysates were prepared and ribonucleotide reductase was assayed.

[b] Specific activity of ribonucleotide reductase as measured by conversion of CDP to dCDP, reported in pmole dCDP formed/h per mg protein. Numbers represent the mean and standard deviation of triplicate assays of a single preparation. Data for two representative experiments for each cell type are given.

[c] Percent decrease in mean specific activity relative to the enzyme activity in extracts from HSV-infected, untreated cells.

[d] Statistically significant changes in ribonucleotide reductase activity relative to that in HSV-1-infected, untreated cells using a one way analysis of variance and the Bonnferroni t-test for multiple comparisons. Significant differences among groups are reported at the level of $p < 0.05$.

3.3. Effects of IFN-α and ACV on tyrosyl radical of the small subunit of ribonucleotide reductase

The peak-to-trough height obtained from the ESR signal of the tyrosyl radical was used as an indicator of the amount of the small subunit of viral ribonucleotide reductase. The tyrosyl free radical signal in HSV-1-infected stromal cells as measured by ESR spectroscopy produced a signal characteristic of the small subunit of the viral enzyme (Fig. 2a) (Sjoberg et al., 1985). An equivalent amount of mock-infected HCS cells did not show a detectable signal (Fig. 2a). The peak-to-trough height of the tyrosyl radical signal was measured upon treatment of HSV-infected cells with IFN-α, ACV or combinations. The combination of IFN-α with ACV decreased the intensity of the tyrosyl radical in HSV-1-infected corneal cells to 50% of that seen in cells treated with ACV (Fig. 2b). HSV-1-infected Vero cells at 6 h after infection showed a 24% decrease in the tyrosyl radical signal upon treatment with 100 IU/ml IFN-α. Treatment with 5 μM ACV alone also showed an 18% decrease in signal and upon treatment with both IFN-α and ACV the decrease in tyrosyl radical signal was 70%, an amount greater than that observed with either treatment alone. These reductions in radical signal were determined as reduction in peak to trough heights in ESR signals which represent the average of 25 scans per sample. Scanning samples in this manner measures the amount of radical within ± 5%.

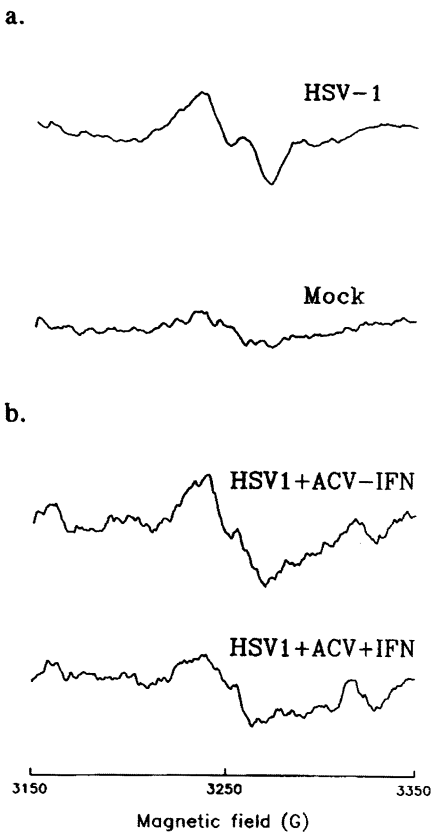

Fig. 2. ESR spectra of tyrosyl radical in the small subunit of ribonucleotide reductase in human cornea stromal cells. Corneal cells were infected with HSV-1 at an MOI of 5 PFU/cell or mock infected and harvested at 6 h after infection. Panel (a) represents the tyrosyl radical signal of the viral ribonucleotide reductase in HSV-1-infected cells and uninfected cells. Panel (b) shows the signal obtained from corneal cells infected and treated with ACV or ACV and IFN-α. Spectrometer conditions were: center field 3250G, scan range 200 Cr, modulation amplitude 5G, scan time 2 min, time constant 0.5 s, microwave power 100 mW.

3.4. Effects of IFN-α and ACV on ribonucleotide reductase mRNA

Northern blots of total cytoplasmic RNA from human corneal stromal cells using a probe specific for the large subunit of HSV ribonucleotide reductase revealed a band of approximately 5.2 kb corresponding to the mRNA encoding the large subunit of ribonucleotide reductase (Anderson et al., 1981; McLauchlan and Clements, 1983; Swain and Galloway, 1986). IFN-α, ACV, or combination treatment had no measurable, reproducible effect on the steady state levels of the mRNA from the UL39 gene (Fig. 3). These Northern blots, when analyzed by densitometric scanning, clearly indicate that the steady-state levels of mRNA from the UL39 gene are not decreased by IFN-α treatment.

4. Discussion

HSV-1 ribonucleotide reductase appears to play an important role in the pathogenesis of HSV in vivo (Cameron et al., 1988; Jacobson et al., 1989; Yamada et al., 1991; Idowu et al., 1992). The

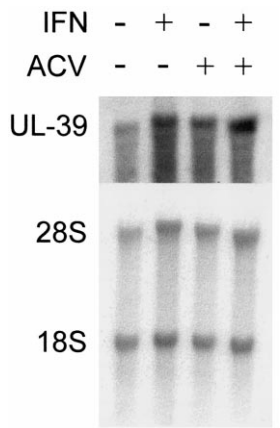

Fig. 3. Northern blots of total cellular RNA from HSV-infected cells. The panels show blots of total cytoplasmic RNA prepared from cells that were mock-treated or treated with 100 IU/ml IFN-α for 24 h prior to infection. Following infections cells were treated with 5 μM ACV as indicated. The upper panel was probed with a ^{32}P-labelled cloned DNA fragment containing sequences of the UL39 gene of HSV-1. The lower panel is a blot prepared by stripping the upper blot and reprobing with a ^{32}P-labelled probe for ribosomal RNA to evaluate relative RNA load.

enzyme has been shown to contribute to ocular virulence in mice (Brandt et al., 1991) while null mutants in ribonucleotide reductase do produce lesions in a guinea pig model (Turk et al., 1989). Controversy also exists as to whether ribonucleotide reductase is a suitable target for antiherpes virus drugs (Prichard and Shipman, 1995).

Specific agents designed to inhibit HSV ribonucleotide reductase activity have been shown not only to act effectively to inhibit HSV-1 replication, but also to act synergistically with ACV to inhibit HSV replication (Spector et al., 1985; Spector and Jones, 1985; Karlsson and Harmenberg, 1988; Spector et al., 1989, 1992; Liuzzi et al., 1994). These agents include thiosemicarbazones which interact with the tyrosyl free radical-iron complex in the small subunit of the ribonucleotide reductase to inactivate the viral enzyme (Spector et al., 1985, 1989, 1991; Shipman et al., 1986; Turk et al., 1986a,b; Porter et al., 1990) and synthetic peptides that inhibit the binding of large and small subunits of the enzyme (Cohen et al., 1986; Dutia et al., 1986; Gaudreau et al., 1987; McClements et al., 1988; Paradis et al., 1988; Moss et al., 1993; Liuzzi et al., 1994). HSV-1 mutants synthesizing enzyme of reduced function or lacking ribonucleotide reductase activity are also extremely sensitive to ACV's antiviral activity (Coen et al., 1989). The synergism between inhibitors of viral ribonucleotide reductase and ACV appears to be due to the decrease in levels of dGTP present in the infected, treated cells. ACV-TP competes with dGTP for utilization by the viral DNA polymerase and when bound, inhibits DNA synthesis. The decrease in dGTP resulting from decreased ribonucleotide reductase activity favors increased utilization of ACVTP and, therefore, increased inhibition of viral DNA synthesis. We have demonstrated a 70% decrease in the size of the dGTP pool in IFN-α-treated, HSV-infected cells compared to untreated cells during the first 6 h of infection (O'Brien et al., 1990). The decrease in dGTP is likely due to the decrease the steady state amounts of ribonucleotide reductase as documented by these studies.

In the studies reported here, IFN-α (100 IU/ml) treatment of corneal or Vero cells reduced the amount of ribonucleotide reductase enzyme activity detectable at 6 h after HSV-1 infection at an MOI of 5. In both corneal and Vero cells, treatment with IFN alone under these conditions did not significantly reduce the yield of virus. This finding is consistent with studies with HSV-1 ribonucleotide reductase deficient mutants showing that ribonucleotide reductase is not required for HSV-1 growth under cell culture conditions where host cells contain large dNTP pools which compensate for the defect in the viral enzyme (Goldstein and Weller, 1988a,b; Jacobson et al., 1989). Combination of 100 IU/ml IFN-α treatment with 5 μM ACV has been shown to decrease virus yields by two to three logs relative to ACV alone (Taylor et al., 1989). The IFN-α-induced decrease in ribonucleotide reductase activity detected in the studies reported here could be an important contributor to this synergism.

The decrease in ribonucleotide reductase activity in IFN-α-treated cells appears to be due to a decrease in the amount of both the small and large subunit of the viral enzyme. Since both subunits are needed for enzyme activity (Bacchetti et al., 1986) a decrease in either subunit could lead

to a decrease in enzyme activity. Conner et al. (1995) reported that the large subunit is present at about twice the concentration as the small subunit in HSV-1-infected cells suggesting that the small subunit is the limiting component in formation of the active enzyme. It may, therefore, be important that the IFN-α/ACV combination-treatment appeared to reduce the level of small subunit more than IFN-α alone based upon ESR detection of the tyrosyl-free radical.

The finding that the reduced amounts of the large subunit are not accompanied by a reduction in RNA levels is consistent with our findings with other HSV early enzymes in IFN-α-treated cells (Taylor et al., 1994, 1998). The mechanism by which IFN-α treatment actually reduces the amount of these early enzymes is unclear, but our data suggest that the effects may be at the level of translation or protein turnover. The induction by IFN-α of a protein kinase (PKR) that inhibits initiation of protein synthesis is well documented (Samuel, 1991; Sen and Ransohoff, 1993), and recent reports suggests it may play a role in IFN-α's action in HSV-1-infected cells (Chou et al., 1995; He et al., 1997). Determination of the mechanism by which IFN-α induces the decrease in production of the HSV-1 ribonucleotide reductase as well as other early viral enzymes involved in nucleoside metabolism could allow development of better strategies of combination treatment for HSV-1 infections.

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