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EVIDENCE FOR AN ALTERED KINETICS OF DNA EXCISION-REPAIR IN CELLS INFECTED BY HERPES SIMPLEX VIRUS TYPE 1

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Summary. – In cells infected with herpesviruses a series of host cell nuclear changes can be observed in a temporal sequence. Such changes include chromosome aberrations. The precise mechanism by which virus infection produces chromosome damage is not known, but we have previously reported that herpes simplex virus type 1 (HSV-1) induces a significant number of single-stranded breaks in the host cell DNA at early hours of infection and in a time-dependent fashion. Here, it is reported that HSV-1-infected cells subjected to irradiation with ultraviolet light, show an altered kinetics in the normal process of DNA excision-repair at early hours of infection.

Key words: herpes simplex virus type 1; DNA breaks; DNA repair

Introduction

In cells infected by herpesviruses a series of nuclear changes can be observed in a temporal fashion. Such changes include: distortion and disappearance of the nucleolus, development of basophilic intranuclear inclusion bodies which are later converted into eosinophilic inclusion bodies, and distortion of the nuclear membrane. During mitosis, the most frequent nuclear aberrations seen in virus-infected cells are chromatid breaks (Hampar and Ellison, 1961; O'Neill and Miles, 1969; Roizman and Sears, 1991). Previous studies have shown interesting modifications in the properties of chromatin from cells infected with HSV-1. These modifications are not due to extensive breakdown of host cell DNA, dissociation of histones from DNA or disruption of nucleosomal structure in bulk host chromatin (Chong, 1980 and personal communication; Peat and Stanley, 1986). These findings suggest that events at other levels than the nucleosomal of chromatin organization are responsible for the chromatin changes in the infected cells.

We have previously shown that infection by HSV-1 induces a significant

number of single-stranded breaks on the host cell DNA (Aranda-Anzaldo, 1992*a*). For this purpose we adapted and modified a highly sensitive and easy-to-handle fluorescence enhancement assay for DNA damage (Kanter and Schwartz, 1982; Aranda-Anzaldo, 1992*a*). Such virus-induced breaks appear in a time-dependent fashion, reaching a peak number at about 3 hr post infection (p. i.), and then almost disappearing at about 8 hr p. i. Such breaks seem to have no direct relationship to the major breakdown of host cell DNA observed in infected cells after 12 hr p. i.

In the present study we report DNA repair experiments carried out on infected cells in an attempt to further clarify the nature of the host cell DNA damage induced by HSV-1 at early hours of infection. DNA excision-repair can be reliably compared in cells of different type or growth state through the use of inhibitors which accumulate strand breaks at repair sites (Collins *et al.*, 1984). The measurement of breaks accumulated in DNA undergoing excision-repair is used as a criterion of the progress and capability of DNA repair.

Inhibitors like hydroxyurea (HU), cytosine arabinoside (ara-C) and aphidicolin can inhibit both DNA replication and repair. HU is an inhibitor of the enzyme ribonucleotide reductase and so indirectly, of DNA polymerization. HU is able to inhibit DNA repair synthesis in UV-irradiated cells held in stationary state, and also is able to cause accumulation of DNA strand breaks at the sites where enzymic incision is not followed by the repair synthesis and ligation (Snyder *et al.*, 1984). The repair synthesis is less sensitive than replication synthesis to the depletion of deoxyribonucleotide pools caused by HU, but still it is affected by the presence of the inhibitor (Collins *et al.*, 1982; Snyder *et al.*, 1984). The incorporation of the cytidine analogue ara-C into cell DNA occurs at chain termini and at internal sites. The internal arabinose groups are alkali-labile, which might possible account for some of the strand breaks detected in alkali-denaturated DNA from cells given ara-C during repair (Fram and Kufe, 1982; Snyder *et al.*, 1984). Continuous incubation of cells with HU and ara-C after UV-induced damage, produces UV dose-dependent stable strand breaks (Collins *et al.*, 1982; Snyder *et al.*, 1984). Aphidicolin specifically inhibits DNA polymerase alpha and thus, has effects on DNA repair. This inhibitor is able to bind to the DNA polymerase alpha molecule at a site near the dCTP binding site, thus blocking the binding of dCTP which results in chain termination at DNA sites requiring cytosine (Collins, 1983). Aphidicolin does not produce alkali-labile sites which might enhance the number of strand breaks detected in alkali-denatured DNA.

Materials and Methods

Cell lines. Mouse L cell fibroblasts were grown as monolayer cultures at 37 °C in tissue culture bottles or Winchester quart bottles with a capacity of aproximately $3x10^7$ and $1x10^8$ cells per bottle, respectively at confluence. Human HeLa cells were grown in suspension at 36 °C in 500 ml cylindrical bottles kept in a roller bottle apparatus. HeLa cultures were usually kept at a density of

about 5–8x10^5 cells/ml. Both cell lines were grown in Eagle's minimum essential medium (MEM) with L-glutamine, supplemented with 5 % tryptose phosphate broth, 5 % newborn calf serum (Gibco), penicillin 100 units/ml and streptomycin sulphate 100 μg/ml (Glaxo). The cell cultures were kept mycoplasma-free by treatment with Tylan (Elanco) at a concentration of 50 μg/ml over a period of 24 hr every 4–6 weeks.

For the purposes of the present study, cultures prepared by subculturing the cells during the 24 hr prior to their use for experiments, are considered as actively growing cultures. For quiescent (stationary phase) cultures, the cells were allowed to reach confluence (L cells) or higher cell densities, about 2x10^6 cells/ml (HeLa), and then deprived of serum overnight before being transferred to full tissue culture medium (TCM) for further experimentation. ^{3}H-thymidine incorporation experiments showed that cells treated in this way have a lag of about 8 hr before entering into exponential growth.

Virus. HSV-1 strains HFEM (var. STH2) and *Fra* were used throughout the present study. Both strains behaved similarly when used in similar experiments. Winchester bottle cultures of L cells grown to confluence, were infected at a multiplicity of infection (m.o.i.) of about 0.01 PFU/cell and kept at 36 °C in a bottle roller. The cell-free virus was harvested and clarified by centrifugation after 3–4 days. Free virus particles dispersed in the cell-free used TCM were kept at 4 °C or frozen at −80 °C. For some experiments, the virus was concentrated by mixing two volumes of clarified TCM supernatant with one volume of 30 % (w/v) polyethyleneglycol 6 000 in phosphate buffered saline (PBS) supplemented with 0.5 mol/l NaCl. The mixture was incubated overnight at 4 °C, the virus precipitate was pelleted by centrifugation at 1000 g for 10 min and resuspended in fresh MEM without serum, broth or antibiotics. Virus infectivity was determined by plaque assay carried out on L cell monolayers. Usually, the non-concentrated virus stocks had titers in the region of 2x10^6 to 1x10^7 PFU/ml.

Experimental infection of cells. L cell monolayers in 50 mm Petri dishes were infected with stock virus at m.o.i. of 5 PFU/cell. Virus was allowed to adsorb to the cells at 37 °C for 30 min. Then the virus inoculum was removed, cell sheets were washed with PBS, supplied with fresh TCM and incubated at 37 °C in the presence of 5 % CO_2. HeLa cell suspensions were gently centrifuged down and resuspended in an appropriate volume of stock virus sufficient to give a m.o.i. of 5 PFU/cell. The virus was allowed to adsorb at 37 °C for 30 min. The inoculum was removed by centrifugation, cell pellet was washed in warm PBS and finally resuspended in fresh TCM for further incubation. Fresh MEM was used for mock infection. The zero time of infection was taken as the time of resuspension of cells in fresh TCM after removal of the viral inoculum.

Fluorescent assay for DNA damage and repair. Bisbenzamide (Hoechst 33 258), a fluorescent dye commonly used as a chromosome stain, complexes with DNA to form a stable, intensely fluorescent product; a less fluorescent product is formed with single-stranded DNA. Thus, the quantitation of DNA damage is based on the property of differential molar fluorescence. The fraction **F** of DNA in duplex form after partial alkaline denaturation proceeding for a fixed time is given by the equation

$$F = \frac{B - C}{A - C} = \frac{\text{duplex DNA}}{\text{total DNA}}$$

(Kanter and Schwartz, 1982). The values **A**, **B** and **C** are obtained from the relative fluorescence determination, where **A** is the fluorescence reading of the non-denatured sample, **B** of the sample subjected to partial alkaline denaturation and **C** of the sample subjected to complete alkaline denaturation. Also the number of breaks **n** in DNA molecules caused by UV-irradiation can be determined using the equations of Rydberg (1975) and expressed as **n**/molecular weight of DNA (Aranda-Anzaldo, 1992*a*).

The following is the general procedure for the bisbenzamide fluorescence enhancement assay as modified by us:

Equal amounts of cells suspended in PBS without Ca^{2+} and Mg^{2+} (PBS-A) at 2–5x10^5 cells/ml, are pipetted (500 μl) into nine disposable borosilicate glass tubes (10x15 mm) kept in ice. To three of these tubes (tubes A) is added 1.0 ml (each) of a solution made by addition of equal volumes of 0.1 N NaOH and 0.1 M KH_2PO_4 (final pH 7.8), followed by the addition of 500 μl of bisbenzamide

bufer solution (0.16% sodium lauryl sarcosinate; 0.2 M KPO_4; 0.04 M disodium EDTA; 1.0 μg bisbenzamide per ml, pH 7.4). The DNA in these tubes is sheared by complete immersion of a microsonicator tip into the cell lysate for 15 sec (Branson Sonifier, setting 3). The cell sonication procedure should produce foaming in the tubes. If not, the tip should be withdrawn and reintroduced until foaming occurs. The DNA in these tubes remains in duplex form (i. e., F the fraction of total DNA in duplex form is equal to 1). To three tubes (tubes B) are added 500 μl of 0.1 N NaOH, such tubes have been kept in ice and then put in a vibration-free area at room temperature just prior to addition of alkali. The tubes are kept in the dark for a carefully timed unwinding period (generally 8 min). After that period, 500 μl of 0.1 M KH_2PO_4 (pH 4.7) are added, followed by the addition of bisbenzamide buffer (500 μl) and the cell lysate is sonicated as above. This set of triplicates contains DNA in a partially denatured form, the extent of denaturation being a function of the size of the unwinding unit and the amount of DNA present. To the final set of three tubes (tubes C) are added 500 μl of 0.1 N NaOH, the alkaline lysate is sonicated (10–15 sec), and 0.1 M KH_2PO_4 pH 4.7 (500 μl) are added after a time which should not be shorter than the time of unwinding of samples B (8 min), and not longer than 2 hr at room temperature, because after this time long stretches of single-stranded DNA could become entagled thus giving a false region of double strandedness. The previous addition is followed by the addition of bisbenzamide buffer (500 μl) and resonication (10 sec). Because extensive DNA fragmentation by sonication at the start of the unwinding procedure, in the tubes C conversion of native DNA to single-stranded DNA is essentially complete (i. e., $F=0$).

The following is a list of the pH ranges expected in each of the different steps of the fluorescent assay: 1) PBS-A + cells pH 7.4; 2) cell suspension + 0.1 N NaOH pH 12.3; 3) cell suspension + 0.1 N NaOH +0.1 M KH_2PO_4 pH 8.0; 4) cell suspension + 0.1 N NaOH + 0.1 M KH_2PO_4 + bisbenzamide buffer pH 7.6. Solution 0.1 N NaOH + 0.1 M KH_2PO_4 pH 7.8; solution 0.1 M KH_2PO_4 pH 4.7.

All the tubes are kept at room temperature inside a drawer, and fluorometric readings are taken after the tubes have equilibrated at ambient temperature (preferably overnight). The fluorescent product once formed is quite stable, samples exposed to normal room lighting for up to two weeks did not show any change in fluorescence. Readings are taken in quartz fluorometry cuvettes (3 ml) in a Perkin-Elmer MPF/44B fluorometer operating in the ratio mode at the following settings which are the optimum conditions for bisbenzamide fluorescence: excitation 353 nm; emission 451 nm; bandpass 5 nm. The fluorescence readings among triplicates are highly reproducible thus giving in all cases standard deviations $\lesssim 5$ % among the triplicates from a sample.

Irradiation of cells. UV-irradiation of L and HeLa cells was carried out in a tissue culture hood under a germicidal lamp emitting UV-light at an output of 1.0 Jm^{-2} sec^{-1} at bench level. L cell monolayers in Petri dishes were irradiated in the absence of culture medium and with the top lid of the Petri dish removed. The culture medium was replaced after irradiation. HeLa cells suspended in MEM were also irradiated in Petri dishes, 3 ml of cell suspension in a 50 mm dish thus giving a liquid layer less than 2 mm thick.

For calibration purposes of our method for DNA damage and repair, X-ray irradiation was carried out on cells in suspension (HeLa) or monolayers (L) in Petri dishes, using General Electric Maxitron 300 therapy machine, at settings of 20 mA, 300 KVP, 1/4 Cu filter and a target-to-radiation source distance of 50 cm. The irradiation rate was 200 rad/min.

For all cases the control could be either a non-irradiated set of cell samples or an irradiated set which was chilled to 4 °C immediately after irradiation. Both types of controls essentially gave similar values of F.

DNA repair inhibitors. In our experiments with DNA repair inhibitors the cells were preincubated in tissue culture medium with the appropriate concentration of inhibitors for 30 min before irradiation, and then further incubated with the same inhibitors for different times after irradiation.

Chemicals. HU was purchased from Boehringer, ara-C from Calbiochem and bisbenzamide from Hoechst. Aphidicolin was a generous gift from Dr. A. Collins. All other chemicals and substances used were reagent grade or tissue culture grade.

Results

Fig. 1 shows the time course of DNA excision-repair in mock-infected and HSV-1-infected HeLa cells that were irradiated with a moderate dose of UV-light (5 Jm^{-2}). The cells were kept in a quiescent state before conducting the experiment. In this plot the data are expressed in terms of percentage of duplex DNA value of the zero-time unirradiated sample; it means that there was an unirradiated control sample for each different time of infection.

At first glance, cells from samples infected for 2 and 3 hr show a poor recovery in the percentage of duplex DNA after 2 hr of incubation post-irradiation. When the same data are plotted in terms of *F* value (absolute fraction of double-stranded DNA per sample for each time point, Fig. 2) and the slopes for the first 30 min of repair activity (initial rates of incision), and for the period between 60 to 120 min post-irradiation (break-sealing period) are calculated, it seems that the 2 and 3 hr p. i. samples are the slowest in both accumulation and sealing of UV-induced DNA breaks, when compared with mock-infected irradiated samples (see the slope values in the legend to Fig. 2).

The difference in the zero-time *F* values (equivalent to unirradiated samples) among all samples at different times post-infection can be seen in Fig. 2. Thus, the 3 hr p. i. sample has the lowest zero-time *F* value in agreement with the fact that it is at this time of infection when the largest number of HSV-1-induced DNA breaks is observed (Aranda-Anzaldo, 1992*a*). However, when the largest number of breaks accumulated after UV-irradiation is calculated at each of the post-infection times tested (either at 30 or 60 min post-irradiation according to

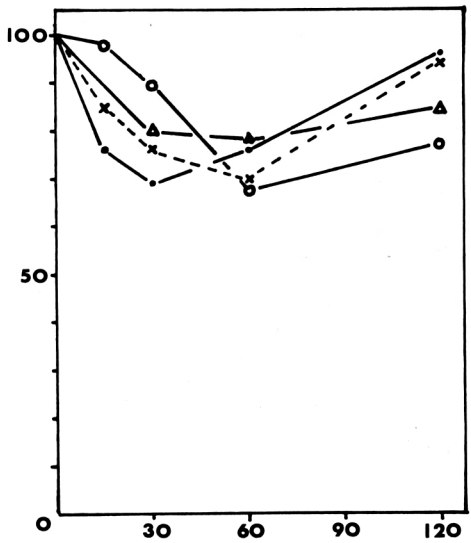

Fig. 1
Kinetics of DNA excision-repair of UV-induced damage in HeLa cells infected by HSV-1
Cells infected with HSV-1 for different times were irradiated with UV-light and incubated in fresh TCM at 37 °C for different times post-irradiation.
(●) mock-infected; (△) 2 hr p. i.; (○) 3 hr p. i.; (×) 5 hr p. i. Abscissa: time post-irradiation (min). Ordinate: % of duplex DNA of unirradiated control.
Maximum number of UV-induced extra DNA breaks/10^9 daltons:
1.82 (mock-infected)
1.31 (2 hr p. i.)
1.88 (3 hr p. i.)
1.77 (5 hr p. i.)

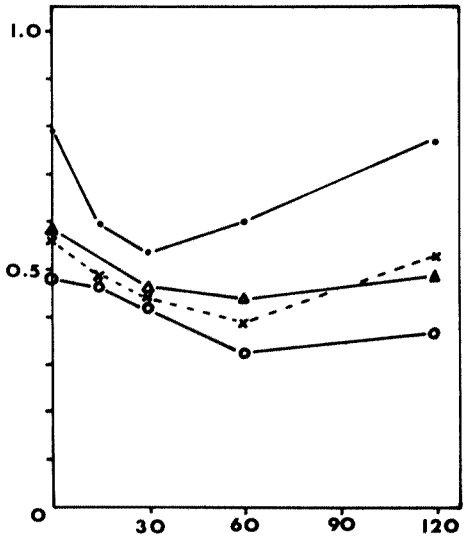

Fig. 2
Kinetics of DNA excision-repair of UV-induced damage in HeLa cells infected by HSV-1
The data from Fig. 1 are plotted in terms of *F* value (asbolute fraction of duplex DNA) at different times post-irradiation.
Abscissa: time post-irradiation (min). Ordinate: *F*.
Initial incision rate (0 to 30 min):
-0.0084 F/min (mock-infected)
-0.0043 F/min (2 hr p. i.)
-0.0040 F/min (3 hr p. i.)
-0.0045 F/min (5 hr p. i.)
Gap-filling rate (60 to 120 min):
+0.0026 F/min (mock-infected)
+0.0006 F/min (2 hr p. i.)
+0.0007 F/min (3 hr p. i.)
+0.0025 F/min (5 hr p. i.)

the particular sample), it becomes clear that all samples accumulate about the same number of DNA breaks after UV-irradiation (see legend to Fig. 1). Thus, it appears that infection with HSV-1 does not increase the accumulation of DNA breaks due to UV-irradiation and actually, it seems that accumulation of such UV-induced DNA breaks was slowed down in the infected samples. Also the

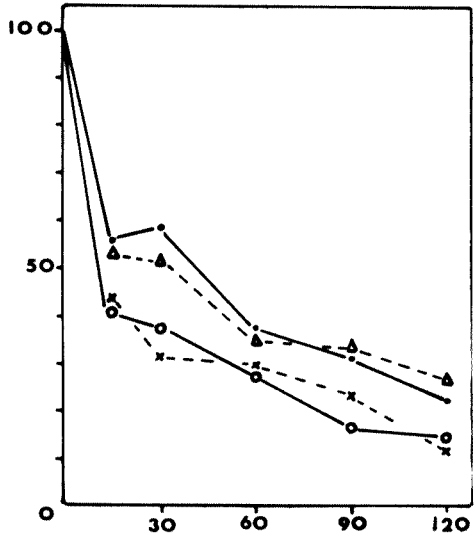

Fig. 3
Kinetics of UV-induced DNA breaks accumulation in HeLa cells infected by HSV-1
Cells infected with HSV-1 for different times were irradiated with UV-light and incubated with repair inhibitors (20μmol/l ara-C + 2 mmol/l HU) to allow accumulation of DNA breaks.
(●) mock-infected; (×) 2 hr p. i.; (○) 3 hr p. i.; (△) 5 hr p. i.
Abscissa: time post-irradiation (min). Ordinate: % of duplex DNA of unirradiated control.
Maximum number of UV-induced DNA breaks/10^9 daltons:
4.4 (mock-infected)
5.0 (2 hr p. i.)
4.9 (3 hr p. i.)
4.1 (5 hr p. i.)

repair of such breaks due to UV-irradiation was slower in the infected samples (see legend to Fig. 2). Therefore, it was decided to use DNA repair inhibitors in order to clarify these observations.

Fig. 3 shows an experiment in which HeLa cells (both mock and virus infected) were incubated in the presence of ara-C and HU in order to promote the accumulation of DNA breaks after being irradiated with UV-light. In this figure, the data are plotted in terms of percentage of duplex DNA from the unirradiated sample (each post-infection time had its own unirradiated control). The legend to the figure shows the maximum number of breaks accumulated for each post-infection time after 2 hr of incubation post-irradiation. From this data it may be concluded that both, mock-infected and virus-infected samples accumulate about the same number of breaks after exposure to a similar amount of UV-light.

The same data are plotted in Fig. 4 but in terms of *F* value (absolute fraction of duplex DNA per sample for each incubation time post-irradiation). The legend to this figure gives the values of the slopes for the first 15 and 60 min of steady accumulation of DNA breaks in the presence of repair inhibitors. From this data it may be concluded that infected samples are slower in accumulating DNA breaks. Nevertheless, all samples (mock and infected) accumulate a similar final number of DNA breaks after exposure to the same UV dose.

Under our culture conditions, the time course of infection for both L and HeLa cells is the same. DNA repair time course experiments with mock-infected and virus-infected L cells kept in a quiescent state showed that cells infected for 3 hr accumulate the largest number of UV-induced DNA breaks in the absence of repair inhibitors. However, the initial rates of incision (first 30 min post-

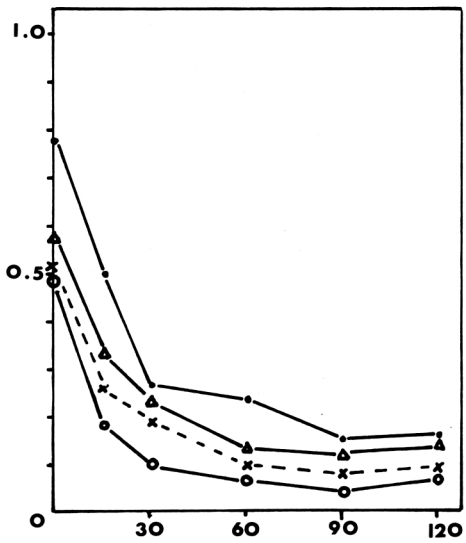

Fig. 4
Kinetics of UV-induced DNA breaks accumulation in HeLa cells infected by HSV-1
The data from Fig. 3 are plotted in terms of *F* value (absolute fraction of duplex DNA) at different times post-irradiation.
Abscissa: time post-irradiation (min). Ordinate: *F*.
Initial incision rate (0 to 15 min):
-0.0230 F/min (mock-infected)
-0.0186 F/min (2 hr p. i.)
-0.0186 F/min (3 hr p. i.)
-0.0180 F/min (5 hr p. i.)
Long-term incision rate (0 to 60 min):
-0.0071 F/min (mock-infected)
-0.0052 F/min (2 hr p. i.)
-0.0048 F/min (3 hr p. i.)
-0.0053 F/min (5 hr p. i.)

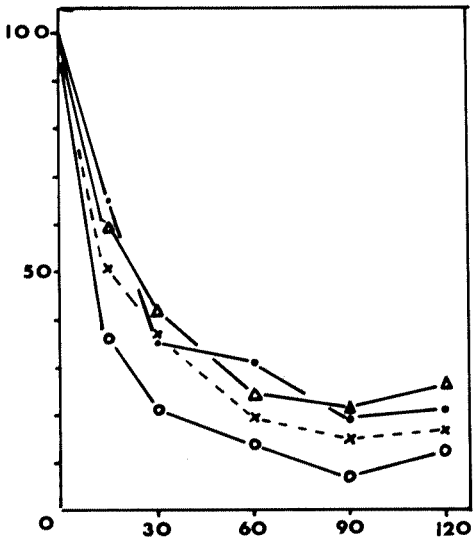

Fig. 5
Kinetics of UV-induced DNA breaks accumulation in the presence of aphidicolin in HeLa cells infected by HSV-1
Cells infected with HSV-1 for different times were irradiated with UV-light and incubated with 5μg/ml aphidicolin for different times post-irradiation to allow the accumulation of breaks.
(●) mock-infected; (×) 2 hr p. i.; (○) 3 hr p. i.; (△) 5 hr p. i.
Abscissa: time post-irradiation (min). Ordinate: % of duplex DNA of unirradiated control.
Maximum number of UV-induced DNA breaks/10^9 daltons:
4.57 (mock-infected)
4.80 (2 hr p. i.)
5.00 (3 hr p. i.)
4.28 (5 hr p. i.)

irradiation) were slower in the infected samples as it was also the case for the rate of DNA damage recovery (60 to 120 min post-irradiation). Experiments performed with L cells incubated in the presence of DNA repair inhibitors (ara-C + HU) showed that the final number of UV-induced breaks accumulated under such conditions was very much the same for both infected and uninfected samples. However, the infected samples (particularly those at 3 hr p. i.) showed

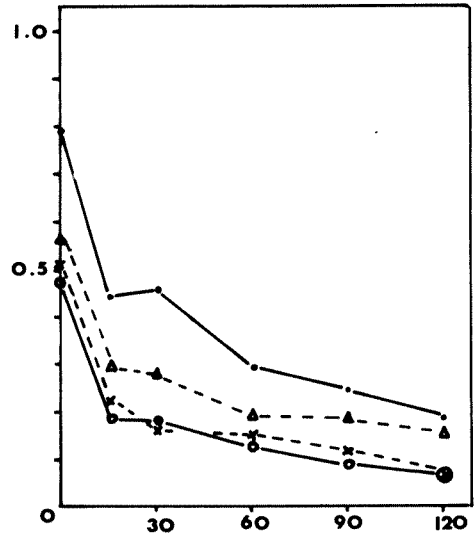

Fig. 6
Kinetics of UV-induced DNA breaks accumulation in the presence of aphidicolin in HeLa cells infected by HSV-1
The data from Fig. 5 are plotted in terms of *F* value (absolute fraction of duplex DNA) at different times post-irradiation.
Abscissa: time post-irradiation (min). Ordinate: *F*.
Initial incision rate (0 to 15 min):
-0.018 F/min (mock-infected)
-0.016 F/min (2 hr p. i.)
-0.020 F/min (3 hr p. i.)
-0.023 F/min (5 hr p. i.)
Long-term incision rate (0 to 60 min):
-0.0087 F/min (mock-infected)
-0.0063 F/min (2 hr p. i.)
-0.0062 F/min (3 hr p. i.)
-0.0068 F/min (5 hr p. i.)

a slower initial rate of break accumulation when compared with the mock-infected samples (data not shown).

All these data suggest that HSV-1 infection slows down the incision steps of the normal DNA excision-repair involved in repairing lesions induced by UV-light. Also, it seems that the viral infection impairs the appropriate sealing (or at least it slows down such a process) of the lesions induced by UV-irradiation on the host cell DNA. Such conclusion is supported by DNA repair experiments carried out in the presence of aphidicolin which is a competitive inhibitor of DNA polymerase alpha and as such an inhibitor of DNA repair. However, in contrast with ara-C it is not susceptible to be incorporated into the DNA at the site of DNA synthesis (Figs 5 and 6).

Discussion

DNA excision-repair involves the transient existence of a break in the strand being repaired. The interval between the creation of the break by the repair endonuclease and its removal by the ligase must be very short, since appreciable numbers of breaks do not appear after moderate UV doses under normal circumstances. Excision repair can be reliably compared in cells of different type or growth state, through the use of inhibitors, in order to accumulate strand-breaks at repair sites. It seems that comparisons are best made between initial rates of break accumulation over a short range of damage doses, and that the rate of break accumulation over a short period after damage is a convenient approximation to the initial rate (Johnson *et al.,* 1984). Given the initial rates of incision, one can apply the conventional techniques of enzymatic analysis on certain assumptions which in case of UV-induced damage are: (a) that the substrates for the endonuclease are pyrimidine dimers; (b) that such substrates are all equally accesible (or that non-accesible sites are uniformly distributed in the DNA); (c) that there is only one species of endonuclease which follows hyperbolic Michelis-Menten dose-dependence, and (d) that the observed breaks represent all incision events. But in fact, all these assumptions are questionable (Collins *et al.,* 1984; Downes, 1984). That is why it would be misleading to plot our data as a simple relationship of ln F versus time, instead of the two parts: incision rates and gap-filling rates actually shown in the graphs.

The initial rate of break formation cannot be determined directly; a close estimation is obtained by measuring break accumulation over a short time (10 to 30 min), after irradiation and assuming that break formation has occurred at a constant rate over that period (Downes, 1984). Since substrate depletion and enzyme inhibition would occur to some extent over such a short time, these direct initial rate approximations must be underestimates. The shorter the time period the less underestimate; but at very short times after irradiation, only few breaks are formed for accurate measurement. The time course of break accumulation over long periods is too complex to be completely understood at present.

Highly accurate, reproducible and easy-to-handle techniques for the assay of DNA damage, like the fluorescent assay described in the present and previous studies (Kanter and Schwartz, 1982; Aranda-Anzaldo, 1992*a*), could speed up our understanding of the kinetics of typical DNA excision-repair. However, to undertake an extensive analysis of such a process from the kinetic point of view is beyond the scope of the present study. Nevertheless, the experiments performed with HSV-1-infected cells subjected to UV-irradiation show that the inital rate of incision seems to be slower in infected cells when compared with non-infected cells. This suggests that the viral infection is not inducing or stimulating an endonuclease activity associated with the normal process of excision-repair. These experiments also point towards a partial and temporal inhibition of the gap-filling steps of DNA excision-repair, as a potential origin for the extra number of single-stranded breaks observed in the DNA of non-irradiated HSV-1-infected cells (Aranda-Anzaldo, 1992*a*). However, the results in the present study do not rule out the possibility that the virus might induce or stimulate a selective endonuclease (either of cellular or viral origin), which is not normally involved in the incision-excision of lesions induced by UV-irradiation. Altogether, the results in the present study suggest that infection by HSV-1 reduces the efficiency of the host cell mechanism involved in normal DNA excision-repair.

Previous studies have suggested that infection by human cytomegalovirus (HCMV) enhances the DNA repair capacity in human cells (Nishiyama and Rapp, 1981). Thus, it is of interest that HCMV is a slow and poorly cytolytic herpesvirus that in contrast with HSV-1 can stimulate and induce DNA replication, transcription and protein synthesis in permissive and non-permissive cells (Tanaka *et al.*, 1971; Stinski, 1977). HCMV seems to enhance DNA repair, while HSV-1 seems to impair the same process. This situation is striking if we consider the fact that most types of repairable damage in DNA are both mutagenic and carcinogenic (Friedberg, 1985), and that HCMV has been associated with various types of cancer while the evidence of an oncogenic role for HSV-1 is scant (Rapp, 1984). Thus, the apparent opposite effects of both HSV-1 and HCMV on host cell DNA repair might be explained by the great differences in growth cycle and pathogenic potential shown by both viruses.

We have carried out studies on the effect of HSV-1-induced early DNA damage upon the higher-order structure of host cell chromatin, which suggest that a temporal reduced efficiency of the DNA excision-repair process is consistent with the modifications observed in host cell chromatin (Aranda-Anzaldo, 1992*b*).

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