BRIEF COMMUNICATION

A COMPARISON OF HERPES SIMPLEX VIRUS PLAQUE DEVELOPMENT AFTER VIRAL TREATMENT WITH ANTI-DNA OR ANTILIPID AGENTS

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ABSTRACT The plaque development of Herpes simplex virus type 1 (HSV) is slower for viruses treated with two anti-DNA agents: ultraviolet radiation (UV) or *n*-acetoxy-2-acetyl-aminofluorene. For HSV treated with three antimembrane agents—butylated hydroxy-toluene, acridine plus near UV radiation, or ether—the plaque development time is the same as for untreated viruses. These differences hold even for viruses that survived treatment that lowered viability below the 1% level. Gamma ray inactivation of HSV produces no change in plaque development even though this agent is believed to preferentially affect viral DNA.

In 1971 Ross et al. reported a "small plaque effect" (SPE) when ultraviolet (UV) irradiated Herpes simplex virus (HSV) was assayed on mammalian cell cultures. This response was dose dependent, virus dependent, and was characterized by the appearance of plaques produced by UV irradiated HSV that were smaller than controls (unirradiated HSV). Further studies showed that this effect was wavelength dependent (Cameron, 1973) and could be attributed to a delay time required for the initiation of virus plaques (Ross et al., 1972). After this delay the surviving virus produced plaques at the same rate as unirradiated virus (Ross et al., 1972). The SPE appeared to be unaffected by agents which inhibit host-cell reactivation but was reversed by photoreactivation (Ross et al., 1972). It follows that the lesions causing the delay are most likely in the viral nucleic acid. Cameron (1975) has reviewed these findings. Our interest in this phenomenon is to make use of the SPE to determine the potential risks of using various antiviral agents. It is reasoned that only those agents that attack viral nucleic acid (and thus cause damage that may delay viral synthesis) should give a SPE. Virucidal agents that act on other viral components (e.g., membranes) should not give rise to such a delay. Nucleic acid damage (and its possible repair) can lead to mutagenesis (Radman, 1975) and mutagenesis to carcinogenesis (Trosko and Chang, 1977).

Reported below are the results of treatment with various antiviral agents on HSV plaque development in mammalian cells. We tested three anti-DNA agents (UV, γ rays, *n*-acetoxy-2-acetylaminofluorene [AAAF] [Roberts, 1978]) and three agents that act on viral

membranes (butylated hydroxytoluene [BHT] [Snipes et al., 1975], acridine plus near UV [Snipes et al. 1979], ether [Andrewes and Horstmann, 1949]). The antimembrane agents tested have been shown by these authors to inactivate enveloped viruses at concentrations and under conditions where lipid free viruses are not affected. No definite grouping of risk potential is apparent for the list of agents used. However, the results suggest that the SPE may measure the degree of reparability of damaged virus.

Details of the procedures for growing mammalian cells and viruses, for virus assay by plaque development, and for viral exposure to UV radiation are as previously described (Coohill et al., 1978). Freshly confluent monolayers of African green monkey kidney cells (line CV-1) in 60-mm plastic petri dishes (Lux, Newbury Park, Calif.) were used for viral assay. HSV-1 macroplaque (MP) strain was the virus used in all of these studies (Hoggan and Roizman, 1959). For UV studies an 8-W General Electric G8T5 germicidal lamp (General Electric Co., Wilmington, Mass.) was placed 10 cm from a suspension of viruses in phosphate buffered saline (PBS). The principal output of this source is at 254 nm. Exposure rates were determined using a UV-sensitive photovoltaic cell (Jagger meter [Jagger, 1967]). For treatment with AAAF viruses were placed in 0.9 ml PBS and AAAF in 10% dimethyl sulfoxide was added in a 0.1 ml aliquot. For γ ray inactivation, viruses were placed in Dulbecco's MEM (Flow Laboratories, Rockville, Md.) and irradiated in a Co^{60} source at a

TABLE I
HERPES SIMPLEX VIRUS PLAQUE DIAMETER AFTER ANTIVIRAL TREATMENT

Antiviral agent	Treatment	Inactivation level as percent survival	Ratio of treated virus plaque size to untreated controls
Anti-DNA agents			
UV (*254 nm*)	100 J m^{-2}	4.0	0.50
	350 J m^{-2}	1.0	0.48
	450 J m^{-2}	0.4	0.45
	800 J m^{-2}	0.1	0.46
	1200 J m^{-2}	0.02	0.44
AAAF	80 μg/ml	4.0	0.87
	100 μg/ml	1.0	0.77
	125 μg/ml	0.2	0.73
	150 μg/ml	0.02	0.79
γ rays (Co^{60})	100 krad	3.0	0.91
	150 krad	1.0	0.96
	200 krad	0.05	1.02
Antimembrane agents			
Acridine, near UV exposure* (*25 μg/ml*)	1.0 min	4.0	0.96
	1.5 min	1.0	1.01
	2.0 min	0.1	0.99
BHT 15-min exposure	1.0 mm	1.0	1.06
	1.5 mm	0.4	0.98
Ether	6%	1.0	0.92
	7%	0.05	1.08
	9%	0.01	0.95

*See text and Snipes et al. (1979).

dose rate of 15 krad/min. BHT inactivation was as described by Snipes et al. (1975). Acridine plus near UV light inactivation was as by Snipes et al. (1979). Viral inactivation by ether was accomplished by adding the appropriate percentage of ether into a viral suspension in PBS. The exposures for each treatment are listed in Table I.

After viral exposure and appropriate dilution, the viruses were inoculated onto CV-1 cell monolayers in growth medium (DMEM plus 10% fetal bovine serum [Grand Island Biological Co., Grand Island, N.Y.]). After an adsorption period of 90 min at 37°C in a 5% CO_2-air atmosphere, the viral inoculum was removed and replaced with 3 ml of growth medium containing 0.25% immune serum globulin to prevent extracellular viral transfer. Plaques were allowed to develop for 48 h after viral transfer. Plaques were allowed to develop

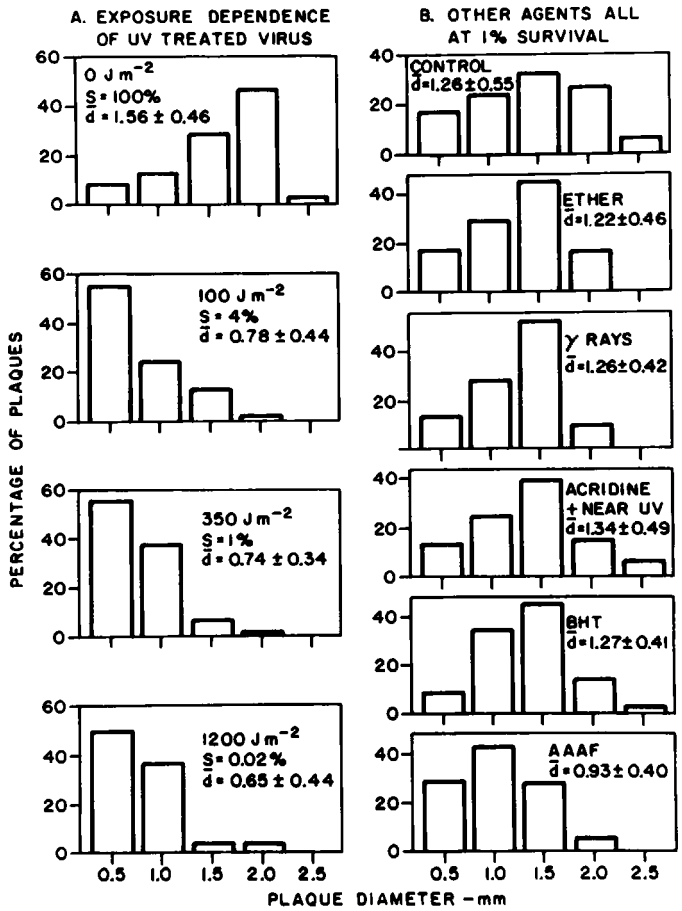

FIGURE 1 Histograms of HSV-MP plaque sizes on CV-1 cells. (*a*) Virus treated with varying amounts of 254-nm UV radiation. S, per cent survival measured by pfa. d, the average plaque diameter in millimeters including a measure of the standard deviation. (*b*) Virus treated with other virucidal agents: control-untreated virus; 6% ether for 15 min; 150 krad of Co^{60} γ rays; 25 μg/ml of acridine plus 1.5 min of near UV radiation (360 nm); 1 mM BHT for 15 min; 100 μg/ml of AAAF for 15 min. All virus was inactivated to the 1% survival level. In both *a* and *b* the ordinate is the percentage of total plaques of a given diameter. The abscissa is the average group diameter (i.e., 1.0 refers to plaques ranging in diameter from 0.75 to 1.24 mm). ~120 plaques were counted for each histogram.

for 48 h after viral inoculation, then the growth medium was removed and the cells stained with 1% crystal violet. Plaque sizes were determined as previously reported (Coohill, et al., 1979) by projecting an enlarged image of the monolayer onto a sheet of paper. Typical plaque size distributions are noted in Fig. 1 and are presented as the unmagnified plaque diameter.

Fig. 1 shows that UV radiation causes HSV-MP to give rise to small plaques as was reported for HSV-1 and HSV-2 by Ross et al. (1971). A dose response for this effect is shown. Fig. 1 also shows that ether, γ rays, BHT, and acridine plus light do not give a SPE at the 1% survival level whereas AAAF does. Table I lists those agents that have been tested for the SPE and shows the range of data collected. In each case virus was isolated from small plaques and reassayed on unirradiated cells. These cloned viruses produced plaques of normal size, that is, small plaques are not due to mutations (Cameron, 1975). Plaque size had no apparent effect on plaque morphology when observed at a magnification of $\times 40$.

The results illustrated in Fig. 1 show that all three antimembrane agents (BHT, ether and acridine plus light) do not give rise to the SPE. It is presumed that inactivation of the viral lipid envelope by these compounds gives rise to two classes of viral particles: those that are inactivated and those that survive. These results show that the surviving virions are equivalent to untreated virus in plaque development timing. UV and AAAF, two anti-DNA agents, give rise to the SPE. It is assumed here that a percentage of the surviving virions were delayed in plaque development presumably due to repair. This delay can cause the observed SPE (Ross et al., 1972). Note that the amount of SPE was less for AAAF treated viruses. The results involving γ-ray inactivation are not easily interpreted. Gamma rays apparently can cause nucleic acid damage to HSV (Powell, 1959). However, Cameron (1975) has pointed out that there is very little information on the molecular changes induced by γ-irradiation of extracellular virus. He also reported that HSV inactivated with electron bombardment did not give a SPE (Cameron, 1975). It would appear from our data that after γ-ray treatment the surviving virions are not hindered in beginning plaque development at the same time as untreated virus. This is true even for viruses inactivated to the 0.05% survival level (see Table I). Whether these data argue that γ-ray damage to HSV is unrepairable by the cell or is repaired immediately to unknown.

These results suggest that the SPE is a rapid (2 d) and reliable (consistent data for each agent) assay for a virus's response to inactivating agents. Whether the SPE measures the degree of repairability and hence mutagenicity of damaged viruses awaits further experimentation involving a mutational assay for HSV.

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