

The Effect of Δ -9-Tetrahydrocannabinol on Herpes Simplex Virus Replication

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SUMMARY

Both herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) failed, in an identical fashion to replicate and produce extensive c.p.e. in human cell monolayer cultures which were exposed (8 h before infection, at infection, or 8 h p.i.) to various concentrations of Δ -9-tetrahydrocannabinol. Similar results were obtained with a plaque assay utilizing confluent monkey cells. Possible mechanisms for this antiviral activity are discussed.

Herpes simplex virus is a frequent infectious agent in man with degrees of severity ranging from mild cutaneous lesions to severe, even life-threatening infections (Rawls *et al.* 1969; Aurelian *et al.* 1970; Melnick & Rawls, 1970; Nahmias *et al.* 1970; Adam *et al.* 1972; Aurelian, 1972; Sprecher-Goldberger *et al.* 1973). The rising incidence of herpes genitalis (Kalinyak *et al.* 1977) and the epidemiological association of HSV-2 infections with the development of cervical cancer in humans further emphasizes the need for the development of more effective chemotherapeutic agents to treat these infections.

The genetic material of HSV consists of double-stranded DNA which is present in the interior of the virion. Previous work by Blevins & Regan (1976) has shown that Δ -9-tetrahydrocannabinol (Δ -9-THC), the principal psychoactive compound extractable from marijuana, depressed DNA, RNA and protein synthesis in human skin fibroblasts and in both human and mouse neuroblastoma cells in culture. Similar observations have been reported in HeLa cells (Blevins & Sholes, 1978). Harris *et al.* (1974) reported that mice, given oral doses of this drug, showed a 75% reduction in Lewis lung carcinoma DNA synthesis. These observations stimulated this research in which the effects of various concentrations of Δ -9-THC on the replication of HSV-1 and HSV-2 were observed in human and monkey tissue culture cells.

The primary human skin fibroblast cells (HSBP), SV-40 virus transformed human embryonic lung (WI-38, VA13) cells (American Type Culture Collection - ATCC - No. CCL-75.1; Rockville, Md.) and the African monkey kidney Vero cells (ATCC No. CCL-81; Rockville, Md.) used throughout this study, were grown to confluence in 75 cm² flasks (Falcon Plastics, Oxnard, Calif.) using standard tissue culture procedures (Eagle, 1959). Cells were grown in either Leibovitz L-15 medium or Eagle's minimum essential medium (MEM; Grand Island Biological Company; Grand Island, N.Y.) supplemented with 10% foetal bovine serum. Herpes simplex virus type 1 and HSV-2 (kindly supplied by Dr Thomas F. Smith, Department of Laboratory Medicine, Mayo Clinic; Rochester, Minn.) were passaged four times on both confluent HSBP cells and confluent WI-38 cells, resulting in specific HSV-1 and HSV-2 virus stock for each cell type. Virus passages were carried out at 37 °C with 15 ml of serum-free (Leibovitz) medium in each culture flask. The resultant virus suspensions in this serum-free medium were harvested when virus-specific c.p.e. was complete (100%), after 3 to 4 days of incubation. Centrifugation at 900 *g* for 15 min removed the cellular debris, and virus stocks containing 8×10^8 p.f.u./ml of either HSV-1 or HSV-2 were stored at -70 °C in sealed ampoules. These HSV-1 and HSV-2 stocks were quantified

by plaque-titrations of infectious virus stock of HSV in Vero cells grown to near confluency in 5 cm plastic dishes containing MEM as previously described (Courtney *et al.* 1970).

Before their use in any of the procedures, the contents of each virus stock were thawed and diluted with serum-free Leibovitz medium (pH 7.6) to a concentration of 1×10^8 p.f.u./ml for HSV-1 and 3×10^7 p.f.u./ml for HSV-2. Confluent cell cultures (HSBP and WI-38) with 2×10^6 cells/flask containing 15 ml of serum-free Leibovitz medium were inoculated in triplicate with an infectivity of 5 to 10 p.f.u./cell of HSV-1 or HSV-2 suspension plus 10 μ l of stock Δ -9-THC solution (lot SSC 69057, Department of Health, Education and Welfare) containing 2 mg of Δ -9-THC/ml of absolute ethanol. Thus, each flask contained 1.3 μ g of Δ -9-THC/ml of serum-free Leibovitz medium. HSBP and WI-38 cell viability under these conditions was determined to be > 90% by the dye exclusion test as described previously (Blevins & Regan, 1976; Blevins & Sholes, 1978). All cultures were microscopically examined for 6 consecutive days for the appearance of virus-specific c.p.e. or any other changes.

Direct exposure of HSV types 1 and 2 to Δ -9-THC was evaluated at 0, 5, 10, 15, 20, 40, 100 and 200 μ g/ml concentrations of serum-free Leibovitz medium containing HSV. The control virus suspensions received the same amounts of absolute ethanol minus the Δ -9-THC. All virus suspensions were incubated in wide-mouth tubes at 22 °C for 1 h with vortex mixing at 10 min intervals. After this incubation, samples containing an infectivity of 5 to 10 p.f.u./cell were inoculated in triplicate from the tubes into flasks (containing 2×10^6 cells) of confluent cells (HSBP and WI-38). Before inoculation, the growth medium in each culture flask of HSBP and WI-38 cells was replaced with 20 ml of the serum-free Leibovitz medium (pH 7.6). The cell cultures were microscopically examined for 21 consecutive days for any signs of residual infectivity marked by the appearance of c.p.e. Every 7 days, 15 ml of the 20 ml serum-free medium containing no additional Δ -9-THC was changed in order to maintain these cells.

Human skin (HSBP) and human embryonic lung (WI-38) cell cultures (2×10^6 cells/ml) were pre-treated for 8 h with Δ -9-THC at 0, 5, 10, 15, 20, 40, 100 and 200 μ g/ml of serum-free MEM. The cell cultures were then infected with 3 to 5 p.f.u./cell of either HSV-1 or HSV-2 in 2 ml MEM. After 1 h absorption at 37 °C in serum-free MEM, the cells were washed three times with colourless Hanks' solution and then incubated for 24 h in fresh serum-free MEM containing no additional virus. The Δ -9-THC was again added to the MEM at 0, 5, 10, 15, 20, 40, 100 and 200 μ g/ml MEM. At 24 h p.i. the cells were scraped into the medium with a rubber policeman and disrupted by freezing (−70 °C), thawing and sonication. The presence of infectious virus was assayed as previously described (Courtney *et al.* 1970, 1973) utilizing Vero cells grown to near confluency in 5 cm plastic dishes containing MEM with 10% calf serum.

Simultaneous exposure of HSBP or WI-38 cells to Δ -9-THC (1.3 μ g/ml medium) and virus (HSV-1 or HSV-2 at 5 to 10 p.f.u./cell) resulted in at least a 75% retardation of observable c.p.e. compared to controls for the 6 consecutive day period. Any viral c.p.e. of the experimental culture was minimal and appeared at least 24 h later than that of the controls. These same results occurred when the Δ -9-THC was added either 8 h before or 8 h after virus inoculation. The Δ -9-THC in the absence of virus had no observable effect at this concentration on either of the cell lines used. The inclusion of 10% foetal bovine serum in the medium, however, blocked the inhibition of virus c.p.e. by the Δ -9-THC and prevented c.p.e. caused by higher concentrations of Δ -9-THC. The observation that Δ -9-THC has a high affinity for serum lipoproteins (Wahlqvist *et al.* 1971) probably accounts for this effect.

The effects of pre-treatment of HSV-1 and HSV-2 with Δ -9-THC are summarized in Table 1. Antiviral effect with human skin fibroblasts was evident (Fig. 1a) at concentrations of 100 and 200 μ g of Δ -9-THC/ml of media containing HSV (5 to 10 p.f.u./cell). In the

Table 1. *Virus c.p.e. and p.f.u. production in primary human epithelium (HSBP) cells after inoculation with herpes simplex virus (HSV) types 1 and 2*

Concentration of Δ -9-THC in μ g/ml of HSV-containing medium* or in pre-treatment medium†	Number of days post inoculation‡					p.f.u./ml†
	2	4	6	12	21	
200	—	—	—	—	—	< 100
100	—	—	—	—	—	< 100
40	—	+§	+	+	+	< 100
20	—	+§	+	+	+	< 100
15	—	+	+	+	+	1.3×10^3
10	—	+	+	+	+	9.0×10^3
5	—	+	+	+	+	1.3×10^4
0	—	+	+	+	+	1×10^8 (HSV-1); 5×10^7 (HSV-2)
Control	—	+	+	+	+	1×10^8 (HSV-1); 5×10^7 (HSV-2)

* Δ -9-THC pre-treated HSV types 1 and 2 containing an infectivity of 5 to 10 p.f.u./cell tested in both HSBP and WI-38 cells, with the data being identical for both cell lines; each datum represents the consistent results of a minimum of 12 determinations.

† HSBP cells were pre-treated with Δ -9-THC and then infected with either HSV type 1 or 2 containing an infectivity of 3 to 5 p.f.u./cell and assayed by the standard plaque method utilizing Vero cells.

‡ Symbols: —, no virus p.f.u. visible; +, c.p.e. with 75 to 100 % of cells affected or sloughed.

§ These flasks had reduced c.p.e. (less than 25 % of cells affected).

|| Absolute ethanol, the solvent for Δ -9-THC, controls at the concentrations utilized in collecting the experimental data.

absence of Δ -9-THC (Fig. 1*b*) the virus caused marked c.p.e. to parallel cultures. Ethanol alone, the solvent used for Δ -9-THC, had no antiviral effect at the concentrations utilized in these experiments (Table 1 and Fig. 1*b*). The yields of both HSV-1 and HSV-2, as judged by plaque assay, were severely depressed by the presence of 5 μ g/ml Δ -9-THC during growth. At concentrations of 20 μ g/ml and above no virus growth could be detected (Table 1).

Delta-9-THC administered to cell cultures 8 h before, simultaneously with, or 8 h after infection with either HSV-1 or HSV-2 resulted in inhibition of c.p.e. of the virus. A standard plaque forming assay confirmed these results, indicating that Δ -9-THC can decrease HSV replication and/or infectivity in human cells. This effect could be mediated with the cells or by a direct effect on the virus. It has been shown (Blevins & Regan, 1976; Blevins & Sholes, 1978) that Δ -9-THC reduces DNA synthesis in human and other mammalian cells; since HSV is a DNA-containing virus, the drug could be blocking the replication of virus DNA. This notion is supported by the observation that Δ -9-THC is effective in preventing c.p.e. in cell cultures when added 8 h after virus inoculation and that maximal DNA synthesis in the host cell occurs 8 h post inoculation by HSV-2 in human cells (Cheng *et al.* 1975).

A direct effect of Δ -9-THC on the virus cannot be ruled out at this time. Gill (1976) has reported that lipophilic cannabinoids (Δ -9-THC) and other compounds with central nervous system activity cause molecular disorder of artificial membranes (liposomes). The comparison of a host-cell plasma membrane to the envelope surrounding the HSV capsid reportedly reveals a similar phospholipid composition and physical configuration (Epstein, 1962; Asher *et al.* 1969). In addition, agents which damage or remove this envelope are known and can greatly reduce the infectivity of the virion (Gentry & Randall, 1973). Thus, the direct antiviral activity of Δ -9-THC that we report could involve structural changes in the lipid matrix of the HSV envelope. This possibility is being investigated presently using isotopically labelled cannabinoids.

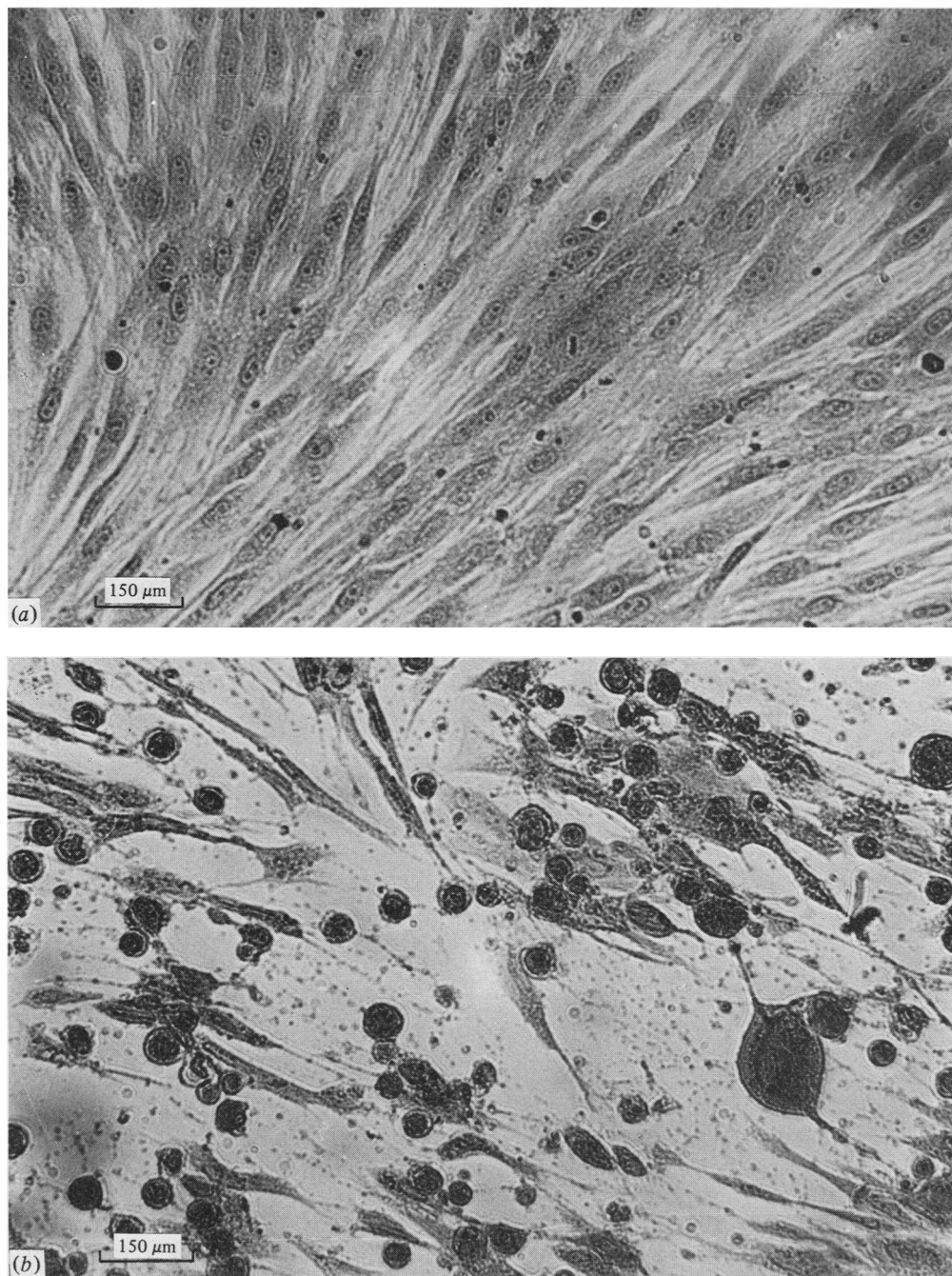


Fig. 1. (a) A 96-h culture of HSBP cells inoculated with 100 µg or 200 µg of Δ -9-THC/ml of pre-treated medium containing HSV-1 (at an infectivity of 5 to 10 p.f.u./cell); these cells are identical to the non-treated Δ -9-THC, non-HSV-1 control. (b) Extensive c.p.e. in a 96-h culture of HSBP cells inoculated only with absolute ethanol (the solvent of Δ -9-THC) pre-treated medium containing HSV-1 (at an infectivity of 5 to 10 p.f.u./cell). Both (a) and (b) are stained by eosin.

The potential of Δ -9-THC as a therapeutic antiviral agent is probably limited to topical application due to the affinity of the drug for adsorption to serum lipoproteins. The need for such an agent, however, should stimulate research to determine the effectiveness of the drug for pharmaceutical use.

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