DELTA-9-TETRAHYDROCANNABINOL AND HERPES SIMPLEX VIRUS REPLICATION

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I. INTRODUCTION

Herpes simplex virus (HSV) is a frequent infectious agent in man with degrees of severity ranging from mild cutaneous lesions to severe, even life-threatening infections (1,3,4,16-18,20). The rising incidence of herpes genitalis (15) and the epidemiological association of HSV-2 infections with the development of cervical cancer in humans further underscores 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 and Regan (6) has shown that delta-9-tetrahydrocannabinol (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 (5). Harris et al. (14) 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 THC on the replication of HSV-1 and HSV-2 were observed in human and monkey tissue culture cells.

II. METHODS

A. Celland Virus Culture

The primary human skin fibroblast cells-HSBP-(18); 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 cm^2 flasks (Falcon Plastics, Oxnard, CA.) using standard tissue culture procedures (10). Cells were grown in either Leibovitz L-15 medium or Eagle's minimum essential medium (MEM) (Grand Island Biological Company; Grand Island, NY.) supplemented with 10% fetal bovine serum. Herpes simplex virus type 1 and HSV-2 (kindly supplied by Dr. Thomas F. Smith, Department of Laboratory Medicine, Mayo Clinic; Rochester, MN.) were passaged four times on both confluent HSBP cells and confluent WI-38 cells, resulting inspecific 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 cytopathic effect was complete (100%), after 3 to 4 days of incubation. Centrifugation (900 X g for 15 minutes) removed the cellular debris, and viral stocks containing 8×10^8 PFU/ml of either HSV-1 or HSV-2 were stored at -70°C in sealed ampules. These stocks were quantitated by conducting plaque-titrations of infectious virus stock of HSV in Verocells grown to near confluency in 5 cm plastic dishes containing MEM as previously described (8).

B. Treatment of Cells and Virus with THC

Before use in any of the procedures described in the remainder of this report, the contents of each viral stock (ampule) were thawed and diluted with serum-free Leibovitz medium (pH 7.6) to a concentration of 1×10^8 PFU/ml for HSV-1 and 3×10^7 PFU/ml for HSV-2. Confluent cell cultures(HSBP and WI-38) with 2×10^6 cell/flask containing 15 ml of serum-free Leibovitz medium were inoculated in triplicate withan infectivity of 5-10 PFU/cell of HSV-1 or HSV-2 suspension plus 10 mcl of stock THC solution (lot SSC 69057, National Institute on Drug Abuse) containing 2 mg of THC/ml of absolute ethanol. Thus, each flask contained 1.3 mg of 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 (5,6). All cultures were

TABLE I. Virus Cytopathic Effect In Primary Human Epithelium (HSBP) Cells After Inoculation With THC Pretreated Herpes Simplex Virus (HSV) Types 1 and 2[a].

Concentration of THC in mcg/ml of HSV-containing medium	Number of days post inoculation				
	2	4	6	12	21
200	-	-	-	-	-
100	-	-	-	-	-
40	-	+[b]	+	+	+
20	-	+[b]	+	+	+
0	-	+	+	+	+
Control[c]	-	+	+	+	+

[a]THC pretreated HSV types 1 and 2 containing an infectivity of 5-10 PFU/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.

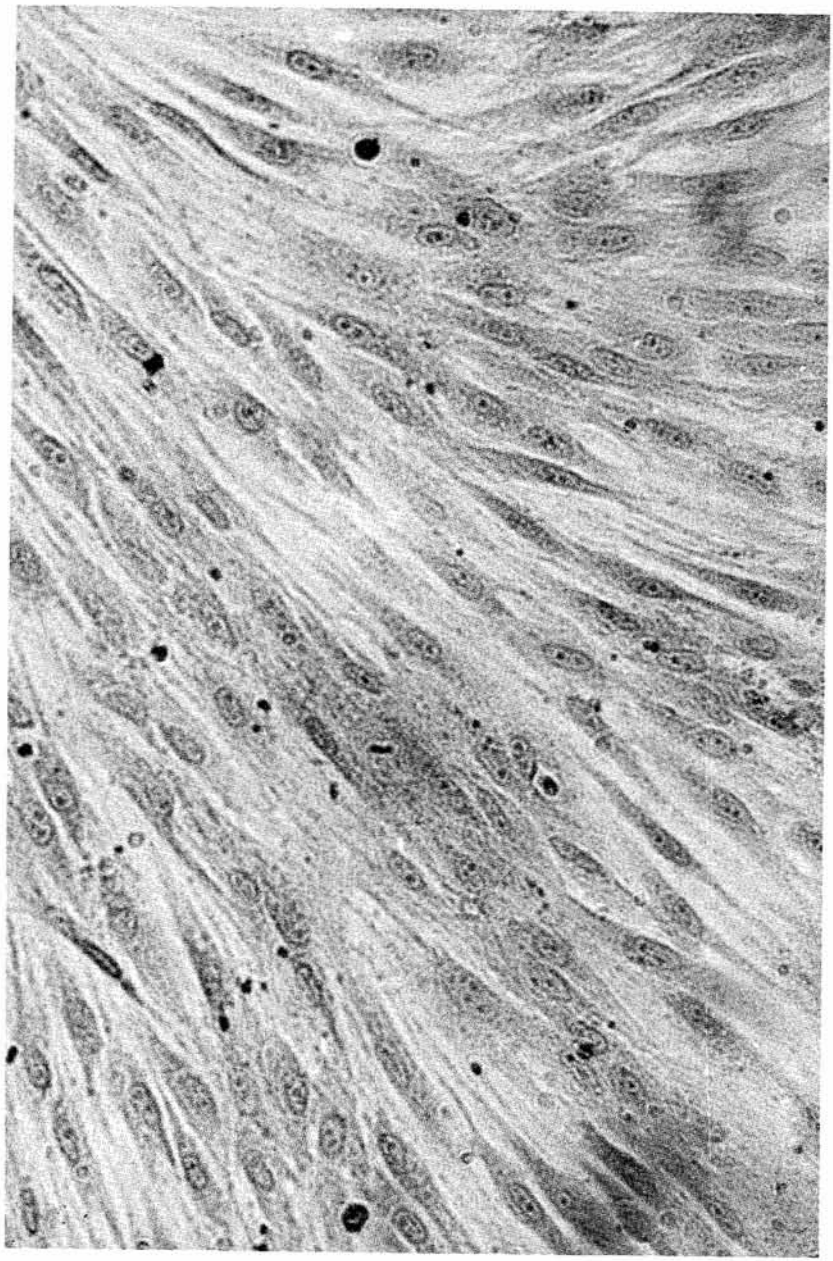
[b]These flasks had reduced cytopathic effect (less than 25% of cells affected).

[c]Absolute ethanol, the solvent for THC, controls at the concentrations utilized in collecting the experimental data.

Symbols: - = no virus cytopathic effect visible;
+ = cytopathic effect with 75 to 100% of cells affected or sloughed.

microscopically examined for 6 consecutive days for the appearance of virus-specific cytopathic effect or any other changes.

Direct exposure of HSV types 1 and 2 to THC was evaluated at 0, 20, 40, 100, and 200 mcg/ml concentrations of serum-free Leibovitz medium containing HSV. The control virus suspensions received the same amounts of absolute ethanol minus the THC. All virus suspensions were incubated in wide-mouth tubes at 22°C for one hour with vortex mixing at 10 minute intervals. After this incubation, aliquots containing an infectivity of 5-10 PFU/cell were carefully inoculated in triplicate from the tubes into flasks (containing 2 X 10^6 cells) of confluent cells (HSBP and WI-38). Before inoculation, the growth medium in each culture flask of HSBPand 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 cytopathic effect. Every 7 days 15 ml of the 20 ml serum-free medium containing no additional THC was changed in order to maintain these cells.

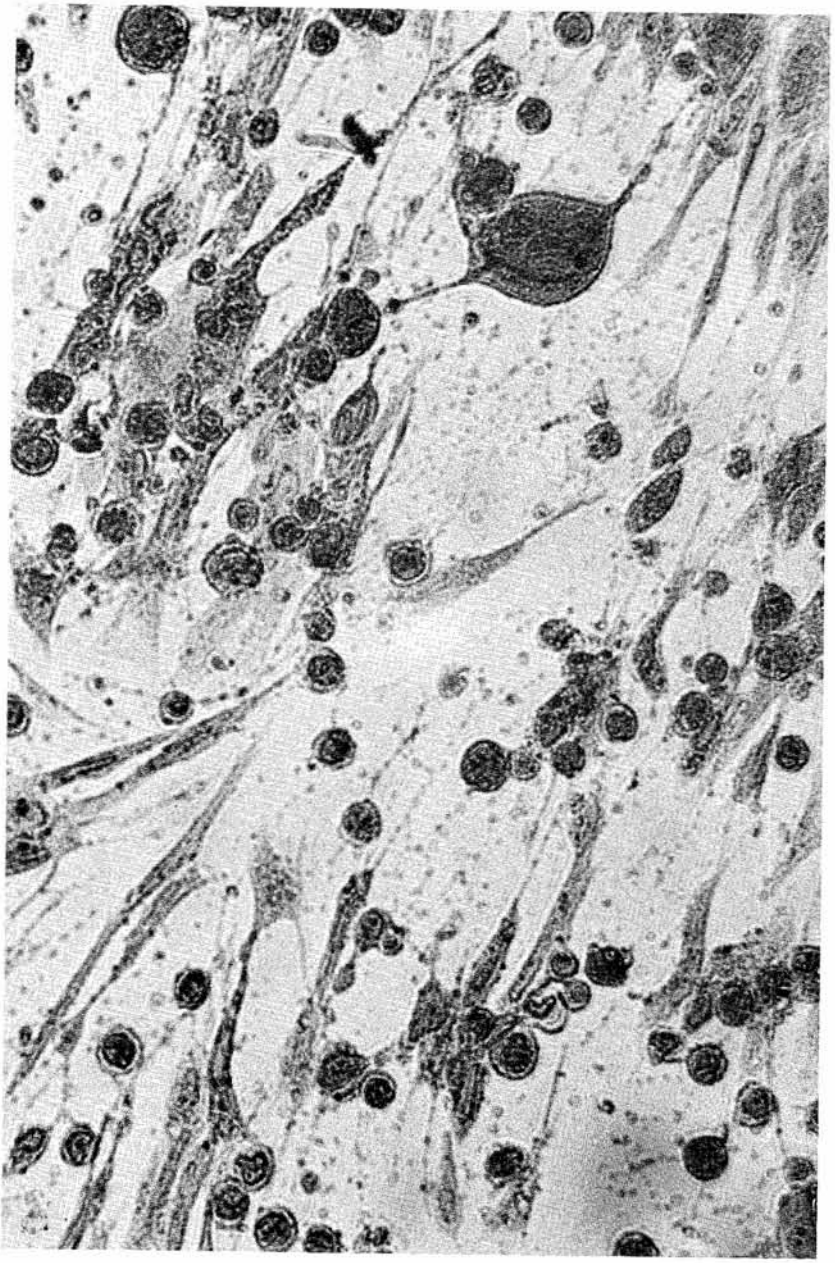
C. Virus Infection of Cells for Plaque Titration withTHC

Human skin (HSBP) and human embryonic lung (WI-38)cell cultures (2 X 10^6 cells/ml) were pretreated for 8 hrs with THC at 0, 20, 40, 100, and 200 mcg/ml of serum-free MEM. The cell cultures were then infected with 3-5 PFU/cell of either HSV-1 or HSV-2 in 2 ml MEM. After 1 hr absorption at 37°C in serum-free MEM, the cells were washed 3 times with colorless Hank's solution and then incubated for 24 hr in fresh serum-free MEM containing no additional virus. The THC was again added to the MEM at 0, 20, 40, 100, and 200 mcg/ml MEM. At 24 hr post-infection 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 (8,9) utilizing Vero cells grown to near confluency in 5 cm plastic dishes containing MEM with 10% calf serum.

III. RESULTS

Simultaneous exposure of HSBP or WI-38 cells to THC (1.3 mcg/ml medium) and virus (HSV-1 or HSV-2 at 5-10 PFU/cell)resulted in at least a 75% retardation of observable cytopathic effects compared to controls for the 6 consecutive day period. Any viral cytopathic effect of the experimental culture was minimal and appeared at least 24 hr later than that of the controls. These same results occurred when the THC was added either 8 hr before or 8 hr after virus inoculation. The THC in the absence of virus had no observable effect at this concentration on either of the cell lines used. The inclusion of 10% fetal bovine serum into the medium, however, blocked the inhibition of virus cytopathic effect by the THC as well as prevented cytopathic effects caused by higher concentrations of THC. The observation that THC has a high affinity for serum lipoproteins (21) probably accounts for this effect.

FIGURE 1. A 96 hr culture of HSBP cells inoculated with 100mcg or 200 mcg of THC/ml of pretreated HSV-1containing medium (an infectivity of 5-10 PFU/cell); these cells are identical to the non-treated THC, non-HSV-1 control. Eosin, 100 X.



The effects of pretreatment of HSV-1 and HSV-2 with THC are summarized in Table I. Antiviral effect with human skin fibroblasts was very evident (Figure 1) at concentrations of 100 and 200 mcg of THC/ml of media containing HSV (5-10 PFU/cell). In the absence of THC (Figure 2) the virus caused marked cytopathic effects to parallel cultures. Ethanol alone, the solvent used for THC, had no antiviral effect at the concentrations utilized in these experiments (Table I and Figure 2).

HSV-1 and HSV-2 were equally sensitive to THC at all concentrations used (200, 100, 40 and 20 mcg/ml of serum-free medium) showing no plague formation in the Vero cells. In the presence of 20 mcg THC/ml, no PFU were seen at a 1:1 dilution of HSV; whereas, control virus preparations untreated with THC had 20 PFU at a dilution of 2^6.

IV. DISCUSSION

THC administered to cell cultures 8 hr before, simultaneously with, or 8 hr following infection with either HSV-1 or HSV-2 resulted in inhibition of cytopathic effects of the virus. A standard plaque forming assay confirmed these results indicating that 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. We have shown (5,6) that THC reduces DNA synthesis in human and other mammalian cells; and since HSV is a DNA containing virus, the drug could be blocking the replication of viral DNA. This notion is supported by the observation that THC is effective in preventing cytopathic effects in cell cultures when added 8 hr after viral inoculation, and that maximal DNA synthesis in the host cell occurs 8 hr post inoculation by HSV-2 in human cells (7).

A direct effect of THC on the virus cannot be ruled out at this time. Recently Gill (13) reported that lipophilic cannabinoids (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 physicalconfiguration

FIGURE 2. Extensive cytopathic effect in a 96 hr culture of HSBP cells inoculated only with absolute ethanol (the solvent of THC) pretreated HSV-1-containing medium (an infectivity of 5-10 PFU/cell. Eosin, 100 X.

(2,11). In addition, agents which damage or remove this envelope are know, and they can greatly reduce the infectivity of the virion (12). Thus, the direct antiviral activity of THC that we report could involve structural changes in the lipid matrix of the HSV envelope. This possibility is being investigated presently using isotopically labeled cannabinoids.

The potential possibility of using 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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