Suppressive Effect of δ-9-Tetrahydrocannabinol on Herpes Simplex Virus Infectivity *In Vitro* (43206)

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Abstract. **δ-9-Tetrahydrocannabinol (THC) was found to reduce the infectivity of herpes simplex virus and was without effect against adenovirus type 2 or poliovirus. The effective THC concentration resulting in an 80% decrement in virus viability was dependent upon the presence or absence of serum in the incubation mixture, as a 5% serum concentration decreased the drug activity by approximately 50-fold. THC-mediated inactivation of herpes simplex virus was both time and dose dependent and did not result in virion disassembly or clumping. The THC-related effect was not influenced by the pH of the suspending medium, suggesting that the mechanism of inactivation differed from that associated with the thermal inactivation of the virus. Thus, the data suggest that THC preferentially reduces the infectivity of the enveloped herpes simplex virus, and that this activity is modulated by the presence of serum proteins.**
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Delta-9-tetrahydrocannabinol (THC) is the major psychoactive ingredient present in marijuana. This substance is a small molecular weight lipophilic compound that binds to serum lipoproteins (1) and is believed to exert much of its psychoactive effects by its ability to bind to and perturb cell membranes in the central nervous system (2).

The health-related effects resulting from exposure to THC may be manifest in two ways: (i) a direct effect of the drug on the physiologic activity or state of the cell or tissue and (ii) as a result of altered immune responses ultimately resulting in enhanced susceptibility to infectious agents or in the development of cancer. Reports linking THC or marijuana to enhanced susceptibility to intracellular pathogens (3), reactivation of latent herpes simplex virus (HSV) (4), altered natural killer and other immune cell function (5–7), and head and neck cancer in humans (8, 9) have appeared. There is, however, a relative paucity of information regarding the effects of THC on viruses and virus infections. Even so, the limited number of studies on the effects of THC on the replication and yield of virus *in vitro* do not agree. Blevins and Dumic (10) reported that an 8-hr pretreatment of human cells with THC in serum-free medium precludes the replication of HSV. In contrast, Cabral *et al.* (11) reported that a 24-hr pretreatment with THC did not affect the yield of HSV in Vero cells, although it enhanced extracellular release of the virus. A reason for this discrepancy was not provided, but may be related to the use of serum-free and serum-containing media during the THC treatment period.

Our initial inquiry into the biologic effects of THC on virus metabolism was to test for direct effects of THC on the infectivity of virion particles in response to data presented by Blevins and Dumic (10), who suggest that high concentrations of THC reduce virus infectivity. Our results indicate that THC does destroy the viability of HSV but not adenovirus or poliovirus. Kinetics of THC inactivation of HSV reveal that the effect is time and dose dependent but is not affected by pH. Finally, the loss of HSV viability is an effect on individual virus particles and is due neither to drug-mediated virion aggregation nor to the disassembly of the virus.

Materials and Methods

Viruses, Cell Lines, and Medium. Virus stocks were prepared by infection of permissive host cells. Following development of maximum cytopathogenic

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effect, the infected cell cultures were frozen and thawed, and the pelleted cells, resuspended in 1 ml of medium, were sonicated (20 kilocycles/sec, 15 sec, 4°C) to release the virus. The mixture of cell sonicate and culture medium was clarified by low-speed centrifugation, and the supernatant fluids, used as virus stocks, were stored at −85°C. Adenovirus type 2 was propagated and quantitated in A549 cells, poliovirus in HeLa cells, and HSV type 1 (strain F) in rabbit skin cells.

Rabbit skin, A549, and HeLa cells were cultured in Eagle's minimum essential medium in Earle's balanced salt solution, supplemented with 5% heat-inactivated (56°C, 30 min) newborn bovine serum (Sigma Chemical Co., St. Louis, MO), 20 m*M* Hepes buffer (pH 7.4), and antibiotics (penicillin, 50 units/ml; streptomycin, 50 μg/ml; and neomycin sulfate, 50 μg/ml (12).

Virus Quantitation. All viruses were quantitated as plaque-forming units (PFU). Serial 10-fold dilutions of the virus samples were prepared, and a 200-μl volume was distributed over the surface of well-drained cell cultures grown in 25-cm^2 flasks. After a 60- to 120-min adsorption period, with intermittent rotation, an overlay was added. Adenovirus was plated on A549 cells, poliovirus on HeLa cells, and HSV on rabbit skin cells. The overlays consisted of medium supplemented with 0.5% agarose except the overlay for HSV, which contained 0.5% methylcellulose. Cell cultures were stained with crystal violet when plaques were apparent on Days 4, 2, and 3 for adenovirus, poliovirus, and HSV, respectively.

Inactivation Studies. Virus stocks at $\geq 10^7$ PFU/ml were diluted into tissue culture medium or buffered water (10 m*M* Hepes, pH 6.95) and incubated for 2 hr at room temperature or for the times shown. Two sets of controls were used: virus suspended in tissue culture medium (or buffered water) and virus suspended in medium (or buffered water) that contained dimethyl sulfoxide (DMSO; diluent for the THC, see below). Plaque counts indicated no difference in these two controls (data not shown). In the THC-treated and DMSO control samples, the amount of DMSO present was held constant in all samples, and at a concentration equivalent to that present in samples receiving the highest concentration of THC. Thus, the maximum DMSO concentration was 0.5% by volume.

Transmission Electron Microscopy. Equal portions of an HSV preparation were treated with THC (100 μg/ml in 0.5% DMSO) or DMSO alone for 1.5 hr as indicated above, resulting in a >80% decrease in virion PFU relative to the control preparation. The virus samples were centrifuged at 58,500*g* for 60 min, 4°C. The pellets containing the virus were fixed first in 0.1 *M* cacodylate-buffered 2.5% glutaraldehyde (pH 7.4), followed by 1% osmium tetroxide and then embedded in Epon 812 and sectioned (800–900 nm). Sections were stained with uranylacetate and lead citrate and viewed with a Philips 301 electron microscope.

Table I. Effect of THC on Virus Viability

THC concentration (μg/ml)	% of control virus PFU[a]				
	Serum-containing medium			Serum-free medium	
	Polio	Adeno 2	HSV (F)	Polio	HSV (F)
1	117	87	89	81	20
10	101	100	85	88	0.4
50	89	108	24	101	0[b]
100	102	95	1	112	0[b]

[a] Virus suspensions were prepared in and THC was dissolved in tissue culture medium or Hepes-buffered water (pH 6.95). Control virus preparations received 0.5% DMSO, which is equivalent to that present in samples receiving 100 μg THC/ml. The virus samples were incubated for 2 hr at room temperature, diluted $\geq 10^{-2}$ to eliminate THC carryover, and the residual virus content (PFU) was determined. The plaque counts on control plates ranged from 100 to 250 for each of the viruses tested. The starting virus concentration was approximately 10^7 PFU/ml in each test sample.

[b] No virus PFU detected; virus titer reduced >2 $\log_{10}$.

Results and Discussion

Inactivation studies were performed at room temperature because the solubility of THC in water-based media decreases significantly at 4°C and to reduce the loss of virus activity that would result from thermal inactivation if higher temperatures (e.g., 37°C) were employed. When virus was suspended in tissue culture medium containing 5% serum and incubated with THC, both poliovirus and adenovirus type 2 were unaffected, whereas the HSV titer was reduced in a dose-dependent manner (Table I). Under these conditions, a THC concentration of 50 μg/ml decreased the viable HSV content by approximately 80%. Since THC has

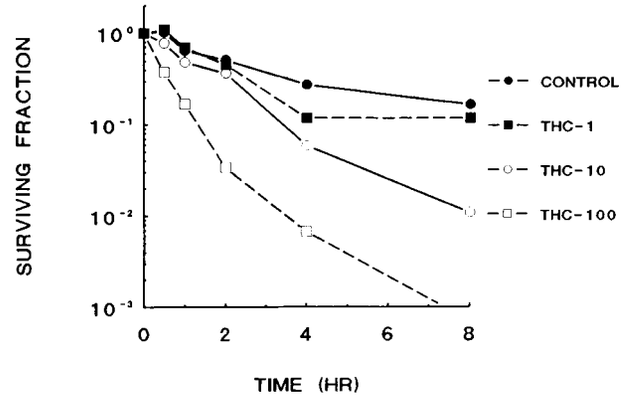

Figure 1. Kinetics of THC inactivation of HSV. HSV was suspended in and THC was diluted into tissue culture medium. The control received DMSO at a concentration equivalent to that present in the sample receiving 100 μg THC/ml. At the times indicated, each sample was assayed for residual viable virus (PFU). The numbers listed in the adjacent legend indicate the THC concentration in μg/ml.

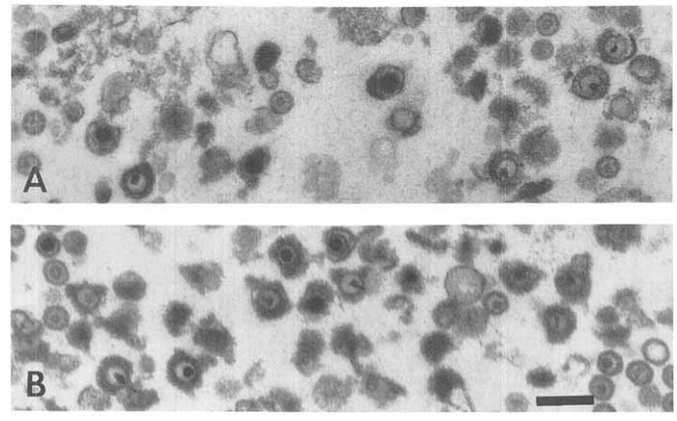

Figure 2. Transmission electron microscopy of THC-inactivated HSV. HSV was incubated with DMSO (control, A) or 100 μg THC/ml (B) resulting in an 80% decrease in viable virus PFU relative to the control preparation. The virus present in the samples was then pelleted and fixed. Sections (800–900 nm) were stained with uranyl acetate and lead citrate. Bar, 250 nm (original magnification ×55, 760).

Table II. Effect of Medium pH on THC Inactivation of HSV[a]

Suspending medium supplement	pH	PFU count	% control
Control (DMSO)	6.4	247	
THC (100 μg/ml)		50	20
Control	6.7	284	
THC		55	19
Control	7.0	250	
THC		69	20
Control	7.2	287	
THC		74	26
Control	7.5	180	
THC		36	20

[a] HSV was diluted into tissue culture medium adjusted to the pH indicated containing THC (100 μg/ml) or DMSO (diluent for THC). The samples were incubated at 22°C for 1 hr, diluted 10^{-4}, and quantitated by plaque counts in rabbit skin cell cultures.

been shown to bind to serum lipoproteins (1), we monitored the THC inactivation of poliovirus and HSV in serum-free medium. The suspending menstruum used was buffered water and not serum-free Eagle's minimum essential medium in Earle's balanced salt solution because previous studies showed that HSV is stabilized when the salt ion concentration is reduced from that present in physiologic saline (13, 14) and that HSV is exceedingly susceptible to inactivation when suspended in serum-free Eagle's minimum essential medium in Earle's balanced solution solution (15). The data indicated that a concentration of 1 μg THC/ml inactivated approximately 80% of the HSV PFU during the 2-hr incubation period and was without effect against poliovirus (Table I). Thus, a 50-fold increase in the drug concentration is required to achieve comparable levels of HSV inactivation if the incubation is carried out in medium containing 5% bovine serum.

The kinetics of THC inactivation at room temperature were examined. Since the incubation period was extended to 8 hr, THC inactivation of HSV was performed in serum-containing medium and using the higher drug concentrations. This was done to take advantage of the ability of serum proteins to stabilize HSV relative to thermal inactivation of the virus during the extended incubation period. The results indicated relatively linear inactivation rates through 4 hr for HSV exposed to 1 and 10 μg THC/ml, and a more rapid and linear rate of inactivation through 2 hr for the HSV incubated with 100 μg THC/ml (Fig. 1). The approximated half-lives for the HSV incubated with 1, 10, and 100 μg THC/ml are 90, 68, and 30 min, respectively, and 134 min for the control. Thus, THC mediates an inactivation of HSV that is time and dose-dependent and that suggests a first-order reaction.

Inquiries into the effects of THC inactivation on the structural integrity of HSV were performed. THC-inactivated and control (DMSO-treated) HSV preparations were pelleted, sectioned, and examined by transmission electron microscopy. The results indicated comparable distributions of enveloped virus and nucleocapsids in both preparations, with respective particle ratios in the range of 1:6–10 (Fig. 2). No gross morphologic differences were apparent in these two preparations. Thus, the process of THC inactivation does not result in virion disassembly, and the loss of PFU in the virus preparation treated with THC is not due to virion aggregation. The drug-mediated loss of HSV viability is the result of a THC effect on individual virion particles.

Previous studies in our laboratory showed that the thermal inactivation rate of HSV is influenced significantly by the composition as well as the pH of the menstruum in which the virus is suspended. This pH

effect is readily apparent when serum-containing tissue culture medium is used, with greater inactivation occurring in media adjusted to an alkaline pH (15–17). When the effect of medium pH on THC inactivation of HSV was examined, the results indicated that the drug-mediated inactivation of virus was not affected by the pH in the range of pH 6.4–7.5 (Table II). This differs significantly from the pH effects on thermal inactivation of HSV and suggests that THC mediates HSV inactivation by a mechanism(s) that is different from that responsible for thermal inactivation of the virus.

A single previous report provides evidence of a direct effect of THC on HSV viability (10). However, it is unclear whether the drug concentrations found to completely preclude HSV viability, i.e., treatment with 100 and 200 μg THC/ml, may have resulted in the carryover of a significant amount of THC from the reaction tubes to the cell cultures during the infection/adsorption period. Thus, it is possible that the lack of development of HSV-associated cytopathology observed was (in part) due to a THC effect on the cycle of HSV replication and not directly to the virus. Our protocol employed a minimum 10^{-2} dilution of the virus-drug test samples to eliminate this possibility and provides evidence confirming the notion of a detrimental effect of THC on HSV viability. Considering the lipophilic nature of THC, it is possible that the drug binds to the HSV envelope, thereby precluding virion attachment. Alternatively, THC treatment may affect virion uncoating and the genetic expression of immediate early α genes, resulting in a loss of virion replicative capability. An envelope-mediated effect would explain the failure of THC to inactivate the naked viruses tested. We are currently investigating the mechanism(s) associated with and responsible for the THC-mediated loss of HSV viability and biologic consequences of this drug-virus inactivation.

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