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Delta-9-Tetrahydrocannabinol Enhances Release of Herpes Simplex Virus Type 2

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SUMMARY

This study was undertaken to determine the effect of micromolar concentrations of delta-9-tetrahydrocannabinol (Delta-9-THC) on herpes simplex virus type 2 (HSV-2) replication in vitro. Virus-infected Vero cells pretreated for 24 h with 10^{-5} M or 10^{-6} M Delta-9-THC yielded 100-fold increases in infectious extracellular virus. Transmission electron microscopy of drug-treated cells revealed plasma membrane dissolution, distension of the smooth and rough endoplasmic reticulum, and the appearance of macrovacuoles in the cytoplasm containing aggregates of virus. These results suggest that Delta-9-THC enhances the release of HSV-2 by perturbing cellular membranes in virus-infected cells.

Delta-9-tetrahydrocannabinol (Delta-9-THC) is the major psychoactive component of marihuana, a substance which induces dysfunction in lymphocyte response to mitogens and particulate antigens (Nahas et al., 1974), decreases T-cell rosette formation (Gupta et al., 1974) and alters alveolar macrophage morphology, function and motility (Mann et al., 1971). The substance has been shown also to inhibit macromolecular synthesis in various culture systems (Nahas et al., 1977; Desoize et al., 1979) and to inhibit herpes simplex virus replication in vitro at doses exceeding 5 μg/ml (Blevins & Dumic, 1980). We have previously demonstrated that Delta-9-THC decreases host resistance to herpes simplex virus type 2 (HSV-2) vaginal infection in both guinea-pigs (Cabral et al., 1985) and mice (Mishkin & Cabral, 1985), resulting in greater severity of herpes genitalis, higher quantities of virus shed from the vagina, and higher mortalities. Since the decreased resistance in mice was accompanied by suppression of alpha/beta interferon (Cabral et al., 1986), by delay in onset of the delayed hypersensitivity response and reduction of humoral immunity to HSV-2 (Mishkin & Cabral, 1985), the greater severity of herpes genitalis may have resulted from drug-induced immunosuppression. However, Delta-9-THC has similarities in structure and pharmacological action to steroids (Munson & Fehr, 1983) which have been shown to potentiate viral replication in vitro (Costa et al., 1974) and in vivo (Baker & Plotkin, 1978). Thus, a direct stimulatory effect of micromolar concentrations of Delta-9-THC on HSV-2 replication has not been ruled out. The objective of this study was to determine the in vitro effect of Delta-9-THC at concentrations ranging from 10^{-5} M to 10^{-7} M on HSV-2 replication.

HSV-2, strain FMC, was isolated from a gynaecological patient and was typed by partial restriction endonuclease analysis of DNA with EcoRI (Hamelin et al., 1984). The virus was propagated in Vero cells as previously described (Cabral & Schaffer, 1976) and had a stock titre of 1 × 10^9 p.f.u./ml by plaque assay using a 2% methylcellulose overlay (Rapp, 1963). Stock HSV-2 was stored at −80 °C until used.

Delta-9-THC (formula weight 316) prepared as a stock solution of 100 mg/ml in 95% ethanol, and Delta-9-THC-1',2',3'H2 (sp. act. 0.0963 Ci/mmol) were provided by the National Institute of Drug Abuse. Delta-9-THC concentrations of 10^{-5} M, 10^{-6} M, 10^{-7} M were prepared by dissolving 10 μl of appropriately diluted (95% ethanol) stock cannabinoid per ml of culture
medium. The vehicle consisted of medium containing 10 μl/ml 95% ethanol. Since Delta-9-THC has affinity for plastic, the percentage of input drug bound to Vero cells was assessed before initiation of experiments. Monolayers were incubated for 24 h in medium containing 3H-Delta-9-THC to give final concentrations of 10⁻⁴ M, 10⁻⁵ M, 10⁻⁶ M or 10⁻⁷ M. Monolayers were then washed with phosphate-buffered saline (PBS), scraped into PBS, subjected to three freeze-thaw cycles, and sonicated at 7 kHz for 1 min. Disrupted pellets were centrifuged at 1000 g. Aliquots (0.1 ml) of the original input media, cell supernatants and cellular pellets were dissolved in liquid scintillation cocktail (Ready-Solv, Beckman) and were monitored for β-emissions using a Beckman LS1800 Beta-Counter. The proportion of recovered Delta-9-THC associated with cellular pellets ranged from 35% to 43%.

Three regimens of Delta-9-THC treatment were initially employed to determine the effect of the drug on the kinetics of infectious virus production. In the first regimen, cells were treated with Delta-9-THC for 24 h prior to virus inoculation. In the second, cells were inoculated with virus and, following the 1 h adsorption period, were maintained in medium containing Delta-9-THC or vehicle. In the third regimen, cells were pretreated with Delta-9-THC or vehicle for 24 h, washed, inoculated with HSV-2, and maintained in medium containing Delta-9-THC or vehicle. Monolayers were inoculated with HSV-2 at a m.o.i. of 5 p.f.u/cell. Qualitatively similar results were obtained with the three drug regimens. Consequently, subsequent experiments entailed incubation of monolayers for 24 h with drug or vehicle.

Replicate cultures, grown in 25 cm² culture flasks, were harvested at various times after virus inoculation and were pooled for infectivity assays. First, supernatants containing the extracellular virus were removed to sterile vials. Cells were then scraped into fresh medium, transferred to conical sterile 15 ml plastic tubes and centrifuged at 1500 r.p.m. for 20 min at 4 °C. Cell pellets containing the intracellular virus were resuspended in 1.0 ml of complete Eagle's MEM, subjected to three cycles of freezing and thawing and sonicated at 7 kHz for 1 min using a cell disruptor (Heat Systems-Ultrasonics Inc., Plainview, N.Y., U.S.A.). These samples were then centrifuged at 2000 r.p.m. for 20 min and the resultant supernatant fractions were recovered. All samples were stored at −80 °C until assayed for infectious virus by plaque assay. For transmission electron microscopy, virus-infected or mock-infected, drug- or vehicle-treated monolayers were scraped into the medium, centrifuged at 1500 r.p.m. at 4 °C, resuspended in cold PBS (4 °C) and centrifuged again. The cell pellets were fixed in 1% OsO₄, dehydrated in ethanol, passed through propylene oxide, and embedded in Poly/Bed 812-Araldite (Polysciences, Warrington, Pa., U.S.A.) using the formulation of Mollenhauer (1964). Thin sections were cut using an LKB Huxley ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM10-CA electron microscope operating at an accelerating voltage of 60 kV.

Results of a typical experiment demonstrating the effects of Delta-9-THC pretreatment on the replication cycle of HSV-2 in Vero cells are shown in Fig. 1. No significant differences in the synthesis of intracellular HSV-2 were observed for cultures treated with vehicle or with 10⁻⁵ M- to 10⁻⁷ M-Delta-9-THC. A significant decrease in production of intracellular virus occurred in cultures treated with 10⁻⁴ M of the cannabinoid. The kinetics of production of extracellular virus in cells treated with 10⁻⁵ M- to 10⁻⁷ M-Delta-9-THC were similar up to 18 h post-inoculation. However, by 48 h post-inoculation a 100-fold increase in extracellular virus was recorded for cells treated with 10⁻⁵ M- or 10⁻⁶ M-Delta-9-THC when compared to vehicle-treated controls. Minimal amounts of extracellular virus were produced at all time periods after inoculation by cells pretreated with 10⁻⁴ M of drug. This low virus production was probably a result of drug-induced cytotoxicity since these cells failed to exclude trypan blue stain in contrast to cells treated with lower concentrations of the drug. Furthermore, cells treated with 10⁻⁴ M-Delta-9-THC lost anchorage to the culture vessel substratum and remained suspended in the culture medium.

Electron microscopy of HSV-2-infected, vehicle-treated cells revealed intranuclear margination of the chromatin, fusion and reduplication of cytoplasmic membranes, disaggregation of the endoplasmic reticulum, and cytoplasmic microvacuole formation (Cabral & Schaffer, 1976). Virus particles were present both within the nucleus and cytoplasm. Cytoplasmic virus was
Fig. 1. Effects of Delta-9-THC on the kinetics of (a) intracellular and (b) extracellular infectious HSV-2 production. Replicate cultures of Vero cells were pretreated for 24 h with Delta-9-THC at concentrations (M) of 0 (△), 10^{-4} (□), 10^{-5} (○), 10^{-6} (●) or 10^{-7} (▲) prior to inoculation with HSV-2 (m.o.i. 5). Virus concentrations were determined by plaque assay.

Fig. 2. Transmission electron micrograph of a Vero cell pretreated with 10^{-5} M-Delta-9-THC for 24 h. Numerous macrovacuoles are present throughout the cytoplasm. Dissolution of the cell-surface membrane (open arrow) and blebbing from the cell surface (closed arrow) are also apparent. Bar marker represents 1 μm.
either free within that compartment or was associated with microvacuoles and microvesicles. Uninfected cells treated with Delta-9-THC at concentrations of $10^{-5}$ M to $10^{-7}$ M demonstrated dissolution of the cell surface membrane, intracytoplasmic macrovacuole formation, and blebbing of the cell surface (Fig. 2). The macrovacuoles in drug-treated cells were considerably larger than those in non-drug-treated, virus-infected cells. Similar macrovacuoles were seen in virus-infected cells pretreated with Delta-9-THC (Fig. 3). These macrovacuoles were especially prominent in cells treated with $10^{-5}$ M or $10^{-6}$ M drug. Numerous enveloped virus particles were observed within the macrovacuoles. Cytoplasmic membrane reduplication and fusion were not observed in virus-infected, drug-treated cells.

The results of this investigation indicate that Delta-9-THC in concentrations ranging from $10^{-5}$ M to $10^{-6}$ M perturbs cell cytoplasmic and plasma membranes. Membrane perturbations were also seen in virus-infected cells pretreated with $10^{-7}$ M-Delta-9-THC. However, these modifications were not as pronounced as those in cells treated with the higher doses of drug. The conclusion that Delta-9-THC perturbs cellular membranes is supported by the observation that drug-treated cells exhibited pronounced vacuole formation and distension of intracytoplasmic channels, denuding of the plasma membrane, and cell surface blebbing. In addition, suppression of cytoplasmic membrane fusion and reduplication was observed in drug-treated, virus-infected cells. The expression of cell surface blebs has been described for cells exposed to various
cytotoxins. For example, lymphotoxin-treated cells bleb prior to lysis (Leopardi et al., 1984). The presence of blebs is consistent with the concept of a direct effect of Delta-9-THC on cell surface membranes. Indeed, Delta-9-THC has been shown to exhibit marked lipophilic properties which facilitate its passage across cellular membranes (Collins et al., 1978). Wing et al. (1985) have suggested that these drug-related hydrophobic interactions impart greater fluidity to cell membranes.

The membrane perturbations probably account for the higher quantities of virus released from cells pretreated with Delta-9-THC. That is, membrane distension and macrovacuole formation may have facilitated virus exit to the extracellular environment. The hypothesis is supported by the apparent absence of a direct stimulatory effect of the drug on virus replication since no significant differences in the kinetics of total infectious virus production were observed. The dissolution of the plasma membrane, the pronounced cytoplasmic channels and vacuoles, and the attendant greater release of HSV-2 infectious particles, may play a role in exacerbation of primary infection in vivo. Because HSV-2 replication and dissemination at the primary site of infection result in a denuding of local epithelium (Becker et al., 1984), enhanced release of HSV-2 in drug-exposed hosts could lead to more rapid exposure of local nerve endings and dissemination of virus to regional nerve ganglia. We have previously shown that Delta-9-THC suppresses alpha/beta interferon induction (Cabral et al., 1985) and delays the delayed hypersensitivity response to HSV-2 (Mishkin & Cabral, 1985). Thus, suppression of host resistance, accompanied by enhanced release of HSV-2 and denuding of surrounding epithelium, could lead to more severe primary HSV-2 infection in vivo.

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