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Effect of Δ^9-Tetrahydrocannabinol on Herpes Simplex Virus Type 2 Vaginal Infection in the Guinea Pig (42325)

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Abstract. The present investigation was undertaken to determine whether Δ^9-tetrahydrocannabinol (Δ^9-THC) decreases host resistance to herpes simplex virus type 2 vaginal infection in the guinea pig. The guinea pig was selected as the host since it has been shown to express a spectrum of primary herpes genitalis which is similar to that in humans. Animals were administered Δ^9-THC or vehicle intraperitoneally on Days 1–4, 8–11, and 15–18. Herpes simplex virus was introduced intravaginally on Day 2. Host resistance to virus infection was assessed by comparing frequency and severity of lesions, virus shedding, and animal mortalities. Virus-infected animals treated with drug at doses of 4 and 10 mg/kg exhibited significantly greater severity of genital disease during the 30-day period of study when compared to virus-inoculated vehicle controls. A direct relationship was noted between dose of Δ^9-THC and cumulative mortalities on Day 14 following primary infection. These results indicate that Δ^9-THC decreases host resistance to herpes simplex virus type 2 vaginal infection in the guinea pig. © 1986 Society for Experimental Biology and Medicine.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of marihuana. This compound inhibits cellular macromolecular synthesis (1, 2) and suppresses the immune system in a number of animal models (3). Δ^9-THC elicits dysfunction in lymphocyte response to mitogens and to particulate antigens (4), decreases T-cell rosette formation (5, 6), suppresses leukocyte migration (7), and affects alveolar macrophage morphology, function, and motility (8). The drug, also, has been shown to decrease resistance of mice to infection with herpes simplex virus type 2 (HSV2) (9, 10). The doses of Δ^9-THC required to impair host resistance of mice are very high compared with exposures of humans to cannabinoids. Thus, an animal model more susceptible to exposure to cannabinoids is needed.

The present investigation was undertaken to determine whether Δ^9-THC decreases host resistance to HSV2 genital infection in the guinea pig. The guinea pig was selected as the host since HSV2 elicits a spectrum of disease in this animal which is similar to that observed in humans (11). The virus was administered intravaginally in order to duplicate the natural route of infection.

Materials and Methods. Female Hartley white guinea pigs, 400–500 g, were purchased from The Charles River Breeding Laboratory (Wilmington, Mass.) and were quarantined for 1 week before initiation of experiments. Δ^9-THC was supplied by the National Institute of Drug Abuse (Rockville, Md.) and was prepared as a stock solution in Emulphor (EL-360, GAF Corp., New York, N.Y.) and ethanol (1:1 w/v) at a concentration of 100 mg/ml. This stock was diluted with 0.85% sodium chloride to yield final concentrations of 25, 10, 4, 2, 1, and 0.2 mg/ml. The vehicle control consisted of Emulphor/ethanol/saline (1/1/8). The drug and vehicle solutions were administered intraperitoneally such that each animal received 1 ml/kg body wt. The virus employed in this study was isolated from a gynecologic patient and was propagated in Green monkey kidney (Vero) cells (Flow Laboratories, McLean, Va.). The isolate was typed as HSV2 on the basis of fingerprinting of viral DNA by restriction endonuclease analysis (12) and was designated HSV2, strain P180/ST(FMC). Stock virus was shown to have an

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LD_{30} of 10^3 PFU/0.2 ml in guinea pigs following intravaginal instillation and subsequent experiments entailed the use of $10^{3.5}$ PFU per inoculum. Animals were bled 1 week prior to the initiation of the study and sera were screened for HSV antibodies by enzyme-linked immunosorbent assay (ELISA) (13). Animals shown to have positive antibody titers to HSV (i.e., reciprocal titer greater than 10) were excluded from the study.

Animals were administered Δ^9-THC or vehicle intraperitoneally on Days 1–4, 8–11, and 15–18. The HSV2 ($10^{3.5}$ PFU) was delivered intravaginally on Day 2 in a 0.2-ml volume of Eagle's minimal essential medium (MEM) using a tuberculin syringe without a needle. Following inoculation, the vaginal orifice was plugged with Gel-Foam (Upjohn Co., Kalamazoo, Mich.) to prevent leakage of virus. The Gel-Foam has been shown to be inert and dissolves in 2–3 days. Animals were observed at the time of dosing for pharmacotoxicologic signs and daily for 30 days following administration of virus for virus shedding, lesion expression, and morbidity and mortality. Animals were scored for stigmata of herpes genitalis using the following scale: 0 = no symptoms; 1 = swelling and erythema; 2 = swelling and erythema with less than 5 lesions; 3 = swelling and erythema with 5–10 lesions; 4 = swelling and erythema, confluent vesicles, mucosal necrosis, and purulent discharge; 5 = severe ulceration and necrosis and/or hindlimb paralysis; 6 = death. Vaginal swabs were obtained from animals on Days 6, 8, 10, 12, 14, 20, and 30 postviral inoculation. Day 6 was selected for the initial time of vaginal swab acquisition since in preliminary experiments vaginal virus was not detected in both vehicle-treated and drug-treated animals until Days 6 and 7 postviral inoculation. Swabs were stored in Eagle's MEM at −80°C until assayed for infectious virus by plaque assay (14). Brain, lung, and liver fragments were obtained from guinea pigs at the time of death. These tissues were stored in Eagle's MEM at −80°C until assayed for infectious virus by cocultivation of tissue fragments with Vero cell monolayers and measurement of cytopathic effect characteristic for HSV. Data on the frequency of lesion expression and mortality were analyzed using the Fisher exact test (15). Analysis of variance and the Newman–Keuls multiple range test (16) were employed for the analysis of lesion scores. Dose-related times until death were analyzed by linear regression analysis (17).

Three independent studies were conducted. The first study consisted of two pooled individual experiments. In the first experiment, animals were given neither drug nor vehicle ($N = 4$), vehicle ($N = 16$), or Δ^9-THC at doses of 0.2 mg/kg ($N = 8$), 1.0 mg/kg ($N = 7$), 2.0 mg/kg ($N = 8$), 4.0 mg/kg ($N = 8$), and 10 mg/kg ($N = 7$). The second experiment was performed to allow for completion of the dose response to the cannabinoid. Animals received neither drug nor vehicle ($N = 4$), vehicle ($N = 16$), or Δ^9-THC at doses of 4.0 mg/kg ($N = 8$), 10.0 mg/kg ($N = 7$), and 25 mg/kg ($N = 6$). Animals were then inoculated with HSV2 and monitored for 30 days for lesion expression and for morbidity and mortality.

The second study was performed to determine whether a dose relationship existed between cannabinoid administration and mortality following HSV2 intravaginal infusion. Animals were administered vehicle ($N = 10$) or Δ^9-THC at doses of 0.2 mg/kg ($N = 8$), 1.0 mg/kg ($N = 7$), 2.0 mg/kg ($N = 8$), and 4.0 mg/kg ($N = 8$). A third study was undertaken to permit quantitation of virus shed from the vagina of cannabinoid-treated guinea pigs inoculated with HSV2. Animals were treated with vehicle ($N = 10$) or with the drug at doses of 1.0 mg/kg ($N = 9$), 2.0 mg/kg ($N = 9$), and 10 mg/kg ($N = 10$).

Results. Results of two independent experiments on the effect of Δ^9-THC on mortality and development of genital lesions in guinea pigs infected with HSV2 and monitored for 30 days are summarized in Table I. Lesions were expressed on the genitalia of 50% of vehicle control animals and of animals receiving neither drug nor vehicle. In contrast, animals receiving Δ^9-THC in doses ranging from 0.2 to 25 mg/kg exhibited higher frequencies of lesion expression (i.e., 63 to 86%). Deaths were recorded for 38% of non-drug-treated animals and for 34% of vehicle controls. Increased mortalities (50–64%) were observed among animals receiving Δ^9-THC at doses of 2 mg/kg or higher. Severity of herpes genitalis also was measured using mean lesion scores (MLS).

TABLE I. EFFECT OF Δ^9-TETRAHYDROCANNABINOL ON MORTALITY, DEVELOPMENT, AND SEVERITY OF GENITAL LESIONS IN GUINEA PIGS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 2

Treatment[a] (mg/kg)	No. animals[b]	% Lesion[c]	% Mortality[c]	MLS[d] (means ± SE[e])
None	8	50	38	2.75 ± 1.06
Vehicle[f]	32	50	34	2.25 ± 0.48
0.2	8	75	38	3.13 ± 0.93
1.0	7	86[g]	29	2.57 ± 0.92
2.0	8	63	50[h]	3.38 ± 1.02
4.0	16	81[g]	63[g]	4.25 ± 0.61[g]
10.0	14	79[h]	64[g]	4.21 ± 0.67[g]
25.0	6	67	50[h]	3.00 ± 1.34

[a] The Δ^9-THC or vehicle was administered intraperitoneally for a total of four consecutive daily treatments per week interspersed with a 3-day rest period for 3 weeks. Intravaginal instillation of $10^{3.5}$ PFU of HSV2 was performed 1 day after the first injection of Δ^9-THC or vehicle.

[b] Female Hartley guinea pigs were acclimated to the animal room for 7 days prior to administration of Δ^9-THC or vehicle.

[c] Percentage of guinea pigs which expressed lesions or which died during the 30-day experimental period. Data on the frequency of lesions and mortality were analyzed by the Fisher exact test.

[d] Mean lesion score.

[e] SE = standard error.

[f] Emulphor/ethanol/saline (1/1/8).

[g] $P < 0.05$.

[h] $P < 0.08$.

Higher MLS were recorded for groups of animals receiving Δ^9-THC at 0.2 mg/kg and at doses ranging from 2.0 to 25 mg/kg.

In order to determine whether Δ^9-THC elicited decreased host resistance to HSV2 vaginal infection in a dose-dependent fashion, a second study was performed in which animals were monitored daily for mortalities and cumulative deaths were calculated on a daily basis. Figure 1 illustrates that a direct relationship was noted by linear regression ($r = 0.997$; $y = 22.75 + 6.88x$) between cumulative deaths and increasing drug doses ranging from 0.2 to 4 mg/kg by Day 14. However, after 14 days cumulative mortalities were observed to plateau.

To further assess the effect of Δ^9-THC on host resistance to HSV2, a third study was undertaken to allow for quantitation of vaginal virus shedding as a function of drug treatment. Results are summarized in Fig. 2. Vaginal virus shedding, as detected by plaque assay of vaginal swabs, occurred in all guinea pigs expressing lesions. In addition, two animals treated with vehicle, but not found to express lesions, shed virus. Infected guinea pigs treated with Δ^9-THC at a dose of 10 mg/kg yielded maximal virus titers on Day 6 postvaginal inoculation. These titers were significantly higher ($P < 0.05$) than those recorded from virus-infected, vehicle control animals. Animals treated with 1.0 or 2.0 mg/kg of drug yielded amounts of virus intermediate between those of vehicle control animals and animals receiving 10 mg/kg Δ^9-THC. Shedding was down by Day 8 for all groups and occurred sporad-

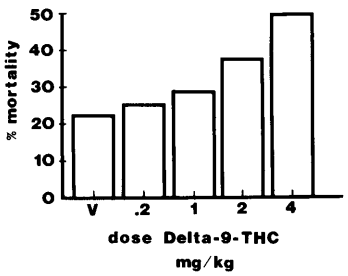

FIG. 1. Dose-dependent guinea pig mortalities recorded by Day 14 using linear regression analysis ($r = 0.997$; $y = 22.75 + 6.88x$). Animals were administered vehicle ($N = 10$) and drug doses of 0.2 mg/kg ($N = 8$), 1.0 mg/kg ($N = 7$), 2.0 mg/kg ($N = 8$), and 4.0 mg/kg ($N = 8$) and were inoculated intravaginally with $10^{3.5}$ PFU of HSV2 as described under Materials and Methods. A direct relationship was noted between dose of drug administered and mortality.

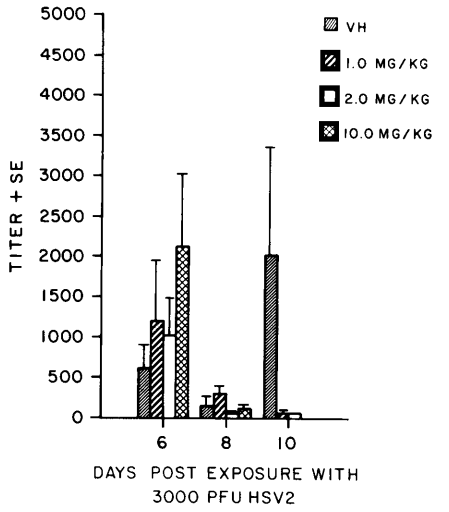

FIG. 2. Shedding of virus in the vagina of guinea pigs inoculated with $10^{3.5}$ PFU of HSV2. Vaginal swabs were obtained from the animals on Days 6, 8, 10, 12, 14, 20, and 30 following virus inoculation. Swabs were employed in plaque assay to determine titers of HSV2 shed. The bars represent mean titers of virus shed by animals treated with vehicle (N = 10) or with 1.0 mg/kg (N = 9), 2.0 mg/kg (N = 9), or 10 mg/kg (N = 10) Δ^9-THC.

ically thereafter. Thus, Δ^9-THC exacerbated HSV2 vaginal infection by eliciting dose-dependent increases in virus shedding. All guinea pigs in this study were bled (1 ml) by cardiac puncture on Day 7 postviral inoculation. Animals which did not succumb to the primary infection were bled on Days 15 and 30. Sera were diluted 1:10 in Eagle's MEM and assayed for infectious virus by plaque assay. Virus was not isolated from any of the sera. Animals which succumbed were necropsied at time of death. Brain, liver, and lung fragments were cocultivated with Vero cell monolayers. In all cases, virus was isolated from brain but not from liver or lung tissues.

Discussion. The results of this study demonstrate that Δ^9-THC decreases host resistance to HSV2 vaginal infection in the guinea pig. This decreased resistance was maximized at drug doses of 4 and 10 mg/kg as evidenced by cumulative data on greater incidence and severity of genital lesions, higher titers of vaginal virus shed, and increased mortalities among drug-treated virus-infected animals when compared with virus-infected vehicle controls. Although some shifts in dose effect were observed (i.e., 86% of animals administered 1.0 mg/kg Δ^9-THC expressed lesions), the two independent experiments gave qualitatively similar results: a dose-dependent increase in susceptibility up to 10 mg/kg Δ^9-THC. Furthermore, it is not unusual to observe shifts in dose effects for infectious models using noninbred animals.

The decreased resistance elicited by the drug occurred in a dose-dependent fashion within 14 days following primary infection based on cumulative mortalities. This "early effect" of Δ^9-THC may have resulted from enhanced virus replication at the primary site of infection, from drug-related immunosuppressive activities, or from accelerated passage of the virus to the central nervous system. Since virus was isolated from brains of all animals which died, but not from the liver or lung, it is suggested that the central nervous system was targeted by the HSV2. Virus access to the brain may have been enhanced as a result of increased virus replication in genital epithelial cells leading to greater numbers and greater severity of localized lesions. These lesions result in denuding of nerve endings, and facilitate invasion of nerve endings by HSV2 (18). Alternatively, suppression of specific or nonspecific early host resistance by Δ^9-THC may account for the higher mortalities in the drug-treated animals since the drug acts as an immunosuppressive agent (3). Indeed, it has been suggested that the cannabinoids, of which Δ^9-THC is the major psychoactive component, elicit some of their immunosuppressive effects by acting on T lymphocytes (19, 20). For example, Morahan *et al.* (9) found that doses and regimens of Δ^9-THC which produced significant decreases in host resistance in mice receiving HSV2 intravenously were similar to those which caused suppression of delayed-type hypersensitivity to sheep erythrocytes. We have demonstrated that doses of the Δ^9-THC which decrease host resistance in B6C3F1 mice inoculated intravaginally with HSV2 result in suppression of the humoral response, and in a delay in onset of the delayed hypersensitivity response to the virus (10). Δ^9-THC may also elicit decreased resistance by acting on α/β interferon (21) or other components of early host resistance.

Δ^9-THC did not significantly suppress resistance to HSV2 vaginal infection at 25 mg/kg. The failure to elicit significant suppression at this dose may be attributed to several factors. First, Δ^9-THC at 25 mg/kg induces hypothermia in guinea pigs. In initial control studies, 25 mg/kg of the drug decreased the mean body temperature of guinea pigs from 39 ± 0.2 to 34.8 ± 0.8°C within 3 hr postintraperitoneal administration. This lower body temperature persisted for an average of 4 days following a single drug inoculation. Drug doses lower than 25 mg/kg (i.e., 10 and 4 mg/kg) did not elicit the decrease in body temperature. This lowering of body temperature may have contributed to a decreased rate of virus propagation within the guinea pig since the rate of virus synthesis *in vitro* is directly related to temperature in ranges compatible with cellular growth. Second, Δ^9-THC in concentrations in excess of 10^{-5} *M* has been shown to inhibit macromolecular synthesis in various mammalian systems (1, 2, 22, 23). Blevins and Dumic (24) have shown that both HSV1 and HSV2 fail to replicate and produce extensive cytopathic effect in cell cultures exposed to Δ^9-THC in doses exceeding 20 μg/ml (i.e., approximately 6×10^{-5} *M*). Finally, animals receiving 25 mg/kg of Δ^9-THC may have developed a degree of tolerance to the drug effect.

These results indicate that Δ^9-THC decreases host resistance to HSV2 vaginal infection in the guinea pig. This decrease was evidenced by higher frequency and greater severity of genital lesions, by acceleration of vaginal virus shedding, and by increased mortality.

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