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Effect of CP-20,961 on genital herpes in guinea pigs

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CP-20,961 (*N*,*N*-dioctadecyl-*N'*,*N'*-bis(2-hydroxyethyl)propanediamine) has been reported to be an interferon inducer, an adjuvant and a macrophage activator. In the present study, this compound was used therapeutically and prophylactically to treat genital herpes simplex virus type 2 (HSV-2) infections in guinea pigs. A significant decrease in the incidence of clinical lesions ($P<0.05$) was observed in animals treated intravaginally with 20 mg CP-20,961 (two doses each containing 10 mg) prior to infection. A single dose of 5 mg CP-20,961 reduced the severity of clinical lesions and inhibited virus shedding from the guinea pig vagina. Preliminary findings indicate that CP-20,961 is a potent agent for prevention of genital herpes infection.

genital herpes; antiviral agents; animal model

Introduction

Genital herpes simplex virus (HSV) infections have been recently recognized as an increasing cause of venereal disease in humans [3]. This disease is different from other venereal diseases because it frequently results in latent infection. After an interval of some time in which the patient is without symptoms, the latent HSV can be reactivated due to some stimulation factors and cause annoying recurrences [17]. In addition, a relationship exists between genital herpes infection and cervical cancer [15,20] and HSV-1 and HSV-2 can transform cells in vitro [7] and induce tumors in vivo [23].

The search for antiviral agents has resulted in the discovery of many compounds with activity against herpes simplex virus infections. A few of them have been widely used. These compounds include 5-iodo-2'-deoxy-uridine (IDU) [12], 9-β-D-arabinofuranosyladenine (ara-A) [24], trifluoro-thymidine (TFT) [9], and acyclovir (ACV) [11].

CP-20,961, *N*,*N*-dioctadecyl-*N'*,*N'*-bis (2-hydroxyethyl) propanediamine, is a lipoidal amine first described by Hoffman et al. [10]. It has been characterized as an interferon inducer which affords prophylactic resistance to both RNA and DNA virus infections [5,6,8,18,21,22]. In addition, several reports showed that CP-20,961 is an

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adjuvant and a macrophage activator [1,16]. In this paper we describe the effect of CP-20,961 on genital herpes infection in guinea pigs.

Materials and methods

Virus strain and cell culture

HSV-2 strain 1868 was used for CP-20,961 treatment studies. Virus stock was prepared in primary rabbit kidney (RK) cells or guinea pig embryo (GPE) cells and characterized as an HSV-2 as described previously [25]. Infectivity titers of stock virus were determined by plaque formation using GPE cell monolayers. They were 10^6 PFU/ml. Primary RK, GPE and chicken embryo (CE) cell cultures were prepared in our laboratory by methods previously described [2]. Cells were grown in Eagle's minimal essential medium (MEM) containing Hanks' balanced salt solution (BSS) and 10% heat inactivated newborn donor calf serum (NDCS). Confluent cell monolayers were maintained with MEM containing Earle's BSS and 2% NDCS.

Animal inoculation

Young female Hartley guinea pigs weighing 450–500 g were used throughout the study. Animals under pentobarbitol sodium anesthesia were inoculated intravaginally with different dilutions of HSV-2 suspension. The vaginal closure membrane was broken and the virus inoculum (0.1 ml) was delivered into the vagina with a tuberculin syringe without a needle. The vaginal canal was then plugged with soluble Gelfoam (Upjohn Co., Kalamazoo, MI, U.S.A.) surgical pads so that it would retain the virus. Guinea pigs were examined daily for clinical manifestations of genital infection. The severity of clinical illness was progressive and was scored as follows: 0, no symptoms; 1, swelling, erythema of the vaginal mucosa, or 1–2 herpetic lesions; 2, 3–10 lesions; 3, 11–20 lesions; 4, 21 confluent lesions; 5, loss of bladder control or paralysis; -1, healing begins (drying crusting of lesions).

Virus recovery from guinea pigs

The vagina of each guinea pig was swabbed with a pre-moistened cotton-tipped applicator which was then placed in a vial containing 1.0 ml of MEM containing Earle's BSS and 2% NDCS with 10% (vol/vol) dimethylsulfoxide. After removal of the cotton-tipped applicator, the samples were frozen at -70°C until assayed for virus infectivity. The suspensions were assayed using either GPE or CE monolayers. After adsorption of virus at 37°C for 1 h, infected cells were overlayed with 0.5% methylcellulose in MEM containing Earle's BSS and 10% NDCS and incubated at 37°C in a 5% CO_2 incubator. Monolayers were fixed and stained 3–4 days post-inoculation. Plaques were enumerated and virus titers were calculated.

Drug treatment

CP-20,961 (lot: ED-G 089-481) was a gift from Dr. William W. Hoffman, Pfizer, Inc., Groton, CT, U.S.A. The drug was a liposome emulsion with a concentration of 50 mg/ml. This emulsion was not virucidal to HSV nor was the drug itself virucidal to vesicular stomatitis virus, vaccinia or Mengo virus [10]. Guinea pigs were treated with one or two doses of CP-20,961, from 7 days before inoculation of virus to 1–3 days after inoculation of virus. Each group of guinea pigs was divided randomly into different subgroups. Animals were weighed daily. Each treated animal received 0.1 or 0.2 ml of CP-20,961 by intravaginal application. Untreated and treated guinea pigs with or without virus infections served as controls. They were housed in separate cages. As an additional control, some animals were also treated intravaginally with 7% ethyl alcohol (the same concentration as in the drug vehicle). Liposome vehicle alone was administered 24 h after HSV infection.

Statistics

Statistical analyses were performed using Student's *t* test.

Results

Effect of CP-20,961 on the incidence of genital lesions in HSV-2 infected guinea pigs

Each experiment included pre-treatment, post-treatment and untreated groups of animals. However, only one dose (5 mg/day) of drug was used for intravaginal administration to infected guinea pigs in two experiments. After inoculation of $10^{5.1}$ PFU HSV-2, the incidence of genital lesions was not different whether drug was given prior to or after virus (Table 1). In the group treated prior to infection (5 mg 1, 2, or 3 days before infection), 86% (31/36) of the guinea pigs showed lesions while the post-infection treated group (5 mg, 1 or 2 days after infection) had 94% (17/18) and the non-treated group 92% (11/12). However, when two doses of drug (10 mg/day, 1 and 2 days before infection) were used for the treatment of the guinea pigs (three separate experiments) and they were infected by inoculation of an average $10^{3.5}$ PFU HSV-2, only 20% of the group showed clinical lesions. The total rate of clinical lesions in this pre-infection treated group was significantly less than in the post-infection treated (3 and 4 days after infection) (75%) and untreated (70%) groups (Table 1). Animals treated with 7% alcohol did not differ from untreated controls. Liposome vehicle alone, administered 24 h after HSV infection, did not affect lesion scores (see Discussion).

Effect of CP-20,961 on the severity of genital lesions in guinea pigs

Single dosage of drug administration Although one dose of drug had no effect on the incidence of genital lesions (see above), observations on various days of pre- and

TABLE 1

Effect of CP-20,961 on the incidence of genital lesions in guinea pigs

Virus inoculum PFU		Method of treatment[a]	Animals studied (No.)	Animals showing lesions (No.)	Animals showing lesions (%)
$10^{5.1}$	One dose (5 mg)	Pre-infection treated	36	31	86
		Post-infection treated	18	17	94
		Untreated	12	11	92
$10^{3.5}$	Two doses (10 mg/dose)	Pre-infection treated	25	5	20[b]
		Post-infection treated	24	18	75
		Untreated	23	16	70

[a] One dose = total from 2 experiments: pre-treatment given 1, 2 or 3 days prior to virus inoculation; post-treatment given 1 or 2 days after virus inoculation. Two doses = total from 3 experiments: pre-treatment given 1 and 2 days prior to virus inoculation; post-treatment given 3 and 4 days after virus inoculation.

[b] $P < 0.01$.

post-infection treatment indicated that the mean lesion scores of 1, 2, and 3 days pre-infection treatment of guinea pigs were significantly less than the post-infection treatment and untreated guinea pigs (Fig. 1). It was apparent from the control untreated animals that lesion scores of 3 or greater at 5 days or thereafter indicated

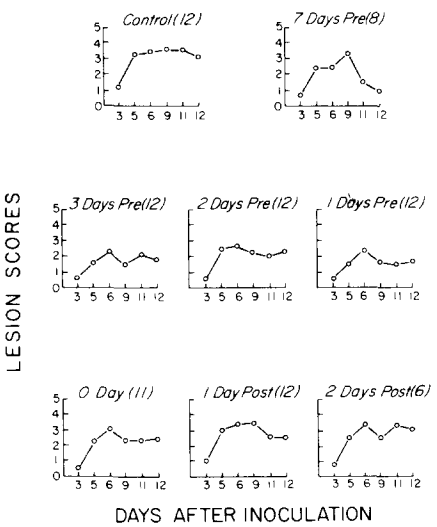

Fig. 1. Prophylactic effect of CP-20,961 on mean genital lesion scores in guinea pigs following intravaginal inoculation with $10^{5.1}$ PFU HSV-2. Each animal received 5 mg drug on indicated days either pre- or post-virus inoculation; all inoculated animals are included. Numbers in parentheses = numbers of animals used in each group.

severe infections. The mean lesion scores of 0-day treatment of guinea pigs which received drugs immediately after virus inoculation were not significantly less than the untreated guinea pigs except on day 9 after virus inoculation. Treatment 7 days before infection had no significant effect on lesion scores during acute infection until day 11 after virus inoculation.

Two doses of drug administration Comparison of lesion scores of guinea pigs showing lesions in the three experiments indicated that the pre-infection treated group had significantly milder lesion scores than post-infection treated and non-treated groups ($P<0.05$, Fig. 2). The rate of lesion healing was also faster in the pre-infection treated group. The mean lesion scores were always higher in the non-treated animals than pre-infection treated animals. On the contrary, the mean lesion scores were not different between post-infection treated and untreated groups.

Effect of CP-20,961 on virus shedding in the genital tract

Intravaginal treatment with CP-20,961 prior to infection was also effective in reducing the excretion of virus from the genital tracts of infected guinea pigs during acute infection. The amount of virus shed after a single dose of drug (5 mg) is shown in Table 2. In the pre-infection treated animals, the mean virus titer in the genital swabs was about 1 log less than the titers from the post-infection treated and non-treated guinea pigs on day 3 after infection with HSV-2. Treatment began on the same day as infection also inhibited virus replication on day 3 after infection. Although day-to-day variations occurred, the average virus titers shed from the pre-infection treated animals were lower than any of the other groups. None of the treatments had an effect on duration of virus shedding.

Effect of CP-20,961 on body weight of guinea pigs following HSV-2 infection

Weight loss or gain is another indicator of the health of the animals. Four compara-

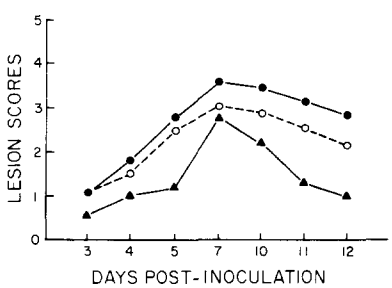

Fig. 2. Prophylactic effect of CP-20,961 on mean genital lesion scores in guinea pigs following intravaginal inoculation with $10^{3.5}$ PFU HSV-2; only those inoculated animals with lesions are included. ●———● control (untreated); ○— — —○ post-infection treatment (10 mg/day for 2 days beginning 24 h after virus inoculation); ▲———▲ pre-infection treatment (10 mg/day for 2 days before virus inoculation); $P<0.05$ (pre- vs. post- or control).

TABLE 2

Effect of CP-20,961 on virus excretion in the genital tract of guinea pigs[a]

CP-20,961 treatment[b]	No. studied	Virus excretion in genital tracts on days post-infection (log PFU/0.1 ml)		
		3	5	Mean
Pre-treated	36	1.85[c] ± 1.12	1.78 ± 1.10	1.82 ± 1.11
0-day treated	11	1.96[d] ± 1.37	2.19 ± 0.81	2.08 ± 1.10
Post-treated	18	3.26 ± 0.91	1.86 ± 1.10	2.56 ± 1.20
Untreated	12	2.95 ± 1.68	2.13 ± 0.73	2.54 ± 1.33

[a] Each animal was inoculated with $10^{5.1}$ PFU of HSV-2 and received 5 mg of drug.

[b] Pre-treatment given 1, 2 or 3 days prior to virus inoculation; post-treatment given 1 or 2 days after virus inoculation; 0 day treatment initiated immediately after virus inoculation.

[c] $P < 0.01$.

[d] $P < 0.05$.

ble experiments showed that, regardless of the amounts of virus inoculated, animals treated with CP-20,961 (5 or 20 mg) prior to infection gained body weight on days 5 and 7 after virus inoculation. At these times, post-infection treated, 0-day treated and untreated guinea pigs had already lost 3–8% of day 0 body weight. Despite the initial weight gain of the pre-infection treated group, these guinea pigs also lost 7–12% of day 0 body weight on days 10–11 after infection. This loss of body weight was less than that of post-infection treated and untreated animals which showed an 18–32% body weight loss. Thus, those animals which were pre-infection treated with drug but developed lesions still showed a beneficial effect on body weight gain when compared with infected animals which were not treated.

Discussion

Epidemiological investigations and virology studies indicate that genital infection with HSV is increasing in incidence in the United States [3]. The limited efficacy of currently available treatment [4] and the ability of herpes infection to become latent and then reactivate make more and more patients subject to the great sufferings of this disease. The genital herpes infection of guinea pigs is a very useful model for studying antiherpes agents and recently has been extensively used in our laboratory for screening possible effective antiviral agents in vivo [13,14,19]. Although there are many antiherpes compounds which have been tested in vitro and in vivo, most of them cannot be used for human studies because of severe toxic effects. Antiviral substances, which are effective in animal models for herpes infections, are not always effective in clinical trials in humans. Therefore, antiviral therapy of human genital herpes requires a suitable animal model for investigation.

The long-chain alkyl propanediamine CP-20,961 is a potential antiviral agent. Previous studies have shown that CP-20,961 has low acute toxicity in mice and has an effective antiviral action on encephalomyocarditis virus, Semliki Forest virus, and vaccinia virus infection in mice but not against pneumonia virus of mice and influenza virus [10]. In our study, 5 and 20 mg of CP-20,961 were used intravaginally for genital herpes treatment in guinea pigs. The data derived from the guinea pig model shows that pre-infection treatment with two 10-mg doses CP-20,961 not only reduced the incidence of clinical lesions in guinea pigs, but also reduced the severity of genital lesions and showed a positive effect on weight gain in treated animals. Though pre-infection treatment with 5 mg CP-20,961 did not prevent the appearance of genital lesions after intravaginal inoculation of $10^{5.1}$ PFU HSV-2, development of lesions was inhibited and more body weight was maintained than in untreated guinea pigs. Also, the pre-infection treatment with 5 mg CP-20,961 reduced HSV-2 excretion from the genital tract. Our data indicated that this single intravaginal treatment was effective if the drug is administered between the day of infection and seven days prior to infection. The post-infection treatment with 5 and 20 mg CP-20,961 was not effective as therapy for genital herpes infection in guinea pigs. However, preliminary experiments indicate that higher doses of drug, administered both intraperitoneally and intravaginally 24 h after HSV infection, did significantly reduce lesion scores of infected animals; also, liposome vehicle alone did not affect lesion scores (data to be reported separately). On the basis of our data, we consider that CP-20,961 is an effective prophylactic agent for herpesvirus infection and a possible therapeutic antiviral agent for genital herpes. The mechanism of the effect of CP-20,961 on HSV infection in vivo, whether physical or biological, is currently under investigation.

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