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Prophylactic and Therapeutic Treatment with Acyclovir of Genital Herpes in the Guinea Pig (41717)

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Abstract. The antiviral drug, acyclovir, was tested on experimentally infected guinea pigs with either of two herpes simplex virus type 1 (HSV-1) isolates following intravaginal inoculation. The drug was continuously infused subcutaneously utilizing an osmotic pump. Infusion was begun either prior to virus inoculation (prophylactic) or after virus inoculation at the time of first appearance of lesions (therapeutic). Prophylactic treatment markedly reduced the severity of the genital lesions, the appearance of acute neurologic sequellae, and the virus excretion in the genital tract of guinea pigs infected with either of the two strains tested. Therapeutic acyclovir treatment, however, did not decrease the incidence of acute neurologic sequellae with one of the two HSV-1 strains tested, nor did it reduce the severity of the genital lesions of either strain. These neurologic sequellae may be due to insufficient levels of ACV in the central nervous system as the concentration of ACV in the dorsal root ganglia was found to exceed that of the plasma, but only trace amounts of acyclovir were present in the brain and spinal cord. Continuous perfusion of ACV gave far higher tissue levels than intermittent injections. These findings suggest that prophylactic ACV is far more effective than therapeutic treatment for genital herpes in the guinea pig model.

Genital infection due to herpes simplex virus is one of the most common venereal diseases today in the United States (1). The increased incidence of genital herpes infection in recent years has been noted by many investigators (2–5). In the past, genital herpes infection was almost exclusively associated with HSV-2 (6), but HSV-1 has been reported to cause primary genital infection in recent years (7–9). As yet there is no efficient treatment of this sexually transmitted viral disease. Following primary infection, painful lesions appear on and around the genitalia lasting about 10–14 days followed by complete healing. During this time, the virus often travels to the dorsal root ganglia where the virus persists in a latent state; from time to time, and in certain individuals at a predictable schedule, the virus reactivates, resulting in an active infection in the genital area (10). The purpose of the present study was to investigate whether or not preventive treatment could control this disease.

The clinical and pathologic features of genital herpetic disease in female guinea pigs is similar to those of humans (11). The pathogenesis of genital herpes in guinea pigs has been studied as an animal model for human disease in this laboratory (12–14) and by others (15, 16). Acyclovir (ACV), administered intraperitoneally after the appearance of lesions ("therapeutic") has been shown to be somewhat effective in the reduction of genital lesions of two strains of HSV-2 (14, 15) infected guinea pigs but ineffective in alleviating the lesions of a strain of HSV-1 (13).

In order to test differences between the virus strains to ACV, two isolates of HSV-1 were compared in the present study. HSV-1 was utilized because inoculation uniformly results in genital lesions and virus excretion, unlike HSV-2 which results in some animals without genital lesions and occasionally without virus excretion (14, 15). It would therefore be extremely difficult to study prophylactic treatment of HSV-2-infected animals. HSV-1 genital infection in guinea pigs is a good model for primary acute infection, although HSV-1 does not cause recurrent disease as frequently as HSV-2 in humans (4) or in guinea pigs (13, 16).

One impediment to ACV efficacy is the short half-life—about 2 hr in the guinea pig

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and about 3 hr in the human which produce a highly variable plasma concentration when applied periodically (17). We have therefore instilled the drug by continuous infusion utilizing implanted osmotic minipumps (18). We chose this method to achieve constant infusion, avoiding the variation intake associated with putting the drug in drinking water or applying the drug locally. The amount of tissue ACV was measured and a consistent concentration of ACV in the various tissues of guinea pigs was noted. The most effective treatment of primary genital herpes infection was obtained when the drug was instituted prophylactically.

Materials and Methods. *Virus stocks.* Two strains of HSV-1 were used. Strain NYU-78 was originally isolated at autopsy from the brain tissue of a 5-month-old girl who died of disseminated herpes (19). Strain D1745 was initially isolated in our clinical laboratory from a genital lesion in a male patient. Two virus isolates from different body sites were chosen because this might improve our chances of finding different biological or pharmacological behvior in the experimental infection. Typing of HSV isolates was performed by cell culture selectivity, drug resistance and plaque reduction neutralization tests (20). Virus stocks were prepared in guinea pig embryo (GPE) cell cultures. When infected cell monolayers showed advanced CPE, they were frozen and thawed and the virus suspensions were clarified by centrifugation. The virus containing supernatant was frozen in aliquots at −70°C with 10% DMSO.

Cell cultures and virus assay. Primary GPE cells were prepared from 20- to 40-day-old Hartley guinea pig embryos as previously described (12). GPE cells were grown for up to four passages in Eagle's minimum essential medium (MEM) containing Hanks' balanced salt solution (HBSS), and 10% newborn donor calf serum. Confluent cell monolayers were maintained in MEM containing Earle's balanced salt solution (EBSS) and 2% calf serum. The HSV-1 infectivity titers were measured by plaque formation in GPE cells growing in plastic plates utilizing a 1% methylcellulose overlay. The infected cells were fixed and stained as previously described (13).

Guinea pig inoculation and virus isolation from the genital tract. Young adult female Hartley guinea pigs weighing approximately 500 g each were inoculated intravaginally with 0.1 ml of HSV-1 containing 10^5 PFU as described (13). Briefly, the vaginal membrane was broken and a dry cotton swab was inserted into the vagina, followed by an inoculum of 0.1 ml of virus delivered by a plastic tuberculin syringe without a needle. One or two pieces (about 0.5 cm^2) of soluble Gelfoam (Upjohn Co., Kalamazoo, Mich.) were placed into the vagina to prevent spillage of the inoculum. Virus excretions from the genital tract were determined by assaying vaginal swabs taken on Days 3, 5, 7, and 10 using GPE cell monolayers.

Assessment of clinical disease. Clinical manifestations of HSV-1 infection were assessed by the genital lesions which were graded from 0 to 4 as follows: 0, neither lesions nor swelling; 1, 1–5 vesicles; 2, 6–10 vesicles; 3, $\geqslant$11 vesicles; 4, $\geqslant$11 vesicles when associated with swelling and purulent discharge. As healing progressed, the crusted and scarred vesicles diminished in number often with progression of swelling and discharge. In such cases the lesion grade was one more than the grade for the number of vesicles. Loss of rectal sphincter control or bladder control were observed as continuous defecation with a flaccid rectal sphincter and continuous urinary dribbling. These findings as well as hindlimb paralysis (usually temporary) and mortality were separately recorded.

Drug administration. For continuous infusion, each Alzet minipump (2ML2, Alza Corp., Palo Alto, Calif.) was loaded with sodium acyclovir diluted in normal saline (one 500-mg ACV equivalent vial plus 2.4 ml saline) to yield an approximate concentration of 192 mg/ml. After a 4-hr start-up period, each pump delivers the drug at a constant rate of 10 μl/hr, resulting in a continuous infusion of 46 mg/day or approximately 92 mg/kg/day. The infusion is continuous for 7 days until the reservoir is exhausted. The pumps were implanted subcutaneously in the paraspinal region of the guinea pig utilizing ether anesthesia.

Each experiment consisted of 22 to 24 guinea pigs randomly divided into three groups: (a) for preinfection treatment, ACV was delivered via the osmotic minipump starting 1 day prior to virus inoculation; (b)

for postinfection treatment, ACV was given via the minipump starting 3 days after virus inoculation; and (c) sham treatment with the minipump containing normal saline 2 or 3 days after virus inoculation.

For intraperitoneal inoculation, 0.5 ml of sodium acyclovir at a concentration of 50 mg/ml, was given twice per day as previously described (13).

Determination of ACV in guinea pig tissues. The concentration of ACV in the various tissues was determined by radioimmunoassay. The measurements were kindly performed by Dr. P. deMiranda's laboratory, Burroughs-Wellcome Company (17, 21). The tissue distribution following continuous delivery was measured 3 or 4 days after implantation with an osmotic minipump delivering ACV at the rate of approximately 92 mg/kg/day. For comparison, the distribution following intraperitoneal injections was measured after animals received ACV twice daily for 11 days at 100 mg/kg/day. Sacrifice was 2 hr after the final injection of ACV.

Results. *Comparison of genital lesions induced by HSV-1 strains following ACV treatment.* The genital lesions induced by HSV-1 strain NYU-78 were significantly and markedly reduced on Days 5, 7, 10, 13, and 17 by prophylactic treatment (Fig. 1A). On Day 7 the genital lesions had become severe in the sham-treated animals, reaching a score of 2.3, while the pretreated animals scored 0.6. There was no significant difference between the post-treated and sham-treated groups. The ineffectiveness of ACV applied postinfection with this strain of virus via continuous infusion was similar to the previous finding when ACV was applied intraperitoneally (13).

The genital lesions induced by HSV-1 strain D1745 were also significantly reduced on Days 3, 5, and 7 by prophylactic treatment (Fig. 1B). On Day 7 the sham-treated animals had severe genital lesions which were rated 3.1, whereas the pretreated group had lesions rating 1.5. At Day 13 the lesions in the sham-treated animals remained poorly healed, averaging a score of 2.2, whereas the pretreated group were largely healed, averaging 0.8. As in the case of the NYU-78 strain, the postinfection treatment on genital lesions induced by D1745 strain by continuous infusion did not show any significant difference from the sham-treated controls.

Each of the animals in the control groups showed lesions and excreted virus indicating a 100% infection rate.

Differences in neurologic dysfunction induced by two HSV-1 strains following ACV treatment. Table I shows that continuous ACV

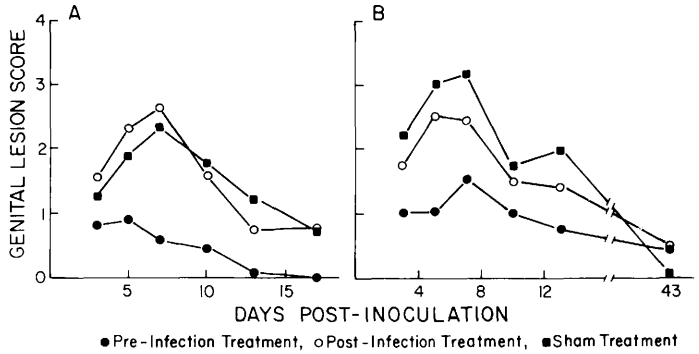

FIG. 1. Lesion score of guinea pigs genitally inoculated with HSV-1, (A) strain NYU-78 and (B) strain D1745, treated by continuous infusion of ACV. Guinea pigs were randomly divided into three groups and the drug or saline in the minipumps was delivered continuously for a duration of 7 days. In (A), 8 were 1-day preinfection treated, 8 were 3-days postinfection treated, and 8 were 3-days postinfection sham-treated. On Days 5 ($P < 0.01$), ($P < 0.001$), 10 ($P < 0.01$), 13 ($P < 0.01$), and 17 ($P < 0.05$). In (B), 8 were 1-day preinfection treated, 7 were 3-days postinfection-treated, and 7 were 2-days postinfection sham-treated. On Days 3 ($P < 0.01$), 5 ($P < 0.01$), and 7 ($P < 0.01$). The significant difference between the preinfection-treated and sham-treated groups are stated above. There was no significant difference between the postinfection-treated and the sham-treated groups. The significance was determined by the Student's t test (two-tailed) with $P < 0.05$ considered significant.

TABLE I. EFFECT OF ACV ON NEUROLOGIC DYSFUNCTION IN GUINEA PIGS FOLLOWING GENITAL INFECTION WITH HSV-1

HSV-1 virus strain	Initiation of ACV treatment	Loss of bladder or rectal control	Hindlimb paralysis	Death
NYU-78	1 Day preinfection	0/8	0/8	0/8
	3 Days postinfection	8/8	0/8	0/8
	Sham treated 3 days postinfection	7/8	0/8	2/8
D1745	1 Day preinfection	0/8	0/8	0/8
	3 Days postinfection	1/7	0/7	0/8
	Sham treated 2 days postinfection	5/7	1/7	1/7

treatment of HSV-1 (NYU-78) instituted before infection reduced the incidence of urinary or rectal incontinence from 88% (sham-treated) to 0% (1 day preinfection), whereas the postinfection treatment was not effective. In contrast, ACV treatment of D1745 was effective both prophylactically and therapeutically. In D1745-infected animals bladder/rectal incontinence, unlike the genital lesions, responded to therapeutic ACV treatment (Fisher exact test, 1 tail, $P < 0.05$). There was little hindlimb paralysis or death in any of the infected animals.

Excretion of virus in the genital tracts following HSV-1 inoculation and continuous ACV infusion pre- and postinfection. Recovery of virus from the genital tracts was reduced in animals receiving ACV prior to virus infection with one HSV-1 strain. Animals infected with NYU-78 strain did not have virus recovered from the genital tracts of 3/8 guinea pigs which received ACV 1 day prior to virus infection (Table II), whereas all eight animals in the post-ACV-treated and eight sham-treated animals showed significant virus titers in their vaginal excretions tested on Days 3 and 5 post-virus inoculations. Slightly lower virus titers were noted in animals receiving ACV 1 day prior to infection with D1745, tested 3 days postinfection, and somewhat lower virus excretions were obtained from animals treated 3 days postinfection with D1745 and tested on Days 5 and 7 (Table II). By Day 10 none of the animals excreted an infectious virus from the genital tracts.

Distribution of ACV in the various tissues of guinea pigs following continuous subcutaneous perfusion and intraperitoneal administration. In an effort to demonstrate that ACV reached a widespread array of tissues, the concentration of ACV in the various tissues was determined. The results are shown in Table III. Regardless of route of drug administration, the drug failed to penetrate the tissues of the central nervous system, i.e., the brain and spinal cord contained undetectable or trace amounts of ACV by both routes. Contrarily, the dorsal root ganglia contained significant levels of ACV which were slightly higher than that in the plasma. The parenchymal organs

TABLE II. VIRUS RECOVERY FROM GENITAL TRACTS DURING ACUTE INFECTION

HSV-1 virus strain	Initiation of ACV treatment	Average virus infectivity titers in genital tract at days postinfection (log PFU/0.1 ml)			
		3	5	7	10
NYU-78	1 Day preinfection	1.8*	<1.0	<1.0	ND
	3 Days postinfection	3.1	2.8	<1.0	ND
	Sham treated 3 days postinfection	3.9	3.2	<1.0	ND
D1745	1 Day preinfection	3.4	4.2	2.4	<1.0
	3 Days postinfection	4.5	2.8	1.5	<1.0
	Sham treated 2 days postinfection	5.0	4.1	3.4	<1.0

* Three of eight animals showed no virus excretion. ND = not done.

TABLE III. DISTRIBUTION OF ACV IN GUINEA PIG TISSUES

Tissue tested	ACV concentration (μg/ml) tissue suspension	
	Intraperitoneal injection*	Subcutaneous perfusion**
Plasma	2.4	11.5
Spleen	7.5	7.3
Kidney	24.0	43.5
Liver	8.0	31.5
Brain	<0.2	<0.2
Spinal cord	0.24	0.9
Dorsal root ganglia	3.4	16.4

* Animal was given ACV intraperitoneally for 11 days as described and sacrificed 2 hr after the last intraperitoneal injection.

** Average of two animals sacrificed 2–4 days after minipump implantations.

tested all contained high levels of the drug. Similar findings were noted in both infected and noninfected guinea pigs.

Discussion. The prophylactic infusion of ACV constituted by far the most effective treatment of genital herpes, greatly reducing lesion scores in both strains of HSV-1 tested. In addition, bladder or rectal incontinence was nearly eliminated by ACV infusion prophylactically. Therapeutic treatment was not effective in reducing these sequellae with strain NYU-78 but some effectiveness was achieved with strain D1745. Thus, our data showed that there are differences in virus strains in response to ACV.

The tissue distribution of ACV was measured to determine accessibility of ACV to the brain, spinal cord, and sensory ganglia. As previously shown, ACV readily distributed throughout the parencymal organs (21). It was worth noting that the dorsal root sensory ganglia were readily accessible to ACV but not the spinal cord and brain. ACV therefore may potentially be effective in reducing the establishment of HSV latency in the sensory ganglia, as we reported in a previous study (14).

The clinical and pathologic features of genital herpetic disease in female guinea pigs is similar to those of humans (11). Our data suggest that by far the most effective treatment was prior to virus infection regardless of virus strains used, and continuous perfusion was in general a more effective method of drug administration. Although this is not feasible in humans in most situations, there are times when viral reactivation can be predicted, for example, genital herpes may recur during the menstrual period. Herpetic reactivation may also occur at time of irradiation for control of malignancy or immunosuppression, commonly used for organ transplantation. The guinea pig model offers encouragement for the prevention and control of herpes lesions by pretreatment, similar to the findings reported for humans (22).

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