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Recurrent genital herpes in the guinea pig augmented by ultraviolet irradiation: effects of treatment with acyclovir

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Summary

The guinea pig model of genital herpes simplex virus infection has proven useful in the evaluation of antiviral drugs. We have recently demonstrated that recurrent herpetic infections can be induced in latently infected guinea pigs by ultraviolet irradiation. In this report we have examined the effect of acyclovir on ultraviolet radiation-induced recurrent genital herpes. Prophylactic topical acyclovir decreased the severity but not the incidence of ultraviolet radiation-induced recurrences while intraperitoneal acyclovir initiated before ultraviolet irradiation reduced both the incidence and severity of induced recurrences. When treatment was begun after ultraviolet exposure, neither topical nor intraperitoneal acyclovir were effective in reducing the incidence or severity of induced recurrent disease. The effectiveness of acyclovir in the control of induced recurrent genital infections in the guinea pig is similar to what has been observed in human trials. This model of ultraviolet radiation-induced recurrent herpes simplex virus infection should prove useful in the evaluation on new putative antiviral drugs.

Acyclovir; Recurrent genital herpes; Herpes simplex virus; Guinea pig model

Introduction

Recurrent herpes simplex virus (HSV) infections result from the reactivation of latent virus in sensory ganglia with transport of virus to the periphery where rep-

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lication produces characteristic herpetic lesions (Klein, 1985). Ultraviolet (UV) radiation has been used to experimentally induce recurrent cutaneous HSV infections in humans and animals (Blyth et al., 1976; Norval et al., 1987; Perna et al., 1987; Spruance, 1985; Spurney and Rosenthal, 1972; Wheeler, 1975). It has been suggested that these models of induced recurrent disease can be used to study the efficacy of antiviral drugs (Spruance, 1988).

The guinea pig model of genital HSV infection has proven useful in the evaluation of putative antiviral compounds (Harrison et al., 1988; Provonost et al., 1982; Richards et al., 1985; Stanberry et al., 1986). After recovery from primary infection the frequency of spontaneous recurrent disease in the guinea pig is initially high (one to two episodes per week); however, the frequency declines over time such that by day 100 post-inoculation recurrences are infrequent (Stanberry et al., 1985). Recently, we have developed a model of UV radiation-induced recurrent genital herpes using the guinea pig (Stanberry, 1989). Employing latently infected animals more than 100 days post HSV-2 inoculation we have successfully used UV radiation to reproducibly induce recurrent genital disease. In this report we have examined the utility of this model in evaluating the efficacy of acyclovir in the control of UV radiation-induced recurrent cutaneous herpetic disease.

Materials and Methods

Animal model

Primary genital infection was produced in 250–300 g female Hartley guinea pigs (Charles River Breeding Laboratories, Wilmington, MA) by intravaginal inoculation with 5.7 $\log_{10}$ pfu HSV-2, strain MS (ATCC VR-540). One hundred days after recovery from primary infection animals were randomized into treatment groups. In the first experiment, the five day drug or placebo regimen was begun on day 108 post-inoculation followed by UV radiation exposure of Metaphane anesthesized animals on day 109. The pattern of recurrent disease was determined from day 110 through day 114. In the second experiment, the same animals were re-randomized into new treatment groups to assess the effectiveness of acyclovir begun after UV exposure. Animals were irradiated on day 130 post-inoculation and the five day drug or placebo regimen was started on day 131. The pattern of recurrent disease was assessed from day 131 through day 135. Evidence of recurrent lesions was sought once per day (at approximately noon) by careful inspection of the guinea pig perineum. Lesion days were defined as days when clinically apparent herpetic lesions were observed on the perineal skin. The incidence of induced disease was quantified by determining the percentage of animals developing recurrent lesions each day for five days after UV radiation exposure. The severity of induced recurrent disease was defined by the duration of lesions and was measured by calculating the mean lesion days per observation period (i.e. five days) for animals experiencing recurrences. The time between UV radiation exposure and development of herpetic lesions was defined as the time to onset of induced lesions.

Utraviolet radiation

The perineal skin of anesthetized guinea pigs was exposed for 10 min to UV-B light produced by transilluminators emitting radiation between 280–320 nm with a peak output of 7000 $\mu W/cm^2$ at 302 nm (UVP, Inc, San Gabriel, CA). The output of the transilluminators was determined using an UV radiometer (UVP, Inc). Mock-irradiated animals were anesthetized and placed on a non-operating transilluminator bed.

Drug treatment

Parenteral treatment consisted of 6 cc of either saline or acyclovir dissolved in saline (2.5 mg/cc) (Burroughs Wellcome Co., Research Triangle Park, NC) administered by intraperitoneal injection twice daily for 5 days. The total dose was approximately 50 mg/kg/day. Topical cutaneous treatment with either polyethylene glycol (PEG) or 5% acyclovir ointment (Burroughs Wellcome Co., Research Triangle Park, NC) was administered by applying 0.1 cc intravaginally and 0.1 cc to the perineal skin four times daily for 5 days.

Results

We first investigated whether antiviral therapy initiated prior to UV irradiation could prevent or ameliorate induced recurrent herpetic disease (Table 1). A five day course of parenteral or topical acyclovir treatment was begun 108 days after intravaginal HSV-2 inoculation, and 24 h later on day 109 post-inoculation (p.i.), the perineal skin of drug and placebo treated animals was exposed to UV radiation. As shown in Table 1, 27% of untreated, non-irradiated animals developed spontaneous recurrent disease and the severity of disease, as measured by the duration of lesions (mean lesion days) was 1.3 ± 0.3 days. Exposure to UV radiation increased the incidence and severity of recurrent disease in both the topical and parenteral placebo groups; however, only the incidence of disease was significantly enhanced by UV irradiation.

Compared to the placebo groups, topical acyclovir begun before UV radiation significantly reduced the severity but not the incidence nor time to onset of induced recurrences ($P = 0.04$, 0.17 and 0.74, respectively). Systemically administered acyclovir, however, significantly reduced both the incidence and severity of UV-induced recurrent disease compared to saline ($P = 0.008$ and 0.02, respectively) and prolonged the time to onset of lesions following UV radiation exposure ($P = 0.05$). The percentage of pretreated animals experiencing recurrent genital HSV-2 lesions each day is shown in Fig. 1, while the cumulative recurrence pattern is presented in Fig. 2.

We next explored whether antiviral therapy started after UV radiation exposure could alter the natural history of induced recurrent genital herpes. A five day regimen of either intraperitoneal or topical acyclovir was begun 24 h after UV irra-

TABLE 1

Effect of acyclovir treatment initiated before ultraviolet radiation exposure on the incidence and severity of induced herpetic disease

Treatment[a]	UV[b]	Incidence[c]		Severity[d]		Onset[e]	
		Animals	*P* Value	Days	*P* value	Days	*P* value
None	–	3/11	–	1.3±0.3	–		
Topical PEG	+	6/8	0.049	1.8±0.2	0.17	2.5±0.5	–
IP. Saline	+	14/16	0.002	2.3±0.3	0.14	1.9±0.2	0.21
Topical PEG	+	6/8	–	1.8±0.2	–	2.5±0.5	–
Topical ACV	+	5/11	0.17	1.2±0.2	0.04	2.2±0.7	0.74
IP saline		14/16	–	2.3±0.3	–	1.9±0.2	–
IP ACV	+	4/11	0.008	1.0±0	0.02	2.7±0.3	0.05

[a]Treatment initiated on day 108 post HSV-2 inoculation, 24 h before ultraviolet radiation exposure and continued for 5 days (see text).
[b]Ultraviolet radiation exposure for 10 min. 7000 μW/cm^2 at 302 nm.
[c]Incidence expressed as number of animals with recurrences over the total number of animals. Data evaluated using Fischer's exact test, values for irradiated placebo groups vs non-irradiated untreated controls; or irradiated placebo vs irradiated treated groups.
[d]Severity expressed as mean lesion days ± standard error for a five day observation period from day 110 through 114 post-inoculation for animals with recurrent disease. Data evaluated using Student's *t*-test.
[e]Time to onset of lesions following UV irradiation, mean days ± standard error for animals with recurrent disease. Data evaluated using Student's *t*-test.

diation. Similar to the previous experiment, 25% of untreated non-irradiated guinea pigs exhibited spontaneous recurrences with an average duration of lesions (severity) of 1.7 ± 0.7 days (Table 2). Compared to the non-irradiated guinea pigs

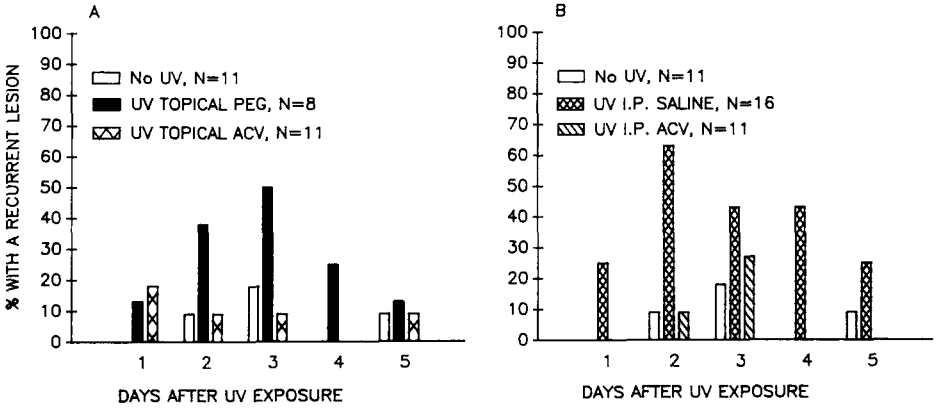

Fig. 1. Effect of prophylactic acyclovir treatment on the incidence of UV radiation-induced recurre disease. (A), topical drug or placebo; (B), intraperitoneal drug or placebo.

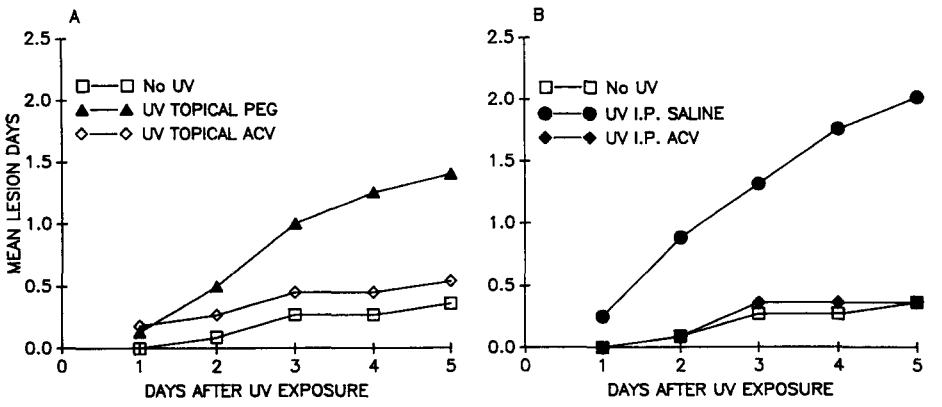

Fig. 2. Cumulative mean number of days when UV radiation-induced recurrent herpetic lesions were observed: Effect of prophylactic acyclovir treatment. (A), topical drug or placebo; (B), intraperitoneal drug or placebo.

UV radiation significantly increased the incidence but not the severity of recurrences in the placebo groups. Topical acyclovir did not reduce the incidence, severity or time to onset of UV radiation induced of recurrent disease while intra-

TABLE 2

Effect of acyclovir treatment initiated after ultraviolet radiation exposure on the incidence and severity of induced recurrent herpetic disease

Treatment[a]	U-V[b]	Incidence[c]		Severity[d]		Onset[e]	
		Animals	*P* Value	Days	*P* value	Days	*P* value
None	−	3/12	−	1.7±0.7	−		
Topical PEG	+	8/12	0.044	1.9±0.4	0.53	2.7±0.4	−
IP saline	+	10/11	0.002	2.0±0.2	0.77	2.7±0.4	1.0
Topical PEG	+	8/12	−	1.8±0.4	−	2.7±0.4	−
Topical ACV	+	10/11	0.16	2.1±0.2	0.59	1.9±0.5	0.16
IP saline	+	10/11	−	2.0±0.2	−	2.7±0.4	−
IP ACV	+	6/11	0.068	1.7±0.3	0.33	2.7±0.5	1.0

[a]Treatment initiated on day 131 post HSV-2 inoculation, 24 h after ultraviolet radiation exposure and continued for 5 days (see text).

[b]Ultraviolet radiation exposure for 10 min; 7000 μW/cm^2 at 302 nm.

[c]Incidence expressed as number of animals with recurrences over the total number of animals. Data evaluated using Fischer's exact test, values for irradiated placebo groups vs non-irradiated untreated controls; or irradiated placebo vs irradiated treated groups.

[d]Severity expressed as mean lesion days ± standard error for a five day observation period from days 131 through 135 post-inoculation for animals with recurrent disease. Data evaluated using Student's *t*-test.

[e]Time to onset of lesions following UV irradiation, mean days ± standard error for animals with recurrent disease. Data evaluated using Student's *t*-test.

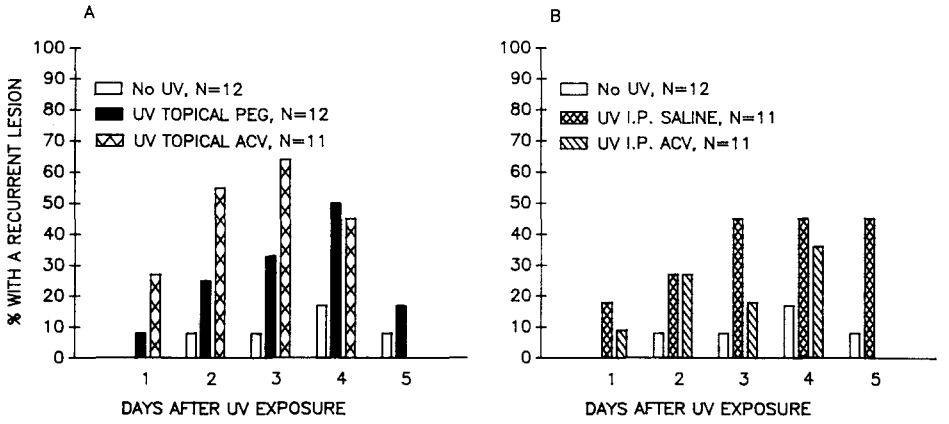

Fig. 3. Effect of acyclovir therapy begun after UV exposure on the incidence of UV radiation-induced recurrent disease. (A), topical drug or placebo; (B), intraperitoneal drug or placebo.

peritoneal acyclovir tended to reduce the incidence of recurrent disease compared to saline ($P = 0.068$) without affecting the severity or time to onset of recurrences. The effect of antiviral treatment initiated after UV irradiation on the percentage of animals experiencing recurrent disease and on the cumulative recurrence pattern is shown in Fig. 3 and 4.

Discussion

In this study we have confirmed our previous report that recurrent genital herpes can be induced in latently infected guinea pigs by exposure to UV radiation (Stan-

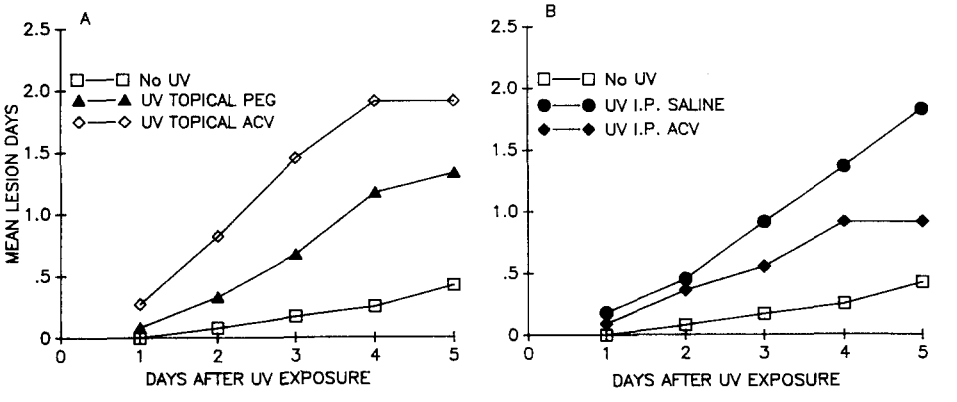

Fig. 4. Cumulative mean number of days when UV radiation-induced recurrent herpetic lesions were observed: effect of acyclovir therapy begun after UV exposure. (A), topical drug or placebo; (B), intraperitoneal drug or placebo.

berry, 1989). Further, we have shown that the administration of antiviral therapy can effectively reduce both the incidence and severity of UV-induced recurrent disease in the guinea pig as well as delay the onset of recurrent infection following UV irradiation. In this study systemically administered acyclovir was more effective than topically applied drug and therapy begun before UV irradiation was more effective than treatment initiated after UV exposure. These results parallel clinical experience using acyclovir for the treatment of spontaneously recurring herpetic disease. For example, oral but not topical acyclovir was effective in shortening the clinical course of recurrent genital herpes (Nilsen et al., 1982; Reichman et al., 1983; Luby et al., 1984). Likewise, early, patient-initiated treatment of herpes labialis with topical acyclovir was ineffective (Spruance et al., 1984), while treatment with oral acyclovir appeared to ameliorate some aspects of herpes labialis (Spruance et al., 1986). More recently it was reported that prophylactic oral acyclovir effectively prevented solar UV radiation-induced herpes simplex labialis in skiers (Spruance et al., 1988) as well as reducing the severity of herpes labialis in human volunteers experimentally exposed to UV radiation (Spruance, 1988). Interestingly, Spruance (1988) noted that the time from irradiation to onset of lesions was reduced in acyclovir treated humans while we noted that prophylactic intraperitoneal acyclovir actually prolonged the time between UV irradiation and the development of herpetic lesions. These differences in the effects of acyclovir on UV radiation induced disease warrant further study.

UV-induced recurrent genital herpes in the guinea pig may be a useful addition to the experimental models currently available for the study of antiviral drugs. Advantages of the model include the ability to reproducibly induce recurrent disease, the modest equipment required, the use of a clinical (inexpensive) rather than a virologic (expensive) measure of efficacy and the fact that latently infected guinea pigs, like humans, can be used in repetitive experiments to screen new antiviral compounds. Additionally, because the effectiveness of a putative antiviral drug in controlling recurrent infection can be determined within five days using a short course of therapy, the model is well suited for the evaluation of drugs available in limited supply. The principle disadvantage of this model is the expense of purchasing guinea pigs and the need to maintain animals for 100 days after intravaginal HSV-2 inoculation before use in UV-induced recurrence experiments.

In conclusion, the guinea pig model of UV radiation-induced recurrent genital HSV-2 infection can be used to assess the effectiveness of antiviral therapy in both the prevention and the treatment of recurrent herpetic disease. The many similarities between genital HSV infection in humans and guinea pigs (Kern, 1984; Stanberry, 1986) suggest that antiviral efficacy in this model may be predictive of efficacy in humans.

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References

Blyth, W.A., Hill, T.J., Field, H.J. and Harbour, D.A. (1976) Reactivation of herpes simplex virus infection by ultraviolet light and possible involvement of prostaglandins. J. Gen. Virol. 33, 547–550.

Harrison, C.J., Jenski, L., Voychehovski, T. and Bernstein, D.I. (1988) Modification of the immunological responses and clinical disease during topical R837 treatment of genital HSV-2 infection. Antiviral Res. 10, 209–224.

Kern, E.R. (1984) Treatment of genital herpes simplex virus infections in guinea pigs. In: F. Rapp (Ed.), Herpesvirus, pp. 617–636. Liss, New York.

Klein, R.J. (1985) Initiation and maintenance of latent herpes simplex virus infections: the paradox of perpetual immobility and continuous movement. Rev. Infect. Dis. 7, 21–30.

Luby, J.P., Gnann, J.W., Jr., Alexander, W.J., Hatcher, V.A., Friedman-Kien, A.E., Klein, R.J., Keyserling, H., Nahmias, A., Mills, J., Schachter, J., Douglas, J.M., Corey, L. and Sacks, S.L. (1984) A collaborative study of patient-initiated treatment of recurrent genital herpes with topical acyclovir or placebo. J. Infect. Dis. 150, 1–6.

Nilsen, A.E., Aasen, T., Halsos, A.M., Kinge, B.R., Tjotta, E.A.L., Wikstrom, K. and Fiddian, A.P. (1982) Efficacy of oral acyclovir in the treatment of initial and recurrent genital herpes. Lancet ii, 571–573.

Noval, M., Howie, S.E.M., Ross, J.A. and Maingay, J.P. (1987) A murine model of herpes simplex virus recrudescence. J. Gen. Virol. 68, 2693–2698.

Perna, J.J., Mannix, M.L., Rooney, J.F., Notkins, A.L. and Straus, S.E. (1987) Reactivation of latent herpes simplex virus infection by ultraviolet light: a human model. J. Am. Acad. Dermatol. 17, 473–478.

Pronovost, A.D., Lucia, H.L., Dann, P.R. and Hsiung, G.D. (1982) Effect of acyclovir on genital herpes in guinea pigs. J. Infect. Dis. 145, 904–908.

Reichman, R.C., Badger, G.J., Guinan, M.E., Nahmias, A.J., Keeney, R.E., Davis, L.G., Ashikaga, T. and Dolin, R. (1983) Topically administered acyclovir in the treatment of recurrent herpes simplex genitalis: a controlled study. J. Infect. Dis. 147, 336–340.

Richards, J.T., Katz, M.E. and Kern, E.R. (1985) Topical butylated hydroxytoluene treatment of genital herpes simplex virus infections of guinea pigs. Antiviral Res. 5, 281–290.

Spruance, S.L. (1985) Pathogenesis of herpes simplex labialis: Experimental induction of lesions with UV light. J. Clin. Microbiol. 22, 366–368.

Spruance, S.L. (1988) Cutaneous herpes simplex virus lesions induced by ultraviolet radiation. Am. J. Med. 85 (Suppl. 2A), 43–45.

Spruance, S.L., Crumpacker, C.S., Schnipper, L.E., Kern, E.R., Marlowe, S., Arndt, K.A. and Overall, J.C., Jr. (1984) Early, patient-initiated treatment of herpes labialis with topical 10% acyclovir. Antimicrob. Agents Chemother. 25, 553–555.

Spruance, S.L., Rowe, N., Stewart, J., Wenerstrom, G., Freeman, D. and Davis, G. (1986) Treatment of herpes simplex labialis with oral acyclovir. Program and abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Abstract No. 623.

Spruance, S.L., Hamill, M.L., Hoge, W.S., Davis, L.G. and Mills, J. (1988) Acyclovir prevents reactivation of herpes simplex labialis in skiers. J. Am. Med. Assoc. 260, 1597–1599.

Spurney, R.V. and Rosenthal, M.S. (1972) Ultraviolet-induced recurrent herpes simplex keratitis. Am. J. Opthalmol. 73, 609–610.

Stanberry, L.R. (1986) Herpesvirus latency and recurrence. Prog. Med. Virol. 33, 61–77.

Stanberry, L.R. (1989) Animal model of ultraviolet-radiation-induced recurrent herpes simplex virus infection. J. Med. Virol. 28, 125–128.

Stanberry, L.R., Kern, E.R., Richards, J.T. and Overall, J.C., Jr. (1985) Recurrent genital herpes simplex virus infections in guinea pigs. Intervirology 24, 226–231.
Stanberry, L.R., Bernstein, D.I. and Myers, M.G. (1986) Evaluation of the herpes simplex virus antiviral activity of pyrethrins. Antiviral Res. 6, 95–102.
Wheeler, C.E., Jr. (1975) Pathogenesis of recurrent herpes simplex infections. J. Invest. Dermatol. 65, 341–346.