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Effects of cytokines and R-837, a cytokine inducer, on UV-irradiation augmented recurrent genital herpes in guinea pigs

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Summary

We have recently reported that latently HSV-2-infected guinea pigs exhibit a three- to four-fold increase in recurrent lesions after exposure to ultraviolet radiation (UV), allowing rapid evaluation of antiviral drugs in treating recurrent HSV disease. In this report we examine the effect of alpha interferon (IFN-α), interleukin-2 (IL-2), and a cytokine inducer (R-837) on UV-induced recurrent genital herpes. We have previously shown that topical R-837 is a biologic response modifier with no in vitro anti-HSV activity, but with potent anti-HSV activity in vivo due to cytokine induction and enhancement of cell-mediated immune responses. Three-day regimens of intravaginal R-837, or five-day intraperitoneal (i.p.) administration of IFN-α or of IL-2 each significantly reduced recurrent genital HSV-2 disease that occurred within 7 days of UV exposure, suggesting that cytokines or cytokine inducers may be useful in the treatment of recurrent HSV disease. This model using ultraviolet radiation to induce recurrent herpes simplex virus infection proved useful in the evaluation of immunoactive agents as putative antiviral drugs.

R-837; Recurrent genital herpes; Biologic response modifier; IL-2; Interferon, UV radiation

Animals used in this study were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Children's Hospital Research Foundation

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Introduction

Recurrent herpes simplex virus (HSV) lesions are manifestations of reactivated HSV that has been transported from sensory ganglia to peripheral sites (Klein, 1985) Experimentally applied ultraviolet radiation (UV) has been observed to increase clinically apparent HSV recurrences in humans and animals (Blyth et al , 1976; Norval et al., 1987. Perna et al , 1987; Spruance, 1985, 1988, Spurney and Rosenthal, 1972) We and others have suggested that animal models of induced recurrent disease could be used to study the efficacy of antiviral drugs (Spruance, 1988, Stanberry et al , 1990)

The guinea pig is a reliable model of human genital HSV infection and has been used to evaluate the pathogenesis of spontaneous recurrent genital disease and predict the efficacy of putative antiviral compounds (Bernstein et al , 1986; Harrison, et al., 1988, Provonost et al , 1982; Richards et al., 1985) In the first six weeks after recovery from primary genital infection, the frequency of spontaneous recurrent disease in the guinea pig is relatively high (one to two recurrences per week) However, by day 80–100 post-inoculation recurrences are infrequent (Harrison et al., 1988; Stanberry et al , 1985). Recently, we reported increased genital recurrences in guinea pigs distantly infected with HSV-2 MS strain up to 7 days after exposure to UV radiation (Stanberry, 1989) and their suppression by prophylactic acyclovir treatment (Stanberry et al , 1990). In this report we used the UV-induced reactivation model to compare the efficacy of three immunomodifying agents administered after UV exposure in reducing UV augmented recurrent HSV-2 strain 333 genital 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 333 (Harrison et al., 1988). Eighty days after recovery from primary infection animals were randomized into treatment groups. Five-day treatment (except R-837 = 3-day) regimens were begun within 6 h of UV radiation exposure of Metaphane anesthetized animals (Stanberry et al , 1990) Evidence of recurrent lesions was sought once per day (at approximately noon) by careful inspection of the guinea pig perineum for 7 days. This was followed by a 10-day washout period The same groups were then randomly crossed over into alternate treatment groups, exposed to UV and begun on five-day treatments (except R-837 = 3-day) within 6 h of UV exposure. The untreated mock-UV-exposed group was not crossed over. The pattern of recurrent disease was again assessed for 7 days after UV exposure.

Lesion days were defined as days when clinically apparent herpetic lesions were observed on the perineal skin. Severe recurrences were defined as those lesion days

when more than one vesicle was observed concurrently. The time between UV radiation exposure and development of herpetic lesions was defined as the time to onset of induced lesions

Ultraviolet radiation

The perineal skin of anesthetized guinea pigs was exposed for 10 min to UV-B light produced by transilluminators emitting radiation between 280 and 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.

Hartley females were divided into treatment groups of 10 each for each of two experiments, resulting in a total of 20 animals for each treatment group:

(1) R-837 (5 mg/kg), kindly supplied as a semisolid creamy paste by 3M Pharmaceuticals, St. Paul, Minnesota, q.d. intravaginally (Harrison et al., 1988) on days 1–3, initiated 4–6 h post UV exposure;
(2) interferon alpha (IFN-α, Hoffman LaRoche) 2×10^6 U/kg q.d. intraperitoneal (i.p.) on days 1–5 initiated 4–6 h post UV exposure;
(3) interleukin-2 (IL-2) kindly supplied by Cetus Corp., Emoryville, California, was administered at 30 000 U/kg i p. b.i d. $\times$ 5 days also begun 4–6 h post UV exposure,
(4) placebo controls received topical placebo (R-837 vehicle without active drug) q.d. on days 1–3, initiated 4–6 h post UV exposure;
(5) unmanipulated controls received mock UV and no treatment as a measure of background spontaneous recurrences

Dosing of each drug was based on preliminary dosing experiments indicating efficacy of these doses for treatment of primary genital HSV.

Statistics

Differences in means were analyzed by analysis of variance, and incidence data was analyzed by Chi square unless cell size was less than 5 when Fisher Exact testing was performed

Results

Animals exhibiting recurrences There was no significant difference in the data obtained during the first and second post UV radiation observation periods (Table 1), therefore data from control groups and from each treatment group from both experiments were combined for analysis. The background rate of clinically identifiable spontaneous recurrences in the latently HSV-infected, untreated, mock-UV-radiated control group was 20% (4/20; Table 2). During the week of observation, significantly more animals in the UV-exposed placebo recipients developed recur-

TABLE 1

Comparison of first and second UV exposures in groups of control and experimentally treated animals

Group	Lesion days for 7 days after UV exposure	
	First exposure	Second exposure
Control	15	20
R-837	7	5
Interferon	7	6
IL-2	5	9

rences than those in the untreated mock-UV-exposed control group (16/20, $P = 0.001$, Table 2) The groups treated topically for three days with R-837, or intraperitoneally for five days with either IFN-α or IL-2 exhibited a significantly reduced incidence of recurrences ($P < 0.03$) in the week after the UV exposure (Table 2) After UV exposure, seven of the placebo recipients and one IFN-α-treated animal, but none of the animals treated with IL-2 or R-837, experienced a severe recurrence (two or more concurrent vesicles on the genital skin, Table 2).

When analyzing only those animals experiencing recurrences, cumulative mean lesion days per group were significantly lower comparing the placebo group and the groups treated with R-837 and IFN-α, but not IL-2 (Table 2). When considering all animals, including those without recurrences, mean cumulative lesion days were significantly lower ($P < 0\ 03$) than UV-exposed placebo recipients in each group treated with an active immunomodifying agent (Fig. 1).

Time to onset of recurrence was different from the placebo group only for the R-837 group, mean±SD = 2 9±1 8 days compared to 4.5±2 0 days respectively ($P < 0.03$) The daily distribution of animals exhibiting lesions for the experimental groups is shown in Fig 2 Genital lesions were fairly uniformly distributed throughout the observation period for all groups except the interferon-treated group

TABLE 2

UV radiation-induced increase in HSV genital recurrence rates in latently HSV infected guinea pigs more than 80 days after primary infection effects of treatment with alpha interferon (IFN-α) interleukin-2 (IL-2), or the immunomodifier R-837

Treatment	UV	Recurrent disease			
	(+/−)	Incidence overall	Severe (N)	Total lesion days [a]	Cumulative mean lesion days[b]
None	−	4/20 (20%)	0	6	1 5±0 3
Placebo	+	16/20 (80%)	7[c]	36	2 2±0 2
R-837 (3-day)	+	9/20 (45%)[d]	0	12	1 3±0 2[c]
IFN-α (5-day)	+	8/20 (40%)[d]	1	13	1 6±0 2[e]
IL-2 (5-day)	+	8/20 (40%)[d]	0	14	1 8±0 2

[a]Days with any lesion

[b]Per animal only for animals with recurrences during observation

[c]$P = 0\ 02$ compared to each treatment group

[d]$P < 0\ 03$ compared to placebo

[e]$P < 0\ 05$ compared to placebo

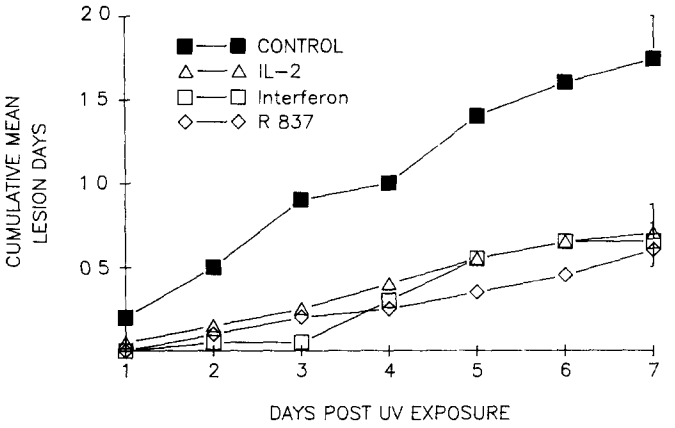

Fig 1 Cumulative mean (± SE) number of lesion days per animal For seven days following UV exposure 20 animals per group were followed daily for the development of recurrent herpetic lesions Mean lesions represent the mean for all 20 animals

in which recurrences occurred predominantly on days 4 and 5 after UV exposure (Fig. 2).

Discussion

In this study we have confirmed our previous reports that recurrent genital herpes can be induced in latently infected guinea pigs by exposure to UV radiation (Stanberry, 1989; Stanberry et al., 1990). Further, we have shown that the administration of cytokines with reported activity against primary HSV-2 genital disease (Kern, 1984; Weinberg, 1986) and an immunomodulator with in vivo but not in

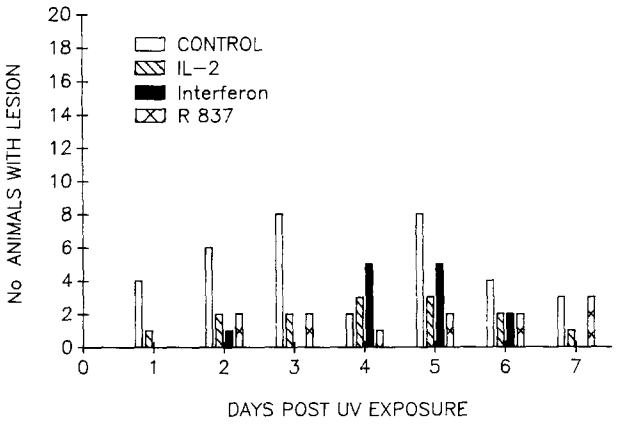

Fig 2 Number of animals developing recurrent herpetic lesions on each day during the seven days following UV exposure

vitro anti-HSV activity (Bernstein and Harrison, 1989, Harrison et al., 1988) can effectively reduce both the incidence and severity of UV-induced recurrent disease Delayed onset of recurrent disease following UV irradiation was noted only in the R-837-treated group.

In the investigations reported here, all treatments were begun after UV exposure. Previously we reported that systemically administered acyclovir was more effective than topically applied drug and acyclovir was effective only if begun before UV irradiation (Stanberry et al., 1990) Thus, the immunomodifying agents evaluated here, including a topically applied compound, begun 4–6 h after UV exposure, appear to be more effective than systemic acyclovir initiated within 2 h of UV exposure These results suggest the need to evaluate topical R-837 in humans likely to experience clinical recurrences after UV exposure, while similar trials of the cytokines may be less attractive due to the need for systemic administration

Cytokines have been postulated as being important in modification of HSV disease. For example, recurrences in HSV disease in humans have recently been associated with decreased HSV-specific in vitro cytokine production, including IL-2 and IFN-α, or altered T lymphocyte subsets (Kuo and Lin, 1990; Sheridan et al , 1982). Likewise, previous reports in animals suggest that increased MHC-unrestricted cytolytic activity against HSV-infected targets induced by IL-2 administration decreased genital HSV-2 disease (Weinberg et al , 1987) Low MHC-unrestricted cytolytic activity at the time of clinical recurrences has also been observed (Kuo and Lin, 1990, Lopez and O'Reilly, 1977) We have also reported enhanced HSV-specific IL-2 production and HSV-specific cell-mediated responses in conjunction with reduced recurrent disease when animals were treated topically with the biologic response modifier, R-837 (Harrison et al., 1988).

Such data implicating a role for antigen-specific cytokine induction and HSV-specific cell-mediated responses suggest that immunotherapy that enhances such responses could be a reasonable strategy for suppression of recurrent HSV disease We also have previously reported that immunotherapy with glycoprotein B and D suppressed recurrent HSV disease independent of antibody titers (Stanberry et al , 1989). It is interesting to postulate that memory-dependent cytokine production and perhaps enhancement of MHC-unrestricted cytolytic activity against HSV-infected cells, which is enhanced by cytokines in vivo (Weinberg et al , 1986) and in vitro (author's unpublished data) may play a role in the protection afforded by gBgD immunization. Further investigations into the effects on recurrent HSV disease of the interplay of cytokines and HSV-specific cytolytic activity in response to R-837 and/or immunotherapy with specific glycoproteins are ongoing

In conclusion, the guinea pig model of UV radiation-induced recurrent genital HSV-2 infection was used to evaluate the effect of cytokine and immunomodulator therapy in the prevention of recurrent herpetic disease. The many similarities between genital HSV infection in humans and guinea pigs (Kern, 1984; Stanberry, 1986) suggest that the observed antiviral effect of immunoactive agents in this model may be predictive of efficacy in humans.

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