Effect of indomethacin on ultraviolet radiation-induced recurrent herpes simplex virus disease in guinea-pigs

D. F. Bratcher, C. J. Harrison, N. Bourne, L. R. Stanberry and D. I. Bernstein

Division of Infectious Diseases, Children’s Hospital Research Foundation, Elland and Bethesda Avenues, Cincinnati, Ohio 45229 and James N. Gamble Institute of Medical Research, 2141 Auburn Avenue, Cincinnati, Ohio 45219, U.S.A.

Exposure to u.v. radiation increases the local level of prostaglandins which may play a role in u.v. radiation-induced herpes simplex virus (HSV) recurrences. We used the guinea-pig model of u.v. radiation-induced recurrent genital HSV-2 disease for examining the effects of indomethacin, a prostaglandin inhibitor, on u.v.-induced recurrences. In the first experiment, performed 100 days after HSV-2 inoculation, treatment with indomethacin for 5 days begun 24 h before u.v.-irradiation decreased the proportion of animals developing HSV disease recurrences from 11/13 (84.6%) to 2/13 (15.4%) (P<0.001). In the second experiment, performed 135 days after HSV-2 inoculation, treatment with indomethacin for 5 days begun 24 h before u.v.-irradiation decreased the number of animals developing recurrences from 12/21 (57.1%) to 5/21 (23.8%) (P<0.05). Five days of indomethacin treatment begun 4 h after u.v.-irradiation, however, did not reduce the percentage of animals developing disease recurrences but did decrease the mean number of days with recurrent lesions in animals that developed recurrences. Our data suggest that indomethacin may modify u.v. radiation-induced recurrent lesions by decreasing viral reactivation when given before u.v. radiation exposure or by reducing prostaglandin-induced immunosuppression when given before or after exposure. Future studies are needed for evaluating indomethacin prophylaxis for recurrent HSV disease when prolonged u.v. radiation exposure is anticipated.

Exposure to u.v. radiation is a known risk factor for induction of recurrent herpes simplex virus (HSV) disease in animals (Blyth et al., 1976; Stanberry, 1989) and humans (Wheeler, 1975; Spruance, 1985, 1988). The mechanisms responsible for this disease induction are poorly understood but may involve prostaglandins. Exposure to u.v. radiation increases local levels of prostaglandins (Greaves & Sondergaard, 1970; Harbour et al., 1983) which could produce local immunosuppression (Hayashi & Aurelian, 1986; Otani & Mori, 1987) allowing the reactivated virus to replicate. Prostaglandins have also been implicated in the reactivation of HSV in vitro (Kurane et al., 1984). Inhibitors of prostaglandin synthesis, such as indomethacin, could thus affect the development of recurrent HSV disease by preventing local immunosuppression or by directly inhibiting reactivation of HSV from latently infected neurons. Inhibitors of prostaglandin synthesis also delay the onset and decrease the intensity of erythema produced by u.v.-irradiation (Snyder & Eaglstein, 1973, 1974) which could alter the erythema-related events involved in the reactivation of HSV (Rooney et al., 1991).

A recent uncontrolled study reported a decreased incidence of spontaneous recurrences in human subjects given indomethacin (Wachsman et al., 1990). Therefore, it was of interest to examine the in vivo effects of indomethacin given prior to and after exposure to u.v. radiation in order to gain insight into the pathogenesis of u.v. radiation-induced HSV disease recurrences and their possible prevention. In this report, we used the well defined guinea-pig model of u.v. radiation-induced HSV recurrent disease (Stanberry, 1989) to evaluate the role of indomethacin. This model has previously proven useful for evaluating the effects of antiviral (Stanberry et al., 1990) and immunomodifying agents (Harrison et al., 1991) on u.v. radiation-induced recurrences.

Primary genital HSV-2 infection was produced in 300 to 400 g female Hartley guinea-pigs (Charles River Breeding Laboratories) by intravaginal inoculation with $10^5$ p.f.u. of HSV-2 strain 333 or MS (ATCC-VR 540) as previously reported (Bernstein et al., 1986). Animals that had recovered from acute genital HSV-2 disease...
Fig. 1. Cumulative mean number of days with recurrent lesions induced by u.v. radiation. Animals were given either indomethacin (■) or saline (▲) beginning 24 h before u.v. exposure and were then observed for 7 days.

were then randomized to receive either indomethacin or placebo at 100 to 135 days after HSV-2 inoculation. Indomethacin (Sigma) was solubilized in 0.2 M-sodium phosphate buffer pH 7.4 at a concentration of 5 mg/ml (Bjornson et al., 1989). Animals received either 10 mg/kg of indomethacin or an equivalent volume of saline administered intramuscularly once daily in alternate thighs for 5 days commencing either 24 h before or 4 h after u.v.-irradiation.

Exposure to u.v. radiation was performed as previously described (Stanberry et al., 1990) when spontaneous recurrent disease would be a rare event. Briefly, the perineal skin of metaphane-anaesthetized guinea-pigs was exposed for 10 min to u.v.-B light produced by transilluminators (UVP Incorporated) emitting radiation at between 280 and 320 nm with a peak output of 7000 μW/cm² at 302 nm.

The guinea-pig perineum was then carefully inspected each day for evidence of recurrent lesions for 7 days after u.v.-irradiation (Stanberry et al., 1990; Harrison et al., 1991). Recurrent 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 7 days after u.v. radiation exposure. The severity of induced disease was evaluated by measuring the number of days with disease recurrences in affected animals. In addition, recurrences with two or more lesions observed concurrently were considered to be severe. Differences in means were analysed by a two-tailed Student's t-test or analysis of variance; P values were adjusted for multiple groups utilizing the Bonferroni correction. Incidence data were analysed by the Fisher exact test.

We first investigated whether indomethacin administered prior to u.v. radiation exposure would prevent or ameliorate the induced recurrent herpetic disease symptoms. One-hundred days after HSV-2 strain 333 inoculation, 26 animals that had recovered from acute genital HSV disease were randomized to receive either indomethacin (n = 13) or placebo (n = 13) for 5 days beginning 24 h prior to u.v.-irradiation (days 100 to 104). Fig. 1 shows the cumulative mean lesion days over the 7 day observation period. Indomethacin reduced the incidence of recurrent disease from 84.6% in the saline placebo group to 15.4% (P < 0.001) (Table 1). Mean lesion days were also significantly decreased from 1.9 ± 1.3 to 0.2 ± 0.6 in the indomethacin-treated group (P < 0.001). Although there was no significant difference in the number of days with disease recurrences in affected animals (Table 1) severe recurrences (more than one lesion) were observed only in the placebo group (n = 2) (data not shown).

Because we speculated that prostaglandin inhibitors could either affect reactivation of latent virus or prevent the local immunosuppression that could lead to the lesion development, we repeated the experiment and included a second treatment group in which indomethacin therapy was delayed until after u.v.-irradiation. Thus, if indomethacin affected reactivation, we hypothesized that beginning treatment after u.v. radiation exposure would not be effective, whereas this regimen could still affect local immunosuppression.

In this second experiment, performed 135 days after vaginal inoculation with HSV-2 strain MS, 34 animals that recovered from acute genital HSV disease were randomized into three groups. Groups 1 and 2 were treated for 5 days with indomethacin (n = 12) or placebo (n = 11), respectively, beginning the day prior to u.v.-irradiation. Group 3 (n = 11) received indomethacin for 5 days starting 4 h after u.v.-irradiation. Animals were examined for recurrent lesions for 7 days after irradiation. Following a 6 day washout period, the groups were

<p>| Effect of indomethacin on u.v. radiation-induced recurrences in HSV latently infected guinea-pigs |</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Incidence (%)</th>
<th>Mean lesion days</th>
<th>Mean lesion days in animals with lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>13</td>
<td>11 (84.6)</td>
<td>1.9 ± 1.3</td>
</tr>
<tr>
<td>Indomethacin*</td>
<td>13</td>
<td>2 (15.4)</td>
<td>0.2 ± 0.6</td>
</tr>
</tbody>
</table>

* Indomethacin was given beginning 24 h prior to u.v. radiation exposure.
then re-randomized and the experiment was repeated. Group 1 now received placebo, while group 2 received indomethacin post-u.v. radiation exposure, and group 3 received indomethacin prior to exposure.

Fig. 2 shows the cumulative increase in mean lesion days during the 7 days of observation. Treatment with indomethacin prior to u.v.-irradiation again decreased the incidence of induced disease recurrences, from 57.1% in the placebo group to 23.8% (P < 0.05) (Table 2) and mean lesion days from 1.2 ± 1.3 to 0.4 ± 0.9 (P < 0.05). The number of days with lesions for affected animals was again not significantly different.

Indomethacin treatment begun after irradiation had different effects. The incidence of recurrences was essentially unchanged (56.6%) compared to the saline placebo group (57.1%). Mean lesion days were decreased but not significantly (P = 0.06, Student's t-test). Animals that developed recurrences, however, had milder recurrences as shown by a reduction in mean lesion days from 2.1 ± 1.0 to 1.2 ± 0.4 (P < 0.05) (Table 2). Again severe recurrences were only observed in the placebo group (n = 3).

It appears that indomethacin when administered prior to u.v. radiation exposure prevented the induction of HSV-2 recurrences, but when administered after it failed to prevent the development of recurrent lesions but reduced the severity of recurrent disease. A number of mechanisms could be operating. In order for recurrent lesions to develop, HSV must be reactivated from the latent state in the dorsal root ganglia, descend the neural pathways, and overcome local immune mechanisms to replicate.

Exposure to u.v. radiation may send a signal that initiates the events leading to reactivation of the virus from latency. Recently, Rooney et al. (1991) reported that topical sunblocking agents significantly decreased the incidence of u.v. radiation-induced HSV disease recurrences in human subjects. They suggested that erythema-related events induced by prostaglandins or other inflammatory mediators are important in the reactivation of HSV infection. Indomethacin may therefore exert its effects by reducing the u.v. radiation-induced erythema and inflammation (Snyder & Eaglstein, 1973, 1974) and thus the reactivation signal. Indomethacin may also act by inhibiting reactivation in the neuron. Kurane et al. (1984) reported that indomethacin inhibited viral reactivation in explanted latently infected mice ganglia.

Finally, there is evidence to suggest that local immunosuppression is another factor. Hill & Blyth (1976) originally proposed that HSV frequently reactivates in ganglia and descends to the skin where subclinical microfoci of infection are produced. These microfoci are, however, routinely eliminated by local defence mechanisms prior to lesion development unless these defences are altered. By increasing local levels of prostaglandin PGE2, exposure to u.v. radiation could produce local immunosuppression (Hayashi & Aurelian, 1986) which would allow HSV to replicate and cause clinical lesions (Harbour et al., 1978; Baker et al., 1982). Indomethacin could reduce the local immunosuppression

Table 2. Effects of indomethacin begun before or after u.v. radiation exposure on u.v. radiation-induced recurrences

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Incidence (%)</th>
<th>Mean lesion days</th>
<th>Mean lesion days in animals with lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>21</td>
<td>12 (57.1)</td>
<td>1.2 ± 1.3</td>
<td>21 ± 1.0</td>
</tr>
<tr>
<td>Immediate indomethacin*</td>
<td>21</td>
<td>5 (23.8)†</td>
<td>0.4 ± 0.9†</td>
<td>16 ± 0.9†</td>
</tr>
<tr>
<td>Delayed indomethacin†</td>
<td>23</td>
<td>13 (56.6)</td>
<td>0.7 ± 0.8</td>
<td>1.2 ± 0.4‡</td>
</tr>
</tbody>
</table>

* Indomethacin was given beginning 24 h prior to u.v. irradiation exposure.
† P < 0.05 compared to placebo.
‡ Indomethacin was given beginning 4 h after u.v. irradiation.
induced by prostaglandins leaving antiviral defence mechanisms intact.

Our data suggest that indomethacin may modify u.v. radiation-induced recurrent lesions by more than one mechanism. Reduction in the incidence of u.v. radiation-induced lesions by indomethacin given prior to irradiation suggests that this compound either altered the reactivation signal or modified an undefined reactivation event in the neuron. Indomethacin given after irradiation did not affect the incidence of induced recurrences probably because the reactivation events were already initiated. Later addition of indomethacin did, however, affect the mean number of days with recurrent lesions probably by diminishing the local immunosuppression induced by u.v. radiation exposure. It is also possible that initiating therapy earlier had more effect on local immunosuppression, thus not only modifying but preventing lesion development. It appears unlikely that recurrent lesions could have been overlooked because of the masking anti-inflammatory activity of indomethacin because delayed indomethacin treatment had no effect on the incidence of recurrent disease.

It would appear that indomethacin should be considered for prophylaxis of recurrent HSV infection when prolonged u.v. radiation exposure is anticipated. Further controlled studies in humans as well as studies to determine the specific mechanism of action of indomethacin also appear warranted.

We thank Fernando Bravo and Alisa Reece for technical assistance and Michelle Becker for preparation of the manuscript. This work was supported by grants AI 23482, AI 22667 and AI 29687 from the NIH.

References


(Received 9 February 1993; Accepted 10 May 1993)