The Influence of PUVA and UVB Radiation on Skin-Graft Survival in Rabbits

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The survival time of full-thickness skin grafts in rabbits was prolonged by administration of methoxsalen and subsequent exposure of the donor and recipient graft sites to longwave ultraviolet radiation (UVA). Erythemogenic doses of radiation were required to prolong graft survival. Similar exposure to mid-ultraviolet radiation (UVB) did not significantly prolong the survival time of grafts.

Exposure to nonionizing radiation has a variety of effects on immune function, including suppression of allergic contact dermatitis and delayed hypersensitivity, alteration of the antigenicity of molecules and induction of altered immune responses to cutaneous neoplasms. These aspects of photoimmunology have recently been reviewed [1]. The aims of the present study were to examine the effects of UV radiation on skin graft rejection. Two types of UV radiation were studied: UVB (280-320 nm) radiation and UVA (320-400 nm) radiation following oral administration of methoxsalen. The latter combination is commonly referred to by the acronym, PUVA.

MATERIALS AND METHODS

Female New Zealand albino rabbits weighing 1.5-2.0 kg were used as the experimental animal.

Grafting Procedure

Each procedure involved a pair of rabbits. Operations were performed under pentobarbital and either anesthesia; grafts were removed by skin punch and scalp el excision. Full-thickness skin grafts, 1.5 cm in diameter, were obtained from the outer aspect of the ear of one animal and transferred to the same site on the ear of the other rabbit. Skin removed to provide the recipient site was transferred to the donor site. The grafts were fixed in position with 4 sutures and covered with a bandage.

Graft Rejection

The grafts were examined daily in the morning. Hardening of the graft, brownish discoloration of the graft or separation of the graft from its bed was accepted as evidence of rejection.

UV Radiation

Irradiation was performed daily until the day of graft rejection. Although the entire animal was exposed, only the ear under study was clipped. The daily exposure dose of each type of radiation required to produce and maintain an erythemal response of the ears of the rabbits was determined in preliminary studies. The erythemal response was evaluated daily on a scale of: 0 = no reaction; 1+ = minimally perceptible erythema; 2+ = pink erythema; 3+ = red erythema; and 4+ = intense erythema with swelling.

Exposures to UVB radiation were administered in a U-shaped bench equipped with FS-40 bulbs (Westinghouse, Bloomfield, NJ). Treatment with PUVA consisted of administration of methoxsalen (24 mg/kg body weight) by gavage followed after a 1 hr interval by exposure to UVA radiation in a U-shaped bench equipped with PUVA fluorescent bulbs (Sylvania, Danvers, MA). The irradiance of each radiator was measured with a cosine-corrected UV spectroradiometer system (IL 783, International Light, Inc., Newburyport, MA). The average irradiance for the UVB radiator was 820 mw/m² (integrated 280-320 nm waveband) and for the UVA radiator, the average irradiance was 45 w/m² (integrated 320-400 nm waveband).

The experimental groups of animals were as follows:

PUVA group 1: Four rabbits were treated daily with PUVA commencing 7 days prior to grafting. The radiant exposure dose of UVA was held constant at 2.7 X 10⁻² J/m².

PUVA group 2: Ten rabbits were treated daily with PUVA commencing 7 days prior to grafting and using an initial radiant exposure dose of 2.7 X 10⁻³ J/m². The erythemal response was evaluated daily and the dose of UV radiation was adjusted with the aim of attaining and maintaining a 3+ to 4+ erythemal response until the graft was rejected. The maximum dose reached was 8.1 X 10⁻² J/m².

UVB group: Daily treatment of 10 rabbits was commenced 7 days prior to grafting using an initial radiant exposure dose of 246 J/m². The erythemal response was evaluated daily and the dose of radiation was adjusted to produce and maintain a 3+ to 4+ erythema.

Control group: The ear was clipped in the 10 rabbits in this group with the same frequency as in other groups but these animals were not exposed to radiation and did not receive psoralens.

The results of the study were analyzed using the Smirnov test [2]. This is a nonparametric test that permits distinction between different experimental samples without making assumptions about the underlying distributions.

RESULTS

The graft healed in all animals and the sutures were removed on the 5th or 6th day. Rejection was found to occur rapidly over a period of 24 hr. The results observed in the individual groups were as follows (Table I):

Control Group

The grafts were rejected in all animals after 7 to 10 days and the mean survival time was 8.7 days.

PUVA Group 1

The maximum erythema achieved was 1+. The grafts were rejected by three animals after 9 days and one animal after 7 days.

PUVA Group 2

All animals showed a 1+ or greater erythemal response on the exposed ear within 4 days of commencing exposures, and a 3+ or 4+ response by the day of grafting. After grafting all animals maintained at least a 3+ response and at some time during the experiment reached a 4+ erythema. The grafts were rejected in 7 to 27 days, the mean survival time being 13.1 days.

UVB Group

Animals in this group showed an erythemal response which was similar to that seen in PUVA Group 2. All animals devel-
Control PUVA 2 10 successful numbers identify the pair of UVB opened an erythema by the 4th day, it reached an intensity of 3+ to 4+ by the time of grafting and a 3+ to 4+ response was control animals.

cant in control animals and 13.1 days in order in which the grafts were performed within the groups.

sites was necessary for prolongation of graft survival. To produce this effect the dose of both the donor and recipient site with the same site served as both a donor and recipient site.

TABLE II. Survival time in days of skin grafts in rabbits exposed to PUVA or UVB radiation and in control nonexposed animals*

<table>
<thead>
<tr>
<th>Pairs of animals</th>
<th>Control group</th>
<th>PUVA group 2</th>
<th>UVB group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>11</td>
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<tr>
<td>4</td>
<td>7</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Mean graft survival:</td>
<td>131</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Mean graft survival:</td>
<td>8.7</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Mean graft survival:</td>
<td>10.0</td>
<td></td>
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</tr>
</tbody>
</table>

* Exposed animals developed a marked erythematous response. Every 2 successive numbers identify the pair of rabbits involved in an exchange of ear skin.

opened an erythema by the 4th day, it reached an intensity of 3+ to 4+ by the time of grafting and a 3+ to 4+ response was maintained in all animals after grafting. All grafts were rejected after 7 to 18 days and mean survival time for the grafts was 10 days.

The survival times for skin grafts in the 30 animals in the control, PUVA 2 and UVB groups are listed in Table II in the order in which the grafts were performed within the groups. The difference between the mean graft survival time of 8.7 days in control animals and 13.1 days in PUVA 2 animals is significant ($p < 0.05$). However, the survival time of UVB-exposed grafts was not significantly different ($p > 0.1$) from that in the control animals.

DISCUSSION

In rabbits, exposure to UVA radiation after administration of methoxsalen prolonged the survival time of full-thickness skin grafts. To produce this effect the dose of PUVA had to be sufficient to produce a marked erythema response (PUVA group 2). The design of the present study provided for treatment of both the donor and recipient site with PUVA because in an individual animal the same site served as both a donor and recipient graft site. A different study design would have to be used in order to investigate whether exposure of either or both sites was necessary for prolongation of graft survival. Furthermore, in this study treatment with PUVA was commenced prior to grafting but it is possible that treatment from the time of grafting, using doses that were markedly erythrogenic, might have inhibited graft rejection. In contrast to the effects of PUVA, exposure to UVA radiation did not produce a significant prolongation of survival time of skin grafts.

Perhaps the most interesting observation of the study was that some grafts in both PUVA and UVB-exposed animals had prolonged survival times. Although the rabbits used in these experiments were outbred, it is possible that the degree of genetic variation differed among pairs of animals. Hence, prolonged survival time might reflect the combined effect of exposure to radiation and relatively minor histocompatibility differences between some, but not between other, pairs of rabbits. This possibility is partly supported by the finding that the grafts exchanged between the last pair of PUVA-exposed animals and the first pair of UVB-exposed animals had long survival times. Variations in the optical properties of the skin may provide an alternative explanation for the few long survival times seen in UV-exposed animals. Transmission of radiation through the skin may have varied between animals so that in some instances a higher dose reached the target site.

Several immunosuppressive agents have been found to prolong skin allograft survival in animals. A study using the rabbit-ear model found that incubation of the graft in a suspension of triamcinolone acetonide for 60 min prior to transplantation prolonged graft survival to 21.4 days as compared to 6.1 days in control animals [3]. High doses of systemic triamcinolone had a similar effect. In mice, systemic administration of cyclophosphamide, methotrexate, azathioprine and antilymphocyte serim prolonged skin graft survival by as much as 3 times the survival in untreated animals [4]. Whole-body exposure to 450 R of ionizing radiation ($^{60}$CO source) more than doubled the graft-survival time in the same study, and it was found that pre-operative exposure was more effective than post-operative treatment. Nonionizing radiation can therefore be added to the list of immunosuppressive agents that prolong skin allograft survival. The findings of the present study are of theoretic interest but, at least under the conditions tested, nonionizing radiation is unlikely to be of practical value for the prolongation of skin allograft survival.

There are several possible mechanisms by which exposure to nonionizing radiation could affect the survival of skin allografts. The function of lymphocytes may be impaired; in humans it has been found that exposure to either UVB [5] or PUVA [6] radiation can alter the function of subpopulations of circulating lymphocytes. The function of lymphocytes may be affected as a result of direct exposure to radiation while these cells percolate through skin capillaries. Alternatively, mediators released from the erythematosus skin might influence lymphocyte function. This latter possibility is supported by the observation that prostaglandin E$_2$, a possible mediator of delayed erythema following exposure to UVB radiation, has been found to prolong skin-graft survival in mice [7]. Another possibility is that antigenic determinants in the graft may be altered by radiation; there is indirect evidence that exposure to UV radiation can cause masking or deletion of antigens in the skin [8,9]. Processing of antigen may also be affected by exposure to radiation; Langerhans cells which appear to be macrophages involved in antigen processing and presentation in the skin are altered by exposure to UVB radiation [10]. Finally, the inflammatory response to the graft may be impaired by the exposure to radiation. A recent study suggests that alterations occur in both the host and graft microvasculature at an early phase of graft rejection [11] and that vessel damage is probably the immediate cause of that rejection. Radiation penetrates to the level of the microvasculature of the skin and may alter the response to these vessels.

REFERENCES

4. Floersheim GL: A study of combined treatment with chemical...
imunosuppressants and antilymphocytic serum to prolong skin allograft survival. Transplantation 8:392-402, 1969

ANNOUNCEMENT

Annual Meeting of ESDR May 24-27 1981

The 1981 Annual Meeting of the European Society for Dermatological Research (ESDR) will be held in the Leuwenhorht Congress Centre, Noordwijk, Holland, 24-27, May 1981.

Attendance at scientific sessions will be open to all members, contributors and guests. Nonmembers who wish to present a paper may submit an abstract. They will be invited to attend the meeting as either contributors or guests.

Abstracts should be presented on the official form. The deadline for submission of abstracts is January 15th, 1981. Abstract forms can be obtained from: Professor M.W. Greaves, Secretary – E.S.D.R., Institute of Dermatology, Homerton Grove, London E9 6BX, England.