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CORRESPONDENCE

Increased susceptibility to β-irradiation of senescent mouse skin irrespective of grafting to young recipients

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1. Introduction

Ageing of an individual is important in determining the outcome of exposure to a carcinogen. This is due firstly, to the often long latent periods (Jones and Grendon 1975), secondly to the time available for chronic exposure to carcinogens (Berenblum and Shubik 1947) (initiator/promoter), and thirdly intrinsic changes in tissue susceptibility to carcinogenic stimuli that may result from ageing, independently of exposure to known carcinogens. Our previous experiments demonstrated an enhanced susceptibility of senescent mouse skin to the effect of a chemical carcinogen (DMBA) to which it had not previously been exposed (Ebbesen 1973), whereas middle-aged skin was not more susceptible to the chemical carcinogen than younger adult skin (Peto et al. 1975, Ebbesen 1977). Here the effect of beta-irradiation on senescent, middle-aged and young adult skin is reported.

2. Materials and methods

Inbred female BALB/c mice were used. Grafting was done with 3 × 4 cm pieces of back skin. After removal of subcutaneous fat, the graft from one donor was sewn onto the back of one recipient. All recipients were 2 months old when grafted.

2.2. Irradiation

Beta-irradiation (β-irr) was carried out 8 months after skin grafting by placing a plate of 20 millicurie 90Sr with an active area of 1 cm² and a surface dose-rate in tissue-equivalent material of 10 rad/sec on the depilated skin for 7 min (4200 rad). The depth doses in tissue were kindly calculated by Mr A. N. Rasmussen, engineer, the Finsen Institute, Copenhagen. At a depth of 33 µm the basal epithelial cells should receive about 98 per cent of the surface dose.

2.3. Chemical carcinogen

7,12-dimethyl-benz[a]anthracene (DMBA) treatment was carried out by applying 15 µg of DMBA in 15 µl acetone on the depilated skin three times at weekly intervals. ‘Simultaneous’ treatment with irradiation and DMBA was done by applying DMBA once, irradiating one week later, and then applying DMBA twice at weekly intervals. The percentage of skin in growth phase (Borum 1954) at start of treatment was nearly identical in the various groups of animals. Malignancy was assessed by grafting minced tumour tissue to syngeneic recipients.
2.4. Statistics

To counter bias due to the heterogeneous mortality pattern of our test groups of different ages, calculations of tumour rates by the chi-square test were performed by the method of Peto (Peto 1974).

Results

3.1. Irradiation alone

Irradiation caused depilation and often benign wounds that healed in 2–5 months. Quite small, pale papillomas (2–4 mm in diameter) appeared after 2 months and regressed after another month. The few malignant tumours were subcutaneous fibrosarcomas and not palpable until the very end of the observation period.

3.2. DMBA alone or in combination with β-irr

These treatments induced larger papillomas measuring up to 10 mm in diameter. They started to appear after 8 weeks, and the maximum number was present 2 months later when progression to malignancy was apparent in some cases.

3.3. Light microscopy

The epidermal changes from the irradiation and DMBA-induced papillomas were hyperplasia, hyperkeratosis and, of course, the folding of epidermis. In some instances, the dermis showed an increase in collagen fibres. Epidermal carcinomas

<table>
<thead>
<tr>
<th>Age of donors at time of grafting to 2-months old recipients</th>
<th>Age of skin at start of treatment</th>
<th>Number of mice with ulceration, benign tumours and malignant tumours versus total number of mice at start of treatment.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
<td>Months</td>
<td>Benign tumours</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>------------</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>(48%)</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>(66%)</td>
</tr>
</tbody>
</table>

† The incidences are uncorrected for intercurrent deaths during the observation period in which 2 per cent of young non grafted, 5–10 per cent of middle aged non-grafted and graft recipients and 40–50 per cent of old non-grafted mice died.

Table 1. Tumour development on skin from old and young donor mice grafted to young recipients and later β-irr or irradiated plus DMBA treated. Observation period was 10 months after start of treatment, except for old non-grafted mice when it was 4 months.
were of the squamous cell type with epithelium infiltrating the deeper structures and ulceration of the tumour surface. They did not produce metastases. Fibrosarcomas also extended from dermis into underlying structures, but metastases were not observed. Skin thickness was slightly less in 26 month old skin than in younger skin, with most of the reduction falling within the subepithelial tissue. Epithelial thickness (surface to basal layer) was about 33 µm.

<table>
<thead>
<tr>
<th>Months after treatment</th>
<th>β-irr alone</th>
<th>DMBA β-irrad.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Young skin on young mice</td>
<td>0/150 5/149</td>
<td>3/149 0/148</td>
</tr>
<tr>
<td>Old skin on young mice</td>
<td>0/150 12/135</td>
<td>11/135 1/135</td>
</tr>
<tr>
<td>non-grafted young mice</td>
<td>0/147 11/146</td>
<td>7/145 0/144</td>
</tr>
<tr>
<td>Non-grafted middle-aged mice</td>
<td>0/142 5/140</td>
<td>6/138 0/130</td>
</tr>
<tr>
<td>Non-grafted old mice</td>
<td>0/67 12/47</td>
<td>6/38 0/28</td>
</tr>
</tbody>
</table>

Table 2. Development of benign papillomas during the first 4 months of observation on mice also shown in table 1. Number of new mice with papilloma observed each month versus number of mice at the beginning of each month.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of mice with papilloma/ total number</th>
<th>Number of mice with carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation</td>
<td>12/149 (8%)†</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Irradiation followed 3 months later by DMBA</td>
<td>80/150 (53%)</td>
<td>22 (16%)</td>
</tr>
<tr>
<td>Irradiation and DMBA simultaneously</td>
<td>80/147 (54%)</td>
<td>8 (6%)</td>
</tr>
<tr>
<td>DMBA</td>
<td>83/150 (55%)</td>
<td>12 (8%)</td>
</tr>
<tr>
<td>DMBA followed 3 months later by irradiation</td>
<td>71/148 (48%)</td>
<td>14 (10%)</td>
</tr>
<tr>
<td>Acetone (control)</td>
<td>0/143 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

†The papillomas seen after irradiation were smaller than those following DMBA treatment.

Table 3. Tumour development on young (2 months) female BALB/c mice receiving 4200 rad β-irr and/or DMBA.
3.4. Incidences of lesions

After β-irr alone, benign papillomas were more common on old grafted skin than on young grafts, and more common on old non-grafted mice than on younger non-grafted animals (tables 1 and 2). Calculated on the basis of data in table 2, according to Peto (Peto 1974), the differences are significant at the 0.1 per cent level. The only two cases of malignant tumours furthermore developed in old skin. Following treatment with chemical carcinogen plus radiation, the papilloma incidence again was higher ($p < 0.001$) on old non-grafted mice than on younger animals, but the difference between the incidence on old grafted and young grafted skin was not significant at the 1 per cent level.

Using the same statistical procedure on the few carcinomas that all emerged in the 4th and 5th month of the observation period, we found that these again had a higher incidence in old non-grafted mice than in younger mice ($p < 0.01$), but the difference between incidences on old and young skin grafts was not significant at the 1 per cent level.

4. Discussion

These results suggest that with advanced age tissue may become more prone to tumour development after β-irr in accordance both with our previous finding for intact skin exposed to a chemical carcinogen (Ebbesen 1974) and with recent work by others on epithelial cultures established from old and young donors (Summerhayes and Franks 1979). As the enhanced susceptibility of senescent skin to β-irr was demonstrated also on grafts carried by young recipients it must reside in local autonomous age-dependent alterations and cannot be explained by age-dependent changes in the general immune (Mackinodan, Albright, Good, Peter and Heidrick 1976), hormone (Dilman 1971) or viral status of the BALB/c mouse (Peters, Hartley, Spahn, Robstein, Whitmire, Turner and Huebner 1972), or by a change in a central growth control mechanism (Burch 1975). This also agrees with our finding for chemical carcinogenesis (Ebbesen 1974, 1977). Middle-aged skin, if anything, appears less prone to tumour development than young skin after β-irr, as is also the case with DMBA tumour induction (Ebbesen 1974).

The low tumour incidence and the long latent period after β-irr is in conformity with the previous results of Hulse (1962). The lack of evidence of a synergistic effect of DMBA and β-irr could be related to the inhibitory effect of irradiation on mitosis. Cell divisions are required for fixation of transformation due to both radiation (Borek and Sachs 1968) and chemical carcinogens (Kakunaga 1975).

Factors of possible importance for the observed enhancement of susceptibility to β-irr with advanced age are: (1) Decrease in skin thickness which will increase the irradiation of the proliferative basal cells, but could also enhance the killing by irradiation of cells that could otherwise have become transformed (Albert, Burns and Heimbach 1967). Skin thickness is not likely to be of much importance considering the 'long' range of the β-irr (1–2 mm) (Duncan and Nias 1977), compared to the thickness of the epithelial layer, and the small change in thickness with ageing. (2) Changes in a local mitosis regulatory system. We have found senescent skin to contain less chalone-like activity (Olsson and Ebbesen 1977) and to respond less well to exogeneous skin chalone than younger skin (Ebbesen, Olsson and Due 1978). The significance of these changes for carcinogenesis is unresolved. (3) An increase in mitotic rate in the proliferative basal layer was reported for senescent rat skin (Bertalanffy, Pusey and Abbott 1965). However, we found no evidence of this with
mouse skin (Olsson and Ebbesen 1977, Ebbesen, Olsson and Due 1978). (4) Spontaneous accumulation with age of variant cells (Prehn 1976) which may be highly susceptible to carcinogens (DiPaolo, Nelson and Donovan 1971, Nana and Ashworth 1974). The well-known accumulation with ageing of non-malignant cells with chromosomal abnormalities supports this hypothesis (Curtis 1963).

As preventive health care hopefully succeeds in reducing our exposure to external carcinogens, individual susceptibility and its possible change with age becomes of increasing importance. So, if enhanced susceptibility to certain carcinogens is a regular feature of ageing of some human tissues (Brown and Doll 1965), the permitted occupational exposure to carcinogen should be age-adjusted in favour of the old.

Acknowledgments
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References