Examination of the Carcinogenic and Cocarcinogenic Effects of Grenz Radiation

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SUMMARY

The effects of the chemical carcinogen 7,12-dimethylbenz(a)anthracene on long-wavelength ionizing radiation (grenz ray)-induced carcinogenesis in the skin of hairless mice were examined. A carcinogenic amount of the grenz energy (20,500 R) was applied over a 12-month period. A single application of 7,12-dimethylbenz(a)anthracene 4 weeks before the initiation of the grenz ray exposures resulted in a significant acceleration of tumor growth and the incidence of deeply invading cancers. These findings simulate the influence of 7,12-dimethylbenz(a)anthracene ultraviolet-induced cancer formation, and confirm the additive effects of physical and chemical carcinogenic stimuli.

INTRODUCTION

The carcinogenic effects of conventional X-rays and UV radiation are well established (3, 6, 9). Recent studies further indicate that the cancer-inducing effects of UV and polycyclic aromatic hydrocarbon carcinogens are additive (8, 10). The relationship between ionizing radiation and chemical carcinogenesis is less clear. In 1950, Mendeleef (15) reported an additive effect of X-radiation and 20-methylcholanthrene. Subsequently, Shubik et al. (19) found that croton oil promotion following a single subcarcinogenic exposure to β-radiation resulted in significant tumor formation. Kawamoto et al. (14) demonstrated that urethan augmented leukemogenesis induced by X-radiation in mice. Berenblum and Trainin (2) found that X-ray-induced leukemogenesis was augmented by urethan only if the chemical was administered after the radiation. However, Argus et al. (1) reported that γ-radiation up to 600 R did not augment chemical carcinogenesis and that X-rays from a 160-kV source actually inhibited tumor growth initiated by the chemicals, apparently as a result of the cytotoxic effects of the irradiation.

This study was designed to evaluate further the relationship between ionizing energy and chemical carcinogenesis. Grenz rays were utilized in order to reduce the cytotoxic properties of the radiation.

MATERIALS AND METHODS

Experimental Animals. In this study, 133 randombred hairless mice (2 months old) were housed in metal cages and fed on unrestricted quantities of Wayne Lab Blox and water. Grenz Ray Source. A Profex grenz ray machine (12 kV and 10 ma) produced 100 R in 15 sec at a distance of 20 cm (half-value layer, 0.017-mm Al).

Chemical Carcinogen. An acetone solution containing DMBA, 2 100 µg/0.05 ml, was used as the carcinogen. The carcinogen and the solvent were applied with a 1-ml syringe.

Chart 1. The development of tumors 4 cu mm or larger in Groups (Gp) I, II, and III.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Incidence</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Acetone + grenz irradiation¹</td>
<td>23/30b</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>DMBA + grenz irradiation³</td>
<td>26/28</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>DMBA</td>
<td>22/23</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Acetone</td>
<td>0/32</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

¹ 20,500 R.
² No. of mice with tumors larger than 2 mm in diameter/no. of survivors.
³ 100 µg/0.05 ml, was used as the carcinogen. The carcinogen and the solvent were applied with a 1-ml syringe.

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Procedure. The hairless mice were divided into 4 groups and were treated as follows. In Group I (35 mice), the back of each mouse received a single application of acetone. Four weeks later, the backs of these animals were exposed to 100 R from the grenz ray source 3 times a week for 4.5 months and 5 times a week for 7 months (a total of 20,500 R). In Group II (32 mice), the back of each mouse received a single 100-μg application of DMBA followed by the irradiation as in Group I. The 25 Group III mice received the DMBA application as in Group II, but no grenz ray exposures. The 41 Group IV mice received the acetone application as in Group I, but were given no irradiation.

The mice were observed regularly, and the number of tumors were tabulated per survivors. Survivors included mice that died or were autopsied with significant tumors at the time of death. When possible, animals were autopsied, and tissue was prepared for light microscopic study as the tumors became large enough to endanger the lives of the mice.

RESULTS

Tumor Formation. Only tumors reaching 2 mm in diameter and persisting for 2 weeks were reported (Chart 1; Table 1) in order to remain consistent with previous reports of tumor induction. In Group I, the 1st tumor was noted at 3.5 months after the initiation of grenz ray exposures (1 tumor on 1 mouse, 4500 R); most tumors appeared between 9 and 14 months. The 1st tumor occurred in Group II at 2 months (1 tumor on 1 mouse, 2400 R), and most tumors appeared at between 6 and 12 months. In Group III, the tumors started at 2.5 months (3 mice with 1 tumor each). The tumors appeared most rapidly between 6 and 12 months. No tumors occurred in the treated areas at any time in Group IV. At 14 months after the onset of irradiation, 77% of the mice pretreated with acetone followed by grenz ray exposures (Group I) had tumors. No growths occurred in the mice that received the acetone application alone (Group IV). Therefore, this amount of grenz radiation was tumorigenic under the conditions of the study. The tumors appeared earlier and at a greater rate in Groups II and III, which received the DMBA. By 14 months, 93% of the mice in Group II and 96% of those in Group III had growths greater than 2 mm in diameter (Table 1).

Effect of DMBA Initiation. As noted in Chart 1 and Table 1, tabulation of the 2-mm tumors did not reveal any variation between the mice treated with both DMBA and grenz rays (Group II) and those given DMBA alone (Group III). However, the tumors grew at different rates in these 2 sets of mice (Chart 2; Table 2).

In Group I, the 1st tumor greater than 50 cu mm was noted 7 months (9,600 R) after the initiation of the radiation. By 14 months (20,500 R) 57% of the mice in this group had developed these large growths. The tumor growth progressed more rapidly in the mice treated with DMBA (Groups II and III), 50-cu mm tumors appearing at 4 months and 3.75 months, respectively. By 12 months, the influence of the grenz radiation on the DMBA effect was noted, since a significantly greater number of mice in Group II had developed these large growths and no growths occurred in the treated areas at any time in Group IV.
growths. This differentiation was emphasized by tabulation of animals with tumors greater than 100 cu mm at the termination of the study (Table 3). Fifty % of the mice that received grenz radiation alone (Group I) and 30% of those treated with DMBA alone (Group III) had tumors of this size, whereas 70% of the mice in Group II, which received both carcinogenic modalities, had growths greater than 100 cu mm.

Tumor Types. Multiple tumors occurred on the mice in all of the groups except Group IV. However, at least 1 of the growths in the tumor bearing mice that received grenz radiation (Groups I and II) was morphologically invasive and bound to underlying tissues. In contrast, the large tumors in 11 of the 14 mice in Group III were exophytic and pedunculated.

Autopsies of animals with large tumors were performed on 16 mice in Group I, on 9 in Group II, and on 8 in Group III, and the tumors greater than 50 cu mm were studied histologically (Table 4). In Groups I and II, all of these growths were malignant, the majority being squamous cell carcinomas (Fig. 1). A few mice in Group I had sarcomas either alone or in combination with a squamous cell carcinoma (Fig. 2). Most of the tumors in Group III were pedunculated papillomatous growths, 75% of which showed carcinomatous invasion of the connective tissue stalk.

DISCUSSION

Grenz radiation is a long-wavelength (1-3A°) type of ionizing energy which occupies a part of the electromagnetic spectrum between conventional X-rays and UV (5, 12, 16). Although, in clinical usage, late sequelae are uncommon and cancer formation is extremely rare (4, 7, 13), this radiation can cause a significant number of cancers in rodents under experimental conditions (18, 20). In this study, grenz radiation and DMBA were utilized for an examination of the relationship of physical and chemical carcinogenic stimuli. Cancer-producing amounts of both modalities were used. However, the combination of their effects resulted in more rapid and continuous tumor growth and in a significant increased incidence of deeply invading, malignant lesions. Thus the cancer-producing effects of grenz ray energy and the chemical carcinogen were additive, under the experimental conditions of this study.

The morphology and histology of the tumors in the animals that received both DMBA and grenz rays were identical with those noted in the mice that were given grenz radiation alone, in contrast to the exophytic growths produced by the DMBA alone. Therefore, the character of the lesions resulting from the combined stimuli was apparently determined by the radiation.

The results of these studies indicate that the cancer-inducing effects of the chemical and physical carcinogen simulated the carcinogenic summation response noted with the combined use of DMBA and UV energy (9). However, the relationship between the effect of a single application of DMBA followed by a physical carcinogenic stimulus and the multistage concept of chemical tumor initiation and promotion is not clear.

The observations of Berenblum and Trainin (2), who used urethan as the promotor, suggested that a 2-stage mechanism may operate in X-radiation-induced leukemogenesis. Shubik et al. (19) reported that croton oil promoted tumor formation, initiated by a single exposure to β-radiation. Similar responses to croton oil have been noted after UV irradiation (11, 17). However, Blum’s (3) extensive calculations strongly suggest that UV-induced tumors follow a pattern of uninterrupted progression to a cancerous form from the 1st exposure. In addition, there is no evidence that DMBA, ionizing radiation, and UV irradiation act on comparable structures or through similar mechanisms to produce tumors. At present we can state only that a single application of a potent chemical carcinogen will accelerate the carcinogenic effects of these 2 types of radiation.

REFERENCES

Fig. 1. a, squamous cell carcinoma from Group 1 invading the underlying musculature. H & E, × 350. b, higher-power view of the squamous cell carcinoma showing anaplastic cells with multiple mitoses. H & E, × 875.

Fig. 2. a, fibrosarcoma from Group 1. H & E, × 350. b, higher-power view of the fibrosarcoma showing anaplasia and multiple cells in mitoses. H & E, × 875.
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