Neoplasms Evoked in Male Sprague-Dawley Rat by Pulse Doses of 7,12-Dimethylbenz(a)anthracene

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Summary

In male rats of the Sprague-Dawley strain a single pulse dose of 7,12-dimethylbenz(a)anthracene elicited a few mammary and sebaceous gland carcinomas but leukemia was not observed. Multiple pulse doses enhanced the yields of these tumors and, in addition, evoked leukemia in rather high incidence.

The induced mammary cancers of male rats did not regress after orchiectomy and hypophysectomy; hence they were hormone-independent.

Introduction

Of the tissues which have been studied, mammary glands of young adult female rats of the S-D strain are especially vulnerable (6, 10) to induction of cancer in vivo by polynuclear aromatic hydrocarbons. Under simple conditions a single feeding (12) or i.v. injection (14) elicited mammary cancer in every female rat; multiple i.v. injections increased the yield of breast cancers (13), and the tumors were evident within a few weeks. The induction of cancer in susceptible rats by i.v. injections of powerful aromatics is one of the most remarkable effects in cancer research.

Male rats are more refractory than females to the induction of mammary cancer by aromatics because, at least in part, hormone-dependent mammary cancers of the rat are destroyed by testosterone (9). In male rats fed 3-MC the incidence of mammary cancer was 0 (2); 0 (16); and 11% (17). Dao and Greiner (3) fed 3-MC to intact male rats, and mammary cancer did not develop. But breast cancers appeared when ovaries were grafted into males, and their yield was increased in castrate males which had received transplanted ovaries before feeding with 3-MC. Bielschowsky (1) incorporated 2-AAF in the diet which was fed to male rats for many months and observed the following classes of tumors: hepatoma, 83%; cancer of periauricular sebaceous glands, 12%; mammary cancer, 7%; and leukemia, 5%.

In the present work, it was found that a massive amount of 7,12-DMBA given on a single occasion by i.v. injection (pulse dose) evoked malignant tumors in low yield in male S-D rats. But reiterated pulse doses elicited, in rather high yields, malignant neoplasms predominantly in 3 classes: leukemia, breast, and sebaceous glands of ear and skin.

Materials and Methods

Three series of intact male rats of the S-D strain, bred at random inter se, were provided a commercial ration3 and water ad libitum; they were housed in groups of 5 in stainless steel boxes kept in an air-conditioned room at 25°C ± 1. At age 27 days holes were punched in the ears for purposes of identification.

A lipid emulsion of 7,12-DMBA (0.5% w/w) was injected in a caudal vein of the experimental animals; 25 control rats were uninjected. The day of the 1st pulse dose is designated Day 0. The dosage of 7,12-DMBA was 35 mg/kg or 6 mg, whichever amount was smaller.

Series 1, comprising 25 rats, was given a single pulse dose of 7,12-DMBA at age 28 days.

Series 2, 95 rats, received 6 pulse doses of 7,12-DMBA at intervals of 14 days beginning at age 28 days.

Series 3, 20 rats, was treated in parallel with Series 2: 11 of the animals developed mammary carcinoma. When the cancers had attained a mean diameter of 2-4 cm, 5 of the rats were subjected to bilateral orchiectomy and 6 other animals were hypophysectomized.

The animals were examined for tumors daily and were weighed 3 times weekly. A sample of venous blood was drawn from each rat which had lost weight on 3 consecutive weighings. Heparinized blood was examined for leukocyte and differential cell counts, HCT, and Hb, using conventional hematologic methods.

The animals were killed when tumors were large, and the experiment was terminated on Day 164. Every rat was subjected to necropsy; classification of tumors was based on histologic examination. Each mammary cancer found at necropsy is designated an “active center”; the mean of active centers is derived from:

\[
\text{No. of mammary cancers} = \frac{\text{Rats with mammary cancer}}{\text{No. of rats}}
\]

The number of rats which died early from toxicity of 7,12-DMBA is deducted from the original number of animals to give the “effective number”; the incidence of tumors is expressed as the percentage of the effective number.

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TABLE 1
INCIDENCE OF NEOPLASMS IN RATS GIVEN SINGLE OR MULTIPLE PULSE DOSES OF 7,12-DMBA*

<table>
<thead>
<tr>
<th></th>
<th>1 pulse dose</th>
<th>6 pulse doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original No. of rats</td>
<td>25</td>
<td>95</td>
</tr>
<tr>
<td>Early deaths</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Effective No. of rats</td>
<td>22</td>
<td>59</td>
</tr>
<tr>
<td>Total rats with neoplasms</td>
<td>7 (32%)</td>
<td>59 (100%)</td>
</tr>
<tr>
<td>No. with multiple classes of neoplasms</td>
<td>1 (5%)</td>
<td>38 (64%)</td>
</tr>
<tr>
<td>No. with single class of neoplasm</td>
<td>6 (27%)</td>
<td>21 (36%)</td>
</tr>
<tr>
<td>Leukemia only</td>
<td>0</td>
<td>13 (22%)</td>
</tr>
<tr>
<td>Mammary cancer only</td>
<td>4 (18%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Periauricular cancer only</td>
<td>2 (9%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* 7,12-DMBA, 7,12-dimethylbenz(a)anthracene.

% of effective number of rats.

Results

Pulse doses of the massive amounts of 7,12-DMBA used in these experiments resulted, before completion of the experiment, in a considerable mortality (Table 1), which was especially high in Series 2, given repeated i.v. injections. All of the “early deaths” occurred between Days 8 and 98 and were associated with aplastic anemia characterized by severe wasting of the tissues generally, low Hb and HCT values, pancytopenia in blood, and atrophic gelatinous bone marrow.

Aplastic anemia and tumors were not observed in 25 control rats studied concurrently with the experimental series but not injected with 7,12-DMBA; necropsy was done at age 180 days.

SINGLE PULSE DOSE OF 7,12-DMBA. In Series 1, 12% of the rats (Table 1) died before Day 50, leaving 22 effective rats for study. Mammary cancer was observed in 23% of this effective number; the mean number of active centers/animal was 1.1. Periauricular cancer was found in 2 rats; leukemia was not detected in this series.

MULTIPLE PULSE DOSES OF 7,12-DMBA. In Series 2, 36% of the rats which received 6 pulse doses of 7,12-DMBA died with aplastic anemia; tumors were not found in these animals. With the exception of these “early deaths,” every rat which received multiple i.v. injections developed 1 or more malignant tumors.

A single class of neoplasm was found in 36% of the effective group (Table 1). These solitary types of tumors were either leukemia (13 rats) or mammary carcinoma (8 rats). Multiple classes of tumors were observed in 38 rats (64%).

Benign tumors (these were exclusively in the mammary gland) were found in 15% of the rats; each host of a benign tumor possessed, in addition, mammary cancer.

The earliest tumor which we recognized was mammary carcinoma on Day 54 (Chart 1). Leukemia was first detected on Day 62. The commonest neoplasms were leukemia, 73%, and mammary carcinoma, 69% (Table 2).

Leukemias were transmitted by cell passage. In 6 rats with leukemia, cardiac puncture was done and 0.2 ml of whole blood was injected i.p. in 9 litters of newborn rats of L-E strain. Leukemia was consistently reproduced in each litter but not in all members of the injected rats. The leukemia was confirmed at autopsy 18–34 days after transmission.

In this series 72 mammary carcinomas were detected, and these were found in 41 rats. The range of active centers/animal was 1–4; mean 1.8 ± 0.9.

Carcinomas of sebaceous glands were found frequently. These were neoplasms of periauricular glands, 41%, and skin, 24%. Cutaneous carcinomas were found only in the dorsal and lateral skin of the trunk.

“Miscellaneous” neoplasms were found in 5 rats (Table 2). These were sarcoma of ear lobe, 2; carcinoma of eyelid, 1; renal sarcoma, 1; hemangiosarcoma, 1.

Testes of all rats in Series 2 were small, soft, and flabby. Ten testes of controls weighed (gm) 2.01 ± 0.09; 62 testes of 7,12-DMBA-injected animals weighed 0.28 ± 0.08. On microscopy the germinal epithelium was completely atrophic in every injected rat and spermatozoa were not found, whereas interstitial cells were abundant. Seminal vesicles and prostates were not atrophic, and secretion was evident on sectioning these glands.
Cancer Induction in Male Hats

**TABLE 2**

<table>
<thead>
<tr>
<th>Classes of Neoplasms Evoked by Single or Multiple Pulse Doses of 7,12-DMBA*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 PULSE DOSE</strong> (EFFECTIVE NO. : 22 RATS)</td>
</tr>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Rats with neoplasms</td>
</tr>
<tr>
<td>Malignant</td>
</tr>
<tr>
<td>Leukemia</td>
</tr>
<tr>
<td>Mammary cancer</td>
</tr>
<tr>
<td>Periauricular cancer</td>
</tr>
<tr>
<td>Skin cancer</td>
</tr>
<tr>
<td>Oral cancer</td>
</tr>
<tr>
<td>Miscellaneous</td>
</tr>
<tr>
<td>Benign</td>
</tr>
<tr>
<td>Mammary</td>
</tr>
</tbody>
</table>

* 7,12-DMBA, 7,12-dimethylbenz(a)anthracene.
* ± S.D.

in the gross. On histologic examination, the secretory epithelium did not differ from that of untreated controls.

**Hormone Independence of Mammary Carcinoma in Male Rat.** In Series 3, 5 rats bearing a total of 6 mammary carcinomas were subjected to bilateral orchiectomy and observed for 28 days. All of the tumors had progressive increase in size of the breast cancers.

Six rats bearing 9 mammary cancers were hypophysectomized and observed for 28–52 days. As in the foregoing group, all of the tumors increased in size and active mammary cancers were found at necropsy.

**Discussion**

Multiple i.v. injections of 7,12-DMBA were more effective than a single dose in evoking tumors in male S-D rats. It has been found (13) in S-D females that multiple pulse doses give rise to a larger number of mammary carcinomas than the number elicited by a single injection. Moreover, in S-D females (11), 2 doses, each of 400 r with a 6-week interval, of total-body irradiation with X-rays doubled the number of breast cancers above the incidence elicited by a single dose of 400 r.

The strain of rat exerts a considerable influence upon the incidence and type of neoplasm elicited in male rats by pulse doses of 7,12-DMBA. In an earlier study (15), male rats of the Long-Evans strain received multiple i.v. injections of 7,12-DMBA, with the same dosage of hydrocarbon and schedule of administration employed as in the present work; the incidence of leukemia was 100% and of mammary carcinoma, 12.5%. In comparison, S-D males in the present experiment had a lower incidence of leukemia with a higher yield of mammary cancer.

It is noteworthy that sarcoma of the ear lobe occurred at the site of perforations of the auricle made for identification; the tissue reaction to recent trauma had localized the carcinogen, and sarcoma developed.

In the rat testis, 7,12-DMBA selectively destroys epithelium of the tubules but *only those germinal cells which synthesize DNA* (5). Germinal cells which proliferate by miosis and the interstitial cells are spared from injury. Hipkin (7) confirmed the observation that 7,12-DMBA had no effect on the endocrine function of the testis. In the present experiments, the findings of secreting prostate and seminal vesicle are additional proof that synthesis of androgenic hormones was not abolished.

It has been found (9) that a large percentage of the hydrocarbon-induced mammary cancers of female rats are hormone-dependent since they regress after ovariectomy or hypophysectomy. Likewise in the human male, orchiectomy (4) and adrenalectomy (8) result in profound regression of mammary cancer in many clinical patients. It is remarkable that mammary cancers in *male rats* differed profoundly from breast cancers in these 2 classes of hosts since no regression occurred after gonadectomy or removal of pituitary. The aromatic-induced cancers of the breast of male rats are, by definition, hormone-independent.

**Acknowledgments**

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