Rapid Induction of Subcutaneous Fibrosarcoma by 7,12-Dimethylbenz(a)anthracene in an Inbred Line of Syrian Hamsters

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SUMMARY

Single s.c. injections of 500 \( \mu g \) 7,12-dimethylbenz(a)-anthracene in tricaprylin were given to 25 males and 25 females of 10 inbred strains of Syrian hamsters. In 9 strains, the average time of latency before palpable tumors appeared was 13 to 16 weeks. In 1 strain, BIO 15.16, the average latency was 10 weeks. These differences are statistically significant. In this line, the first palpable tumors appeared in both sexes 5 weeks following carcinogen injection. All animals of this line developed tumors within 15 weeks after carcinogen injection [there was a single exception in a male (included in the average latency) wherein a tumor developed 34 weeks after injection]. Histologically, the tumors were fibrosarcomas invading the subcutaneous musculature and morphologically indistinguishable from similarly induced tumors in other strains. There were few metastases; all tumors tested grew upon subcutaneous transplantation into hamsters of the same as well as of unrelated strains.

INTRODUCTION

It has long been known that the susceptibility of mice to polycyclic hydrocarbon carcinogens is dependent upon the genetic background of such animals. Thus, among the most susceptible strains of mice are the C3H, C, M, C57BL/6, A, and DBA and among the most resistant are I, Y, and NHO (4). Although Syrian hamsters have been subjects for the study of chemical carcinogenesis since the early 1940's, their genetic variability of susceptibility to carcinogens remains unknown (5, 6).

This paper describes the first in a series of studies on strain variability of susceptibility to carcinogens and is limited to observations on the effect of s.c. administration of a standard dose of DMBA\(^2\) in 10 inbred strains of Syrian BIO hamsters, namely, 15.16, BT, 1.5, 4.22, 7.88, 12.14, 45.5, 54.7, 82.73, and 87.20.

MATERIALS AND METHODS

The animals used were BIO hamsters, pedigreed descendants of the breeders described in Table 1. The hamsters were identified by earmarks and housed 3 to 5 of the same sex per cage in an air-conditioned animal room, fluorescent lighting being the sole source of light from 7:00 a.m. to 9:00 p.m., on Ab-Sorb-Dri bedding (Old Mother Hubbard Dog Food Co., Lowell, Mass.), with Guilford breeder chow and tap water \( \text{ad libitum} \). The cages were changed weekly, at which time all animals were examined and injection sites were palpated.\(^3\)

The carcinogen studied was DMBA purchased from Eastman Kodak Company, Rochester, N. Y. (Batch 5149). Its purity was ascertained by means of ultraviolet spectrophotometry, thin-layer chromatography, and melting point determination. Tricaprylin purchased from Eastman Kodak (Batch 2097) was used as a medium; its purity was identically determined.

Five hundred \( \mu g \) of the carcinogen in 0.1 ml of tricaprylin were injected s.c. into the groin of 25 male and 25 female hamsters of each strain. The animals were 90 days old.

Tumors which developed were permitted to reach at least 2 cm in diameter before these animals were sacrificed and autopsied. At autopsy, no less than 1 tumor from each strain was removed for trocar transplantation into hamsters of the same as well as unrelated strains, and for histological study. All organs were inspected and sections were taken of anomalous lesions. Occasionally, animals were found dead and had to be discarded.

In a previous aging study, 80 to 100 animals of 7 of the strains used in this study were observed throughout their natural life-span without occurrence of spontaneous subcutaneous sarcomas.

RESULTS

The carcinogen was well tolerated in all strains, as seen from the survival rates in Chart 1. Weekly palpation shortly following injection revealed the formation of resilient cysts. There was no significant difference among strains in the rate of development of this early response to the injection of carcinogen. There was considerable variability between several strains with regard to the rate of formation of solid masses, clearly palpable as tumors, which occurred as early as 5 weeks in some animals and much later in others. The average time of...
Table 1

Origin, degree of inbreeding, and description of inbred BIO hamsters used in carcinogenesis study

<table>
<thead>
<tr>
<th>Line designation</th>
<th>Source of original breeders</th>
<th>Minimum No. generations inbred by brother X sister mating</th>
<th>Coat color</th>
<th>Known characteristics and diseases</th>
<th>Longevity under standard conditions (time in days at which 50% of animals survived)</th>
<th>Average body weight at time of carcinogen injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 15.16</td>
<td>Warren</td>
<td>17</td>
<td>Tawny</td>
<td>Highly susceptible to s.c. DMBA</td>
<td>Unknown</td>
<td>100</td>
</tr>
<tr>
<td>2. BT</td>
<td>Toth</td>
<td>12</td>
<td>White</td>
<td></td>
<td>Unknown</td>
<td>109</td>
</tr>
<tr>
<td>3. 1.5</td>
<td>NIH</td>
<td>18</td>
<td>Acromelanic White</td>
<td>Dental caries susceptible</td>
<td>590</td>
<td>104</td>
</tr>
<tr>
<td>4. 4.22</td>
<td>Schwentker</td>
<td>41</td>
<td>Agouti</td>
<td></td>
<td>481</td>
<td>100</td>
</tr>
<tr>
<td>5. 7.88</td>
<td>Toolan-Gulf</td>
<td>29</td>
<td>Cream</td>
<td>Hindleg paralysis</td>
<td>473</td>
<td>89</td>
</tr>
<tr>
<td>6. 12.14</td>
<td>Warren</td>
<td>20</td>
<td>Cream</td>
<td></td>
<td>Unknown</td>
<td>150</td>
</tr>
<tr>
<td>7. 45.5</td>
<td>Ingham-Toolan-Gulf</td>
<td>22</td>
<td>Cream</td>
<td></td>
<td>562</td>
<td>147</td>
</tr>
<tr>
<td>8. 54.7</td>
<td>Warren</td>
<td>19</td>
<td>Amber</td>
<td>Heterochromia iridis</td>
<td>509</td>
<td>101</td>
</tr>
<tr>
<td>9. 82.73</td>
<td>Warren</td>
<td>20</td>
<td>Lilac</td>
<td></td>
<td>502</td>
<td>95</td>
</tr>
<tr>
<td>10. 87.20</td>
<td>Ingham-Toolan-Gulf</td>
<td>18</td>
<td>Rust</td>
<td></td>
<td>670</td>
<td>118</td>
</tr>
</tbody>
</table>

Chart 1. Average time of latency (in weeks) and standard deviation of development of palpable subcutaneous sarcomas in inbred Syrian hamsters following s.c. injection of 0.5 mg of DMBA.

Latency elapsing between carcinogen injection and palpation of an occurring tumor varied from 9 weeks in females of the BIO 15.16 strain to 17.5 weeks in males of the BIO 82.73 strain. The latency of the BIO 15.16 strain was significantly different from all others at the 0.001 level. The differences between latent times of all strains other than the BIO 15.16 strain were not statistically significant at the 0.05 level. The Iball (8) index of carcinogenicity takes into account not only average latency but the percentage of tumors found among surviving animals. This index is calculated by dividing the percentage number of tumors by the average latent period in days and multiplying the resulting ratio by 100. The Iball indices shown in Chart 1 also reveal the BIO 15.16 hamsters to be the most responsive to DMBA. The survivors in all strains eventually developed tumors which were fibrosarcomas with somewhat greater polymorphism of cells than is generally seen with chemically induced fibrosarcoma in mice. In addition to the typical spindle-shaped, elongated cells which form swirling strands interspersed with scant fibrous stroma, nests of foamy polygonal cells surrounded by basophilic polysaccharide were present. These observations are illustrated in Fig. 1. In each strain, 1 of the early appearing tumors in each group of animals (1 in males and 1 in females) was transplanted subcutaneously into hamsters of the same strain, as well as into most of the unrelated strains; all of these transplants grew rapidly. The morphology of the tumors of 2nd, 3rd, and, in some cases, more numerous transplant generations was similar to that seen with induced tumors. In some animals, metastases of induced tumors were observed; however, this was a rare occurrence which appeared in only 6 animals, so that statistical analysis as to strain susceptibility to metastasis was not feasible. Those
organ showing metastases were the lungs (4 cases) and diaphragm (1 case). In 1 male of the BIO 15.16 strain, a thymoma was observed; no other tumors were found.

DISCUSSION

The observations of Nettleship and Smith (9) suggest that subcutaneous hamster fibroblasts are transformed with great rapidity into fibrosarcomas by injection of polycyclic hydrocarbons. This observation has been confirmed in our studies with benz(rst)pentaphene (dibenzo-pyrene) (7). Berwald and Sachs (1) demonstrated that malignant transformation in vitro occurs rapidly in cells from this species. The few studies reported in the literature on the s.c. injection of polycyclic hydrocarbons in hamsters [with the exception of Nettleship and Smith (9)] do not report particularly rapid tumor formation.

Benzpyrene in the hands of Halberstaedter (3) caused 93% tumors with an average latency of 3 months, as well as a high incidence of lymph node metastases. In the studies of Nettleship and Smith (9), 500 μg of methylcholanthrene produced palpable tumors within 10 to 12 weeks, although examination of injection sites 24 hr following s.c. injection revealed fibroblasts morphologically different from normal.

Crabb (2) obtained sarcomas in 4 hamsters in from 11 to 14 weeks following injection of 2 mg of DMBA. Wodinsky et al. (10) used benz(rst)pentaphene (dibenzo-pyrene) in tricaprylin in doses ranging from 0.25 to 2 mghamster and observed from 55 to 100% tumors, depending on the dosage, with the 1st tumor appearing as early as 8 weeks following administration of the highest dosage.

Our studies, therefore, reveal the most rapid tumor production until now described in hamsters and, as far as we are able to ascertain, in any rodent (1st tumor in 5 weeks; mean latency of 9 weeks). However, this rapid response is limited so far to only 1 (BIO 15.16) of the 10 inbred lines studied. All others have significantly longer latency times (ranging from 13 to 16 weeks on the average) which are not significantly different from each other.

It may be concluded that in the hamster, as in the mouse, genetic factors govern the responsiveness of subcutaneous fibroblasts to polycyclic hydrocarbons and that the BIO 15.16 line is "supersensitive" to the carcinogen studied (DMBA). Whether the fibroblasts in this line are sensitive to other types of carcinogens or possibly that other cells in these animals possess similarly great susceptibility to oncogenic stimuli remains for further research to determine. The inbred lines included in this study do not have spontaneous neoplasms as far as we are presently able to determine.

In all the lines studied, serum antibodies were present against PVM and Sendai viruses. Antibodies against reovirus type 3 were present in all strains except 87.20 and 4.22. Antibodies against mouse adenovirus were present in strains 15.16, BT, 1.5, 7.88, 54.7, and 82.73 and absent in all others. No antibodies were found against the following viruses: GD VII, K, polyoma, minute virus of mice, Kilham rat virus, H1, SV5, ectromelia, mouse hepatitis, and LCM.4

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Mrs. Lois B. Ramsey.

REFERENCES


4 The virus antibody studies were performed by Microbiological Associates, Inc., Bethesda, Md. 20014.

Fig. 1. A, high-power (× 400) photomicrograph of a subcutaneous fibrosarcoma induced in a Syrian hamster (BIO 7.88 line) by a single s.c. 0.5-mg dose of DMBA in tricaprylin. Note anomalous fibroblasts with large nuclei arranged in swirling strands (bottom right) with atypical cells of varying shape and nuclear size and density elsewhere in field shown. B, low-power (× 100) photomicrograph of lung metastasis of fibrosarcoma induced in a Syrian hamster (BIO BT line) by a single s.c. 0.5-mg injection of DMBA. Note parenchymal metastasis and hematogenous spread with tumor in blood vessels. C, low-power (× 100) photomicrograph of lung metastasis peppered with multiple metastases. D, low-power (× 100) photomicrograph of metastasis to diaphragm of subcutaneous fibrosarcoma induced in a Syrian hamster (BIO 7.88 line) by a single s.c. 0.5-mg injection of DMBA. Same animal from which subcutaneous tumor shown in A was taken. Note invasion of muscle by sarcoma. E, high-power (× 400) photomicrograph of diaphragmatic metastasis shown in D. Note necrotic muscle fibers (top).
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