The Induction of Malignant Melanomas in Syrian White Hamster by Neonatal Exposure to Urethan

S. D. Vesselinovitch, N. Mihailovich, and W. R. Richter

Division of Oncology, Institute for Medical Research, The Chicago Medical School, and Department of Pathology, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60637

SUMMARY

Urethan was administered to newborn Syrian white hamsters to disclose their neoplastic competence. The first treatment was given within 24 hr of birth, and four subsequent injections were given at 3-day intervals. The survivors were sacrificed at 120 weeks of age.

The urethan-treated animals developed malignant melanomas which metastasized to regional lymph nodes, lungs, kidneys, and liver. None of the untreated controls showed any melanomas either clinically or at autopsy. The melanoma-bearing hamsters had significantly shorter life-spans than did the untreated controls. A variety of other primary tumors, such as stomach papillomas, hepatocarcinomas, and kidney adenomas, which were not seen in the controls developed in the carcinogen-treated animals, although with a low frequency.

INTRODUCTION

Melanotic tumors have been successfully induced in hamsters by topical application of 7,12-dimethylbenz[a]anthracene (5-15) and by p.o. administration of urethan (13, 18). These lesions showed morphological similarity to the cellular blue nevi of man (12, 14, 15), arising apparently from melanocytes surrounding some of the pilosebaceous structures in the skin (5). Nakai and Rappaport (11) and Rappaport et al. (14) indicated that the carcinogen-induced melanotic tumors grew slowly, metastasized rarely, and had no appreciable effect upon the life expectancy of the host.

As an integral part of our comparative studies on the neonatal carcinogenesis in various species (20-25), we exposed newborn Syrian white hamsters to a limited number of urethan treatments. The short neonatal administration of this carcinogen resulted primarily in the induction of malignant melanomas. The present paper deals with biological behavior and morphology of the former lesions and discusses possible factors responsible for their successful induction.

MATERIALS AND METHODS

Hamsters. Randomly bred Syrian white hamsters were used in the present experiment. This variant of the Syrian hamster is not a true albino, having pigmented skin of the ear and the scrotum but being devoid of the perifollicular collections of pigmented melanocytes around the pilosebaceous follicles. The original animals were obtained from Abrams Small Stock Breeders, Chicago, Ill., in 1961 and bred in this laboratory. The animals were mated at 3 months of age, and the newborn hamsters were treated as indicated below. The animals were weaned and separated by sex when 30 days old, following which they were weighed and inspected at 2-week intervals. Animals were housed in plastic cages in sets of 5 in a temperature-controlled laboratory at 78°F; they were fed Rockland diet and given water ad libitum.

The experiment was terminated when the animals were 120 weeks old. The time of appearance of the external tumors and their anatomical locations were recorded. At autopsy, specimens were taken from tumors and all internal organs. The ceca were not taken. All enlarged lymph nodes were preserved and studied histologically. The tissues were fixed in buffered 10% formalin. Sections were routinely stained with hematoxylin and eosin.

Treatment. The solution of urethan (10% w/v in redistilled water) was injected i.p., 0.005 ml (0.5 mg of urethan)/g of body weight at each injection. The treatment was initiated when the animals were less than 24 hr old. The injections were given at 3-day intervals, and animals received a total of 5 treatments, the last on the 13th day of age (Groups 1 and 3, males and females, respectively). Control groups received no treatment (Groups 2 and 4, males and females, respectively). Control groups received no treatment (Groups 2 and 4, males and females, respectively).

RESULTS

The animals showed a good survival rate and maintained average body weights within normal range throughout the experiment (Table 1). Sixty-two % of the males (Group 1 versus Group 2, p < 0.001) and 40% of the females (Group 3 versus Group 4, p < 0.01) developed at least 1 primary tumor following the neonatal exposure to urethan. Three males (12.5%) and 1 female (3.3%) were found with stomach papillomas and 1 female had hepatocarcinoma (3.3%). Cortical kidney adenomas were seen in 1 male (4.2%) and 2 female (6.6%) hamsters. Although none of these tumors were...
Table 1

The survival and the average weight of untreated controls and urethan-treated Syrian white hamsters

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals and sex</th>
<th>Treatment with urethan</th>
<th>No. of survivors/average weights (g) at age (wk) below:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>24, M</td>
<td>Yes</td>
<td>22/110</td>
</tr>
<tr>
<td>2</td>
<td>22, M</td>
<td>No</td>
<td>22/103</td>
</tr>
<tr>
<td>3</td>
<td>30, F</td>
<td>Yes</td>
<td>29/120</td>
</tr>
<tr>
<td>4</td>
<td>28, F</td>
<td>No</td>
<td>26/115</td>
</tr>
</tbody>
</table>

*At the time of weaning.

seen in the untreated controls, no statistical significance could be attributed regarding the induction of any of the above tumors by urethan due to their low frequencies.

However, the urethan-treated animals developed melanomas (Table 2) with statistically significant frequencies ($p < 0.001$, males; $p < 0.01$, females). Primary melanotic lesions appeared at an average age of 46 weeks, and the animals having these tumors died 34 weeks later on the average. At autopsy, these animals had neoplasms, not only in the skin, but also in the lungs, spleen, kidneys, and submandibular salivary glands. Histological studies showed that the skin was the primary site, while the other tumors were metastases of malignant melanomas. In 11 animals, primary tumors were located in the head and neck region, and in 8 animals, tumors were located in the midback region. The metastases were found in 82% of males and 25% of female hamsters (Table 3). Regional lymph nodes were involved in 50% of melanoma-bearing animals. The lungs were most frequently involved in male animals. The primary skin tumors were presented clinically as black or brown to pink, elevated, and circumscribed tumors. Tumors generally grew slowly, reaching an average weight of 2.5 g at the time of animal death. The animals bearing melanotic tumors were, on the average, 80 weeks old at the time of death. The untreated control animals lived significantly longer, dying at an average age of 93 weeks ($t = 2.85; p < 0.01$).

Morphology of primary and metastatic malignant melanomas has been illustrated in Figs. 1 to 6. These tumors were characterized by great variation in abundance of melanin, growth patterns, and cellular pleomorphism. Primary tumors grew expansively and were heavily (Fig. 2) to moderately laden with melanin, the latter containing amelanotic regions (Fig. 1), while metastases tended to be only slightly pigmented to amelanotic. The melanin was seen in large polygo-

Table 2

Tumor response of Syrian white hamsters treated with urethan during the neonatal period

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals and sex</th>
<th>Treatment with urethan</th>
<th>Animals with melanomas</th>
<th>Average age (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>At appearance ± S.E.</td>
</tr>
<tr>
<td>1</td>
<td>24, M</td>
<td>Yes</td>
<td>15</td>
<td>62.5</td>
</tr>
<tr>
<td>2</td>
<td>22, M</td>
<td>No</td>
<td>12</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>30, F</td>
<td>Yes</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>28, F</td>
<td>No</td>
<td>2d</td>
<td>7.1</td>
</tr>
</tbody>
</table>

*aAt the time of weaning.

bAnimals bearing melanotic tumors or malignant melanomas clinically and/or at autopsy.

cAverage age of melanoma-bearing hamsters (Groups 1 and 3) and all untreated animals (Groups 2 and 4) at the time of death.

dTwo cholangioma-bearing animals (male and female) and 1 hemangioma-bearing hamster (female) were observed.
Malignant Melanoma Induction by Neonatal Exposure to Urethan

Table 3

Anatomical distribution of primary melanomas and the frequency of metastatic lesions found at various sites in urethan-treated Syrian white hamsters

Urethan, 0.5 mg/g of body weight, was delivered i.p. at each injection; treatment began on the first day of life (<24 hr) and continued 5 times at 3-day intervals (total dose 2.5 mg/g of body weight).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>No. of animals with melanomas&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Animals with metastases</th>
<th>Frequency of metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Location</td>
<td>Animals with metastases</td>
<td>Various organs&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Head and neck</td>
<td>Total</td>
<td>Lungs</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>9</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Animals bearing melanotic tumors or malignant melanomas recorded at inspection and/or at autopsy.

<sup>b</sup>In each instance, several metastatic foci were found in organs involved.

Primary tumors were covered by a thin layer of epithelium which was not invaded by neoplastic cells, although skin adnexa were atrophic (Fig. 1). Metastatic lesions in the regional lymph nodes, lungs, and kidneys were circumscribed but not encapsulated. In the liver, the tumor was infiltrative, not well circumscribed, and thus distorted the architecture of the organ.

Neoplastic cells at times showed a mesenchymal structure (Fig. 3) forming whorls, bands, or bundles and thus resembling fibrosarcomas. On other occasions, cells are polygonal, epithelial-like (Fig. 6), and arranged in random pseudoglandular pattern (Figs. 5 and 6), in cords, or around blood vessels. An intermediate type of tumor has also been observed (Fig. 4).

DISCUSSION

As anticipated, several types of tumors developed in urethan-treated Syrian white hamsters. These animals were exposed to a lower total dose than formerly given to either mice (20—23) or rats (24, 25) because they failed to tolerate larger amounts of this agent. Apparently due to this limitation on administered dose, the incidence of the majority of tumor types which developed was low (26) and statistically not significant compared to controls. Nevertheless, the development of the malignant melanomas was biologically significant, especially since the malignant melanomas were not previously induced by any carcinogen in this variety of hamster (7, 15). In contrast, although the Syrian golden hamsters developed malignant melanomas spontaneously late in life (4), various carcinogens had in general no effect on the malignant transformation of the readily induced benign melanotic lesions (11, 14—16).

Nakai and Rappaport (11) concluded on the basis of their comparative studies on golden and white Syrian hamsters that “nonpigmented dermal melanocytes or dermal melanocytes with nascent pigment granules are susceptible to the carcinogenic effects of DMBA,<sup>3</sup> while melanocytes in which the granules are completely saturated with melanin are resistant to its effect.” In the white hamster, the pigmentation of the melanin granules begins on the 21st day of life (11). Present data further indicate the high susceptibility of white hamsters to melanoma carcinogenesis during the period prior to melanogenesis. Although one-half of the melanotic tumors emerged by the 46th week of life, animals with malignant melanomas did not die, on the average, until the 80th week of life. Thus, apparently the long life-span of animals permitted progression of the majority of benign melanotic tumors to malignant melanomas within the life-span of the carcinogen-treated host.

Melanotic lesions have been induced previously in the iris of August hooded rats (17) and in the skin of C57BL X C3H F<sub>1</sub> mice treated neonatally with urethan (S. D. Vesselinovitch, unpublished study). The hamsters, however, appeared to be the only species prone to the neonatal induction of malignant melanomas. From the viewpoint of comparative carcinogenesis, it has been shown that the newborn mice were highly susceptible to hepatogenesis and leukenogenesis (1, 2, 8—10, 19—21, 23), and the newborn rats were highly susceptible to hepato- and gliomagenesis, gliomagenesis, and development of Anitschkow cell sarcoma of the heart (24, 25). Obviously, a given chemical may trigger carcinogenesis in several animal species, although the tumor profile and the incidence need not be identical in each instance. The state of the neoplastic competence of the biological system, apparently, may be of greater importance than the carcinogen in determining the occurrence and the type of carcinogenesis. This is of theoretical and applied significance.

<sup>3</sup>The abbreviation in the quotation is: DMBA, 7,12-dimethylbenz[a]anthracene, or 9,10-dimethyl-1,2-benzanthracene.
Presented by: Vesselinovitch, Mihailovich, and Richter

ACKNOWLEDGMENTS

We thank Dr. P. Shubik for his interest in the work. The competent technical assistance of A. Davis, C. Montalvo, and B. Romero is greatly appreciated.

REFERENCES


Fig. 1. Primary s.c. melanoma of the ear. Note the relatively amelanotic character and the absence of the skin adnexa in this region of the tumor. Tumor was first observed when hamster was 49 weeks old. Animal died when 76 weeks old. H & E, X 210.

Fig. 2. A heavily pigmented region of another primary tumor from an animal that died at 49 weeks of age. H & E, X 560.

Fig. 3. Kidney metastasis showing interlacing fascicles of spindle cells simulating a fibrosarcomatous growth pattern. Primary tumor was observed at 37th week in midback region. Animal died at 72 weeks of age. H & E, X 270.

Fig. 4. Lung metastasis showing growth pattern and cell type intermediate to mesenchymal and epithelial. Observe tendency of tumor to random growth and reduced cellularity as compared to Fig. 3. Animal was 66 weeks old at the time the primary tumor was detected on the tip of the right ear. This animal died at 94 weeks of age. H & E, X 370.

Fig. 5. Lung metastasis. Note the clusters of polygonal cells within a matrix with eosinophilic properties. Another area of the tumor illustrated in Fig. 4. H & E, X 225.

Fig. 6. Metastasis to the submaxillary lymph node. Cells are arranged at random or in pseudoglandular patterns showing an epithelial type of growth. Primary tumor which was heavily pigmented was found on the eyelid when animal was 55 weeks old. H & E, X 350.
Malignant Melanoma Induction by Neonatal Exposure to Urethan

![Image of tissue sections](image-url)

Research...
The Induction of Malignant Melanomas in Syrian White Hamster by Neonatal Exposure to Urethan

S. D. Vesselinovitch, N. Mihailovich and W. R. Richter