TUMOR INDUCTION IN THE JAPANESE HOUSE MUSK SHREW, *SUNCUS MURINUS* (INSECTIVORA), BY 1, 2-DIMETHYLHYDRAZINE (DMH)

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**Abstract:** The carcinogenic effects of 1, 2-dimethylhydrazine (DMH) were investigated in virgin female Japanese house musk shrews, *Suncus murinus* (family: Soracidae, order: Insectivora). From 6 weeks of age, 15 shrews were given 14 doses of DMH (40 mg per kg, s.c.), administered weekly (group 1) and another 15 animals were given 28 doses of DMH (20 mg per kg, s.c.), also administered weekly (group 2); 5 untreated shrews served as controls. A high incidence of musk gland tumors, and some other tumors (carcinomas of the small intestine, pulmonary adenoma, liver adenoma, and leukemia) were induced in DMH-treated shrews, whereas no such tumors were seen in untreated shrews up to 60 weeks of age. Musk gland tumors developed in 82% (9/11) of animals in group 1 and in 92% (12/13) of animals in group 2 at 36 to 60 weeks of age. DMH, a colonotrophic carcinogen in rodents, did not evoke colon cancers in shrews. (*J Toxicol Pathol 6: 181~186, 1993*)

**Key words:** Shrew, *Suncus murinus*, Insectivora, DMH, Musk gland tumor

**Introduction**

From the phylogenetic point of view, insectivora are considered to be the most primitive class of primates. One member of this class, the house musk shrew (*Suncus murinus*; family Soracidae), which is small and has a short lifespan, has been bred under laboratory conditions. Shrews, being primitive primates, represent a more suitable animal model than the rodents that are used extensively and findings may easily be extrapolated to humans. Shrews possess a pair of musk glands which, in turn consist of many sebaceous glands. Cutaneous pilosebaceous tumors develop spontaneously at a high incidence, with the musk glands rarely being involved, and with a marked preponderance being shown in males. As other spontaneous tumors are rarely seen in these animals, the females are convenient to use for chemical carcinogenic studies. Although only a limited number of studies have been performed to date, it has been shown that shrews are sensitive to butylated hydroxyanisole (BHA), which induces pulmonary tumors, to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), which induces esophageal carcinomas, and to 7, 12-dimethylbenz(a)anthracene (DMBA), which induces leukemia and intestinal tumors.

Weekly s.c. injections of 1, 2-dimethylhydrazine (DMH) have been shown to induce colon carcinomas in rats, mice, and hamsters. DMH-induced colon carcinomas in rodents are widely accepted as a valuable model that has great reproducibility and marked organ specificity. However, to our knowledge, thus far no attempts have been made to develop tumors in shrews by the use of DMH. Accordingly, in this study, we...
attempted to determine whether DMH has carcinogenic potency in female shrews, since this animal has a clearer phylogenetic relationship to humans than do rodents.

Materials and Methods

Animals

Thirty-five virgin female 4-week-old outbred Japanese house musk shrews, Jic: SUN strain², were purchased from Clea Japan Inc. (Osaka). The animals were housed in plastic cages, five per cage, with sterilized white pine chips as bedding. Room temperature was 22±2°C and relative humidity was 60±10%. The animals were fed a special pellet diet for shrews (CIEA-305, Clea Japan Inc., Osaka) and had free access to water.

Treatment and experimental procedures

A solution of 1, 2-dimethylhydrazine dihydrochloride (DMH; Aldrich Chemical, Milwaukee, Wis., USA) was prepared in a 0.9% saline solution that contained 1.5% EDTA (Nacalai Tesque, Kyoto, Japan) and was adjusted to pH 6.5 with 4% NaOH. The solution was always freshly prepared just before use. Animals were given weekly s.c. injections in the back, beginning at 6 weeks of age. Fifteen shrews were given 14 weekly doses of 40 mg/kg body weight (group 1), and another 15 animals were given 28 weekly doses of 20 mg/kg body weight. Injections were stopped at the 14th injection in group 1 due to the death of two experimental animals during the week before the 14th injection, and we also stopped at the 28th injection in group 2 to adjust the total dose of DMH (560 mg/kg/shrew). Five animals that received no injections served as controls (Group 3). Shrews were weighed weekly until the termination of the experiment (60 weeks of age). The animals were sacrificed when they became moribund, when tumors were visible, or when they reached 60 weeks of age. The number of viable shrews was taken to be the number that survived until the end of DMH administration.

Histological examination

All animals were carefully autopsied. The whole digestive canal was opened longitudinally and examined flattened out on a card with the mucosa upward. The small intestines were coiled up in a 'swiss-roll' fashion. Both gross and histological examination of all other organs was performed. All tumors and a variety of tissues were fixed with methacarn, dehydrated, and embedded in paraffin. The colon was sectioned at at least four different levels. All blocks were sectioned at 4 μm, and routinely stained with HE, and with PAS if necessary.

Results

The increases in body weight in the DMH-treated and control shrews were comparable, and the animals showed no acute toxicity after DMH treatment. In addition to the two shrews that died in group 1, two animals each in groups 1 and 2 died after the carcinogen treatment was completed; however, as no histopathological examination could be made due to cannibalism, they were excluded from the calculation. Therefore, as shown in Table 1, the numbers of viable shrews were 11 in group 1 and 13 in group 2. Tumors developed in DMH-injected animals, whereas in the 5 control shrews, no tumors were seen when they were sacrificed at 60 weeks of age when the experiment was terminated. The mean age when killed was 55.9 weeks in group 1 animals and 53.1 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>DMH administration</th>
<th>No. of shrews</th>
<th>Mean age when killed (weeks)</th>
<th>Type of tumor (no.; %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Viable</td>
<td>Musk gland tumor</td>
</tr>
<tr>
<td>1.</td>
<td>40 mg DMH/kg/wk * 14</td>
<td>15</td>
<td>11</td>
<td>55.9±5.8</td>
</tr>
<tr>
<td>2.</td>
<td>20 mg DMH/kg/wk * 28</td>
<td>15</td>
<td>13</td>
<td>53.1±7.6</td>
</tr>
<tr>
<td>3.</td>
<td>No treatment</td>
<td>5</td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>
Fig. 1. Musk gland tumor. Sharply circumscribed multilobular tumor cell nests are seen. HE ×12

Fig. 2. Musk gland tumor. Ulceration and bleeding occur frequently. HE ×20

Fig. 3. Musk gland tumor showing sebaceous adenoma. The tumor consists of basaloid cells and mature sebocytes. Holocrine secretion is seen. HE ×200
in group 2. All these animals exhibited similar tumor induction, so that the following descriptions apply to both groups. Musk gland tumors, usually bilateral, were induced in 9 animals in group 1 (82%), and in 12 in group 2 (92%) between 36 and 60 weeks of age. Microscopically, the musk gland tumors, which were multilobular, were sharply circumscribed, and they often exhibited ulceration and bleeding (Figs. 1 and 2). In some tumor cell nests, they were rimmed by basaloid cells and fully developed sebocytes were seen near the center (sebaceous adenoma) (Fig. 3), this often being accompanied by holocrine secretion. Other tumor cell nests consisted of basaloid cells only (sebaceous epithelioma) (Fig. 4). Mitosis was seen, but the lack of local invasion indicated its benign nature.

Besides the musk gland tumors, the DMH-treated shrews developed leukemia\(^1\), liver adenoma\(^1\), pulmonary adenoma\(^1\), and intestinal tumors\(^2\). The leukemia developed in a 50-week-old shrew showing splenomegaly and was lymphoblastic in type. The pulmonary adenoma and liver cell adenoma occurred in shrews that were 50 and 60 weeks of age, respectively. In general, the livers of the DMH-treated shrews showed signs of toxic damage. Although mitosis was not seen, variable shapes of hepatocytes

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**Fig. 4.** Musk gland tumor showing sebaceous epithelioma.
The tumor is homogeneous, consisting of basaloid cells only. HE ×200

**Fig. 5.** Megalocytosis. Variable shapes of hepatocytes with enlarged nuclei are seen in DMH-treated shrews. HE ×200
(megalocytes) with enlarged nuclei were observed (Fig. 5). Additionally, the liver parenchymal cells became progressively less PAS-positive, indicating loss of glycogen storage. During the experiment, no diarrhea or rectal bleeding was seen. Although we observed two carcinomas originating in the small intestine (Fig. 6), no macroscopic or microscopic alterations were found in the colons of DMH-treated shrews. No metastasis was observed in any of these tumor-bearing shrews within the experimental period.

Discussion

This study showed that a high incidence of musk gland tumors was induced in female shrews by DMH injection. Musk gland tumors are androgen-dependent and contain androgen receptors. It is interesting to note that the induced tumors were restricted to the musk glands and that no tumors developed in the cutaneous pilosebaceous glands. Although the spontaneous occurrence of pilosebaceous tumors is frequently seen in male shrews, these tumors are rarely seen in females. This remarkable sexual dimorphism indicates that male sex hormones may favor the development of these tumors. Sebaceous tumors have been experimentally induced in rats exposed to various carcinogens, e.g., DMBA, DMH and its metabolites such as azoxymethane (AOM), the tumors usually being localized in the sebaceous glands of the ear ducts.

In previous studies of shrews, a high incidence of leukemia and pulmonary tumors was seen after DMBA and BHA administration, respectively. In the present study, we found that DMH also evoked these tumors but at a much lower incidence. DMH had toxic effects on the liver; similar toxic effects have been observed in rats, mice, and hamsters, wherein hepatocytes became greatly enlarged and exhibited pleomorphic nuclei (megalocytes). Adenoma of the liver was induced in one shrew, this being similar to that reported to be induced in rats by AOM.

DMH consistently induces a high specific colon tumor in rodents, and in these species, DMH has proven to be a colonotrophic carcinogen. In contrast, although carcinoma of the small intestine was induced in two animals, similar to that induced by DMBA treatment, the present results showed that DMH appears to have no enhancing effect on tumorigenesis in the colon in shrews. This is probably due to a different DMH metabolism, or to the short length of the colon in shrews, i.e., they may not have sufficient numbers of target cells for neoplastic transformation. In conclusion, the organotrophism of DMH in shrews was different from that in rodents, in that high frequencies of musk gland tumors were seen in DMH-treated female shrews, whereas no colon cancers were seen, i.e., DMH-induced tumors revealed species-specific differences.
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References


