Effects of Prednisolone and Thalidomide on Induced Submandibular Gland Tumors in Hamsters

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Summary

No significant difference in the incidence of induced fibrosarcomas in hamster submandibular glands was noted when the animals were given a single intraglandular injection of 100, 200, 500, and 1000 µg of prednisolone concurrently with administration of 0.05 ml of 0.5% 7:12-dimethylbenz(a)anthracene (DMBA) in liquid petrolatum. There was, however, an inhibitory trend when, in addition to the initial intraglandular injection of 500 µg of prednisolone, the same amount was injected i.p. every week throughout the experiment. There was a higher but insignificant incidence of metastasis in prednisolone-treated hamsters as compared to the controls.

Thalidomide failed to show any influence on induced tumors when given to hamsters in a similar procedure using 3 dosage levels, a single 5- or 10-mg dose, and a 5-mg initial injection plus 5 mg/week.

Introduction

There are conflicting reports in the literature regarding the effects of corticoids upon tumorigenesis in animals. Some investigators have observed a stimulating influence (10, 16, 17), others report the drug has no influence (4, 8), and still others have seen an inhibitory effect of cortisone on experimental carcinogenesis (3, 5, 6, 9, 15, 18). Sabes and his co-workers (14) have recently reviewed the literature on this subject. The divergent results reported by various investigators have stemmed from the differences in the experimental design, the methodology, the species and strain of animals used, the dose of cortisone, its mode and duration of administration, the nature of the carcinogen, its dose and mode of administration and, lastly, the type of tumors induced.

Since the inhibitory effects of cortisone on fibroblasts and intercellular substances are known, it is assumed that induced connective tissue tumors in the submaxillary glands of the hamsters may provide a useful biologic system for testing the influence of corticosteroids upon induced fibrosarcomas. The present investigation is therefore designed to assess the role of prednisolone upon induced fibrosarcomas in the submandibular glands of hamsters. It is further intended to study the effects of the drug on the metastatic behavior of these chemically induced autochthonous tumors.

Thalidomide is a well-known teratogenic agent which produces congenital malformations by selective inhibitory action on coordinated fetal growth. It would be interesting, therefore, to find its influence on the uncoordinated cellular growth in induced malignant tumors. The present study is concerned with the investigation of the influence of thalidomide, or its breakdown products, on rapidly growing fibrosarcomas induced in the submandibular glands of the hamsters.

Materials and Methods

Ninety-four Golden Syrian hamsters were matched on the basis of weight and were randomly assigned to the various groups. Animals were singly caged in a room with no environmentally controlled conditions of light, temperature, humidity, or noise. They were fed carrots, Purina laboratory chow, and water was available ad libitum.

The hamsters were anesthetized with veterinary pentobarbital sodium and their submandibular glands exposed surgically. Two separate 1-ml tuberculin syringes were used for the injections in each group; 1 syringe was used to administer 0.5% 7:12-dimethylbenz(a)anthracene (DMBA) in liquid petrolatum and the other, prednisolone in varying concentrations. The needles were inserted simultaneously into the gland but the injection of carcinogen preceded that of prednisolone. Following injections, the neck wounds were closed in layers with silk sutures. Starting at 8:00 A.M., and ending at 2:00 p.m., the injections were given in rotation in order to minimize the possibility of time factor differences among treatments and to compensate for any diurnal differences in steroid levels among animals. The groupings of the animals and their respective treatments are summarized in Table 1. Groups I through IV were given intraglandular injections of 0.05 ml of 0.5% DMBA and the same volume of prednisolone in concentrations of 100, 200, 500, and 1000 µg, respectively. Group V animals were injected with the same volume of DMBA and 500 µg of prednisolone in the gland. In addition, they were given weekly i.p. injections of 500 µg of prednisolone. The control group was injected with carcinogen and prednisolone vehicle, which was essentially normal saline with added buffers.

Another study was conducted in which 50 hamsters were divided into 4 groups of 14, 13, 15, and 8 animals each. They were injected with 0.05 ml of 0.5% DMBA into the submandibular gland. In addition, Groups I and II were given 5 and 10 mg of...
The tumors were excised and fixed in 10% formalin for routine examination and examined for any gross or microscopic evidence of metastases. Since Group V animals showed an apparent tumor inhibition and were on a regimen of weekly injections of prednisolone, a comparison of the combined experimental groups and the control similarly showed no significant difference indicating that thalidomide at the above levels exerts no influence on tumorigenesis.

There were 67 prednisolone-treated animals in which the visceral organs were available for microscopic examination. Of these, 44 developed fibrosarcomas in the submandibular glands of which 8 metastasized to different organs, comprising an incidence of 18%. The control group contained 22 animals of which 14 developed fibrosarcomas but only 1 metastasized to the lung and kidney (7%). Thus, there was roughly a 2.5:1 ratio of metastases in experimental animals as compared to the controls.

### Discussion

No definite conclusions can be drawn from the data of this investigation regarding the influence of corticoids on induced fibrosarcomas in the submandibular glands of the hamster. No significant difference was found when the animals were given a single injection of 100, 200, 500, and 1000 µg of prednisolone concurrently with an intraglandular injection of DMBA. There was, however, an inhibitory trend noted when the drug was administered in 500-µg weekly doses for the subsequent 21 weeks of the experiment. A large series of animals with higher repeated weekly doses may help to determine the effects of the corticoids on induced fibrosarcoma. Ghose (7), using methylrholanthrene, noted reduced but insignificant differences in the cortisone-induced fibrosarcomas in the subcutaneous tissue of mice, and noted reduced but insignificant differences in the cortisone-treated animals as compared to the controls. Sabes and his co-workers (14) noted a similar inhibitory trend, but they attributed this partially to the time of the day the injections were administered.

Certain types of biologic activities are shared both by the teratogenic and the carcinogenic agents, particularly the antimitotic activity, the tumor-inhibitory activity, and the mutagenicity. The hypothesis that thalidomide may possess tumor-inhibitory activity was not supported within the limitations of the experimental design used in the present investigation. Bach and his co-workers (2) saw no inhibition of 2 types of transplantable mouse tumors. Roe and Mitchley (13) noted no inhibition in growth of the transplantable Walker rat carcinosarcoma 256 in response to thalidomide administered orally or i.p. in repeated large doses. Luers (11) found no evidence that thalidomide causes mutations in Drosophila.

It should be reiterated that the data on metastases were obtained from the routine 1–2 microscopic sections of the organs. No attempt was made to cut serial sections of those organs in which gross evidence of a tumor was lacking.

It seemed that corticoid-treated animals had a higher yield of pulmonary metastases but this was not substantiated statistically, and this, again, is in agreement with the findings of Ghose. The exact mechanism by which corticoids may cause spread of cancer is highly speculative. Pomeroy (12) attributed this action of the drugs to their destructive influence on the lymphoid tissue and reticuloendothelial cells, thereby disturbing the immune response. Agosin (1) compared the metastasizing action of cortisone to that of hyaluronidase which is known to favor the propagation of the tumors.

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**TABLE 1**

**Effect of Prednisolone on Induced Tumors**

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals/Group</th>
<th>Animals Survived</th>
<th>Treatment: DMBA + prednisolone</th>
<th>Animals with Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>17</td>
<td>DMBA + 100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
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<td>17</td>
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</tr>
<tr>
<td>III</td>
<td>14</td>
<td>14</td>
<td>DMBA + 500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>IV</td>
<td>13</td>
<td>12</td>
<td>DMBA + 1000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>9</td>
<td>DMBA + 500&lt;sup&gt;b&lt;/sup&gt; + 500 weekly&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>VI</td>
<td>23</td>
<td>23</td>
<td>DMBA + vehicle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15</td>
</tr>
</tbody>
</table>

<sup>a</sup> DMBA, 7:12-dimethylbenz(a)anthracene.<br>
<sup>b</sup> Intraglandular.<br>
<sup>c</sup> Intraperitoneal.

**TABLE 2**

**Effect of Thalidomide on Induced Tumors**

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals/Group</th>
<th>Animals Survived</th>
<th>Treatment: DMBA + Thalidomide</th>
<th>Animals with Tumors</th>
</tr>
</thead>
<tbody>
<tr>
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<td>13</td>
<td>DMBA + 5 mg&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>DMBA + 10 mg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>11</td>
<td>DMBA + 5 mg&lt;sup&gt;b&lt;/sup&gt; weekly</td>
<td>8</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>6</td>
<td>DMBA + vehicle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> DMBA, 7:12-dimethylbenz(a)anthracene.<br>
<sup>b</sup> Intraglandular.<br>
<sup>c</sup> Intraperitoneal.
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The pathway by which the malignant cells from cancer can propagate in the body.

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References


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