Vitamin A and Benzo(a)pyrene Carcinogenesis in the Respiratory Tract of Hamsters Fed a Semisynthetic Diet

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INTRODUCTION

Male Syrian golden hamsters were fed a semisynthetic diet, given 12 weekly intratracheal instillations of 3 mg benzo(a)pyrene adherent to 3 mg Fe₂O₃, and then given either 100, 1600, or 2400 μg retinyl acetate (RA) per week intragastrically in 2 divided doses. One-half of the animals in each group were housed conventionally; the other half were housed in laminar flow units.

Hepatic and serum vitamin A levels were markedly increased in hamsters given 1600 or 2400 μg RA per week compared to controls given 100 μg, which is adequate for growth and maintenance.

In hamsters housed conventionally, increased RA intake was associated with an increased incidence of benign respiratory tract tumors. In all groups of hamsters housed in laminar flow units there was a longer period to death with respiratory tract tumor than in conventionally housed hamsters; increased RA intake was associated with a somewhat lower incidence of respiratory tract tumors. Laminar flow housing significantly reduced the incidence of the respiratory tract infection in non-tumor-bearing hamsters. Squamous papillomas of the forestomach were significantly reduced in all groups of hamsters given high levels of RA, regardless of housing.

SUMMARY

Male Syrian golden hamsters were fed a semisynthetic diet, given 12 weekly intratracheal instillations of 3 mg benzo(a)pyrene adherent to 3 mg Fe₂O₃, and then given either 100, 1600, or 2400 μg retinyl acetate (RA) per week intragastrically in 2 divided doses. One-half of the animals in each group were housed conventionally; the other half were housed in laminar flow units.

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MATERIALS AND METHODS

Male Syrian golden hamsters (Dennen Animal Industries, Inc., Gloucester, Mass.) were derived from dams fed a semisynthetic diet, containing 10 μg retinoic acid per g, during lactation (8). Retinoic acid is not stored in the liver, so the weanlings entered the experiment with minimal vitamin A stores. Hamsters were housed individually in plastic tubs with cellulose filter tops; half were placed on racks in air-conditioned rooms, and the other half were contained in laminar flow units, which have a dual air filtration system (Cardworth, New York, N. Y.) (1). Bedding was 1/8-inch to 1/4-inch ground corn cobs (Bed-O-Cobs; Anderson Cob Mills, Inc., Maumee, Ohio). All animals were fed the semisynthetic diet, which contained 2 μg RA (Hoffman-LaRoche, Inc., Nutley, N.J.) per g, until the end of BP-hematite (Fe₂O₃) instillation.

When the hamsters reached an average weight of 100 g, weekly intratracheal instillation of BP-Fe₂O₃ suspended in 0.2 ml of 0.15 M sodium chloride was started and continued for 12 weeks (10, 15). One week after the 12th BP-Fe₂O₃ treatment, vitamin A was removed from the diet and hamsters were assigned to 1 of 3 groups to receive either 100, 1600, or 3300 μg RA intragastrically in 0.2 ml carotene-free cottonseed oil per week, divided into 2 doses. Signs of vitamin A toxicity (weight loss, lethargy, and rough hair coat) appeared in hamsters given 3300 μg RA 24 weeks after the last BP instillation, so the RA dosage was reduced to 2400 μg/week.
Moribund hamsters were killed and autopsied; dead hamsters were autopsied. Animals to be killed were anesthetized with ether and bled from the retroorbital capillary plexus, and the trachea was exposed and ligated at full inspiration. The larynx, trachea, and lungs were removed en bloc and fixed in 10% neutral buffered formalin. The 5 pulmonary lobes were separated, processed by standard histological methods, and stained with hematoxylin and eosin. Sections of larynx and trachea were stained also with periodic acid-Schiff. Liver, kidney, forestomach, duodenum, ileum, and testes also were examined microscopically in hematoxylin and eosin sections. Samples of liver and serum were frozen for assay of vitamin A content by fluorometric techniques (5, 6). Cumulative probability of death with respiratory tract tumor was calculated (12).

RESULTS

Vitamin A. At weaning, the average hepatic vitamin A content was low, 0.80 ± 0.4 (S.E.) µg/g. Feeding the semisynthetic diet containing 2 µg RA per g for 6 to 8 weeks increased hepatic vitamin A to 20 ± 2 µg/g prior to BP-Fe₂O₃ instillations; at the end of BP treatment the level was 17 ± 2 µg/g. The hepatic vitamin A content was maintained at that level in hamsters given 100 µg RA per week. In hamsters given 3300 and then 2400 µg RA per week, hepatic vitamin A increased rapidly for 30 weeks to 650 ± 25 µg/g; it continued to increase at a slower rate for the duration of the study. In hamsters given 1600 µg RA per week, hepatic vitamin A increased for 60 weeks to a level of 600 ± 25 µg/g.

Serum vitamin A content increased rapidly in hamsters given the highest dose of vitamin A to 475 ± 50 µg/g at 30 weeks and then decreased to 250 ± 50 µg/g at 40 weeks. In hamsters given 1600 µg RA per week, serum vitamin A content was 275 ± 40 µg/g at 40 weeks and remained at that level. In hamsters given 100 µg RA per week, the serum content was 150 ± 15 µg/ml. Housing did not affect vitamin A stores.

Respiratory Tract Tumors. The incidence of respiratory tract tumors in conventionally housed hamsters was higher in animals given the 2 larger doses of RA than in those given 100 µg/week (Table 1). If the 2 high-dose groups are combined, the difference is significant (p < 0.05). There was no consistent effect of RA on the incidence of malignant epithelial tumors or on the number of tumors per hamster; the differences occurred in the incidence of benign tumors and sarcomas. There was no effect of RA dosage on tumor type (Table 2). Squamous cell carcinomas comprised 26% of all respiratory tract tumors in hamsters given 100 µg RA, 31% in hamsters given 1600 µg, and 37% in hamsters given 2400 µg. Small cell tumors comparable to human oat cell tumors were found in a few animals. The cumulative probability of death with a respiratory tract tumor was greater in the 2 groups of hamsters given high doses of RA than in hamsters given 100 µg/week (Chart 1).

Hamsters maintained in laminar flow housing had a median life-span of 57 weeks, which was considerably longer than that of conventionally housed hamsters (37 weeks). Body weight gain was the same in the 2 groups. The incidence of respiratory tract infection was significantly reduced in non-tumor-bearing hamsters housed in laminar flow units, 34% compared to 55% in non-tumor-bearing hamsters housed conventionally (p < 0.005). There was no other difference in gross or microscopic pathology in the 2 housing groups.

Hamsters housed in laminar flow units, regardless of their RA dosage, died with tumor after a considerably longer period than did conventionally housed hamsters (Chart 1). Since the tumors were no more advanced at death, it may be assumed that they developed at a slower rate than in animals housed conventionally. The cumulative probability of death with respiratory tract tumor was not consistently related to the RA dosage, although hamsters given high doses of RA had lower tumor incidence than did hamsters given 100 µg/week; the differences were not significant (Table 1). Forty-eight percent of the hamsters given 100 µg RA, 19% of hamsters given 1600 µg, and 29% of hamsters given 2400 µg developed multiple tumors of the respiratory tract.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Hamster given 100 µg RA/ wk&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hamsters given 1600 µg RA/ wk&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Hamsters given 2400 µg RA/ wk&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>LF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Benign epithelial alone</td>
<td>10 ± 17</td>
<td>14 ± 27</td>
<td>12 ± 21</td>
</tr>
<tr>
<td>Malignant epithelial</td>
<td>22 ± 39</td>
<td>22 ± 42</td>
<td>28 ± 48</td>
</tr>
<tr>
<td>Malignant mesenchymal</td>
<td>2 ± 4</td>
<td>1 ± 2</td>
<td>5 ± 9</td>
</tr>
<tr>
<td>Total</td>
<td>34 ± 60</td>
<td>37 ± 71</td>
<td>45 ± 78</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fifty-seven hamsters in conventional and 52 in laminar flow housing at initiation of RA administration. In conventional housing, tumor incidence was significantly less than in the other 2 groups combined (p < 0.05); in laminar flow housing, differences not significant.

<sup>b</sup> Fifty-eight hamsters in conventional and 53 in laminar flow housing at initiation of RA administration.

<sup>c</sup> Fifty-eight hamsters in conventional and 49 in laminar flow housing at initiation of RA administration.

<sup>d</sup> C, conventional housing; LF, laminar flow housing.
Types of respiratory tract tumors in hamsters in conventional or laminar flow housing

<table>
<thead>
<tr>
<th>Histological type of tumor</th>
<th>100 µg/week C</th>
<th>100 µg/week LF</th>
<th>1600 µg/week C</th>
<th>1600 µg/week LF</th>
<th>2400 µg/week C</th>
<th>2400 µg/week LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma</td>
<td>23</td>
<td>25</td>
<td>20</td>
<td>16</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Polyp</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>10</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Adenoma</td>
<td>5</td>
<td>14</td>
<td>10</td>
<td>2</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>26</td>
<td>25</td>
<td>31</td>
<td>36</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>18</td>
<td>11</td>
<td>11</td>
<td>16</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>3</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>7</td>
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<tr>
<td>Oat cell carcinoma</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>14</td>
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<tr>
<td>Large cell undifferentiated carcinoma</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
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<td>0</td>
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<tr>
<td>Carcinosarcoma</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>0</td>
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<td>Fibrosarcoma</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>2</td>
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<tr>
<td>Hemangiosarcoma</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Chondrosarcoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* C, conventional caging; LF, laminar flow housing.

Incidence of forestomach papillomas induced by BP in hamsters

<table>
<thead>
<tr>
<th>µg RA/wk</th>
<th>No. of hamsters</th>
<th>% with papilloma</th>
<th>No. of papillomas/papilloma-bearing hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>109</td>
<td>50</td>
<td>2.9 ± 0.2*</td>
</tr>
<tr>
<td>1600</td>
<td>111</td>
<td>25*</td>
<td>2.2 ± 0.3*</td>
</tr>
<tr>
<td>2400</td>
<td>107</td>
<td>26*</td>
<td>3.1 ± 0.2*</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Squamous cell carcinomas again were the most common tumors found in all 3 groups, comprising 25% of all respiratory tract tumors in hamsters given 100 µg RA, 36% in hamsters given 1600 µg, and 22% in hamsters given 2400 µg (Table 2).

**FORESTOMACH PAPILLOMAS.** Hamsters given either 1600 or 2400 µg RA per week had a significantly lower total incidence of papillomas of the forestomach than did hamsters given 100 µg RA (p < 0.005) (Table 3). The incidences were not different in the 2 housing groups, and the results were combined for analysis. RA dosage had no effect on the average number of papillomas per papilloma-bearing hamster.

**DISCUSSION**

Under the experimental conditions described, we have found no significant suppression by vitamin A of the induction of respiratory tract tumors by BP in hamsters. In hamsters housed in laminar flow units, hypervitaminosis A inhibited development of respiratory tract tumors somewhat, but the result was not statistically significant. In conventionally housed hamsters, hypervitaminosis A enhanced respiratory tract tumor formation. A similar finding in hamsters fed a stock diet has been reported from our laboratory and led to the present experiments in which diet composition was controlled by the use of a semisynthetic diet.

The incidence of respiratory tract tumors in our studies was approximately twice that reported by Saffiotti et al. (11) in a study in which protection by vitamin A was found. We may have overcome any potentially protective effect of RA by giving the animals a more effective exposure to the carcinogen. The induction and maintenance of high hepatic and serum content of vitamin A assured that the tissues were exposed to high levels of the vitamin and demonstrated that a significant difference existed between the control animals given 100 µg RA per week and the animals given high doses.

BP-induced papillomas of the forestomach were inhibited by high doses of RA. The forestomach epithelium was exposed to small doses of BP administered through the respiratory tract and to large doses of RA given intragastrically. The concentration ratios of BP and RA were therefore quite different than in the respiratory tract.

Hamsters in laminar flow units developed tumors more slowly than did hamsters in conventional housing. Their decreased incidence of pneumonia may have contributed to this effect, since respiratory tract infection may be cocarcinogenic for the lung (13). Animals in laminar flow housing...
were exposed to a lower concentration of airborne particulate matter.

A role for vitamin A in protection of the upper gastrointestinal tract against chemical carcinogenesis has been demonstrated previously (3, 11). The importance of adequate vitamin A intake in protecting the colon against chemical carcinogenesis was evident in studies that demonstrated enhanced colon carcinogenesis in vitamin A-deficient rats, but there was no further protection by hypervitaminosis A (7, 9). To protect epithelial surfaces of adequately nourished animals against chemical carcinogenesis, local application of vitamin A in high doses may be required; the most consistent results with vitamin A have been obtained in studies of inhibition of gastric or cutaneous carcinogenesis (2, 3, 11).

REFERENCES


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