Differences in Tumor Types and Organ Susceptibility in BALB/c and RF Mice following Dimethylnitrosamine and Diethylnitrosamine

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SUMMARY

Total doses of 300 mg/kg of dimethylnitrosamine (DMN) and 515 and 1010 mg/kg of diethylnitrosamine (DEN) were given to adult BALB/c mice in their drinking water. The oncogenic results for this strain were compared with previously reported data on a noninbred subline of RF/Un mice handled under similar conditions. DEN induced forestomach and esophageal squamous cell carcinomas in both strains but induced liver hemangiosarcomas in BALB/c mice and liver hepatomas in RF mice. In addition, DEN induced a high incidence of lung adenomas in the RF but was only slightly effective (3%) in BALB/c mice. In both strains, DMN induced lung adenomas and liver hemangiosarcomas. The liver sensitivity of both strains to DEN, in developing different tumor cell types between strains, suggests that different liver cells may metabolize DEN in the two strains and offers an excellent tool for metabolic studies. These carcinogens induced high incidences of tumors in the low-tumor-incidence BALB/c strain, with the exception of DEN, which had no oncogenic effect on the lung. Tissue sensitivity does not appear to relate directly to the spontaneous tumor incidence of the strain. No leukemogenic effect was observed following either DMN or DEN in either strain.

INTRODUCTION

Differences in the oncogenicity of DMN and DEN have been shown in many species and several strains of mice (see reviews in Refs. 2 and 3). In the RF mouse, DMN induces high incidences of lung adenomas and liver hemangiosarcomas, while DEN induces lung adenomas, liver hepatomas, and forestomach squamous cell carcinomas, suggesting organ and perhaps even cellular specificity of metabolism for each nitrosamine (2, 3). Recent studies have shown the existence of dose dependence with DEN liver carcinogenesis (4), while a strict dose dependence was not observed after DMN treatment (5). A difference in sensitivity of the target organs has been suggested for DEN with lung most sensitive, stomach intermediate, and liver least sensitive (5). Strain differences in spontaneous tumor incidences, however, suggest that genetic influence may predispose certain strains to tumors and may be absent in low tumor strains; this difference in spontaneous incidence may affect susceptibility to tumorigenesis by carcinogens.

This study was made to evaluate tumorigenesis and leukemogenesis of DMN and DEN in BALB/c mice, a strain in which there are very low spontaneous incidences of tumors, and to compare the results with previous studies in RF mice, a strain in which there are high spontaneous incidences of lung tumors and reticulum cell sarcoma, a late-occurring leukemia (4, 5).

MATERIALS AND METHODS

Five groups of 12-week-old male BALB/c mice were given DMN or DEN daily in their drinking water for varied treatment periods with total doses of 300 mg DMN/kg and 515 and 1010 mg DEN/kg body weight. A total of 109 mice survived the treatment periods; 62 untreated male BALB/c mice were handled similarly and served as controls. Cumulative doses were calculated from consumed water volume measurements as previously reported (2). The animals were housed 8 per cage and received Purina laboratory chow ad libitum.

Mice were routinely necropsied when they died or were killed after reaching a moribund state. Tissues for histological examination were taken from approximately 80% of the mice, fixed in Zenker-formol solution, embedded in paraffin, and stained with hematoxylin and eosin. In order that tissues might be preserved for microscopic examination, the remaining treated mice were killed between 160 and 200 days after treatment was begun when most of the mice were obviously sick and near death. These data are reported as mean age at observation rather than mean survival time. A total of 44 control mice were killed from 290 to 715 days of age while the remaining controls were allowed to die, with ages at death ranging from 290 to 715 days. Since all control mice were observed after the last treated mice were killed, the observations were combined to represent control data.

RESULTS

The incidences of primary tumors in the various organs of BALB/c mice following DMN and DEN are shown in Table 1.
Mouse Strains and Oncogenicity

Table 1
Primary tumor incidences in DMN- and DEN-treated adult male BALB/c and RF mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Treatment</th>
<th>Cumulative dose (mg/kg)</th>
<th>Daily dose (mg/kg)</th>
<th>Experiment terminated (age in days)</th>
<th>Meantime at observation (days)</th>
<th>No. of mice</th>
<th>Tumor-positive mice/no. histologically examined</th>
<th>Lung (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Liver (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Hepato-cellular tumors</th>
<th>Hemangiosarcomas</th>
<th>Stomach&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Esophagus&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>A</td>
<td>BALB/c</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>715&lt;sup&gt;d&lt;/sup&gt;</td>
<td>520</td>
<td>82</td>
<td>0/30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>BALB/c</td>
<td>DEN</td>
<td>1010</td>
<td>6.7</td>
<td>245</td>
<td>238</td>
<td>73</td>
<td>49/60</td>
<td>3</td>
<td>0</td>
<td>23</td>
<td>58</td>
<td>(2/60)</td>
<td>(14/60)</td>
</tr>
<tr>
<td>C</td>
<td>BALB/c</td>
<td>DEN</td>
<td>515</td>
<td>3.6</td>
<td>285</td>
<td>266</td>
<td>18</td>
<td>14/15</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>87</td>
<td>(4/15)</td>
<td>(13/15)</td>
</tr>
<tr>
<td>D</td>
<td>BALB/c</td>
<td>DMN</td>
<td>300</td>
<td>1.7</td>
<td>285</td>
<td>266</td>
<td>18</td>
<td>10/15</td>
<td>47</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>(7/15)</td>
<td>(3/15)</td>
</tr>
<tr>
<td>E&lt;sup&gt;f&lt;/sup&gt;</td>
<td>RF</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>615&lt;sup&gt;d&lt;/sup&gt;</td>
<td>262</td>
<td>39</td>
<td>0/15</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>F&lt;sup&gt;h&lt;/sup&gt;</td>
<td>RF</td>
<td>DEN</td>
<td>943</td>
<td>6</td>
<td>270&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65</td>
<td>74</td>
<td>90</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G&lt;sup&gt;f&lt;/sup&gt;</td>
<td>RF</td>
<td>DMN</td>
<td>243</td>
<td>0.91</td>
<td>360&lt;sup&gt;d&lt;/sup&gt;</td>
<td>94</td>
<td>99</td>
<td>2</td>
<td>96</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Primarily adenomas and papillary adenomas.
<sup>b</sup> No. of histologically examined mice with tumors/no. of mice histologically examined.
<sup>c</sup> Primarily squamous cell carcinomas and some papillomas.
<sup>d</sup> There were 38 dead and moribund mice 290 to 715 days of age; 44 mice were killed at 290 to 700 days of age.
<sup>e</sup> Esophagus was not consistently observed.
<sup>f</sup> See the work of Clapp et al. (5).
<sup>g</sup> Mean survival time.
<sup>h</sup> See the work of Clapp et al. (4).

and are compared with mice of the same age and sex from a noninbred subline of the RF/Un strain (hereafter called RF) that received similar doses of DMN and DEN under comparable conditions (4, 5). Nontreated BALB/c mice did not develop tumors despite survival times that exceeded those of treated mice by 6 to 8 months. These observations confirm previous reports of extremely low incidences of spontaneous tumors in BALB/c mice (11).

Liver hemangiosarcomas, closely resembling those described previously (3), were induced by both carcinogens in BALB/c mice and in DMN-treated RF mice, while DEN induced liver hepatomas in RF mice. DMN induced lung adenomas in 7 of 15 histologically examined BALB/c mice, while DEN was only slightly effective, inducing tumors in 2 of 75 histologically examined BALB/c mice. In contrast, high incidences of lung tumors were induced in RF mice by both carcinogens. DEN induced squamous cell carcinomas of the forestomach and esophagus in BALB/c mice, as in RF mice, while DMN had no detectable effect on these 2 organs in either strain. The tumors induced in BALB/c mice were remarkably similar upon gross and microscopic examination to those reported previously in the same organs of RF mice (2—5). Only 1 BALB/c mouse in Group B died with reticulum cell sarcoma (2% incidence), indicating the absence of any obvious leukemogenic effect in BALB/c mice by either DMN or DEN and resembling the results in RF mice (4, 5).

DISCUSSION

While confirming the susceptibility of the liver to DMN and DEN, our data show an intriguing difference in strain sensitivity to DEN. DEN induced tumors in the liver of both strains, but different tumor types developed, hemangiosarcomas in BALB/c mice and hepatomas in RF mice, suggesting that different liver cells may metabolize DEN in the 2 strains. These results suggest an excellent tool for metabolic studies with DEN showing strain differences in liver sensitivity between BALB/c mice and RF mice as well as the differences in liver tumor types induced by DMN and DEN in RF mice (2—5).

The relative resistance of the BALB/c lung to DEN was somewhat surprising, especially since DMN induced tumors in approximately 50% of the mice treated in concurrence with a previous report (11). This suggests (a) that different enzyme systems may be utilized by the cells to metabolize DEN and DMN to the proximate carcinogens, or (b) that the system for metabolizing DEN is not present in sufficient quantity in the BALB/c lung. In contrast, the lung of the RF mouse is sensitive to both DMN and DEN as shown by high incidences of tumors following both carcinogens (2—5).

The organ most susceptible to DEN oncogenesis in BALB/c mice appears to be the forestomach, where a maximum tumor incidence of 87% was seen; this approaches incidences of 100% observed in RF mice after DEN treatment (2). The esophagus was not always observed for tumors but resembled the RF mouse in being susceptible to DEN oncogenesis. In contrast, DMN did not induce tumors of the forestomach or esophagus in either strain. No kidney tumors were found in DMN- or DEN-treated BALB/c or RF mice (4, 5), in contrast with observations in ddN, ICR, and C3H mice (8, 9), Swiss mice (10), and the rat (1, 6, 7) and suggesting that refractory organs may not be able to metabolize DMN or DEN.

The data reported in this study suggest that the strain susceptibility to spontaneous tumor induction does not necessarily relate directly to the carcinogenic results following treatment with a powerful carcinogen. The organs of BALB/c mice, a very-low-tumor-incidence strain, resemble in susceptibility to DMN and DEN tumorigenesis the response of mice from the RF strain, in which there is a high spontaneous
incidence of lung tumors, the exception being the refractoriness of the BALB/c lung to DEN oncogenesis. If any correlation does exist between spontaneous tumor incidences in a strain and the susceptibility to tumor induction, it is seen where DEN induces high percentages of lung tumors in RF mice, in which there is a high spontaneous incidence of such tumors, but DEN does not induce lung tumors in the low-incidence BALB/c strain. Diversities in oncogenesis are seen in the stomach, where DEN has a marked effect in both strains while DMN has none in either strain, and in the liver, where it appears that even certain cells within an organ differ in their sensitivity to these 2 compounds. Thus, the susceptibility of a particular organ or of a particular cell system within an organ may more closely relate to the ability of the organ or cell to metabolize the carcinogen to a proximate carcinogen than to the observed incidence of spontaneous tumors in a strain. Our data suggest that a compound-enzyme specificity may exist and that only certain cells within an organ are capable of carrying out this process.

REFERENCES

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