Intestinal Tumors Induced by a Single Intraperitoneal Injection of Methyl(acetoxymethyl)nitrosamine in Three Strains of Rats

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ABSTRACT

Methyl(acetoxymethyl)nitrosamine (DMN-OAc) when injected i.p. in Sprague-Dawley Charles River CD rats selectively induces epithelial tumors of the intestines. Males are more severely affected than females. To determine whether the strain of rat determines the quantity or type of tumors induced, 5-week-old male and female rats of Sprague-Dawley (SD), Buffalo (BUF) and Fischer (F344) strains were given a single i.p. injection of DMN-OAc. The dose used, 13 mg (0.1 mmol)/kg body weight, was one-half of the acute i.p. median lethal dose determined for 5-week-old male SD rats. In each strain treated with DMN-OAc, a large number of intestinal epithelial tumors developed. The histological features of these tumors were the same in all strains and both sexes. In each strain, females had fewer induced tumors of the small and large intestines than had males of the same strain. The greatest difference between males and females in frequency of small intestine tumors induced was observed in F344 rats. There were 1.7 small intestine tumors/male F344 rat and only 0.2 small intestine tumors/female F344 rat. The lowest induced tumor incidence was seen in BUF rats (0.3 small intestine tumors/male rat and 0.2 small intestine tumors/female rat). In SD rats, there were 2.0 small intestine tumors/male and 1.3 small intestine tumors/female. In all strains and both sexes, there were many more small intestine tumors induced than tumors of the large intestine. Tumors occurred throughout the small intestine and colon and in the cecum, with a tendency to occur most frequently in the distal ileum. Localization was the same in all strains and sexes studied. Other tumors induced with high frequency by i.p. administration of DMN-OAc were schwannomas (primary in peritoneum), testicular mesotheliomas, and splenic angiosarcomas.

INTRODUCTION

DMN-OAc,2 an ester of the presumed carcinogenic metabolite of dimethylnitrosamine, has been shown to induce a high incidence of intestinal tumors in Sprague-Dawley (Charles River CD) rats after a single i.p. injection (5, 12), and to be more highly carcinogenic for males than for females (5). We have now repeated this experiment and extended our studies to Fischer (F344) and Buffalo rats of both sexes to investigate further the roles of sex and genetic background as determinants of susceptibility to carcinogenesis in the intestinal tract in rats.

Genetic differences in susceptibility to chemical induction of tumors of the intestines have been studied principally in mice (2–4) by using agents such as 1,2-dimethylhydrazine, which require several successive metabolic oxidation steps for conversion to a chemically reactive ultimate carcinogen. Accordingly, genetically determined differences in tissue levels of any of the necessary enzymes, in addition to any intrinsic differences in susceptibility of the intestinal mucosa, could account for differences in tumorigenic response between different strains.

Not only hydrazine derivatives, but also a variety of other metabolism-dependent carcinogens have been reported to induce intestinal tumors in rats of various strains (6). The majority of investigators have studied only a single strain. For example, the effects of 3,2′-dimethyl-4-aminobiphenyl have been investigated in the Wistar rat (9), and 1,2-dimethylhydrazine (11) and azoxymethane (13) have been studied in the Fischer F344 rat. However, when 2 or more strains of rats have been subjected to the same agent, major quantitative differences in tumorigenic response have frequently been observed. 3,2′-Dimethyl-4-aminobiphenyl, for example, induced intestinal tumors with a much higher frequency in Wistar rats than in Slonaker rats (10), whereas another aromatic amine, 2-acetylaminofluorene, induced intestinal tumors much more efficiently in piebald than in Wistar rats (1). DMN-OAc differs significantly from the hydrazine derivatives and the aromatic amines in that no oxidative metabolism is required for its activation; it is subject to hydrolysis by esterases (7), and we have found that it behaves much like a direct-acting alkylating agent, inducing local sarcomas at the site of s.c. injection, gastric carcinomas when given p.o., and tumors of other organ systems when injected i.v.3 Accordingly, genetically determined differences among rats in susceptibility to intestinal carcinogenesis by DMN-OAc may be fundamentally different in nature from those which determine susceptibility or resistance to metabolism-dependent carcinogens.

MATERIALS AND METHODS

DMN-OAc (M.W. 132) was synthesized as previously described (7); it was distilled twice before use. Chemicals were dissolved in phosphate buffer at pH 7.0 (0.15 m Na+). All solutions were prepared within 2 hr of administration.

Sixty rats of each sex from each of 3 strains were used: Sprague-Dawley (SD; Charles River Breeding Laboratories, Inc., Wilmington, Mass., Buffalo (Texas Inbred Mice Co., Houston, Texas), and Fischer 344 (NIH, Bethesda, Md.). All rats were housed 3/polycarbonate cage (13 x 15 inches) on 0.125-inch corn cob bedding.

For carcinogenesis studies, 30 rats of each sex and strain...
were lightly anesthetized with Fluothane to reduce activity and ensure the instillation of carcinogen into the peritoneal cavity. Rats were given an injection of DMN-OAc in aqueous buffer solution [13 mg (0.1 mmol)/kg body weight], equivalent to one-half of the acute i.p. median lethal dose for 5-week-old SD males. Thirty control rats of each sex and strain were given an injection of buffer only.

Rats were fed Purina laboratory chow and had access to water ad libitum. They were weighed weekly for the first month and once a month thereafter. All rats were inspected and any deaths were recorded daily. Animals with intestinal tumors frequently became rapidly anemic due to bleeding from the tumors. Rats that appeared seriously ill were killed, and surviving rats were killed 83 to 90 weeks following treatment. Necropsies were performed on all rats except for 6 from various groups, which were cannibalized, grossly autolyzed, or had died within the first week of treatment. An additional 25 rats from various groups died within 3 months of treatment and were excluded from the data summaries as they died before they were considered at risk for induced tumors. At autopsy, the entire gastrointestinal tract from oral cavity to anus was removed, opened, and examined for lesions. Selected viscera and all abnormal organs and tissue masses were studied microscopically. Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for routine histological examination. Special stains, including periodic acid-Schiff, mucicarmine, Verhoeff’s elastic, and the Tibor Pap silver impregnation for reticulin were used as necessary.

Significance of differences in incidence and multiplicity of intestinal epithelial tumors was estimated by the \( \chi^2 \) test for proportions, the Student \( t \) test for means of populations with different variances, and the Wilcoxon (Mann-Whitney) rank method for unpaired measurements, as described in a standard text (8).

Synthesis and histopathological evaluation of tissue sections were conducted at the National Cancer Institute. Long-term animal holding, necropsies, and histological processing of tissues were done at Microbiological Associates, Inc.

RESULTS

Intestinal tumors were induced in both small and large intestines in both sexes in all 3 strains of rats (Table 1). No intestinal tumors were found in control rats of any sex or strain. The frequency of intestinal tumors induced by a single i.p. injection of DMN-OAc varied significantly among the strains of rats used. Greater, however, was the variation between the males and females of each strain.

SD rats were in general the most susceptible of the 3 strains. The proportion of male SD rats with 1 or more epithelial tumors of any segment of the intestinal tract (26 of 29) was very significantly greater by \( \chi^2 \) test than was the proportion of BUF males (7 of 26; \( p < 0.005 \)), but it was not significantly greater than the proportion of tumor-bearing F344 males (23 of 28). SD females, however, were significantly more responsive (25 of 30) than either F344 females (5 of 28; \( p < 0.005 \)) or BUF females (3 of 27; \( p < 0.005 \)). SD males had as many as 8 tumors in a single animal, and 16 of the 29 rats at risk developed 2 or more tumors, for an average of 2.4 intestinal epithelial tumors per treated rat. F344 males developed as many as 5 tumors in a single animal, and 13 of the 28 rats at risk developed 2 or more tumors, for an average of 1.9 tumors per treated rat. This response was not significantly different from that of SD males.

In each strain, there were fewer induced tumors of both the small and large intestines in females than in males. This difference was not due to differences in the age at death among females, for in each strain, treated females outlived the males, thus extending the time during which tumors might arise. The difference between male and female susceptibility to intestinal carcinogenesis was greatest in F344 rats, with a nearly 5-fold difference between the sexes in tumors induced and the number of rats with tumors (Table 2). The fraction of F344 males with 1 or more intestinal tumors (23 of 28) was significantly greater than was the corresponding fraction of females (5 of 28; \( p < 0.005 \)). In SD rats, there was no significant difference in incidence between the sexes, but the mean number of tumors per rat at risk was significantly greater in males (2.4) than in females (1.4) by the Student \( t \) test (\( p < 0.025 \)). The tendency of individual males compared to individual females to have more tumors was, however, only marginally significant by the Wilcoxon rank test (\( p = 0.055 \)). The sex difference in BUF rats was not statistically significant.

There were no sex or strain differences in the types or sites of localization of intestinal tumors. In all strains combined, there were 165 small intestine epithelial tumors found in the at-risk population, of which 123 or 75% were carcinomas. The percentage of malignant large intestine tumors was much smaller. Among a total of 21 large intestine tumors observed in all treated groups, 13 or 62% were carcinomas. Histological features of these tumors have been previously described (5, 12). Tumors occurred throughout the intestinal tract in all strains but were especially frequent in the terminal portion of the ileum, as previously noted in the SD strain (12). The tendency was most marked in F344 males: of 53 tumors, 32 (60%) were found in the terminal one-fourth of the small intestine and another 5 (9%) in the cecum.

As reported earlier (5), mesenchymal tumors of the intestines were also observed in the groups given DMN-OAc but at a much lower frequency than that seen for tumors arising from the intestinal mucosa. For the most part, these tumors arose

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of rats with tumors at risk</th>
<th>Age at death (mos.)</th>
<th>No. of rats with small intestine tumors</th>
<th>No. of rats with large intestine tumors</th>
<th>Tumors/No. of treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>Male</td>
<td>15.0</td>
<td>25/29</td>
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<td>70/26</td>
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<tr>
<td></td>
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<td>16.1</td>
<td>25/30</td>
<td>0/28</td>
<td>41/25</td>
</tr>
<tr>
<td>F344</td>
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<td>22/28</td>
<td>0/15</td>
<td>53/23</td>
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<td>17.2</td>
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<td>0/29</td>
<td>8/5</td>
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<td>Male</td>
<td>16.0</td>
<td>6/26</td>
<td>0/24</td>
<td>10/7</td>
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<tr>
<td></td>
<td>Female</td>
<td>16.1</td>
<td>3/27</td>
<td>0/22</td>
<td>4/3</td>
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</tbody>
</table>

* Includes cecum.
Neurinomas (schwannomas) arising from nerves lining the intestinal wall and serosa of peritoneal viscera were also observed (Table 2). These tumors comprised 3 basic histological and biological variants. (a) Tumors were large and consisted chiefly of Antoni type B tissue with areas of type A tissue and cysts (Figs. 1 and 2); these tumors spread along the peritoneal surfaces, often filling the abdomen but rarely invading abdominal organs. (b) Tumors consisted of densely cellular Antoni type A tissue growing in a swirling pattern and often seen within and surrounding normal nerves. These lesions usually contained prominent Verocay bodies and were found most often near the periprostatic ganglia in male rats (Figs. 3 and 4). Always quite small, these lesions might be the precursor for the other types of schwannomas observed. (c) In the third, more anaplastic variant, tumors consisted of a densely cellular, almost sarcomatous pattern. This type frequently contained Verocay bodies, invaded muscle, and metastasized to viscera.

Angiosarcomas of the spleen were also seen in rats treated with DMN-OAc. In toto, 9 cases of this tumor rarely reported in rats were observed (Table 2). These tumors were highly hemorrhagic, and animals with this variety of tumor occasionally died of massive splenic hemorrhage.

Mesotheliomas were seen, almost always arising from the peritoneal extension of the peritoneal mesothelium. However, one case of splenic mesothelioma was seen in a F344 female treated with DMN-OAc. In all strains combined, there was a 27% incidence of this tumor in treated male animals. Several types of neoplasms not covered in Table 2 were seen exclusively in treated rats at frequencies too low to be statistically significant. However, taken as a group, these uncommon neoplasms were seen overwhelmingly in the treated animals.

Notable for their rarity in treated rats were liver and kidney tumors. Four hepatocellular carcinomas (3 in SD rats; 1 in an F344 rat) and 2 renal adenomas (both in SD rats) were found among all treated rats. None were seen in control rats. Zymbal’s gland tumors were conspicuously absent; none at all were seen in treated or untreated rats.

A wide variety of tumors and neoplastic conditions was seen in the two sexes. Although there were no remarkable qualitative differences among the strains with regard to types of tumors induced, there were great differences in the quantity of induced intestinal neoplasms. In each strain, females developed fewer intestinal tumors than did males, this difference being greatest in F344 rats (0.3 intestinal tumors/female treated; 1.9 intestinal tumors/male treated).

For the first time with DMN-OAc, high incidences of testicular mesothelioma, splenic angiosarcoma, and peritoneal neurinoma (schwannoma) were noted. All of these tumors were seen originally with i.p. injections of DMN-OAc (5) but were quite uncommon in that study. The reason for the discrepancy in the quantity of these tumors in the different experiments is due perhaps to the use in the present study of an anesthetic administered prior to DMN-OAc injection. Absorption of carcinogen may have been delayed slightly by the anesthetic, presumably as a result of decreased peristalsis and motion of the animal. More time may have been allowed for the carcinogen to bathe the spleen, intestinal ganglia, and peritesticular mesothelium.

Most abdominal viscera were affected by DMN-OAc, as tumors were found in intestines, spleen, liver, kidney, stomach, and prostate. However, no tumors of prostatic epithelial origin were seen.

The high intestinal specificity of DMN-OAc when injected i.p. is an interesting phenomenon which so far has escaped explanation. Preliminary observations indicate that hydrolysis of DMN-OAc proceeds much less rapidly in bile than in serum, which may in part account for this selectivity. Alternatively, it may be possible that DMN-OAc can diffuse through the intest-

Table 2

<table>
<thead>
<tr>
<th>No. of control rats with</th>
<th>Strain</th>
<th>Total no. of</th>
<th>Small intestine&lt;sup&gt;a&lt;/sup&gt; (epithelial)</th>
<th>Large intestine&lt;sup&gt;a&lt;/sup&gt; (epithelial)</th>
<th>Intestines (mesenchymal)</th>
<th>Neuroinoma (peri toneal)</th>
<th>Splenic angiosar coma</th>
<th>Testicular mesothelioma</th>
<th>Total no. of these tumors</th>
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</thead>
<tbody>
<tr>
<td>CD</td>
<td>Male</td>
<td>29</td>
<td>59</td>
<td>11</td>
<td>4</td>
<td>6</td>
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<td>5</td>
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<tr>
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<td>30</td>
<td>38</td>
<td>3</td>
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<td>4</td>
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<td>0</td>
<td>29</td>
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<td>4</td>
<td>3</td>
<td>7</td>
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<td>9</td>
<td>16</td>
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<tr>
<td></td>
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<td>7</td>
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<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>30</td>
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<tr>
<td>Buffalo</td>
<td>Male</td>
<td>26</td>
<td>8</td>
<td>2</td>
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<td>30</td>
</tr>
<tr>
<td></td>
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<td>27</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes cecum.
<sup>b</sup> Neurinoma.
<sup>c</sup> Mesothelioma.
tinal wall and enter the intestinal mucosa, which because of its high mitotic rate is intrinsically highly susceptible to carcinogens.

The lower susceptibility of female rats of the different strains to intestinal carcinogenesis poses another problem awaiting resolution. Further insight into the organotropism of DMN-OAc and the observed female resistance to DMN-OAc-induced intestinal tumors may come from experiments in which DMN-OAc is administered by several different routes (p.o., i.v., s.c.) to rats of both sexes.

In 3 successive studies by i.p. injection in SD rats, the mean intestinal tumor yield from a single exposure to 0.1 mmol DMN-OAc per kg body weight has been extremely reproducible, ranging from 2.7 to 3.6 tumors/tumor-bearing rat in males and from 1.6 to 2.1 in females (5, 12). DMN-OAc provides, in several different rat strains, a tool for the study of carcinogenesis in intestinal mucosa which appears well suited to investigation of genetically determined susceptibility and resistance that is not attributable to differences in oxidative metabolism of the carcinogen.

ACKNOWLEDGMENTS

The authors thank Russell Bracken for histological preparations; Lee Dove, Debbie Devor, Cynthia Molello, Sharon Martin, and Robert Shores for excellent technical assistance; and Maxine Bellman for preparing the typescript.

REFERENCES

Fig. 1. Large i.p. neurinoma. Large cysts lined by tumor cells were numerous in areas of Antoni type B tissue. H & E, x 130.

Fig. 2. Large intraabdominal neurinoma consisting of mixed Antoni A and B tissue. Palisading nuclei form lengthy Verocay bodies in this area of type A tissue. Sprague-Dawley male, 9 months postinjection. H & E, x 130.

Fig. 3. Early neurinoma (upper right) originating in small nerve near urethra (center left) consists exclusively of fibrous Antoni type A tissue. F344 male, 11 months postinjection. H & E, x 55.

Fig. 4. Higher magnification of tumor tissue illustrates tendency of nuclei to form whorls and irregular palisades. H & E, x 220.
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