INDUCTION OF COLON ADENOCARCINOMAS BY 1,2-DIMETHYLHYDRAZINE IN (C3H×MSM) F1 MICE

Osamu Noguchi, Masahiro Tsutsumi, Shunji Okita, Toshifumi Tsujiuchi, Eisaku Kobayashi, Kohsuke Horiguchi, and Yoichi Konishi
Department of Oncological Pathology, Cancer Center, Nara Medical University
Nobumoto Miyashita
Genetic Stock Research Center, National Institute of Genetics
Kazuo Moriwaki
Department of Cell Genetics, National Institute of Genetics

Abstract: The possibility of producing colon tumors by administration of 1,2-dimethylhydrazine (DMH) to (C3H×MSM) F1 hybrids of inbred and wild strains mice, was investigated in an attempt to establish an experimental model for detection of DNA polymorphism markers for colon carcinogenesis in mice. DMH at doses of 0, 10, and 15 mg/kg body weight was given intra-peritoneally once a week for 10 weeks and the experiment was terminated 46 weeks after the initial administration. The first colon tumors appeared after 30 weeks and final incidences reached 38.5% in female mice receiving 10 mg DMH and 90.9 and 100%, respectively, in male and female mice receiving 15 mg DMH/kg body weight. Tumors were multiple, mainly located in the distal colon and primarily adenomas and adenocarcinomas.

Key words: DMH, (C3H×MSM) F1 mouse, Colon adenocarcinoma

The incidence of colorectal cancer which has recently been increasing in Japan so that it is second only to carcinoma of the lung in men, is third after breast and lung cancer in women in Western industrialized societies1. Adenocarcinoma of the colon in humans is considered as a particularly useful neoplasm with which to study the progression of molecular events, including mutation of oncogenes and tumor suppressor genes, that is generally thought to underlie malignant transformation2. However, detailed studies of genetic alterations occurring during colon chemical carcinogenesis in experimental animals are required in view of the inherent limitations with human studies. So far, colon tumors caused by DMH in mice have only been studied in inbred strains which are not suitable for detection of DNA polymorphism markers. MSM, a wild mouse strain trapped in Mishima City, Shizuoka Prefecture, Japan, and C3H strain mice have been mated to give (C3H×MSM) F1 hybrids with DNA polymorphism and these offer a way to overcome this problem. In the present experiment, the possibility of inducing colon tumors by DMH in (C3H×MSM) F1 mice was ascertained with a view to future studies on detection of DNA polymorphism markers for colon carcinogenesis in mice.

Six-to 10-week-old (C3H×MSM) F1 mice raised in the National Institute of Genetics were used. They were maintained individually in stainless steel cages in an air-conditioned room at 24°C...
and 60% humidity with alternating 12 h periods of light/darkness, fed Oriental MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and given water ad libitum. DMH dihydrochloride (Aldrich Chemical Company, Wisconsin, U.S.A) was dissolved in saline containing 0.4 mM EDTA at a concentration of 2 mg/ml and the pH of the solution adjusted to 6.6 with 1 M sodium hydroxide. Both male and female mice received DMH intra-peritoneally at doses of 0, 10 and 15 mg/kg body weight once a week for 10 weeks. All mice were daily monitored and sacrificed moribund. Surviving animals at 46 weeks after the beginning of the experiment were also killed by exsanguination under ether anesthesia. The colons were removed, opened longitudinally from the anus to the ileocecal valve, fixed in ice-cold absolute ethanol, and examined macroscopically. Routine processing for histological examination was performed and lesions in the colon were histologically diagnosed according to published criteria for colon tumors in mice.

During the experimental period, it proved impossible to measure body weights because of the very wild behavior of the mice but the final body weights measured under anesthesia were all in the range of 20 to 25 g. The first colon tumors were detected 30 weeks after the beginning of the experiment in both male and female mice receiving 15 mg DMH/kg body weight, with symptoms of anal bleeding and a distended abdomen without signs of severe malnutrition. Table 1 summarizes details for final induction of colon tumors in the treated (C3H × MSM) F1 mice. High incidences were observed in female mice receiving 10 mg DMH/kg body weight and both male and female mice receiving 15 mg DMH/kg body weight. Tumors were multiple and located mainly in distal colon. Histologically, they were adenomas (Fig. 1) or adenocarcinomas (Fig. 2) but attempts to transplant the latter to syngeneic or nude mice proved unsuccessful, probably due to infections. Anal carcinomas were only rarely induced and their histology was squamous cell type.

The first experimental model for induction of adenocarcinomas of the colon in female CF1 mice by DMH was established by Thurnherr et al.3. Subsequently, extensive studies of colon carcinogenesis by DMH in various inbred strains of mice have been performed and it is now clear that a substantial differential susceptibility exists in the induction of colorectal tumors by this carcinogen4-7. The inbred ICR/Ha strain appears most susceptible with a 100% yield4. However, the DBA/2, C57BL/Ha, and GR strains of mice have proven completely resistant4,5 and differences even between inbred and randombred ICR/Ha mice4 and among sublines of C57BL/6 mice, have been demonstrated.

Table 1. Induction of Colon Tumors by Dimethylhydrazine in (C3H × MSM) F1 Mice

<table>
<thead>
<tr>
<th>DMH dose (mg/kg)</th>
<th>Sex</th>
<th>Initial</th>
<th>Effectivea)</th>
<th>No. of tumors (%)</th>
<th>No. of tumors</th>
<th>No. of tumors located at</th>
<th>Tumors incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Male</td>
<td>2</td>
<td>2</td>
<td>0/2 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>3</td>
<td>0/3 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>10</td>
<td>8</td>
<td>0/8 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14</td>
<td>13</td>
<td>5/13 (38.5)</td>
<td>5</td>
<td>1.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>12</td>
<td>11</td>
<td>10/11 (90.9)</td>
<td>25</td>
<td>2.5</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>Female</td>
<td>15</td>
<td>13</td>
<td>13/13 (100)</td>
<td>39</td>
<td>3.0</td>
<td>17</td>
</tr>
</tbody>
</table>

a): Those for which histological examination was possible.
The incidence of colon tumors in female C3H animals given DMH subcutaneously at the dose of 8 mg/kg body weight once a week for 25 weeks reached 75%7 and while the doses used in the present experiment were higher, at 10 and 15 mg/kg body weight, the experimental period was almost the same (46 as compared with 50 weeks). In the Turusov's Turusov experiment7 C3H showed the highest survival among various strains of mice. Colon tumor induction in (C3H × MSM) F1 strain is presumably genetically influenced by the C3H pedigree. The sex dependence, with no induction of colon tumors in male mice receiving 10 mg DMH/kg body weight remains to be explained. Differential susceptibility in various strains of mice to DMH has been proposed to depend on variation in DNA damage, presence or absence of particular nuclear proteins, and proliferative characteristics of colonic epithelial cells8–12. In mice with a low susceptibility to DMH (C57BL/Ha), concentrations of 7-methylguanine and 06-methylguanine in DNA of colon, ileum, and kidney were 40 to 60% less than in mice with a high incidence of colonic tumors (ICR/Ha)8. A correlation between colonic DNA breaks and susceptibility to DMH in various mouse strains has also reported8–10. Boffa et al.11, using one-dimensional and two-dimensional gel electrophoretic analyses revealed the presence of number of prominent protein peaks in the tumor nuclei of DMH–sensitive SWR/J mice which were not evident, or present at much lower concentrations, in normal SWR/J or AKR/J colonic nuclei or in nuclei of DMH–treated but tumor free AKR/J mice. Deschner et al.12 examined the proliferative characteristics of the colonic mucosae of various mouse strains to determine their value in forecasting differential susceptibility to DMH–induced tumor formation and found that endogenous conditions with influence are level of DNA synthesis and the dimensions of the proliferation compartment.

Recently, Thibodeau et al.13 reported microsatellite instability only in cancers located in the proximal colon and suggested that some colorectal cancers may arise through a mechanism that does not necessarily involve loss of heterozygosity. The present results for induction of colon tumors by DMH in (C3H × MSM) F1 mice suggests that this should provide a useful model for studying microsatellite instability by using DNA polymorphism markers for colon carcinogenesis in mice.

Acknowledgements: This study was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and by a Grant from the Ministry of Health and Welfare for a Comprehensive 10-year strategy for Cancer Control, Japan.

References


