PROMOTION OF THYROID TUMORS IN F344 MALE RATS GIVEN A LOW IODINE DIET AFTER TREATMENT WITH N-METHYL-N-NITROSOUREA IN THEIR DRINKING WATER

Takahiko Gotoh, Hiromitsu Watanabe, Midori Tanizaki, Yoko Sakai, Nariaki Fujimoto, and Akihiro Ito
Department of Cancer Research, Research Institute for Radiation Biology and Medicine, Hiroshima University

Abstract: The present study was designed to examine the effects of a low iodine diet (LID) on tumorigenesis in F344 rats treated with N-methyl-N-nitrosourea (MNU). Four-week-old male F344 rats were exposed to 100 ppm MNU in their drinking water for 15 weeks. Thereafter Group 2 animals received a low iodine diet LID while Group 1 received no further treatment after MNU. Non-carcinogen control animals received the LID without prior initiation (Group 3). At sacrifice, 36 weeks after starting MNU administration, nerve tumors were present in 16 of 20 (80%) animals, and 19 of 32 (59%) in Groups 1 and 2, respectively, most being malignant schwannomas. Thymic lymphomas appeared in 7 of 20 (35%) rats in Group 1 and 15 of 32 (47%) in Group 2, and also gastric tumors were present in 6 of 20 (30%) in Group 1 and 10 of 32 (31%) in Group 2. There were no significant intergroups differences regarding these lesions. However, the incidence of thyroid carcinomas in Group 2 was significantly higher than that in Group 1 (P<0.01). Also cumulative incidence curves indicated a significantly earlier appearance in Group 2 and the number of rats bearing 3 different tumors was significantly increased in this group (P<0.01). Moreover, administration of the LID was itself associated with a small incidence of thyroid lesions. (J Toxicol Pathol 9: 191-197, 1996)
Key words: MNU, Thyroid tumors, Low iodine diet, F344 rat

Introduction

N-methyl-N-nitrosourea (MNU) is well known to be a potent mutagen and direct acting carcinogen producing tumors in several species in a variety of organs, including the central nervous system, intestine, kidney, stomach, mammary gland, and skin. Hirota et al. reported that MNU in the drinking water selectively induces glandular stomach carcinomas at a high incidence in F344 rats. This was confirmed by Fujita et al. and the MNU-F344 rat model has now become firmly established for investigations of gastric carcinogenesis. Recently, Shibutani et al. reported induction of anaplastic astrocytomas and glioblastomas in adult F344 male given MNU in their drinking water. We also reported that gastric tumors, spinal cord tumors, thymic lymphomas, and thyroid tumors develop in response to MNU application in the drinking water. The present investigation was performed to determine whether a low iodine diet may be a thyroid tumor promoter in the male F344 rat MNU tumorigenesis model, when given subsequent to carcinogen withdrawal.

Materials and Methods

Animals

Seventy-three male F344 (Charles River Japan Co. Ltd., Hino) four-weeks-old at the commencement, were used in the present study. They were housed four or five to a polycarbonate cage and kept under constant conditions of temperature (24±2°C), and relative humidity (55±10%) with a 12 h light/12 h dark cycle. The animals were maintained under the guidelines set forth in the “Guide for the Care and Use of Laboratory Animals” established by Hiro-
They were fed commercial diet MF (Oriental Yeast, Co. Ltd., Tokyo) or low iodine diet (LID, Oriental Yeast Co. Ltd.) and were provided with distilled water *ad libitum* except during the MNU treatment.

**Chemicals**

MNU was purchased from Sigma Chemical Co., St Louis and dissolved in distilled water at a concentration of 100 ppm. Rats were given this solution in light-opaque bottles as their drinking water for 15 weeks. The MNU solution was changed at 3 to 4 day intervals.

**Experimental design**

Animals were divided into 3 groups. In Group 1, MNU was given alone. In Group 2, LID was added after MNU treatment. In Group 3, animals received LID without the prior carcinogen. All had free access to food and water throughout.

**Examination of animals**

All animals were regularly observed and killed upon observation of paralysis of one or two legs or at the termination of the experiment 36 weeks after the start of MNU treatment. At the time of necropsy, the body, liver, and other major organs were weight and prepared for histopathology. Skulls were opened and animals were observed for brain and pituitary lesions. The back bones were placed in fixative for more than 24 h and the spinal cord was opened. The stomach was cut open along the greater curvature, stretched out and pinned on a board with the mucosal surface facing upward and washed several times with physiological saline before gross examination and fixation in 10% neutral formalin. Strips of stomach were cut perpendicularly to the mucosal surface, two at the lesser curvature and four at the greater curvature. The strips were embedded in paraffin and serially sectioned at 3 μm. Sections from all tissues were routinely stained with hematoxylin and eosin, and with alcian-blue-periodic acid Schiff. Additional sections were also stained for mucin with high-iron diamine-alcian blue whose appropriate.

**Statistical analysis**

The significance of differences in numerical data was evaluated by the chi-squared and Student's *t* tests and by the generalized Wilcoxon test for Kaplan Meier survival rates.

**Results**

The mean body weights of the rats at the time of autopsy in Groups 1 and 2 were significantly decreased as compared to those in Group 3. Liver,
kidney, testis, and adrenal weights in Groups 1 and 2 were significantly decreased as compared to those in Group 3 (Table 1). Relative testis weights in Groups 1 and 2 were significantly increased as compared to that in Group 3 (Table 2).

The first sarcoma had appeared by 120 days from the beginning of MNU treatment in Group 2. A thymic lymphoma was detected at 126 days, both adenocarcinoma of the glandular stomach and thyroid adenoma were observed at 140 days and paralysis of both legs appeared at 149 days. In Group 1, the first thymic lymphoma was detected at 150 days from the beginning of MNU treatment and a thyroid tumor was observed at 153 days. In Group 3 a thyroid tumor was detected at 245 days. Incidences of total tumors were 100, 94, and 14% in Groups 1-3, respectively at the 36 week time-point. Spinal cord tumors (Fig. 2), thymic lymphomas (Fig. 3), and gastric tumors (Fig. 4) were the predominant lesions in the MNU-treated groups. Brain tumors also developed as well as sarcomas. Although the incidences of lesions other than those in the thyroid did not significantly differ between the MNU-treated groups, that for thyroid carcinomas (Fig. 5, 6) in Group 2 was

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean survival (days)</th>
<th>Body (g)</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
<th>Testis (g)</th>
<th>Spleen (g)</th>
<th>Adrenal (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>MNU</td>
<td>188±30</td>
<td>230±35</td>
<td>7.2±2.9</td>
<td>1.82±0.29</td>
<td>2.38±0.34</td>
<td>1.19±1.27</td>
<td>0.052±0.023</td>
</tr>
<tr>
<td>Group 2</td>
<td>MNU+LID</td>
<td>178±32</td>
<td>254±36</td>
<td>6.6±1.6</td>
<td>1.75±0.42</td>
<td>2.44±0.42</td>
<td>1.19±1.74</td>
<td>0.050±0.017</td>
</tr>
<tr>
<td>Group 3</td>
<td>LID</td>
<td>161±47N</td>
<td>359±57N</td>
<td>10.4±2.0N</td>
<td>2.43±0.44N</td>
<td>2.73±0.46N</td>
<td>0.64±0.11</td>
<td>0.068±0.018NG</td>
</tr>
</tbody>
</table>

*: Significantly different from the MNU case (P<0.05)
#: Significantly different from the MNU+LID case (P<0.01)
*: Significantly different from the MNU case (P<0.01)
#: Significantly different from the MNU+LID case (P<0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testis</th>
<th>Spleen</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>MNU</td>
<td>3.08±1.12</td>
<td>0.791±0.015</td>
<td>1.033±0.124W</td>
<td>0.521±0.582</td>
<td>0.0232±0.0112</td>
</tr>
<tr>
<td>Group 2</td>
<td>MNU+LID</td>
<td>2.66±0.67</td>
<td>0.705±0.229</td>
<td>0.981±0.172W</td>
<td>0.470±0.660</td>
<td>0.0210±0.0105</td>
</tr>
<tr>
<td>Group 3</td>
<td>LID</td>
<td>3.00±1.38</td>
<td>0.696±0.276</td>
<td>0.792±0.282</td>
<td>0.183±0.058</td>
<td>0.0201±0.0095</td>
</tr>
</tbody>
</table>

*: Significantly different from the LID case (P<0.01)

Fig. 2. Spinal cord tumor, malignant schwannoma.  HE ×100
Fig. 3. Thymic lymphoma. HE × 200

Fig. 4. Gastric tumor, well-differentiated type. HE × 100

Fig. 5. Thyroid tumor, follicular adenocarcinoma. HE × 100
significantly increased as compared to the Group 1 value (P<0.01) (Table 3). Cumulative incidence curves of thyroid tumors in Group 2 also indicated a significantly earlier appearance than in Group 1 (Fig. 1) and the number of rats bearing 3 different tumors was also significantly elevated in Group 2 (P<0.01) (Table 4). Small numbers of thyroid lesions also developed in Group 3 from 250 days.

Gastric tumors appeared in 30% and 31% of animals of Groups 1 and 2, respectively, without any significant intra-group difference. Morphologically, most were well-differentiated types with a few moderate or poorly differentiated types. Larger sized lesions demonstrated both gastric mucosal and intestinal components. Most of them were adenocarcinomas.

All animals with paralysis demonstrated spinal cord tumors. Incidences of these were 30% and 28% in Groups 1 and 2, respectively. Morphologically, most of the spinal cord tumors in Group 1 had typical features of malignant schwannomas, positive for S-100 protein, but in Group 2, 5 oligodendrogliomas were encountered. Mitoses were frequently observed and growth rates were very rapid. Spinal cord tumors were located around the lumbar but not cervical or thoracic segments. Malignant schwannomas appeared in both groups, with no significant differences in incidence or growth rate.

Table 3. Incidence of Tumors (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective animal No.</th>
<th>Tumor bearing animal</th>
<th>Gastric Adenocarcinoma</th>
<th>Thyroid Tumor</th>
<th>Thymic lymphoma</th>
<th>Nerve tumor</th>
<th>Sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 MNU</td>
<td>20</td>
<td>20 (100)</td>
<td>6 (30)</td>
<td>4 (20)</td>
<td>0</td>
<td>4 (20)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Group 2 MNU+LID</td>
<td>32</td>
<td>31 (94)</td>
<td>10 (31)</td>
<td>12 (38)</td>
<td>15 (47)</td>
<td>27 (84)</td>
<td>15 (47)</td>
</tr>
<tr>
<td>Group 3 LID</td>
<td>21</td>
<td>3 (14)</td>
<td>0</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>3 (14)</td>
<td>0</td>
</tr>
</tbody>
</table>

: Significantly different from the MNU or LID cases (P<0.01)

Table 4. Numbers of Tumors Per Rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of tumors per rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 MNU</td>
<td>0</td>
<td>8 (40) 10 (50) 1 (5)</td>
</tr>
<tr>
<td>Group 2 MNU+LID</td>
<td>1 (3)</td>
<td>8 (25) 8 (25) 13 (40.6)</td>
</tr>
<tr>
<td>Group 3 LID</td>
<td>18 (86)</td>
<td>3 (14) 0 0 0</td>
</tr>
</tbody>
</table>

: Significantly different from the MNU+LID case (P<0.01)
nomas were also observed near the stomach in the abdomen. Brain tumors were diagnosed as oligodendroglioma (Table 5).

Discussion

Nerve and gastric tumors, as well as thymic lymphomas, predominated in the present experiment, in line with the results described previously. Thus, MNU is a potent mutagen and direct acting carcinogen producing tumors in several species in a variety of organs. Shibutani et al. also reported anaplastic astrocytomas and glioblastomas can be induced in F344 rats given MNU in their drinking water. So that the carcinogen can clearly be absorbed across the gastrointestinal mucosa, and reach its target organs through the circulation.

In this present experiment, thyroid carcinomas were also induced, and their development was significantly increased and accelerated by a LID. Plasma TSH and thyroid weight have both been reported to increase in animals on restricted iodine diet. TSH is the primary hormonal mitogen of thyroid cells and stimulates proliferation of thyroid epithelium in vivo and in vitro. There is growing evidence that locally produced growth factors also may play an important role in regulation of cell proliferation and differentiation in the thyroid. Recently we reported that gastric tumors in the animals with elevated serum growth hormone appeared earlier than in animals of normal GH level by using the same system in the present experiment. The present system thus offers a good model for ascertaining promoter function in carcinogenesis.

References


