Induction of Duodenal Tumors in F344 Rats by Continuous Oral Administration of N-Ethyl-N-nitrosourea 1, 2

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ABSTRACT—Forty male and 40 female inbred F344 rats were given a solution of 400 mg N-ethyl-N-nitrosourea/liter in their drinking water. Digestive tract tumors were induced in 32 males (an incidence rate of 80%) and in 28 females (an incidence rate of 70%). Among these digestive tract neoplasms, duodenal tumors occurred most frequently. Most were of the epithelial type, such as adenoma or adenocarcinoma. Tumors in hematopoietic organs were also found in 15 males (38% incidence) and in 17 females (43% incidence).—JNCI 64: 613-616, 1980.

1-Alkyl-1-nitrosoureas are strong carcinogens. In our studies in which female Donryu rats were used, 1-methyl-1-nitrosourea induced neurogenic tumors (1), ENU rapidly induced erythroblastic leukemias (2), 1-propyl-1-nitrosourea induced leukemias and digestive tract tumors (3, 4), and 1-buty1-1-nitrosourea induced leukemias and mammary tumors (5, 6). ENU is a potent carcinogen not only in adult rats but also in fetal rats (7, 8).

In this paper the results of oral administration of ENU to inbred male and female F344 rats are described. Digestive tract tumors, especially epithelial tumors of the duodenum, were induced in many rats.

MATERIALS AND METHODS

Rats and diet.—Five-week-old male and female F344 rats (Charles River Japan, Inc.) were maintained on a basal diet, CE-2 (CLEA Japan Inc.), until they were 11 weeks old, at which time oral administration of ENU was started.

ENU.—ENU was synthesized according to the procedure of Arndt and Amende (9) for preparation of 1-methyl-1-nitrosourea. ENU, sensitive to light, heat, and humidity, was refrigerated at 5°C until used.

Experimental procedure.—A 400-mg ENU solution/liter in distilled water was administered in the drinking water to 40 male and 40 female rats. Animals took the solution ad libitum. The average intake of the solution was 5.1 and 3.6 ml/day/rat in the male and female groups, respectively. Four rats each were kept in a plastic cage (38x32x18 cm) in an air-conditioned room (room temperature, 24±1°C; relative humidity, 55±5%). The freshly prepared ENU solution was given every day for the first 4 weeks and 5 days a week thereafter. This regimen was continued until the rats were killed for autopsy. Many animals developed tumors and were killed before the 38th experimental week, when the 3 surviving female rats were autopsied. The body weights of the rats were determined every week, and peripheral blood smears were examined every 2 weeks.

Histologic examination.—All rats were autopsied when they were moribund or died. Tissues were fixed in 10% neutral buffered Formalin, and sections were routinely stained with H & E.

RESULTS

The first autopsy of a rat with a tumor was done at the 15th experimental week. Before that time, no rats died from intoxication.

Incidence of tumors and average survival times.—The number of rats with tumors that developed in various organs is given in table 1. Generally, rats with hematopoietic neoplasms died early in the experiment, and the association of tumors in other organs was seldom observed. In contrast, digestive tract tumors were dominant in animals with multiple primary tumors.

The number of rats with tumors in the digestive tract are listed in table 2. Tumors were observed in all parts of the digestive tract but most frequently in the duodenum.

The average survival times of the animals are also given in tables 1 and 2.

Histology of digestive tract tumors.—The histology of the tumors is given in table 2. Most digestive tract tumors were of the epithelial type. The duodenal tumors were classified according to the criteria of Prozharisski (10). Most of the adenomas in the duodena were of the pedicle type; the sessile type was rare. Tumor cells were cylindrical, and the basement membrane was lined with a small amount of connective tissue and a few goblet cells. In duodenal adenocarcinomas, the papillotubular type was the most frequent and this type was found in many adenomas. Tumor cells were cylindrical or cubical; a few were goblet cells. Nuclei were large and polymorphic and occupied a large part of the cytoplasm. The connective tissue was minimal (fig. 1). Three typical signet-ring cell carcinomas were observed in the stomach, duodenum, and jejunum. Carcinoma cells full of mucus proliferated in epithelial tumors.

ABBREVIATIONS USED: ENU = N-ethyl-N-nitrosourea; H & E = hematoxylin and eosin.
TABLE 1.—Tumors induced in various organs of F344 rats by ENU

<table>
<thead>
<tr>
<th>Sex of rat</th>
<th>No. of rats examined</th>
<th>Average survival time, wk</th>
<th>No. of rats with tumors (%)</th>
<th>No. of rats with tumors in</th>
<th>Digestive tract (%)</th>
<th>Hematopoietic organs (%)</th>
<th>Mammary glands (%)</th>
<th>Ear duct (%)</th>
<th>Other organs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>40</td>
<td>23.8±4.2</td>
<td>23.8±4.2</td>
<td>36 (90)</td>
<td>32 (80)</td>
<td>15 (38)</td>
<td>0 (15)</td>
<td>5 (15)</td>
<td>12 (38)</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>29.1±4.2</td>
<td>29.1±4.2</td>
<td>39 (98)</td>
<td>28 (70)</td>
<td>17 (43)</td>
<td>11 (28)</td>
<td>5 (15)</td>
<td>15 (38)</td>
</tr>
</tbody>
</table>

* Mean±SD.

** Four tumors in the lung and 1 tumor each in the pituitary gland, submaxillary gland, thyroid gland, heart, spleen, adrenal gland, testis, and preputial gland.

*** Four tumors each in the kidney and preputial gland, 2 tumors in the subcutaneous tissue, and 1 tumor each in the parotid gland, thyroid gland, lung, spinal cord, and adrenal gland.

TABLE 2.—Histology of tumors and number of F344 rats with ENU-induced tumors in various parts of the digestive tract

<table>
<thead>
<tr>
<th>Organ affected with tumor (No. of rats with tumors)</th>
<th>Histologic type of tumor</th>
<th>No. of tumors by histologic type</th>
<th>Males*</th>
<th>Females*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue (13, 07)</td>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Esophagus (25, 62)</td>
<td>Papilloma</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Forestomach (25, 39)</td>
<td>Papilloma</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Glandular stomach (75, 72)</td>
<td>Adenoma</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fibroma</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hemangiendothelioma</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Leiomyoma</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Duodenum (26, 162)</td>
<td>Adenoma</td>
<td>11</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>21</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Jejunum (85, 39)</td>
<td>Adenoma</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ileum (15, 19)</td>
<td>Adenoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Colon (20, 19)</td>
<td>Adenocarcinoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Of 40 rats examined, 32 had tumors in the digestive tract.

** Of 40 rats examined, 28 had tumors in the digestive tract.

was observed in 10 rats. In female rats, hyperplasia was found in 3 and fibroadenomas, adenomas, and/or adenocarcinomas were observed in 11.

Other tumors.—Sebaceous squamous cell carcinomas of the ear duct, adenomas of the lung, adenocanthonomas of the preputial gland, and nephroblastomas of the kidney were found in 10, 5, 5, and 4 rats, respectively. The incidence of other tumors was less than 3%.

DISCUSSION

The results of this experiment show that digestive tract tumors were often induced in F344 rats that received a solution of ENU in their drinking water. Of these tumors, duodenal tumors occurred most frequently. Druckrey et al. (11) induced duodenal tumors in 7 of 13 rats by sc injection of 1,2-dimethylhydrazine. However, upon autopsy of the rats, they found large neoplasms of the colon in 10 rats and of the rectum in 8 rats and additional smaller tumors in the duodena in 7 rats (11). Druckrey (12) also induced digestive tract tumors with 1-propyl-l-nitrosourea, and the incidence of duodenal tumors was 25%. Ogiu et al. (3) obtained similar results with 1-propyl-l-nitrosourea (3). Nakamura et al. (13) and Matsuyama et al. (14) induced adenocarcinomas of the duodena in about 70% of animals with N-ethyl-N'-nitro-N-nitrosoguanidine, but only in mice. Therefore, the results obtained in the F344 rats administered ENU are unique in regard to the high incidence and the short induction time of duodenal tumors.

The histology and the morphogenesis of the duodenal tumors in the F344 rats given ENU were also interesting. Matsuyama et al. (14) reported that no erosion, ulcer, or benign tumor anteceded the malignant lesion. They considered from these morphologic features that N-ethyl-N'-nitro-N-nitrosoguanidine carcinogenesis in the duodenum occurred in one step. In the present experiment, all duodenal tumors were epithelial, and most were adenomas or well-differentiated adenocarcinomas. Although a signet-ring cell tumor was induced, nonepithelial tumors were not observed. Macroscopically, these neoplastic lesions were found in the mucosa and were polypous. Histologically, ade-
nomas and adenocarcinomas were observed as different nodules in the duodena of some rats. In most of the tumors diagnosed as adenocarcinomas, the lesions were surrounded by an adenoma, although the border was not always clear. Therefore, some of these adenocarcinomas could be said to have converted from adenoma. These findings show that carcinogenesis with ENU in the duodena of F344 rats occurs in one and/or more steps. These experimental systems are useful for the analysis of the morphogenesis of intestinal tumors in man.

The strain of rat and the route of administration of ENU are also important factors in the determination of the target organ of ENU. Druckrey et al. (4) reported many neurogenic tumors and a few nephroblastomas in BD rats after receiving a single sc injection of ENU and also leukemias and gliomas in the brains of BD rats after receiving iv pulse doses of ENU. Druckrey (15) administered a single oral intubation of ENU to 10-day-old BD rats and induced mainly neurogenic tumors in these rats. Hadjiolov (16) injected ENU ip (50 mg/animal) into adult noninbred Wistar rats and induced thymic lymphomas and leukemias. Ogiu et al. (2) administered to female Donryu rats ENU in the drinking water, which rapidly induced erythroleukemias at a high incidence. Pelfrene et al. (7) administered ENU in the drinking water to MRC Wistar rats and obtained digestive tract tumors, mammary tumors, and myelocytic leukemias. In all these studies, duodenal tumors were not observed.

In the present experiment, duodenal tumors were induced with ENU at high incidences in male and female F344 rats. The method described in this paper is therefore useful for the study of carcinogenesis in the duodenum.

REFERENCES

FIGURE 1.—Adenocarcinoma in the duodenum of a male rat given an ENU solution for 20 wk. H & E. × 80
FIGURE 2.—Signet-ring cell carcinoma in the jejunum of a female rat given an ENU solution for 32 wk. H & E. × 80