Carcinogenesis in Rat Esophagus by Intraperitoneal Injection of Different Doses of Methyl-n-amylNitrosamine

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ABSTRACT

The carcinogenicity of methyl-n-amylnitrosamine in MRC-Wistar rats was determined after i.p. injection at a variety of dose schedules. After 6 weekly methyl-n-amylNitrosamine injections of 25 mg/kg or 12 weekly injections of either 12.5 or 25 mg/kg, the incidence of esophageal squamous cell papillomas was 85 to 100% and that of esophageal squamous cell carcinomas was 40 to 65%. With 12 injections, the mean survival time was 25 to 31 weeks. Treatment with 1 or 2 doses of 50 mg/kg produced a lesser incidence (<20%) of esophageal tumors, with a longer survival time of 67 to 77 weeks. One 85-mg/kg injection caused esophageal carcinomas in 5 of 7 rats. The treated groups also had squamous cell papillomas and carcinomas in the nasal cavity (up to 50% incidence) and trachea (up to 30% incidence). Hence, a 6- or 12-week treatment schedule was adequate for inducing esophageal tumors and could be used for studies on agents modifying esophageal tumor induction by methyl-n-amylNitrosamine.

INTRODUCTION

Unsymmetrical dialkylnitrosamines (e.g., MNAN, N-methyl-N-nitrosobenzyamine and N-methyl-N-nitrosouanilime) and certain cyclic nitrosamines (e.g., N-nitrosopiperidine) induce esophageal tumors in rats (2). The effect of chronic ethanol treatment (8) and of deficiencies in zinc (3) and vitamin A (9) on nitrosamine-induced esophageal carcinogenesis has been determined.

We wish to test possible enhancers of esophageal carcinogenesis such as croton oil and Bidens pilosa, given to rats in the diet (7). In such tests, the primary carcinogen should be administered for only a short time and should produce only a moderate incidence of esophageal tumors (10). The present experiments involved various dose schedules with MNAN and were designed to find out whether this nitrosamine might serve such a function. The test systems could also be useful for studying pathological changes in the esophagus leading to neoplasia. We chose MNAN because it was reported to affect the esophagus rather specifically (2) and because we have studied MNAN inhibition of [3H]thymidine incorporation into rat esophageal DNA (5).

MATERIALS AND METHODS

MRC-Wistar rats from the Eppley Institute were given Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.) and water ad libitum. Rats were housed in groups of 5 in plastic cages on granular cellulose bedding and kept under standard laboratory conditions. Mean body weight during the experiment was 400 g for males and 250 g for females.

MNAN was synthesized from commercial methyl-n-amylnitrosamine as before (5) and stored at —15°, and its purity was checked by UV absorption of aqueous solutions. Freshly prepared MNAN solutions in distilled water were injected i.p. at a level of 10 ml solution per kg, except for the dose of 85 mg/kg, where 15 ml/kg were used. One to 12 injections of 12.5 to 85 mg/kg were given, starting at an age of 7 to 8 weeks (Table 1). Multiple injections were given at weekly intervals. Survival times were measured from the start of the experiment.

Since MNAN is volatile and may be expired unchanged (6), the injected rats were kept in a chemical hood during and for 2 days after each injection. Thereafter, they were returned to the animal room. Analysis (4) showed that MNAN was undetectable in the air of the animal room but was present in the air of the chemical hood while the rats were being given injections (0.25 ppb). Two days later, apparent concentration in the hood was insignificant (<0.02 ppb).

The rats were allowed to die spontaneously or were killed when moribund, and they then were completely autopsied. The cause of death was not determined. The esophagus was dissected longitudinally and spread out on paper, and gross pathological lesions were recorded on a sketch. In addition to the esophagus, tissues of the upper and lower respiratory tract, pharynx, stomach, liver, kidneys, and any other tissues with gross abnormalities were taken for histology. The tissues were fixed in 10% buffered formalin, embedded in Paraplast, and stained with hematoxylin and eosin. About 3 sections were prepared of the top, middle, and bottom thirds of each esophagus; these included most or all of the tumors. Lesions were not termed squamous cell carcinomas unless they were invasive.

RESULTS AND DISCUSSION

An i.p. injection of 85 mg MNAN per kg was acutely toxic, with 8 of 16 rats dying within 1 week. [The 50% lethal dose when administered p.o. or s.c. to BD rats was 120 mg/kg (2).] The surviving rats were maintained for life (Table 1, Group 2). The highest individual dose chosen for the remaining groups of the chronic test was 50 mg/kg. Table 1 shows the survival times for the chronic test. MNAN most commonly produced squamous cell papillomas and carcinomas of the esophagus (Table 2). No sex differences were observed for these tumors.

Tumor incidence in the esophagus was relatively low in the rats given 1 or 2 injections (Groups 1 to 3). An exception was that 5 of 7 rats treated once with 85 mg/kg (Group 2) developed esophageal carcinomas (mean survival time, 76 weeks). Almost all rats in Groups 4 to 6 (injected 6 or 12 times) showed....

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4 The abbreviation used is: MNAN, methyl-n-amylNitrosamine.
### Table 1

<table>
<thead>
<tr>
<th>MNAN treatment</th>
<th>No. of rats alive at Wk</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
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<tr>
<td>Dose (mg/kg)</td>
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<td>No. of injections</td>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Tumor distribution</th>
<th>No. of rats with tumors of</th>
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<tr>
<td></td>
<td>Esophagus</td>
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### basement esophageal papillomas, and about one-half also had esophageal carcinomas. The mean number of esophageal tumors per rat was 1 to 3 in Groups 1 to 3, 5 to 8 in Group 4, and 9 to 11 in Groups 5 and 6 (mean values refer to each sex separately). The mean survival time for rats with esophageal tumors was 58 to 105 weeks for Groups 1 to 3, 42 to 50 weeks for Group 4, and 25 to 31 weeks for Groups 5 and 6. Hence, increasing the number of MNAN injections caused an increase in esophageal tumor incidence and multiplicity and a decrease in survival time of the tumor-bearing rats.

Squamous cell papillomas and carcinomas also occurred in the nasal cavity and trachea (Table 2). The incidence of nasal cavity tumors was highest in Groups 4 to 6, in which it reached 50%. Group 4 had a higher incidence of nasal cavity tumors than did Group 6; this is attributed to the longer survival of Group 4, which allowed more time for these tumors to develop. Tracheal papillomas and carcinomas occurred in Groups 4 to 6, with a maximum incidence of 30%. Other tumors (Table 2)
were similar to those in control rats of the present experiment (Group 7) and of previous studies (1).

In the study by Druckrey et al. (2), MNAN was administered to rats p.o. continuously or by weekly s.c. injections for life, and an 80 to 90% incidence of esophageal tumors was observed, with survival times of 30 to 40 weeks. In the present study, the time of treatment with MNAN was much shorter than that used by Druckrey et al. Nevertheless, we also obtained a high incidence of esophageal tumors when 6 to 12 weekly injections were given, with survival times similar to those observed by Druckrey et al. In these groups, the total dose of 150 to 300 mg/kg was only slightly less than that used by Druckrey et al. (330 to 600 mg/kg).

MNAN was not as specific a carcinogen for the esophagus as had been hoped, since tracheal and nasal cavity tumors were also induced. Esophageal tumors might predominate more strongly if enhancers specific for the esophagus were administered in addition to MNAN. For tests of such tumor enhancers, the treatment in Group 4 (6 x 25 mg/kg) may be the most suitable, except that the MNAN dose per injection should probably be lowered.

ACKNOWLEDGMENTS

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REFERENCES


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