ABSTRACT

Dose-response relationships for the induction of mammary tumors by a single i.v. injection of N-methyl-N-nitrosourea (MNU) were studied. At 50 days of age, groups of 20 virgin female Sprague-Dawley rats received single doses of 50, 45, 40, 35, 30, 25, 20, 15, or 10 mg MNU per kg body weight; a group of 10 control rats received 0.85% NaCl solution only. Animals were observed for the appearance of mammary tumors over their life span or until 600 days after carcinogen administration. Both malignant and benign mammary tumors appeared in all groups; however, malignant tumors appeared earlier and at a faster rate than did benign tumors. Incidence of cancer and number of cancers per animal increased with increasing MNU dose; the latent period for cancer increased with decreasing dose. The number of benign tumors induced as a percentage of total tumors increased with decreasing dose, ranging from approximately 10% in groups receiving more than 30 mg MNU per kg to 58% in the group receiving 10 mg/kg. Foci of metastatic mammary carcinoma were found in lungs of animals in several MNU dose groups. Data from the present study indicate that a single i.v. administration of MNU induces mammary cancer in a dose-related fashion, with little toxicity and a short latent period; induced cancers metastasize to distant sites. The single-dose MNU model thus appears to be superior to both the 7,12-dimethylbenz(a)anthracene and multiple-dose MNU models, particularly for use in studies of modification of mammalian carcinogenesis.

INTRODUCTION

The induction of mammary tumors in the female rat by i.v. injection of MNU has provided an experimental model for breast cancer that appears to satisfy many of the deficiencies of mammary tumor models which use polycyclic aromatic hydrocarbons such as DMBA and 3-methylcholanthrene as chemical carcinogens (2). The DMBA model has many advantages which make it a useful experimental system for the study of breast cancer: tumors can be induced in high incidence with a short latent period and little toxicity (3); tumor response is dose dependent (4, 11); a high percentage of the tumors is hormone dependent (3); and a single carcinogen dose is sufficient for tumor induction (4). However, the model is deficient in several aspects, indicating the need for additional experimental models which may better simulate the human disease. Specifically, major deficiencies in the DMBA model are (a) lack of local tumor invasion and metastasis to distant sites and (b) the relatively high proportion of induced benign lesions compared to carcinomas, especially in the Sprague-Dawley strain (5, 10).

The MNU-induced mammary tumor model as developed by Gullino et al. (2) appears to satisfy these deficiencies. Using a 3-dose regimen in which the MNU doses were administered at 4-week intervals to BUF/N rats, Gullino et al. induced a high incidence of mammary tumors with a short latent period and with little toxicity. The majority of induced tumors were hormone dependent; all were classified histologically as adenocarcinomas or papillary carcinomas, and metastasis to spleen, liver, and lung was reported. Subsequent studies by Rose et al. (9) have provided biological characterization of MNU-induced mammary tumors in the Sprague-Dawley strain.

Although the 3-dose MNU model as used by Gullino et al. (2) appears to have distinct advantages over the single-dose DMBA model in terms of the characteristics of the induced tumors, administration of the carcinogen in multiple doses precludes accurate temporal definition of the stages of carcinogen availability and postcarcinogen tumor development and growth. This complicates data interpretation, particularly in studies involving agents which modify the initiation or progression stages of mammary carcinogenesis. For this reason, the development of a breast cancer model using a single dose of MNU is desirable. Chan et al. (1) have reported that a single dose of 50 mg MNU per kg body weight is effective in the induction of mammary tumors in F344 and Sprague-Dawley rats; the present paper reports a lifetime dose-response study of the induction of mammary tumors in female Sprague-Dawley rats by single doses of MNU.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats were obtained at 42 days of age from ARS/Sprague-Dawley, Madison, Wis.; a total of 190 rats was used in the study. Animals were housed 3 to a cage in a room illuminated 14 hr each day and maintained at a temperature of 22 ± 1°C (S.D.). All animals had free access to Wayne Lab Blox (Allied Mills, Chicago, Ill.) and drinking water throughout the experiment.

Crystalline MNU was purchased from Ash-Stevens, Inc. (Detroit, Mich.) and was dissolved in 0.85% NaCl solution acidified to pH 5.0 with acetic acid. At 50 days of age, the rats were lightly anesthetized with ether and received a single i.v. injection of either 50, 45, 40, 35, 30, 25, 20, 15, or 10 mg MNU per kg body weight via the jugular vein. Control animals received only a single i.v. injection of the 0.85% NaCl (pH 5.0) solution (Table 1).

Beginning 4 weeks after MNU administration, animals were palpated twice weekly to monitor mammary tumor appearance. Animals were weighed weekly throughout the experiment. When moribund, rats were killed by CO₂ asphyxiation. At 600 days after MNU administration, the experiment was terminated,
and the remaining rats [5 rats from the MNU (10 mg/kg) group and 4 rats from the control group] were killed. Animals killed or found dead were necropsied promptly; mammary tumors were removed and coded as to location. Additionally, liver, spleen, kidneys, lungs, and any grossly abnormal tissues were removed and prepared for histological study. Tissues were fixed in 10% buffered formalin, stained with hematoxylin and eosin, and classified histopathologically.

Values for tumors per animal were calculated by dividing the number of new tumors observed in each 10-day interval by the number of rats at risk in that interval; this calculation thus accounts for intercurrent mortality. Values determined for each 10-day interval were summed over the entire experiment.

RESULTS AND DISCUSSION

Administration i.v. of MNU at doses ranging from 10 to 50 mg/kg induced no acute toxicity in treated animals. No short-term mortality was observed, nor were animal weights depressed as a result of MNU administration. In general, animal survival throughout the study was inversely associated with MNU dose. The values in Table 1 for time to 50% mortality indicate that this value for individual dose groups increased with decreasing dose; mortality curves for representative dose groups are shown in Chart 1.

Intercurrent mortality had little effect on tumor incidence in the high-dose groups. In groups receiving doses of 30 mg MNU per kg or more, final cancer incidences were all ≥90%, indicating little, if any, competing risk in terms of mortality. In lower-dose groups, however, cancer incidence decreased to levels as low as 45% (10-mg/kg dose group). These groups, then, had significant mortality due to factors other than mammary cancer; mortality in non-tumor-bearing animals generally was associated with chronic respiratory infection or pneumonia.

Administration of MNU resulted in the induction of both mammary cancers and benign mammary tumors in all dose groups. We define cancers here to include both adenocarcinomas and papillary carcinomas; a significant number of lesions showed a mixed adenopapillary pattern, and a few showed regions of spindle cell sarcoma. Areas of sarcoma were characterized by numerous bundles of spindle-shaped cells with irregular orientation, variability of cell shape and size, and hyperchromatic nuclei with normal and abnormal mitotic figures. Benign mammary tumors were defined to include fibroadenomas, fibromas, and lobular hyperplasia; mammary cysts were not included. Mixed tumors with areas of carcinoma and fibroadenoma were scored as cancers.

The temporal patterns of cancer and benign tumor appearance are illustrated in Chart 2, in which tumor data from all groups are pooled. It can be seen that, although both malignant and benign mammary tumors were induced in large numbers, the benign tumors had a greatly increased latent period in comparison to carcinomas. Linear regression of the log-log plots indicates that 0.1 palpable carcinoma per rat developed by approximately 45 days post-MNU, and 1.0 cancer per rat appeared by 110 days postcarcinogen. By contrast, the induction of 0.1 benign tumor per rat was delayed until approximately 140 days post-MNU administration, and 1.0 benign tumor per rat was not seen until the end of the experiment at 600 days.

It is also of interest to note that the slope of the regression line for cancer appearance between 0.1 and 1.0 tumor per rat is 2.47 (r = 0.97), while the slope for benign tumor appearance is 1.53 (r = 0.98). This indicates that cancers not only appeared earlier in the study than did benign tumors, but they
also were induced at a faster rate after the time of first tumor appearance.

A distinct point of inflection in the rate of cancer appearance is seen at a value of approximately 1.25 tumors per animal; after this point (approximately 120 days post-MNU), the rate of cancer appearance is greatly decreased compared to the rate before the point of inflection (slope of line = 0.61 versus 2.47 before the inflection point). This suggests that the appearance of each mammary cancer is not an independent event; the rate of tumor appearance is decreased after approximately 1.25 tumors per rat, indicating that the presence of palpable cancers decreases the likelihood of appearance of additional lesions. Were the cancers independent of one another, their distribution among the experimental animals would be expected to follow a Poisson distribution; analysis of the distribution of tumors (8), however, shows that it is not consistent with a Poisson distribution ($p < 0.001$). Therefore, some interference of early cancers in the rate of appearance of later tumors is implied.

Dose-response relationships with respect to cancer incidence and carcinoma multiplicity are illustrated in Charts 3 and 4. It can be seen in both charts that the induction of malignant mammary tumors by MNU is dose related, with cancer response decreasing and latency period increasing as a function of decreasing MNU dose. While all groups receiving doses of 30 mg MNU per kg or more had a final cancer incidence of ≥90%, the time required to reach 90% incidence increased with decreasing dose. Final cancer incidence was decreased at MNU doses of 25 mg/kg or less, and the latency period of the induced cancers was greatly increased compared to the higher-dose groups. This trend is also evident in values for the median tumor induction time for each group (Table 1).

The number of cancers per animal decreased as a function of decreasing dose. Even the lowest MNU dose (10 mg/kg) was effective in cancer induction, however, as this group had significantly more cancers per animal ($p < 0.05$) and a decreased tumor latency in comparison to the “spontaneous” tumor response in 0.85% NaCl solution-treated controls.

The number of benign mammary tumors induced as a percentage of total mammary tumor response also was dose related, increasing with decreasing dose (Table 1). Groups receiving 35 mg MNU per kg or more developed approximately 90% cancers and 10% benign tumors, while the percentage of benign lesions showed a consistent increase in lower dose groups, reaching a value of 58% in the 10-mg/kg group. Fifty % of the tumors found in 0.85% NaCl solution-treated controls were benign. As the median life span of the treated groups also increased with decreasing dose, it might be inferred that the increased fibroadenoma response is an age effect, with older animals developing more benign tumors.

Tumor metastasis was observed in animals from several MNU dose groups. In their study with BUF/N rats, Guillon et al. (2) noted frequent metastasis to spleen, liver, and lung. No metastases to spleen or liver were found in the present study, but 7 animals showed metastasis of mammary cancers to the lung (Table 2; Fig. 1). Of the 7 animals bearing lung metastases, one had metastatic foci from 2 separate mammary tumors (one adenocarcinoma, one sarcoma). Other studies performed in this laboratory (6) have noted metastasis of MNU-induced mammary carcinoma to the kidney, although none was found in the current study.

Aside from metastatic mammary cancers, the administration of MNU did result in a number of tumors in organs other than the mammary gland. In high-dose groups, the kidney was a susceptible target: 8 of 120 animals receiving MNU doses of 25 mg/kg or more developed renal tumors of various histological types (Table 2). At lower doses, most nonmammary tumors were sarcomas of undetermined abdominal origin, although 2 uterine tumors (one leiomyoma and one adenocarcinoma) were...
found. Two primary lung tumors were noted in addition to the 8 metastatic mammary lesions.

As a proportion of total tumor response, however, nonmammary tumors were infrequent occurrences. Over 74% of the animals receiving MNU developed mammary cancer, and 87% developed a mammary tumor; nonmammary tumors were found in 10% of treated animals. Total tumor response consisted of over 400 mammary cancers, 88 benign mammary tumors, and 18 tumors in organs other than the mammary gland. Of the 18 animals bearing primary nonmammary tumors, 16 also had mammary tumors.

The data from the present study indicate that the induction of mammary cancer in the female Sprague-Dawley rat by a single i.v. dose of MNU provides a useful model for the experimental study of breast cancer. Tumors can be induced rapidly in a dose-dependent manner with little toxicity. Tumor induction is relatively specific for the mammary gland, the induced carcinomas or papillary carcinomas, and the appearance of the tumors induced by high (>30 mg/kg) doses of MNU are adenocarcinomas or papillary carcinomas, and the appearance of cancers will metastasize to distant sites, and previous studies in this laboratory have found that approximately 75% of the tumors induced by a single dose of MNU are hormone dependent (7). Although both benign tumors and mammary cancers develop as a result of MNU administration, approximately 90% of the tumors induced by high (≥30 mg/kg) doses of MNU are adenocarcinomas or papillary carcinomas, and the appearance of benign tumors occurs long after the appearance of cancers. The MNU-induced mammary cancer model satisfied the major deficiencies of the DMBA model, i.e., lack of tumor metastasis and high yield of benign lesions. Use of a single dose of carcinogen allows accurate temporal definition of the periods of carcinogen availability and the subsequent postcarcinogen phase of tumor development. Thus, the single-dose MNU model appears to be an improvement over multiple-dose models, particularly for use in studies involving modification of initiation or progression stages of mammary carcinogenesis.

REFERENCES


Table 2

Nonmammary tumors induced by MNU

Tumors of sites other than the mammary gland appeared in virgin female Sprague-Dawley rats which received a single i.v. administration of MNU at age of 50 days.

<table>
<thead>
<tr>
<th>Dose group (mg MNU/kg)</th>
<th>Lung tumors</th>
<th>Kidney tumors</th>
<th>Other sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2 transitional cell carcinomas of renal pelvis</td>
<td>1 abdominal sarcoma*</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>1 spindle cell carcinoma 1 sarcoma</td>
<td>1 abdominal sarcoma*</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>2 metastatic mammary adenocarcinomas 1 sarcoma 1 fibrosarcoma</td>
<td>1 abdominal sarcoma*</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>2 metastatic mammary adenocarcinomas 1 metastatic mammary sarcoma</td>
<td>1 abdominal sarcoma*</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2 metastatic mammary adenocarcinomas</td>
<td>1 abdominal sarcoma*</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1 primary bronchial adenocarcinoma 1 nephroblastoma 1 hemangioma</td>
<td>1 mesothelioma</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1 metastatic mammary adenocarcinoma</td>
<td>1 rhabdomyosarcoma*</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1 adenocarcinoma of uterus</td>
<td>1 abdominal mesenchymoma* 1 leiomyoma of uterus</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1 metastatic mammary adenocarcinoma</td>
<td>1 adenocarcinoma of uterus</td>
<td>1 abdominal sarcoma*</td>
</tr>
<tr>
<td>0</td>
<td>1 abdominal sarcoma*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Abdominal mass of undetermined origin.
Fig. 1. a, lung metastasis of adenocarcinoma from MNU-treated Sprague-Dawley rat. H & E, x 30. b, high-power view of metastatic mammary cancer in a. H & E, x 250.
Lifetime Dose-Response Relationships for Mammary Tumor Induction by a Single Administration of N-Methyl-N-nitrosourea

David L. McCormick, Christine B. Adamowski, Arsen Fiks, et al.

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