Induction of Mammary Gland Carcinomas by the Subcutaneous Injection of 1-Methyl-1-nitrosourea

Henry J. Thompson and L. David Meeker

ABSTRACT

The efficacy of s.c. administration of 1-methyl-1-nitrosourea (MNU) for the induction of mammary carcinomas was compared with the i.v. method of carcinogen injection in female Sprague-Dawley rats. Group-housed animals fed a laboratory chow diet and distilled water ad libitum throughout the study were injected at 50 days of age with 50 mg MNU per kg body weight. The carcinogen was given either s.c. or i.v., via the jugular vein, to one of the two groups of 20 rats each. Animals were palpated for tumor detection weekly and necropsied 180 days after injection with the carcinogen. At the termination of the study, 180 days postcarcinogen, cancer incidences were similar, 95 versus 90% in animals given MNU either s.c. or i.v. with an average of 3.9 and 3.9 cancers per rat, respectively. Time of tumor appearances were essentially identical under both treatment conditions. Using either method of carcinogen administration resulted in the induction of approximately 2.4 times more carcinomas in the cervical-thoracic mammary glands than in the abdominal-inguinal glands with no differences observed in cancer occurrence in the left versus the right mammary gland chains. The data indicate that s.c. administration of MNU is as effective and specific in the induction of mammary carcinomas as is i.v. administration. The s.c. method has the advantage of being easier and faster to perform and permits reproducible treatment of large numbers of rats by a small technical staff.

INTRODUCTION

The majority of research in rat mammary carcinogenesis has been conducted using the DMBA-induced tumor system. The DMBA model of breast cancer, developed by Huggins et al. (5), has contributed significantly to the understanding of mammary carcinogenesis; however, unlike breast cancers, the tumors rarely invade surrounding tissues or metastasize (4). There has been considerable recent interest in the induction of mammary gland carcinomas by MNU since this model has been found to contribute significantly to the understanding of mammary carcinogenesis; however, unlike breast cancers, the tumors rarely invade surrounding tissues or metastasize (4). There has been no metabolic activation, whereas DMBA is a lipophilic agent which must be further metabolized to become a carcinogen. The carcinogenic process. MNU is an alkylating agent which requires no metabolic activation, whereas DMBA is a lipophilic agent which must be further metabolized to become a carcinogen. The primary purpose of this investigation was to confirm our preliminary, unpublished observation that s.c. administration of MNU is as effective as i.v. administration in the induction of mammary gland carcinomas.

MATERIALS AND METHODS

Forty virgin female Sprague-Dawley rats obtained from Taconic Farms, Germantown, N. Y., at 35 days of age were used. The animals were housed in stainless steel, wire mesh-bottomed cages (4 rats/cage) in a controlled environment with temperature maintained at 24° and a 12-hr light-dark cycle. Animals were fed Purina rodent chow (No. 5001) and distilled water ad libitum throughout the study. At 50 days of age, animals were randomized into one of 2 groups of 20 rats and received 50 mg MNU dissolved in acidified 0.9% NaCl solution per kg body weight either s.c. or i.v. via the jugular vein. Thereafter, animals were palpated for the detection of mammary tumors twice each week and were weighed weekly.

The study was terminated 180 days after administration of MNU. At necropsy, the skin of each rat was transilluminated, and all grossly observable tumors were removed and processed for histological evaluation. Statistical evaluations for differences in the incidence, number, and latency of appearance of mammary gland carcinomas were as follows: differences in incidence by the \( \chi^2 \times 2 \times 2 \) contingency method without the conservative Yates correction for continuity (1, 2); differences in tumor number by analysis of variance following square root transformation of tumor counts (3, 12); differences in the time to appearance of all tumors by the Mann-Whitney test (7); and differences in latency of first tumor appearance by the procedure of Mantel (8).

RESULTS

The incidence and number of mammary carcinomas induced by the i.v. and s.c. administration of MNU are shown in Table 1. There were no statistically significant differences in these parameters between the 2 treatment groups. Similarly, the latency of cancer appearance and the rate of multiple cancer occurrences were essentially identical between groups. Using either route of injection of the carcinogen resulted in the induction of an average of 2.4 times as many mammary carcinomas in the left and right mammary gland chains, and tumors began to appear at about the same time in all sites. As has been reported by several laboratories, the carcinomas induced were more aggressive and locally invasive than are DMBA-induced mammary cancers, and the fibrous component in the MNU-induced lesions is less prominent than in those induced with DMBA. Using the mammary tumor classification system recently proposed by Komitowski et al. (6), the most frequently induced lesions were the compound fibropithelial tumors which have been reported to progress to anaplastic carcinomas. There were no significant differences in the various histopathological forms of tumors induced by either i.v. or s.c. routes of administration. The number of benign lesions induced in the mammary glands in each group was lower than that reported using the DMBA system (6). These included 5 tubular adenomas, 2 cystic adenomas, 2 fibroadenomas, and 1 fibroma (Table 1). Neither primary tumors of nonmammary organ sites nor metastatic lesions were observed in any of the animals. All major tumors were compound fibropithelial tumors. Some of the tumors were classified as carcinoma in situ, whereas others were invasive, and metastatic to the lungs.

1 Supported by USPHS Grant CA28109 from the National Cancer Institute. Scientific Contribution 1185 from the New Hampshire Agricultural Experiment Station.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; MNU, 1-methyl-1-nitrosourea.

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1628
DISTRIBUTION WITHIN THE BODY SIMILAR TO THAT ACHIEVED BY ITS I.V.

Figures 1 and 2. Mean number of cancers per cancer-bearing rat surviving until the end of the study.

Table 1

<table>
<thead>
<tr>
<th>MNU treatment</th>
<th>No. of rats</th>
<th>Survivors</th>
<th>No. of cancer-bearing rats</th>
<th>Total no.</th>
<th>Mean tumor latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>16</td>
<td>12</td>
<td>18 (60)</td>
<td>105</td>
<td>3.9</td>
</tr>
<tr>
<td>i.v.</td>
<td>17</td>
<td>13</td>
<td>18 (90)</td>
<td>105</td>
<td>3.9</td>
</tr>
</tbody>
</table>

a MNU was injected at a dose of 50 mg/kg body weight at 50 days of age as outlined in "Materials and Methods."

b Initial number of rats given carcinogen.

c Number of animals which lived until the end of the study.

d Number of animals bearing at least one palpable cancer.

e Total number of benign lesions induced.

f Differences among groups were not statistically significant.

g Median tumor-free time.

h Total number of cancers induced in animals surviving until the end of the study.

i Mean number of cancers induced in animals surviving until the end of the study.

j Mean number of cancers per cancer-bearing rat surviving until the end of the study.

k Number in parentheses are the number of animals which survived carcinogen treatment.

l Numbers in parentheses are the number of animals which survived carcinogen treatment, expressed as a percentage.

DISCUSSION

Several laboratories have reported that i.v. administration of MNU induces a high incidence of mammary carcinomas (4, 9–11, 13). The biological characteristics of these lesions, particularly with regard to the hormone dependency of the tumors, have been found to vary depending upon the number of carcinogen injections and the total dose of MNU administered (11). In giving the carcinogen i.v., at least 3 different sites of administration have been used: the footpad; the tail vein; and the jugular vein. All 3 approaches require a high degree of technical skill in order to guarantee quantitative administration of carcinogen to all treated animals. Differences in technical precision of administration of carcinogen could account at least in part for the lack of agreement among laboratories in the incidence and number of carcinomas reported to be induced by a given dose of MNU. Using the same techniques and doses of MNU, Rose et al. (11) induced fewer tumors with a lower incidence of cancer than that reported by Moon et al. (9, 10, 13). Our laboratories have found that the administration of the carcinogen via the jugular vein to be the most reliable method when large numbers of animals (greater than 200) are to be injected on the same day, since in our hands there is no decline in accuracy of administration. However, the procedure does suffer from its requirement of a large technical staff and the anesthesia of the animals. Furthermore, the need to suture the incision made to expose the jugular vein gives rise to scar tissue which can be mistaken during palpation for an induced tumor. In order to overcome these deficiencies, our laboratory has sought an alternative method of carcinogen administration with the hope that variations in tumor response observed in different laboratories can be reduced. It is widely known that s.c. injection of an agent leads to distribution within the body similar to that achieved by its i.v. injection. Therefore, in an initial pilot experiment, this approach was evaluated, and a 100% incidence of mammary carcinomas was induced in 15 animals. In the present study, the s.c. administration of MNU induced a 95% incidence of mammary gland carcinomas within 180 days of carcinogen treatment. Ninety-three % of the tumors induced were classified as mammary carcinomas.

In conclusion, the data presented suggest that s.c. administration of MNU is as effective and specific as its i.v. administration in inducing mammary gland carcinomas. This method is quantitative, accurate, and technically effective since it is faster than the administration of MNU i.v. via a jugular vein cutdown procedure.

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REFERENCES

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