Effect of Age at Treatment on Incidence and Type of Renal Neoplasm Induced in the Rat by a Single Dose of Dimethylnitrosamine

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ABSTRACT

The effect of age at treatment on the incidence and type of kidney tumor induced by dimethylnitrosamine was investigated in an established model of experimental renal carcinogenesis. Groups of Porton albino Wistar rats were dosed with carcinogen at 24 hr; at 3 weeks; and at 1, 1.5, 2, 3, 4, and 5 months of age. In all groups except the neonates, the animals were preconditioned by feeding a no-protein, high-carbohydrate diet for 5 days prior to the i.p. injection of dimethylnitrosamine (30 mg/kg). In the neonatal group, it was necessary to reduce the dose of carcinogen to 20 mg/kg in order to achieve approximately equivalent numbers of survivors. Notwithstanding the loss of strict comparability of data between the newborns and the remaining age groups, kidney tumor incidence showed a bimodal distribution represented by the occurrence of two separate entities, renal mesenchymal tumor and cortical epithelial tumor. Mesenchymal tumor proved to be a neoplasm of the neonatal and immature rat, the susceptibility to which fell rapidly after one month of age to nil at five months of age. In contrast, susceptibility to tumors of cortical tubule epithelium increased with ensuing sexual maturity, but it declined by five months of age. Age at dosing also influenced to some degree the grade of tumor induced. An altered grade of mesenchymal tumor was illustrated by the emergence in older age groups of a fibroma-like variant not encountered in earlier groups while epithelial tumors graded as carcinoma were not induced in rats dosed at five months of age. The adenomas in this group also presented a less active appearance than did equivalent lesions in earlier groups. No neoplasms conforming to the classification of renal lipomatous tumor were among the one hundred mesenchymal neoplasms induced in this study, but a single lesion consistent with an early nephroblastoma was found in one rat dosed at birth. The exclusive occurrence of two predominant types of kidney tumor emphasizes once again the specificity of the host-tumor interaction elicited by dimethylnitrosamine.

INTRODUCTION

In recent years, the induction of kidney tumors by means of a single i.p. dose of DMN in dietary-conditioned rats has been used as a potent model of chemical carcinogenesis for the study of cancer biology in general and of renal cancer in particular. The experimental system involves the dosing of 6-week-old Wistar-derived rats that have been preconditioned for several days with a diet high in carbohydrate but lacking in protein. Renal tumors of 2 types are induced in high incidence (4, 13): tumors of connective tissue origin now classified as mesenchymal tumors, and cortical epithelial tumors conforming to the adenoma, adenocarcinoma, and carcinoma series (2, 4, 22).

Although tumor induction with DMN in 5- to 6-week-old rats has been relatively well studied in the above system, little is known about the effect that the age of the animal at the time of carcinogen administration has on the incidence and type of resultant renal neoplasms. When Terracini and Magee (19) dosed newborn Wistar rats at 1 day of age, no epithelial kidney tumors were induced, all of the tumors being of the mesenchymal type then described as anaplastic. In contrast, the report of Murphy et al. (15) using 5-month-old Wistar rats described the occurrence of only cortical epithelial tumors with no lesions corresponding to the mesenchymal neoplasm. In 6-week-old Porton albino Wistar rats, application of the high-carbohydrate, no-protein dietary regimen with a dose of 60 mg DMN per kg has induced mesenchymal tumors consistently in up to 100% incidence concomitantly with a lower incidence, 30 to 50%, of cortical epithelial tumors (4). Notwithstanding any differences which may stem from the variations in the strain of rat used, from the dose of DMN administered, and from the dietary preconditioning, these isolated results suggest that the age at which the carcinogen is administered to the animal may influence markedly the type of renal neoplasm and its relative incidence.

Any difference in susceptibility to cancer induction that is a function of age may have significance with respect to the dynamics of target cell populations. Therefore, the age-related effect in this established model of nitrosamine carcinogenesis was explored by comparing kidney tumor induction in groups of rats receiving a single treatment of DMN at varying times after birth.

MATERIALS AND METHODS

Animals and Diet. Random-bred male and female rats of Porton albino Wistar stock were housed in plastic cages and maintained on a conventional pellet diet with water ad libitum. For 5 days prior to carcinogen administration, all rats with the exception of the neonatal group were placed in wire grid cages and fed a powdered diet consisting exclusively of glucose: sucrose (50:50) with 20% aqueous glucose solution as drinking water. After carcinogen treatment, animals were returned to conventional housing and diet.

Carcinogen. DMN (Eastman Organic Chemicals, Rochester, N. Y.) was purified by distillation and prepared as a 3.0% solution in 0.9% NaCl solution. The carcinogen was administered as a single i.p. injection of 30 mg/kg of body weight to all groups except the neonates which received an injection of 20 mg/kg of body weight (see below).
Experimental Plan. Altogether, 324 rats were dosed with the carcinogen. This was administered at various ages according to the following schedule: Group A, 24 hr (neonates), 24 males, 20 females; Group B, 3 weeks (weanlings), 17 males, 37 females; Group C, 1 month, 14 males, 22 females; Group D, 1.5 months, 15 males, 18 females; Group E, 2 months, 15 males, 13 females; Group F, 3 months, 16 males, 22 females; Group G, 4 months, 27 males, 26 females and Group H, 5 months, 10 males, 14 females. For obvious reasons, the neonatal animals in Group A could not be preconditioned with the high-carbohydrate, no-protein diet, a maneuver which is known to increase survival and predisposition to DMN-induced renal tumors (4, 13); they did not tolerate, therefore, a dose of DMN, 30 mg/kg. Consequently, the dose rate for this group was dropped to an amount (20 mg/kg of body weight) which permitted survival of a percentage of rats equivalent to the number of survivors in the weanling group. The experimental animals were observed at regular intervals, and those in which palpation disclosed a large abdominal mass were killed forthwith. The experiment was terminated at 9 months after carcinogen administration when all surviving animals in the group were perfused under ether anesthesia with 10% formol:0.9% NaCl solution selectively for the kidneys via the abdominal aorta. For microscopic examination, each kidney was sectioned sagittally in 5 or 6 planes, and the resulting step sections were stained with Harris’ hematoxylin and eosin. Certain lesions were stained also with hematoxylin-phloxine-saffron, Van Gieson’s collagen stain, Mallory’s phosphotungstic acid-hematoxylin, and Gomori’s trichrome.

Statistical analysis of the data was performed with the use of both the difference between 2 Poisson means test and the normal theory z test. For the latter, the rats were divided into early (Groups A to C) and late (Groups D to H) treatment groups.

RESULTS

No consistent difference between the sexes in renal tumor incidence or type was observed in this study. In consequence, the group data for male and female rats have been presented in a pooled form. In considering the results, it must be remembered that the neonatal group is not strictly comparable with the other groups in terms of DMN dose or dietary preconditioning. Nevertheless, the information obtained from this group is relevant to the overall perspective of tumor type and distribution through the series of age-related groups. In Table 1, the numerical data relating to the survival of the rats in each group and to the predominant renal tumors and associated lesions induced by DMN are summarized. Rats of 1 to 2 months of age tolerated the dose of carcinogen best with only 7 to 15% of the animals dying in the interim period of 5 months before macroscopic tumor diagnosis was possible. The majority of these deaths occurred in the acute phase of hepatotoxicity between 2 and 4 days. Renal tumors were induced in all 8 age groups and at total incidences that were not significantly different in any but the group dosed at birth. However, depending upon the age of the rats at treatment, these group tumor incidences reflected significant differences in predisposition to 2 predominant tumor types. Based on the difference between 2 Poisson means test, there were significantly more cortical epithelial tumors occurring in rats dosed at the latest ages than mesenchymal tumors and vice versa. This age-dependent distribution of tumors is displayed for easier visual comprehension in a stylized form in Chart 1 where the data are expressed as percentages of tumor-bearing animals.

Not only was there a between-group variation in the numbers of tumor-bearing animals, but there was also a variation in the numbers of tumors present in the kidneys of individual rats. The tumor multiplicity data are tabulated as tumor indices for each of the predominant tumor types in Table 2.

Further elaboration of the results is presented under subheadings of lesion type.

Mesenchymal Tumors. These neoplasms were most frequent in the 3 youngest age groups (z test, p < 0.001) with greatest susceptibility occurring in the 1-month group with an incidence of 70%. Beyond this, predisposition to mesenchymal tumor induction declined markedly to be absent in rats dosed at 5 months of age. The pattern of mesenchymal tumor incidence was accentuated further by the multiplicity of these

<table>
<thead>
<tr>
<th>Age at treatment</th>
<th>Total rats in group</th>
<th>Effective survivors</th>
<th>Total no. of tumor-bearing rats</th>
<th>Rats with mesenchymal tumors</th>
<th>Rats with cortical epithelial tumors</th>
<th>Rats with adenomas</th>
<th>Rats with carcinomas</th>
<th>Rats with hyperplastic tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>44</td>
<td>33 (75)</td>
<td>11 (33)</td>
<td>8 (24)</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Weanling</td>
<td>54</td>
<td>39 (72)</td>
<td>25 (64)</td>
<td>18 (46)</td>
<td>16 (41)</td>
<td>13 (33)</td>
<td>5 (13)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>1 mos.</td>
<td>36</td>
<td>33 (92)</td>
<td>25 (76)</td>
<td>23 (70)</td>
<td>12 (36)</td>
<td>11 (33)</td>
<td>2 (6)</td>
<td>11 (33)</td>
</tr>
<tr>
<td>1.5 mos.</td>
<td>33</td>
<td>28 (85)</td>
<td>17 (63)</td>
<td>5 (19)</td>
<td>14 (52)</td>
<td>13 (48)</td>
<td>1 (4)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>2 mos.</td>
<td>28</td>
<td>26 (93)</td>
<td>13 (50)</td>
<td>2 (8)</td>
<td>11 (42)</td>
<td>11 (42)</td>
<td>1 (4)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>3 mos.</td>
<td>38</td>
<td>27 (71)</td>
<td>18 (67)</td>
<td>3 (11)</td>
<td>18 (67)</td>
<td>18 (67)</td>
<td>10 (37)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>4 mos.</td>
<td>53</td>
<td>52 (60)</td>
<td>22 (69)</td>
<td>7 (22)</td>
<td>21 (66)</td>
<td>17 (53)</td>
<td>7 (22)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>5 mos.</td>
<td>24</td>
<td>14 (58)</td>
<td>7 (50)</td>
<td>0 (0)</td>
<td>6 (43)</td>
<td>6 (43)</td>
<td>0 (0)</td>
<td>4 (29)</td>
</tr>
</tbody>
</table>

a Rats surviving for a minimum period of time (5 months) commensurate with the diagnosis of tumor development.

b Because mesenchymal and epithelial tumors could occur coincidentally in the same animals, the data in this column are not necessarily the sum of the data in the next 2 columns.

c Because adenomas and carcinomas could occur coincidentally in the same animals, the data in this column are not necessarily the sum of the data in the next 2 columns.

d Numbers of rats expressed as percentage of effective survivors in each group.

Table 1

Age-dependent induction by DMN of renal tumors and associated renal lesions

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Chart 1. Distribution of predominant renal tumor types induced by DMN according to age of rat at treatment. ●, mesenchymal tumors; ○, cortical epithelial tumors.

Table 2

<table>
<thead>
<tr>
<th>Age at treatment</th>
<th>Tumor index a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesenchymal tumor</td>
</tr>
<tr>
<td>Neonate</td>
<td>0.33</td>
</tr>
<tr>
<td>Weaning 1 mo.</td>
<td>0.64</td>
</tr>
<tr>
<td>1.5 mos.</td>
<td>1.37</td>
</tr>
<tr>
<td>2 mos.</td>
<td>0.22</td>
</tr>
<tr>
<td>3 mos.</td>
<td>0.08</td>
</tr>
<tr>
<td>4 mos.</td>
<td>0.11</td>
</tr>
<tr>
<td>5 mos.</td>
<td>0.28</td>
</tr>
</tbody>
</table>

* Tumor index = Total no. of tumors of each type per group/No. of effective survivors in group

There was no difference in the mesenchymal tumors induced in the 4 youngest age groups with respect to histology or size range. The tumors in these rats were actively growing neoplasms (Fig. 1) with a complex histological spectrum conforming to earlier detailed descriptions (2, 4, 7). These were very heterogeneous connective tissue neoplasms consisting of fibroblast-like spindle cells, primitive mesenchyme, smooth muscle, areas suggestive of vascular neoplasia, deposits of collagen and reticulin, and tubule profiles or cysts representing sequestered nephric elements from the preexisting parenchyma (Fig. 2). However, in the older age groups when predisposition was declining, there was a noticeable tendency for the mesenchymal tumors to be smaller and less active in histological appearance. Thus, in the 2-month age group, one of the 2 tumors found closely resembled a fibroma while 3 of the 7 mesenchymal tumors in the 4-month age group were of similar fibroma-like character. In addition, some of the other mesenchymal tumors in this and the 3-month group were unusually small. Unlike the rapidly growing, complex mesenchymal tumors of the younger age groups, the fibroma-like variant was a circumscribed nodule (Fig. 3) consisting for the greater part of poorly cellular, homogeneous, collagenous matrix containing a few tubule profiles and surrounded peripherally by compressed normal parenchyma (Fig. 4) indicating some growth by expansion but with only scattered pockets of peripherally located, basophilic spindle cells indicative of local invasion. This histological variant with its more organized pattern and less heterogeneous spectrum therefore displayed a rather benign appearance contrasting with the mesenchymal tumors of younger rats.

Cortical Epithelial Tumors. The proportions of cortical epithelial tumor-bearing rats were significantly higher in the older age groups than in the younger (z test, p < 0.001). Thus, sensitivity to these neoplasms was lowest in the neonates (incidence 6%) and highest in the 3- and 4-month-old rats (66 to 67%).

Cortical epithelial tumors were divisible on a somewhat arbitrary basis of size into adenomas on the one hand and adenocarcinoma-carcinoma (hereafter referred to as carcinoma) on the other. The latter neoplasms exceeded 0.3 mm in diameter and displayed mitotic activity, cellular pleomorphism, development of a vascular supporting stroma, and focal necrosis. Tumors of a size definable as adenomas, i.e., less than 0.3
mm in diameter, usually lacked both necrosis and a well-developed vascular stroma, but they sometimes displayed mitotic activity and cellular pleomorphism. This categorization was not intended to imply any discrimination based on lesion size between benign and malignant behavior but served to draw attention to differences between certain age groups.

In keeping, multiplicity of lesions designated as adenomas was highest in rats dosed at 3 months of age but lowest in the neonates. In the 5-months age group, it was noticeable that the adenomas were generally smaller than equivalent lesions in earlier groups and lacked obvious mitotic activity. Likewise, the peaks of carcinoma incidence and multiplicity also occurred in the 3-months age group. However, lesions of this size and form were absent from the 5-months age group.

**Hyperplastic Tubules.** The occurrence of single-tubule profiles in the cortex, showing proliferation of epithelial-lining cells without causing obliteration of the tubule lumen or distortion of surrounding architecture, was remarkably consistent through the age groups with the exception of the neonatal group in which only one animal possessed a single abnormal tubule.

**Other Renal Tumors.** Two pelvic carcinomas were encountered, one a small lesion with the characteristics of transitional cell carcinoma in situ in the neonates and the other a well-developed squamous cell tumor in rats dosed at 5 months of age. Of the total of 100 renal mesenchymal neoplasms induced in this study, none was referable to the lipomatous tumor complex, substantiating a probable ontogenetic distinction between these 2 forms of connective tissue tumor (2).

A single, small lesion found in a rat dosed at birth conformed to nephroblastoma in a relatively early stage of development. The 1-mm-diameter lesion situated at the inner stripe of the outer medulla consisted of ball-like clusters of deeply basophilic, epithelioid cells within a supporting connective tissue stroma. It was quite distinct histologically from renal mesenchymal tumor and resembled closely aggregates of primitive metanephrogenic blastema (8).

**Hepatic Tumors.** Neoplasms of the liver were found in decreasing incidence in the youngest age groups. Of the surviving neonates, weanlings, and 1-month-old rats, liver tumors were found in incidences of 18, 8, and 2%, respectively, but in no other groups. The tumors conformed to the classifications of hepatocellular carcinomas, adenomas, and in one case (a 1-month-old rat) bile-duct adenoma.

**DISCUSSION**

Several previous studies (10, 20, 21) indicate that the newly born animal is more susceptible than its older counterparts to chemically induced neoplasia but that this age-related effect does vary from organ to organ. Whereas the results for lung tumorigenesis were equivocal, neonates were found to be more susceptible to the development of thymic lymphomas and liver cell tumors than were their weaned counterparts while tumors of the forestomach were unrelated to age at treatment (10, 20, 21). The complexity of the issue has been stressed in a critical review by Toth (23). The present study utilizing a DMN model of renal carcinogenesis extends the observations to demonstrate that the age-dependent effect within a single target organ varies also with the type of tumor induced. Based on the tumor incidences per group and the multiplicity of neoplasms per rat, the data show a clearly bimodal distribution of renal tumors related to age at treatment in which the bimodality represents the occurrence of 2 differing types of neoplasm. Essentially, renal mesenchymal tumor proves to be a neoplasm of the neonatal and immature rat with an apparent peak of predisposition at 1 month of age while susceptibility to cortical epithelial tumors increases as the rats attain maturity to peak at 3 months of age. Thus, the newborn with its predilection for mesenchymal tumors following a single high dose of DMN stands in contrast to the 5-month-old rat which appears to be susceptible only to the induction of epithelial kidney tumors. These results correlate the isolated observations on DMN-induced renal carcinogenicity (see above) made by Terracini and Magee (19) using neonates and those of Murphy et al. (15) with 5-month-old rats.

Age at dosing influences also to some degree the grade of tumor induced. With ensuing sexual maturity, there is a tendency for renal mesenchymal tumors induced by DMN to be less active, culminating in the emergence of a variant in older age groups which by virtue of its circumscribed form, uniform collagen distribution, and relatively poor cellularity is more fibroma-like than are mesenchymal tumors induced in the immature rat. A similar age-related influence on cortical epithelial tumors is evident with neoplasms graded as carcinoma decreasing in incidence from the most susceptible age group at 3 months to nil in the 5-month group. Even the adenomas developing in rats dosed at 5 months of age contrast with adenomas of the 3-month age group in degree of mitotic activity and size. Such trends in histological grade support the bimodal pattern of age-related tumor distribution. On the other hand, lesions designated as hyperplastic tubules, which are believed to be a precursor stage of cortical epithelial tumors (1, 18), showed little variation in incidence for any of the age groups except the neonates.

It is possible that a varying tumor incidence correlating with increasing age could be due to an altered capacity for the high-carbohydrate, no-protein diet to inhibit metabolism of the carcinogen by the liver. However, microsomal enzyme system studies provide _in vitro_ evidence which indicates that the capacity for modifying the liver's response to DMN does not decrease with age in the rat (11, 24). Furthermore, recent tests in this laboratory show that the metabolism of DMN by 3- and 6-month-old rats that have been fed the high-carbohydrate, no-protein diet follows precisely the same course as in 4- to 6-week-old rats on the same diet, suggesting that this dietary preconditioning has an identical effect in both the younger and older rats.

The most probable explanation for the characteristic age-dependent distribution of renal mesenchymal tumor is the stem-cell theory of cancer which proposes the specific involvement of undifferentiated stem cells in tumor genesis (12, 16, 17). In the rat kidney model, the results imply the persistence in the immature organ of embryonic mesenchymal stem cells, representing the specific target of the carcinogen, capable of differentiation into a wide range of connective tissue cell types. Presumably, these cells have disappeared by 5 months of age or have reached a stage in the differentiative sequence which is refractory to the critical molecular lesion induced by the carcinogen. Certainly, the metanephrogenic blastema must have only very limited sensitivity to the carcinogenic action of this nitroso compound for, despite the persistence of primitive metanephric tissue at birth in the rat kidney (14), only one lesion conforming histologically to the classification of nephe-
roblastoma (2, 8) was induced in this series. This is the first time such a lesion has been encountered in many hundreds of rats dosed with DMN in our laboratory. Thus, in terms of renal target cell populations, the study emphasizes the specificity of the carcinogen for mesenchymal cells of the interstitial space and cells of the cortical tubule epithelium.

Utilization of immature rats in this model of DMN carcinogenesis with its very high induction rate of renal mesenchymal tumors (up to 100% with a dose of DMN, 60 mg/kg of body weight) has proven to be a suitably potent system for investigation of the developmental biology of mesenchymal neoplasia both in vivo (5, 6) and in vitro (3, 9). However, as epithelial carcinogenesis is the predominant neoplastic process in humans as well as in animals, there is a need to create an equivalently potent, single-dose, high-incidence model of epithelial kidney cancer in order to permit in-depth study of the epithelial tumor induction process in this organ. The results reported here indicate that the 3- to 4-month-old rat may provide a probable basis for developing such a system. Experiments to explore this approach are currently in progress.

ACKNOWLEDGMENTS

The author gratefully acknowledges Dr. Martin S. Rosenzweig of the Biometry Department at Temple University Medical School for statistical evaluation of the data and John Lee for expert technical assistance.

REFERENCES

Fig. 1. Mesenchymal tumor from a rat dosed at birth. The neoplastic tissue has proliferated through the whole kidney and has invaded the renal pelvis (*). Gomori’s trichrome, × 5.

Fig. 2. Mesenchymal tumor from a rat dosed at 1 month of age. This histological profile shows a fibrosarcomatous zone surrounding a solid cluster of smooth muscle fibers (*). Dilated and collapsed tubules represent preexisting parenchyma that has been sequestered by the proliferating neoplastic mesenchymal cells. H & E, × 200.

Fig. 3. Fibroma-like variant of a renal mesenchymal tumor from a rat dosed at 4 months of age. Compared with the tumor shown in Fig. 2, the lesion is circumscribed and consists predominantly of homogeneous collagen. Gomori’s trichrome, × 7.

Fig. 4. Fibroma-like mesenchymal tumor consisting of poorly cellular, homogeneous collagen compresses adjacent renal parenchyma indicating some growth by expansion. A few collapsed epithelial profiles representing preexisting renal tubules are incorporated within the tumor mass. H & E, × 160.
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