Stromal Nephromas and Renal Cell Tumors in Suckling and Weaned Rats

James S. Campbell, G. Stuart Wiberg, Harold C. Grice, and Peter Lou

SUMMARY

Renal neoplasms with induction times ranging from 218 to 505 days appeared in 51 or 64% of 80 male Wistar rats in 3 groups which, when 1 or 7 days old, were given single s.c. injections of dimethylnitrosamine in dosages of 125 μg/animal or 10 mg/kg. In 44 male rats so treated after weaning at 21 or 70 days of age, tumor yields were scant and induction times exceeded 480 days, but dimethylnitrosamine dosage levels of 20 or 30 mg/kg produced renal tumors with induction times ranging from 286 to 550 days in 33 or 53% of 62 males in 4 groups also treated at 21 or 70 days of age. The hepatoma yield was small. Abdominal palpation was highly accurate for relatively early diagnosis of renal neoplasms. Ninety-two % of renal neoplasms in rats treated when sucklings were stromal nephromas; 41% of renal tumors in rats treated after weaning were of renal cell type. One stromal nephroma produced epithelial-containing pulmonary metastases.

INTRODUCTION

Yields of murine renal neoplasm can be expected to follow single DMN dosages (6-9, 15, 17). This study of murine renal carcinogenesis was to determine whether age of animals at the time of single DMN injections would influence morphological types (1, 5-9, 11, 12, 14, 15, 17, 20-25), as well as incidence and induction times of induced tumors (22-24).

Adult rats are large enough for small visceral lesion masses to be detected by palpation (12, 25, 30). In these experiments, therefore, simple digital examination was relied upon for detection of renal tumors.

MATERIALS AND METHODS

Albino rats derived from Wistar stock, housed in groups of 10 or less in stock cages, were fed Master Fox Cubes (Toronto Elevators Limited, Toronto, Ont., Canada.). All animals tested received 0.25% DMN (Eastman Kodak Co., Rochester, N.Y.) in distilled water by single s.c. injections in dorsolumbar s.c. tissues. DMN was administered to suckling via 30-gauge needles. To prevent cannibalism, care was taken not to excite the dams; sucklings were handled throughout all procedures with rubber gloves. Blood leakage at injection points was scrupulously swabbed away. Injections were made into skin folds raised between the fingers and gently compressed to prevent leakage of the carcinogen, while the needles, which had been inserted to full length, were slowly withdrawn. Following injections, sucklings were returned to the mothers in the colony nursing room. Weaned at 21 days, they were segregated according to sex and transferred to stock cages.

Only males were treated after weaning. Weaned male rats received DMN in dorsolumbar regions via 23-gauge needles. To detect variation in renal carcinogenic response attributable to DMN dosage variation and variation in age of rats at the time of DMN injection, rats were grouped and treated as follows.

DMN in dosages of 125 μg/rat was administered to 33 male and 31 female sucklings aged 1 day, 38 male and 28 female sucklings aged 7 days, 30 weaned male rats aged 21 days, 32 male rats aged 70 days, 28 male rats aged 6 months, and 27 male rats aged 1 year.

DMN in dosages of 10 mg/kg was administered to 30 male and 24 female sucklings aged 7 days, 30 weaned male rats aged 21 days, 30 male rats aged 70 days, 28 male rats aged 6 months, and 35 male rats aged 1 year.

DMN in dosages of 20 mg/kg was administered to 34 weaned male rats aged 21 days and to 40 male rats aged 70 days.

DMN in dosages of 30 mg/kg was administered to 30 weaned male rats aged 21 days and to 30 male rats aged 70 days.

DMN dosages greater than approximately 20 mg/kg (i.e., 125 μg/5- to 6-g sucklings, aged 1 day) were not administered to sucklings because such levels are frequently lethal to perinates of the strain concerned.

Under the same colonial conditions, equal numbers of untreated male and female control animals were maintained for full life-spans in groups corresponding to those of tested rats.

Examination of rats for carcinogenic response was begun 60 days after DMN administration. Abdominal palpation for indurative renal and hepatic enlargement was carried out weekly.
Rats were usually allowed to live their full lifespan, but those overtly ill of cancer or other diseases were immediately sacrificed. To assess the accuracy of diagnostic observations, some rats were necropsied within 24 hr after evidence of neoplasms was first detected. Other animals with palpable visceral masses were allowed to survive in attempts to identify neoplastic lesion by observing progressive growth.

Kidney, liver, lung, and other tissues for histological examination were fixed in 10% buffered neutral formalin, embedded in paraffin, cut at 5 µm, and routinely stained with hematoxylin, phloxine, and saffron. Selected blocks of kidney tumors were examined with the use of Masson's trichrome, Verhoeff's elastic, Weigert's reticulum, periodic acid-Schiff, alcian blue, mucicarmine, oil red O, and sudan black staining techniques.

RESULTS

In 90% of the rats, the presence or absence of renal neoplasms was correctly assessed by abdominal palpation (Table 1). Producing protuberances when superficial and induration when deep, renal neoplasms as small as 0.5 cm in diameter were palpable. In 6% of animals, hydronephrosis or hepatic neoplasms invading paracolic gutters were initially confused with renal neoplasms. Tumors at first causing only minor induration or deformity of kidneys were correctly identified as neoplastic, once progressive growth became obvious by repeated examinations.

Incidence, induction times, and histological types of DMN-induced renal neoplasms were influenced by age of animals when treated but were uninfluenced by sex in sucklings (Tables 2 and 3). Of 159 renal tumors (Table 3), 31 were of renal cell origin; 118 were stromal nephromas. One of the latter invaded renal veins and produced pulmonary metastases containing epithelium (Figs. 5 and 6). Tumor incidence in response to DMN, 10 mg/kg, was greater and induction time was shorter in male animals treated when suckling than in males so treated after weaning (Table 3). Male animals receiving DMN, 20 mg/kg, when aged 21 days, or 30 mg/kg when aged 70 days, yielded more renal tumors more quickly than males of similar ages receiving 10 mg/kg. DMN in dosages of 125 µg/rat quickly produced renal tumors only when administered to sucklings.

Hepatoma yields were scant but significantly less so in rats treated when suckling (Table 3).

In weaned rats receiving 125 µg DMN when aged 21 days, 1 hepatoma appeared 574 days after treatment among 12 surviving animals. In rats receiving 125 µg DMN when aged 70 days, 1 stromal nephroma appeared 534 days after treatment among 8 surviving animals.

In rats receiving 125 µg DMN when aged 6 months, 1 hepatoma was found 567 days after treatment among 3 surviving animals; median survival time was 367 days. In rats receiving 125 µg DMN when aged 1 year, no tumors were found; median survival time was 327 days.

In animals receiving DMN, 10 mg/kg, when aged 6 months, 1 renal cell carcinoma was found in the only rat surviving to 658 days; median survival time in this group was 429 days. In rats receiving 10 mg/kg when aged 1 year, no tumors were found; median survival time was 293 days.

Table 1

<table>
<thead>
<tr>
<th>Age at injection (days)</th>
<th>Controls</th>
<th>125 µg</th>
<th>70 µg</th>
<th>210 µg</th>
<th>700 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats tested</td>
<td>240</td>
<td>33</td>
<td>38</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>No. of survivors when tumor first detected: effective no.</td>
<td>24</td>
<td>33</td>
<td>23</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>No. of survivors that developed renal tumors</td>
<td>0</td>
<td>13</td>
<td>14</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Median induction times (days)</td>
<td>237</td>
<td>218</td>
<td>225</td>
<td>286</td>
<td>286</td>
</tr>
<tr>
<td>Range of induction times (days)</td>
<td>144-351</td>
<td>151-505</td>
<td>159-373</td>
<td>253-521</td>
<td>257-550</td>
</tr>
</tbody>
</table>

Table 2

Incidence and induction time of kidney tumors in male rats receiving single injections of DMN at varying ages, not exceeding 70 days

Induction times for tumors in suckling rats receiving DMN in dosages of 10 µg/kg when aged 1 or 7 days were significantly shorter than in weaned rats similarly treated; p less than 0.1, as tested by the rank sum method of Wilcoxon and Wilcox (28). To avoid possible error in induction time estimates as a result of confusion of renal with hepatic neoplasms on abdominal palpation, rats with hepatomas were excluded.
Table 3

Neoplastic responses: effect of age of rats at time of injection on incidence and type of renal tumor

Differences in renal responses between suckling and weaned rats were significant at 99% confidence limits calculated to 2 decimal places in accord with Tables for Use with Binomial Samples by Mainland et al. (16). Differences in hepatoma incidence between suckling and weaned rats were significant at 95% confidence limits. Differences in response between male and female sucklings were not significant.

<table>
<thead>
<tr>
<th>Rats with renal tumors</th>
<th>Total nephromas</th>
<th>Stomatal nephromas</th>
<th>Renal cell tumours</th>
<th>Hepatomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective no.</td>
<td>No. %en\textsuperscript{a}</td>
<td>No. %nt</td>
<td>No. %nt</td>
<td>No. %nt</td>
</tr>
<tr>
<td>All males treated when sucklings</td>
<td>80</td>
<td>63.75</td>
<td>61</td>
<td>91.80</td>
</tr>
<tr>
<td>All rats treated when sucklings</td>
<td>141</td>
<td>62.41</td>
<td>103</td>
<td>92.23</td>
</tr>
<tr>
<td>All rats treated when aged 21, 70 days</td>
<td>106</td>
<td>37.73</td>
<td>56</td>
<td>58.92</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Includes multiple and bilateral tumors.
\textsuperscript{b} %en, effective number; nt, number of tumors.

DISCUSSION

For early detection of renal neoplasms, abdominal palpation with or without anesthesia combined simplicity of method with 90% diagnostic accuracy. Similar results may be anticipated in other models using rats or larger animals in which visceral neoplasms are the chief yield of carcinogens. During a recent study here, splenic enlargement in rats with a transplantable leukemia was easily detected by abdominal examination under anesthesia (30).

Abundant tumor yield and relatively quick responses to small DMN dosage gave suckling rats an advantage over weaned rats. Ranges of tumor incidence and induction times such as those that followed the administration of DMN in 10 mg/kg dosages to sucklings were achieved in weaned rats, treated when juvenile, only by 2- and 3-fold increases in DMN dosages.

Renal neoplasms in this study (Figs. 1 to 6) closely resembled DMN-induced murine renal neoplasms previously described (1, 5–9, 11, 12, 14, 15, 17, 20–25). In previous reports, rats and mice treated with DMN when juvenile or adult produced renal adenomas (11, 23); suckling rats so treated more often yielded stromal nephromas (22). Also, in this study, renal cell tumors were frequent only in animals treated after weaning; rats treated when suckling usually developed stromal nephromas.

Stromal nephromas induced by DMN in Food and Drug strain Wistar rats appeared more variegated but generally more differentiated in light-microscopic appearance than nephroblastomas induced by dimethylbenzanthracene in castrated pubescent Sprague-Dawley rats (13) or those rarely occurring in rats not exposed to experimental carcinogens (10, 19, 25). No control rat displayed renal neoplasia. In the 15-year history of the colony concerned, about 25,000 rats of Wistar stock have been necropsied, but spontaneous nephroblastomas have been observed in only 4 of these rats (25).

DMN-induced stromal nephromas are reputedly mesenchymal, with epithelial entrapment giving nephroblastic appearances that are spurious (7, 9). Of contrary import are epithelium-containing intravascular growths and pulmonary metastases (Figs. 5 and 6).

The stromal nephromas in this study were, by light microscopy, very similar to human infantile nephromas or renal hamartomas and differentiated or partly differentiated nephroblastomas, some of which are congenital (2–4, 26, 27). Having histological features in common with stromal nephromas, “mixed” renal neoplasms occasionally develop in the offspring of rats receiving DMN during pregnancy (18). However, no analogous mechanism has yet been incriminated that relates nitrosamines to renal carcinogenesis in the human fetus (29).

REFERENCES

Fig. 1. Bilateral stromal nephromas in male rat, aged 7 days when treated with 125 mg of DMN. Lesions were palpable 190 days after injection.

Fig. 2. a, proliferating smooth muscle in stromal nephroma of rat, aged 7 days when treated with 125 mg of DMN. Hematoxylin, phloxine, and saffron, X 130. b, tubules and microcysts, one with dysplastic epithelial lining, in stromal nephroma of rat, aged 7 days when treated with DMN, 10 mg/kg; lower left, border zone including engulfed glomerulus. Hematoxylin, phloxine, and saffron, X 325.

Fig. 3. Renal cell carcinoma in male rat, aged 70 days when treated with DMN, 30 mg/kg. Hematoxylin, phloxine, and saffron, X 325.

Fig. 4. Stromal nephroma with undifferentiated stroma, "glomeruloid bodies," and epithelial tubule in female rat, aged 1 day when treated with 125 mg DMN. Hematoxylin, phloxine, and saffron, X 325.

Fig. 5. Extension of stromal nephroma in renal vein of male rat, aged 70 days when treated with DMN, 30 mg/kg. Differentiated tubules are enclosed in neoplastic stroma. Hematoxylin, phloxine, and saffron, X 325.

Fig. 6. Metastatic nephroma to lung in rat with lesions illustrated in Fig. 5. Differentiated tubules and epithelial-lined clefts lie amid neoplastic stroma. X 260.
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