Oncogenic Effects of 4-(p-Dimethylaminostyryl)quinoline (4M20) in Mice

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SUMMARY

RF and 101C3F1 mice which survived midlethal amounts of 4-(p-dimethylaminostyryl)quinoline (4M20), received when they were 3 months of age, showed the following long term changes: (a) acute hepatotoxic effects followed by marked liver abnormalities, which included a high incidence of hepatoma, liver nodularity, fatty liver, irregularities of parenchymal cell and nuclear size, and an increased incidence of nuclear inclusion bodies; (b) shortening of the mean life span (~16%) in RF mice but not in 101C3F1 mice; (c) small increase in incidence of reticular, ovarian, and lung neoplasms, giving a rather marked over-all increase in total neoplasm incidence in 101C3F1 mice; (d) an increase in the frequency of thymic lymphomas and ovarian tumors and a decrease in the frequency of reticulum cell tumors in RF mice.

Studies on the acute pathologic effects of 4-(p-dimethylaminostyryl)quinoline (4M20) in mice (3, 10) indicated that the compound had certain radiomimetic effects. It was also noted that lens cataracts occurred in animals surviving the acute effects (5). A striking nonradiomimetic feature of the acute lesions was the marked hepatotoxic effect of the drug. This was manifested by fatty liver and necrosis of liver cells during the 1st week, followed by repair in the second week. Residual liver changes persisted in survivors of the acute toxicity. The fate of such survivors later in life is reported here.

MATERIALS AND METHODS

The mice were young adult females of the RF and 101 x C3H F1 strains weighing 21–28 gm. The RF mice were from a noninbred production colony in this laboratory. The 101 x C3H F1 mice, hereafter designated 101C3F1, were obtained from Cumberland View Farms, Clinton, Tennessee. These strains were chosen because of extensive experience with the spontaneous diseases in them. The 4M20 was synthesized and kindly supplied by Dr. Carl T. Bahner, Carson Newman College, Jefferson City, Tennessee. It was given via the tail vein in doses of 2, 3, 4, or 4.4 mg/mouse. Each dose was dissolved in 0.2 ml of distilled water with a final pH of about 1.4. One group of mice received 0.2 ml of dilute HCl at pH 1.4 as an acid (vehicle) control. Control mice received no injections.

Survivors of the acute toxicity were caged in groups of 5–10 and maintained under standard colony conditions. Some of the agouti brown 101C3F1 were checked at intervals for graying of the fur. At the time of death, necropsies were performed and all gross abnormalities recorded. If the animals were not decomposed (about 25%), they were studied by histologic examination of major organs.

RESULTS

SURVIVAL

Drug toxicity with the 4-mg dose of 4M20 caused approximately 40% mortality within 3 days after injection. There was no further mortality to 90 days after injection. Only those animals still alive 90 days after injection are included in this report. In RF mice both dose levels of 4M20 resulted in significant shortening of mean survival time (Table 1), by approximately 16%. The acid-control group had a longer mean survival time than unirradiated controls, but the group was so small that the difference is of doubtful significance. Injection of 4M20 into 101C3F1 mice did not significantly reduce the mean survival time.

LIVER ABNORMALITIES

In both strains of mice, injection of 4M20 was followed by marked increase in the frequency of liver abnormalities as seen at necropsy. These abnormalities included hepatomas, nodularity of the liver with scarring, capsular indentation over the scars, and fatty liver (Table 2). The hepatomas were often multiple, and those seen at gross necropsy ranged in size from 5 to 30 mm; the average size was 16 mm in 30 cases in which size was recorded (Figs. 1, 2). The gross appearance and color were quite variable. Many projected from the liver surface on broad
or narrow pedicles. Almost all were obvious at a glance because of a difference in color from that seen in the non-neoplastic liver parenchyma. The color varied from yellow-white to dark red and was dependent on the amount of congestion, hemorrhage, and fatty change in the liver tumor. Many of the larger ones were partly infarcted and hemorrhagic. In 7 instances the hepatoma was found on microscopic examination of the liver slides when it had not been seen at autopsy. These were smaller lesions, usually less than 5 mm in diameter.

Histologically, the cells of the hepatomas were not malignant in appearance, although more swelling, granulation, fatty change, and irregularity of size were noted (Figs. 3–6). There was no distinct capsule of connective tissue. The border between neoplastic and non-neoplastic liver was sharply defined, often with compression of the adjacent non-neoplastic liver. The hepatomas tended to show the same general microscopic changes as those present in the non-neoplastic liver. For example, fatty liver, leukemic infiltrates, or presence of nuclear cytoplasmic inclusions would be seen in both neoplastic and non-neoplastic portions of the liver.

Neither the gross nor the histologic appearances of the hepatomas differed between the two mouse strains used, nor were they different from those seen in mice in other leukemic infiltrates, or presence of nuclear cytoplasmic inclusions would be seen in both neoplastic and non-neoplastic portions of the liver.

Histologically, the cells of the hepatomas were not malignant in appearance, although more swelling, granulation, fatty change, and irregularity of size were noted (Figs. 3–6). There was no distinct capsule of connective tissue. The border between neoplastic and non-neoplastic liver was sharply defined, often with compression of the adjacent non-neoplastic liver. The hepatomas tended to show the same general microscopic changes as those present in the non-neoplastic liver. For example, fatty liver, leukemic infiltrates, or presence of nuclear cytoplasmic inclusions would be seen in both neoplastic and non-neoplastic portions of the liver.

Neither the gross nor the histologic appearances of the hepatomas differed between the two mouse strains used, nor were they different from those seen in mice in other long term studies in this laboratory.

The scarring, nodularity, and capsular indentation (Fig. 7) appeared to be the late result of necrosis of liver cells seen in the early postinjection period since the distribution was the same. This distribution was along the course of subcapsular and central venous branches. The degree of severity was quite variable, but never approached that of a severe cirrhosis. The extent of fatty liver likewise varied, involving most cells in some mice but only those near the central lobular vein in most instances.

Livers from 4M20-injected mice showed marked variation in cellular and nuclear size, and enlarged cells were scattered throughout (Figs. 8–10).

Eosinophilic nuclear inclusion bodies were encountered in some hepatic parenchymal cells (Fig. 10). The number of animals in which these inclusions were noted was markedly increased after 4M20 injection in both strains of mice (Table 2).

Other lesions of the liver were rare and included focal necroses and small biliary cysts.

### Neoplasms

Table 3 lists the percentage of mice in each group with 1 or more neoplasms present at the time of death. In the 101C3F1 strain there was an increased over-all incidence of neoplasms in animals injected with 4M20, chiefly reflecting increases in reticulum cell, ovarian, and lung neoplasms. In the RF strain, injection of 4M20 resulted in a decrease in over-all neoplasm incidence, due chiefly to a marked reduction in reticulum cell tumors.

An increased incidence of thymic lymphoma occurred in 4M20-injected RF mice at both dose levels ($P < 0.05$ with 4 mg of 4M20 and $P < 0.01$ with 2–3 mg of 4M20) (Table 4). Injection of 4M20 at both dose levels resulted in a significant decrease in the incidence of reticulum cell neoplasms in RF mice ($P < 0.01$), but the mean age at death of mice with reticulum cell neoplasms remained unchanged. The incidence of myeloid leukemia in this strain did not differ significantly between groups.

In the 101C3F1 mouse, the incidence of reticular neoplasms was not significantly changed by 4M20 injection. These neoplasms were principally of the reticulum cell type; there were no cases of thymic lymphoma or of myeloid leukemia (Table 4). The gross, microscopic, and clinical features of reticular neoplasms in these strains have been reported previously (8, 14, 17).

Other common neoplasms involved the ovary and lung (Table 3). There was a tendency toward an increased

### Table 1

**Mean Survival Time for 101C3F1 and RF Mice Receiving 4M20 i.v.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>Material</th>
<th>Dose (mg/mouse)</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101C3F1</td>
<td>34</td>
<td>4M20</td>
<td>3.0—4.4</td>
<td>752 ± 27*</td>
</tr>
<tr>
<td>101C3F1</td>
<td>29</td>
<td>None</td>
<td></td>
<td>750 ± 36</td>
</tr>
<tr>
<td>RF</td>
<td>62</td>
<td>4M20</td>
<td>4.0</td>
<td>495 ± 25</td>
</tr>
<tr>
<td>RF</td>
<td>95</td>
<td>4M20</td>
<td>2.0—3.0</td>
<td>484 ± 22</td>
</tr>
<tr>
<td>RF</td>
<td>30</td>
<td>Acid vehicle+a</td>
<td>0.2</td>
<td>662 ± 29</td>
</tr>
<tr>
<td>RF</td>
<td>98</td>
<td>None</td>
<td></td>
<td>590 ± 15</td>
</tr>
</tbody>
</table>

*a ±1 standard error of the mean.

*b Dilute HCI, pH 1.4.

### Table 2

**Abnormalities of the Liver in 101C3F1 and RF Mice Receiving 4M20 i.v.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Material</th>
<th>Dose (mg/mouse)</th>
<th>Hepatoma Incidence (%)</th>
<th>Nodularity (%)</th>
<th>Fatty Liver (%)</th>
<th>Other Lesions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101C3F1</td>
<td>4M20</td>
<td>3.0—4.4</td>
<td>45 ± 9</td>
<td>74 ± 8</td>
<td>16 ± 7</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>101C3F1</td>
<td>None</td>
<td></td>
<td>7 ± 5</td>
<td>3 ± 3</td>
<td>6 ± 4</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>RF</td>
<td>4M20</td>
<td>4.0</td>
<td>22 ± 6</td>
<td>80 ± 5</td>
<td>13 ± 4</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>RF</td>
<td>4M20</td>
<td>2.0—3.0</td>
<td>11 ± 3</td>
<td>56 ± 5</td>
<td>13 ± 4</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>RF</td>
<td>Acid vehicle+a</td>
<td>0.2</td>
<td>0</td>
<td>6 ± 6</td>
<td>6 ± 6</td>
<td>0 ± 3</td>
</tr>
<tr>
<td>RF</td>
<td>None</td>
<td></td>
<td>0</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>30 ± 4</td>
</tr>
</tbody>
</table>

*a ±1 standard error of the mean (estimated by assuming the incidence to be binomially distributed).

*b Dilute HCI, pH 1.4.
incidence of ovarian tumors in all groups of 4M20-injected mice of both strains, although the differences were not statistically significant. The incidence of lung tumors and mean age at death with these neoplasms in 4M20-injected animals did not vary significantly from the values in controls.

**Non-neoplastic Diseases**

In the RF mouse, glomerulosclerosis was common in old animals, and the incidence was lower in animals injected with 4M20 or acid vehicle (Table 5). The mean age at death in mice with this disease was approximately the same in injected and control groups. Glomerulosclerosis

### TABLE 3

**INCIDENCE OF MICE WITH 1 OR MORE NEOPLASMS AFTER RECEIVING 4M20 i.v. WITH A BREAKDOWN OF NONRETICULAR NEOPLASM TYPES**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Mice</th>
<th>Material</th>
<th>Dose (mg/mouse)</th>
<th>Mice with 1 or More Neoplasms (%)</th>
<th>Ovarian</th>
<th>Lung</th>
<th>Uterine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Mean age at death (days)</td>
<td>Incidence (%)</td>
<td>Mean age at death (days)</td>
</tr>
<tr>
<td>101C3F1</td>
<td>31</td>
<td>4M20</td>
<td>3.0–4.4</td>
<td>76 ± 8</td>
<td>35 ± 9</td>
<td>796</td>
<td>20 ± 8</td>
</tr>
<tr>
<td>101C3F1</td>
<td>29</td>
<td>None</td>
<td>4.0</td>
<td>48 ± 9</td>
<td>21 ± 8</td>
<td>889</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>RF</td>
<td>55</td>
<td>4M20</td>
<td>4.0</td>
<td>56 ± 7</td>
<td>24 ± 6</td>
<td>686</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>RF</td>
<td>82</td>
<td>4M20</td>
<td>2.0–3.0</td>
<td>51 ± 6</td>
<td>18 ± 4</td>
<td>643</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>RF</td>
<td>17</td>
<td>Acid vehicle</td>
<td>0.2</td>
<td>65 ± 12</td>
<td>12 ± 8</td>
<td>612</td>
<td>18 ± 9</td>
</tr>
<tr>
<td>RF</td>
<td>97</td>
<td>None</td>
<td>0.2</td>
<td>74 ± 4</td>
<td>11 ± 3</td>
<td>616</td>
<td>23 ± 4</td>
</tr>
</tbody>
</table>

* ±1 standard error of the mean.

b Dilute HCl, pH 1.4.

### TABLE 4

**RETICULAR NEOPLASMS IN RF AND 101C3F1 MICE RECEIVING 4M20 i.v.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Material</th>
<th>Dose (mg/mouse)</th>
<th>Thymic</th>
<th>Myeloid</th>
<th>Reticulum cell</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Mean age at death (days)</td>
<td>Incidence (%)</td>
<td>Mean age at death (days)</td>
</tr>
<tr>
<td>101C3F1</td>
<td>4M20</td>
<td>3.0–4.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>101C3F1</td>
<td>None</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RF</td>
<td>4M20</td>
<td>4.0</td>
<td>13 ± 5</td>
<td>298</td>
<td>2 ± 2</td>
<td>538</td>
</tr>
<tr>
<td>RF</td>
<td>4M20</td>
<td>2.0–3.0</td>
<td>16 ± 4</td>
<td>353</td>
<td>1 ± 1</td>
<td>464</td>
</tr>
<tr>
<td>RF</td>
<td>Acid vehicle</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RF</td>
<td>None</td>
<td>2 ± 1</td>
<td>525</td>
<td>2 ± 1</td>
<td>53 ± 5</td>
<td>620</td>
</tr>
</tbody>
</table>

* ±1 standard error of the mean.

b Dilute HCl, pH 1.4.

### TABLE 5

**SOME COMMON NON-NEOPLASTIC DISEASES IN RF AND 101C3F1 MICE TREATED WITH 4M20 i.v.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Material</th>
<th>Dose (mg/mouse)</th>
<th>Glomerulosclerosis</th>
<th>Pyelonephritis</th>
<th>Hypertrophy of Uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Mean age at death (days)</td>
<td>Incidence (%)</td>
</tr>
<tr>
<td>101C3F1</td>
<td>4M20</td>
<td>3.0–4.4</td>
<td>0</td>
<td>6 ± 3</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>101C3F1</td>
<td>None</td>
<td>4.0</td>
<td>3 ± 2</td>
<td>55 ± 9</td>
<td>693</td>
</tr>
<tr>
<td>RF</td>
<td>4M20</td>
<td>4.0</td>
<td>16 ± 5</td>
<td>7 ± 3</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>RF</td>
<td>4M20</td>
<td>2.0–3.0</td>
<td>26 ± 5</td>
<td>9 ± 2</td>
<td>38 ± 5</td>
</tr>
<tr>
<td>RF</td>
<td>Acid vehicle</td>
<td>0.2</td>
<td>6 ± 6</td>
<td>0</td>
<td>35 ± 12</td>
</tr>
<tr>
<td>RF</td>
<td>None</td>
<td>47 ± 5</td>
<td>591</td>
<td>2 ± 1</td>
<td>65 ± 5</td>
</tr>
</tbody>
</table>

* ±1 standard error of the mean.

b Dilute HCl, pH 1.4.
was uncommon and was not induced by 4M20 injection in 101C3F1 mice.

Pyelonephritis was common in untreated 101C3F1 mice and was considerably reduced after 4M20 injection. This was uncommon in all groups of RF mice (Table 5).

In the RF mouse, the incidence of hypertrophy of the uterus was approximately halved in all the injection groups (Table 5), but the mean age at death with this diagnosis was the same as in controls. In the 101C3F1, the incidence was not altered by 4M20 injection.

In the 101C3F1 mouse, mild graying of the fur was noted at 4–6 months after injection in the majority of the animals receiving 4M20. The controls did not gray at all.

**DISCUSSION**

Survivors of 4M20 injection showed a marked increase in liver abnormalities, consisting of hepatomas, liver nodularity due to scarring, fatty liver, variation in the size and morphology of liver cells, and inclusion bodies in liver cell nuclei. These lesions were presumably a sequel to acute parenchymal cell damage in the liver after injection. The scarred areas of liver were in the regions involved in early postinjection necrosis, at which time fatty liver and variations in cell and nuclear size were also noted (10). The size variations in liver cells and liver cell nuclei were much more pronounced in the late survivors than they were in the acute phase of the toxicity. The incidence of eosinophilic nuclear inclusions was increased in late survivors in 4M20 injection, but the significance of these bodies is not known. A number of reports of similar inclusion bodies and speculations as to their nature have been made [see discussions in Boecker (4), Thompson et al. (15)].

Increased pleomorphism of liver cells and an increased incidence of hepatoma have been noted after massive irradiation of the liver (19) and after urethan treatment (11). These changes may be accelerated by addition of other liver insults to the radiation effect (7). The enlarged cells are probably polyploid [discussion of polyploidy in mouse liver is given by Inamdar (12)].

Other studies report shortening of the life span in mice treated with radiomimetics (2, 18), although Curtis and Gebhard (9) did not observe it after administration of large single, or many small, doses of HN2. These differences may be due to difference in drug, drug dosage, age of animal, and strain variation. Life in our RF mice, but not in 101C3F1 mice, was shortened by 4M20.

Walpole (20) reviewed the tumorigenic properties of some of the radiomimetic drugs. Clayson (6) reviewed the chemical induction of liver tumors. Our experience here, as well as that of others (2, 13, 16) indicates tumorigenic activity of a variety of alkylating agents and points out the variability of tumor incidence, depending on the drug used and the susceptibility of different organs and strains of mice. With the exceptions of thymic lymphoma and myeloid leukemia, which occurred earlier, 4M20-injected animals dying with neoplasms in our experiments had a mean age of death which approximated that in controls with the same diagnosis.

The decreased incidence of glomerulosclerosis, uterine hypertrophy, and reticulum cell sarcoma in RF mice after 4M20 injection cannot be explained conclusively. However, it may be attributable to intercurrent mortality and life shortening from other diseases. The mean age at death in mice with these diseases was not reduced, whereas there was a marked reduction in the mean age at death of the over-all population. Since there was no decrease in the mean age at death in 110C3F1 injected with 4M20, this explanation is not valid for the decreased incidence of pyelonephritis in injected mice of that strain.

The mild graying of fur in 101C3F1 mice after 4M20 injection approximated the level of graying in this mouse after 350 r of whole-body X-rays (Cosgrove, unpublished data).

The cellular alterations possibly accounting for varied late effects of radiomimetic drugs have been discussed by Curtis and Gebhard (9), Koller (13), Alexander (1), Alexander and Connell (2), and Upton et al. (18) among others. The delayed effects encountered may result only in part from early cell killing by the drugs. Early cell killing is probably not the mechanism for tumor induction by these compounds, except possibly in the ovary. The bulk of the effects reported remain, therefore, to be explained.

**REFERENCES**


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Fig. 1.—Photograph of the viscera of a 101C3F1 mouse dying 12 months after a 3-mg dose of 4M20. There is a hepatoma (arrow) which is lighter in color than the adjacent liver.

Fig. 2.—Photograph of the viscera of a 101C3F1 mouse dying 25 months after a 4.2-mg dose of 4M20. There is a large dark hepatoma (large arrow) with a lighter necrotic portion (small arrow).

Fig. 3.—Photomicrograph of the liver of an RF mouse dying 26 months after a 3.8-mg dose of 4M20. There is a sharp demarcation between a hepatoma (upper) and non-neoplastic liver (lower). H & E, X 40.

Fig. 4.—Photomicrograph of the liver of an RF mouse dying 25 months after a 4-mg dose of 4M20. A pedunculated hepatoma (right) is attached to the liver. Both neoplastic and non-neoplastic liver show fatty changes. H & E, X 40.

Fig. 5.—Photomicrograph of the liver of the animal in Fig. 1. The section is from the junction of the hepatoma (upper) and non-neoplastic liver. H & E, X 300.

Fig. 6.—Photomicrograph of the liver in Fig. 3. The section is from the junction of the hepatoma (upper) and non-neoplastic liver. The cells in the hepatoma are markedly vacuolated. H & E, X 300.
Fig. 7.—Depression of the liver capsule over parenchymal scar in an RF mouse aged 28 months, injected with 4M20 25 months previously. H & E, X 125.

Fig. 8.—The appearance of liver cells and nuclei in a control mouse at age 21 months. The cells and nuclei are fairly uniform in size and staining and there is only occasional giantism. Compare with Figs. 9 and 10. H & E, X 500.

Figs. 9, 10.—Marked irregularity and giantism of liver cells and nuclei in an RF mouse at age 25 months—22 months after 4M20 injection. Eosinophilic nuclear inclusions are indicated by arrows. Note the marked variation in nuclear size by comparison with Fig. 8. H & E, X 500.
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