Regression of myelocytic leukemia in rats after hypophysectomy

(N-nitroso-N-methylurea/primary myelocytic leukemia/secondary myelocytic leukemia/myelocytic sarcoma/mammary carcinoma)

CHARLES B. HUGGINS* and NORIFUMI UEDA†

*The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, IL 60637; and †Department of Pathology, Kobe University School of Medicine, Kobe 650, Japan

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ABSTRACT A standard series of five intravenous injections of N-nitroso-N-methylurea (NMU) elicited selective patterns of neoplasms in male rats of the Sprague–Dawley strain; the neoplastic pattern was related to the age of the recipients. When the injections of NMU were started in weanling male rats, a specific triad of neoplasms developed that consisted of mammary carcinoma, primary myelocytic leukemia, and ear duct cancer. When the injections of NMU were started in males at age 100 days, a specific dyad of neoplasms was evident that consisted of primary myelocytic leukemia and ear duct cancer, whereas mammary cancer and other neoplasms were not evident. Secondary myelocytic leukemia and myelocytic sarcoma were produced when blood from the foregoing rats with advanced primary myelocytic leukemia was injected in a subcutaneous site in newborn allogeneic rats. In a proportion of animals, primary and secondary myelocytic leukemia and myelocytic sarcoma underwent dramatic and continued regression after hypophysectomy.

Primary leukemias of two sorts, erythroleukemia (EL) and myelocytic leukemia (ML), are readily, selectively, and reproducibly induced in the rat by a series of doses of powerful chemical carcinogens. Of these leukemogens, the lipid-soluble carcinogens preferentially induce EL, whereas the water-soluble compounds selectively cause ML in high yields.

Among the lipid-soluble leukemogens are emulsions (1) or lipid solutions (2) of 7,12-dimethylbenz(a)anthracene (Me2BA), 7,12-trimethylbenz(a)anthracene (Me3BA), and 3-methylcholanthrene (3-MC). A single dose of these highly potent carcinogens seldom evokes leukemia, whereas a series of big doses administered by intragastric instillation or intravenous (i.v.) injection selectively elicits EL in high incidence, while ML rarely develops.

Shay et al. (3) found that intragastric instillation of a lipid solution of 3-MC, 2 mg daily, for many months was leukemogenic for an occasional rat of the Wistar strain but the incidence of leukemia was low (4); in a series of 422 rats at risk, 3 cases of ML (0.7%) were detected. Transfer of ML to other rats was obtained by intraperitoneal injection of blood from rats with advanced ML into newborn Wistar rats. Schultz et al. (5) isolated verapomeroxidase, a green enzyme, from leukocytes of rats with Shay leukemia.

Huggins et al. (6) studied rats of the Long–Evans strain and found that a series of eight intragastric instillations of Me2BA at intervals of 14 days elicited leukemias reproducibly and in high yields: females, 82%; males, 70%. Studied histologically were 56 cases of rat leukemia elicited by administering Me2BA by gavage and these were classified as 55 EL and 1 ML. Newborn allogeneic rats were given subcutaneous (s.c.) injections of blood from Long–Evans donors in which advanced EL had been induced by Me2BA producing erythrosarcomas (ES); these sarcomas developed as solid tumors at the site of injection of blood from the leukemic donors. The ES (7) grew rapidly and possessed distinctive characteristics; they became manifest as rapidly growing firm lobulated grape-age masses that were easily distinguishable from other tumors by physical examination.

In rats of the Sprague–Dawley strain, a series of five i.v. injections of a water-soluble carcinogen, N-nitroso-N-methylurea (NMU), induced ML in high yields, whereas EL was not elicited (8). Conversely, a set of i.v. injections of Me2BA specifically induced EL in high incidence in Sprague–Dawley rats, whereas ML was not elicited.

To be described, it was found that primary and secondary ML as well as myelocytic sarcoma (MS), induced by blood from rats with primary ML, underwent regression after hypophysectomy.

MATERIALS AND METHODS

Chemical. A refined sample of NMU, mp 122–123.5°C, was synthesized (9) and stored in a refrigerator at 4°C. NMU (0.25 g) was dissolved in saline (25 ml) with magnetic stirring for ca. 20 min prior to i.v. injection.

Biological Materials. Heterozygous Sprague–Dawley rats were obtained from King Animal Laboratories (Oregon, WI) and housed 9–12 rats of the same age, designated a set, in large stainless steel cages in air-conditioned laboratories at 25°C. The rats were random bred inter se and maintained as a closed colony. In the experiments male rats were studied exclusively. The animals were fed a commercial ration [Rockland Mouse/Rat Diet (Teklad, Monmouth, IL)] with tap water ad lib as drinking fluid.

In our standard series sets of rats received five i.v. injections of NMU, 35 mg/kg, in a caudal vein with an interval of 14 days between injections; the first injection of NMU is designated day 0. Newborn Sprague–Dawley rats, age 1–5 days, were injected s.c. in the interscapular region with heparinized blood from donors with advanced ML. Mammary cancer detected in the gross is designated an active center.

Biopsy of liver, MS, and mammary carcinoma were performed with aseptic precautions on animals under ether anesthesia. Histological examination of paraffin sections stained with heparinized blood from donors with advanced ML. Mammary cancer detected in the gross is designated an active center.

Hypophysectomy. The pituitary was removed surgically through a parapharyngeal approach from rats anesthetized with ether; the day of operation is denoted hypox-day 0. The hypophysectomized rats were kept in well-ventilated stainless steel boxes [10 × 6 × 16 inches (1 inch = 2.54 cm)]. A solution of 10% sucrose, oranges, and a salt lick were supplements to the usual maintenance ration; the supplements were discontinued after hypox-day 10.

Hematology. Once each week by cardiac puncture (8), using a hypodermic needle (26 gauge, ½ inch), 0.1 ml of blood

Abbreviations: EL, erythroleukemia; ML, myelocytic leukemia; MS, myelocytic sarcoma; ES, erythrosarcoma; 3-MC, 3-methylcholanthrene; Me2BA, 7,12-dimethylbenz(a)anthracene; Me3BA, 7,8,12-trimethylbenz(a)anthracene; NMU, N-nitroso-N-methylurea; i.v., intravenous; s.c., subcutaneous.
Table 1. Detection of mammary cancer, ear duct cancer, and leukemia elicited in weanling and mature Sprague-Dawley rats by series of i.v. injections of NMU

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Age at first injection, days</th>
<th>No. of rats</th>
<th>Mammary cancer</th>
<th>Ear duct cancer</th>
<th>Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of rats</td>
<td>Mean</td>
<td>Days</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>25</td>
<td>11</td>
<td>58–81</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>100</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

Intact male rats were given five i.v. injections of NMU, 35 mg/kg of body weight, with an interval of 14 days between injections. "Days" refers to when cancer or leukemia was first detected.

RESULTS

Induction of Neoplasms in Male Rats with NMU. Two series of intact male rats were given i.v. injections of NMU, 35 mg/kg of body weight, on five occasions with intervals of 14 days between injections.

In a series of 12 rats (Table 1) the first injection of NMU was given at age 25 days (day 0) and the experiment was terminated on day 125. Three sorts of neoplasms were found: mammary carcinoma was observed in 11 rats (100%), ear duct cancer in 4 rats (36%), and leukemia in 5 rats (45%). A total of 24 mammary carcinomas (with one to seven active centers per rat) was found. The mammary tumors possessed abundant alkaline phosphatase (Fig. 1) in myoepithelial cells and malignant cords of mammary epithelium. The mammary carcinomas grew slowly. All of the leukemias were myelocytic in type with vivid green color visible in collections of leukemic cells.

In a series of nine mature male rats (Table 1), the first i.v. injection of NMU was given at age 100 days (day 0) and the experiment was terminated on day 162. Two sorts of neoplasms were found: ear duct cancer in two rats (22%) and leukemia in seven rats (78%). All of the leukemias were myelocytic and, in the gross, were bright green. Mammary cancers were not present.

Regression of Primary ML. Hypophysectomy was dangerous to life for rats with advanced ML induced by NMU. The pituitary was removed from seven rats with primary ML; four rats died within 7 days and three animals survived 12–25 days postoperatively.

Beginning at age 102 days, an intact male Sprague-Dawley rat was given five i.v. injections of NMU, 35 mg/kg of body weight, with intervals of 14 days between injections. ML was detected on day 122; the spleen was easily palpable, smooth, and very large. The leukocyte count of cardiac blood was 31,600/mm³ and examination of blood films revealed a profusion of immature myelogenous cells containing peroxidase granules and alkaline phosphatase in the cytoplasm. On day 125 hypophysectomy was performed. On hypox-day +4, the spleen had decreased in size and was no longer palpable. Hematologic changes after hypophysectomy are given in Table 2. It was found that the leukocyte count on hypox-day +12 was 7,758/mm³. The experiment terminated on hypox-day +25.

In two additional cases of primary ML the leukocyte counts of cardiac blood on hypox-day −3 were respectively, 26,900 and 40,000/mm³, whereas on hypox-day +12 the counts were, respectively, 17,200 and 18,800/mm³.

Regression of Secondary ML and MS. Secondary ML and MS were produced by inoculation of blood from a donor rat with advanced primary ML into a s.c. site in allogeneic newborn rats. Hypophysectomy was performed on 13 rats with secondary ML and MS; 4 of these animals succumbed before hypox-day +2 and 9 rats survived longer than 70 days.

The donor of primary ML was a Sprague-Dawley male

<table>
<thead>
<tr>
<th>Hypox-day</th>
<th>Body weight, g</th>
<th>Hematocrit, %</th>
<th>Hb, g/100 ml</th>
<th>Leukocytes/mm³</th>
<th>Packed platelets, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>−3</td>
<td>470</td>
<td>40</td>
<td>11.6</td>
<td>31,600</td>
<td>0.76</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+12</td>
<td>361</td>
<td>37</td>
<td>10.5</td>
<td>7,758</td>
<td>0.92</td>
</tr>
<tr>
<td>+19</td>
<td>363</td>
<td>35</td>
<td>10.2</td>
<td>7,493</td>
<td>0.86</td>
</tr>
<tr>
<td>+24</td>
<td>331</td>
<td>33</td>
<td>10.2</td>
<td>8,960</td>
<td>0.64</td>
</tr>
</tbody>
</table>

A male Sprague-Dawley rat was injected i.v. with NMU, 35 mg/kg of body weight, on five occasions at biweekly intervals starting at age 102 days; ML was detected 122 days after the first dose of NMU and hypophysectomy was performed 3 days later. Blood (0.1 ml) was obtained from the lightly ether-anesthetized rat by cardiac puncture.
that had received a series of five i.v. injections of NMU, 35 mg/kg of body weight, at intervals of 14 days starting at age 96 days (day 0). On day 146 the leukocyte count of cardiac blood was 152,400/mm$^3$, with many immature myeloblasts and myelocytes in the blood film; polymorphonuclear leukocytes were not present. Alkaline phosphatase and peroxidase were strongly positive in the cytoplasm of the immature myelogenous cells. The donor rat was exsanguinated on day 146. At necropsy the spleen was greatly enlarged. All of the lymph nodes that were examined were big and green and the marrow was vivid green.

Each of five Sprague–Dawley rats age 2 days was injected s.c. in the interscapular region with blood (0.2 ml) from the aforementioned donor with advanced primary ML. Palpable tumors at the site of injection were evident in all recipients at age 56 days. Biopsy of the dorsal masses revealed MS, green

Fig. 2. MS elicited by s.c. injection of blood from a rat with advanced primary ML into a newborn. (×500.)

and with smooth surface; it was composed of immature myelocytes (Fig. 2).

On day 57 the group of five rats was subjected to hypophysectomy; one rat succumbed on hypox-day +4 and one on hypox-day +20. Three rats had regression of MS (Fig. 3) and of secondary ML. These animals were living on hypox-day 68. The hematologic changes in one of the hypophysectomized rats with regression are shown in Table 3; on hypox-day −1 the leukocyte count was 17,300/mm$^3$, whereas on hypox-day +68 the leukocyte count was 9,511/mm$^3$.

**DISCUSSION**

Removal of the pituitary of rats is followed by a moderately severe anemia (13). Berlin et al. (14) found that after hypophysectomy there was a decline of erythrocyte volume from 2.87 ml to 1.18 ml/100 g of body weight; the latter was ca. 45% of the value for normal rats of a commensurate age. Crafts and Meineke (15) found that posthypophysectomy anemia is accompanied by hypoplasia of the marrow and an increase of the leukocyte count in peripheral blood.

Huggins and Oka (7) observed regression in 31% of rats with EL and ES after hypophysectomy. In the favorable cases, evidence of improvement was prolongation of life, diminution or disappearance of histological evidence of leukemia, and complete regression of ES. In light of the beneficial effect of hypophysectomy on EL in the rat, the central question in the present experiments was the possibility of regression of ML. It was found that removal of the pituitary caused benefit of rats with primary and secondary ML and MS. Hypophysectomy was dangerous to life for rats with advanced primary ML but the operation was rather well tolerated by rats with secondary ML and MS. It was significant that "spontaneous" regression of ML or MS was not observed in this experiment.

Age is of critical significance in the induction of mammary cancer by chemical compounds in the rat. The influence of age on the incidence of cancer of the breast was investigated by Huggins et al. (16), who fed a single massive but tolerable dose of 3-MC by gastric instillation to Sprague–Dawley female rats of different ages. In brief, it was found that every female that was fed 3-MC at age 50 days developed mammary carcinoma, whereas cancer of the breast was elicited only in a small percentage of those mates that were fed 3-MC at age 100–365 days. Sinha and Dao (17) gave i.v. injections of Me$_2$BA to female rats age 60 days and observed that 80% of the animals developed mammary cancer, whereas rats injected at age 120 days or older failed to develop cancer of the breast.

Gribs et al. (18) administered i.v. injections of NMU to female Sprague–Dawley rats of various ages and found that the induction of mammary cancer was greatest in young rats
but declined as the age of recipients at the time of treatment increased. Rats receiving NMU at a young age exhibited a greater number of active centers per rat than those treated at later ages.

In the present experiments on male rats of Sprague-Dawley strain, the initiation of a series of injections of NMU at age 25 days evoked a triad of neoplasms, including mammary carcinoma, whereas starting the carcinogenic series at age 100 days resulted in a dyad of neoplasms composed exclusively of ear duct cancer and leukemia; mammary cancer was not evident. By carrying out experiments on leukemia in the older males, the complicating factor of cancer of the breast was eliminated.

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