Regression of Stem-Cell Erythroblastic Leukemia After Hypophysectomy

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SUMMARY

Profound regression followed hypophysectomy in 31% of rats with stem-cell erythroblastic leukemias induced by a set of pulse doses of a lipid emulsion of 7,8,12-trimethylbenz(a)anthracene. In the favorable cases, evidence of improvement following hypophysectomy comprised prolongation of life, decreased leukocytosis in circulating blood, diminution or disappearance of histological signs of leukemia, and complete regression of stem-cell erythroblastic sarcomas in 50% of rats inoculated s.c. with leukemic blood as newborns.

INTRODUCTION

It has been found that a set of i.v. injections of homogenized lipid emulsions of 7,12-DMBA2 (9) or of 7,8,12-TMBA (7) in Long-Evans rats elicits leukemias rapidly and in high yield. The predominant (>80%) sort is a stem-cell leukemia with erythrobastic differentiation. In the rat, hydrocarbon-induced erythroblastosis preferentially invades the liver, where it grows diffusely and replaces the endothelium of the hepatic sinusoids. The liver grows to a huge size in a few weeks.

The erythroblastic leukemia is transmitted readily by allogeneic transplantation i.p. of whole leukemia cells (9) to newborns; s.c. inoculation of blood of leukemic donors results preferentially in erythroblastic sarcomas (8) at the site of injection, whereas leukemia occurs less commonly. In this work, it was found that hypophysectomy frequently resulted in significant regression of the primary erythroblastic leukemia of the donors and of the derived erythroblastic sarcomas in recipients of transplanted leukemia cells.

The following considerations led us to investigate the effect of hypophysectomy on erythroblastic leukemia. (a) Dougherty (3) found that the thymus and spleen were diminished greatly in size after hypophysectomy. The profound diminution in size of these hemopoietic structures is one of the most impressive anatomic findings in the hypophysectomized rat; bovine growth hormone restored their size (12). (b) Van Dyke et al. (17) observed that the red cell mass of the rat decreased to one-half of its original volume within 3 months after hypophysectomy. (c) There is general hypoplasia of bone marrow (2, 5, 14) accompanied by anemia in the hypophysectomized rat. (d) Moon et al. (12) noted the absence of spontaneous neoplasms in hypophysectomized rats maintained more than 1 year, whereas lymphosarcomas (11) developed in many rats injected with bovine growth hormone. (e) The neoplastic response of hypophysectomized rats to 7,12-DMBA was significantly depressed (10), as manifested by delayed appearance and low incidence of sarcoma at the site of injection of the hydrocarbon. The administration of bovine growth hormone reinstated this carcinogenic response.

MATERIALS AND METHODS

The experimental animals were Long-Evans rats. We have maintained our closed colony by breeding the animals inter se for more than 12 years. The rats were housed in metal cages in air-conditioned rooms at 25 ± 2°C. They were fed a commercial ration (Rockland Mouse/Rat Diet, Teklad, Inc., Monmouth, Ill.) and were given drinking water ad libitum.

Heparinized blood (0.2 ml) was obtained by cardiac puncture at intervals of 7 to 14 days for hematological study by conventional methods. Biopsy of liver (7) and spleen was performed on Day 50. For biopsy purposes, we excised 30 to 50 mg of the edge of the liver and splenic nodules when present. Paraffin histological sections of these tissues were stained with hematoxylin and eosin.

Stem-cell erythroblastic leukemia was studied exclusively; all of the leukemic rats that were investigated had histologically verified leukemia in liver and spleen. The leukemic rats were divided into 2 groups: experimental and control. These groups were closely matched according to time of detection of leukemia after Day 0 and its extent in the hepatic biopsy. One group was hypophysectomized under ether via the parapharyngeal route; the 2nd matched group of leukemic rats was intact. All of the operations were performed by one of us (H. O.). After operation, both groups were fed the powdered commercial ration plus fresh oranges; water containing 5% sucrose was provided for drinking.

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Leukemia was transmitted by inoculation of whole blood (0.2 ml) s.c. or i.p. in rats on the day of birth. Blood plasma was filtered through a Millipore filter (HA 0.45 µ) and injected similarly, s.c. or i.p., in other groups of newborns.

The statistical probability, p, of a significant effect was derived from Fisher's (4) table of t values.

RESULTS

Incidence and Characteristics of Stem-Cell Erythroblastic Leukemia. Leukemias were detected before Day 100 in 18 of 22 rats (82%) that were given injections of 4 pulse doses of 7,8,12-TMBA; the time of detection was 62.1 ± 12 days. In a cumulative series of 134 consecutive leukemic rats, we classified the leukemias as follows: stem-cell erythroblastic, 130; myelogenous, 2; stem-cell thymus-lymph node leukemia, 2.

Leukemia was transmitted to newborn rats with the whole blood but not with the filtered plasma of the leukemic donors. Thirty groups, each consisting of 5 newborn rats, were inoculated i.p. or s.c. with the whole blood (0.2 ml) of 15 leukemic donors with erythroblastosis and were observed for 7 weeks subsequently. The results (Table 1) were: leukemia in 15/15 groups that received i.p. injections of whole blood; local sarcoma formed in 6/15 groups that received s.c. injections. Neither leukemia nor sarcoma was detected (Table 1) in similar groups of newborn rats that were given injections of filtered plasma (0.2 ml) of the leukemic donors and observed for more than 6 months.

Effect of Hypophysectomy on Transmitted Stem-Cell Erythroblastic Sarcomas. Neoplasms of this sort followed s.c. injection of whole blood, 0.2 ml, of leukemic donors. These erythroblastic sarcomas in the 1st generation were firm, lobulated (Fig. 1), and pale pink. They grew rapidly. On microscopic examination, confluent masses of peroxidase-negative leukemic cells were found. Mitoses were abundant and erythroblasts and normoblasts were frequent.

Twenty rats with erythroblastic sarcomas of the 1st generation were subjected to hypophysectomy. In 10 cases, there was either slight or no diminution in size of the neoplasms. In 10 cases of erythroblastic sarcoma, there was complete disappearance of the tumor (Figs. 2 and 3).

Forty rats bearing erythroblastic sarcomas of the 1st generation were selected at random as controls and were untreated: there was spontaneous regression of sarcomas in 3 rats (7.5%).

Effect of Hypophysectomy on Primary Stem-Cell Erythroblastic Leukemia. Twenty-six rats with primary erythroblastic leukemia survived the operation of hypophysectomy. There were 40 matched controls. The hypophysectomized leukemic rats (Table 2) lived significantly longer (p < 0.02) than the matched, untreated leukemic controls, and there was decreased leukocytosis (Table 3) in the circulating blood.

The extent of leukemic involvement in the liver was estimated histologically in hepatic biopsies that were obtained both prior and subsequent to hypophysectomy. In the group

| Table 1 |
| Leukemia transmission to newborn rats |
| Newborn rats were treated by i.p. or s.c. injection on the day of their birth. The 15 donors had advanced stem-cell erythroblastic leukemia induced by 7,8,12-TMBA. There were 5 recipients in each group. Time of observation: 6 months. |

| Incidence of No. of recipient groups affected |
| Leukemia After whole blood i.p. 15 After filtered plasma i.p. 0 Sarcoma After whole blood s.c. 6 After filtered plasma s.c. 0 |

| Table 2 |
| Survival of rats after detection of erythroblastic leukemia |
| Each rat had received 4 injections of 7,8,12-TMBA at biweekly intervals, starting at age 25 days. The groups of leukemic rats were matched before hypophysectomy, which was performed 2 to 3 days after detection of leukemia. |

<p>| Untreated leukemia | Hypophysectomy |</p>
<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Survival (days)</th>
<th>No. of rats</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>20</td>
<td>13.6 ± 12a</td>
<td>16</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>12.3 ± 9</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>13 ± 11</td>
<td>26</td>
</tr>
</tbody>
</table>

a Mean ± S.D.

| Table 3 |
| Effect of hypophysectomy on erythroblastic leukemia in rats |
| Decreased leukocytosis and histological regression of leukemia after hypophysectomy. Mean values are given. |

| Circulating leukocytes/cu mm | Histological regression |
|---|---|---|---|
| No. ofrats | Before hypophysectomy | After hypophysectomya | No. | % |
| Female | 16 | 9,700 | 6,200 | 3 | 19 |
| Male | 10 | 16,900 | 10,500 | 5 | 50 |

a Ten to 14 days after hypophysectomy.
of 26 leukemic rats surviving hypophysectomy (Table 3), evidence of regression was demonstrated in 8 cases (31%). In 4 of the cases, there was complete disappearance (Figs. 4 and 5) of histological signs of leukemic involvement of the liver. There was no spontaneous regression of leukemia in any rat in a matched group of 40 untreated companion rats with primary stem-cell erythroblastic leukemia that were maintained as controls.

DISCUSSION

In newborn rats, s.c. inoculation of whole blood from donors with primary stem-cell erythroblastic leukemia preferentially evoked erythroblastic sarcoma at the injection site; filtered plasma from the same leukemic donors did not transmit erythroblastosis.

The sarcomas transmitted by inoculation of whole blood grew rapidly at the site of injection. Spontaneous regression of the derived sarcomas occurred in 7.5% of the animals. We did not observe spontaneous regression in any rat with primary stem-cell erythroblastic leukemia.

Leukemia is one of the neoplasms the course of which is profoundly influenced by endocrine procedures. Heilman and Kendall (6) discovered that administration of cortisone to mice bearing a highly malignant transplanted lymphosarcoma often resulted in rapid regression of the neoplasm. Pearson et al. (13) found that glucocorticoids influenced certain cases of human leukemia, Hodgkin's disease, and lymphosarcoma.

Huggins and Kuwahara (8) observed that dexamethasone caused regression of the stem-cell leukemia which has preferential growth in thymus and lymph nodes in the rat; erythroblastosis was not influenced by glucocorticoids.

A new finding in the present study is that hypophysectomy often resulted in rapid and extensive regression of stem-cell erythroblastic leukemia. Regressions of great magnitude occurred in both the primary leukemias and the erythroblastic sarcomas derived therefrom by allogeneic transplantation of whole blood into the newborn.

In normal animals, erythropoiesis is considerably influenced by both steroid and protein hormones; males have more hemoglobin than do females. It is not yet possible to identify the hormonal activity that maintains erythroblastosis in the rat.

It has been demonstrated (16) that erythropoietin is essential for the susceptibility of bone marrow cells to the chromosomal aberration inflicted by a single pulse dose of 7,12-DMBA. Sugiyama (16) also found that the induction of leukemia in the rat by multiple pulse doses of 7,12-DMBA is strikingly influenced by the erythropoietin level.

ACKNOWLEDGMENTS

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REFERENCES

Figs. 1 to 3. Transplanted stem-cell erythroblastic sarcoma in 1 rat subjected to hypophysectomy (hypox).
Fig. 1. Age, 37 days; hypophysectomy at Day 0.
Fig. 2. Eight days after hypophysectomy (hypox).
Fig. 3. Sixteen days after hypophysectomy (hypox).

Figs. 4 and 5. Biopsy of the liver of a rat with stem-cell erythroblastic leukemia subjected to hypophysectomy.
Fig. 4. Leukemic infiltration with erythroblastic leukemia subjected to hypophysectomy; 2 days before hypophysectomy. H & E, X 100.
Fig. 5. Disappearance of leukemic infiltration; 6 days after hypophysectomy. H & E, X 100.
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