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Rapid and Selective Induction of Erythroleukemia in Female Donryu Rats by Continuous Oral Administration of 1-Ethyl-1-nitrosourea[1]

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SUMMARY

Three groups of female Donryu rats were given continuously 1-ethyl-1-nitrosourea, 400, 200, or 100 mg/liter, in their drinking water. Leukemias developed in 94 of 104 (92%) rats surviving more than 6 experimental weeks. Of the leukemias, the erythroleukemias were induced most frequently, rapidly, and selectively. Other types of leukemias were found in a few rats of the high-dose group and, in some cases, in rats of the low-dose group. Tumors were also induced in the digestive tract, mammary glands, ear duct, and other organs, but their incidences were lower than 24%.

INTRODUCTION

Among various chemical leukemogens (6, 7, 10-13, 15), 7,12-dimethylbenz(a)anthracene is the most potent chemical for induction of erythroblastic leukemia in Long-Evans rats (7). BNU[2] and PNU are also strongly leukemogenic in Donryu rats, and induce myeloblastic leukemia and myelocytic leukemia, respectively, in many of the rats (11-13).

On the other hand, ENU is also a strong carcinogen in many species, not only in adult but also in fetal animals. However, its leukemogenic activity is not high in many strains of rats (3, 5, 14).

In this paper, the results of continuous p.o. administration of ENU to female Donryu rats are described. Briefly, leukemias were induced very rapidly in many rats, and erythroleukemia was found most frequently, followed by myeloblastic and myelocytic leukemias.

MATERIALS AND METHODS

Rats and Diet. Five-week-old female Donryu rats (Nihon Rat Co., Tokyo, Japan) were maintained on the basal diet CE-2 (CLEA Japan Inc., Tokyo, Japan) until they were 11 weeks old, when continuous p.o. administration of ENU was started.

ENU. ENU was synthesized according to the procedure for preparation of 1-methyl-1-nitrosourea given by Arndt and Amende (1). The yield of ENU was 68.0%, and its melting point was 101-102°. The structure of ENU was confirmed by infrared and nuclear magnetic resonance spectra. ENU, sensitive to the effects of light, heat, and humidity, was stored at 5° in a refrigerator until used.

Experimental Groups. Eleven-week-old rats were divided into 3 groups, Groups 1, 2, and 3, each consisting of 36 animals. Rats were housed 4/hanging cage, and were kept in an air-conditioned room. Freshly prepared ENU solution, 400, 200, or 100 mg/liter (in distilled water), was placed daily in a shielded plastic bottle in each cage of Groups 1, 2, and 3, respectively. The ENU solution was given everyday for the 1st 5 weeks, and 5 days a week after the 6th experimental week continuously until the rats were killed for autopsy. Many animals developed tumors and were killed before the 36th week, when the surviving 1 rat in Group 2 was autopsied.

Hematological Experimentation. The number of WBC and RBC was estimated on a hemocytometer when the animals were autopsied, and blood smears were examined every 2 weeks after the 6th experimental week and terminally. The smears were treated with Wright's solution and Giemsa stain.

Cytologically, leukemias were classified into 5 types, erythroblastic, myeloblastic, myelocytic, lymphoblastic, and aleukemic, according to the criteria published in a previous paper (13).

Histological Experimentation. All the rats were killed for autopsy when moribund. Tissues were fixed in 10% neutral buffered formalin, and sections were stained with hematoxylin and eosin.

RESULTS

The number of tumors that developed in various organs in each group is given in Table 1.

The 1st autospy of a rat with tumor was done at the 6th experimental week. Before then, 0, 0, and 2 rats died in Groups 1, 2, and 3, respectively, due to intoxication. They were excluded from the results.

Incidence of Tumors. In Group 1 (400 mg/liter), 35 of 36 (97%) rats developed leukemias and 4 had papillomas of the forestomach. Other tumors were not observed.

In Group 2 (200 mg/liter), 31 of 35 (89%) rats developed

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[2] The abbreviations used are: BNU, 1-butyl-1-nitrosourea; PNU, 1-propyl-1-nitrosourea; ENU, 1-ethyl-1-nitrosourea.

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Table 1
Leukemias and tumors in various organs

Group	Concentration of ENU (ppm)	No. of rats examined	No. of rats with tumors	No. of rats with leukemia	No. of rats with tumors in: Digestive tract	Mammary glands	Ear duct	Others
1	400	36	35 (97)[a]	35 (97)	4 (11)	0	0	0
2	200	35	31 (89)	28 (80)	7 (20)	3 (9)	2 (6)	1[b] (3)
3	100	33	28 (85)	24 (73)	8 (24)	4 (12)	1 (3)	7[c] (21)

[a] Numbers in parentheses, percentages.
[b] In lower jaw.
[c] Two in pancreas, 1 each in lower jaw, kidney, brain, meninges, and vagina.

Table 2
Cytological types of leukemia

Group	No. of rats with leukemia	Av. survival period (weeks)	Erythroleukemia	Myeloblastic leukemia	Myelocytic leukemia	Lymphoblastic leukemia	Aleukemic leukemia
1	35	12.1 ± 1.7[a]	30 (86)[b]	1 (3)	1 (3)	0	3 (9)
2	28	24.1 ± 3.2	20 (71)	2 (7)	3 (11)	0	3 (11)
3	24	21.6 ± 4.6	11 (46)	7 (29)	3 (13)	1 (4)	2 (8)

[a] Mean ± S.D.
[b] Numbers in parentheses, percentages.

tumors, *i.e.*, leukemias in 28 (80%), digestive tract tumors in 7 (20%), and mammary tumors in 3 (9%) rats. In addition, ear duct tumors and tumor of the lower jaw were found in 2 and 1 rats, respectively.

In Group 3 (100 mg/liter), 28 of 33 (85%) rats had tumors, *i.e.*, leukemias in 24 (73%), digestive tract tumors in 8 (24%), and mammary tumors in 4 (12%). In addition, pancreatic tumors were found in 2 rats; tumors of the ear duct, lower jaw, brain, meninges, vagina, and kidney were detected in 1 rat each.

Leukemias. The number of rats bearing each type of leukemia is shown in Table 2.

In total, 61 of 87 (70%) leukemic rats had erythroleukemia, 10 had myeloblastic leukemia, 7 had myelocytic leukemia, and 1 had lymphoblastic leukemia. The other 8 leukemic rats were aleukemic. As shown in Table 2, erythroleukemia was found predominately in the highest dose group, and myeloblastic leukemia was observed in many rats in the lowest dose group, although erythroleukemia was found most frequently in the latter group.

Average survival period of the leukemic rats was shorter in the highest dose group than in the other 2 groups (Table 2).

Average WBC count and average weight of the liver and spleen of the rats with each type of leukemia are given in Table 3.

Invasive patterns of leukemic cells in the liver of the rats were characteristic of each leukemic type. Erythroleukemia, forming small clusters of tumor cells, tended to invade the sinusoids; myeloblastic leukemia invaded widely and extensively into the acini; and myelocytic leukemia tended to invade in and around Glisson's capsule.

Table 3
Average WBC count and average weight of the liver and spleen of the rats with various types of leukemia

	WBC count (10^3/cu mm)	Liver wt. (g)	Spleen wt. (g)
Erythroleukemia	31 ± 42[a]	16.7 ± 5.2	1.4 ± 1.2
Myeloblastic leukemia	18 ± 10	12.7 ± 5.6	5.2 ± 4.9
Myelocytic leukemia	118 ± 95	12.6 ± 3.3	3.0 ± 2.0
Aleukemic leukemia	6 ± 3	15.0 ± 8.0	2.7 ± 4.4

[a] Mean ± S.D.

The number of rats with tumors in extrahematopoietic organs is given in Table 4.

Digestive Tract Tumors. Among 19 rats with digestive tract tumors, 9 had tumors only in the forestomach; 3 had tumors in both the forestomach and duodenum; 2 had tumors in the glandular stomach; 2 had tumors in the colon; and 1 had a tumor in the duodenum. One rat had tumors in the forestomach and colon and another rat had tumors in the glandular stomach, duodenum, and jejunum. In total, 13 forestomach tumors, 3 glandular stomach tumors, 5 duodenal tumors, 1 jejunum tumor, and 3 colon tumors were observed in 19 rats.

Mammary Tumors. Each of 7 rats with mammary tumors in Groups 2 and 3 had 1 to 5 tumor nodules; average, 2.1. Most of these mammary tumors were well capsulated with fibrous membrane, and necrotic areas and ulceration of the superficial skin were commonly observed in large nodules. Histologically, 4 rats had papillary or nodular adenocarcinoma, 1 had fibroadenoma, and 2 had adenocarcinoma and fibroadenoma as separate nodules.

Ear Duct Tumors. All 3 ear duct tumors were solid, fria-

Table 4
Histology of tumors in various organs

Organs	Histology	No. of rats with tumors after dose of ppm		
		400 ppm	200 ppm	100 ppm
Stomach	Papilloma	4	5	4
	Adenoma	0	0	1
	Adenocarcinoma	0	1	0
	Malignant endothelioma	0	0	1
Duodenum	Adenoma	0	0	2
	Adenocarcinoma	0	1	0
	Fibroma	0	1	0
	Fibrosarcoma	0	1	0
Jejunum	Adenocarcinoma	0	0	1
Colon	Adenoma	0	0	3
Mammary glands	Fibroadenoma	0	0	3
	Adenocarcinoma	0	3	3
Ear duct	Squamous cell carcinoma	0	2	1
Lower jaw	Squamous cell carcinoma	0	1	0
	Hemangiosarcoma	0	0	1
Pancreas	Adenoma	0	0	2
Kidney	Fibrosarcoma	0	0	1
Brain	Astrocytoma	0	0	1
Meninges	Meningioma	0	0	1
Vagina	Papilloma	0	0	1

ble, and yellowish gray, and histologically squamous cell carcinomas.

Other Tumors. Tumors were found in the lower jaw, pancreas, kidney, brain, meninges, and vagina in 2 or less rats each.

DISCUSSION

The results of this experiment show that female Donryu rats that received ENU continuously in the drinking water developed a high incidence of leukemias rapidly, and most were erythroleukemia.

The total incidence of various leukemias in this experiment was higher and the induction time shorter than in any other previous reports in the literature (6, 7, 10–13, 15). 7,12-Dimethylbenz(a)anthracene (7) induced leukemias at between 12 and 18 experimental weeks, and BNU (11) induced leukemias between 18 and 33 weeks; average, 20 weeks. In this study, leukemias developed as early as 6 to 15 weeks (average, 12 weeks) in the rats given the highest dose of ENU. PNU induced leukemias later than these 3 chemicals; *i.e.*, 20 to 38 weeks; average, 24 weeks (13). Therefore, ENU is considered the strongest leukemogen that induces a high frequency of leukemias in a short period of time.

BNU induced myeloblastic leukemia (11, 12), PNU induced myelocytic leukemia (12, 13), and ENU induced erythroleukemia in the majority of female Donryu rats. On the other hand, 1-methyl-1-nitrosourea induced tumors in the nervous system of female Donryu rats, primarily in the roots of the peripheral nerves, and the incidence of leukemias was very low.

The strain of rats and route of administration are also important factors in determining the target organ by ENU. Druckrey *et al.* (4) administered a single s.c. injection of ENU to newborn or 10-day-old BD rats, and got many neurogenic tumors and a few nephroblastomas. He also gave i.v. pulse doses of ENU to the same strain of rats and obtained leukemias and gliomas in the brain (3). However, the incidence of the former was about 56%. Ten-day-old BD rats were administered a single p.o. intubation of ENU and developed mainly neurogenic tumors, both in peripheral and central nervous system (4). Pelfrene *et al.* (14) administered ENU in drinking water to MRC Wistar rats, 5 days a week for 52 weeks, from 8 weeks of age. They obtained digestive tract tumors, mammary tumors, and myelocytic leukemia. The number of leukemic rats was lower than one-third of the effective number of rats. Hadjiolov (5) gave ENU (50 mg/animal i.p.) to adult Wistar rats and got thymic lymphoma and myeloid leukemia; the incidence of the latter was 25%. Transplacental administration of ENU induced neurogenic tumors in Sprague-Dawley (9, 17), BD (2, 8), Wistar (17), and DA agouti rats (16), but it did not cause leukemias. In this experiment, erythroleukemia was induced very rapidly with a high yield in female Donryu rats. It may be said, therefore, that Donryu rats are most sensitive to the leukemogenic action of ENU.

In this experiment, erythroleukemia was induced selectively. Its incidence was as high as 86% of all leukemias that were induced in the rats receiving 400 mg of ENU per liter in the drinking water. The incidence of erythroleukemia decreased in the rats receiving lower concentration of ENU. On the other hand, the incidence of other types of leukemias tended to increase in the lower dose groups. A similar relationship was observed in the BNU experiments (11, 12), but it was not clear in the case of PNU (12, 13).

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