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Effect of an Androgen on the Radiosensitivity and Recovery after Irradiation of Mouse Hemopoietic Cells

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An injection or injections of an androgen resulted in several fold increase of the hemopoietic spleen colonies in 600 R irradiated mice. As one of the mechanisms explaining this phenomenon radiosensitivity of the hemopoietic stem cells in androgen injected mice was studied by irradiating the cells in vivo and in vitro with 300 R. Approximately 3 fold, at maximum, resistence to radiation was demonstrated. The mice injected with an androgen before and after 400 R irradiation showed an earlier recovery of the leucocyte and granulocyte counts than the control.

Androgens have been known to stimulate erythropoiesis. Reports on their effect, however, on the pluripotent hemopoietic stem cell (CFU-S or CFU) and the granulocyte progenitor cells (CFU-C or CFC) are not many. The present communication is to report our preliminary studies on this subject.

MATERIAL AND METHOD

Female DS mice, 10 to 14 weeks old, supplied from Shionogi Pharmaceutical Company were used. Nandrolone decanoate (NAN-D) was used as an androgen. NAN-D has the strongest, per weight basis, erythropoietic activity among commercially available parenteral androgens.[1] Exogenous spleen colony method of Till and McCulloch[2] was used to estimate the number of transplantable CFU. Endogenous spleen colony method of Marsh *et al.*[3] was used to study the effect of an androgen on endogenous CFU. A compact ^{60}Co irradiation facility of The Institute for Chemical Research of Kyoto University was used to irradiate mice or bone marrow and spleen suspensions.[4] Polycythemic mice were made by intraperitoneal transfusions of 0.45 to 0.5 ml of packed RBC per day on two successive days. Only mice whose hematocrit above 55% at the time of sacrifice or whose reticulocyte count less than 0.1% before irradiation in the experiments of leucocyte recovery were used. In vitro irradiation of bone marrow and spleen cells was performed by irradiating bone marrow and spleen suspensions in Tyrode solution with 300 R. Following this, their aliquots (25×10^5 and 15×10^6 per mouse with bone marrow cells and spleen cells, respectively) were injected into 800 R irradiated syngeneic mice. Seven to eight days later the mice were sacrificed, the spleens were removed and fixed in Bouin's solution, and the surface colonies were counted. In vivo irradiation of bone marrow and spleen was performed by irradiating mice with 300 R, the femurs and spleens were removed within a few hours after irradiation, and the aliquots of their

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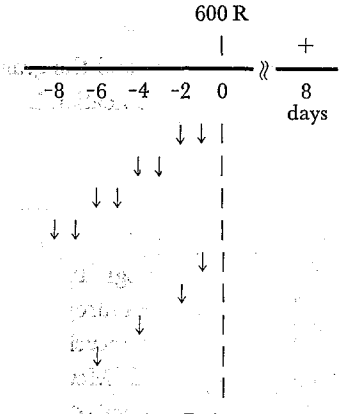
suspensions were injected into 800 R irradiated syngeneic mice. Subsequent procedures were the same as in vitro irradiation.

RESULTS

1) Effect of NAN-D on the Hematopoietic Spleen Colony Formation in Irradiated Mice

Each of experimental mice was injected subcutaneously with NAN-D once or twice (once a day for two successive days) and 1 to 7 days after the last injection the animals were irradiated with 600 to 640 R of ^{60}Co gamma-rays. The mice injected with 2.5 mg of NAN-D 5 to 2 days before irradiation significantly increased the number of endogenous spleen colonies, the maximum increase being more than 7 fold (Table I). In subsequent studies NAN-D was injected between 2 and 3 days before irradiation unless specified.

Table I. Effect of Androgen on Hematopoietic Spleen Colonies in Irradiated Mice



Colony count (No. of mice) ± S.D.		Ratio to control
11.6 ± 3.3	(10)	7.3*
10.7 ± 3.5	(10)	6.7*
5.6 ± 3.7	(10)	3.5*
1.7 ± 1.1	(10)	1.1
3.0 ± 2.5	(7)	1.9
11.2 ± 6.1	(6)	7.0*
9.4 ± 4.4	(8)	5.9*
1.9 ± 2.0	(7)	1.2
1.6 ± 1.4	(9)	

↓ : NAN-D 2.5 mg
* : significant

2) Effect of NAN-D on the Radiosensitivity of CFU Cells

As one of possible mechanisms of this increase of spleen colonies in NAN-D injected and irradiated mice, radiosensitivity of CFU cells was tested.

Table II. Sensitivity of CFU-S in Androgen-injected Mice to 300R *in vitro* Irradiation

Group	Cells	Ratio survived (%)	Ratio to control
NAN-D	Bone marrow	2.52 ± 0.32 (S.D.)	2.19*
	Spleen	2.46 ± 0.62	1.48*
HT+NAN-D	Bone marrow	3.38 ± 0.56	2.91*
	Spleen	3.06 ± 0.56	1.84*
Sesame oil	Bone marrow	1.16 ± 0.26	
	Spleen	1.66 ± 0.48	

HT : Hypertransfusion
* : significant

Table III. Sensitivity of CFU-S in Androgen-injected Mice to 300R *in vivo* Irradiation

Group	Cells	Ratio survived (%)	Ratio to control
NAN-D	Bone marrow	1.77 ± 0.92 (S.D.)	1.27
	Spleen	1.72 ± 0.22	1.67*
Sesame oil	Bone marrow	1.39 ± 0.31	
(control)	Spleen	1.02 ± 0.50	

*: significant

a) In vitro irradiation. The results is shown in Table II. CFU cells in both bone marrow and spleen of NAN-D injected mice, whether hypertransfused or not, showed a significantly less sensitivity.

b) In vivo irradiation. In vivo irradiation checks not only radioprotective effect upon the cell itself but also effect on the environment which leads to cellular protection. The result is shown in Table III. In the NAN-D injected mice, significantly more CFU cells in the spleen survived whereas those in the bone marrow did not.

3) Recovery of Leukocyte after Irradiation

Changes of leukocyte, granulocyte and lymphocyte counts after 410 R irradiation is shown in Figs. 1 & 2, which shows an earlier recovery of leukocyte and granulocyte

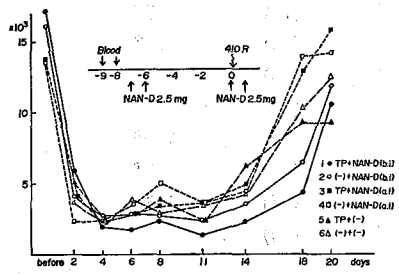


Fig. 1. Effect of Androgen on Recoery of WBC (1)

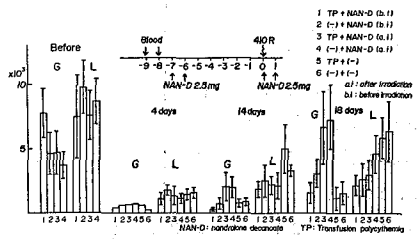


Fig. 2. Effect of Androgen on Recovery of Granulocytes and Lymphocytes (1)

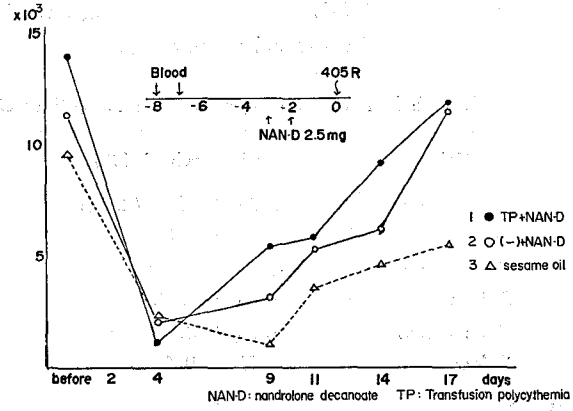


Fig. 3. Effect of Androgen on Recovery of WBC (II)

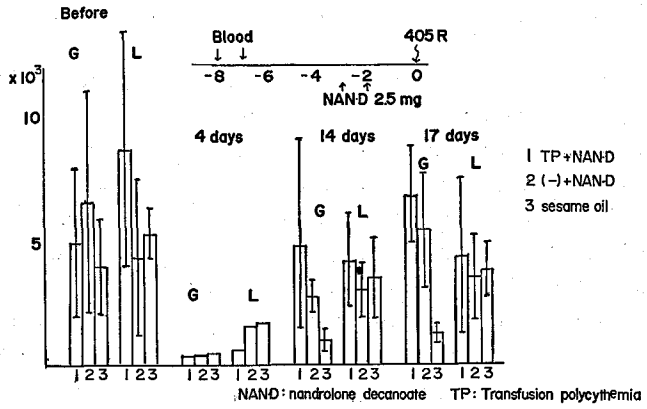


Fig. 4. Effect of Androgen on Recovery of Granulocytes and Lymphocytes (II)

counts in mice given NAN-D after irradiation irrespective of whether they are hypertransfused or not. Injections of NAN-D 7 and 6 days before irradiation did not give beneficial effect on the recovery of leucocyte count. However, as shown in Figs. 3 & 4, injections 3 & 2 days before irradiation resulted in earlier leucocyte and granulocyte recovery as in the case of injections immediately after irradiation.

DISCUSSION

The increase of endogenous spleen colonies in NAN-D injected and irradiated mice is by no means specific. Boggs, Marsh and their group reported a similar effect following injections of foreign plasma, phytohemagglutinin, vaccines, sheep red cells and various erythropoietic stimuli such as erythropoietin, testosterone or testosterone plus cobalt.[5,6] They also studied the mechanism of the increase of endogenous spleen colonies in plasma injected and irradiated mice.[7] Experiments designed to support or deny various hypotheses were negative except for an increase in the total body pool of colony forming cells. The hypothesis that plasma injection increased the fraction of CFU cells which survive a given dose of irradiation as compared to untreated cells was negative. In the present study, CFU cells in the mice given NAN-D before irradiation is more resistent to radiation than the control, which may explain at least part of the increase in number of endogenous spleen colonies after irradiation. In in vivo irradiation, femoral marrow was found not to have more number of transplantable CFU cells remaining after irradiation than the control. This does not necessarily show bone marrow CFU cells remaining after irradiation were not increased, because CFU cells might have moved from the bone marrow to the spleen during the time after irradiation and removal of bone marrow for CFU assay. The hemopoietic stem cells have been known mostly in the Go and enter the S phase after an androgen administration.[8] Murine bone marrow cells in the S phase were shown to become radioresistent as much as 10 fold.[9] However, radioresistence cannot explain whole increase of endogenous spleen colonies, because in our study less than 3 fold radioresistency was obtained. The kinetics of the stem cell system appear complex, and as stated by Boggs *et al.*[7] it would be hazardous to draw any con-

clusions concerning the effect of any given stimulus upon the compartment unless studies include more than one type of CFU assay.

The mechanism of earlier recovery of granulocyte count after 400 R irradiation in the mice given NAN-D may be: 1) less damage of the stem cells, 2) earlier recovery of the stem cells, 3) earlier recruitment of CFC to granulocytes by enhanced production of colony stimulating factor (CSF), and 4) shift of granulocytes from the marginal to the circulating pool. All of the four may not be mutually exclusive. Radiosensitivity of the stem cells in mice injected with androgens has been described. Increase of CSF production was shown in mice injected with androgens.[10] Further studies are needed to clarify the mechanism(s) of this earlier recovery of granulocyte count.

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