19-NORTESTOSTERONE DECANOATE INDUCED ERYTHROPOIESIS IN THE ACTINOMYCIN-D-TREATED HYPEROXIC MOUSE*

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SUMMARY

The erythropoietic effect of an androgen, 19-nortestosterone decanoate (19-ND), on the actinomycin-D- (ACT) treated mouse has been investigated. The toxic effect of a daily administration of 60 µg/kg body weight of ACT on hematopoietic tissue was partially overcome by a daily 1.25 mg injection of the androgen. When an equivalent amount of ACT was administered in fractionalized doses (20 µg/kg body weight at 8-hour intervals), significant levels of erythropoiesis were maintained by identical androgen therapy. These data suggest that 19-ND increases the absolute number of erythroid precursor cells, thus increasing the absolute number of these elements which remain unaffected by the amount of ACT injected. Such unaffected cells will mature to normal erythrocytes, thereby maintaining significant erythropoiesis.

INTRODUCTION

Although androgenic steroids play an important role in the regulation of many metabolic processes, the mechanism of these compounds remains obscure. 19-nortestosterone decanoate (19-ND) has been shown to enhance erythropoiesis in mice (1,2) and man (3). In addition to the ability to increase erythropoietin production (4), the proposed mechanism by which androgens enhance erythropoiesis suggests a direct stimulation of uncommitted G_0 stem cells (5,6). These elements are

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subsequently triggered to a G_1 cycle responsive to erythropoietin (7). This hypothesis gains support in the present study which investigates whether androgen therapy could counteract the toxic effect of actinomycin-D (ACT) on hematopoietic tissue.

MATERIALS AND METHODS

Swiss Webster virgin female mice, pretreated with 1-2 mg iron as iron-dextran solution (Imferon - Lakeside) to insure adequate iron stores, were randomized into groups of eight. The mice were maintained on a diet of Purina Rodent Chow and water ad libitum. Endogenous erythropoietin was suppressed, as previously described (5), by the 60 per cent O_2 hyperoxic system in order to prepare an animal more senitive to exogenous erythropoietic simulation.

19-ND, dissolved in propylene glycol (25 mg/ml), was supplied by Dr. Henry Strade of Organon, Inc. ACT (Lyovac Cosmegen - Merck, Sharp and Dohme) was freshly prepared every third day and maintained in a dark refrigerator. Twenty four hours prior to sacrificing, 0.5 µC ^{59}Fe as ferrous citrate (Ferrutope - Squibb) was injected via the tail vein. By cardiac puncture technique, 0.5 ml of blood was collected per mouse. Red cell radioactivity was counted in a Baird Atomic well-type scintillator and ^{59}Fe incorporation was calculated as a percentage of injected isotope. The procedures used are depicted in Figure 1.

RESULTS

Effect of 19-ND on mice receiving 60 µg/kg body weight of actinomycin-D in a single daily injection.

Mice receiving both 60 µg/kg body weight of ACT and 1.25 mg of 19-ND each in a single daily injection from time 0 of hyperoxic exposure (Fig. 1) were compared to control groups receiving ACT alone, or appropriate vehicle on the same injection schedule. A highly significant elevation in the ^{59}Fe incorporation for mice treated with both ACT

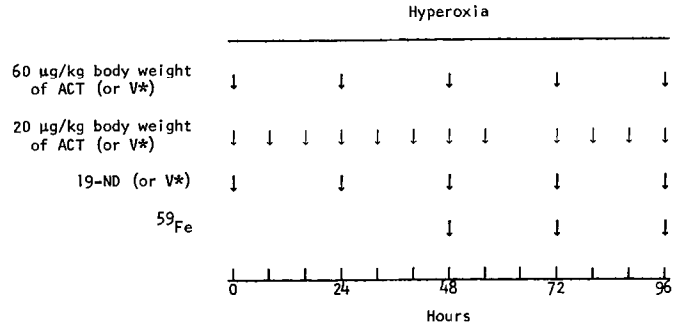

Fig. 1. Procedures used to study the erythropoiesis induced by 19-ND

*Vehicle

and 19-ND was observed 48 hours following the initial injection (Table 1). However, by 72 hours ^{59}Fe incorporation was suppressed and remained so for the duration of the study.

Effect of 19-ND on mice treated with 60 µg/kg body weight of actinomycin-D in divided doses.

Following the protocol described above (Fig. 1), but substituting 20 µg/kg body weight ACT injections at 8-hour intervals for the single

Table 1. Effect of 19-ND on ^{59}Fe Incorporation in Hyperoxic Mice Receiving a Single Daily 60 µg/kg Body Weight Injection of Actinomycin-D*

Hrs Following 1st Injection	Experimental	Control			
	ACT and 19-ND	ACT	p	V**	p
48	20.9 ± 2.6	4.0 ± 0.9	<0.001	6.1 ± 0.9	<0.001
72	1.5 ± 1.0	1.3 ± 0.3	NS***	2.1 ± 0.8	NS
96	0.4 ± 0.1	0.3 ± 0.08	NS	3.2 ± 2.0	NS

*Values represent mean ± S.E.M.

**Vehicle.

***Not statistically significant.

daily 60 µg/kg body weight dose, a significant elevation of ^{59}Fe incorporation in mice receiving both ACT and 19-ND was again observed 48 hours following the initial injection. However, on this dosage schedule, the elevation of ^{59}Fe incorporation was sustained for the duration of the study (Table 2).

Table 2. Effect of 19-ND on ^{59}Fe Incorporation in Hyperoxic Mice Receiving 60 µg/kg Body Weight of Actinomycin-D in Daily Divided Doses*

Hrs Following 1st Injection	Experimental	Control			
	ACT and 19-ND	ACT	p	V**	p
48	21.6 ± 3	7.5 ± 3.0	<0.01	10.6 ± 1.0	<0.02
72	12.2 ± 5	2.2 ± 0.4	<0.05	4.8 ± 0.9	<0.02
96	11.7 ± 1	1.5 ± 0.4	<0.001	4.0 ± 0.8	<0.001

*Values represent mean ± S.E.M.

**Vehicle.

DISCUSSION

The ability of the androgen, 19-ND, to overcome the erythropoietic eradication caused by ACT, a powerful cytotoxic agent, and 60 per cent hyperoxia was demonstrated in the present study. The androgen was less effective in maintaining erythropoiesis when a single 60 µg/kg body weight dose of ACT was administered daily, as compared to the fractionalized regimen. In the latter study, it was found that the same amount of androgen could successfully sustain erythropoiesis.

Based on the half-life of erythropoietin, Kretchmar et al. (7) postulated that cells having a G_1 length of four hours are maximally responsive to this hormone. Furthermore, Reismann and Ito (9) have shown that a daily 60 µg/kg body weight ACT injection eliminates erythropoiesis in mice without affecting normal production of megakary-

ocytes or leukocytes. This implies that cells in a 4-hour G_1 phase are also maximally sensitive to the damaging effects of ACT. It would appear that 19-ND rapidly triggers large numbers of G_0 or G_1 cells to a 4-hour G_1 phase, thereby increasing the number of these elements which escape the toxicity of a given dose of ACT. This results in the normal maturation of more erythroid elements as compared to the non-androgen-treated control.

ACT inhibits DNA mediated RNA synthesis in G_1 cells, preventing these cells from entering the S phase of cell cycle (10,11). The ability of the single daily 60 µg/kg body weight injection of ACT to more effectively eradicate the erythropoietic effect of the androgen as compared to the divided regimen is in accord with recent observations by Skipper (12). He demonstrated that the dose-response curve for cancer chemotherapeutic agents is very steep, suggesting that a large increase in cell-kill occurs for a proportionately smaller increase in drug dosage. This observation implies that a single large dose of the cancer chemotherapeutic agent would affect a greater number of cells than an equivalent dose given in a fractionalized regimen over a prolonged period. Hence, the damaging effect (i.e., blocking cells triggered to the 4-hour G_1 cycle by 19-ND) of 60 µg/kg body weight of ACT administered in single daily injections was greater than three daily 20 µg/kg body weight injections of ACT. The divided dose schedule would not be powerful enough to counteract the influx of cells to the 4-hour G_1 cycle.

The observation of a direct effect on stem cells by the androgen is in agreement with the recent works of Byron (13,14) who demonstrated a stem cell effect of androgenic steroids using the ^{3}H-thymidine-kill technique.

In conclusion, the following deserves mention. Cancer chemotherapeutic agents which interfere with DNA synthesis or damage the DNA

macromolecule of rapidly dividing cells unfortunately damage normal tissue with high rates of proliferation as well as those tissues which are malignant. The bone marrow, gastrointestinal tract, and hair follicles are tissues which most often bear the brunt of the toxic side effects. The damage to hematopoietic tissue is clinically manifested as anemia, leukopenia, and thrombocytopenia. In the above report, the potential of 19-nortestosterone decanoate to counteract the toxic effect of actinomycin-D on the erythroid cell line was demonstrated. Although measurements of myelopoiesis and thrombopoiesis were not taken, it is feasible that an increased stem cell pool, resulting from androgen therapy, will result in an increased production of all cell lines. Hence, the treatment of patients receiving cancer chemotherapy with anabolic steroids could render the accompanying toxic side effects more tolerable. The clinical implications of these animal studies merit further investigation.

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