Erythropoietin and Erythropoiesis in Anemic Man
Following Androgens

By Raymond Alexanian

That androgenic hormones stimulate erythropoiesis in animals and man has been known for many years.1,2 The principle mechanism for this activity probably results from an increased production of erythropoietin since both serum and urine levels are elevated by androgens.3-5 Even when plasma erythropoietin content is high, as in most anemias, further enhancement may occur after administration of testosterone.6 In man, the relationship between androgen dose and erythropoietin response has not been clarified, and the degree of erythropoietin stimulation in anemic patients has not been compared with the effect in normal man. These problems were studied in order to define a more rational basis for the use of androgens in the treatment of patients with anemia due to bone marrow failure. This report correlates the urinary excretion of erythropoietin with changes in red cell volume in normal, hypogonadal, and anemic patients after different doses of androgenic hormones. Results suggested that further stimulation of erythropoietin production by a synergistic effect between androgens and anemia accounts for the increased erythropoiesis produced in some anemic patients by large doses of androgens.

Materials and Methods

Urinary erythropoietin excretion was measured serially during androgenic hormone therapy of 26 patients ranging in age from 30 to 78 years. Red cell indices were normal bone marrow hemosiderin was present, and creatinine clearance exceeded 70 ml./min. in all subjects. Serum iron and transferrin concentrations were determined by the method of Ramsay.7 Transaminase levels were normal before therapy, and were measured serially after institution of androgens. No red cell transfusions were given during the study period. When required for underlying malignant disease (3 patients), the nature, dose, or schedule of daily maintenance chemotherapy was not altered. Red cell volume was measured using the 51Cr technic8 at least 3 months after institution of androgens; control measurements were made at least 2 months after cessation of therapy. Results were expressed in ml./m.2, and compared with ranges derived in this laboratory for normal men and women. The urinary excretion of erythropoietin was assessed in polycythemic mice, and expressed in Standard B units/day, as described previously.5

All subjects were divided, according to their hematologic and endocrine status, into 3 treatment groups. Six males constituted the control group. Ages ranged from 43 to 61 years. All had indolent malignancies in complete remission that did not require any therapy during the study period. Diagnoses included nodular lymphoma (3 patients), localized

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This work was supported by U.S.P.H.S. Grant AM 09155.

First submitted August 9, 1968; accepted for publication November 4, 1968.

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Hodgkin's disease (2 patients), and localized reticulum cell sarcoma (1 patient). Bone marrow cellularity on clot section was normal; erythropoiesis was considered normal on the basis of normal hematocrit (42 to 48 volumes percent), normal red cell volume (1060 to 1220 ml./m.²), and normal erythropoietin excretion (0.7 to 2.2 St. B units/day).

Eight males were hypogonadal with decreased testosterone production rate (< 2 mg./day) and/or 17-ketosteroid excretion (<3 mg./day). Hypophysectomy for pituitary adenoma had been performed on all at least 6 months previously. Replacement therapy with thyroid and prednisone was continued during the study period. With one exception basal hematocrit was less than 41 volumes per cent (median 39), and red cell volume was less than 1060 ml./m.² (median 950). In all, control erythropoietin excretion was less than 0.8 units/day.

Six males and 6 females formed the group with anemia due to bone marrow failure. Diagnoses included advanced Hodgkin's disease (4 patients), multiple myeloma (4 patients), lymphosarcoma (2 patients), and myelofibrosis (2 patients). Impairment of marrow function from prior, large- or multiple-field radiotherapy was the major factor contributing to anemia in 8 subjects. Marrow infiltration by malignant cells was considered the major cause of anemia in 4 patients. Hematocrits ranged from 29 to 40 volumes per cent (median 36), and red cell volume ranged from 64 to 79 per cent of the normal mean value for each sex. Basal erythropoietin excretion was either elevated (7 patients), or at the upper limit of the normal range for each sex (5 patients). Fluoxymesterone™ was given to all patients for at least 3 months. The daily oral dose was 10 m./meter² for hypogonadal males and anemic females, and 40 mg./meter² for control and anemic males.

RESULTS

Following androgenic treatment, all subjects demonstrated an increase in urinary erythropoietin; two thirds of the patients studied developed an elevation in red cell volume of at least 15 per cent. The kinetics of the erythropoietin response differed in each treatment group.

Control Males

In males forming the control group, median erythropoietin excretion increased eightfold from 1.0 to 8.5 St. B units/day after one month of fluoxymesterone treatment (Fig. 1). This level was greater (p < .01) than previously induced in normal men by lower doses of androgen. Subsequent values were less, decreasing to a median of 2.3 units/day by the third month. At this time, the median increase was 5 volumes per cent (range 1 to 8) in hematocrit and 15 per cent (range 7 to 25 percent) in red cell volume (Fig. 2). There was no correlation between the maximum level of erythropoietin excretion and the degree of subsequent erythrocytosis. Within one week after ceasing androgenic therapy, erythropoietin excretion fell to or below the control range (Fig. 1).

Hypogonadal Males

Basal erythropoietin excretion in hypogonadal males ranged from 0.3 to 0.7 St. B units/day, values less than found previously in normal men. After one month of androgen therapy, all patients showed erythropoietin stimulation of 2 to 8 times control, with a median maximum excretion of 1.9 units/day (range 1.0 to 4.8) (Fig. 3). This level was not different (p = .4) from values

*Upjohn Company, Kalamazoo, Michigan.
Fig. 1.—Erythropoietin excretion in control men during fluoxymesterone therapy in a daily dose of 40 mg./m.². The shaded area includes 85 per cent of all values.

Fig. 2.—Red cell volume before and after fluoxymesterone in control and hypogonadal men, and in patients anemic from marrow failure. The shaded areas indicate the normal range for each sex.
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found previously in normal men, but was less \( (p < .01) \) than found in normal women after similar doses of androgen.\(^5\) The maximum level in hypogonadal males was less than that measured in the control group of males after larger doses \( (p < .01) \) (Fig. 1). In hypogonadal males, median hematocrit increased from 39 (range 38 to 45) to 53 (range 44 to 58) volumes per cent, and red cell volume increased by 26 per cent (range 18 to 54 per cent) (Fig. 2). In all but one patient, the red cell volume reached normal or polycythemic levels. The exception was the most anemic subject who required treatment with Dilantin and had megaloblastic changes in the bone marrow. One week after cessation of androgens, urinary erythropoietin returned to the control value or less (Fig. 3). When the response in individual patients was compared, there was no correlation between the degrees to which erythropoietin and red cell mass were stimulated.

**Males and Females Anemic from Marrow Failure**

Twelve anemic patients received fluoxymesterone; 6 women received a daily dose of 10 mg./m.\(^2\) and 6 men received 40 mg./m.\(^2\). Control levels of erythropoietin excretion ranged from 0.9 to 3.5 units in women and 1.2 to 10 units in men. The median level for all patients was 2.1 units/day. After one month of therapy, erythropoietin excretion increased in all at least fivefold to more than 10 units/day to a median maximum excretion of 33 units/day (Fig. 4). This level was significantly greater than found previously in normal men and women after similar doses of androgen \( (p < .01) \) (Fig. 1). Subsequent values were less, with a median excretion of 10 units/day after 3 months. Termination of therapy was followed by return to the control range or less, but not to the undetectable levels occasionally found in normal and hypogonadal males. Red cell volume increased at least 15 per cent in 3 of 7 anemic patients studied (Fig. 2); one patient’s red cell volume increased to the normal range. One additional patient, in whom no blood volume studies were done, increased his hematocrit 7 volumes per cent. Thus, at least one third of the anemic patients in this series developed some erythropoietic response subsequent to an enhanced erythropoietin excretion. There was no correlation between basal or maximum erythropoietin levels and the subsequent elevation in red cell volume, an observation consistent with the variable degrees of anemia and bone marrow reserve present in these patients.

**Miscellaneous Effects**

No change was found in reticulocyte concentration, white blood cell count, or platelet count. Mild anabolic effects, with improved appetite and weight, occurred in most subjects. Transferrin saturation fell from a median percentage of 29 (range 16 to 46) to 18 (range 12 to 25) in 14 normal and hypogonadal males after 1 or 2 months of fluoxymesterone treatment. Marked elevations of transferrin concentration accounted for most of this change. In one hypogonadal male, serum transferrin level increased from 340 to 610 \( \mu g \) per cent.

Fluoxymesterone was well tolerated in more than 90 per cent of subjects. Slight dose reductions were required for two women, in one because of hirsutism and in the other because of irritability. Many patients experienced
occasional, but mild, leg cramps. No patient developed jaundice. Transaminase levels exceeded 50 mg. per cent on more than one measurement in 20 per cent of males receiving large doses of fluoxymesterone (i.e., 40 mg./meter²/day), but cessation of therapy was required in only one. This patient developed nausea and weakness associated with an SGOT content of 290 mg. per cent and an SGPT level of 620 mg. per cent, changes which returned to normal within 2 weeks after therapy was terminated.

**Discussion**

Erythropoietin production was evaluated by assessing changes in the urinary excretion of erythropoietin. The renal clearance of erythropoietin is low, and androgens may influence erythropoietin excretion by a direct effect on renal blood flow. Thus, only marked changes in erythropoietin excretion (i.e., > 3 X control) in groups of patients were considered in this evaluation of erythropoietin production. With less variability in erythropoietin excretion and when individual patients are compared, renal and other factors are more likely to obscure significant differences in erythropoietin production. This may explain our failure to detect any correlation between the degrees to which erythropoietin and red cell mass were stimulated among the patients in the control and hypogonadal treatment groups. Plasma was not used because of the difficulty in demonstrating erythropoietin in normal plasma or in anemic plasma when the urinary excretion was less than 10 St. B units/day. Previous studies ruled out an indirect stimulation of endogenous erythropoiesis in assay animals by fluoxymesterone or its metabolites. Neutralization by an anti-human erythropoietin antibody confirmed the identity of the stimulating sub-
Fig. 4.—Erythropoietin excretion in 12 anemic men and women during fluoxymesterone therapy. The shaded area includes 85 per cent of all values.

stance in urine concentrates with human erythropoietin.\textsuperscript{5} In view of the slow and small increment in erythropoiesis induced by androgens,\textsuperscript{2} changes in red cell volume were used to assess an effective marrow response after at least 3 months of therapy.

Fluoxymesterone was given because of the convenience of oral administration, the established capacity to stimulate red cell production in man,\textsuperscript{13} and the desire to avoid the serious hepatic toxicity reported with other anabolic agents.\textsuperscript{14} Using lower doses, masculinizing side effects in women were infrequent and minimal in degree. With the larger doses given to men, hepatic toxicity was uncommon, mild, and reversible. Since marrow iron stores were adequate, the observed decrease in per cent saturation of transferrin was attributed to an effect of androgens on transferrin metabolism in a manner unrelated to a change in erythropoiesis.

The close relationship between androgenic hormones and erythropoiesis has been known for many years. The anemia following orchidectomy is corrected by testosterone.\textsuperscript{1} Large doses of androgenic hormones induce an erythrocytosis in certain animals and human beings,\textsuperscript{1,2} an effect preceded by increased levels of erythropoietin.\textsuperscript{3-6} Erythropoietin antibodies suppress the enhancement of erythropoiesis induced by testosterone.\textsuperscript{15} Gordon has demonstrated that andro-
gens augment the production of a renal factor which reacts with a serum component in order to elaborate active erythropoietin. These observations support the conclusion that the marrow stimulation induced by androgens results primarily from an increased production of erythropoietin. Whether androgens also exert a direct effect on the bone marrow independent of erythropoietin was not evaluated in this study.

The detection of erythropoietin in the urine of normal human beings and its suppression by increased numbers of circulating red blood cells indicates that steady-state erythropoiesis in normal man results from continuous erythropoietin production and activity. The higher level of erythropoiesis and erythropoietin excretion in adult men, in contrast to women and prepubertal boys, presumably results from a higher level of androgenic hormone stimulation. This explanation is supported by the subnormal erythropoietin excretion and red cell volume found in hypogonadal men, with restitution to normal and elevated levels by androgen replacement. This effect in subjects hypophysectomized for pituitary tumors indicates that the erythropoietin-stimulating capacity of androgens is not mediated or dependent upon those pituitary hormones with similar erythropoietic activity, such as growth hormone and vasopressin. Thus, the mild anemia usually found in hypogonadal males may be attributed primarily to decreased erythropoietin production. The greater erythropoietin response found in normal women, in comparison with the effect in hypogonadal males after similar androgen doses, is consistent with observations by Jepson that other endocrine factors may also influence erythropoietin production.

Less erythropoietin stimulation developed in normal males than in normal females after a standardized dose of androgen. Normal men required about four times larger doses of androgen to achieve a level of erythropoietin excretion comparable to that induced in normal women. A similar sex difference and dose-response relationship has been observed in mice. Thus, maximum tolerated doses of androgenic hormones are justified in male patients when indicated in the treatment of certain anemias due to bone marrow failure.

Significant elevation in red cell volume was induced by androgens in several patients with mild anemia caused by impaired marrow function. Despite erythropoietin values higher than those induced in normal or hypogonadal males, the frequency and degree of red cell stimulation in these anemic patients was less. Similar to observations by others, these studies suggest that further enhancement of a previously elevated rate of erythropoietin production accounts to a major degree for the increased erythropoiesis that occurs in some anemic patients receiving androgen treatment. The marked degree of erythropoietin elevation in anemic patients, to levels higher than those predicted from the response in normal man, suggests that androgens and anemia are synergistic in their stimulation of erythropoietin production. Similar synergism between androgens and hypoxia has been demonstrated in animals. Thus, enhancement of erythropoietin production to maximum levels may provide a better opportunity for significant marrow response in some patients with anemia resulting from marrow failure.
CONCLUSION

Erythropoietin excretion was correlated with red cell volume during androgenic hormone therapy of selected patients with normal and decreased erythropoiesis. Normal men required larger doses of androgen to achieve the same degree of erythropoietin stimulation as that previously induced in women with lower doses. In hypogonadal males, low erythropoietin excretion was enhanced, and subnormal red cell volume reached normal or polycythemic levels. Thus, mild anemia in hypogonadal males probably results from decreased erythropoietin production. In patients with anemia due to narrow failure, previously elevated levels of urinary erythropoietin increased markedly within one month to at least 10 X normal. The degree of erythropoietin enhancement conformed with a stimulation of erythropoietin production by a synergistic effect from androgens and anemia. Results suggested that further elevation of erythropoietin production accounts to a major degree for the increased erythropoiesis that occurs in some anemic patients with large doses of androgenic hormones.

ACKNOWLEDGMENT

The author is indebted to Dr. V. W. Cole, Dr. C. C. Shullenberger, and Dr. J. F. Gamble for contributing patients to this study and to Brenda Doherty for her technical assistance.

REFERENCES


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