The Effect of Testosterone on Erythropoietin Levels in Anemic Patients

By N. Rishpon-Meyerstein, T. Kilbridge, J. Simone and W. Fried

Documentation of consistent differences in the red blood counts of males and females has suggested the possibility that sex hormones can influence erythropoiesis. Androgens have since been shown to stimulate erythropoiesis when administered to normal, hypophysectomized, castrated and plethoric rodents. McCullagh reported that prolonged therapy with testosterone corrected the mild anemia of eunuchs indicating that androgens can also stimulate erythropoiesis in humans. Since then, testosterone has been used in the therapy of various types of anemia. However, the mechanism by which androgens influence erythropoiesis remains unknown.

Testosterone has been shown to increase erythropoietin production in rodents and lower primates. The data to be reported here describe the effects of androgen therapy on plasma erythropoietin levels in five anemic patients.

Methods

Five patients with various types of anemias were studied. Table 1 summarizes the pertinent clinical data in these patients. At the various times indicated in Figures 1-5, 10 ml of blood was obtained by venepuncture into a heparinized syringe. The plasma was separated by centrifugation and immediately stored at -5 C. for erythropoietin assay. At the same time, capillary blood obtained from a finger was used for determination of the hematocrit by the microcapillary tube method and for determination of the reticulocyte count.

The erythropoietin content of the plasma was measured using the polycythemic mouse assay as described by DeGowin et al. The plasma erythropoietin level will be expressed as the percentage Fe uptake into newly formed RBC's after injection of one-half ml. of plasma into assay mice. Assay of plasmas from non-anemic patients consistently resulted in Fe uptakes of less than 1 percent. The values depicted in the Figures represent the average of determinations from six assay mice.

Results

Studies performed on M.M., a 75 year old male with multiple myeloma, are described in Figure 1. Prior to receiving androgen therapy, his hematocrit
Table 1.—Clinical Data on Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Weight</th>
<th>Diagnosis</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. N.</td>
<td>75</td>
<td>M</td>
<td>58 kgm.</td>
<td>Multiple Myeloma</td>
<td></td>
</tr>
<tr>
<td>L. H.</td>
<td>39</td>
<td>M</td>
<td>55 kgm.</td>
<td>Multiple Myeloma</td>
<td>Chronic Renal Disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BUN 40 mgm. percent</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Creatinine 2.3 mgm. percent</td>
</tr>
<tr>
<td>K. K.</td>
<td>3</td>
<td>F</td>
<td>10 kgm.</td>
<td>Aplastic Anemia</td>
<td>Secondary to choramphenicol toxicity</td>
</tr>
<tr>
<td>D. N.</td>
<td>7</td>
<td>M</td>
<td>16 kgm.</td>
<td>Idiopathic Normochromic Normocytic Anemia</td>
<td>Idiopathic Mental Retardation</td>
</tr>
<tr>
<td>D. L.</td>
<td>7</td>
<td>M</td>
<td>20 kgm.</td>
<td>Idiopathic Normochromic Normocytic Anemia</td>
<td>Idiopathic Mental Retardation</td>
</tr>
</tbody>
</table>

Fig. 1.—Effects of testosterone on hematologic values of patient M. M. Black bars represent Fe\(^{59}\) uptakes. Broken lines connect reticulocyte values. The drug doses, unless otherwise indicated, refer to daily doses.

was 25 percent and assay of his plasma for erythropoietin resulted in 7 percent Fe\(^{59}\) incorporation. The patient then began to receive injections of testosterone enanthate\(^*\) (200 mgm. IM twice weekly). Eight days later, assay of his plasma resulted in 16.2 percent Fe\(^{59}\) uptake. The plasma erythropoietin level continued to be elevated while he was receiving injections of testosterone. M.M.’s hematocrit rose during androgen therapy to a peak level of about 34 percent. After discontinuing the testosterone injections, the plasma erythropoietin content returned to the pretreatment level; and over the next 12 weeks, the hematocrit slowly declined to 25 percent. The plasma erythropoietin

\(^*\)Delatestryl.
TESTOSTERONE AND ERYTHROPOIETIN LEVELS

Fig. 2.—Effects of testosterone on hematologic values of patient L.H. Black bars represent Fe\(^{59}\) uptakes. Broken lines connect reticulocyte values. The drug doses, unless otherwise indicated, refer to daily doses, except for testosterone, which was given twice weekly.

level then remained constant at a level resulting in an Fe\(^{59}\) incorporation of about 7 percent. The only other form of therapy which M.M. received consisted of a short course of cyclophosphamide (50 mgm. daily). This was started four weeks after discontinuing the testosterone injections, after the plasma erythropoietin titer had already returned to the pretreatment level. No change in the plasma erythropoietin level was observed subsequent to cyclophosphamide therapy.

Figure 2 describes the studies on L.H., a 39 year old male with multiple myeloma and chronic renal disease. Prior to receiving androgens, his plasma erythropoietin level resulted in an Fe\(^{59}\) incorporation of only about 1 percent. Three weeks after beginning androgen therapy (100 mgm. Testosterone Propionate twice weekly), his plasma erythropoietin titer rose to a level capable of increasing Fe\(^{59}\) uptake to over 6 percent. The dose of testosterone was then increased (200 mgm. testosterone propionate twice weekly) and the plasma erythropoietin titer rose further to a value resulting in over 16 percent Fe\(^{59}\) incorporation. The hematocrit varied between 21 and 25 percent and the reticulocyte count fluctuated for the most part between 2 and 6 percent. The latter, however, rose to a peak value of over 9 percent coincident with the maximal rise of the plasma erythropoietin titer. In addition to testosterone, L.H. received a course of x-irradiation consisting of 2500 r directed to a 6" × 12" area over the cervical and upper thoracic spine. He also received
Fig. 3.—Effects of testosterone on hematologic values of patient K.K. Black bars represent Fe\(^{59}\) uptakes. The drug doses, unless otherwise indicated, refer to daily doses.

2 mgm. of melphalan daily throughout the period of observation and received doses of prednisone which rose initially from 20 to 40 mgm. daily and which subsequently were reduced to 5 mgm. daily. The plasma erythropoietin titer began to rise after the increase in the dose of prednisone but continued to rise to its maximal values as the dose of prednisone was reduced. It is therefore unlikely that the prednisone influenced the plasma erythropoietin level significantly.

Figure 3 describes the results of studies on K.K., a three year old girl with an aplastic anemia caused by chloramphenicol toxicity. Her hematocrit prior to starting therapy was 27 percent and assay of her plasma for erythropoietin resulted in an Fe\(^{59}\) uptake of 10 percent. She was then treated with Methyl testosterone (30 mgm. orally, daily). One month later, her hematocrit was still 27 percent but assay of her plasma resulted in an Fe\(^{59}\) incorporation of over 20 percent. The patient expired one month later and no other determinations were made.

D.L. and D.N. are both 7 year old males with mental retardation resulting from birth injury. Both have idiopathic normochromic normocytic anemias of moderate severity. The results of studies on D.N. are illustrated in Figure 4.
Prior to therapy, assay of his plasma resulted in 2.8 percent Fe$^{59}$ uptake. He then began to receive injections of testosterone (testosterone propionate 100 mgm. twice weekly). One week later his plasma erythropoietin level began to rise and reached a maximal level after four weeks which resulted in over 28 percent Fe$^{59}$ uptake. The reticulocyte count during this time reached a peak value of about 3 percent. Plasma erythropoietin titers measured 16, 17 and 18 weeks after discontinuing therapy had returned to the pretreatment level.

Figure 5 illustrates the results of studies performed on D.L. Assay of the plasma for erythropoietin prior to any treatment resulted in an Fe$^{59}$ uptake of 2.7 percent. After three weeks of treatment with testosterone, D.L.'s plasma erythropoietin level began to rise and after four weeks reached a maximal value which resulted in over 14 percent Fe$^{59}$ uptake. This was accompanied by reticulocyte counts as high as 4.7 percent. Plasma erythropoietin titers measured two weeks after discontinuing all therapy had returned to the pretreatment level. The hematocrit values ranged between 30 and 37 percent.

**DISCUSSION**

The plasma erythropoietin titers of all five patients rose above the pretreatment level while receiving testosterone. Two patients (K.K. and M.M.) had elevated titers before receiving testosterone and in both the titers rose substantially higher during treatment. In most patients the plasma erythropoietin levels reached their highest values 3-4 weeks after beginning androgen therapy, remained high for the duration of treatment, and returned to the
pretreatment value shortly after stopping the therapy. The hematocrit of M.M. rose while he was receiving testosterone and returned to the pretreatment level several weeks after discontinuing therapy. Both D.L. and D.N., developed elevations of their reticulocyte counts during testosterone therapy. In both, the hematocrit values varied considerably, obscuring any rise in red cell mass which might have occurred. The fluctuations in the hematocrit values within short periods of time probably resulted from changes in the state of hydration of these two patients. L.H.'s pretreatment erythropoietin level was very low, although his hematocrit was only about 22 percent. His BUN ranged between 36 and 45 mgm. percent and his serum creatinine between 2.1 and 3.1 mgm. percent. His bone marrow aspirate revealed marked plasma cell infiltration with diminished erythropoiesis. The low plasma erythropoietin levels prior to beginning androgen therapy most likely resulted from chronic renal disease. His failure to respond to the elevated plasma erythropoietin levels following testosterone therapy probably was due to the extensive infiltration of the marrow with myeloma. Both M.M. and L.H. received other forms of therapy including cyclophosphamide, melphalan, prednisone, and local x-irradiation. As was pointed out, however, these did not seem to influence the plasma erythropoietin titer.

These results are consistent with those reported by Alexanian who observed increases in the 24 hour urinary erythropoietin excretion after administration of testosterone to normal males and females; and with those of Moores et al. who observed increases in both urinary and plasma erythro-
Testosterone and Erythropoietin Levels

It is highly unlikely that the erythropoietic-stimulating activity in the plasma of these patients was the result of testosterone circulating in the plasma. We have previously shown that testosterone must be given in large doses to stimulate erythropoiesis in plethoric mice. Furthermore, the effect of testosterone on erythropoiesis cannot be detected unless it is given at least 72 hours prior to injecting Fe59. The assay for erythropoietin was performed by injecting Fe59 48 hours after giving the test plasma. These data indicate that testosterone in pharmacologic doses increases the plasma erythropoietin level, and suggest that this is the mechanism whereby testosterone stimulates erythropoiesis in anemic patients. This would imply that anemias which are responsive to androgens are the result of a defect which renders the erythropoietin-responsive-cell relatively but not absolutely refractory to erythropoietin. A definitive test of this concept must await the development of concentrated erythropoietin preparations which are suitable for human use. A similar type of defect has however, been reported in at least two varieties of hereditary anemias in mice. Both mice with genotype WW,15 and with genotype SIS,16 have high plasma erythropoietin titers and respond to very large doses of erythropoietin but not to physiologic doses. Furthermore, when testosterone is given to WW mice, plasma erythropoietin levels rise even higher and the anemia is corrected.17

The data presented here do not exclude the possibility that testosterone also acts directly on the bone marrow to stimulate erythropoiesis. In support of this action, Reisner18 has shown that testosterone can directly stimulate bone marrow cells grown in tissue culture. Whether these studies are applicable to the action of androgens in vivo and in humans remains to be determined.

Summary

Studies in five patients with various types of anemias have shown that testosterone can increase plasma erythropoietin levels in humans. We suggest that testosterone stimulates erythropoiesis in some anemic patients by this mechanism.

Summario in Interlingua

Studios in cinque patientes con varie typos de anemia ha demonstrate que testosterona pote augmentar le concentrationes plasmatic de erythropoietina in humanos. Es postulate que isto es le mechanismo per le qual testosterona stimula le erythropoiese in certe patientes con anemia.

Acknowledgment

We are indebted to Dr. Irving Schulman, Dr. Charles Abilgaard and Dr. Paul Heller for their cooperation and advice.

References

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