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DEPRESSION OF STIMULATED ERYTHROPOIETIN PRODUCTION IN MICE WITH ENHANCED ERYTHROPOIESIS

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ABSTRACT

Background. **The reports of lower plasma erythropoietin (EPO) in anemic patients with active erythropoiesis (hyperplastic) than in comparably anemic subjects with erythroid hypoplasia have generally been interpreted as the result of EPO utilization by the target cells of the hormone. An alternative explanation could be that there is a feedback mechanism through which EPO formation by EPO-producing cells is modulated by the erythroid activity of the erythropoietic organs. The present study was thus designed to investigate EPO production during acute hypoxemia in a mouse model in which the oxygen-carrying capacity of blood, the plasma EPO level, the blood viscosity and the plasma EPO half-life are within normal values in spite of an intense stimulation of erythropoiesis.**

Materials and Methods. **Adult female mice of the CF1 strain with either normal or increased rates of erythropoiesis were used in this study. Erythropoiesis was stimulated by two injections of 10 units of rhEPO given 24 h apart. All experimental determinations were performed 24 h after the second EPO injection. Erythropoiesis was measured by the percent of a tracer dose of ^{59}Fe incorporated into the spleen. Hypobaric hypoxemia was induced by exposing mice to atmospheric air maintained at 50% atmospheric pressure for 6 h. Plasma EPO concentration was determined by RIA. Plasma disappearance of radiolabeled rhEPO was determined by i.v. injection of the hormone and sampling by cardiac puncture every hour for 6 h.**

Results. **Administration of rhEPO to mice increased splenic ^{59}Fe uptake significantly without affecting the hematocrit, the plasma EPO level or the plasma disappearance of radiolabeled EPO. Plasma EPO titer after 6 h of exposure to hypobaric air was about 70% lower in mice with EPO-induced stimulation of erythropoiesis than in mice with normal erythropoiesis.**

Conclusions. **The results of this study suggest that there is an inverse relationship between the rate of stimulated EPO production and erythropoietic marrow activity. They also suggest that the variations in plasma EPO levels during periods of rapidly increasing erythropoiesis are the reflection of a decrease in the rate of production rather than an increase in the rate of utilization by a proliferating pool of erythroid cells.**

Key words: erythropoiesis, erythropoietin, hypoxia

Erythropoietin (EPO) is a glycoprotein hormone, produced mainly by the kidneys in an inverse correlation with the oxygen content of arterial blood, that is part of the feedback mechanism involved in the control of erythropoiesis.[1]

The reports of higher serum EPO titers in patients suffering from hypoplastic anemias than in those with hyperplastic anemias at similar hemoglobin levels[2-5] have generally been interpreted as the result of EPO consumption by erythroid progenitor/precursor cells.[6,7] An alter-

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native explanation could be that the signals produced in the erythropoietic organs as a function of erythroid activity could be sent back to the EPO-producing cells and modulate EPO formation. The existence of this feedback system has been suspected for a long time.[8-11]

We recently reported[12] that plasma EPO titers in both hypoxemic and cobalt-injected mice are lower in animals with high rates of erythropoiesis than in those with normal rates of red cell production. EPO concentration in the plasma compartment depends on the balance between EPO formation and EPO disappearance rates. Consequently, for the above findings to be meaningful, information is required concerning the plasma half-life of EPO in the above experimental conditions. Therefore the present study was designed to investigate both plasma EPO titer (during stimulation of hormone formation by hypoxemia) and plasma EPO half-life in mice in which the rate of erythropoiesis was increased by injection of recombinant EPO.

Our results suggest that there is an inverse relationship between the rate of erythropoiesis and stimulated EPO production, as derived from the finding of below normal plasma EPO titers in the presence of unmodified plasma EPO half-lives.

Materials and Methods

Animals

Female CF1 mice (body wt 24-28 g) were used in this study. They were fed a standard rodent chow and water *ad libitum*.

Hypoxic stimulation of EPO production

Hypobaric hypoxemia was used as the stimulus for EPO formation. To this end animals were exposed to air maintained at 50% atmospheric pressure for 6h in a high-altitude chamber. Within 15 min of hypoxic exposure animals were bled through cardiac puncture under ether anesthesia for pEPO determinations.

Stimulation of erythropoiesis

Erythropoiesis was stimulated by the s.c. injection of 10 units recombinant EPO (Bio Sidus S.A., Argentina) on two consecutive days. Animals were studied 24h after the last EPO injection.

Measurement of erythropoiesis

Erythropoiesis was measured by the percent of a tracer dose of ^{59}Fe incorporated into the spleen.[13]

Mice were given ^{59}Fe 0.2 uC i.v. as ferric citrate, and radioiron uptake was determined 6 h later on the whole spleen using a well-type scintillation counter. Hematocrits were determined by the micromethod.

Determination of the plasma disappearance of radiolabelled recombinant human EPO

For the EPO life span studies, 30 mice for each determination were injected with approximately 600,000 cpm of recombinant EPO that had been previously iodinated by the chloramine-T technique.

Mice were bled by cardiac puncture in groups of five every hour for 6 h; TCA was added to 100 ul of plasma obtained from each sample and the samples were incubated on ice for 20 min. They were then spun at 3,000 rpm for 15 min and decanted. The radioactivity of the precipitate was measured in a gamma counter. It was plotted semilogarithmically vs. time; the corresponding regression line was derived by the least-squares method.

Determination of plasma EPO levels

EPO was determined by radioimmunoassay as previously described,[14] using an antiserum raised against rhEPO and ^{125}I-rhEPO as tracer. This antiserum is cross-reactive with mouse EPO but displacement curves for mouse and human EPO are different.

Therefore serial dilutions of a mouse plasma pool, enriched in EPO by total body irradiation of donor animals followed by phenylhydrazine administration,[15] were used as the standard. This plasma pool had been previously calibrated against the Second International Reference Preparation (II-IRP, World Health Organization) in the post-hypoxic polycythemic mouse assay. EPO concentration was 25 U/mL.

Statistical analysis

Data were analyzed by the Student's t-test. $p < 0.05$ was considered significant.

Results

Table 1 lists the hematologic parameters measured in the mice with either normal or stimulated erythropoiesis. As expected, administration of rhEPO increased splenic ^{59}Fe uptake significantly without affecting the hematocrit, the plasma EPO titer or the plasma disappearance of radiolabeled rhEPO. Figure 1 shows that plasma EPO after 6 hours of exposure to hypobaric air was about 70% lower in mice with EPO-induced stimulation of erythropoiesis than in control mice with normal erythropoiesis.

Table 1. Hematologic parameters and labelled recombinant EPO half-lives in mice with either normal or stimulated erythropoiesis.

Group	*Hematocrit (%)*	*Splenic ^{59}Fe uptake (%)*	*pi EPO (mU/mL)*	*$t^{1}_{/2}$ (min)*
Normal	44.3±2.6	9.1±1.5	37.2±5.2	182.8±14.4
Stimulated	44.0±1.8	22.1±1.7*	42.8±3.7	178.6±9.2

Values are mean±SEM.
**indicates measurements with statistical significance. (p<0.05 as compared to normal values.*

Discussion

The kidney is the major production site of EPO in mammals during adult life. Because it probably contains a sensor for local tissue oxygen, when oxygen tension falls EPO production is increased. However, while many experimental studies have confirmed that altered blood oxygen availability is the principal stimulus controlling EPO production, in a number of situations EPO formation during anemic and hypobaric hypoxia is also apparently modified by the ability of hemopoietic cells to respond to EPO[11,16,17] or by the erythropoietic activity of the bone marrow.[5,8,12] It was of interest therefore to see whether there is an association between the erythrocyte production rate and the EPO response to hypobaric hypoxia in normal mice. To this end, the rate of erythropoiesis was stimulated in mice by two injections of rhEPO given 24h apart. Hematologic parameters measured 24h after the second injection of EPO revealed an intense stimulation of splenic erythropoiesis, which was about 2.4 times greater in EPO-stimulated than in control mice. This figure should be close to that of total erythropoiesis since it has been previously demonstrated that the erythropoietic response to both endogenous and exogenous EPO in adult mice is primarily splenic.[18] Because of the time lag that normally exists between EPO injection and the appearance of newly-formed red cells in circulation, the hematocrit value in the group of mice with stimulated red cell production did not increase but remained similar to that of the control group. Since the EPO half-life in the mouse is approximately 3h, plasma EPO in EPO-stimulated and control mice did not differ significantly. Furthermore, the EPO half-life in mice with EPO-induced erythropoietic stimulation was not found to be significantly different from the half-life of normal mice. This agrees with previous reports and extends to mice the concept, demonstrated in dogs[19] and rats,[20,21] that a hyperplastic bone marrow does not influence

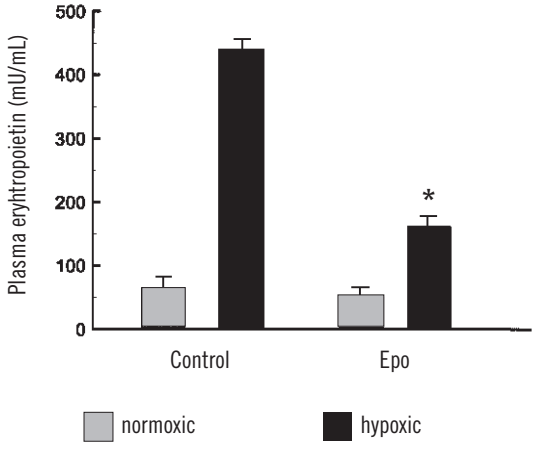

Figure 1. Plasma immunoreactive erythropoietin in mice with either normal (control) or EPO-stimulated erythropoiesis (EPO) under normoxic and hypoxic conditions. Erythropoiesis was stimulated in experimental mice (EPO) by administration of 10 units of rhEPO on 2 consecutive days. Exposure to 50% atmospheric pressure for 6h was performed 24 h after the last EPO injection. Values are the mean (±SEM) of 12 mice per group. *$p < 0.01$ compared to control value.

attributed to an increased erythropoietic rate. As expected from previously reported findings from our laboratory[12] plasma EPO was about 70% lower in mice with above normal red cell production than in control mice in response to the hypoxic stimulation. This low pEPO in the presence of a normal plasma EPO half-life can be interpreted as a truly depressed EPO response to hypoxia. Therefore, although this study does not provide an answer to the nature of the operating mechanism, it shows that an inverse realtionship exists between the rate of stimulated-EPO production and erythroid marrow activity. The results also suggest that the variations in plasma EPO levels during periods of rapidly increasing erythropoiesis are the reflection of a decrease in the rate of production rather than an increase in the rate of utilization by a proliferating pool of erythroid cells.

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