Endocrine, *vol. 8, no. 3, 331–333, June 1998* 0969–711X/98/8:331–333/$8.75

Plasma Disappearance of Exogenous Erythropoietin in Mice Under Different Experimental Conditions

C. E. Lezón, M. P. Martínez, M. I. Conti, and C. E. Bozzini

Department of Physiology, University of Buenos Aires, Faculty of Odontology, Buenos Aires 1122, and Bio Sidus S.A., Buenos Aires 1254, Argentina

Erythropoietin (EPO) is a glycoprotein hormone produced primarily in the kidneys and to a lesser extent in the liver that regulates red cell production. Most of the studies conducted in experimental animals to assess the role of EPO in the regulation of erythropoiesis were performed in mouse models. However, little is known about the in vivo metabolism of the hormone in this species. The present study was thus undertaken to measure the plasma $t^1/_2$ of radiolabeled recombinant human EPO (rh-EPO) in normal mice as well as in mice with altered erythrocyte production rates (EPR), plasma EPO (pEPO) titer, marrow responsiveness, red cell volume, or liver function. Adult CF-1 mice of both sexes were used throughout. For the EPO life-span studies, 30 mice in each experiment were intravenously injected with 600,000 cpm of ^{125}I-rh-EPO and bled by cardiac puncture in groups of five every hour for 6 h. Trichloroacetic acid (TCA) was added to each plasma sample and the radioactivity in the precipitate measured in a γ-counter. EPO, pEPO, marrow responsiveness, or red cell volume were altered by either injections of rh-EPO, 5-fluorouracil, or phenylhydrazine, or by bleeding, or red cell transfusion. Liver function was altered by Cl_4C administration. In the normal groups of mice, the estimated $t^1/_2$ was 182.75 ± 14.4 (SEM) min. The estimated $t^1/_2$ of the other experimental groups was not significantly different from normal. These results, therefore, strongly suggest that the clearance rate of EPO in mice is not subjected to physiologic regulation and that pEPO titer can be really taken as the reflection of the EPO production rate, at least in the experimental conditions reported here.

Key Words: Erythropoietin; clearance; erythropoiesis.

Received April 24, 1997; Revised October 3, 1997; Accepted October 17, 1997.

Author to whom all correspondence and reprint requests should be addressed: Carlos E. Bozzini, PhD, Cátedra de Fisiología, Facultad de Odontología, Marcelo T. de Alvear 2142, Buenos Aires (1122), Argentina. E-mail: fisio@odon.uba.ar

Introduction

The hormone erythropoietin (EPO) is the primary regulator of mammalian erythropoiesis, being produced by the kidney and to a lesser extent by the liver in an oxygen-dependent manner *(1)*. Most of the studies conducted in experimental animals to asses the role of EPO in the regulation of red cell production were performed on mouse models. However, little is known about the disappearance rate of the hormone from the plasma compartment (EPO-PDR) in this species. On the contrary, EPO-PDR in the rat has been extensively studied, either under normal *(2–4)* or altered *(5–7)* physiological conditions.

In addition to its inherent physiological interest, knowledge of the EPO-PDR in the mouse has another important implication. Plasma EPO titer is usually taken as the expression of the EPO production rate in different conditions. EPO concentration in the plasma compartment depends on the balance between EPO formation and EPO disappearance rates. Consequently, plasma EPO titer values could be taken as representative of EPO production rates only in such cases in which EPO-PDR values are also known.

The present investigation was thus designed to measure EPO-PDR (EPO $t^1/_2$) in mice under conditions of altered erythropoiesis, plasma EPO titer, marrow responsiveness, red cell volume, or liver function. The results obtained strongly suggest that the clearance rate of EPO in mice is not subjected to physiologic regulation and that plasma EPO concentration can be taken as the reflection of the EPO secretion rate, at least in the experimental conditions reported here.

Results

Table 1 lists the measured hematological parameters as well as the plasma disappearance ($t^1/_2$) in mice under different experimental conditions, which induced changes in the hematocrit value, the splenic ^{59}Fe uptake, and/or the plasma EPO titer. The EPO $t^1/_2$ was 182.8 ± 14.4 min in the group of normal mice. The $t^1/_2$ of each of the other groups was not significantly different from normal (ANOVA, $F = 0.8571$).

Table 1
Hematologic Parameters and Labeled rh-EPO Disappearance ($t^1/_2$) in Mice Under Different Experimental Conditions[a]

Grupo	Hematocrit, %	Splenic ^{59}Fe uptake, %	piEPO, mU/mL	rh-EPO $t^1/_2$, min
Normal	44.3 ± 2.6	9.1 ± 1.5	37.2 ± 5.2	182.2 ± 14.4
Phenylhydrazine	21.2 ± 1.3[e]	24.2 ± 2.3[e]	1116.5 ± 102.1[e]	186.0 ± 12.6[b]
5-FU	42.4 ± 1.8[b]	0.28 ± 0.3[d]	41.5 ± 4.8[b]	200.0 ± 16.1[b]
rh-EPO (2 h)	43.6 ± 1.7[b]	8.7 ± 0.8[b]	852.3 ± 47.1[e]	204.3 ± 15.4[b]
rh-EPO (3 d)	44.0 ± 1.8[b]	22.1 ± 1.7[e]	42.8 ± 3.7[b]	178.1 ± 11.8[b]
Transfusion	61.8 ± 0.9[e]	0.31 ± 0.02[d]	10.6 ± 1.1[c]	207.8 ± 15.2[b]
Bleeding	20.7 ± 1.1[e]	23.8 ± 2.8[e]	960.4 ± 67.1[e]	194.6 ± 16.1[b]
Cl_4C	42.4 ± 0.7[b]	6.6 ± 1.2[b]	39.4 ± 3.2[b]	168.2 ± 15.4[b]

[a]Values are mean ± SEM.
[b]$p > 0.05$ ns.
[c]$p < 0.05$.
[d]$p < 0.01$.
[e]$p < 0.001$ (Dunnet Multiple Comparisons Test, normal vs experimental).

Discussion

The PDR of both endogenous *(2,5,8)* and exogenously administered EPO, in the form of either EPO-enriched homologous plasma or EPO preparations of variable purity, radioiodinated or not *(4,6,7)*, has been extensively studied in the rat. Analysis of available data indicates that the plasma half-life of all these materials approximates 2–3 h once the equilibrium is achieved throughout the distribution space *(2)*.

In the present study, EPO-PDR has been estimated in mice by measuring the $t^1/_2$ of radioiodinated recombinant human EPO (rh-EPO) after a single pulse injection. Labeling did not affect rh-EPO bioactivity (data not shown). Moreover, previously reported studies indicate that it does not affect the PDR either *(7,9)*.

The plasma clearance curves of normal mice in the present study were biphasic with a $t^1/_2$ of 22 ± 3 min for the first component. This phase was followed by a slower disappearance phase, which has been considered as the hormone's true metabolic clearance *(4)*. After exclusion of the early data points that represent distribution effects, disappearance of ^{125}I-rh-EPO from the circulation could be approximated by a single exponential function that showed a $t^1/_2$ of 182.8 ± 14.4 min in normal mice. This value is close to that reported previously for the rat *(3,4,6,7)* and confirms for the mouse the sluggish metabolism of EPO when compared with other protein hormones.

The metabolic fate of EPO remains largely unknown. Hormone consumption by erythropoietic tissue has been proposed, since it is internalized and degraded after binding to its target cell *(10,11)*. However, studies in laboratory animals have not supported this hypothesis, and EPO catabolism appears independent of marrow activity *(5,8)* or marrow responsiveness to the hormone *(7)*. The liver has also been implicated in EPO catabolism. Recent studies demonstrated, however, that in vivo ^{125}I-EPO elimination is unchanged following liver bypass *(12)*. It has also been proposed that renal handling of EPO involves both excretion in the urine and a modest degree of degradation by the renal parenchyma *(6)*, thus affecting EPO catabolism. Although a small amount of EPO is excreted in the urine, this accounts for only 10% or less of endogenously produced or intravenously administered EPO in man *(13,14)*. Since pharmacokinetic profiles of intravenously injected rEPO are not significantly different from patients with normal or impaired renal function, renal elimination of EPO appears to have a minor role in plasma clearance *(15)*. Furthermore, renal uptake of EPO is very low, which makes renal degradation being an important fate of plasma EPO less likely.

In order to extend some of these observations to mice, PDR was measured in normal animals as well as animals with experimentally induced changes in the red cell mass, the erythrocyte production rate, the plasma EPO titer, the responsiveness to EPO, or the liver function. PDR in each experimental model was found not to be significantly different from the value of normal mice. These results, therefore, strongly suggest that the clearance rate of EPO in mice is not subject to physiologic regulation and that plasma EPO concentration can be taken as the reflection of the EPO production rate, at least in the experimental conditions reported here.

Materials and Methods

Adult CF-1 mice of both sexes were used throughout the experiments. They were kept in a temperature-controlled room, and fed standard chow and water ad libitum. They were divided in groups of 30 animals each and treated as follows: group 1 = untreated control; group 2 = phenylhydrazine-induced hemolytic anemia. A solution of 1% phenylhydrazine HCl was neutralized with 1.0 *N* sodium hydroxide immediately before administration and given at a dose of 60 mg/kg subcutaneously on two consecutive days. The EPO $t^1/_2$ was measured 24 h after the second injection; group 3 = bleeding anemia. Mice were bled

25 mL/kg by cardiac puncture. Volume was replaced with normal saline injected intraperitoneally. EPO $t^1/_2$ was determined 48 h later; group 4 = 5-fluorouracil (5-FU) induced marrow unresponsiveness to EPO. 5-FU was given in a single ip injection of 150 mg/kg. EPO $t^1/_2$ was measured 48 h later; group 5 = supranormal plasma EPO (pEPO) level. Mice were given a single ip injection of 10 IRP unit of rh-EPO. The EPO $t^1/_2$ was measured 2 h later; group 6 = supranormal erythrocyte production rate (EPR). Mice were subcutaneously injected with 10 IRP unit of rh-EPO on two consecutive days. The EPO $t^1/_2$ was measured 24 h later. Group 7 = subnormal erythrocyte production rate. Mice were intraperitoneally injected with 0.6 mL of packed red cells on two consecutive days. The EPO $t^1/_2$ was determined on the 4th day after the last transfusion on mice whose hematocrit values were >0.55; group 8 = acute liver damage. Mice were injected with 0.15 mL Cl_4C subcutaneously just once. The EPO $t^1/_2$ was measured 48 h later.

Hematologic parameters were determined on each group at the time of the study. PEPO titer were determined by radioimmunoassay as previously described *(16)*. The 6-h spleen uptake of ^{59}Fe was used as a measure of the erythrocyte production rate *(17)*. At zero hour, mice received 0.2 µCi of ^{59}Fe intravenously; 6 h later, they were sacrificed by ether overdose and the radioactivity of the entire spleen was measured. For the EPO half-life studies, 30 mice in each group were injected intravenously with approx 600,000 cpm of ^{125}I-rh-EPO. At 1-h intervals during 6 h, blood from 5 mice was collected by cardiac puncture and centrifuged to obtain cell-free plasma. The radioactivity that was precipitable by ice-cold 10% trichloroacetic acid was measured for each sample in a γ-counter. With this time schedule, the early phase of the disappearance curve, determined in other groups of normal mice and reflecting distribution effects, was excluded. The radioactivity of the precipitate was plotted semilogarithmically vs time; the corresponding regression line was derived by the least-squares method. The plasma clearance of rh-EPO was thus defined by a single exponential, which represents the hormone's true metabolic clearance.

Statistically significant differences between control and experimental groups were assessed by analysis of variance (ANOVA) and subsequently Dunnett Multiple Comparisons Test. All data are presented as mean group values ± SE.

Acknowledgments

This work was partially suported by research grants from the University of Buenos Aires (OD-009) and FONCYT 82/OC-AR. C. E. L. is a postdoctoral fellow from the University of Buenos Aires; C. E. B. is a Career Investigator from CONICET.

References

1. Jelkmann, W. (1994). *Clin. Invest.* **72,** S3–S10.
2. Reissmann, K. R., Diederich, D. A., Ito, K., and Schmaus, J. W. (1965). *J. Lab. Clin. Med.* **65,** 967–975.
3. Bozzini, C. E. (1966). *Nature* **209,** 1140,1141.
4. Spivak, J. L. and Hogans, B. B. (1989). *Blood* **73,** 90–99.
5. Naets, J. P. and Wittek, M. (1969). *Am. J. Physiol.* **217,** 297–301.
6. Emmanouel, D. S., Goldwasser, E., and Katz, A. I. (1984). *Am. J. Physiol.* **247,** F168–F176.
7. Piroso, E., Erslev, A. J., Flaharty, K. K., and Caro, J. (1991). *Am. J. Hematol.* **36,** 105–110.
8. Bozzini, C. E. (1966). *Acta Physiol. Latinoam.* **16,** 313–317.
9. Kinoshita, H., Ohishi, N., Kato, M., Tokura, S., and Okazaki, A. (1991). *Arzneim-Forsch./Drug Res.* **41,** 1004–1007.
10. Mufson, R. A. and Gesner, T. G. (1987). *Blood* **69,** 1485–1492.
11. Sawada, K., Krantz, S. B., Sawher, S. T., and Civin, C. I. (1988). *J. Cell. Physiol.* **137,** 337–345.
12. Widness, J. A., Kisthard, J. A., Veng-Pederson, P., Peters, C., Pereira, L. M., Schmidt, R. L., et al. (1994). *Exp. Hematol.* **22,** 704 (abstract).
13. Rosse, W. F. and Waldmann, T. A. (1964). *J. Clin. Invest.* **43,** 1348–1354.
14. Flaharty, K. K., Caro, J., Erslev, A., Walen, J. J., Morris, E. M., Bjornsson, T. D., et al. (1990). *Clin. Pharmacol. Ther.* **47,** 557–562.
15. Kindler, J., Eckardt, K. U., Ehmer, B., Jandeleit, K., and Kurtz, A. (1989). *Nephrol. Dial. Transplant.* **4,** 345–351.
16. Alippi, R. M., Boyer, P., Leal, T., Barceló, A. C., Martínez, M. P., and Bozzini, C. E. (1992). *Haematologica* **77,** 446–449.
17. Bozzini, C. E., Barrio Rendo, M. E., Devoto, F. C. H., and Epper, C. E. (1970). *Am. J. Physiol.* **219,** 724–728.