Erythropoietin Kinetics in Rats: Generation and Clearance

By Stephen E. Steinberg, Joseph F. Garcia, Gary R. Matzke, and Jeanette Mladenovic

Detailed studies to analyze the early events of erythropoietin (Ep) secretion and clearance were performed in a rat model using a double antibody radioimmunoassay. Ep clearance was determined following intravenous injection of 1 mL of Ep-rich plasma, 1,080 mU/mL, obtained from phlebotomized rats. Analysis revealed a disappearance curve that conformed to a two-compartment model with an α half-life of 3.6 minutes and a β half-life of 86 minutes. The volume of distribution was similar to the calculated plasma volume. In anephric animals, there was no change in the plasma clearance rate or the volume of distribution. Rapid Ep secretion was elicited by a single 15 mL/kg phlebotomy (hematocrit decrement 45% to 30%), so that levels reached 20 to 30 times baseline (524 ± 76 μg/mL) at five hours, whereas they plateaued for at least 33 hours. The increase in the rate of secretion was geometric, from 9.9 mU/1 baseline secretion to 429 mU/h. These data identify a very sensitive and rapidly responsive system for Ep modulation in the rat.

Erythropoietin (Ep), a glycoprotein hormone of renal origin, stimulates red cell proliferation and differentiation in mammals in response to hypoxia. As studies begin to demonstrate the potential therapeutic usefulness of this hormone in such disease states as chronic renal failure, an understanding of its pharmacokinetic properties becomes important. In the past, detailed studies of Ep secretion and clearance have been limited by the lack of an assay sensitive enough to detect small changes in hormone levels. The recent development and availability of a radioimmunoassay (RIA) for Ep have made these studies feasible. We used the RIA in a rat model to determine the clearance rate of homologous Ep and to describe the early events of Ep secretion.

MATERIALS AND METHODS

Sprague Dawley rats, weighing 200 to 250 g, were used in all experiments. The source of Ep for clearance studies was plasma obtained from donor rats made severely anemic by every-other-day phlebotomy until the hematocrit reached 12% to 18%. The animals were then exsanguinated by aortic puncture, the heparinized blood was centrifuged at 2,500 rpm for 30 minutes, and the plasma was pooled. This Ep-rich plasma, although impure, represented a physiological form of the hormone that would be subject to endogenous clearance. Ep activity was assayed by the double-antibody RIA using a rat standard curve. The intra-assay coefficient of variation was 8.4%. The Ep titer of the plasma used for all studies was 1,080 mU/mL. The bioactivity of the pooled Ep was demonstrated by intravenous (IV) injection of 1.0 mL into three animals followed by twicedaily determinations of the reticulocyte response. This resulted in a rise in reticulocyte count from a baseline of 0.6% ± 0.2% (SEM) to 5.8% ± 1.2% on day 3.

For clearance studies, animals were anesthetized with Innovar-Vet, and a polyethylene catheter was placed in the right atrium through the jugular vein for vascular access. One milliliter of Ep-rich plasma was given as an IV bolus. Serial 0.5-mL samples of heparinized blood were obtained through the central venous catheter from 30 seconds to two hours. Following each sample, volume was replaced with normal donor blood. Separated plasma samples were frozen at −20°C until assayed by RIA. To study the effect of renal clearance on Ep disappearance, animals were anesthetized with Innovar-Vet and nephrectomized immediately before Ep-rich plasma was injected. This approach minimized volume changes related to diminished renal function.

To investigate Ep secretion, 12 animals underwent a 3-mL phlebotomy (15 mL/kg) after insertion of a central venous catheter as above. Immediate volume replacement with pooled plasma from normal donor animals (Ep = 18.5 ± 2.5 SEM mU/mL) resulted in a fall in hematocrit from an initial mean value of 45 ± 2.8 SEM to 29.5% ± 3.1%. Serial samples were again removed at intervals for Ep determination. An equivalent volume of normal donor blood adjusted to an hematocrit of 30 was reinfused after each sampling to avoid hemodilution and hypovolemia.

Analyses. The plasma clearance data were analyzed by the following equation based on minimization of the residual sum of squares.

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}, \quad [1]$$

where $C(t)$ is the concentration in serum at time $t$, $A$ and $B$ are the $y$ intercepts, and $\alpha$ and $\beta$ are the disposition rate constants obtained from the first and second phases of the plot of log serum Ep concentration vs time. Initial estimates of the kinetic parameters were generated by standard curve stripping techniques. Final estimates were obtained by nonlinear regression analysis with the program KINA on a Control Data digital computer (University of Minnesota, Minneapolis). The volume of distribution of the central compartment, the steady-state volume of distribution, the area under the serum concentration curve from zero to infinity, and the total body clearance were calculated by standard techniques.

Stimulated secretion data were analyzed as follows. The baseline Ep serum concentration data were used to determine the unstimulated secretion rate (Ep Base) in 12 animals. The baseline Ep concentrations were assumed to reflect steady-state conditions, with the amount of Ep formed per unit of time equal to the amount of Ep removed during the same period of time. The concentration time curve from 0 to six hours was then analyzed on a Hewlett-Packard 9845 desktop computer by linear regression analysis and also by polynomial regression analysis from 0 to 60 hours [Equation 2]. The maximal Ep secretion rate (Ep max) was estimated from the ratio of the stimulated plateau Ep concentration (C_{stim}) to baseline Ep concentration (C_{base}), assuming that total body clearance remained constant [Equation 3].

$$C(t) = m_1(t) + m_2(t^2) + b \quad [2]$$

$$Ep_{max} = \frac{C_{stim}}{C_{base}} \cdot Ep_{base} \quad [3]$$

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RESULTS

Erythropoietin clearance following IV bolus of 1 mL of Ep-rich plasma (1,080 mU) conformed to a two-compartment model (Fig 1 and Table 1). An initial rapid α disappearance, t½α = 3.6 minutes, was followed by a longer β phase, t½β = 86.4 minutes (r = 0.97, P < .01). The steady-state volume of distribution (4.27 mL) was similar to the plasma volume of these rats (5.1 mL).7

When the clearance study was repeated in anephric animals, similar results were obtained (Fig 2 and Table 1). Again the data conformed to a two-compartment model. When compared with clearance in normals, Ep clearance in anephric animals had an α phase that was several minutes longer, although the difference was not statistically significant. Although the β disappearance was prolonged due to the increased volume of distribution, total body clearance for anephric animals was slightly greater than normal, but the difference was not statistically significant (Table 1).

The early events of erythropoietin secretion are shown in Fig 3. In response to a single 15-mL/kg phlebotomy, a 21-fold increase in Ep levels was observed, from a baseline of 21 mU/mL to 525 mU/mL. Levels increased within minutes, began to plateau at five hours, and remained elevated for at least 33 hours. Using the homologous clearance data generated in the first portion of this study, the rate of secretion required to maintain the normal Ep level of ~20 mU/mL was calculated to be 53 mU/h. In response to a single phlebotomy, this basal rate of secretion doubled approximately every hour until it reached 450 mU/h at plateau.

DISCUSSION

These experiments detail the kinetics of Ep secretion and clearance, using homologous hormone, assaying levels by a sensitive RIA, and incorporating concurrent clearance data into a secretion model and vice versa. Following the bolus administration of EP-rich plasma, a two-phase clearance curve was obtained, as with previous studies.8-11 Although studies using nonhomologous and/or biochemically altered Ep have yielded a slow equilibrium phase (ascribed to a large Ep molecule dispersing into a sizable extravascular space),8,11 in this study the mean distribution phase t½d was 3.6 minutes. This rapid distributional phase might be expected in view of the volume of distribution, which was equivalent to the plasma volume of the animal.7 This result is consistent with studies in sheep, also using homologous Ep, in which the Ep space was the same as the measured plasma volume.12 In contrast, using a heterologous protein (sheep Ep injected into rats), Reissmann found that the volume of distribution approximated twice the plasma volume.8 It is possible that the initial rapid clearance of a foreign protein or of Ep bound to a heterologous carrier protein could yield an inordinately large volume of distribution, perhaps accounting for the discrepancy.11

Following the distribution phase, the second phase, that of plasma clearance, was linear from ten minutes to the end of the two-hour assay period, with a t½ of 86 minutes. Naets and Wittek, also using a biologic assay and homologous, exogenous Ep,13 obtained a t½ of 1.5 hours. Slightly longer t½s have been obtained in studies of Ep disappearance following the withdrawal of a hypoxic stimulus.8,13,14 This difference may be due to ongoing Ep secretion, which persists even after withdrawal of the stimulus, thereby prolonging the apparent t½.8 Other clearance data have been published, with Ep t½s ≤24 hours.8,12,14-21 In addition to species variability,16,17,22 there are several methodological differences between these studies and the present one. In some studies, the use of nonhomologous and/or iodinated serum Ep or even urinary Ep could have resulted in nonphysiologic clearances of "foreign" proteins or Ep aggregates.8,9,11,13,21 As suggested by Emmanoel et al, it is possible that heterologous 125I-Ep is metabolized differently than endogenous Ep, reflecting either the heterologous nature of the protein or the possibility that the iodination process, which renders the protein biologically inactive, also alters its clearance characteristics.11 Although the use of 125I-labeled Ep obviates confusion in the data due to ongoing endogenous production of the hormone,11 this difficulty may also be resolved by incorporating the experimentally determined secretory rate into the calculations of Ep clearance.

There has been controversy regarding the role of the kidney in Ep clearance.3 Early studies demonstrated that

Table 1. Erythropoietin (Ep) Clearance Data Following Intravenous Bolus of 1 mL of Ep-Rich Plasma

<table>
<thead>
<tr>
<th></th>
<th>Normal (Mean ± SEM)</th>
<th>Anephric (Mean ± SEM)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α t (min)</td>
<td>3.62 ± 0.51</td>
<td>7.80 ± 4.4</td>
<td>NS</td>
</tr>
<tr>
<td>β t (min)</td>
<td>86 ± 16</td>
<td>111 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>Vd (mL)</td>
<td>1.87 ± 0.19</td>
<td>2.55 ± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td>Vm (mL)</td>
<td>4.27 ± 0.29</td>
<td>4.78 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Total body (mL/min)</td>
<td>2.3 ± 0.31</td>
<td>2.48 ± 0.89</td>
<td>NS</td>
</tr>
</tbody>
</table>

Clearance data for 1 mL (1,080 mU) of erythropoietin in normal and anephric animals. Vd, central compartment volume of distribution; Vm, steady state volume of distribution.
12 rats.

648 of disappearance rate and distribution space on plasma concentr-
erthropoietin. Blood change in the total body clearance. Mladenovic et al found no

cally insignificant prolongation of the plasma t\text{2}', and no
current study, nephrectomized animals had a slight, statisti-
body contributing to the plasma t\text{2} and or catabo-

Fig 3. Ep levels (mU/mL) following 15-mL/kg phlebotomy in

12 rats.

urinary excretion parallels plasma concentration, but that
urinary excretion constituted only a small portion of clear-
ance. \textsuperscript{10} Subsequent studies, nevertheless, have reported pro-
longation of Ep clearance after nephrectomy. \textsuperscript{11,13-14,22,23}

Recently, Emmanoel et al, using a highly purified erythro-
poietin, reported a very slight but statistically significant contribution of the kidneys to total body clearance. \textsuperscript{11} In the current study, nephrectomized animals had a slight, statistically insignificant prolongation of the plasma t\text{1/2} and no change in the total body clearance. Mladenovic et al found no difference in Ep clearance in sheep both prenephrectomy and postnephrectomy. \textsuperscript{13} It seems likely that the renal contribution to Ep clearance is less than 10%. It is possible that the loss of a renal contribution to Ep clearance and/or catabo-

lism results in a compensatory increase in Ep clearance by other tissues.

In the past, it has not been possible to determine the rate of
Ep secretion because of the requirement for accurate basal

levels and the incorporation of plasma disappearance data. \textsuperscript{3}

In this study, the availability of the RIA and homologous plasma clearance data have allowed an accurate determination of the kinetics of Ep production. The normal Ep level of 19 mU/mL was maintained by a basal Ep secretory rate of 53 mU/h. When the animals were rapidly rendered anemic, the rate of Ep production increased geometrically, to at least nine times baseline levels. The Ep level rose almost instantane-
ously, reached a plateau at five to six hours and remained at this elevated level for up to 33 hours, suggesting persistent Ep secretion. The continuing Ep secretory response to the experimentally-induced anemia in this study may correspond to the clinical situation in which elevation of the Ep level is proportional to the severity of the anemia. \textsuperscript{1} Although some studies of the time course of the Ep response are consistent with our data, \textsuperscript{25,26} others have shown an early peak and rapid falloff of Ep levels by 24 hours. \textsuperscript{24,25,27,28} These differences may be partially explained by differences in the various methods used to induce hypoxic stress (eg, phlebotomy, phenylhydra-
zine-induced anemia, hypoxia, and irone deficiency anemia). Miller et al and Cohen et al have further suggested that acid-base disturbances associated with both hypoxia and volume depletion could play a role in the early fall in Ep levels after stimulation.\textsuperscript{27-29}

This study demonstrates the existence of a very sensitive system for Ep modulation in the rat. The immediate secretory response to anemic stress and the limitation of distribution to the plasma compartment work to initiate a rapid bone marrow response to changes in oxygen delivery. The relatively slow rate of clearance maintains stimulation of a slowly responsive end organ.

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