The In Vivo Metabolism of Recombinant Human Erythropoietin in the Rat

By Jerry L. Spivak and Beth B. Hogans

We compared the in vivo plasma clearance and organ accumulation in anesthetized rats of $^{125}$I-labeled, recombinant human erythropoietin and $^{131}$I-labeled, desialated recombinant erythropoietin. The immediate volume of distribution of $^{125}$I-labeled, recombinant erythropoietin approximated that of the plasma volume. Its plasma clearance was multiexponential, with an initial rapid distribution phase ($t_{1/2} = 53$ minutes) and a slower elimination phase ($t_{1/2} = 180$ minutes). Organ accumulation of labeled recombinant erythropoietin, as compared with $^{125}$I-labeled human albumin, was negligible until 30 minutes after injection when small amounts appeared in the kidneys and bone marrow. Only 24% of the $^{125}$I-labeled, desialated recombinant erythropoietin was recovered immediately after injection, and 96% of the hormone was cleared from the plasma with a $t_{1/2}$ of 2.0 minutes. The bulk of the desialated hormone accumulated in the liver where it was rapidly catabolized and its breakdown products released back into the plasma. Significantly, in contrast to unmodified erythropoietin, there was also early accumulation of desialated hormone in the kidneys, marrow, and spleen. Desialated orosomucoid but not orosomucoid, yeast mannann, or dextran sulfate 600 inhibited the rapid plasma clearance and hepatic accumulation of desialated erythropoietin. Oxidation of the desialated hormone restored its plasma recovery and clearance to normal but rendered it biologically inactive, and accumulation in organs other than the kidney was negligible.

ERYTHROPOIETIN, the hormone that regulates erythropoiesis, has been purified and molecularly cloned and the recombinant protein successfully used to correct anemia in patients with end-stage renal disease. In spite of these remarkable achievements, however, little is known about the in vivo metabolism of the hormone. Erythropoietin, which has a molecular weight (mol wt) of 30,400, is heavily glycosylated and is particularly rich in sialic acid residues. The sialic acid residues of the hormone are not required for the expression of biologic activity in vitro but are required for its expression in vivo. It has generally been assumed, based on experiments using crude preparations of sheep plasma erythropoietin and an in vivo bioassay, that the hormone’s sialic acid residues serve to prevent its removal prematurely from the circulation by the liver. This assumption has not been examined directly, nor have there been any studies of the in vivo metabolism of recombinant human erythropoietin. In this paper, we describe the plasma clearance and organ distribution of pure, recombinant human erythropoietin and its desialated derivative in the rat.

MATERIALS AND METHODS

Animals. Male albino Sprague-Dawley rats weighing 200 to 350 g were obtained from Harlan Industries (Indianapolis), and RJDJF, female mice weighing 20 to 35 g were obtained from the Jackson Laboratories (Bar Harbor, ME). The animals were housed in groups of five and fed standard chow and water ad libitum.

Erythropoietin. Pure recombinant human erythropoietin produced in Chinese hamster ovary cells (Amgen, Thousand Oaks, CA; specific activity, 125,000 U/mg protein) was used for the in vivo plasma clearance studies. The erythropoietin was iodinated by the lactoperoxidase technique using commercially available reagents (Enzymo-Beads, Bio-Rad Laboratories, Richmond, CA, and carrier-free $^{125}$I, Amersham Corp, Arlington Heights, IL) to a specific activity of 33 to 100 $\mu$Ci/µg protein. On average, 80% of the radioactivity in these preparations was precipitated by ice-cold 10% trichloroacetic acid (TCA). The quality of the iodinated erythropoietin preparations was monitored most easily by sodium dodecyl sulfate (SDS)-urea/7.5% polyacrylamide gel electrophoresis using tube gels and counting 1-mm gel slices in a gamma counter as previously described or with SDS/12.5% polyacrylamide slab gels and autoradiography. Human serum albumin labeled with $^{125}$I (specific activity, 8.3 $\mu$Ci/µg protein, Mallinckrodt, St Louis) was used for in vivo plasma clearance studies.

Desialation of erythropoietin. Once labeled with $^{125}$I, the erythropoietin was desialated chemically by heating at 80°C for 60 minutes in 0.1 mol/L HC1. The solution containing the erythropoietin was adjusted to pH 7.4 with 0.1 mol/L NaOH and 0.01 mol/L sodium phosphate, pH 7.0, and dialyzed for 18 hours against phosphate-buffered saline (PBS), pH 7.4, before use. Recovery of $^{125}$I-labeled, desialated erythropoietin was 91% as determined by acid-precipitable radioactivity. On the basis of a sialic acid content of 13% and the electrophoretic behavior of the protein in SDS-urea/polyacrylamide gels, desialation was essentially complete (Fig 1).

In some experiments, the iodinated erythropoietin preparation was desialated by using immobilized neuraminidase (Clostridium perfringens, Type VI, Sigma Chemical Co, St Louis). The labeled erythropoietin, in PBS, pH 7.4, was applied to a 1 mL agarose-bound neuraminidase—containing column preequilibrated with PBS, pH 7.4; column flow was then stopped and the column incubated for 30 minutes at 37°C. Following incubation, the erythropoietin solution was washed from the column with PBS and concentrated to its original volume. Recovery of erythropoietin from the agarose-neuraminidase columns was 72% as determined by recovery of acid-precipitable radioactive material.

Oxidation of desialated erythropoietin. To oxidize its penultimate galactose residues, erythropoietin desialated by exposure to neuraminidase was incubated in the dark at 4°C in the presence of 0.03 mol/L sodium metaperiodate and 0.1 mol/L sodium acetate, pH 5.0. After 30 minutes, the reaction was quenched by the addition of 0.05 vol of ethylene glycol and incubation for 30 minutes. The solution containing the desialated, oxidized erythropoietin was then dialyzed against PBS and 1% bovine serum albumin for 18 hours.

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with several changes of the dialysis fluid. The recovery of labeled erythropoietin following this procedure, as determined by acid-precipitable radioactivity, was 43%.

*Plasma clearance studies.* Rats were anesthetized by intraperitoneal injection of 40 mg/kg sodium pentobarbital (Abbott Laboratories, North Chicago, IL) and placed on a heating pad to maintain their body temperature between 37°C and 38°C during the course of the clearance study. Body temperature was monitored rectally with a digital thermometer. The labeled protein to be studied was injected as a bolus in a total volume of 0.3 to 0.5 mL into the femoral vein. At selected time intervals, 0.2-mL aliquots of blood were collected from the tail veins into heparin and centrifuged to obtain cell-free plasma. Total radioactivity and radioactivity that was precipitable by ice-cold 10% TCA was measured for each sample. Total injected radioactivity was established by subtracting the radioactivity remaining in the needle and syringe from the total radioactivity before injection. The plasma volume for each rat was calculated from its actual body weight by using a value of 3.93 mL/100 g body weight for the plasma volume of the rat.18 On average, approximately $2 \times 10^6$ cpm was injected for each clearance study, which represents 22 to 90 ng (2.7 to 11.2 units) of erythropoietin.

For a determination of the tissue accumulation of labeled proteins, rats were killed by cervical dislocation at selected time intervals after injection and the liver, spleen, and kidneys removed, weighed, and homogenized in 20, 5, and 5 mL of PBS, pH 7.4, respectively. Both femurs were also removed and stripped of muscle and connective tissue and the ends cut off to collect the marrow, which was weighed and homogenized in 2 mL of PBS, pH 7.4. Aliquots of each homogenate were counted in a gamma counter to measure total radioactivity, mixed with an equal volume of ice-cold 20% TCA, and incubated on ice for 15 minutes. The precipitate was harvested for gamma counting by centrifugation at 2,700 g for ten minutes at 4°C. A simultaneously obtained plasma sample at each time interval was also processed for total and acid-precipitable radioactivity.

*Bioassay of erythropoietin.* The in vitro incorporation of triitated thymidine (3H-Tdr) by splenic erythroblasts17 was used as a measure of the biologic activity of both native and chemically or enzymatically modified erythropoietin preparations. Briefly, BDF1 mice received an intraperitoneal injection on two consecutive days of 60 mg/kg of neutralized phenylhydrazine to induce anemia. Four days after the initial injection, the mice were killed and the spleens removed for preparation of single-cell suspensions. The isolated spleen cells were plated at 2.5 x 10^6 cells/well in a 96-well microtiter dish (Linbro Scientific Co, New Haven, CT) in α medium with 10% fetal calf serum, 2 mmol/L β-mercaptoethanol and the sample to be tested in a final volume of 0.1 mL. The cells were incubated at 37°C in a 95% air–5% CO₂ atmosphere for 24 hours, at which time 1 μCi 3H-Tdr (specific activity, 42 to 52 Ci/mmoll, Amersham) was added in a volume of 10 μL. After an additional four hours of incubation, the cells were harvested on prewet (PBS, pH 7.4) glass fiber filters and washed once with PBS and once with ice-cold 10% TCA followed by PBS and then 95% ethanol. The filters were dried under infrared light, and the radioactivity remaining on the filters was measured.

Statistical analysis. The plasma clearance of the labeled albumin and the various erythropoietin preparations was analyzed by the method of residuals by using a standard curve-stripping technique.18 The plasma clearance of albumin was defined by a single exponential indicating a single compartment of distribution. For erythropoietin, however, the plasma clearance curve was complex and best defined by a two-compartment model according to the equation

$$C(t) = Ae^{-at} + Be^{-bt}$$

where $C(t)$ represents the concentration of labeled protein at time $t$ and $A$, $α$, $B$, and $β$ are the paired constants for compartments 1 and 2, respectively.

The half life, $(t_{1/2})$, of the albumin and the various erythropoietin preparations in each compartment was calculated from the formula $t_{1/2} = -0.693/slope$.

### Table 1. In Vitro Bioassay of Iodinated, Intact or Desialated Recombinant Human Erythropoietin

<table>
<thead>
<tr>
<th>Additions to Cultures</th>
<th>3H-Tdr Incorporation Into Splenic Erythroblasts (cpm/μg x 10⁶ Cells)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8,000 ± 600</td>
</tr>
<tr>
<td>r Erythropoietin</td>
<td>75,000 ± 5,000</td>
</tr>
<tr>
<td>(0.5 U/mL)</td>
<td>75,000 ± 4,000</td>
</tr>
<tr>
<td>125I-r Erythropoietin</td>
<td>158,000 ± 17,000</td>
</tr>
<tr>
<td>(0.5 U/mL)</td>
<td>138,000 ± 8,000</td>
</tr>
</tbody>
</table>

*Pure, carrier-free, recombinant human erythropoietin was iodinated by the lactoperoxidase technique and desialated by acid hydrolysis as described in Materials and Methods. The biologic activity of the various erythropoietin preparations was tested by using 3H-Tdr incorporation into mouse splenic erythroblasts as described in Materials and Methods. Abbreviation: r, recombinant.

*Mean ± SEM.
where the slope was determined by least-squares regression for ln $C_v t$.

RESULTS

Effect of iodination on the electrophoretic behavior and biologic activity of unmodified and desialated recombinant erythropoietin. Iodination of carrier-free, pure recombinant human erythropoietin by the lactoperoxidase technique yielded a single band of radiolabeled protein when analyzed by either electrophoresis in SDS-urea/7.5% polyacrylamide tube gels or 12.5% SDS–polyacrylamide slab gels (data not shown). Desialation, however, changed the apparent mol wt of the recombinant erythropoietin from 34,000 to 29,000 (Fig 1). Based on a sialic content of approximately 13%, desialation was essentially complete. Neither the iodination procedure nor desialation by acid hydrolysis impaired the biologic activity of the hormone as measured by a $^3$H-TdR incorporation assay using mouse splenic erythroblasts (Table 1).
Fig 3. (A) Plasma clearance of iodinated, chemically desialated recombinant human erythropoietin. The labeled hormone was injected into the femoral vein of an anesthetized rat and blood samples obtained at the indicated time intervals from the tail veins for determination of total and acid-precipitable radioactivity as described in Materials and Methods. Similar results were obtained in six separate experiments (Table 2). (B) Plasma clearance of iodinated, enzymatically desialated recombinant human erythropoietin. Similar results were obtained in three separate experiments (Table 2).

Table 2. Plasma Clearance of Unmodified and Desialated Recombinant Human Erythropoietin in the Rat

<table>
<thead>
<tr>
<th>Hormone Preparation</th>
<th>Percent Recovery (Acid-Precipitable Fraction)</th>
<th>Distribution Phase $t_{1/2}$ (min)$^*$</th>
<th>Elimination Phase $t_{1/2}$ (min)$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified</td>
<td>88.0 ± 7.5 (66.3-100)</td>
<td>53.1 ± 5.6 (30.2-81.5)</td>
<td>180.1 ± 16.3 (124-258)</td>
</tr>
<tr>
<td>Desialated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemically</td>
<td>23.7 ± 3.3 (12.9-39.4)</td>
<td>2.0 ± 0.4 (0.7-3.9)</td>
<td>89.7 ± 22.5 (36.8-159)</td>
</tr>
<tr>
<td>Enzymatically</td>
<td>25.2 ± 6.0 (19.2-37.3)</td>
<td>4.9 ± 1.3 (3.2-7.5)</td>
<td>90.3 ± 18.3 (55-117)</td>
</tr>
</tbody>
</table>

$^*$Mean ± SEM.

Plasma clearance of iodinated human albumin and recombinant erythropoietin. As a control for the behavior of a human plasma protein in the circulation of the rat, we examined the plasma clearance of $^{125}$I-labeled human albumin. All of the injected acid-precipitable radioactivity was present in the plasma within two minutes of injection, which indicated that the initial volume of distribution of the albumin approximated that of the rat's plasma volume. As shown in Fig 2A, the plasma clearance of the labeled albumin could be described by a single exponential with a $t_{1/2}$ of 210 minutes. During the period of observation, there was essentially no increase in acid-soluble plasma radioactivity. When $^{125}$I-labeled, recombinant human erythropoietin was injected intravenously (IV), the average recovery of acid-precipitable radioactivity at two minutes after injection was 88% (range, 66% to 100%), thus indicating that its volume of distribution also approximated that of the rat's plasma volume. However, in contrast to albumin, the clearance of the labeled erythropoietin was complex (Fig 2B). When analyzed by the method of residuals,$^9$ the plasma clearance had two components: an initially rapid distribution phase...
with a $t_{1/2}$ of 53 minutes (range, 30.2 to 81.5) and a slower elimination phase with a $t_{1/2}$ of 180 minutes (range, 124 to 258) (Table 2). During the period of observation, over 90% of the plasma radioactivity remained acid precipitable, and the quantity of hormone injected (22 to 90 ng) did not influence the clearance kinetics.

**Plasma clearance of iodinated, desialated recombinant erythropoietin.** Figure 3A illustrates the plasma clearance of chemically desialated recombinant erythropoietin. As in the case of intact recombinant erythropoietin, the plasma clearance was complex, but the behavior of the desialated derivative differed markedly not only with respect to kinetics but also recovery and metabolism. Within two minutes after injection, on average only 24% of the acid-precipitable radioactivity injected could be recovered in the plasma, and as shown in Table 2, the initial component of plasma clearance was 25-fold faster than that of intact erythropoietin. The in vivo behavior of the desialated protein was not a consequence of the method of desialation since, as shown in Fig 3B and Table 2, the plasma clearance of enzymatically desialated erythropoietin was identical. Furthermore, the effects could not be ascribed to nonspecific proteolysis since, as shown in Fig 3C, the initial recovery of the acid-precipitable fraction of trypsinized erythropoietin was threefold greater (74%) and the initial phase of plasma clearance $t_{1/2}$ (24.4 minutes) was 12-fold greater than for the two desialated hormone preparations (data not shown).

As illustrated in Fig 3A and B, the metabolism of desialated hormone was also markedly different from unmodified recombinant erythropoietin. The initial rapid increase in plasma acid-precipitable radioactivity was followed by a rapid increase in acid-soluble plasma radioactivity that was maximal at 30 minutes, accounted for 57% of total plasma radioactivity, and appeared to reach a steady state over the period of observation. The acid-precipitable material remaining in the plasma at this time represented on average only 4% of the injected labeled protein.

**Plasma clearance of iodinated, desialated, oxidized recombinant erythropoietin.** To determine whether the plasma clearance of the desialated erythropoietin was related to exposure of the penultimate galactose residues, two experiments were performed. First, the exposed galactose residues of the enzymatically desialated erythropoietin were oxidized with sodium metaperiodate. As shown in Fig 4, oxidation of the exposed galactose residues restored both the recovery (100%) and plasma clearance ($\alpha = 66.7 \pm 4.2$ and $\beta =$...
METABOLISM OF RECOMBINANT ERYTHROPOIETIN

286.3 ± 18.3 minutes) of the erythropoietin to normal. As shown in Table 3, however, oxidation of the exposed galactose residues was associated with a loss of biologic activity.

Next, rats were injected simultaneously with iodinated, desialated recombinant erythropoietin and the unlabeled, desialated derivative of another human plasma protein, orosomucoid. As shown in Fig 5A, 1 mg of orosomucoid did not alter the plasma clearance kinetics of desialated erythropoietin, while 1 mg of asialoorosomucoid increased the recovery of the desialated erythropoietin from 24% to 74% and retarded its plasma clearance and the appearance of acid-soluble radioactivity in the plasma but not its magnitude (Fig 5B). Neither unmodified or desialated orosomucoid influenced the plasma clearance of unmodified recombinant erythropoietin (data not shown).

Effect of reticuloendothelial system blockade on the plasma clearance of iodinated, desialated recombinant erythropoietin. Although the previous experiments suggested that desialated erythropoietin was removed from the circulation through recognition of exposed galactose residues, presumably by hepatocytes, we also examined the possibility that plasma clearance of the hormone might be mediated by either phagocytic cells or cells with receptors for other sugars such as mannose or N-acetyl glucosamine, both of which are abundant in erythropoietin. To this end, we first examined the plasma clearance of labeled desialated erythropoietin in animals injected IV two hours before with

Table 4. Effect of Yeast Mannan or Dextran Sulfate 500 on the Plasma Clearance of Desialated, Recombinant Human Erythropoietin in the Rat

<table>
<thead>
<tr>
<th>Competitive Inhibitor</th>
<th>Distribution Phase (T1/2, min)*</th>
<th>Elimination Phase (T1/2, min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.9</td>
<td>122</td>
</tr>
<tr>
<td>Yeast mannan</td>
<td>4.8</td>
<td>113</td>
</tr>
<tr>
<td>Dextran sulfate 500</td>
<td>3.3</td>
<td>108</td>
</tr>
</tbody>
</table>

Rats were injected with labeled desialated erythropoietin alone or with the erythropoietin preparation and yeast mannan. Dextran sulfate 500 was injected two hours before injecting the desialated erythropoietin. Plasma clearances were determined as described in Materials and Methods.

*Mean ± SEM.

Fig 5. (A) Effect of an IV injection of 1 mg of orosomucoid on the plasma clearance and metabolism of 125I-labeled, desialated recombinant human erythropoietin in the rat. Similar results were obtained in two separate experiments. (B) Effect of an IV injection of 1 mg of asialoorosomucoid on the plasma clearance and metabolism of labeled, desialated recombinant human erythropoietin in the rat. Similar results were obtained in two separate experiments.
dextran sulfate 500 to block the reticuloendothelial system. As shown in Table 4, reticuloendothelial cell blockade had no effect on the plasma clearance of the desialated hormone. Injection of yeast mannan simultaneously with desialated erythropoietin also did not influence the plasma clearance of the hormone, thus indicating that neither N-acetyl glucosamine nor mannose residues were involved in the plasma clearance of the hormone.

Organ distribution of iodinated, unmodified and desialated recombinant erythropoietin. To study the organ distribution of labeled unmodified or desialated erythropoietin, we injected animals with these proteins and at selected time intervals killed them and obtained samples of plasma, liver, kidneys, spleen, and femoral bone marrow for the measurement of acid-precipitable radioactivity. Simultaneous studies were also performed with 125I-human albumin to control for contaminating plasma radioactivity. As shown in Fig 6, over a period of 30 minutes the uptake of iodinated, unmodified recombinant erythropoietin by the liver was not different from that of iodinated albumin, thus indicating that the initial rapid distribution component of its plasma clearance was not due to hepatic sequestration. By contrast, for labeled desialated erythropoietin (Fig 7), there was a reciprocal increase in hepatic acid-precipitable radioactivity as plasma acid-precipitable radioactivity declined. At the peak organ accumulation, 87% of the acid-precipitable radioactivity was in the liver, 2.8% in the kidneys, 0.6% in the spleen, and 0.3% in the bone marrow (means of data pooled from two experiments). Following the uptake of radiolabeled protein by the liver, there was a rapid increase in the proportion of intracellular radioactivity that was acid soluble, and this was followed by a similar increase in plasma acid-soluble radioactivity after a delay of approximately 14 minutes.

In Fig 8, the accumulation of acid-precipitable radioactivity for labeled, unmodified and desialated erythropoietin in various organs in addition to the liver is illustrated and expressed as a fraction of the simultaneous plasma radioactivity. An increase in this ratio above 1.0 indicates tissue accumulation of the labeled protein. Labeled human albumin, which exhibited no organ-specific accumulation, served as a control for organ contamination by plasma radioactivity. Within two minutes after the injection, there was a twofold increase in liver accumulation of desialated erythropoietin as compared with plasma. Accumulation of radioactivity in the liver was reduced by the simultaneous injection of desialated orosomucoid, which resulted in an increase in the accumulation of the desialated hormone in the kidneys, a process that was more marked with time not only in the presence of desialated orosomucoid but also in its absence. With time, unmodified recombinant erythropoietin also accumulated in the kidneys and bone marrow but not to the same relative
extent as its desialated derivative. Interestingly, splenic accumulation of desialated erythropoietin was similar to that of the bone marrow.

Organ distribution of iodinated, desialated, oxidized erythropoietin. To examine whether determinants other than sialic acid, galactose, or N-acetyl galactosamine residues might be involved in the tissue recognition and uptake of erythropoietin, the organ distribution of recombinant desialated, oxidized erythropoietin was measured. As shown in Table 5, in comparison to unmodified recombinant erythropoietin, tissue accumulation of the desialated, oxidized erythropoietin was negligible except for some uptake by the kidneys 30 minutes after injection.

**DISCUSSION**

The carbohydrate residues of glycoproteins subserve a variety of functions important for their biologic activity such as intracellular transport, metabolic stability, receptor recognition, and signal transduction. Erythropoietin is a heavily glycosylated protein with three N- and one O-linked carbohydrate side chains representing over 30% of its mass. Recent studies indicate that both the carbohydrate content and side-chain structure of human urine erythropoietin and recombinant erythropoietin as well as their amino acid composition are identical. Goldwasser and Kung were the first to demonstrate that desialylation of erythropoietin was associated with the loss of in vivo biologic activity while in vitro biologic activity remained intact. From experiments using desialated crude sheep plasma erythropoietin and asialoorosomucoid or stachyose as competitive inhibitors, it was concluded that the sialic acid residues of the hormone prevented its premature removal from the circulation by hepatic galactosyl receptors. In the present study, we sought to examine this issue directly by using pure recombinant erythropoietin.

The initial volume of distribution of IV administered iodinated recombinant erythropoietin approximated that of the plasma volume. Thereafter, the labeled hormone was cleared from the plasma in a manner similar to that observed for both pure human urine erythropoietin and homologous rat plasma erythropoietin.

When using labeled albumin as a marker for the vascular space, there was insufficient accumulation of recombinant erythropoietin in the liver, kidney, spleen, or bone marrow to account for the initial component of the plasma disappearance curve. This suggests that this component was due to distribution of the hormone between the plasma and the extravascular space from which elimination subsequently occurred as defined by the second slower component of the disappearance curve. Since there was little accumulation of acid-soluble material in the plasma during the period of observation, neither the distribution phase nor the elimination phase was associated with significant catabolism of the erythropoietin. Because the quantity of hormone injected exceeded the quantity of circulating endogenous erythropoietin by 13- to 50-fold, it is unlikely that significant catabolism would have been missed, and since the rate of plasma clearance was unaffected by the quantity of hormone injected, it appears that catabolism of the hormone was independent of its concentration.

Accumulation of iodinated human recombinant erythropoietin by the kidney was remarkably similar to that of labeled human urine erythropoietin at two and 180 minutes after injection when calculated on the basis of total radioactivity (0.20 and 0.48 cpm · g tissue−1 · cpm · mL plasma−1)
for recombinant erythropoietin as compared with 0.23 and 0.47 cpm · g tissue−1/cpm · mL plasma−1, respectively, for urine erythropoietin). This suggests that the kidney is involved to a small extent in the catabolism of the hormone. This contention is also supported by the studies with desialated, oxidized recombinant erythropoietin, which also appeared to accumulate in the kidneys as opposed to other organs.

Desialation markedly altered the plasma clearance kinetics of recombinant erythropoietin. In contrast to the fully sialated hormone, only 24% of the desialated erythropoietin could be recovered within two minutes after injection, and the initial plasma clearance of the desialated hormone was 25-fold faster than for unmodified erythropoietin. The bulk of the desialated erythropoietin was sequestered in the liver where it was rapidly metabolized.

The uptake of desialated, recombinant erythropoietin by the liver appeared to be mediated by hepatic galactosyl receptors. Thus, oxidation of the penultimate galactose residues of desialated erythropoietin blocked hepatic sequestration of the hormone and restored both its recovery and plasma clearance kinetics to normal. Asialoorosomucoid but not its fully sialated derivative, orosomucoid, had a similar but less marked effect. Yeast mannan also did not alter the plasma clearance of desialated erythropoietin, thus indicating that hepatic mannosyl/N-acetyl glucosamine receptors were not involved in its metabolism.

The pattern of organ accumulation of desialated erythropoietin differed significantly from that of the intact hormone. Thus, the relative accumulation of desialated erythropoietin in the kidneys, bone marrow, and spleen was greater than that of unmodified erythropoietin. Competition experiments with asialoorosomucoid emphasized these differences. Although accumulation of the desialated hormone in the marrow was not unexpected considering the reduction in the charge hindrance of the molecule and the documented presence of galactosyl receptors on marrow endothelial cells,26,27 its accumulation in the spleen was unexpected. Since neither asialoorosomucoid nor prior administration of dextran sulfate 500 influenced the organ clearance of desialated erythropoietin, it is unlikely that splenic accumulation of the modified hormone was a consequence of either galactose-specific receptors or phagocytosis. Rather, since the spleen is an erythropoietic organ in rodents, the accumulation of erythropoietin there may reflect the presence of a pool of erythropoietic progenitor cells.

Another unexplained finding of the present study was the behavior of the desialated, oxidized recombinant erythropoietin, which had the same plasma clearance kinetics as unmodified recombinant erythropoietin even though it lacked biologic activity. By contrast, desialated, oxidized orosomucoid is cleared rapidly from the plasma by liver mannosyl/N-acetyl glucosamine receptors.28 This suggests that determinants other than carbohydrate residues may serve as recognition sites for the metabolism of erythropoietin.

From the data in this and previous studies, it appears that the plasma clearance of erythropoietin is best described by a two-compartment model. What is unclear, however, is the mechanism for the difference in the initial plasma clearance of human recombinant or urine erythropoietin26 and rat plasma erythropoietin synthesized during severe anemia.25 Endothelial cells have galactosyl receptors,26,27 and it is possible that erythropoietin synthesized under stress is incompletely sialated and, therefore, more rapidly cleared from the plasma. This would explain the rapid initial clearance observed by Steinberg et al for newly synthesized rat plasma erythropoietin.25 Partially desialated erythropoietin would not only be more rapidly delivered to the bone marrow, but excess hormone would be rapidly cleared by the liver. Selective differences between hepatic and endothelial cell galactosyl receptors could also explain the differential organ uptake of desialated erythropoietin in the presence of the competitive inhibitor, asialoorosomucoid. The possibility that partially desialated erythropoietin is the most active form of the hormone has therapeutic implications.

ACKNOWLEDGMENT

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