IMMUNOLOGICAL RESPONSE TO REPEATED ADMINISTRATION OF RECOMBINANT HUMAN ERYTHROPOIETIN IN RATS

Biphasic Effect on Its Pharmacokinetics

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ABSTRACT:

We studied changes in the pharmacokinetics of 125I-recombinant human erythropoietin (125I-rh-EPO) after repeated subcutaneous administration once a week for 4 weeks. The plasma level of trichloroacetic acid-precipitable radioactivity after the fourth administration of 125I-rh-EPO was minimal in 8 of 10 rats, whereas in the other two rats, the plasma level was almost the same or somewhat higher than that in control rats that had received the vehicle solution 3 times instead of the first three sequential administrations. Antibody against rh-EPO in serum was detected in all 10 rats receiving multiple administrations of 125I-rh-EPO. However, the binding capacity for 125I-rh-EPO in the latter two rats, assessed by an in vitro serum binding study, was lower than for the other eight rats, suggesting that the antibody level in these two was lower. The effect of intravenous preinjection of various volumes of anti-rh-EPO antiserum on the pharmacokinetics of 125I-rh-EPO was examined. The half-life in the β-phase was prolonged at lower doses of antiserum. When the pretreatment dose of antiserum was further increased, the half-life in the β-phase rather shortened and the total body clearance (CLtotal) increased. These results suggest that repeated administration of rh-EPO induces the production of antibody against rh-EPO that affects the pharmacokinetics of rh-EPO in a biphasic manner; CLtotal was reduced when a small amount of antibody was produced, and CLtotal was increased when a large amount of antibody was produced.

EPO is one of the hematopoietic growth factors, such as granulocyte colony-stimulating factor and granulocyte macrophage colony stimulating factor; it is a 34 kDa glycoprotein mainly produced by the kidney, and it stimulates the proliferation and differentiation of colony-forming unit erythroid (1). rh-EPO is currently used as a treatment for anemia in patients with endstage renal diseases. Such patients with renal failure have anemia because of a low production of EPO. Treatment of rh-EPO improves this type of anemia in such patients (2).

It has been shown that the pharmacokinetics of many biologically active polypeptides exhibits nonlinearity due to saturation of their disposition mainly governed by RME (3, 4). In our previous study, we showed the extent of the contribution of RME to the nonlinear elimination of rh-EPO from the circulation in rats (5). Our study also indicated that repeated administration of rh-EPO caused up- and downregulation of receptor-mediated uptake by target tissues (5). Not only the saturation of receptor binding and/or receptor-mediated uptake, but also such up- and downregulation of the receptor may affect the pharmacokinetics of rh-EPO.

Since our previous study (5) was performed over a short period (<1 week), specific antibody against the injected rh-EPO might have not been produced. However, in the preclinical studies performed for a longer period in experimental animals, it may be possible that specific antibody is produced against the injected biologically active polypeptides because recombinant human polypeptides are used in the experimental animals. It is possible that the production of antibody against them causes a reduction in their pharmacological effect. In fact, a significant reduction in pharmacological effect was observed after multiple administration of rh-EPO (6). To evaluate the pharmacological and toxicological effects of such an antibody in vivo, it is important to understand the pharmacokinetics of these peptides in the presence of antibody. The present study showed that the production of antibody against rh-EPO exhibits interindividual differences in rats, producing a biphasic effect on the pharmacokinetics of rh-EPO.

Materials and Methods

rh-EPO was produced using Chinese hamster ovarian cells transfected with expression vector harboring the human erythropoietin cDNA at Production Technology Laboratories, Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). 125I-sodium iodine (17.4 Ci/mg) was obtained from Amersham plc (Amersham, UK). Iodo-Gen (1,3,6-tetrachloro-3,6-diphenylglycouril) was obtained from Pierce Chemical Company (Rockford, IL). Protein A was obtained from Behring Diagnostics (La Jolla, CA). All other reagents were obtained as the purest grade available.

Radiolabeling. 125I-rh-EPO was prepared by the Iodo-Gen method described previously (7). The specific radioactivity was 6.87 µCi/µg as determined by gel filtration assay. The radiochemical purity was 95.2% as determined by gel filtration.
Animals. Male Sprague-Dawley rats (JCL-SD, Clea Japan, Inc., Tokyo, Japan) were allowed to acclimatize to the laboratory environment for 1 week, and then the experiment was started at 7 weeks of age when the animals had a body weight of 240–290 g. Animal rooms were maintained at constant ambient temperature and relative humidity of 24°C and 55%, respectively, throughout the experimental period. A standard rodent feed in pellet form (CE-2, Clea Japan, Inc.) and tap water ad libitum were available throughout the study.

Multiple Administration Study. 125I-rh-EPO was administered subcutaneously to the dorsal back region of 10 animals at dose of 1 μg/kg polypeptide equivalent to rh-EPO once a week for 4 weeks. The control rats received the vehicle solution 3 times instead of the first three administrations (from first to third) of 125I-rh-EPO. Blood was withdrawn from each rat through the tail vein into a heparinized tube at 4, 8, 10, 12, 18, 24, 34, and 48 hr after injection of 125I-rh-EPO. Blood was centrifuged at 15,000 rpm for 3 min. Anti-rh-EPO antiserum was added to 100 μl of plasma. The mixture was allowed to stand for 4 hr at room temperature. They were then incubated for 1 hr with 400 μl of Bio Mag Goat Anti-Rat IgG (Advanced Magnetics, Inc., Cambridge, MA) and then centrifuged for 10 min at 3,000 rpm. Five hundred microliters of supernatant and the precipitated fraction were counted in a gamma counter. Nonspecific binding was measured by the aforementioned procedure without addition of antiserum.

Gel Filtration of Plasma Samples. Gel filtration of plasma samples was performed on a column of TSK gel G3000SWXL at a flow rate of 0.5 ml/min with 0.1 M phosphate buffer (pH 7.2) was added to 100 μl of diluted (1,000-fold) anti-rh-EPO antiserum. The mixtures were allowed to stand for 4 hr at room temperature. Then they were then incubated for 1 hr with 400 μl of Bio Mag Goat Anti-Rat IgG (Advanced Magnetics, Inc., Cambridge, MA) and then centrifuged for 10 min at 3,000 rpm. Five hundred microliters of supernatant and the precipitated fraction were counted in a gamma counter. Nonspecific binding was measured by the aforementioned procedure without addition of antiserum.

Effect of Antiserum on Pharmacokinetics of rh-EPO. Anti-rh-EPO antiserum was obtained from rabbits that had been immunized with 100 μg of rh-EPO. Anti-rh-EPO antiserum was added to the plasma samples at a concentration of 1:10. The mixtures were allowed to stand for 4 hr at room temperature and then centrifuged for 5 min at 3,000 rpm, followed by measurement of the radioactivity in the TCA-precipitated fractions. For immunoprecipitation assay, 200 μl of plasma. The reaction mixture was allowed to stand for 10 min at room temperature and then centrifuged for 5 min at 3,000 rpm, followed by measurement of the radioactivity in the TCA-precipitated fractions. For immunoprecipitation assay, 200 μl of phosphate buffer (pH 7.2) was added to 100 μl of diluted (1,000-fold) anti-rh-EPO antiserum. The mixtures were allowed to stand for 4 hr at room temperature. They were then incubated for 1 hr with 400 μl of Bio Mag Goat Anti-Rat IgG (Advanced Magnetics, Inc., Cambridge, MA) and then centrifuged for 10 min at 3,000 rpm. Five hundred microliters of supernatant and the precipitated fraction were counted in a gamma counter. Nonspecific binding was measured by the aforementioned procedure without addition of antiserum.

TCA Precipitation Assay and Immunoprecipitation Assay. For a TCA precipitation assay, 200 μl of 25% TCA solution and 150 μl of 1 M NaF were added to 50 μl of plasma. The reaction mixture was allowed to stand for 10 min at room temperature and then centrifuged for 5 min at 3,000 rpm, followed by measurement of the radioactivity in the TCA-precipitated fractions. For immunoprecipitation assay, 200 μl of phosphate buffer and 200 μl of diluted (100-fold) anti-rabbit serum were added to 50 μl of plasma. The mixture was allowed to stand for 16 hr at room temperature and then incubated for 1 hr with 100 μl of a suspension containing 5% protein A. Then 200 μl of 0.9% NaCl was added after centrifugation at 15,000 rpm using a microcentrifuge for 3 min. The supernatant was removed, and the precipitated fraction was counted in a gamma counter.

Data Analysis. The plasma concentration data after intravenous administration were fitted to eq. 1 using a nonlinear regression program MULTI (9). The plasma concentration declined biexponentially and could be described by a two-compartment open model according to eq. 1:

\[ C = A \exp(-\alpha t) + B \exp(-\beta t), \] (1)

where \( C \) is the plasma concentration with time; and \( A, B, \alpha, \) and \( \beta \) correspond to the coefficients and exponents of the biexponential equation. Akaike’s information criteria was used to judge the appropriateness of the models (9). The calculated SD (percentage of coefficient of variation) for \( A, B, \alpha, \) and \( \beta \) was 12.0 ± 4.3%, 11.7 ± 6.6%, 27.2 ± 9.7%, and 14.6 ± 2.9% (mean ± SD), respectively. Half-lives in \( \alpha \) - and \( \beta \)-phases were calculated as In2/\( \alpha \) and In2/\( \beta \), respectively. The \( V_1 \) was calculated as dose/(A + B). The AUC was calculated by the trapezoidal rule with extrapolation to infinity. \( C_{\text{meas}} \) was calculated as dose/AUC. AUC \( \text{sub} \) was estimated by trapezoidal rule. The plasma concentration data at 18, 24, 34, and 48 hr after subcutaneous administration were fitted to \( C = A \exp(-\alpha t) \). Half-life was calculated as In2/\( \alpha \).

Statistical Method. Comparison of \( B_{\text{max}} \) was performed using Mann-Whitney test. Comparisons of pharmacokinetic parameters were performed using a one-way analysis of variance followed by Scheffé’s test. Statistical significance was taken as \( p < 0.05 \).

Results

The pharmacokinetics of 125I-rh-EPO was examined after the first and fourth subcutaneous administrations of 125I-rh-EPO in rats (fig. 1). In all rats, the plasma levels of immunoreactive radioactivity reached a maximum 10–12 hr after the first dose and declined with a half-life of ~10 hr (fig. 1). On the other hand, the plasma levels of TCA-precipitable radioactivity after the fourth administration of 125I-rh-EPO reached ~10 hr after the first dose and declined with a half-life of ~10 hr (fig. 1).
rh-EPO was minimal and increased little in any of the rats, except nos. 6 and 9, which exhibited an AUC after the administration comparable with both that in control rats and after the first administration (fig. 1). The plasma levels of TCA-precipitable radioactivity after administration of \( ^{125}\text{I-} \text{rh-EPO} \) in the control rats, which received the vehicle solution 3 times instead of three sequential administrations of \( ^{125}\text{I-} \text{rh-EPO} \), were almost identical to those of immunoreactive radioactivity after the first administration of \( ^{125}\text{I-} \text{rh-EPO} \) in the 10 rats (fig. 1).

The antibody against rh-EPO in serum was detected in all rats receiving multiple administration of \( ^{125}\text{I-} \text{rh-EPO} \), whereas in control rats no antibody was observed. The gel filtration chromatogram of plasma obtained from rat 9, in which the plasma concentration of TCA-precipitable radioactivity rose even after the fourth administration, showed that the most of the radioactivity was eluted in the higher molecular weight fraction (232–669 kDa) than free \( ^{125}\text{I-} \text{rh-EPO} \) (34 kDa) (fig. 2B). The gel filtration chromatogram of plasma obtained from rat 5, in which plasma concentration of TCA-precipitable radioactivity did not increase after the fourth administration, showed only one peak corresponding to free iodide ion and no peak corresponding to higher molecular weight material (fig. 2A). The gel filtration chromatogram of plasma obtained from control rats showed two peaks: the major one corresponding to \( ^{125}\text{I-} \text{rh-EPO} \) and the other, minor, one corresponding to iodide ion, respectively (fig. 2). Binding of \( ^{125}\text{I-} \text{rh-EPO} \) to immunoglobulin in individual rat serum was determined in the in vitro serum binding study using goat anti-rat IgG antibody (fig. 3). The obtained \( B_{\text{max}} \) values in rats in which plasma levels of radioactivity did not increase after the fourth administration (rats 1–5, 7, 8, and 10) were higher (\( \geq 490 \text{ ng/ml} \)) than those in the other two rats, nos. 6 and 9 (254 and 156 ng/ml, respectively) (table 1). In these two rats (nos. 6 and 9), the specific binding (\( B_{\text{max}}/K_d \)) was also much less (0.1 and 0.4, respectively) than in the other rats (0.9–6.8) (table 1). AUC\( _{0–48h} \) after the fourth administration was plotted against \( B_{\text{max}} \) (fig. 4). Only two rats (nos. 6 and 9) showed an AUC\( _{0–48h} \) comparable with the control level. The \( B_{\text{max}} \) values in these two rats were lower than those in the other eight rats showing smaller AUC\( _{0–48h} \) values (\( p < 0.05 \)) (fig. 4). Thus, there seems to exist a threshold for the \( B_{\text{max}} \) beyond which the AUC\( _{0–48h} \) suddenly fell as the \( B_{\text{max}} \) slightly increased (fig. 4).

Figure 5 shows plasma concentrations of TCA-precipitable radioactivity after intravenous administration of 0.1 \( \mu \text{g/kg} \) \( ^{125}\text{I-} \text{rh-EPO} \) to rats pretreated with anti-rh-EPO serum from rat 5 (0, 5, 50, and 500 \( \mu \text{L} \)). The \( t_{1/2b} \) was prolonged from 3.15 to 4.66 hr, and 7.30 hr as the volume of anti-rh-EPO antiserum increased from 0 to 5 and 50 \( \mu \text{L} \), respectively. However, \( t_{1/2b} \) was reduced to 3.81 hr in rats receiving 500 \( \mu \text{L} \) of anti-rh-EPO antiserum (table 2). \( CL_{\text{renal}} \) decreased significantly from 16.6 ml/hr/kg to 10.5 ml/hr/kg, and 6.70 ml/hr/kg as the volume of anti-rh-EPO antiserum increased from 0 to 5 and 50 \( \mu \text{L} \), respectively. However, \( CL_{\text{renal}} \) rather increased to 21.4 ml/hr/kg in rats receiving 500 \( \mu \text{L} \) of anti-rh-EPO antiserum (table 2). The \( V_c \) did not change after administration of anti-rh-EPO anti-serum (table 2).

**Discussion**

Recently, rapid advances in recombinant DNA technology have made it possible to use human biologically active polypeptides for therapeutic purposes. However, human polypeptide is administered to experimental animals in preclinical studies, and this may cause the production of antibody against it. In this study, we have shown that such production of antibody shows interindividual differences, and the effect of antibody on the pharmacokinetics of rh-EPO is biphasic, depending on the level of antibody in plasma.
half-life of TCA-precipitable radioactivity after administration of $^{125}$I-
BSA in rats that had been immunized with BSA to produce anti-BSA
antibody (IgG) was 4 min and significantly shorter than that in control
rats where the half-life was 24 hr (10). In addition, neutralizing
antibodies were produced in most of the rats receiving multiple
intramuscular administrations of rIFN-α2A for 28 days (see ref. 12),
and the plasma clearance of rIFN-α2A after its administration was
much larger in rats with a high antibody titer, compared with that in
the rats with a low antibody titer (12). However, Tagliaro et al
(11) reported that the plasma concentrations of eel calcitonin in patients
with the antibody against it after repeated treatment with eel calcitonin
were significantly greater than those in the patients without this
antibody. These findings also support the hypothesis that the effect of
antibody on the pharmacokinetics of polypeptides takes two forms:
case 1—an antigen is rapidly eliminated from the circulation; and case
2—an antigen becomes more stable in circulation.

Such an interindividual difference in the effect of antibody on the
pharmacokinetics of rh-EPO may come from different binding char-
acteristics between rh-EPO and antibody. Therefore, the plasma pro-
tein binding of $^{125}$I-rh-EPO was examined by gel filtration (fig. 2).
The radioactivity in plasma from rat 9 was found in the high molec-
ular fraction ($\geq 232–669$ kDa), corresponding to the dimer or trimer
of the immune complex, and the free form of $^{125}$I-rh-EPO (34 kDa)
was not observed (fig. 2). This result suggests that the $^{125}$I-rh-EPO is
stable in blood when $^{125}$I-rh-EPO exists as a dimer or trimer immune
complex (fig. 2). In addition, $B_{\text{max}}$ and specific binding ($B_{\text{max}}/K_d$)
for serum from rats 6 and 9 was much lower than that for other rats ($p<
0.05$) (table 1). Thus, rh-EPO is stable when the plasma level of
antibody is relatively lower. On the other hand, most of the radioac-
tivity was recovered as a degradation product in rat 5 (fig. 2), imply-
ing that the stability of $^{125}$I-rh-EPO is markedly lower in the circu-
lating blood of this rat. Antibody against rh-EPO was also detected in
the blood of rat 5, and the $B_{\text{max}}$ was much higher than that in rats 6
and 9 (table 1). Thus, although the immune complex could not be
detected in the gel filtration chromatogram (fig. 2), there is a high
level of antibody against rh-EPO in the blood of rat 5. These results
indicate that the plasma clearance of $^{125}$I-rh-EPO is large when the
antibody concentration is high, and the clearance is smaller when the
antibody concentration is low.

This hypothesis was also supported by the following finding.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>$K_d$</th>
<th>$B_{\text{max}}$</th>
<th>$B_{\text{max}}/K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>697 ± 47</td>
<td>3,270 ± 460</td>
<td>4.69</td>
</tr>
<tr>
<td>2</td>
<td>538 ± 165</td>
<td>490 ± 90</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>1,150 ± 260</td>
<td>1,360 ± 200</td>
<td>1.18</td>
</tr>
<tr>
<td>4</td>
<td>825 ± 121</td>
<td>900 ± 86</td>
<td>1.09</td>
</tr>
<tr>
<td>5</td>
<td>872 ± 89</td>
<td>4,030 ± 270</td>
<td>4.62</td>
</tr>
<tr>
<td>6</td>
<td>2,120 ± 2,360</td>
<td>254 ± 200</td>
<td>0.12</td>
</tr>
<tr>
<td>7</td>
<td>451 ± 58</td>
<td>3,060 ± 240</td>
<td>6.78</td>
</tr>
<tr>
<td>8</td>
<td>491 ± 76</td>
<td>1,450 ± 130</td>
<td>2.95</td>
</tr>
<tr>
<td>9</td>
<td>386 ± 180</td>
<td>156 ± 44</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>555 ± 42</td>
<td>1,450 ± 60</td>
<td>2.61</td>
</tr>
</tbody>
</table>

$^a$Mean ± calculated SD.

intramuscular administrations of rIFN-α2A for 28 days (see ref. 12),
and the plasma clearance of rIFN-α2A after its administration was
much larger in rats with a high antibody titer, compared with that in
the rats with a low antibody titer (12). However, Tagliaro et al
(11) reported that the plasma concentrations of eel calcitonin in patients
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were significantly greater than those in the patients without this
antibody. These findings also support the hypothesis that the effect of
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2—an antigen becomes more stable in circulation.

Such an interindividual difference in the effect of antibody on the
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acteristics between rh-EPO and antibody. Therefore, the plasma pro-
tein binding of $^{125}$I-rh-EPO was examined by gel filtration (fig. 2).
The radioactivity in plasma from rat 9 was found in the high molec-
ular fraction ($\sim 232–669$ kDa), corresponding to the dimer or trimer
of the immune complex, and the free form of $^{125}$I-rh-EPO (34 kDa)
was not observed (fig. 2). This result suggests that the $^{125}$I-rh-EPO is
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antibody is relatively lower. On the other hand, most of the radioac-
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ing that the stability of $^{125}$I-rh-EPO is markedly lower in the circu-
lating blood of this rat. Antibody against rh-EPO was also detected in
the blood of rat 5, and the $B_{\text{max}}$ was much higher than that in rats 6
and 9 (table 1). Thus, although the immune complex could not be
detected in the gel filtration chromatogram (fig. 2), there is a high
level of antibody against rh-EPO in the blood of rat 5. These results
indicate that the plasma clearance of $^{125}$I-rh-EPO is large when the
antibody concentration is high, and the clearance is smaller when the
antibody concentration is low.

This hypothesis was also supported by the following finding.

<table>
<thead>
<tr>
<th>AUC$_{0-48h}$</th>
<th>$B_{\text{max}}$ (ng eq. of rh-EPO/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 6</td>
<td>30</td>
</tr>
<tr>
<td>No. 9</td>
<td>40</td>
</tr>
<tr>
<td>No. 2</td>
<td>10</td>
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<td>No. 3</td>
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<td>No. 4</td>
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<td>25</td>
</tr>
<tr>
<td>No. 5</td>
<td>30</td>
</tr>
</tbody>
</table>

AUC$_{0-48h}$ was calculated by the trapezoidal rule from the individual rat data
shown in fig. 1. $B_{\text{max}}$ values are taken from table 1.
ligand binding to the receptor, it is possible that no pharmacological effect should be reduced when the antibody that inhibits polypeptide binding to the receptor—the so-called neutralizing antibody—is produced. However, even when the antibody produced cannot inhibit the receptor, the so-called neutralizing antibody—that is, the antibody produced against rIFN was administered before administration of rIFN (14).

It has been reported that the disappearance from the circulating plasma of the antigen–antibody complex of large size (more than a trimer) is fast, compared with that of a small one (monomer or dimer). In this case, the clearance of 125I-rh-EPO is small because such an immune complex can escape from the glomerular filtration in kidneys. On the other hand, when the amount of antibody in plasma is relatively large, the immune complex is taken up rapidly from the circulation if a lot of antibodies are produced and the resulting immune complexes have high molecular weights. On the other hand, when the amount of antibody is small, resulting in formation of small immune complexes and an increase in the stability of rh-EPO in blood, there are two possibilities: in one case, no pharmacological effect can be observed because the antibody inhibits the receptor binding of the polypeptide; in the other, antibody cannot inhibit receptor binding and, therefore, a pharmacological effect is still observed. The plasma concentrations and pharmacological effect of eel calcitonin in patients with an antibody against eel calcitonin after treatment with eel calcitonin were greater than those in patients without antibody (11). This finding suggests that the immune complex can exert a pharmacological effect when the immune complex is stable in the circulating blood and can still bind to receptor. Therefore, to evaluate the pharmacological effect of biologically active polypeptides, it is by all means important to monitor the production of the antibody and its effect on both receptor binding and pharmacokinetics.


TABLE 2
Pharmacokinetic parameters after intravenous administration of 125I-rh-EPO at the dose of 0.1 µg/kg to rats treated with rat anti-rh-EPO serum, the volumes being used 0, 5, 50, and 500 µl

<table>
<thead>
<tr>
<th>Dose Volume</th>
<th>t½a</th>
<th>t½b</th>
<th>Vc</th>
<th>AUC</th>
<th>CLtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>µl</td>
<td>hr</td>
<td>hr</td>
<td>ml/kg</td>
<td>ng·eq hr/ml</td>
<td>ml/hr/kg</td>
</tr>
<tr>
<td>0</td>
<td>0.23</td>
<td>3.15</td>
<td>49.1</td>
<td>6.18</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.49)</td>
<td>(4.1)</td>
<td>(1.16)</td>
<td>(3.0)</td>
</tr>
<tr>
<td>5</td>
<td>0.23</td>
<td>4.66</td>
<td>46.6</td>
<td>9.53*</td>
<td>10.5*</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(1.04)</td>
<td>(3.0)</td>
<td>(0.37)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>50</td>
<td>0.25</td>
<td>7.30*</td>
<td>46.6</td>
<td>15.0*</td>
<td>6.70*</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td>(1.92)</td>
<td>(3.7)</td>
<td>(1.3)</td>
<td>(0.58)</td>
</tr>
<tr>
<td>500</td>
<td>0.17</td>
<td>3.81</td>
<td>51.6</td>
<td>4.73</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.56)</td>
<td>(5.5)</td>
<td>(0.56)</td>
<td>(2.5)</td>
</tr>
</tbody>
</table>

Each value represents the mean (SD) of 3 or 4 animals.

* Significant difference from control (p < 0.05).

Namely, t½b was prolonged significantly as the pretreatment volume of anti-rh-EPO antiserum increased from 0 to 50 µl, whereas it became shorter in rats receiving 500 µl of anti-rh-EPO antiserum (fig. 4, table 2). Additionally, CLtotal decreased significantly as the volume of anti-rh-EPO antiserum increased from 0 to 50 µl and increased to 21.4 ml/hr/kg in rats receiving 500 µl of anti-rh-EPO antiserum (fig. 4, table 2). In our preliminary study, CLtotal also increased to 47.2 ml/hr/kg in the rat receiving antiserum (2 ml) (data not shown). These results suggest that elimination of rh-EPO is slower when there is only a small amount of antibody in plasma and more rapid when there is a large amount.

It has been reported that the disappearance from the circulating plasma of the antigen–antibody complex of large size is small because such an immune complex can escape from the glomerular filtration in kidneys. On the other hand, when the amount of antibody in plasma is relatively large, 125I-rh-EPO bound to the antibody in plasma is in the monomeric or dimeric form. In this case, the clearance of 125I-rh-EPO is small because such an immune complex can escape from the clearance mechanism of EPO, such as RME in target organs and glomerular filtration in kidneys. On the other hand, when the amount of antibody is relatively large, the immune complex is taken up rapidly by the liver because 125I-rh-EPO and the antibody form an immune complex with a molecule weight greater than a trimer. It has also been reported that the disappearance of plasma rIFN was delayed and the AUC increased ~ 15 times when the monoclonal antibody against rIFN was administered before administration of rIFN (14). Sato et al. (15) reported that the CLtotal of rhIL-2 after administration of rhIL-2 mixed with its monoclonal antibody is only one-sixth that of 125I-rh-EPO. Moreover, it has been reported that the elimination of small immune complexes and the increase in the stability of rh-EPO in blood. Namely, when the amount of antibody is small, resulting in the formation of small immune complexes and an increase in the stability of rh-EPO in blood, there are two possibilities: in one case, no pharmacological effect is observed because the immune complexes may be eliminated rapidly from the circulation if a lot of antibodies are produced and the resulting immune complexes have high molecular weights. On the other hand, when the amount of antibody is small, resulting in the formation of small immune complexes and an increase in the stability of rh-EPO in blood, there are two possibilities: in one case, no pharmacological effect can be observed because the antibody inhibits receptor binding of the polypeptide; in the other, antibody cannot inhibit receptor binding and, therefore, a pharmacological effect is still observed. The plasma concentrations and pharmacological effect of eel calcitonin in patients with an antibody against eel calcitonin after treatment with eel calcitonin were greater than those in patients without antibody (11). This finding suggests that the immune complex can exert a pharmacological effect when the immune complex is stable in the circulating blood and can still bind to receptor. Therefore, to evaluate the pharmacological effect of biologically active polypeptides, it is by all means important to monitor the production of the antibody and its effect on both receptor binding and pharmacokinetics.

References


