Effect of Daily Subcutaneous Administration of Recombinant Erythropoietin on Chronic Anemia in Rheumatoid Arthritis

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Mean ($\pm$ SD) serum erythropoietin (EPO) levels were 18.6 ± 5.6 mU/ml in 180 normal Japanese subjects. Serum EPO levels were elevated with a negative correlation on a log scale ($r = -0.864$, $P < 0.005$) to hematocrit (Ht) values in anemic patients not associated with rheumatoid arthritis (RA) or chronic renal failure (CRF). Serum EPO levels in patients with RA (31.6 ± 16.4 mU/ml) were relatively lower than those in normal subjects and anemic patients without RA or CRF when matched for comparative Ht values. Seven anemic patients with RA were treated by daily subcutaneous (sc) injection of recombinant EPO (rEPO, 500–1,000 U/day) for 4 weeks. The patients had initial Ht values of 25.1 % or less and maintained stable clinical status. The treatment with rEPO raised serum EPO levels (53.8 ± 15.2 mU/ml, $P < 0.05$), which resulted in an increase in Ht values (more than 3%) in 6 out of 7 patients with RA. The mean ($\pm$ SD) Ht values at the end of the treatment with rEPO (500–1,000 U/day) were greater than those before the treatment in the 7 patients with RA (28.5 ± 4.6 vs. 22.7 ± 2.5 %, $P < 0.05$). These findings suggest that chronic anemia associated with RA may be corrected by daily sc injection of a small dose of rEPO. (Internal Medicine 33: 193–197, 1994)

Key words: hematocrit, hemoglobin, human erythropoietin, subcutaneous injection

Introduction

The pathogenesis of anemia in rheumatoid arthritis (RA) remains to be fully investigated. The principle abnormality may be the failure of the erythropoietic response to compensate for a reduction in mean red cell life span (1). Initial studies using the polycythemic mouse bioassay suggested that serum erythropoietin (EPO) levels were low or absent in RA (2, 3). Recent studies using a specific radioimmunoassay clearly demonstrated that serum EPO response to anemia in RA is relatively blunted compared to that for anemia of other etiologies except for pregnancy and renal disease (4). Recombinant EPO (rEPO) has been documented to be effective in correction of anemia in patients with chronic renal failure (CRF), which is characterized by insufficient production of EPO (5–7).

It was recently reported that rEPO stimulates correction of anemia in most patients with RA (8, 9). In these studies, rEPO was injected iv in a large dose of 50–200 U/kg three times per week in combination with oral ferrous sulfate. It was also reported that iv injection of rEPO in a smaller dose of 1,500–6,000 U three times a week was partly effective in anemic

patients with RA (10). However, the minimum effective dose of EPO for correction of anemia in RA remains to be further clarified. In the present study, we investigated the effect of daily subcutaneous (sc) administration of a small dose of rEPO (500–1,000 U/day) in anemic patients with RA.

Materials and Methods

Patients with RA

Seven patients with RA (2 males and 5 females; age 60 to 76 yr) were investigated. All patients met the 1958 American Rheumatism Association criteria for RA (11). The mean duration of disease was 3.4 yr, with a range of 2 to 7 yr. All patients had stable initial hematocrit levels with a mean value of 22.7 %, and a range of 19.9 to 25.1 %. RA in these patients was being treated only with nonsteroidal anti-inflammatory drugs, and no patient was taking gold, methotrexate, azathioprine, penicillamine or prednisolone for 3 months prior to or during rEPO administration.

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Control normal subjects and anemic patients

One hundred and eighty normal subjects, 76 males and 104 females, aged from 21 to 80 yr, were examined as normal controls. They were selected from ongoing epidemiological studies based on physical examination, chest roentgenography, electrocardiography, routine hematology and serum chemistries. Eighty-five patients with anemia of varying etiologies not associated with RA were also examined as a control anemic patients. They consisted of 17 chronic renal insufficiency, 62 iron deficiency, 7 acute leukemia, 4 multiple myeloma, 6 myelodysplastic syndrome and 4 aplastic anemia. Anemia was defined as a hemoglobin (Hb) concentration of less than 13.0 g/dl for males and less than 12.0 g/dl for females.

Recombinant human EPO administration

Recombinant human EPO (rEPO, KRN5702) was provided by Kirin Brewery Co. (Tokyo, Japan). The patients with RA were initially entered into the treatment with 500 U/day rEPO, which was administered as a single subcutaneous (sc) injection every morning for 4 weeks. When an increase in the Hb concentration of more than 0.75 g/dl or the hematocrit (Ht) values of more than 3% was not obtained at two weeks after the start of rEPO treatment, the rEPO dose was increased to 1,000 U/day for the last two weeks of the treatment period. Informed consent was obtained from each patient and the protocol was approved by the Clinical Investigation Committee of our institute.

Blood sampling

Blood samples were obtained by venipuncture at bed rest after overnight fasting in all the subjects. After blood cell counting was performed, serum was promptly separated and kept at -20°C until assayed for EPO.

In patients with RA treated with rEPO, blood sampling was conducted in the early morning every week, immediately before the sc injection of rEPO during the treatment, for determination of serum EPO level, Ht value, Hb concentration, white blood cell and differential counts, platelet count, reticulocyte count, and erythrocyte sedimentation rate as well as routine chemistries such as serum iron and ferritin. Clinical status of RA was monitored weekly using a standardized self-report questionnaire and objective physical examination. All data were analyzed 8 weeks after the start of the rEPO treatment.

Radioimmunoassay

Serum EPO levels were measured by specific radioimmunoassay using the Recombigen EPO RIA kit (Japan DPC, Chiba) as previously reported (12). Recombinant human EPO was used as the standard. The minimum detectable quantity of EPO was 5.0 mU/ml. Inter- and intra-assay coefficients of variation were 3.5% and 8.9%, respectively. Diluted human serum showed that the displacement curve was parallel to the standard rEPO. The mean recovery of rEPO added was 93.3%.

Statistical analysis

Statistical significance was evaluated by one way analysis of variance in combination with paired t-test. Linear regression

analysis was used for evaluating the correlation between two parameters. $P < 0.05$ was considered significant.

Results

Mean ($\pm$ SD) serum EPO levels were 18.6 ± 5.6 mU/ml in 180 normal subjects. The normal range was 7.4 to 29.8 mU/ml, corresponding to the mean ± 2 SD values. There was no difference in serum EPO levels between both sexes. Serum EPO levels were elevated above the normal range in 78 out of 83 anemic patients not associated with RA or CRF. Linear regression analysis revealed a negative correlation between the logarithm of serum EPO levels and Ht values in patients with anemia without RA or renal dysfunction as shown in Fig. 1.

In 7 patients with RA, mean ($\pm$ SD) Ht values and Hb concentrations were $22.7 \pm 2.5\%$ and 7.6 ± 1.3 g/dl, respectively. Serum EPO levels ranged from 17.7 to 68.6 mU/ml with the mean ($\pm$ SD) values of 31.6 ± 16.4 mU/ml in these patients. The

Fig. 1. Correlation between serum EPO levels and Ht values in 7 anemic patients with RA ($\bullet$) and 83 other anemic patients ($\circ$) not associated with RA or chronic renal insufficiency. Serum EPO levels are shown on a logarithmic scale. Linear regression was obtained between log scaled serum EPO levels and Ht values in anemic patients not associated with RA or renal insufficiency ($Y = -0.827X + 4.608$, $r = -0.864$, $P < 0.005$). Both serum EPO levels and Ht values moved toward the regression line in all 7 patients with RA after the treatment with rEPO as indicated by arrows. The square outlines the normal range of serum EPO levels and Ht values obtained from 180 normal subjects.

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serum EPO levels were lower than those in normal subjects and other anemic patients not associated with RA or CRF when matched for comparable Ht concentrations (Fig. 1).

Daily sc administration of rEPO in a dose of 500 U per day for the initial two weeks resulted in a considerable increase in Hb concentrations (more than 0.75 g/dl) in two cases (Cases 6 and 7) and in Ht values (more than 3%) in three cases (Cases 3, 6 and 7) (Table 1). Both Hb concentrations and Ht values were considerably increased in the other three cases (Cases 1, 2 and

4) two weeks later when the dose of rEPO was raised to 1,000 U per day (Table 1, Fig. 2). An increase in Hb concentration and Ht value was preceded by an increase of reticulocyte counts (Fig. 2). At the end of daily sc administration of rEPO for 4 weeks, the mean ($\pm$ SD) Hb concentrations and Ht values were significantly greater than those before the treatment with rEPO (Hb 9.1 ± 1.7 vs. 7.6 ± 1.3 g/dl, $P < 0.05$; Ht 28.5 ± 4.6 vs. $22.7 \pm 2.5\%$, $P < 0.05$) (Figs. 1 and 2, Table 1). The serum EPO levels were increased in all of the patients after the start of the treatment with

Table 1. Effect of rEPO Treatment in 7 Anemic Patients with RA

Case No.	Before treatment	During treatment with rEPO*		After treatment
		2nd week	4th week	
Serum EPO (mU/ml)				
1	19.7	34.0	41.0 ^a	20.0
2	33.4	56.7	51.7 ^a	37.2
3	17.7	41.6	42.5	16.7
4	68.6	76.6	85.6 ^a	68.3
5	21.3	24.0	37.5 ^a	22.8
6	24.2	43.8	53.4	24.2
7	38.2	49.1	59.9	38.2
Mean $\pm$ SD	31.6 $\pm$ 16.4	45.8 $\pm$ 15.3	53.8 $\pm$ 15.2 ^b	32.5 $\pm$ 16.5
Hb concentrations (g/dl)				
1	8.3	8.8	10.3 ^a	9.7
2	7.9	8.5	9.1 ^a	8.3
3	10.1	10.5	12.0	12.9
4	7.3	7.1	8.0 ^a	6.6
5	6.1	6.6	6.6 ^a	6.1
6	6.9	8.1	9.9	8.6
7	6.3	7.7	7.9	7.0
Mean $\pm$ SD	7.6 $\pm$ 1.3	8.2 $\pm$ 1.2	9.1 $\pm$ 1.7 ^b	8.5 $\pm$ 2.2
Ht values (%)				
1	25.1	29.0	32.0 ^a	25.6
2	25.0	26.7	28.0 ^a	25.4
3	25.0	32.3	37.2	32.8
4	24.1	23.1	27.2 ^a	21.6
5	19.9	21.3	22.8 ^a	19.6
6	21.2	24.2	28.9	26.4
7	18.9	22.9	23.1	20.4
Mean $\pm$ SD	22.7 $\pm$ 2.5	25.4 $\pm$ 3.4	28.5 $\pm$ 4.6 ^b	24.5 $\pm$ 4.2
Serum Fe (μ g/dl)				
1	35	32	34 ^a	40
2	30	28	27 ^a	51
3	40	53	66	71
4	29	27	28 ^a	27
5	27	18	23 ^a	26
6	32	55	44	37
7	35	28	19	29
Mean $\pm$ SD	32.6 $\pm$ 4.1	34.4 $\pm$ 13.0	34.4 $\pm$ 14.9	40.1 $\pm$ 15.0
Serum Ferritin (ng/ml)				
1	203	164	187 ^a	186
2	142	112	82 ^a	109
3	461	423	385	452
4	84	82	86 ^a	101
5	177	180	133 ^a	127
6	220	179	193	216
7	135	128	124	116
Mean $\pm$ SD	203.1 $\pm$ 113.4	176.9 $\pm$ 105.6	146.0 $\pm$ 109.5	186.7 $\pm$ 115.4

^arEPO (1,000 U/day), ^b $P < 0.05$ vs. before treatment.

*The rEPO treatment was initiated by daily sc injection [500 U per day; dose was increased to 1,000 U per day in 4 of 7 patients (Cases 1, 2, 4 and 5) during the last two weeks of the treatment].

Fig. 2. Effect of the treatment with rEPO on serum EPO level, Ht value, Hb concentration and reticulocyte count in a patient with RA. rEPO was daily injected sc at a dose of 500 U per day for the initial two weeks and then increased to 1,000 U per day for the last two weeks.

rEPO and the elevated serum EPO levels were sustained during the rEPO treatment (Figs. 1 and 2, Table 1). The mean serum EPO levels, Hb concentrations and Ht values at two weeks after the end of the rEPO treatment were not significantly different from those before the treatment.

Discussion

It was reported that the logarithm of serum immunoreactive EPO levels was inversely correlated with the Hb concentration, indicating the integrity of the physiological response of EPO to anemia in RA (13). However, the EPO response was relatively blunted compared to that for anemia not associated with RA, suggesting an impaired serum EPO response to anemia in patients with RA (4, 11). In the present study, we confirmed that serum EPO levels in anemic patients with RA were lower than those in normal subjects and other anemic patients not associated with RA or renal insufficiency when matched for comparable Ht values.

Pincus et al (9) previously reported that severe anemia in RA can be partly corrected by supplementation with rEPO without

meaningful toxicity. They intravenously injected a large dose of rEPO (50–150 U/kg BW) three times a week. Takashina et al (10) reported that an iv injection of rEPO in a dose of 1,500–6,000 U three times a week effectively corrected anemia in 4 out of 6 patients with RA. In the present study, we effectively treated anemia in 6 of 7 patients with RA by a daily sc injection of a smaller dose of rEPO (500–1,000 U/day) for 4 weeks. The effect lasted for a short period and returned to the basal levels 2–4 weeks after the end of the rEPO treatment. Therefore, the increased serum EPO levels do not reflect endogenous EPO responses in these patients. The mean Hb concentrations and Ht values were significantly increased by daily sc administration of 1,000 U rEPO but not by a smaller dose of rEPO (500 U/day) in 3 of 4 patients examined, suggesting that the minimum effective dose of rEPO is about 1,000 U/day for the initial sc treatment for anemia in RA. The treatment with rEPO moved both serum EPO levels and Ht values in these patients toward the regression line obtained by serum EPO levels and Ht values in anemic patients not associated with RA or CRF. These findings suggest that the anemic patients with RA are associated with insufficient production of EPO and considerable sensitivity to the supplement of rEPO. Although hypertension and headache are known to be rarely encountered during the rEPO treatment of patients with CRF (5–7), no considerable side effect was recorded in the present study. However, the effect of the EPO treatment of RA for a longer period remains to be further investigated.

Iron deficiency was seen in some patients with RA; iron has been administered concomitant with rEPO in previous studies (9). Iron release from the reticuloendothelial system is known to be impaired in RA (14). It was reported that the effect of rEPO is blunted in the anemic patients associated with both RA and iron deficiency, in which serum ferritin is lowered and serum EPO is rather increased (10). Although serum iron was low in all 7 cases with RA in the present study as described in other chronic inflammatory diseases (15), serum ferritin ranged from 84 to 461 ng/ml, suggesting that iron was not deficient in these patients. However, supplementation of iron may be considered to correct the effect of rEPO in these patients since it was reported that supplement of iron raises functional availability of serum iron (16) and corrected the effect of rEPO in the case with CRF in which serum ferritin was normal (17).

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