Effects of Recombinant Human Erythropoietin (rHuEPO) Treatment on Plasma Insulin-like Growth Factor-I (IGF-I) and Hemoglobin Concentrations in Patients with Type 2 Diabetes Mellitus Associated with Nephropathy and Anemia of Chronic Renal Failure.

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Abstract

Both insulin-like growth factor-I (IGF-I) and erythropoietin (EPO) have been found to stimulate erythropoiesis, and IGF-I levels are decreased in patients with chronic renal failure (CRF). Decreased IGF-I levels might contribute to the progression of anemia of CRF. However, no studies have examined the effects of rHuEPO therapy on plasma IGF-I levels and Hb concentration in predialysis diabetic patients with CRF and anemia. Therefore, we investigated the effects of rHuEPO treatment on plasma IGF-I levels and Hb concentrations in patients with diabetes and anemia of CRF.

Seven patients with type 2 diabetes mellitus accompanied by advanced nephropathy (renal failure stage) were studied. The mean (±SE) age was 62.6±6.2yrs. Serum creatinine and creatinine clearance levels were 327.1±44.2 µmol/L and 0.261±0.022 ml/s, respectively. Recombinant human EPO (rHuEPO) was subcutaneously infused into the abdomen for 4 weeks at a flow rate of 6,000 IU/2.5ml/week using a portable infusion pump.

Plasma EPO levels were increased from 19.4±2.2 IU/L to 69.6±16.9 IU/L 1 week after the start of rHuEPO administration, and were maintained at a steady state. Plasma IGF-I levels at time 0 decreased to 72.7±8.4 µg/L compared with age-matched diabetic patients without nephropathy (166.0±15.5 µg/L). Plasma IGF-I levels were increased 1-3 weeks after the start of rHuEPO administration, followed by an increase in Hb concentrations 3-4 weeks after rHuEPO administration.

We have therefore hypothesized that rHuEPO administration increases plasma IGF-I levels followed by Hb concentrations. These findings suggest that rHuEPO treatment has a stimulatory effect on IGF-I production, and that increases in IGF-I might be a good indicator of improved Hb concentrations.

Key words: Insulin-like growth factor-I, Erythropoietin, Diabetes Mellitus, Diabetic nephropathy, Anemia

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Introduction

Erythropoietin (EPO) is a 34-kilodalton glycoprotein hormone and a potent regulator of erythropoiesis, that is secreted primarily from the endothelium and interstitial fibroblasts of renal tubules, and to a lesser extent from the liver in the rat [1-3]. Chronic renal failure (CRF) is associated with anemia, which is accompanied by relatively decreased EPO secretions from the kidney. The anemia of CRF accompanied by diabetic nephropathy is more severe than that of glomerulonephritis [4].

Insulin-like growth factor I (IGF-I) plays an important role in erythrocyte production (5), stimulating proliferation of erythroid precursor cells in vitro [6-11]. IGF-I is required for and essential to erythroid development [12,13].

Recently, some investigations have suggested that both IGF-I and EPO play an important role in erythropoiesis in vitro [9,12,14]. The addition of IGF-I with EPO leads to enhanced heme synthesis and moderate cellular proliferation in the late erythroblasts in vitro [9]. Brox et al. have suggested that anemia of CRF may present both EPO-deficient and functional IGF-I-deficient states, and that subtherapeutic doses of EPO and IGF-I cause a significant increase in hemoglobin (Hb) levels in the mouse [14]. In a human study, plasma IGF-I levels were found...
to be increased in patients with dialysis and kidney transplantation treated with recombinant human EPO (rHuEPO) therapy compared without rHuEPO therapy [15]. However, no studies have examined the effects of rHuEPO therapy on plasma IGF-I levels and Hb concentrations in predialysis diabetic patients with CRF and anemia.

In the present study, we investigated the effects of rHuEPO treatment on plasma IGF-I levels and Hb concentrations in diabetic patients with nephropathy and anemia of CRF.

Materials and Methods

Subjects

Seven patients with type 2 diabetes mellitus accompanied by advanced nephropathy (renal failure stage) were studied. The mean (±SE) age was 62.6±6.2 yrs (4 males and 3 females). BMI was 22.5±1.9 kg/m². HbA1c levels were 6.7±0.2%. Serum creatinine and creatinine clearance levels were 327.1±44.2 µmol/L and 0.261±0.022 ml/s, respectively. Serum albumin levels were 32±5 g/L. Hb concentrations were 78±3 g/L. Urine albumin excretions for 24 hours were 2,613±578 mg/day. Type 2 diabetes mellitus was defined by criteria for the diagnosis of diabetes in non-pregnant adults shown in American diabetes association. Anti-glutamic acid decarboxylase (GAD) antibody was negative in all patients. All patients were treated with insulin, and none underwent hemodialysis.

Study Protocol

Recombinant human EPO (rHuEPO) (Epoetin beta, Chugai Pharmaceutical Co.) was subcutaneously infused into the abdomen for 4 weeks at a flow rate of 6,000 IU/2.5ml/week using a portable infusion pump (SP-3HQ, Nipro Co., Japan). Both subjective and objective symptoms were evaluated each day. Blood pressure and body weight were measured weekly. Blood samples were also obtained weekly in the morning after overnight fasting for 4 weeks before and after the start of rHuEPO administration. Plasma samples were stored at -20°C until assay. Hb concentrations as well as plasma IGF-I and plasma EPO levels were measured.

The present study was carried out in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all subjects.

Assays

Plasma IGF-I levels were measured by specific radioimmunoassay after acid/ethanol extraction, as previously described [16]. The minimal detectable quantity was 30 µg/L using a 50 µL plasma sample. The coefficients of variation for intra- and inter-assays were 6.3% and 7.2%, respectively. The levels of plasma IGF-I were found to be 202.8±70.6 µg/L and 198.6±91.8 µg/L in males and females, respectively [16]. Plasma EPO levels were measured by sensitive enzyme immunoassay (EIA), as previously described [17]. The sensitivity was 0.75 IU/L using 20 µl of plasma sample. The coefficients of variation for intra- and inter-assays were 5.3% and 7.2%, respectively.

Hb concentrations, serum and urine creatinine levels, serum and urine albumin levels and HbA1c levels, as well as common blood cell counts (CBC) and other blood chemistry were measured by conventional methods in our laboratory. The normal ranges of Hb concentration in our laboratory were found to be 136-183 g/L and 112-152 g/L in males and females, respectively.

Statistical analysis

Statistical analysis of the data was performed by ANOVA in combination with the Student's t-test. P<0.05 was considered significant. Data are expressed as the mean± SD.

Results

As shown in Fig. 1, plasma EPO levels were increased from 19.4±2.2 IU/L to 69.6±16.9 IU/L 1 week after the start of rHuEPO administration, and were maintained at a steady state. The effects of rHuEPO treatment on plasma IGF-I and Hb concentrations are shown in Fig. 2. Plasma IGF-I level at time 0 were 72.7±8.4 µg/L, lower than IGF-I levels in age-matched diabetic patients without...
nephropathy (166.0±15.5 µg/L). Plasma IGF-I levels were increased 1-3 weeks after the start of rHuEPO administration, followed by an increase in Hb concentrations 3-4 weeks after rHuEPO administration. Hb concentrations were increased from 78±3 g/L to 86±3 g/L at 4 weeks after the start of rHuEPO administration. There were no significant changes in serum creatinine levels and blood urea nitrogen levels. Blood pressure and body weight did not change during the experimental period.

Discussion

We have hypothesized that rHuEPO administration increases plasma IGF-I levels, followed by Hb concentrations. There have been few reports regarding the contribution of both rHuEPO therapy and IGF-I levels to the prevention of anemia. Brox et al have described that combination therapy with EPO and IGF-I induces a large increase in Hb concentrations in the mouse (14). They have suggested that anemia of CRF may represent both an EPO- and a functional-IGF-I deficient state. In human studies, there has been no report of the effects of rHuEPO therapy on plasma IGF-I and Hb concentrations in predialysis CRF patients. In the present study, plasma IGF-I basal levels were decreased in diabetic patients with anemia of CRF compared with diabetic patients without anemia and diabetic nephropathy. Plasma IGF-I levels were increased to a normal range 1 - 3 weeks after rHuEPO administration. Such increases in plasma IGF-I levels were rapid. These findings may suggest that rHuEPO has a direct stimulatory effect on erythropoiesis as well as IGF-I production in humans. Although this study was small in scale and not a placebo control study,

Our results suggest that increased IGF-I levels might be a good indicator of improved Hb concentrations. Therefore, a large-scale study is needed to clarify the relationship between rHuEPO treatment and IGF-I production.

IGF-I production is affected by diabetes mellitus in vivo. Plasma IGF-I levels are decreased in streptozotocin-induced diabetic rats (18), and the abundance of total hepatic IGF-I mRNA decreases after streptozotocin administration [19]. Circulating IGF-I levels decrease in patients with diabetes mellitus [20]. In addition, IGF-I levels are low in patients with diabetic complications, negatively correlating with HbA1c levels [21]. In the present study, plasma glucose control was good in all subjects (data not shown).

Plasma IGF-I levels decrease in patients with CRF (22,23), making plasma IGF-I a good indicator of the nutritional status of CRF patients [22]. Plasma IGF-I levels in CRF patients are lower than those in normal subjects and can be improved by nutritional therapy. Therefore, the increase in plasma IGF-I levels in the present study and the improvement of nutritional status due to rHuEPO administration might contribute in part to the increases in plasma IGF-I levels. However, it is unknown whether rHuEPO has a direct stimulatory effect on improvements in nutritional status.

Plasma IGF-I levels might be affected by the GH status in patients with chronic renal failure. The GH paradoxical response was observed in patients with CRF. rHuEPO administration might affect hypothalamus and pituitary function in patients with chronic renal failure (24-26). We could not assess GH response by GH stimulation tests during rHuEPO administration. However, there has been no report that rHuEPO has a direct stimulatory effect on GH secretion.

We administered rHuEPO by means of continuous subcutaneous infusion (CSI). This method could maintain constant plasma EPO levels, which we believe is the physiological state. Intravenous injection resulted in transient increases in plasma EPO levels, producing non physiological conditions. We could not carry out a comparative study of CSI and SC injections. Therefore, a further comparative study is needed.

In summary, we found that rHuEPO administration increases plasma IGF-I levels followed by Hb concentrations. It also appears that rHuEPO may have a stimulatory effect on IGF-I production. These findings suggest that decreased IGF-I levels might play a role in the progression of renal anemia in diabetic patients with renal failure and that increases in IGF-I might be a good indicator of improved Hb concentrations.
Acknowledgments

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References


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