Stimulation of erythropoietin secretion by continuous subcutaneous infusion of recombinant human GH in anemic patients with chronic renal failure

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Abstract

We have investigated the effect of human GH on erythropoietin (EPO) secretion in eight anemic patients with chronic renal failure (CRF) (three males and five females, aged from 46 to 83 years). Recombinant human GH was infused subcutaneously at a flow rate of 2 $\mu\text{g/kg}$ body weight per 0.1 ml/h for 72 h using a portable infusion pump. Blood samples were obtained immediately before and 2, 4, 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 and 168 h after the start of GH infusion. Storage urine samples were obtained before and 24, 48 and 72 h after the start of the infusion. The mean ($\pm$ S.E.M.) basal plasma GH levels increased from 1.9 ± 0.3 to $18.8 \pm 0.7 \mu\text{g/l}$ during the GH infusion. Plasma IGF-I levels increased 12 h after the start of GH treatment and the mean peak values of $403.6 \pm 38.5 \mu\text{g/l}$ were obtained at 72 h. Plasma EPO levels increased 6 h after the start of GH infusion, and the peak values of $38.4 \pm 11.6 \text{ IU/l}$ were obtained at 96 h ($P < 0.05$ vs basal values $24.5 \pm 7.2 \text{ IU/l}$). Reticulocyte counts increased from $28.7 \pm 5.2 \times 10^3/\mu\text{l}$ to $40.3 \pm 8.0 \times 10^3/\mu\text{l}$ at 108 h, $43.6 \pm 9.2 \times 10^3/\mu\text{l}$ at 120 h and $41.7 \pm 7.7 \times 10^3/\mu\text{l}$ at 160 h ($P < 0.05$). Serum urea nitrogen decreased at 72 h ($P < 0.05$), whereas there was no significant change in urinary excretion of nitrogen. Hemoglobin levels were not significantly changed throughout the experimental period. These findings indicate that human GH has a stimulating effect on EPO secretion in anemic patients with CRF.

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Introduction

Erythropoiesis is mainly regulated by erythropoietin (EPO), a glycoprotein with a molecular mass of 34 000 Da, and hypoxia-induced erythropoiesis is mediated by an increase of EPO secretion (1). EPO is mainly produced in the kidney, but partly in the liver in the rat (2–5). Anemia accompanied by chronic renal failure (CRF) is characterized by relatively blunted EPO secretion from the kidney (6).

The endocrine system also plays a role in the regulation of erythropoiesis (7, 8). Hypopituitarism is often accompanied by normochromic and normocytic anemia, and erythroid hypoplasia of the bone marrow, resulting in normochromic and normocytic anemia, which is partly corrected by replacement with growth hormone (GH) (9). It was considered that GH had no direct effect on hemopoiesis, but rather influenced the bone marrow function indirectly through improved general metabolism (10, 11). It has been recently reported that GH has a direct stimulating effect on hematopoietic cells *in vitro* (12). It has also been reported that GH stimulates erythropoiesis via insulin-like growth factor-I (IGF-I), a GH-dependent growth factor (13). It remains to be

elucidated, however, whether GH stimulates EPO secretion in the human.

In the present study, we investigated the effect of human GH on plasma EPO levels in anemic patients with CRF.

Materials and methods

Subjects

Eight patients with CRF, three males and five females, were studied. The mean ($\pm$ S.E.M.) age was 65.9 ± 5.2 years (range 46–83 years). They included six patients with diabetic nephropathy, one patient with chronic nephritis and one with renovascular renal failure. Body mass index was $20.7 \pm 1.6 \text{ kg/m}^2$. All the patients had high serum creatinine (Cr) levels of $265.2 \pm 56.3 \mu\text{mol/l}$ (normal range: 61.9 to $106.1 \mu\text{mol/l}$) and high serum urea nitrogen (UN) levels of $15.7 \pm 7.8 \text{ mmol/l}$ (normal range: 1.8 to 7.1 mmol/l). All the patients had low hemoglobin (Hb) levels of $8.4 \pm 0.8 \text{ g/dl}$ (normal range: 13.0 to 16.0 g/dl) and relatively low plasma EPO levels of $24.3 \pm 7.3 \text{ IU/l}$ (normal range in a control group without anemia: 7.9 to 30.3 IU/l). Plasma IGF-I

levels were $184.3 \pm 17.4 \mu\text{g/l}$ (normal range: 124.0 to $197.4 \mu\text{g/l}$ (male) and 80.9 to $212.7 \mu\text{g/l}$ (female)). Serum tri-iodothyronine, thyroxine (T_4), free T_4 and thyroid-stimulating hormone (TSH) levels were $117.2 \pm 8.5 \text{ ng/dl}$ (normal range: 90 to 195 ng/dl), $7.6 \pm 0.4 \mu\text{g/dl}$ (normal range: 5.4 to $13.5 \mu\text{g/dl}$), $1.1 \pm 0.1 \text{ ng/dl}$ (normal range: 0.9 to 1.9 ng/dl) and $2.81 \pm 0.76 \text{ mU/l}$ (normal range: 0.32 to 3.70 mU/l) respectively. Serum cortisol levels were $12.0 \pm 1.3 \mu\text{g/dl}$ (normal range: 2 to $18 \mu\text{g/dl}$). All patients were maintained on a diet containing 30 kcal/kg body weight (BW) per day including 1.0 g/kg BW of protein during the admission. BW and blood pressure did not change during the study. Written informed consent was obtained from all subjects.

Protocol

After overnight fasting, an indwelling catheter was placed into a cubital vein for blood sampling. The 22 kDa form of recombinant human GH (Sumitomo Co., Tokyo, Japan) was dissolved in 0.9% saline containing 0.3% human albumin and subcutaneously infused into the abdominal wall for 72 h at a flow rate of $2 \mu\text{g/kg BW}$ per $100 \mu\text{l/h}$ using a portable syringe infusion pump (SP-3HQ, Nipro Co., Osaka, Japan). Blood samples were obtained immediately before and 2, 4, 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 and 168 h after the start of GH infusion. Plasma samples were kept frozen at -20°C until assayed. The storage urine samples were obtained before and 24, 48 and 72 h after the start of GH infusion. Urine samples were kept at -20°C until assayed. Plasma GH, EPO, IGF-I, Cr, serum UN, Hb concentration and reticulocyte counts (RC) as well as urine Cr and nitrogen were determined.

Assays

Plasma GH levels were measured by a highly sensitive enzyme immunoassay in duplicate as previously described (14–16). The minimum detectable quantity was 3 ng/l using $20 \mu\text{l}$ plasma. The intra- and inter-assay coefficients of variation were 6.0% and 9.8% respectively. Plasma EPO levels were determined by a commercially available RIA kit (Recombigen EPO, Japan DPC Co., Chiba, Japan) as described previously (17). The minimum detectable quantity was 5 IU/l. Plasma IGF-I levels were measured by specific RIA after acid-ethanol extraction as previously described (18). Plasma and urinary Cr were measured by an autoanalyzer (TBA50S, Toshiba, Tokyo, Japan). Urinary nitrogen was measured by the decomposition chemiluminescence method. Serum UN, Hb and RC were determined by conventional methods.

Statistical analysis

Statistical differences were evaluated by analysis of variance in combination with the paired *t*-test. A *P* value less than 0.05 was considered significant.

Results

As shown in the upper panel of Fig. 1, mean ($\pm$ S.E.M.) plasma GH levels increased from 1.9 ± 0.3 to $18.8 \pm 0.7 \mu\text{g/l}$ during the subcutaneous infusion of human GH. At 12 h after the end of GH infusion, plasma GH returned to the basal level ($1.6 \pm 0.2 \mu\text{g/l}$). As shown in the lower panel of Fig. 1, mean plasma IGF-I levels increased at 12 h after the start of GH infusion ($211.5 \pm 15.0 \mu\text{g/l}$ vs basal value $184.4 \pm 23.3 \mu\text{g/l}$, $P < 0.01$). The peak value of plasma IGF-I was obtained at 72 h after the start of GH infusion ($408.4 \pm 43.4 \mu\text{g/l}$, $P < 0.001$ vs basal value). After the end of GH infusion, plasma IGF-I levels gradually decreased but were still greater than the basal level at 168 h ($234.3 \pm 45.4 \mu\text{g/l}$,

Figure 1 Plasma GH and IGF-I levels after GH constant subcutaneous infusion (CSI) in eight anemic patients with CRF. GH was infused at $2 \mu\text{g/kg BW}$ per h for 72 h. Data are means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$ vs 0 h.

$P < 0.05$). Plasma EPO levels were considerably elevated at 6 h after the start of GH infusion (27.5 ± 7.5 IU/l vs basal level 24.5 ± 7.2 IU/l, $P < 0.01$) and the peak value of plasma EPO was obtained at 96 h after the end of GH infusion (38.4 ± 11.6 IU/l, $P < 0.05$ vs basal level) (Fig. 2, upper panel). Plasma EPO levels remained increased at 168 h (33.4 ± 8.1 IU/l, $P < 0.05$ vs basal level). As shown in the lower panel of Fig. 2, RC increased at 108, 120 and 168 h after the start of GH infusion ($40.3 \pm 8.0 \times 10^3/\mu\text{l}$, $43.6 \pm 9.2 \times 10^3/\mu\text{l}$ and $41.7 \pm 7.7 \times 10^3/\mu\text{l}$ respectively, $P < 0.05$ vs basal level $28.7 \pm 5.2 \times 10^3/\mu\text{l}$). However, there was no significant change in Hb levels. Mean serum UN levels decreased at 72 h after the start of GH infusion (5.47 ± 1.00 mmol/l vs basal level 6.06 ± 1.04 mmol/l, $P < 0.05$). Urinary nitrogen excretion was not significantly decreased during 72 h (basal level 582.2 ± 57.8 mmol/day, 537.9 ± 107.3 mmol/day at 72 h). There was no change in serum Cr levels and 24 h Cr clearance during the GH infusion.

Figure 2 Plasma EPO levels and RC after GH constant subcutaneous infusion (CSI) in eight anemic patients with CRF. Data are means $\pm$ S.E.M. * $P < 0.05$ vs 0 h.

Discussion

The relationship between the endocrine system and hemopoiesis has been known for nearly half a century (7, 10, 11, 19, 20). Hypopituitarism is often accompanied by normochromic and normocytic anemia. The appropriate replacement therapy with glucocorticoid and T_4 increases Hb concentration, which does not reach the normal range in some cases. Hypoplastic bone marrow is restored by GH treatment, but not by adrenocorticotrophic hormone, follicle-stimulating hormone, luteinizing hormone and TSH (7, 21). Androgen and thyroid hormone are known to stimulate erythropoiesis (22).

EPO is the major humoral regulator of erythropoiesis, which is mainly secreted from the endothelium and interstitial fibroblasts of renal tubules, and partly secreted from the liver in the rat (2, 4, 5). EPO secretion is mainly controlled by oxygen pressure (23). Hypoxia rapidly stimulates EPO secretion and EPO-encoding mRNA appears in the kidney and the liver within 1 h of inducing anemia in mice and rats (3, 24).

There have been a few reports on endocrinological regulation of EPO secretion. It was previously reported that GH treatment increased Hb levels and urine excretion of EPO in patients with hypopituitarism, in which EPO activity was determined by bioassay using polycythemic mice (20). However, the effect of GH treatment on plasma EPO levels remained to be elucidated.

In the present study, we found that plasma EPO levels increased within 6 h of the start of GH infusion in anemic patients with CRF. The rapid increase in plasma EPO suggests a direct stimulating action of GH on EPO secretion. GH stimulated erythropoiesis in nephrectomized and hypophysectomized rats (25) and in rat bone marrow perfused with GH (26). Furthermore, GH directly stimulated the proliferation of erythroid progenitor cells *in vitro* (12). Kotzmann *et al.* (27) recently reported that erythroid and myeloid progenitor precursor cells increased after GH replacement in adult patients with GH deficiency whereas peripheral red blood cells, reticulocytes and EPO remained almost unchanged. IGF-I also has a stimulating activity on erythropoiesis *in vitro* (28) and *in vivo* (29). Merchav *et al.* (13) indicated that IGF-I plays a role as a mediator of erythropoiesis induced by GH since the effect was attenuated by monoclonal antibody against IGF-I. It has also been reported that IGF-I has stimulatory effects on EPO secretion in patients with insulin resistance (30). In the present study, plasma EPO levels increased earlier than did those of plasma IGF-I, which was detected at 12 h after the start of GH infusion, indicating that GH has a directly stimulating effect on EPO secretion.

The stimulating effect of GH on erythropoiesis could be explained, at least partly, by anabolic action rather than a direct effect. The anabolic effect of GH induces an increase in metabolic activity and necessity for oxygen transport to peripheral tissue, resulting in an increase of oxygen transportation and Hb levels (9). Erythropoiesis

was really stimulated by androgen (19, 22). Malgor & Fisher (31) reported that androgen stimulated EPO secretion from isolated and perfused dog kidney. These findings indicate that the stimulating effect of androgen on erythropoiesis is not simply due to anabolic effects. Subcutaneous injection of GH produced nitrogen retention in catabolic subjects at a dose of 100 µg/kg per day (32). On the other hand, it was reported that 66 µg/kg per day of GH did not show any anabolic effects in catabolic status (33). We administered GH as a continuous subcutaneous injection at the lower dose of 2 µg/kg per h, which is equivalent to 48 µg/kg per day. Serum UN levels slightly decreased at 72 h, whereas urinary nitrogen excretion was not significantly decreased, indicating that anabolic effects were not evident during the continuous subcutaneous infusion of GH at this dose for 72 h.

We found that EPO secretion was stimulated by GH administration in these patients. However, it remains to be elucidated where the EPO originated from in these subjects. The liver is the major site of EPO production in the ovine fetus, whereas the kidney plays a major role in EPO production in adult sheep (34). In the neonatal rat, EPO was found to originate from the liver rather than the kidney, and hypoxia induced EPO production in the kidney and the liver (35). Although there is no report suggesting that EPO is secreted from the liver in the adult human, it may be possible that EPO is partly produced in the liver when the kidney function is impaired in patients with CRF. As IGF-I is mainly produced in the liver, EPO secretion from the kidney might be locally stimulated by IGF-I; as a result the prolonged EPO secretion after the discontinuation of GH might be partly mediated by IGF-I stimulated by GH infusion.

In summary, constant subcutaneous infusion of recombinant human GH for 72 h raised plasma EPO levels and RC in anemic patients with CRF. An increase of plasma EPO was obtained earlier than an increase of plasma IGF-I, suggesting that GH has a directly stimulating effect on EPO secretion. Although Hb concentrations were not changed in the present study, longer administration of GH needs to be studied for its effect on anemia in these patients with renal disease. However, as GH administration for a longer term might induce hypertension and hyperglycemia in aged subjects (36), careful observation is required to avoid secondary side effects.

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