CLINICAL STUDY

Effect of recombinant human GH on circulating granulocyte colony-stimulating factor and neutrophils in patients with adult GH deficiency

Motoi Sohmiya, Ippei Kanazawa and Yuzuru Kato

Department of Endocrinology, Metabolism and Hematological Oncology, Shimane University School of Medicine, Izumo 693-8501, Japan

(Correspondence should be addressed to M Sohmiya; Email: motoi@med.shimane-u.ac.jp)

Abstract

Background: We previously reported that short-term continuous subcutaneous infusion (CSI) of recombinant human growth hormone (rhGH) increased plasma erythropoietin levels and hemoglobin concentrations in patients with adult GH deficiency. In the present study, we investigated the effect of rhGH on plasma granulocyte colony-stimulating factor (G-CSF) levels and neutrophil counts in patients with adult GH deficiency.

Methods: rhGH was administered for 1 year in six patients with adult GH deficiency (age range, 24–69 years; mean $\pm$ S.E.M., 51.7 ± 5.8 years; two males and four females) by means of CSI at a rate of 0.25 U/kg per week. Blood samples were obtained in the morning after overnight fasting every month before and after the start of rhGH administration. Plasma GH, insulin-like growth factor I (IGF-I) and G-CSF levels, and neutrophil counts, were measured.

Results: Mean ($\pm$ S.E.M.) plasma GH levels increased from 0.26 ± 0.14 to 2.28 ± 0.20 μ g/l 1 month after the start of rhGH administration. An increase of the plasma GH levels was accompanied by an increase in the plasma IGF-I levels from 64.7 ± 8.5 to 293.3 ± 80.6 μ g/l. Plasma G-CSF levels increased at 2, 3, 8, 9 and 10 months after the start of rhGH administration compared with 28.6 ± 11.0 ng/l at time 0. The neutrophil counts increased at 2, 3, 7, 8, 9, 11 and 12 months after the start of rhGH administration compared with 2822 ± 377 neutrophils/ μ l at time 0.

Conclusion: rhGH administration increased plasma G-CSF levels and neutrophil counts. GH and/or IGF-I might stimulate neutrophil production and/or release via G-CSF.

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Introduction

Growth hormone (GH) exerts an influence on the hematopoietic system as regards the normal differentiation and function of erythroid, myeloid and lymphoid cells (1–4). GH deficiency is often associated with anemia, leukopenia and thrombocytopenia (2–4). Furthermore, GH administration increased hemoglobin concentration in patients with adult GH deficiency (5, 6), and enhanced the function of myeloid cells and neutrophils (3, 4). We previously reported that continuous subcutaneous infusion of recombinant human GH (rhGH) increased plasma erythropoietin levels and hemoglobin concentrations in patients with adult GH deficiency (6). However, the effect of GH on neutrophil production *in vivo* in patients with adult GH deficiency is not yet fully understood.

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor, a glycoprotein with a molecular mass of about 20 000 Da that stimulates the

production and functional activation of neutrophils (7–9). Recent studies have demonstrated that recombinant G-CSF is effective in the treatment of patients with neutropenia. G-CSF is known to be produced by monocytes (10), endothelial cells (11), fibroblasts (12) and bone marrow stromal cells (13). However, the main source of circulating G-CSF remains to be elucidated. G-CSF secretion is stimulated by interleukin-1 (IL-1) (11–13), tumor necrosis factor α , bacterial endotoxin (14) and prednisolone (15), and inhibited by IL-4, IL-10 and IL-13 (16). However, there are no reports on the relationship between GH and G-CSF.

GH stimulates the development and function of granulocytes (17). The GH receptor is also present on monocytes, fibroblasts, neutrophils and lymphocytes (18). Therefore, GH administration might exert an influence on both G-CSF secretion and the neutrophil count. However, the relationship between neutrophil production and plasma G-CSF levels during rhGH administration has not been reported.

In the present study, we investigated the effect of rhGH on plasma G-CSF levels and the neutrophil count in patients with adult GH deficiency. This first report is a pilot study on the relationship between plasma G-CSF levels and rhGH treatment in patients with adult GH deficiency.

Materials and methods

Subjects

Six patients with adult GH deficiency (age range, 24–69 years; mean $\pm$ S.E., 51.7 ± 5.8 years; two males and four females) were studied. The subjects consisted of five patients with pan-hypopituitarism after pituitary tumor resection, and one additional patient with Sheehan's syndrome. The tumors were completely resected when they were evaluated histologically and by magnetic resonance imaging (MRI) after the surgery.

After insulin-induced hypoglycemia and arginine loading, peak GH levels were less than $3 \mu\text{g/l}$ in all of the subjects. In addition, all subjects had been diagnosed with a GH deficiency more than 18 months before the study began. Hormones other than GH were maintained within the normal range. Thyroid hormone levels and adrenocortical hormone levels were maintained within the normal range by appropriate replacement with L-thyroxine and hydrocortisone, respectively, for more than 1 year in all of the patients. The dosage of these drugs was not altered during the experimental period. None of the patients were given any ferric medicine during the experimental period. Bone marrow examination revealed normal myelograms in all of the subjects. The present study was in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all of the subjects prior to participation in the study.

Study protocol

22 kDa rhGH (Norditropin; Novo Nordisk Co., Bagsværd, Denmark) was dissolved in 0.9% saline and administered to the subjects by means of continuous subcutaneous infusion (CSI) at a flow rate of 0.25 U/kg per week (1.79 U/day per 50 kg body weight) for 12 months using a portable syringe pump (SP-3HQ; Nipro Co., Osaka, Japan) that had been inserted into the abdominal wall.

Blood samples were obtained in the early morning after an overnight period of fasting. These samples were obtained both before and every week after the start of rhGH administration for 1 month, and then the same protocol was followed for each of the next 11 months. Plasma GH, insulin-like growth factor I (IGF-I) and G-CSF levels, and neutrophil counts were monitored once a month. Lymphocyte, monocyte and eosinophil counts were also measured. Complete

blood count (CBC), C-reactive protein, erythrocyte sedimentation rate and other blood-chemistry analyses were carried out on a monthly basis. Body weight, blood pressure, and subjective and objective symptoms were also evaluated every month.

Assays

Plasma GH levels were measured by enzyme immunoassay as described previously (19). The minimum detectable quantity was $0.01 \mu\text{g/l}$ using a $20 \mu\text{l}$ plasma sample. Intra- and inter-assay coefficients of variation were 4.3 and 5.2%, respectively.

Plasma IGF-I levels were measured by specific RIA after acid-ethanol extraction, as described previously (20). The minimal detectable quantity was $30 \mu\text{g/l}$ using a $50 \mu\text{l}$ plasma sample. Intra- and inter-assay coefficients of variation were 6.3 and 7.2%, respectively. Normal ranges of plasma IGF-I levels in our laboratory were 202.8 ± 70.6 and $198.6 \pm 91.8 \mu\text{g/l}$ in males and females, respectively.

Plasma G-CSF levels were measured by sensitive enzyme immunoassay as described previously (15). The minimum detectable concentration was 1 ng/l using a $20 \mu\text{l}$ plasma sample. Intra- and inter-assay coefficients of variation were 2.7 and 5.6%, respectively. The mean recovery was 96.8%. There was no cross-reactivity with macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interleukin-1 β or erythropoietin at a concentration of $1 \mu\text{g/ml}$.

Neutrophil counts as well as CBC, C-reactive protein, erythrocyte sedimentation rate and other blood-chemistry analyses were measured by conventional methods in our hospital laboratory.

Statistical analysis

All values are means $\pm$ S.E.M. The data were analysed with a Dunnett *post-hoc* test in combination with ANOVA. $P < 0.05$ was considered significant.

Results

As shown in Fig. 1, plasma GH and IGF-I levels were increased significantly ($P < 0.0001$). Plasma GH levels (mean $\pm$ S.E.M.) increased from 0.26 ± 0.14 to $2.28 \pm 0.20 \mu\text{g/l}$ at 1 month after the start of rhGH administration, which was accompanied by an increase in plasma IGF-I levels from 64.7 ± 8.5 to $293.3 \pm 80.6 \mu\text{g/l}$. Steady-state plasma GH and IGF-I levels were obtained during the next 11 months. As shown in Fig. 2, plasma G-CSF levels were increased significantly ($P = 0.030$). Plasma G-CSF levels increased at 2, 3, 8, 9 and 10 months after the start of rhGH administration compared with $28.6 \pm 11.0 \text{ ng/l}$ at time 0. As shown in Fig. 3, the neutrophil counts were increased

Figure 1 Effect of rhGH on plasma GH (●) and IGF-I levels (■). Mean $\pm$ S.E. values are shown. * $P < 0.05$ versus time 0 (GH); # $P < 0.05$ versus time 0 (IGF-I).

significantly ($P = 0.038$). The neutrophil counts increased at 2, 3, 7, 8, 9, 11 and 12 months after the start of rhGH administration compared with 2822 ± 377 neutrophils/ μ l at time 0. There were no changes in lymphocyte, monocyte or eosinophil counts. There were no abnormal findings of C-reactive protein or erythrocyte sedimentation rate during the study period.

Discussion

We demonstrated in this study that circulating G-CSF levels and neutrophil counts increased following rhGH administration in patients with adult GH deficiency. rhGH is commonly administered by means of a bolus subcutaneous injection once a day. CSI of GH is more useful for some metabolic effects than a daily GH injection (21, 22). Laursen *et al.* (21) reported that CSI of GH for 4 weeks resulted in constant and increased serum GH levels, and higher levels of IGF-I and IGF-binding protein (IGFBP)-3 compared with a daily injection of GH, and that it did not affect glucose

Figure 2 Effect of rhGH on plasma G-CSF levels. Mean $\pm$ S.E. values are shown. * $P < 0.05$ versus time 0.

Figure 3 Effect of rhGH on neutrophil counts. Mean $\pm$ S.E. values are shown. * $P < 0.05$ versus time 0.

tolerance and produced lower insulin levels than with a daily GH injection. We reported previously that CSI of GH effectively increased erythropoietin secretion in anemic patients with chronic renal failure (23) and in malnourished predialysis anemic patients with diabetic nephropathy (24). As the present study was performed on a small number of patients, further investigation is required.

Based on the present study we would expect patients with acromegaly to have elevated or high normal neutrophil counts. However, as far as we know, few patients with acromegaly had elevated or high normal neutrophil counts. We think that acromegalic patients with very high plasma GH levels were associated with increased circulating plasma volume. Therefore, neutrophil counts might not reflect the true neutrophil counts for hemodilution.

IGF-I is also known to potentiate the stimulating effect on hematopoietic cells (25–27). It was reported that IGF-I promoted granulocyte functions by increasing granulocyte longevity (28) and that IGF-I administration enhanced lymphoid and myeloid reconstitution after allogeneic bone marrow transplantation (29). However, there is no report about the relation between IGF-I and G-CSF. The present study suggests that GH and/or IGF-I may stimulate neutrophil production via G-CSF stimulation. As many cells secreting G-CSF possess both GH and IGF-I receptors, it was impossible to hypothesize whether these two hormones possess independent direct and indirect (via G-CSF production) granulopoietic effects *in vivo*.

We demonstrated that long-term treatment with rhGH raised plasma G-CSF levels within the initial 2 weeks in patients with adult GH deficiency. Subsequently, neutrophil counts increased to the normal range in association with suppressed plasma G-CSF levels in these patients treated with rhGH. The phenomenon that plasma G-CSF levels fall with an increase of neutrophil count after G-CSF elevation is often presented (30–33). Layton *et al.* (31) found a

decrease in circulating G-CSF levels on neutrophil regeneration in patients with intravenous continuous G-CSF infusion after chemotherapy. A similar reaction between neutrophil counts and circulating G-CSF levels was found when recombinant G-CSF was administered by daily subcutaneous injections in patients (32) and healthy subjects (33). Endogenous G-CSF levels were regulated under the potential feedback mechanism of the neutrophil counts (34). It was suggested that the number of neutrophils regulates the biological activity of G-CSF by absorbing and metabolizing the G-CSF (35), and incubation experiments on recombinant G-CSF with neutrophils *in vitro* resulted in decreased G-CSF concentration (36). However, the precise mechanism was not fully elucidated.

The possibility that GH exerts a negative influence on leukocytic systems has been reported (37, 38). Previous clinical studies have demonstrated an increased incidence of leukemia occurring in pediatric subjects treated with GH (37, 38). However, other recent studies have provided no evidence of an increased incidence of leukemia or extracranial nonleukemic malignancies among patients without prior risk factors (39–41). In the present study, GH stimulated neutrophil production within the normal range via G-CSF secretion *in vivo*.

The neutrophil counts might be affected by redistribution, premature release and decreased clearance from the blood, and G-CSF is known to lead to premature release as well as to increased production. We could not clarify, based on the present study, whether redistribution of neutrophils contributed to the change in neutrophil count. Therefore further large-scale study is necessary.

Two important limitations of our study merit further discussion. First, the present study was not designed by placebo control study because of difficulty of long-term CSI using physiological saline in patients with adult GH deficiency. Plasma G-CSF levels showed no sex difference, and no apparent correlation with age, hematocrit or white blood cell counts, but significant correlation was observed between the plasma G-CSF level and neutrophil count in normal subjects. Plasma G-CSF level was lower in the morning than in the afternoon or at night (42). However, there is no report of a seasonal change in plasma G-CSF levels or neutrophil counts. Therefore, further placebo control studies are required. Second, the present study was carried out with only a few patients. Therefore, it may be thought of as a pilot study.

In summary, we demonstrated that CSI of rhGH for 12 months raised plasma G-CSF levels at 2 months after the start of rhGH administration in patients with adult GH deficiency. Neutrophil counts were changed with plasma G-CSF levels. These findings suggest that GH and/or IGF-I might stimulate neutrophil production via G-CSF.

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