Serum Levels of 20 Kilodalton Human Growth Hormone (20K-hGH) in Patients with Acromegaly before and after Treatment with Octreotide and Transsphenoidal Surgery

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Abstract. Circulating human GH (hGH) consists of several molecular isoforms. It was previously reported that the proportion of 20 kilodalton hGH (20K-hGH) was elevated in the serum of patients with active acromegaly. In this study, we investigated the effects of octreotide and transsphenoidal adenomectomy on the proportion of 20K-hGH in the serum of 7 acromegalic patients. To achieve an acute effect, octreotide (100 μg) was subcutaneously injected as a bolus. To observe the chronic effects of octreotide therapy and surgery, serum samples were obtained by repetitive blood sampling before and 3 to 8 weeks after treatment. Serum levels of 20K-hGH and 22 kilodalton hGH (22K-hGH) were determined by specific enzyme-linked immunosorbent assay. A bolus injection of octreotide elicited a parallel decrease in serum 22K-hGH and 20K-hGH, resulting in an unchanged proportion of 20K-hGH to total circulating hGH. The proportion of 20K-hGH was decreased in 4 of 4 patients 4 to 7 weeks after surgery and in 2 of 4 patients after chronic treatment with octreotide for 3 to 8 weeks. The proportion of serum 20K-hGH was positively related to mean serum 20K-hGH as well as serum total hGH levels, but not with serum IGF-I levels. These findings suggest that high serum levels of 20K-hGH or total hGH per se might elicit a chronic change in the clearance kinetics of 20K-hGH and increase the proportion of 20K-hGH in acromegalic patients.

Key words: Human growth hormone, Acromegaly, Octreotide

CIRCULATING human growth hormone (hGH) consists of several molecular isoforms, in which 22 kilodalton hGH (22K-hGH) is the most abundant [1]. Twenty kilodalton human GH (20K-hGH), a naturally occurring isoform of 22K-hGH, arises from the same gene (hGH-N) as 22K-hGH by alternative splicing of mRNA [2]. 20K-hGH lacks 15 amino acids at its binding site 1 to hGH receptor [3], resulting in a reduction of its binding affinity [4]. 20K-hGH also differs from 22K-hGH in some of its metabolic actions. 20K-hGH has weaker diabetogenic and early insulin-like activities than 22K-hGH [5].

There has been growing evidence for the regulation of 20K-hGH secretion. It was reported that molecular forms of hGH secreted in vivo are not specific for physiological and pharmacological secretory stimuli [6–11]. In addition, we have reported that 20K-hGH and 22K-hGH are secreted in toto from GH-producing pituitary adenoma cells in vitro [12].

There have been conflicting reports on metabolic clearance of 20K-hGH. Although 20K-hGH is poorly trapped by hGH-binding proteins [4], the plasma survival time of administered 20K-hGH is longer than that of 22K-hGH [13]. In contrast, a pharmacokinetic study demonstrated that endogenous kinetics of 20K-hGH was comparable with those of 22K-hGH in normal men [14].

In this study, we investigated possible changes of the proportion of 20K-hGH in circulating hGH when
serum hGH levels were decreased in 7 patients with acromegaly after octreotide treatment as well as transsphenoidal surgery.

Subjects and Methods

Patients

Seven Japanese acromegalic patients were recruited in this study after obtaining informed consent. The study procedures were approved by the ethical committee of Shimane Medical University. Acromegaly was diagnosed based on characteristic clinical features, elevated serum levels of hGH and IGF-I, and pituitary mass on brain MRI images. Plasma hGH levels were not suppressed below 5 µg/L by oral administration of 75 g oral glucose. Basal serum GH level as determined by a commercial EIA kit was 2.3 µg/L in case 2. A nadir value after 75 g oral glucose was 2.2 µg/L whereas a peak value of 12.4 µg/L was obtained after TRH stimulation in case 2. Four of 7 patients (cases 1, 5, 6 and 7) underwent transsphenoidal surgery. The other three patients (cases 2, 3 and 4) were treated with octreotide (Novartis Pharma, Tokyo) (20 µg sc, every 2 h) using a portable infusion pump for 3 to 8 weeks. Case 1 underwent transsphenoidal surgery after preceding treatment with octreotide for 3 weeks.

Single octreotide suppression test

Single octreotide suppression test was performed in 6 patients (cases 1, 2, 4, 5, 6 and 7) before the treatment with octreotide or surgery. After overnight fasting, an indwelling catheter was inserted into the antecubital vein for blood sampling. Blood samples were obtained immediately before, and 1, 2, 3 and 4 h after the administration of octreotide (100 µg) which was injected subcutaneously as a bolus.

Repetitive blood sampling

After overnight fasting, an indwelling catheter was inserted into the forearm vein. The catheter was kept patent with heparinized saline. Patients were allowed to take meals and engage in daily activities. Repetitive blood samplings were performed through the catheter before therapy, after octreotide therapy (duration of the therapy: 3 weeks in cases 1 and 2, 4 weeks in case 4 and 8 weeks in case 3) and after surgery (4 weeks after in cases 5 and 7, 6 weeks after in case 6 and 7 weeks after in case 1). In cases 1, 2 and 3, blood samples were obtained every 1 h for 24 h (24 samples). In cases 4, 5, 6 and 7, blood samples were obtained every 20 min for 3 h (10 samples). In cases 4 and 7, blood samples were obtained at an interval of 20 min for 2 h (7 samples) during treatment with octreotide and after surgery, respectively.

Assay

Serum 20K-hGH and 22K-hGH levels were measured by specific enzyme-linked immunosorbent assays (ELISA) as previously described [6]. The minimal detectable concentrations of 20K-hGH and 22K-hGH were 5 ng/L and 50 ng/L, respectively. The precision of the assays is as described elsewhere in detail [6]. Serum IGF-I was measured using an immunoradiometric assay kit (Chiba-Corning Inc., Tokyo, Japan) according to the manufacturer’s recommendations.

Statistics

The data were expressed as mean ± SD. Serum concentrations of 20K-hGH plus 22K-hGH were designated as serum total hGH. Proportion of serum 20K-hGH to serum total hGH (%20K-hGH) was calculated as [20K-hGH]/[total hGH] ×100. Statistical differences were evaluated by ANOVA and Fisher’s test as appropriate. Correlation between two variables was analyzed by least square method. A P value less than 0.05 was considered significant.

Results

Serum 22K-hGH and 20K-hGH values and IGF-I levels are shown in Table 1. The pretreatment values of %20K-hGH were ranged from 6.59 to 12.35% with the mean (± SD) of 9.14 ± 2.02% in these patients (Table 1). As shown in Fig. 1, a bolus subcutaneous injection of 100 µg octreotide resulted in a rapid decrease in both serum 22K-hGH and 20K-hGH in 5 of 6 patients examined (cases 1, 2, 4, 5 and 6). In patient 7, octreotide failed to decrease either 22K-hGH or 20K-hGH. When the data of 5 responders were analyzed as the percent change of the basal serum values, it was shown that serum 22K-hGH and 20K-hGH were
decreased in parallel (Fig. 2, left panel). Consequently, %20K-hGH value did not change throughout the observation period (Fig. 2, right panel).

Mean serum levels of 22K-hGH and 20K-hGH as well as IGF-I considerably decreased in all the patients treated with octreotide for 3 to 8 weeks (cases 1, 2, 3 and 4) and surgery (cases 1, 5, 6 and 7) (Table 1). Mean (± SD) values of %20K-hGH decreased in all the 4 patients after surgery whereas they decreased in 2 of 4 patients treated with octreotide. The value after octreotide treatment was not different from the pretreatment value in patient 2 in whom the basal %20K level was as low as 6.59%. In patient 1, %20K value as well as serum 22K- and 20K-hGH levels was significantly lower after surgery compared with the value after octreotide therapy.

When all the data obtained by repetitive blood samplings were analyzed as a whole, positive correlations were observed between %20K-hGH and serum 20K-hGH (Fig. 3, left panel), and between %20K-hGH and
serum total hGH (Fig. 3, middle panel). There was no correlation between %20K-hGH and serum IGF-I (Fig. 3, right panel).

Discussion

It was first reported that the proportion of non-22K hGH isoforms in total circulating hGH was increased in acromegalic patients [15]. Furthermore, it was previously demonstrated that the proportion of 20K-hGH to total hGH was increased in the patients with active acromegaly [6]. In the present study, the mean values of serum %20K-hGH ranged from 6.59 to 12.35% in 7 acromegalic patients before treatment. These values are similar to those described in our previous report [6].

The mechanisms involved in the higher proportion of 20K-hGH in the sera of acromegalic patients remains to be elucidated. Since the proportion of non-22K-hGH was decreased after pituitary surgery, it was postulated that GH-producing adenoma secretes more non-22K-hGH [15]. However, we previously reported that the %20K-hGH values were greater in serum than in perfusion effluent in vitro, suggesting that the proportion of 20K-hGH in the serum total hGH might be affected by the difference in metabolic clearance of hGH isoforms [12].

In the present study, we found that %20K-hGH was chronically decreased after surgery in all the 4 patients with acromegaly examined. This observation is in line with a previous report that plasma %20K-hGH values were not different in inactive acromegalic patients treated by surgery from those in normal subjects [6]. It is notable that a small but significant decrease in %20K-hGH was also observed after the treatment with octreotide for 3 to 8 weeks in 2 of 4 patients. In case 4, the difference did not reach statistical significance presumably due to a small number of blood samplings. The other 1 patient (case 2) had low %20K-hGH values before octreotide treatment. The decrease of %20K-hGH in the presence of GH-producing adenoma tissues and of still high levels of serum IGF-I suggest that the secretion of more 20K-hGH from adenoma cells is not solely responsible for the high proportion of 20K-hGH in the sera of acromegalic patients, and that a change in clearance of 20K-hGH is also implicated.

The serum %20K-hGH was correlated with mean serum 20K-hGH and mean serum total hGH levels whereas IGF-I levels were not correlated with the proportion of serum %20K-hGH. These observations...
suggest that high 20K-hGH or total hGH level per se rather than metabolic effects of hGH hypersecretion including increases in serum acid-labile subunit and IGF binding protein-3 levels [16] might be related to the increase in proportion of 20K-hGH. Bearing this in mind, it is not surprising that pretreatment %20K-hGH was similar to the normal levels [6] in patient 2 in whom pretreatment total hGH levels were less than 1 μg/L. This notion also makes it possible to explain the high proportion of 20K-hGH in the sera of patients with anorexia nervosa in whom a large amount of hGH originates from non-tumorous somatotrophs [6].

Interestingly, we found that a single subcutaneous injection of octreotide resulted in a parallel decrease of 20K-hGH and 22K-hGH in acromegalic patients. Consequently the proportion of 20K-hGH to total hGH did not change. These observations indicate that clearance rates of 20K-hGH and 22K-hGH are similar in acromegalic patients in the study design to see the acute effect of a single octreotide injection. The reasons for the discrepancy between the similar clearance rates and chronic high %20K-hGH values remain to be elucidated. Poorer interaction of 20K-hGH than 22K-hGH with circulating GH-binding protein may affect clearance rate from plasma [4]. The difference in clearance rates of 20K- and 22K-hGH, if any, should be too small to be detected in the acute study of a single subcutaneous octreotide injection. The possibility cannot be ruled out, however, that a small difference in clearance rates of 20K- and 22K-hGH in association with possible secretion of more 20K-hGH from adenoma cells could lead to the high proportion of 20K-hGH in the sera of acromegalic patients.

The present study has several limitations. The number of patients was small and control groups of healthy subjects and placebo-treated patients were not included. However, this study might provide information regarding the proportion of 20K-hGH in the sera of acromegalic patients in relation to therapy.

In conclusion, the proportion of serum 20K-hGH to serum total hGH decreased in all the acromegalic patients who underwent surgery and in some patients treated with octreotide for 3 to 8 weeks. The proportion of 20K-hGH was positively correlated with mean serum 20K-hGH and total hGH levels, which per se might change the clearance kinetics of 20K-hGH and increase the proportion of 20K-hGH in acromegalic patients.

References


