NOTE

Pharmacokinetics and Metabolic Effects of High-Dose Growth Hormone Administration in Healthy Adult Men

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Abstract. To evaluate pharmacokinetics of growth hormone (GH) and its effects on IGF-I, glucose, insulin, non-esterified fatty acid (NEFA) and triglyceride (TG), fifteen Japanese healthy adult male volunteers (20–27 years old) were studied. The subjects were divided into three groups, and received with a single s.c. injection of 0.075, 0.15 and 0.30 IU/kg of GH, respectively. The subjects assigned to receive 0.30 IU/kg were administered for additional 6 days. After a single administration of GH, C_{max} and AUC of GH were increased in a dose-dependent manner. There was a significant positive correlation between the AUC and the T_{1/2} ($r=0.516$, $P<0.05$). Total body clearance was significantly greater in 0.075 IU/kg group than the other groups and showed a significant negative correlation with C_{max} ($r=-0.694$, $P<0.005$) and AUC ($r=-0.723$, $P<0.005$). After a single administration of each dose, serum IGF-I concentrations were increased gradually. In the repeated administered group (0.30 IU/kg), IGF-I concentrations almost reached a plateau at a significantly high level four days after the start of administration and remained at a high level (786–405.4 ng/ml) until day 8. There was no significant difference in diurnal change of blood glucose and serum insulin after a single administration of GH among three groups. In the 0.3 U/kg group, there was no significant difference in diurnal change of blood glucose between day 1 and day 7, but serum insulin level was significantly higher in day 7 than in day 1 ($P<0.01$). Serum concentrations of NEFA were increased over time after administration in all subjects administered once or repeatedly. TG concentrations showed no changes after single administration of each dose level, but were significantly increased on day 7 in the subjects repeatedly treated with 0.30 IU/kg/day. This effect is speculated to be caused by high dose GH treatment. The above findings demonstrated that higher GH dose significantly influences on carbohydrate and lipid metabolism. It remains necessary to elucidate what kinds of effects of the long-lasting increased levels of insulin and triglyceride, even if reversible, would have on glucose and lipid metabolism.

Key words: High-dose GH, Insulin, Triglyceride, Healthy adult

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SINCE human growth hormone (GH) has been produced by genetic recombinant technology in place

of extraction from the pituitary gland, there is no limitation of supply for GH treatment. It is now a consensus that GH treatment with replacement dose (0.5 IU/kg/week used in Japan) is not enough to treat growth hormone deficiency (GHD) [1] as well as non-GHD short children [2]. Satisfactory therapeutic results have been reported with a high dose of 0.30 IU/kg/day in children with non-GHD short stature and with intrauterine growth retardation

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(IUGR) without serious adverse events [3–5].

In vivo and in vitro studies have shown that growth hormone (GH) has the effect on carbohydrate metabolism and lipid metabolism. Since clinical trials with high dose GH on non-GHD children have been conducted, it is necessary to investigate the effect of high dose GH on carbohydrate and lipid metabolism.

In the present study, the pharmacokinetics and metabolic effects of high dose GH was studied in healthy adult volunteers.

Subjects and Methods

Subjects

The subjects consisted of healthy volunteers with respect to previous history, weight (within $\pm 20\%$ of standard weight), medical interview, blood pressure and pulse rate, body temperature, electrocardiography and laboratory tests (hematology, serum chemistry and routine urinalysis). Fifteen Japanese adult males aged from 20 to 27 years were recruited as the study subjects with an informed consent. They were divided into three groups and administered by a single subcutaneous injection of 0.075, 0.15 and 0.30 IU/kg ($n=5$ at each dose level) of recombinant GH (Norditropin; Novo Nordisk Pharma, Tokyo, Japan). The subjects assigned to receive 0.30 IU/kg were administered for additional 6 days.

Study design

The GH was administered at 9:00 a.m. after overnight fasting. A lunch, evening meal and snack were served at 13:00, 18:00 and 21:00, respectively. In the 0.3 IU/kg group, a breakfast was served at 9:00 before blood sampling on the day 2–6.

The subjects of the 0.3 IU/kg group took approximately 2500 cal/day of meals containing 80 g protein and 80 g fat on average from day 2 to day 6.

Blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 hours after single injection. In the 0.30 IU/kg group, blood sampling was also performed at 24 hours after administration on days 2–6, and 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 hours after administration on day 7. Blood glucose, serum GH, serum IGF-I

and serum insulin were measured in these samples and triglyceride (TG) and non-esterified fatty acid (NEFA) were measured in the samples before and till 4 hours after GH administration on day 1 and day 7. GH was measured by IRMA (GH kit [Daiichi], Daiichi Radioisotope), IGF-I (Somatomedin C [Chiba Corning], Chiron) and insulin by RIA (Insulin[Eiken], Eiken), and glucose by glucosedehydrogenase method. In the 0.30 IU/kg group, anti-GH antibody titer was determined by charcoal method before the initial administration and 14 days after the study period.

This clinical trial was approved by the Institutional Review Board at Waseda Clinic.

Statistical analysis

Results are expressed as mean and standard deviation (SD) in the figures. The time interval from GH injection to maximum GH level was recorded as T_{max} . To determine the elimination half-time ($T_{1/2}$), a linear regression analysis was performed on the GH levels starting from 1 h after T_{max} . The total body clearance of GH was calculated from the injected dose divided by the area under the curve (AUC) of GH.

The data obtained were statistically analyzed by the following methods: (i) homogeneity of background factors: Kruskal-Wallis test, (ii) comparison of laboratory data between the pretreatment and post treatment levels: Wilcoxon's signed rank sum test, (iii) comparison of changes in blood concentrations of each parameter between the dose groups: two-way analysis of variance and Tukey multiple comparison, and (iv) Pearson's correlation coefficient.

Results

The demographic data of the subjects are shown in Table 1. There was no significant difference among three groups in each parameter.

Serum GH concentrations after single administration and some of the calculated variables of the GH profiles are shown in Fig. 1 and Table 2. There was a significant, dose-dependent increase of the C_{max} and the AUC. However, there was no significant difference in T_{max} and $T_{1/2}$ among the three groups. Total body clearance was significantly

Table 1. Demographic data of subjects

Group	0.075 IU/kg (n=5)	0.15 IU/kg (n=5)	0.30 IU/kg (n=5)	Significance*
Age (year)	22.4±1.3	22.4±1.5	24.4± 2.6	NS
Height (cm)	171.5±7.5	170.2±5.8	170.2± 2.3	NS
Weight (kg)	62.8±6.5	65.1±7.5	61.7± 7.3	NS
% overweight	-2.2±6.6	3.0±8.1	-2.3±11.7	NS

*Kruskal-Wallis test NS: not significance

Fig. 1. Change in serum GH concentration after GH injection.**Table 2.** Pharmacokinetics of GH administration

Group	0.075 IU/kg	0.15 IU/kg	0.30 IU/kg
Cmax (µg/L)	55.4 (38~82)	93.8 (80~130) ^a	180.0 (160~210) ^{ab}
AUC (µg/Lx24 h)	311.5 (251.8~344.1)	836.9 (681.5~909.5) ^a	1778.5 (1619.1~1958.1) ^{ab}
Tmax (hr)	3.6 (2~5)	4.4 (3~6)	4.1 (3~5)
T1/2 (hr)	2.28 (1.47~3.00)	3.08 (1.99~3.87)	3.57 (2.59~5.38)
Clearance (mL/min)	327.2 (274.8~412.8)	249.1 (231.6~262.2) ^a	228.1 (196.2~258.0) ^a

^a Significant vs 0.075 IU/kg at $P < 0.05$ ^b Significant vs 0.15 IU/kg at $P < 0.05$

greater in 0.075 IU/kg group than the other groups. In the 0.30 IU/kg group, no significant difference was observed in diurnal change of serum GH concentrations between days 1 and 7 (Fig. 1). During the treatment period of 7 days, serum GH concentrations returned to the baseline 24 hours after each administration.

After single sc injection, IGF-I concentrations did not significantly change until 5–6 hours after the administration and increased gradually toward 24 hours

(Fig. 2), when the levels were significantly greater than those before injection. There was a significant difference in serum IGF-I level between the 0.075 and 0.30 IU/kg groups ($P < 0.005$) 24 hours after injection. There was a positive correlation between the AUC and IGF-I value 24 hours after a single injection, but it does not reach a significance ($r = 0.474$, $P = 0.074$). In the 0.3 IU/kg group, serum IGF-I concentrations increased and reached nearly a plateau on day 4, after which they remained

Fig. 2. Change in serum IGF-I after GH injection.

high until day 8 (Fig. 2). On day 8, IGF-I attained a significantly higher serum concentration of 405–786 ng/ml than on day 1, which was comparable to the concentration of IGF-I normally seen during puberty.

There was no significant difference in diurnal change of blood glucose and serum insulin after a single administration of GH among three groups as shown in Fig. 3(a) (b). In the 0.3 U/kg group,

there was no significant difference in diurnal change of blood glucose between day 1 and day 7, either (Fig. 3(a) (b)). However, blood insulin level was significantly higher in day 7 than in day 1 ($P < 0.01$).

Serum concentrations of NEFA were increased over time after administration in all subjects administered once or repeatedly (Fig. 4). The time-related changes were not significantly different among the three dose groups ($P = 0.33$). Intragroup com-

Fig. 3(a). Diurnal change of blood glucose.

Fig. 3(b). Diurnal change of serum IRI.

parison in the repeatedly treated subjects also showed no significant difference in changes in NEFA concentrations between days 1 and 7.

There was no significant difference in TG concentration among three groups after a single injection of GH (Fig. 5). In the 0.3 U/kg group, serum TG concentrations gradually increased during 7-day administration (Fig. 6) and serum TG level before GH injection on day 7 was significantly higher than the corresponding value on day 1. But the TG level gradually declined in four hours after administration on day 7 (Fig. 6).

In the repeated administered group, anti-GH antibodies were not detected in any of the subjects 14 days after the treatment period.

No adverse events were found after single administration of 0.075 and 0.15 IU/kg. In the 0.3 IU/kg group, one subject complained of mild pain in the both knees on day 1, which disappeared without any treatment on day 2. The same subject exhibited papules all over the both legs. This subject showed a negative response to DLST (drug-induced lymphocyte blastogenesis test) in all the tests using solvent, lyophilized powder and drug solution. At the fol-

Fig. 4. Change in NEFA after GH injection.

Fig. 5(a). Change in serum triglyceride after GH injection.

Fig. 5(b). Change in serum triglyceride in 0.3 IU/kg group.

low-up tests, the fraction of eosinophils in the blood was also within the reference range. Therefore, the causal relation to the drug was not definite. There were no abnormal laboratory findings after a single administration. In the 0.3 IU/kg group, changes in serum ALT, changes in serum TSH and urinary occult blood were recorded in 2, 1 and 1 subjects, respectively. However, these changes were not clinically significant.

Discussion

We have shown a significant dose-dependent increase of the C_{max} and the AUC. This relation, to some extent, reflected in the serum IGF-I levels 24 hours after an injection. But the effect of the dose on IGF-I should be followed after several days, since serum IGF-I concentrations did not

reach a plateau until on day 4 in 0.30 IU/kg group. The plateau level was compatible to the pubertal level and to the level of patients with intrauterine growth retardation (IUGR) treated with 0.3 IU/kg/day (5). In the IURG study (5), the high dose GH treatment significantly increased growth velocity and increased height SD score more than 2 SD in two years. These findings suggest that high IGF-I level by high dose GH closely relates to increased height velocity. Since high dose trial has been conducted in children, it is reasonable to conduct this study in children. However, because of the ethical problem and the amount of blood drawn, high dose trial was conducted in adults.

A dose-dependent increase of the C_{max} and the AUC is in agreement with the reports in GH deficient children [6] and in Turner syndrome [7]. However, maximum concentration of GH is higher in our study than in the others, though accurate dose comparison is difficult; they used 2~6 IU/day or 4~8 IU/m²/day. This difference may be caused by the difference in underlying diseases and/or in age, or even it may be explained by the different assays for GH measurement. Although there was no significant difference in T_{max} and T_{1/2} among three groups, there was a tendency that T_{1/2} became longer in higher dose group and clearance was significantly smaller in higher dose groups than 0.075 IU/kg group. Even this smaller clearance at high dose, GH accumulation was not observed in 0.30 IU/kg group and the later plateauing of IGF-I was observed. This may indicate some saturation of the clearance mechanisms and some saturation of biological activities.

For glucose and insulin, only physiological changes in glucose concentrations due to food intake were observed with no significant difference being noted between days 1 and 7 in the 0.30 IU/kg group, but significant increased insulin level was observed on day 7 in the 0.30 IU/kg group. There are many reports that GH treatment increases plasma insulin in GH deficient children [6], GH deficient adults [8, 9], non-GH deficient short children [4] and Turner syndrome [10], but glucose tolerance is not impaired. Our study is compatible with these reports. Prevalence of clinical diabetes is reported not to be increased in GH-treated children [11]. It is reasonable that replace dose of GH in

GH deficiency does not impair glucose tolerance. However, high dose GH treatment in Turner syndrome produced a significant increase in fasting and insulin levels [12]. It is possible that long term treatment with high dose of GH in non-GH deficient short children impair glucose tolerance, since acromegaly is associated with an increased prevalence of diabetes [13–15].

It is known that NEFA is increased in response to GH [16, 17]. Also in this study, NEFA concentrations were increased over time, but not significantly different among the three dose groups. Comparison of this parameter in the repeated dose group also showed no significant difference between days 1 and 7. These results show that lipolytic effect of GH is not dose dependent in the dose range studied and NEFA accumulation is not observed in repeated injection of high dose GH.

TG concentrations showed no changes after single administration of each dose level, but were significantly increased on day 7 in the subjects repeatedly treated with 0.30 IU/kg/day. It has been reported that GH replacement therapy did not show any significant changes in TG concentration in adult GHD [8, 9]. In acromegalic patients, however, a mild or moderate hypertriglyceridemia and decreased lipoprotein lipase activity have been observed [18]. GH is known to stimulate the release of free fatty acid from fat cells resulting in an increased flux of free fatty acids to the liver. GH stimulates the esterification of oleic acid into triglyceride in hepatocytes from hypox rats [19] and decreases lipoprotein lipase release from rat epididymal fat *in vitro* [20]. Therefore, it is speculated that increased TG level observed in repeated administered group is caused by high dose GH treatment, since replacement GH treatment dose not influence on lipid metabolism in adult GHD [8, 9] and in GHD children [6]. Since hypertriglyceridemia is clinically important as a risk factor for atherosclerosis, further evaluation of the effects on TG is required in the long-term use of high-dose GH.

The above findings demonstrated that higher GH dose significantly influences on carbohydrate and lipid metabolism. It remains necessary to elucidate what kinds of effects of the long-lasting increased levels of insulin and triglyceride, even if reversible, would have on glucose and lipid metabolism.

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