NOTE

Effects of sc Administration of Recombinant Human Insulin-Like Growth Factor I (IGF-I) on Normal Human Subjects

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Abstract

Recombinant human insulin-like growth factor I (IGF-I) was administered subcutaneously to each of 5 normal human subjects at doses of 0 mg/kg (control), 0.06 mg/kg, or 0.12 mg/kg successively at one week intervals. After 0.06 mg/kg or 0.12 mg/kg IGF-I injections, plasma IGF-I levels increased from 185 ± 17 ng/ml (Mean ± SEM) to maximal levels of 396 ± 21 ng/ml at 3 hours and from 169 ± 14 ng/ml to 480 ± 27 ng/ml at 4 hours, respectively. These two peak values were statistically different (p<0.05). After 0.06 mg/kg and 0.12 mg/kg IGF-I administration, blood glucose levels decreased from 85 ± 2 mg/dl to minimal levels of 73 ± 3 mg/dl at 3 hours and from 83 ± 1 mg/dl to 50 ± 4 mg/dl at 2 hours, respectively. These two minimal values were statistically different (p<0.001).

Serum insulin and C-peptide levels were decreased in a dose dependent manner after IGF-I administration. There were no changes between blood urea nitrogen levels before and 4 hours after IGF-I administration. The urinary GH concentration decreased after 0.06 mg/kg IGF-I administration, but increased and maintained normal values after 0.12 mg/kg IGF-I administration.

Insulin-like growth factor I (IGF-I) is an important mediator of growth hormone (GH) action (Daughaday et al., 1957). However, it is structurally similar to insulin and shares many of its biological properties (Rinderknecht and Humbel 1978a, 1978b, Froesch and Zapf 1985, Zapf et al., 1984). IGF-I is now produced by recombinant-DNA technology and thus available for laboratory and clinical use (Schaleh et al., 1984, Niwa et al., 1986, Zapf et al., 1986, Scheiwiller et al., 1986, Guler et al., 1987, 1989, Laron et al., 1988). Insulin-like growth factor elicits two types of biological effects, an insulin-like effect and a growth-promoting effect. We have already reported that recombinant IGF-I administration showed growth promoting effects in both normal and hypophysectomized rats (Hizuka et al., 1986, 1988). In our previous papers, we also noted an anabolic effect of IGF-I.

In this paper, the results of our study
on the effects of recombinant IGF-I on normal human subjects are reported.

Materials and Methods

Subjects and study design

Five healthy young adults aged between 22 and 24 participated in this study. None of the subjects had clinical evidence of illness or was taking any drugs. All were within 20 percent of their ideal body weight. Informed consent was obtained from each volunteer and the experimental protocol was approved by the Human Subjects Investigation Committee of our medical school.

Each subject underwent three experiments, one with saline and the other two with 0.06 mg/kg and 0.12 mg/kg IGF-I, respectively. The experimental interval was 7-14 days. The subjects fasted overnight and remained in a recumbent position from 30 minutes before to 4 hours after each experiment. Before each injection, a catheter was placed in an antecubital vein for the first 4 hours. IGF-I 0.06 mg/kg, 0.12 mg/kg, or saline was injected at 8 a.m. (time 0). Blood samples were drawn before and at every succeeding hour for 6 hours, then at two hour intervals for the following 12 hours, and finally at 24 hours after injection (total 11 times). Vital signs such as pulse rate, respiratory rate, body temperature, and blood pressure were measured at the same time as each blood sampling throughout the study. Food was first taken at 4 hours after injection (lunch). A light snack and dinner were eaten at 6 and 10 hours after injection, respectively. All blood samples were immediately placed on ice and centrifuged within 3 hours. Serum and plasma were stored at -20°C until they were assayed. Urine was collected for 24 hours to measure urinary GH.

Recombinant human IGF-I preparation

Recombinant human insulin-like growth factor I (IGF-I) was kindly provided by Fujisawa Pharmaceutical Co., Ltd. Osaka, Japan. The IGF-I preparation was synthesized by recombinant DNA technology as described (Niwa et al., 1986). The amino acid sequence of the IGF-I preparation is the same as that of natural IGF-I. The purity of the preparation (lot 1A6685K) was 97.1% as determined by reverse phase high pressure liquid chromatography. IGF-I was dissolved in physiological saline at a concentration of 0.6% just before use.

Assays

Plasma IGF-I was measured by radioimmunoassay as described previously (Miyakawa et al., 1986). Blood glucose was measured with an "Autoanalyzer". Serum insulin, C-peptide, and plasma glucagon were measured with commercially available RIA kits. Minimal detectable levels of insulin, C-peptide, and glucagon were 2.5 μU/ml, 0.2 ng/ml, and 30 pg/ml, respectively. A routine blood chemistry study was performed before and at 24 hours after injection in the laboratory center of our hospital. Blood urea nitrogen was measured before and at 4 and 24 hours after injection in the laboratory center of our hospital. Urinary GH was measured by enzyme immunoassay as described earlier (Sukegawa et al., 1988).

Statistics

Student's t-test and paired t-test were used for statistical analysis of the data.

Results

Plasma levels of total IGF-I

The individual and the mean plasma levels of IGF-I are shown in Fig. 1 and Fig. 2. Plasma IGF-I levels did not change throughout the study after saline administration. After 0.06 mg/kg IGF-I administration, mean plasma IGF-I increased from 185 ± 17 ng/ml (Mean ± SEM) to a maximal level of 396 ± 21 ng/ml at 3 hours and then gradually decreased. However, the level did not return to the basal value, even at 24 hours after injection. After 0.12 mg/kg IGF-I administration, the levels rose further; the peak levels reached at 4 hours had a mean value of 480 ± 27 ng/ml. These two peak values were statistically significantly different (p < 0.05). The percent increase in IGF-I was statistically greater after 0.12 mg/kg IGF-I administration at 2, 3, 4, 5, 6 and 12 hours than that after 0.06 mg/kg
IGF-I injection.

**Blood glucose concentration**

The individual and the mean blood glucose concentrations are shown in Fig. 1 and Fig. 2. Blood glucose levels did not change throughout the study after saline administration. After 0.06 mg/kg IGF-I injection, the blood glucose levels fell gradually and reached the minimal value of 73 ± 3 mg/dl at 3 hours, which was maintained at 4 hours. After lunch, the levels returned to the basal value. After 0.12 mg/kg IGF-I administration, the blood glu-

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**Fig. 1.** Individual plasma IGF-I levels and blood glucose levels after 0 mg/kg (○--○), 0.06 mg/kg (■●), or 0.12 mg/kg (□○) IGF-I sc administration in five normal human subjects. M in the figure indicates lunch and dinner, respectively.
Fig. 2. Mean plasma IGF-I and blood glucose levels after 0 mg/kg (○--○), 0.06 mg/kg (●--●), or 0.12 mg/kg (□--□) IGF-I sc administration in five normal human subjects. M in the figure indicates lunch and dinner, respectively.

Serum insulin, C-peptide (CPR), and plasma glucagon levels

The mean levels of these peptides throughout the study are shown in Fig. 3. After saline injection, serum insulin levels did not change until 4 hours; after lunch, the level rose significantly. After 0.06 mg/kg IGF-I administration, the insulin level fell to below the detection level of the assay (2.5 µU/ml) at 1 hour in three subjects and remained low until 4 hours. As we treated any value lower than 2.5 µU/ml as 2.5 µU/ml, we could not obtain statistically lower values for insulin during these 4 hours than those observed following saline injection. The insulin values increased after lunch similarly to those obtained in the control study. After 0.12 mg/kg IGF-I injection, plasma insulin decreased further; at 2 hours, the level fell lower than the minimum detectable level and remained there until 4 hours. Then once again the levels rose after lunch in a manner similar to that observed after saline injection throughout the rest of the study.

The serum C-peptide (CPR) level changes were very similarly to insulin levels. After 0.06 and 0.12 mg/kg IGF-I administration, plasma CPR decreased to significantly lower values until 4 hours as compared to the values obtained before 0.06 mg/kg and 0.12 mg/kg IGF-I injection (p<0.01). The three mean values at 5 and 6 hours after injection did not differ significantly; however, the values obtained at 8, 10 and 12 hours after IGF-I administration at both doses were greater than those after saline injection.
After saline injection, plasma glucagon significantly decreased from 133 ± 12 to 101 ± 13 pg/ml (p < 0.01) at 2 hours; however, the values returned to the basal level at 5 hours. After 0.06 mg/kg and 0.12 mg/kg IGF-I administration, plasma glucagon again decreased. The levels observed at 1
hour after injection (107±15 pg/ml and 98±11 pg/ml, respectively) were significantly lower than that obtained after saline injection (127±11 pg/ml). Otherwise, the values for glucagon did not differ significantly throughout the study with different dosages of IGF-1.

**Blood urea nitrogen (BUN) and urinary GH concentration**

Blood urea nitrogen levels before, 4 hours, and 24 hours after 0.06 mg/kg and 0.12 mg/kg IGF-I administration were 13.2±1.2 and 15.3±1.3 mg/dl (0 h), 11.9±1.0 and 13.5±1.5 mg/dl (4 h), and 13.9±1.4 and 13.8±1.7 mg/dl (24 h), respectively. There were no statistical differences between any of the values after IGF-I administration.

The urinary GH concentration in the control study ranged from 6.5 to 20.5, with a mean value of 13.1±2.4 ng/day. The value decreased significantly (p<0.01) after 0.06 mg/kg IGF-I injection; it ranged from 0.9 to 7.4 with a mean value of 4.2±1.2 ng/day. After 0.12 mg/kg IGF-I administration, the value increased and ranged from 3.6 to 16.4 with a mean of 10.6±2.3 ng/day, which is not different from that obtained during the control study.

**Clinical symptoms and others**

All the subjects felt pain after IGF-I injections, but not after saline injection. However, the pain disappeared within 1 or 2 minutes and there was no redness, swelling, or warmth at the injection site thereafter. Clinical symptoms such as drowsiness (Nos. 1, 3), hunger (Nos. 1, 3), heat sensation in the front of the head (No. 2), palpitation (No. 3), dizziness and hand tremor (No. 3) were observed after the 0.12 mg/kg IGF-I injection. Subject No. 3 had several symptoms but was the only one out of the 3 subjects who had decreased blood glucose. His blood glucose levels before and at 1, 2, 3 and 4 hours after 0.12 mg/kg IGF-I injection were 82, 45, 46, 55 and 58 mg/dl, respectively. The symptoms disappeared within 10 minutes. The body temperature, pulse rate, respiratory rate, and blood pressure did not change significantly throughout the study. There were no significant changes in the blood cell count, urinalysis, or routine chemistries before and 24 hours after IGF-I administration.

**Discussion**

After sc administration of IGF-I, the plasma IGF-I level peaked at 3–4 hours in a dose dependent manner and decreased gradually thereafter. However, the levels did not return to the basal value even at 24 hours after the injection. We have already reported that plasma IGF-I did return to the basal level at 48 hours after 0.1 mg/kg sc administration of IGF-I (Hizuka et al., 1989). The prolonged half-life of IGF-I is due to the fact that IGF-I in the circulation is bound to IGF-I binding proteins (Hintz et al., 1984, Wilkins & D’Ercole 1985, Hossenlopp et al., 1986, Hardouin et al., 1987). The blood glucose concentration decreased in a dose-dependent manner after IGF-I administration. However, there were no correlations between plasma IGF-I and blood glucose levels. After 0.12 mg/kg IGF-I administration, low blood glucose concentrations of 54±5, 50±4, and 56±2 mg/dl were observed at plasma IGF-I concentrations of 399±24, 457±19, and 474±26 mg/dl, respectively. Similar plasma IGF-I levels were observed at 5, 6, 8 and 10 hours. However, at these times the blood glucose levels were above 88±6 mg/dl. These results may be explained by the amounts of free IGF-I in circulation. We have reported that the free IGF-I level reached a peak at one hour, decreased gradually, and returned to the basal value at 8 hours after 0.1 mg/kg IGF-I sc injection (Hizuka et al., 1989). Guler et al. (1989)
reported that free IGF-I levels above 100 ng/ml are likely to cause hypoglycemia.

Serum insulin and C-peptide decreased in a dose-dependent manner during the first 4 hours after IGF-I administration. The levels increased after food intake. The insulin levels after 0.12 mg/kg IGF-I administration were below the detection limit of the assay, so we could not obtain accurate measurements of their concentrations. However, from the much lower blood glucose levels we could estimate that the levels were lower than those observed after 0.06 mg/kg IGF-I administration. This decreased insulin secretion might have been responsible for the increase in blood glucose (137 ± 18 mg/dl) after lunch, because we could not find any difference in insulin and glucagon levels at one hour after lunch. Other counter-insulin hormones might also play a role in this phenomenon, but further study will be required to solve this problem. The exact mechanisms by which IGF-I decreases serum insulin and C-peptides levels are not clear. We speculate that hypoglycemia plays an important role in suppressing these hormone secretions; however, a direct effect of IGF-I could not be ruled out, because we found one subject (No. 4) who did not show signs of hypoglycemia, even though his insulin levels were below the detection limit. Serum C-peptides were also decreased in this subject. Plasma glucagon concentrations are regulated by insulin and glucose. In this study, we found statistically decreased values at 1 and 3 hours after 0.06 mg/kg IGF-I and at 1 hour after 0.12 mg/kg IGF-I administration. Therefore, we think that the suppressive effect of IGF-I on glucagon might be only very slight.

IGF-I has been reported to inhibit GH secretion from the pituitary in animals in vitro and in vivo (Abe et al., 1983, Berelowitz et al., 1981, Yamashita et al., 1986). Guler et al. (1987) reported that iv injection of 100 μg/kg IGF-I in human subjects induced both hypoglycemia and a plasma GH increase. However, in another study of 20 μg/kg/hour IGF-I sc administration for 6 days where euglycemia was maintained (Guler et al., 1989), plasma GH secretion after GRF stimulation and spontaneous GH secretion during the night were suppressed. We previously observed that urinary GH measurements are useful for evaluating endogenous GH secretion (Sukegawa et al., 1988). Therefore, we measured the urinary GH concentration in the present study. We found decreased GH in urine after 0.06 mg/kg IGF-I injection, which reflects decreased GH secretion during the day. On the other hand, GH in urine increased after 0.12 mg/kg IGF-I injection and maintained levels similar to those in the control study. These results suggest that IGF-I provides a negative feedback for GH secretion; however, the severe hypoglycemia induced by higher doses of IGF-I resulted in increased GH secretion. This theory could also explain the two different results observed by Guler et al. (1987, 1989).

Hypoglycemia, where blood glucose levels are less than 50 mg/dl, were observed in three subjects (Nos. 3, 4 and 5). However, clinical hypoglycemic symptoms were seen only in one subject when his blood glucose levels were between 45–55 mg/dl. Two other subjects showed much lower blood glucose levels of 38 mg/dl and 41 mg/dl, respectively; however, no special clinical signs were noted. Because of the small number of subjects, we cannot stress the asymptomatic hypoglycemia of IGF-I, but we must pay attention to this phenomenon during further study of repeated administration of IGF-I.

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References


