0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(3):799–805

doi: 10.1210/jc.2005-1536

Prolonged Stimulation of Growth Hormone (GH) and Insulin-Like Growth Factor I Secretion by CJC-1295, a Long-Acting Analog of GH-Releasing Hormone, in Healthy Adults

Sam L. Teichman, Ann Neale, Betty Lawrence, Catherine Gagnon, Jean-Paul Castaigne, and Lawrence A. Frohman

WinPharm Associates (S.L.T., A.N.), Alamo, California 94507; ConjuChem, Inc. (B.L., C.G., J.-P.C.), Montréal, Québéc, Canada; and Section of Endocrinology, Department of Medicine, University of Illinois (L.A.F.), Chicago, Illinois 60612

Context: Therapeutic use of GHRH to enhance GH secretion is limited by its short duration of action.

Objective: The objective of this study was to examine the pharmacokinetic profile, pharmacodynamic effects, and safety of CJC-1295, a long-acting GHRH analog.

Design: The study design was two randomized, placebo-controlled, double-blind, ascending dose trials with durations of 28 and 49 d.

Setting: The study was performed at two investigational sites.

Participants: Healthy subjects, ages 21–61 yr, were studied.

Interventions: CJC-1295 or placebo was administered sc in one of four ascending single doses in the first study and in two or three weekly or biweekly doses in the second study.

Main Outcome Measures: The main outcome measures were peak concentrations and area under the curve of GH and IGF-I; standard pharmacokinetic parameters were used for CJC-1295.

Results: After a single injection of CJC-1295, there were dose-dependent increases in mean plasma GH concentrations by 2- to 10-fold for 6 d or more and in mean plasma IGF-I concentrations by 1.5- to 3-fold for 9–11 d. The estimated half-life of CJC-1295 was 5.8–8.1 d. After multiple CJC-1295 doses, mean IGF-I levels remained above baseline for up to 28 d. No serious adverse reactions were reported.

Conclusions: Subcutaneous administration of CJC-1295 resulted in sustained, dose-dependent increases in GH and IGF-I levels in healthy adults and was safe and relatively well tolerated, particularly at doses of 30 or 60 μg/kg. There was evidence of a cumulative effect after multiple doses. These data support the potential utility of CJC-1295 as a therapeutic agent. **(*J Clin Endocrinol Metab* 91: 799–805, 2006)**

THE USE OF GH for the treatment of children with impaired linear growth has been accepted as an important therapeutic modality for more than 50 yr (1). An unlimited supply of the hormone, made possible by the availability of recombinant GH in the 1980s, permitted expansion of the target population to include GH-deficient adults. Most adults receiving GH today have primary pituitary disease with impaired GH secretory capacity. However, most children being treated with GH have no evidence of pituitary disease and are believed to have an impaired hypothalamic signaling mechanism due to a GHRH neurosecretory dysfunction. GH has also been used for therapy of disorders in children and adults in which pituitary function is either intact or only slightly impaired, such as chronic renal failure and Turner syndrome (in children) and HIV-related wasting and lipodystrophy and burn therapy (in adults).

In patients with intact pituitary function, there has been interest in the use of GHRH rather than GH in the hope of producing a more physiological pattern of tissue exposure to GH than occurs by a single daily injection of the hormone. In fact, several studies in both children and adults have suggested that comparable or near-comparable results can be achieved with GHRH therapy (2–4).

A major limitation in the use of GHRH for therapy, however, is its short half-life. Native GHRH, a 44-amino acid peptide, has a half-life of 7 min (5), which is even shorter than that of GH (12 min) (6), necessitating daily or even more frequent injections. Polyethylene glycol-conjugated GHRH has been studied in an effort to overcome this limitation (7).

A synthetically modified form of GHRH has been linked to a reactive chemical that enables binding to endogenous serum albumin after sc administration. The chemical structure of this compound, drug affinity complex-GH-releasing factor (DAC-GRF; CJC-1295, ConjuChem, Inc., Montréal, Canada) is shown in Fig. 1. The core therapeutic moiety is GHRH-(1–29)NH_2, which contains the full biological activity of GHRH-(1–44)NH_2 modified by substitution of four amino acids that serve to render the compound more resistant to proteolytic cleavage (herein called GRF). GRF is linked by the amino acid, lysine, to a reactive chemical [maleimidopropionic acid (MPA)] that binds to unpaired thiol (sulfhydryl)

First Published Online December 13, 2005

Abbreviations: AUC, Area under the curve; C_{max}, peak plasma concentration; DAC-GRF, drug affinity complex-GH-releasing factor; MPA, maleimidoproprionic acid; T_{max}, time to peak plasma concentration.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

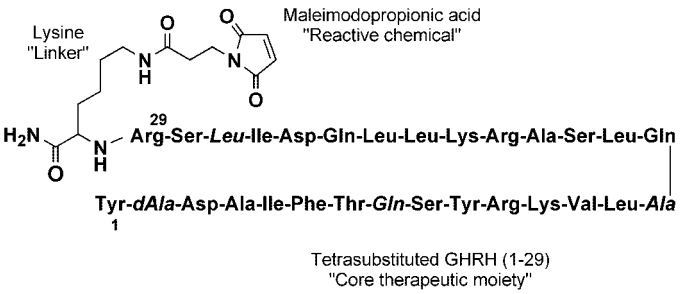

FIG. 1. Chemical structure of the CJC-1295 (DAC-GRF). The core therapeutic moiety is a tetrasubstituted GHRH-(1–29)NH_2. The substituted amino acids are shown in *italics*. The linker is lysine, and the reactive chemical is maleimidoproprionic acid that binds covalently to the single unpaired cysteine (cysteine 34) in serum albumin.

groups. The predominant free thiol group available for binding after parenteral administration is the single unpaired cysteine (cysteine 34) in serum albumin. At least 90% of CJC-1295 binds covalently to albumin in this fashion, with trace amounts found bound to fibrinogen and IgG. No other chemical species have been found bound to DAC-GRF after administration (data on file, ConjuChem, Inc.). This binding extends the half-life of the active pharmacophore, resulting in a markedly prolonged duration of action in several animal species (8). Moreover, studies in both dogs and pigs indicate that physiological GH secretion is maintained, and IGF-I levels are enhanced for several days after a single administration.

We assessed the safety, tolerability, pharmacokinetic profile, and effect of CJC-1295 on circulating concentrations of GH and IGF-I in two randomized, placebo-controlled, double-blind, dose-escalating studies in healthy adult subjects.

Subjects and Methods

The subjects consisted of healthy men and women, ages 21–61 yr, with a body mass index of 30 kg/m^2 or less and IGF-I levels in the normal range for age and gender. Appropriately constituted independent ethics committees reviewed and approved each of the studies, and written informed consent of all subjects was obtained before participation.

Study design

Study 1 was an ascending, randomized, double-blind, placebo-controlled single-dose trial performed at SFBC International, Inc. (Miami, FL). Study 2 was an ascending, randomized, double-blind, placebo-controlled multiple-dose trial performed at Kendle International BV (Utrecht, The Netherlands).

In study 1, four sequential, dose-escalation groups were evaluated. These dose levels were 30 μg/kg (n = 6, five active and one placebo), 60 μg/kg (n = 6, five active and one placebo), 125 μg/kg (n = 6, five active and one placebo), and 250 μg/kg (n = 6, five active and one placebo). An additional cohort of 18 subjects (15 active and three placebo) subsequently received 125 μg/kg.

Serum GH was measured on d 0 at 60, 30, and 15 min before study drug dosing; at 15, 30, and 60 min and 2, 3, 4, 6, 8, 10, 12, and 24 h after dosing; and then every 8 h on d 2–3, then daily on d 4, 5, 6, 7, 9, 11, 14, 21, and 28. Serum IGF-I and CJC-1295 were measured on d 0, 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 21, and 28.

In study 2, 24 subjects were enrolled in one of four sequential dosing cohorts. Group 1 (n = 6, five active and one placebo) received two injections of 30 μg/kg (d 0 and 14), group 2 (n = 6, five active and one placebo) received two injections of 60 μg/kg (d 0 and 14), group 3 (n = 6, five active and one placebo) received three injections of 30 μg/kg (d 0, 7, and 14), and group 4 (n = 6, five active and one placebo) received three injections of 20 μg/kg (d 0, 7, and 14). Sample collection was similar to that in study 1, with the addition of more frequent pre- and postinjection sampling on d 7 (groups 3 and 4 only) and 14 as well as a final sample collection on d 49 in all subjects.

Serial clinical evaluations (vital signs, adverse events, and physical examination) and laboratory safety assessments (serum chemistry, hematology, and urinalysis) were performed ending on d 28 in study 1 and on d 49 in study 2.

Laboratory methods

GH. Serum GH was measured by a double antibody RIA (Esoterix Laboratory Services, Inc., Calabasas Hills, CA). The assay sensitivity was 0.3 ng/ml, and the coefficient of variation was 10%.

IGF-I. Serum IGF-I was measured by a double antibody RIA by Esoterix Laboratory Services, Inc., after ethanol extraction and with the addition of IGF-2 as a blocking agent. The assay sensitivity was 10 ng/ml, and the coefficient of variation was 5.4%. Normal ranges for the assay are age and gender adjusted.

Other hormones. Serum cortisol, prolactin, TSH, and LH concentrations were measured in patients receiving 60 μg/kg CJC-1295 in study 1 by Esoterix Laboratory Services, Inc.

CJC-1295. Plasma CJC-1295 concentrations were measured by RIA at PPD Development, LP (Richmond, VA), using a rabbit anti-(tetrasubstituted)GRF-(1–29) coupled to keyhole limpet hemocyanin and radioiodinated GRF-(1–29). The antibody exhibited 100% cross-reactivity with albumin-bound DAC of the tetrasubstituted GRF-(1–29). There was no cross-reactivity with native GRF-(1–29), native GRF-(3–29), or the DAC of the fragment 12–29 of the tetrasubstituted GRF. There was 25% cross-reactivity with free (nonalbumin-bound) tetrasubstituted GRF-(1–29). The lower limit of detection was 0.2 nmol/liter, and the mean intra- and interassay coefficients of variation were 5.5% and 8.2%, respectively.

Pharmacokinetic analysis. Pharmacokinetic parameters [peak plasma concentrations (C_{max}), time to peak plasma concentrations (T_{max}), and area under the curve (AUC)] of CJC-1295, GH, and IGF-I were calculated from the concentration *vs.* time values for each patient using a compartment model in the single-dose study (WinNonlin Professional version 4.1, Pharsight Corp., Mountain View CA) and a noncompartmental model in the multiple-dose study (WinNonlin Professional version 4.0.1).

Antibody formation. A validated immunoradiometric assay was used to determine the presence of antibodies to CJC-1295. The anti-CJC-1295 antibody was raised in rabbits by immunization with a CJC-1295 analog [the tetrasubstituted GRF-(1–29)] to which a cysteine residue was added at position 30 to permit direct conjugation to keyhole limpet hemocyanin to make the molecule more immunogenic. This antibody was also used in the assay for plasma CJC-1295 concentrations. In this immunoradiometric assay, tubes are coated with CJC-1295 bound to inactivated MPA. Test samples or affinity-purified rabbit anti-CJC-1295 antibody controls in human serum were added. After incubation, tubes were washed and [^{125}I]protein LA (Sigma-Aldrich, St. Louis, MO) was added. After incubation, tubes were washed again, radioactivity was determined in a γ-counter, and the specific binding of the samples was calculated.

Statistical analysis

Mean and variance estimates were calculated for all pharmacokinetic parameters by dose group. C_{max} and AUC to the last sampling time (AUC_t) were log transformed before analysis, and AUC values were calculated using the linear trapezoidal rule. Differences in GH and IGF-I levels and AUC between groups were compared by ANOVA and/or one-tailed *t* test; $P < 0.05$ was considered significant. All statistical analyses were performed using SAS version 8.02 (SAS Institute, Cary, NC).

Because all enrolled subjects received at least one dose of the study drug, all available data are included in analyses of safety, pharmaco-

kinetic, and pharmacodynamic parameters. No effort to estimate missing data was made, with the exception of AUC calculations.

Subjects receiving placebo in all dosing groups in each study were pooled for comparison with groups treated with active drug.

Results

Subject characteristics and disposition

The distribution of subjects in the study groups by age and gender is shown in Table 1. The mean overall age of subjects in the two studies was 49.3 ± 1.1 (±SE) yr (range, 21–61 yr), and 44% of subjects were men. Although the dosing cohort groups in both studies were generally similar in mean age, gender, height, weight, and BMI, subjects in group 2 in study 2 were younger (mean age, 33 yr), and 80% were men. Two subjects in study 1 (both randomized to the 125 μg/kg dosing cohort) and one subject in study 2 (in group 2) discontinued the study prematurely. The subjects in study 1 withdrew 5 and 22 d after dosing because of mild injection site reactions. The subject in study 2 withdrew 6 d after experiencing multiple mild adverse effects following a single injection of 30 μg/kg.

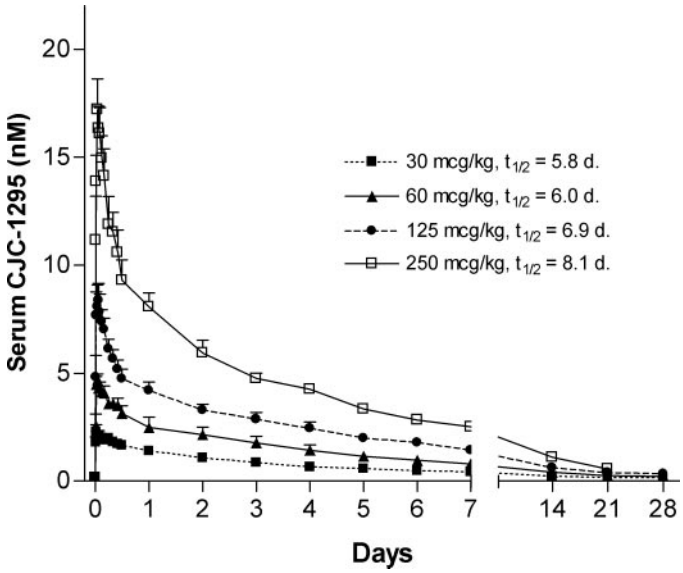

FIG. 2. Plasma disappearance curves of CJC-1295 after a single sc injection. Shown are the mean ± SD half-life. Drug concentrations were generally measurable for at least 12–14 d after injection.

Plasma CJC-1295 concentrations

In study 1, the mean C_{max} of CJC-1295 increased with dose, with values of 2.17, 5.19, 8.16, and17.1 nmol/liter in the 30, 60, 125, and 250 μg/kg single-dose groups, respectively (Fig. 2). Maximum drug concentrations were reached between 1 and 1.5 h in all groups. The mean AUC_t in the 30, 60, 125, and 250 μg/kg dose groups were 143, 355, 669, and 1276 h·nmol/liter, respectively, with dose-proportionality observed in the 60, 125, and 250 μg/kg dose groups. Terminal elimination rate, systemic clearance, and volume of distribution gradually increased with dose. The mean half-life ranged from 5.8–8.1 d; mean systemic clearances were 0.04, 0.04, 0.05, and 0.05 liters/h/kg, and mean volumes of distribution were 8.1, 9.7, 11.6, and 13.8 liter/kg in the four dose groups, respectively.

In study 2, maximum CJC-1295 plasma concentrations were 11–32% higher after the injection on d 7 than on d 0 in the two groups that received weekly injections (data not shown). After the d 14 injection, maximum CJC-1295 concentrations were 29–70% higher than those on d 0 in all four groups. Similar increases occurred in $AUC_{0-24\ h}$ on d 7 (12% and 15%) in groups 3 and 4 and in all four dosing groups on d 14 (31–57%). The 0–24 h AUC values were dose dependent.

Maximum drug concentrations were typically reached within 0.5–2.0 h after injection, but there was a high degree of individual variability. The variability increased with subsequent doses, but did not appear to be dose dependent. Drug concentrations were measurable for 10–14 d. In subjects in whom samples were obtained for up to 14 d, the mean estimated half-life of CJC-1295 ranged from 5.4–9.2 d, and the mean clearance was between 1.1 and 3.3 liters/h. The pharmacokinetic parameters were independent of body weight.

Serum GH concentrations

In study 1, mean preinjection GH concentrations ranged from 0.7–1.1 ng/ml. Mean GH concentrations increased by 2- to 10-fold after single-dose injection of CJC-1295 through d 6 (Fig. 3A). In contrast, mean GH concentrations remained

TABLE 1. Study drug doses and regimens with subject information

	Total no.	Age ± SD	No. of men	No. of women	No. of doses	Dosing days
Study 1						
Dose						
30 μg/kg	5	55 ± 8	2	3	1	
60 μg/kg	5	53 ± 7	2	3	1	
125 μg/kg	20	50 ± 6	7	13	1	
250 μg/kg	5	53 ± 3	1	4	1	
Pooled placebo	7	52 ± 7	3	4	1	
Study 2						
Dose						
20 μg/kg	5	43 ± 14	3	2	3	d 0, 7, 14
30 μg/kg	5	33 ± 15	4	1	3	d 0, 7, 14
30 μg/kg	5	40 ± 11	3	2	2	d 0, 14
60 μg/kg	5	57 ± 1	2	3	2	d 0, 14
Pooled placebo	4	51 ± 13	2	2	2 or 3	d 0, 14, or d 0, 7, 14

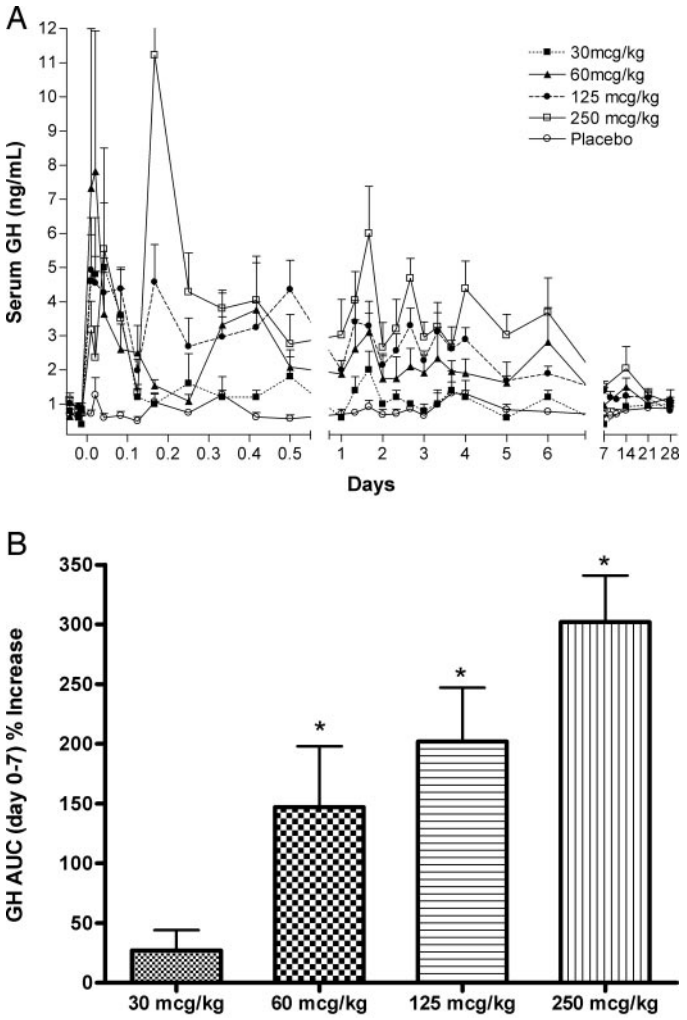

FIG. 3. GH responses to a single sc injection of CJC-1295. A, Serum GH concentrations (mean ± SD) are shown and suggest that pulsatile hormone secretion is maintained. B, Mean GH $AUC_{0-7\ d}$, expressed as a percent increase over placebo. *, $P < 0.05$ *vs.* placebo. Shown are the mean ± SD. Mean maximum concentrations of GH were 6.6, 9.6, 9.9, and 13.3 ng/ml in the 30, 60, 125, and 250 μg/kg groups; mean AUC were 758, 969, 977, and 1370 ng/ml·h, respectively.

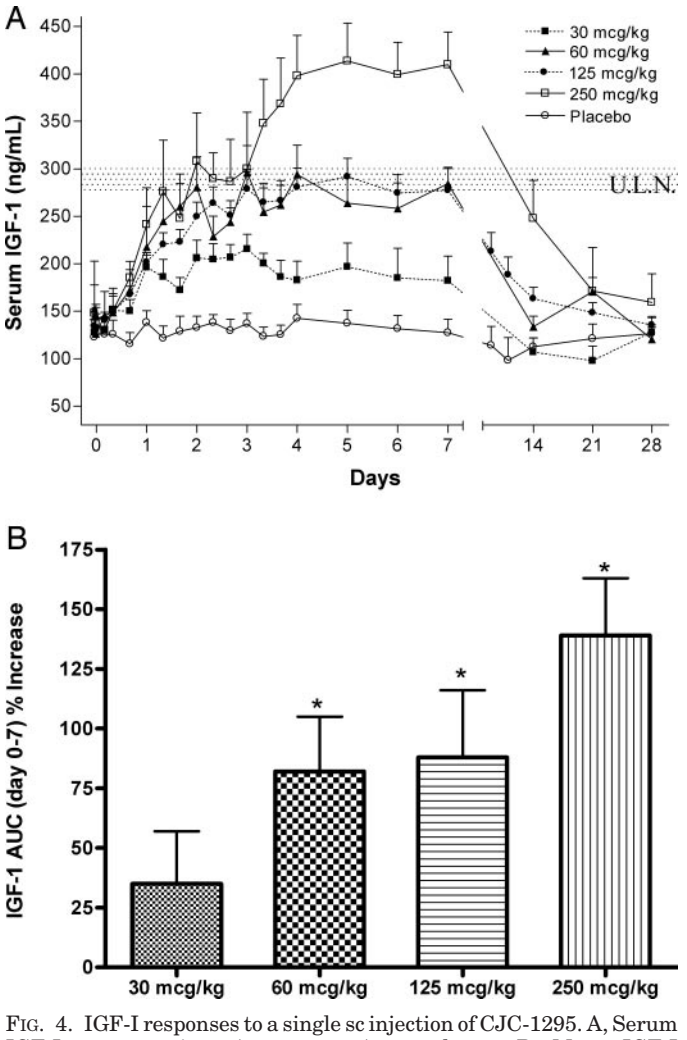

FIG. 4. IGF-I responses to a single sc injection of CJC-1295. A, Serum IGF-I concentrations (mean ± SD) are shown. B, Mean IGF-I $AUC_{0-7\ d}$, expressed as a percent increase over placebo. *, $P < 0.05$ *vs.* placebo. Shown are the mean ± SD. The *shaded area* marked is the upper limit of normal (U.L.N.) for age- and gender-matched cohorts. Mean maximum concentrations of IGF-I were 232, 319, 328, and 435 ng/ml in the 30, 60, 125, and 250 μg/kg groups; mean AUC were 91, 127, 119, and 172 μg/ml·h, respectively.

stable in the placebo group. The mean GH $AUC_{0-7\ d}$ values were elevated in a dose-dependent manner, although only the groups receiving 60, 125, and 250 μg/kg had significant increases compared with the placebo group (Fig. 3B).

The median peak GH level occurred within 1 h in all dosing groups in both studies, with the exception of the group receiving a single 250 μg/kg dose of CJC-1295, in which the median peak GH level occurred at 4 h. The mean peak GH levels were more variable and occurred 0.5–4 h after dosing. The variability was neither dose dependent nor progressive.

Serum IGF-I concentrations

Mean preinjection IGF-I concentrations ranged from 123–152 ng/ml in study 1. IGF-I levels remained elevated compared with baseline for 9–11 d after a single injection of CJC-1295 in all dosing groups in study 1, and mean levels increased by 0.5- to 3-fold over baseline (Fig. 4A). Mean IGF-I $AUC_{0-7\ d}$ values were elevated in a dose-dependent manner, reaching statistical significance compared with baseline in the groups receiving 60, 125, and 250 μg/kg (Fig. 4B). IGF-I levels exceeded the age- and gender-adjusted normal ranges only in subjects receiving 250 μg/kg CJC-1295. In contrast, mean IGF-I levels in the placebo group remained relatively stable during the same period. The time to peak IGF-I levels was dose dependent, occurring 2–3 d after administration of the lowest three doses, but not until 4 d after the highest dose. IGF-I levels remained at a plateau for up to 7 d, after which they gradually declined toward baseline. IGF-I levels remained elevated for at least 2 wk after injection in patients receiving the two highest doses.

In study 2, mean IGF-I levels increased within 8 h of CJC-1295 injection and remained above baseline levels through d 28 (Fig. 5). Mean IGF-I values remained elevated above baseline before the second and/or third doses in all CJC-1295-treated groups, although this reached significance

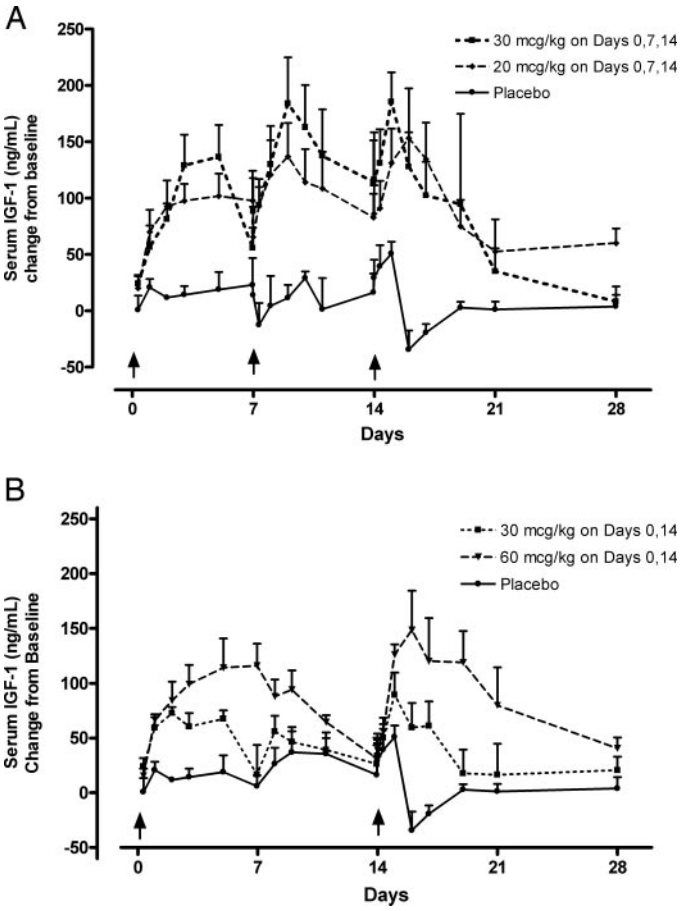

FIG. 5. IGF-I responses to multiple sc injections of CJC-1295. Serum IGF-I concentrations (mean ± SE) are shown. *Arrows* indicate days of injection. A, Changes in serum IGF-I levels after three weekly injections of CJC-1295 or placebo. B, Changes in serum IGF-I levels after two biweekly injections of CJC-1295 or placebo. In both A and B, for d 0–7 and 14–21, the entire pooled placebo group (n = 4) is plotted, because all these subjects received placebo injections on d 0 and 14. In A, for the period from d 7–14, only the two subjects who received placebo injections on d 7 are plotted (*i.e.* three weekly injections). In B, for the period from d 7–14, only the two subjects who received placebo injections on d 0 and 14 are plotted (*i.e.* two biweekly injections).

only for group 4 ($P < 0.03$ for group 4 and $P < 0.07$ for group 3, by paired t test). Maximum IGF-I levels after the second and/or third injections were progressively greater than after the initial injection (data not shown). In addition, the T_{max} for IGF-I was progressively shorter after subsequent injections (data not shown).

Mean $AUC_{0-7\ d}$ and $AUC_{0-14\ d}$ for IGF-I were significantly increased in group 2 (60 μg/kg) compared with group 1 (30 μg/kg; $P = 0.041$ and $P = 0.043$, respectively) and were significantly greater in both groups compared with the placebo group ($P = 0.003$ and $P = 0.005$, respectively). Mean $AUC_{0-7\ d}$, $AUC_{7-14\ d}$, and $AUC_{14-21\ d}$ were all significantly higher in group 3 (30 μg/kg) than in group 4 (20 μg/kg; $P = 0.010$, $P = 0.020$, and $P = 0.026$, respectively).

Other than age, no significant predictors of IGF-I or GH response, including gender, baseline weight or body mass index, and lipid or glucose levels, were observed.

Other hormonal effects

There were no significant increases in serum cortisol, prolactin, TSH, or LH levels after a single injection of CJC-1295 (60 μg/kg), thus confirming the specificity of CJC-1295 for GH secretion (data not shown).

Safety

In the single-dose study, adverse events were reported in 33 (94%) *vs.* two (29%) subjects in the active and placebo groups, respectively; all were of mild to moderate severity, and none required medical intervention to resolve. Injection site reactions (irritation, erythema, induration, pain, or itching) occurred transiently (up to several hours) in approximately 70% of subjects receiving CJC-1295 and rarely in subjects receiving placebo. Injection site reactions tended to be more severe and/or prolonged after higher doses, with residual induration lasting up to 5 d. No local reaction exceeded 10 cm in diameter, and all resolved spontaneously. Transient urticarial rashes at the injection site occurred in almost 30% of subjects and were not dose related. Other adverse events reported in actively treated subjects included headache (63%), diarrhea (43%), and systemic vasodilatory reactions (flushing, warmth, and transient hypotension; 30%); all were more common at higher doses (125 or 250 μg/kg). Headache and diarrhea occurred occasionally at various times during the 7 d after dosing. Of these adverse events, only headache occurred in the placebo-treated group (14%). Overall, the adverse events observed at 250 μg/kg were moderate in severity and resolved spontaneously after a few hours, whereas the 125 μg/kg dose of CJC-1295 was considered well tolerated.

Injection site reactions (irritation, erythema, induration, pain, or itching) were reported in all actively treated subjects in study 2; all were mild in severity. Reactions were up to 10 cm in diameter. Mild injection site erythema (<2–3 cm) was reported in three of four placebo-treated subjects (75%) as well as induration and urticaria (<1–2 cm; 25%). Flushing occurred only in actively treated subjects, occurred within 30 min of injection, and resolved within 1–2 h. The incidence of flushing was dose dependent, with an incidence of 40% after low-dose and 100% after high-dose injections.

Other adverse events included transient loose stools/diarrhea (45% and 100% incidence in the 125 and 250 μg/kg groups, respectively), headache (non-dose dependent and ranging from 20–80% depending on the dose group), and nausea or abdominal pain (20%). Of these adverse events, only headache occurred in the placebo-treated group (14% in study 1 and 50% in study 2).

The incidence and severity of all adverse events were gender independent. There were no consistent changes in blood or urine laboratory values, including glucose levels and liver function studies, or in electrocardiographic findings in either study. No significant antibody formation was detected in subjects who received the active study drug.

A single subject in study 2 reported mild, transient (resolving fully and spontaneously in <24 h) involuntary leg muscle contractions and some loss of coordination after re-

ceiving the second biweekly injection of 30 μg/kg. Two subjects experienced transient dizziness and hypotension after the first injection of 30 μg/kg that resolved spontaneously and did not recur after subsequent injections of CJC-1295.

Discussion

This report describes the safety, pharmacokinetic profile, and pharmacodynamic effects of CJC-1295, a synthetic analog of GHRH that permanently and covalently binds to serum albumin after administration. Results of the single-dose and multiple-dose studies demonstrate a prolonged half-life of CJC-1295 (~6–8 d) after sc administration, with measurable drug concentrations for 10–13 d after single or multiple doses. In addition, there was clear evidence of a dose-responsive and sustained biological effect, with elevated GH and IGF-I serum concentrations persisting for at least 6 and 14 d, respectively, after single doses of CJC-1295. In the multiple-dose study, there was a cumulative effect after two or three injections of CJC-1295 administered weekly or biweekly, with elevated levels of both GH and IGF-I above baseline on d 14 in most subjects. CJC-1295 was safe and generally well tolerated, particularly at doses of 30 and 60 μg/kg.

Treatment with human GH typically consists of a single daily injection of the hormone, resulting in transient supraphysiological levels, followed by a decline to baseline. However, failure to mimic the physiological pulsatile nature of GH secretion may preclude optimal therapeutic effects and may contribute to some of the adverse effects that have been observed even in the presence of normal serum IGF-I levels. In contrast, injections or infusions of GHRH stimulate the pulsatile release of GH (9, 10), but the short plasma half-life (7 min) (5) renders GHRH impractical for therapeutic use. Therefore, the availability of a GHRH preparation with sustained effect has important therapeutic potential.

The half-life of CJC-1295, as predicted from preclinical animal studies (8), was substantially prolonged compared with that of native GHRH, ranging from 5.8–8.1 d in the single-dose study and from 5.4–9.2 d in the multiple-dose study. Maximum concentrations were typically reached within 2 h after injection and exhibited a slow exponential decrease over several days. The disappearance rates were not dose dependent, although serum CJC-1295 concentrations were proportional to the dose injected. In the multiple-dose study, C_{max} and $AUC_{0-24\ h}$ were 17% greater on d 7 than on d 0 and from 30–70% greater on d 14 than on d 0.

Administration of single doses of CJC-1295 resulted in a 2- to 10-fold increase in mean serum GH levels in all dosing groups, which was dose incremental and persisted for up to 6 d. Similarly, a dose-related increase in mean serum IGF-I levels was observed at all dose levels, ranging from 1.5- to 3-fold and persisting for up to 14 d. Administration of ascending multiple doses of CJC-1295 resulted in elevated levels of GH, similar to those observed after a single dose.

In contrast, elevations in IGF-I levels showed a progressive effect over time, particularly in subjects receiving CJC-1295 every 7 d. Results of the multiple-dose study suggest both a cumulative pharmacokinetic effect [*i.e.* persistence of elevated predose levels of IGF-I in all dosing groups except group 1 (*i.e.* two injections of 30 μg/kg)] and a pituitary priming effect (*i.e.* progressively greater C_{max} and progressively shorter T_{max} after serial dosing). The data indicate that a minimum dosing interval of 7 d appears reasonable. The most appropriate dosing interval will be determined based on actual efficacy and safety data from longer-term therapeutic studies in patients with various clinical conditions.

No serious adverse reactions were reported in either study. The most frequently reported adverse events in subjects receiving CJC-1295 were injection site reactions, consisting of transient pain, swelling, and induration that were sometimes accompanied by local urticaria. Injection site reactions tended to be more severe and/or prolonged at higher dose levels. Headache, diarrhea, and flushing were also observed, with occasional transient and mild hypotension, but occurred primarily at higher doses.

Adverse effects complicate the use of GH in the treatment of HIV-associated metabolic conditions such as wasting and lipodystrophy. Although increases in the daily dose of GH from 1 to 6 mg are associated with dose-responsive benefits (11–13), doses of 2–3 mg/d or greater are associated with edema, arthralgias, and glucose intolerance. These side effects can become dose limiting. In the current studies, none of the subjects experienced these adverse effects. Future clinical trials on this disorder will confirm whether the use of GHRH rather than GH will circumvent these problems, as has been suggested in recent publications (4, 14).

In summary, a single sc administration of CJC-1295 produced sustained elevations of serum GH and IGF-I levels in normal subjects for nearly 2 wk. Weekly or biweekly administration of CJC-1295 resulted in stimulation of GH and IGF-I secretion for at least 7 d. Both single and multiple doses of CJC-1295 over 2 wk were safe and generally well tolerated, particularly at doses of 30 and 60 μg/kg. Future studies are indicated to evaluate the clinical utility of treatment with CJC-1295 in patients with intact GH secretory capacity.

Acknowledgments

Received July 11, 2005. Accepted December 6, 2005.

Address all correspondence and requests for reprints to: Dr. Lawrence A. Frohman, Section of Endocrinology, Department of Medicine, University of Illinois, 1819 West Polk Street (M/C 640), Chicago, Illinois 60612. E-mail: frohman@uic.edu.

This work was supported by ConjuChem, Inc. (Montréal, Canada).

References

1. **Raben MS** 1958 Treatment of a pituitary dwarf with human growth hormone. J Clin Endocrinol Metab 18:901–903
2. **Brain C, Hindmarsh PC, Pringle PJ, Brook CGD** 1989 Use of continuous subcutaneous growth hormone-releasing hormone (GHRH (1–29)NH2) infusions to augment growth hormone secretion and to promote growth. Acta Paediatr Scand 78:109–113
3. **Thorner M, Rochiccioli P, Colle M, Lanes R, Grunt J, Galazka A, Landy H, Eengrand P, Shah S** 1996 Once daily subcutaneous growth hormone-releasing hormone therapy accelerates growth in growth hormone-deficient children during the first year of therapy. Geref International Study Group. J Clin Endocrinol Metab 81:1189–1196
4. **Koutkia P, Canavan B, Breu J, Torriani M, Kissko J, Grinspoon S** 2004 Growth hormone-releasing hormone in HIV-infected men with lipodystrophy: a randomized controlled trial. JAMA 292:210–218
5. **Frohman LA, Downs TR, Williams TC, Heimer EP, Pan Y-CE, Felix AM** 1986 Rapid enzymatic degradation of growth hormone-releasing hormone by

plasma in vitro and in vivo to a biologically inactive, N-terminally cleaved product. J Clin Invest 78:906–913
6. **Bright GM, Veldhuis JD, Iranmanesh A, Baumann G, Maheshwari H, Lima J** 1999 Appraisal of growth hormone (GH) secretion: evaluation of a composite pharmacokinetic model that discriminates multiple components of GH input. J Clin Endocrinol Metab 84:3301–3308
7. **Munafo A, Nguyen TXQ, Papasouliotis O, Lécuelle H, Priestley A, Thorner MO** 2005 Polyethylene glycol-conugated growth hormone-releasing hormone is long-acting and stimulates GH in healthy young and elderly subjects. Eur J Endocrinol 153:249–256
8. **Benquet C, Castaigne J-P, Pham K, Robitaille M, Frohman LA,** CJC-1295 (DAC-GRF), a long acting GRF analog, enhances pulsatile GH secretion, increases IGF-I levels, and restores linear growth. Program of the 86th Annual Meeting of The Endocrine Society, New Orleans, LA, 2004, p 491 (Abstract P3-109)
9. **Webb CB, Vance ML, Thorner MO, Perisutti G, Thominet J, Rivier J, Vale W, Frohman LA** 1984 Plasma growth hormone responses to constant infusions of human pancreatic growth hormone releasing factor: intermittent secretion or response attenuation. J Clin Invest 74:96–103
10. **Vance ML, Kaiser DL, Evans WS, Furlanetto R, Vale W, Rivier J, Thorner MO** 1985 Pulsatile growth hormone secretion in normal man during a continuous 24-hour infusion of human growth hormone releasing factor (l-40). J Clin Invest 75:1584–1590
11. **Andersen O, Haugaard SB, Flyvbjerg A, Andersen UB, Orskov H, Madsbad S, Nielsen JO, Iversen J** 2004 Low-dose growth hormone and human immunodeficiency virus-associated lipodystrophy syndrome: a pilot study. Eur J Clin Invest 34:561–568
12. **Engelson ES, Glesby MJ, Mendez D, Albu JB, Wang J, Heymsfield SB, Kotler DP** 2002 Effect of recombinant human growth hormone in the treatment of visceral fat accumulation in HIV infection. J Acquired Immune Defic Syndr 30:379–391
13. **Burgess E, Wanke C** 2005 Use of recombinant human growth hormone in HIV-associated lipodystrophy. Curr Opin Infect Dis 18:17–24
14. **Falutz J, Allas S, Kotler D, Thompson M, Koutkia P, Albu J, Trottier B, Routy J-P, Cote P, Abribat T, Grinspoon S** 2005 A placebo-controlled, dose-ranging study of a growth hormone releasing factor in HIV-infected patients with abdominal fat accumulation. AIDS 19:1279–1287

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.