Pharmacodynamic hormonal effects of anamorelin, a novel oral ghrelin mimetic and growth hormone secretagogue in healthy volunteers

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A R T I C L E   I N   F O

Article history:
Received 28 June 2008
Revised 3 December 2008
Accepted 17 December 2008
Available online xxxx

Keywords:
Ghrelin
Anorexia
Growth hormone secretagogues
IGF-1
Growth hormone

A B S T R A C T

Objective: Activation of ghrelin receptors stimulates GH secretion and appetite, increasing lean body mass and body weight. However, clinical use of ghrelin is limited because it has a short half-life and must be administered parenterally. Anamorelin is a novel, orally active, non-peptidic ghrelin mimetic and growth hormone secretagogue. Our objective was to evaluate its hormonal effects in healthy subjects.

Design: A double-blind, randomized, placebo-controlled study evaluated the short-term effects of anamorelin on GH, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), prolactin, ACTH, LH, FSH, TSH, cortisol, insulin and glucose. Normal healthy volunteers (n = 32) recruited from the general population were administered escalating doses of anamorelin (25, 50, and 75 mg daily) vs. placebo.

Results: Anamorelin significantly increased GH levels at all doses (p < 0.01). Effects on the somatotropic axis were maintained, as evidenced by sustained increases in IGF-1 and IGFBP-3 compared to placebo following 5–6 days of treatment. Negligible effects on other anterior pituitary hormone profiles and on fasting glucose were noted and all mean hormone levels remained within normal range. Some degree of insulin resistance as assessed by HOMA-IR was evident after treatment with 75 mg dose but not with the 25 or the 50 mg doses. Significant dose-related increases in body weight were recorded. Changes in body weight directly correlated with changes in IGF-1 levels. Anamorelin was well tolerated.

Conclusions: Anamorelin increases GH, IGF-1, IGFBP-3 and body weight with good tolerability and selectivity, without affecting other anterior pituitary axes or fasting glucose levels.

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1. Introduction

Growth hormone secretagogues (GHS) are small molecules with growth hormone (GH)-secreting properties that act, at least partially, through GHRIH-independent mechanisms. Using reverse pharmacology, a receptor for these GHS was cloned in 1997 and named growth hormone secretagogue receptor (GRLN) [1]. The endogenous ligand for this receptor was later identified as a 28-amino acid peptide isolated mainly from the stomach and named ghrelin [2,3]. As expected, ghrelin was shown to induce GH secretion but more recently it also was shown to play an important role in regulating metabolic balance, decreasing fat utilization via GH-independent mechanisms and increasing appetite through activation of neuropeptide Y neurons in the arcuate nucleus of the hypothalamus [4–8].

Based on these anabolic and orexigenic properties, ghrelin has been postulated as a therapeutic agent in anorexia and wasting conditions. This would be clinically relevant because weight loss and lack of appetite have been associated with poor prognosis in this setting [9]. Acute administration of ghrelin in humans has been shown to effectively increase appetite and food intake in healthy volunteers and in subjects suffering from cancer-anorexia [10]. In the setting of chronic-obstructive pulmonary disease [11] and congestive heart failure (CHF) [12], ghrelin not only increased appetite and body weight but also improved lean body mass, muscle strength and exercise capacity. However, long-term clinical use of ghrelin is limited because it is a protein with a very short half-life and must be administered by injection.

Recently it was unambiguously demonstrated through experiments on GH secretagogue receptor (GRLN)-KO mice that the GRLN mediates ghrelin’s GH-releasing and orexigenic properties [13]. Hence, the development of GRLN agonists (GHS) may be useful in the treatment of wasting conditions offering the advantage of longer half-life and oral bioavailability. Several compounds in this class have been studied to this date. However, not all of them exhibit the same selectivity with some of them also increasing prolactin levels or affecting the hypothalamic-pituitary-adrenal axis [14–18].
The study reported here was conducted in healthy volunteers to assess GH response to the novel growth hormone secretagogue and ghrelin mimetic anamorelin, including response of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3); evaluate possible effects on the serum hormone profile (prolactin, ACTH, LH, FSH, TSH, cortisol), and carbohydrate metabolism (insulin, glucose).

2. Materials and methods

2.1. Study design and subjects

This single-center study was randomized, double-blind, and placebo-controlled. Subjects were randomized using a computer-generated schedule into three sequential panels of healthy volunteers as previously described [19]. Subjects remained sequestered at the study center for the duration of the trial and were instructed to avoid strenuous exercise from the time of screening throughout the study. The subjects’ wake and sleep cycle was not controlled. Subjects were not allowed to take any concomitant medication within 48 h of study treatment. The protocol and the informed consent form were reviewed and approved by the study center’s Institutional Review Board before subjects were enrolled in the study. All subjects signed the written consent form, and all clinical investigations were conducted in accordance with the guidelines in the Declaration of Helsinki. Subjects were compensated for their participation in the study.

Entry criteria were healthy males or surgically sterile females; ages 18–40 years; body weight 50–100 kg; and body mass index (BMI) within 18–29 kg/m². Study subjects were to have no significant condition or medical abnormality, including any condition that may have interfered with the absorption, metabolism, or elimination of the study drug, and did not participate in a clinical trial with an investigational agent within the 30 days prior to this study. Subjects also were screened for any dietary extreme (weight gaining or losing diets or other interventions) that could confound the impact of anamorelin on body weight.

2.2. Study compound: anamorelin

Anamorelin was prepared as the hydrochloride salt for clinical use. The chemical name for anamorelin hydrochloride is (3R)-1-(2-methylalanyl-D-tryptophyl)-3-(phenylmethyl)-3-piperidinecarboxylic acid (C31H42N6O3-Cl). The molecular formula is C31H42N6O3·HCl (molecular weight = 583.16). Subjects received anamorelin as a capsule or matching placebo. Anamorelin and placebo were provided by Sapphire Therapeutics (BridgeWater, NJ). Placebo capsules contained only excipients and were indistinguishable from the active compound with regards to size, shape, color and smell.

2.3. Dosing and study procedures

Study subjects (n = 32) were divided into three panels with different treatment durations and dosing regimens. Hormonal evaluations took place after the morning doses while fasting. Subjects in all panels received the morning dose at 8:00 AM ±1 h; subjects in panel B who received 25 mg twice daily (bid) received the evening dose 12 h after the morning dose. Panel A (n = 8) received placebo or anamorelin 25 mg once daily (qd) in the morning for 5 days. Panel B (n = 15) received placebo twice daily (morning and evening) for 11 days; or anamorelin 50 mg in the morning and placebo in the evening for 6 days with subsequent crossover to anamorelin 25 mg twice daily (morning and evening); or anamorelin 25 mg twice daily for 6 days with subsequent crossover to anamorelin 50 mg in the morning and placebo in the evening for 5 days (no washout period between crossover treatments). Panel C (n = 9) received placebo or anamorelin 75 mg once daily for 6 days. Dose escalation to the next panel was based on review of the clinical safety data, including adverse events, electrocardiograms (EKGs), vital signs, and laboratory safety studies. Blood and urine samples were obtained pre-study and during the study at specified time intervals to measure the effects of anamorelin on GH, IGF-1 and IGFBP-3; and effects on other hormones (ACTH, cortisol, prolactin, TSH, LH, and FSH) and carbohydrate metabolism (insulin and glucose). Fasting insulin sensitivity was assessed using the homeostasis model assessment (HOMA-IR). HOMA-IR [HOMA-IR = fasting glucose (mmol/L) × fasting insulin (µU/mL)/22.5] was calculated as previously described [20]. Estimates of insulin resistance from this index correlate well with estimates from the “gold standard” hyperinsulinemic euglycemic clamp method (r = 0.88). Morning body weights were obtained using a calibrated scale to assess weight changes.

Tolerance was assessed by recording adverse events, clinical laboratory values, vital signs, and EKG. Adverse events were defined as any unfavorable event that occurred after administration of study drug, whether or not it was considered to be related to the study drug, or worsening of a pre-existing condition. Adverse events were categorized as asymptomatic (a laboratory abnormality or physical sign evident only on diagnostic testing), mild, moderate, or severe. Post-study assessments were performed 10 to 14 days after discharge and included follow-up medical history, vital signs, body weight, clinical laboratory values, and any adverse events since discharge. The investigators did not have access to hormonal values (IGF-1, IGFBP3, GH, insulin, pituitary panel) during the study to ensure blinding.

2.4. Hormonal assays

All hormonal evaluations were performed with the morning dose while fasting. Samples were processed immediately after collection and kept at 4°C during processing. Samples were then aliquoted into polypropylene vials and stored at −20°C until assayed. All assays were performed no later than 2 weeks after specimen collection.

On day 1 (all panels), day 5 (Panels A and C) and Days 6 and 11 (Panel B), standardized meals were provided 4 and 10 h postdose in order to study the effect of anamorelin on GH, glucose and insulin while fasting. On these days, subjects were allowed to eat and drink non-alcoholic beverages ad libitum starting 12 h postdose. On all other days, subjects were allowed to eat ad libitum after hormonal evaluations and dosing. Levels of GH, IGF-1, and IGFBP-3 were measured by a solid-phase, enzyme-labeled chemiluminescent immunoassay with the IMMULITE® 2000 analyzer (EURO/DPC Ltd.; United Kingdom). Blood samples (4 mL) for determination of serum GH profiles were collected at predose and at 30, 60, 90, 120, 240 and 480 min postdose on Day 1 in all panels and again on Day 5 in Panel A, Days 6 and 11 in Panel B, and Day 5 in Panel C to assess changes during study drug treatment. IGF-1 and IGFBP-3 were measured in the morning before dosing at baseline and daily during treatment through 24 hours following the final dose of study drug. On Day -1 prior to treatment, blood samples for determination of serum anterior pituitary hormones, insulin and glucose were collected at the same time points indicated above. These profiles were repeated on Day 5 in Panel A, Days 6 and 11 in Panel B, and Day 5 in Panel C to assess changes during study drug treatment. The data from all other anterior pituitary hormones in the hormone profiles were prolactin, ACTH, LH, FSH, and TSH. Serum cortisol concentrations were also determined in Panel C only.
2.5. Statistical methods

All statistical analyzes were performed using the SAS for Windows statistical package (Version 8.2 or higher). All statistical tests were performed as 2- tailed tests unless otherwise specified, and all effects were considered to be statistically significant if \( p \leq 0.05 \). Growth hormone, IGF-1, IGFBP-3 and hormone profile levels were summarized descriptively; calculated parameters (area under the curve [AUC], AUC change from baseline, maximal concentration \([C_{\text{MAX}}]\), and average concentration \([C_{\text{AVE}}]\)) were analyzed by ANOVA. Pearson correlations were obtained between continuous variables.

3. Results

No relevant differences were noted in subject demographics and characteristics at enrollment except for race and BMI (Table 1). The results for body weight and tolerability have been published previously [19]. Subjects had a stable body weight between screening and the first visit.

3.1. Effect on growth hormone

Baseline GH levels were quite variable but there were no significant differences between groups (Table 1). This GH variability is a well-described phenomenon and it is likely to be due to its pulsatile nature [21,22]. GH levels were not significantly different from baseline in the placebo group but were increased after all anamorelin doses (Fig. 1). Peak level occurred approximately 1 hour postdose when measured on Day 1 and Day 5/6. Calculated GH response for \( \text{AUC}_{0-4} \), \( C_{\text{MAX}} \), and \( C_{\text{AVE}} \) on Day 1 were significantly increased \(( \text{p} \leq 0.01 \)) in the anamorelin 50 mg and 75 mg dose groups compared with placebo (Table 2). A decrease in GH response was observed in all active treatment groups when the dose groups compared with placebo (Table 2). A decrease in GH response for \( \text{AUC}_{0-4} \), \( C_{\text{MAX}} \), and \( C_{\text{AVE}} \) on Day 1 were significantly increased \(( \text{p} \leq 0.01 \)) in the anamorelin 50 mg and 75 mg dose groups compared with placebo (Fig. 2A).

3.2. Effect on IGF-1 and IGFBP-3

Baseline IGF-1 and IGFBP-3 levels were no different within groups (Table 1). Mean increases in IGF-1 and IGFBP-3 levels were calculated using the mean of the last 2 days of treatment. Mean increase from initial IGF-1 levels was significant \(( \text{p} < 0.001 \)) for anamorelin 50 mg and 75 mg qd compared with placebo (Fig. 2A). Mean IGFBP-3 levels increased from initial levels in all anamorelin dose groups, with significant increases compared with placebo in the 25 mg daily, 25 mg twice a day, and 75 mg daily groups \(( \text{p} \leq 0.03 \)) (Fig. 2B). IGF-1 and IGFBP-3 levels were no significantly different between the 50 mg qd and 75 mg qd dosing groups. Mean post-treatment IGF-1 and IGFBP-3 values were still within the age-adjusted reference range. Although mean increase in IGFBP-3 from initial levels to end of treatment was higher with anamorelin 25 mg twice a day compared with 50 mg daily, the difference was not statistically significant.

3.3. Effect on the anterior pituitary axis

Anamorelin had a negligible effect on prolactin, ACTH, LH, FSH, TSH, and cortisol at all doses, even at the highest dose tested (75 mg qd for 6 days) (Fig. 3). The average values for hormone levels measured at 30, 60, 90, 120, 240 and 480 min postdose remained within normal range throughout anamorelin dosing.

3.4. Effect on carbohydrate metabolism

Glucose \( \text{AUC}_{0-2} \) levels and glucose \( C_{\text{MAX}} \) were not significantly different at any dose compared to placebo or to baseline values. Insulin \( \text{AUC}_{0-2} \) levels were not significantly different at any dose compared to placebo or to baseline values. However, insulin \( C_{\text{MAX}} \) was elevated at 6 days in the 75 mg qd dose group (mean \( 13 \pm 3.82 \mu \text{U/mL} \)) compared to placebo (7 \( \pm 2 \mu \text{U/mL}; \text{p} = 0.02 \)).

### Table 1

**Subject demographics and characteristics at baseline.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment</th>
<th>Placebo (n = 6)</th>
<th>Anamorelin 25 mg QD (n = 6)</th>
<th>Anamorelin 25 mg BID, then 50 mg QD (n = 6)</th>
<th>Anamorelin 50 mg QD, then 25 mg BID (n = 6)</th>
<th>Anamorelin 75 mg QD (n = 8)</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n,%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6 (100)</td>
<td>5 (83)</td>
<td>6 (100)</td>
<td></td>
<td>7 (87.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1 (17)</td>
<td>0</td>
<td></td>
<td>0</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Race (n,%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3 (50.0)</td>
<td>5 (83)</td>
<td>1 (17)</td>
<td>5 (83)</td>
<td>1 (12.5)</td>
<td>4 (50.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Black</td>
<td>3 (50.0)</td>
<td>1 (17)</td>
<td>5 (83)</td>
<td>1 (17)</td>
<td>3 (37.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>21.2 ± 1.5</td>
<td>27.0 ± 7.6</td>
<td>27.0 ± 5.6</td>
<td>27.2 ± 6.7</td>
<td>27.8 ± 4.3</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 ± 7.1</td>
<td>72 ± 11.1</td>
<td>80 ± 6.4</td>
<td>78 ± 10.4</td>
<td>85 ± 10.6</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>66–85</td>
<td>63–94</td>
<td>71–88</td>
<td>66–95</td>
<td>67–96</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>BMI (kg/m²²)</td>
<td>24 ± 1.7</td>
<td>23 ± 2.8</td>
<td>24 ± 3.4</td>
<td>25 ± 2.8</td>
<td>28 ± 2.0</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>1.8 ± 2.9</td>
<td>1.2 ± 1.7</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>1.4 ± 3.3</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.1–7.6</td>
<td>0.1–4.4</td>
<td>0.0–0.4</td>
<td>0.1–0.3</td>
<td>0.0–9.6</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>213 ± 49</td>
<td>182 ± 64</td>
<td>192 ± 60</td>
<td>168 ± 44</td>
<td>194 ± 60</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>169–281</td>
<td>90–249</td>
<td>107–291</td>
<td>111–223</td>
<td>124–290</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>IGFBP-3 (µg/ml)</td>
<td>4.6 ± 0.4</td>
<td>4.4 ± 0.6</td>
<td>4.5 ± 0.6</td>
<td>4.4 ± 0.8</td>
<td>4.7 ± 0.7</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>4.1–5.4</td>
<td>3.7–5.3</td>
<td>3.8–5.3</td>
<td>3.1–5.4</td>
<td>3.5–5.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*BID = twice daily; BMI = body mass index; N = number of subjects; QD = once daily; SD = standard deviation; NS = not significant; NR = not reported.

* p-values for continuous variables from a one-way ANOVA with a term for sequence; p-values for categorical variables from Fisher's Exact test; p-values for IGF-1 and IGFBP-3 from ANOVA.
Pre-dosing insulin resistance as assessed by HOMA-IR was elevated on day 6 in the 75 mg qd group (2.76 ± 0.9) compared to placebo (1.26 ± 0.62, \( P < 0.01 \)). However, changes from baseline were not significant for any group compared to placebo (\( P = 0.5 \)).

### 3.5. Effect on body weight and correlations

Significant (\( p < 0.05 \)) dose-related increases in body weight were recorded after 5 or 6 days of treatment compared with placebo. Mean increase in weight from baseline after 50 mg daily was 1.08 kg (\( p < 0.001 \) vs. placebo), and after 75 mg qd was 1.36 kg (\( p < 0.0001 \) vs. placebo). In subjects receiving anamorelin, there was a negative correlation between GH response and baseline body weight (Table 3). Changes in body weight were strongly correlated with peak and AUC GH on Day 1 and changes in IGF-1. The correlation between IGF-1 changes and GH peak or AUC was weak and did not reach statistical significance. HOMA-IR correlated directly with changes in body weight and IGF-1 levels. These variables were not correlated in the placebo group.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Calculated parameters for GH on Day 1 (( p )-values are vs. placebo).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time point</td>
<td>Statistic</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0–4} (ng·h/mL)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>( p )-value</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
</tr>
<tr>
<td>C_{average} (ng)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>( p )-value</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
</tr>
<tr>
<td>C_{max} (ng)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>( p )-value</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
</tr>
</tbody>
</table>

Fig. 1. Changes in mean GH levels at baseline and 0.5, 1.0, 1.5, 2.0, and 4.0 hours postdose on Day 1 and Day 5/6. Standard deviations (SD) are not shown because of the wide range in SDs; wide range is most likely due to the pulsatile nature of GH release and the small number of subjects. The magnitude of SDs varied between <1 to >70. Number of patients at each dose on each day: 25 mg qd, \( n = 6 \); 50 mg qd, \( n = 6 \); 25 mg bid, \( n = 6 \); 75 mg qd, \( n = 8 \). The scale used for day 1 and day 5/6 are different.

Fig. 2. Changes in trough (A) IGF-1 and (B) IGFBP-3 levels from initial levels to end of treatment as measured each morning (Values are mean ± SEM). * \( p < 0.05 \), ** \( p < 0.0005 \) compared to placebo. Number of patients at each dose on each day: 25 mg qd, \( n = 6 \); 50 mg qd, \( n = 6 \); 25 mg bid, \( n = 6 \); 75 mg qd, \( n = 8 \).
Table 3
Correlations between efficacy variables of interest [r (p-value)].

<table>
<thead>
<tr>
<th>Baseline Body weight</th>
<th>Peak GH on Day 1</th>
<th>GH AUC on Day 1</th>
<th>Change in IGF-1</th>
<th>Day 5/6</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in body weight</td>
<td>0.25 (0.19)</td>
<td>0.45 (0.006)</td>
<td>0.43 (0.01)</td>
<td>0.54 (&lt;0.001)</td>
<td>0.58 (0.004)</td>
</tr>
<tr>
<td>Change in IGF-1</td>
<td>0.07 (0.67)</td>
<td>0.3 (0.08)</td>
<td>0.33 (0.052)</td>
<td>0.65 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Peak GH on day 1</td>
<td>−0.45 (0.006)</td>
<td>−0.34 (0.11)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Change is the average of the last 2 days of treatment minus baseline. Only data from the active Anamorelin groups is included.

3.6. Tolerance

Dose-related adverse events did not occur. Except for 1 event of severe headache reported in the 75 mg qd group that was not considered related to study drug, all adverse events were considered mild or moderate in intensity. One subject who received anamorelin 50 mg (Panel B: 25 mg twice daily followed by 50 mg daily) had moderate elevations in AST and ALT levels (3–4 times above the upper limit of normal), assessed by the Investigator as related to study drug treatment. ALT and AST normalized upon discontinuation of the drug.

4. Discussion

Anamorelin is a novel, potent, orally-available ghrelin mimetic and GH secretagogue. In the study reported here, single 25-mg, 50-mg, and 75-mg doses of anamorelin produced increases in circulating GH, with peak effects approximately 1 h postdose on all dosing days. The magnitude of this effect was attenuated with continued dosing of anamorelin, as illustrated by the change from baseline to Day 5 or 6 in calculated GH parameters (AUC₀–₆, CMAX, and C₆). This attenuation in GH response is most likely related to the increase in IGF-1 levels that, by a negative feedback mechanism mediated through somatostatin, suppresses GH secretion at the hypothalamic and pituitary level [23,24]. Another possibility is that this may represent desensitization of the GRLN to the effects of the drug. However, this is less likely because long-term treatment (6 months) with the growth hormone secretagogue MK-677 has been shown to increase IGF-1 levels by 84% compared to a 17% increase with placebo [25], in spite of showing a similar decline in GH response after its initial administration [26]. In the latter study, the serum peak total GH concentration was 23.5 mcg/L after the first MK-677 administration, 6.2 mcg/L at 2 weeks, and 5.1 mcg/L at 8 weeks. Furthermore, in a group of GH-deficient adults responding to the ghrelin mimetic NN-703, the GH AUC₀–₆ was dramatically increased after the first dose but its response decreased by 42% after one week of administration [27]. Although a direct comparison cannot be made between these agents because the research subjects and the setting were different, taken together the evidence suggests that even in the presence of negative feedback mechanisms, GH secretagogues stimulate GH secretion leading to a significant activation of the somatotropic axis as indicated by a sustained increase in IGF-1 levels.

The more integrated and stable GH markers, IGF-1 and IGFBP-3, were also monitored during the study period. A robust response was observed for increases in IGF-1 with clinically meaningful increases occurring after administration of the 50 mg and 75 mg doses of anamorelin. Interestingly, the 25 mg twice a day dosing did not show the same increase in GH and IGF-1 levels than the 50 mg once daily dose. Other GH secretagogues have been shown to induce a greater increase in IGF-1 levels when administered in the morning compared to evening dosing [28]. Although our study was not designed to detect differences between morning and evening dosing, we postulate that this may explain the different GH responses seen. Other hormones including testosterone and cortisol have a marked circadian variation with their secretion being highest in the morning. GH secretion may be enhanced by testosterone administration [29,30] and also by higher cortisol levels [31] suggesting that the interaction between GH and these hormones may also be responsible for the decrease in efficacy with split dosing.

Among the parameters assessed, the strongest predictor of change in body weight was change in IGF-1. This finding is consistent with data generated in animal models that have shown that the same receptor (GRLN) is responsible for both the orexigenic and GH-secreting effects of ghrelin [13]. This is important because, if these results are replicated in larger studies involving undernourished subjects, it would suggest that change in IGF-1 may prove to be a reasonable surrogate for predicting desired increases in total body mass in this patient population. Also of interest was the negative correlation between GH response upon completion of treatment and body weight, which indicates that pituitary responsiveness to anamorelin may be enhanced in individuals with...
lower BMI. These findings are particularly interesting because others have reported that body weight does not affect the release of GH elicited by single doses of ghrelin or the ghrelin mimetic GHRP-2 [32,33]. Whether these differences are due to the different dosing regimens (single dose in other studies vs. multiple doses in ours) or the result of other drug-specific properties remains to be determined and it should be the focus of larger studies in the future.

Given the effect that other GH secretagogues have on anterior pituitary hormones including prolactin and cortisol [14–18], it was of interest to test the effects of anamorelin on plasma concentrations of prolactin, LH, FSH, TSH, ACTH and cortisol. All hormones remained within the normal range throughout anamorelin administration, even with the highest dose (75 mg daily); which indicates that anamorelin selectively increases GH without altering the anterior pituitary axis.

In this small study population of healthy volunteers, fasting insulin levels were variable at baseline among the treatment groups. This is probably due to differences in BMI between groups at baseline, where the 75 mg qd group was significantly heavier than the other groups. Changes from baseline in glucose, insulin and insulin resistance levels were not significantly different for any of the groups compared to placebo. However, fasting insulin Smax and HOMA-IR were significantly higher for the 75 mg qd group compared to placebo after treatment while glucose levels remained stable. We hypothesize that the higher insulin and HOMA-IR levels in the 75 mg qd group on treatment day 6 without significant changes from baseline may be related to the higher BMI in this group since higher body weight is associated with higher insulin and HOMA-IR levels. However, a true effect of anamorelin cannot be completely excluded. Studies with other GH secretagogues and with GH have shown a worsening glucose tolerance acutely.

In general these changes have been transient and have improved after several weeks of treatment due to changes in body composition [17,34]. Although, no evidence of clinically meaningful effects of anamorelin on carbohydrate metabolism was found in this study, further studies would be needed to assess its long-term effect on glucose metabolism.

Results of this study demonstrated that anamorelin significantly increases GH, other markers of activation of the somatotropic axis and body weight with good selectivity. Effects on other anterior pituitary axes were negligible, as were effects on fasting glucose levels. Further studies in patients suffering from wasting disorders are warranted and they should include body composition analysis, appetite, and food intake measurements.

Disclosure

This study was supported by a grant from Sapphire Therapeutics, Inc., Bridgewater, NJ. Dr. Garcia has received research support from Sapphire Therapeutics and Dr. Polvino owns stock in Sapphire Therapeutics.

Acknowledgements

Dr Garcia Receives support from a MERIT Review Entry Program Grant from the Department of Veterans Affairs, a South Central Network Career Development Award from the Department of Veterans Affairs.

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