The effect of treatment with the oral growth hormone (GH) secretagogue MK-677 on GH isoforms

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Abstract

Growth hormone (GH) consists of several isoforms. We have studied the proportion, expressed as percentage of total GH concentration, of non-22 kDa (non-22K) GH isoforms and 20K GH during 8-week oral treatment with MK-677 25 mg daily in 12 obese males. The proportion of non-22K GH isoforms in peak total GH samples after the initial MK-677 administration was higher than that after 2 and 8 weeks ($p < 0.01$ and $p < 0.05$, respectively). In selected non-peak total GH samples after the initial MK-677 administration, however, the proportion of non-22K GH isoforms was similar to that in the peak total GH samples after 2 and 8 weeks. The proportion of 20K GH in 2-h samples after the initial MK-677 administration was lower than that after 2 and 8 weeks ($p < 0.01$ and $p < 0.05$, respectively). We concluded that the proportion of non-22K GH isoforms was higher in peak, but not in non-peak, total GH samples after the initial MK-677 administration than that observed after multiple doses. The proportion of 20K GH in 2-h samples after the initial MK-677 administration was lower than that after 2 and 8 weeks. These moderate changes in the proportion non-22K GH isoforms are likely of small importance for the clinical response to MK-677 treatment.

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1. Introduction

The work by Bowers [1–3] resulted in the discovery of growth hormone-releasing peptides (GHRPs) including the hexapeptide GHRP-6. Later research has led to the development of the GHRPs GHRP-1, GHRP-2, and hexarelin [4,5], and non-peptidyl GH secretagogues (GHSs) such as the orally active spiroindoline sulfonamide MK-677 [6]. A cell membrane G-protein coupled receptor has been cloned and shown to be a target for the GHSs [7]. An endogenous ligand for the GHS receptor, ghrelin, has been isolated [8].

The main GH isoform produced by the GH-N gene in the pituitary is 22K GH [9]. Another isoform, the 20K GH, which lacks residues 32–46 of 22K GH, is produced in the pituitary by alternative splicing of 22K GH messenger ribonucleic acid [10–12]. Studies show that 20K GH enhances linear growth in hypophysectomised rats [13] and exerts lipolytic activity in vitro [14]. 20K GH binds to GH receptors similarly to 22K GH [15], but differs in acute insulin-like activity [16] and binding to lactogenic receptors [17,18]. Furthermore, exogenous 20K GH administration suppresses endogenous 22K GH secretion in normal men [19].

In addition to 22K and 20K GH, several other GH molecular isoforms exist [9,13–15]. These can arise in the pituitary through pre-transcriptional and posttranslational mechanisms, or in peripheral tissues, where 22K GH may be degraded to various recirculating GH fragments [20–23]. The physiological role of these various GH isoforms is not fully known. However, these non-22K GH isoforms may differ from 22K GH regarding biological activity, binding properties, and metabolic clearance [9,23]. 22K GH binds to one GH receptor by a first binding site, and then to another GH...
receptor by a second binding site, resulting in receptor homodimerisation and activation [24,25]. Thus, GH isoforms, partially or completely lacking one of these two binding sites, may act as weak agonists or antagonists of the GH receptor [26,27]. It has also been suggested that some non-22K GH isoforms and fragments may bind to specific receptors [26,28].

Little is known about the regulation of non-22K isoforms. In a study by Baumann et al. [29], the relative proportion of circulating GH isoforms was not changed after administration of GH-releasing hormone (GHRH) or L-dopa in healthy subjects, or after administration of thyroid stimulating hormone in acromegalic patients [29]. However, in acromegaly, several other studies have shown an increased proportion of various non-22K GH isoforms [30–33]. In a study by Tsushima et al. [34], the ratio between 20K and 22K GH was unchanged after administration of insulin or GHRH in healthy subjects, whereas it was increased in acromegaly and anorexia nervosa [34].

We have previously reported that 8-week MK-677 treatment elicited a sustained increase in serum peak total GH levels, although the initial serum peak total GH response to MK-677 was higher than the peak GH responses to MK-677 at 2 and 8 weeks of treatment [35]. The aim of the present study was to determine the released proportion of non-22K GH isoforms and 20K GH after MK-677 administration in healthy obese males using the 22K GH exclusion assay (GHEA) [36] and a 20K-specific enzyme-linked immunosorbent assay (ELISA) [34].

2. Subjects and methods

2.1. Subjects

Twelve males, 19–49 years of age, with body mass index (BMI) >30 kg/m² and waist:hip ratio >0.95 were studied. At study start, mean age was 36.8 (2.7) years and mean BMI was 32.0 (0.4) kg/m². They were recruited using advertisements in local newspapers. Exclusion criteria included fasting blood glucose >6.4 mmol/L and blood pressure >165/95 mm Hg. Except for obesity, all subjects were in good general health and none used concomitant medication.

One subject was discontinued from the study after approximately 1–2 weeks due to a threefold increase in serum alanine-aminotransferase (ALT) and aspartateaminotransferase (AST), both of which decreased spontaneously to pre-study values after discontinuation of MK-677 treatment. Of note, this subject had violated the protocol by ingesting alcohol around the time of the elevation of AST and ALT. The discontinued subject was replaced with a new one who received the same treatment as the subject he replaced.

2.2. Study design

The present study was performed as an 8-week, randomised, double-blind, parallel, and placebo-controlled trial of the oral administration of MK-677 in healthy obese subjects. However, non-22K and 20K GH isoforms were only analysed in the MK-677 treatment group (below). Subjects were randomised to receive oral MK-677 25 mg or matching placebo daily for 8 weeks (N = 12 per group). The dose was administered with 150 mL water between 08.00 and 09.00. Compliance was checked by weekly tablet counts and indicated >99% compliance.

No samples after placebo administration were analysed with the GHEA or with the specific 20K GH ELISA. The reason for this was that total GH concentrations were generally low or undetectable after placebo administration. In consequence of this, most non-22K GH values in the placebo group would have been below the detection limit of the GHEA of 0.25 µg/L. Furthermore, ratios between 22K or 20K and total GH would not be meaningful due to the many zero total GH values in the placebo group.

2.3. Study protocol

The subjects were studied as out-patients. Prior to study start, subjects underwent a complete physical examination with laboratory safety assessments. The subjects were instructed not to change their ordinary daily caloric intake or physical activity during the study period. At study start, the subjects returned to the research unit. After an overnight fast, venous blood samples were drawn predose and at 30, 60, 90, 120, 240, and 480 min after administration of the daily dose of MK-677. Blood sampling was performed after the first intake of study drug and after tablet intake at 2 and 8 weeks. The results of the GH determinations in these samples, measured by a polyclonal immunoradiometric assay (IRMA; Pharmacia and Upjohn, Uppsala, Sweden), have previously been reported [35].

In the present study, peak GH samples after MK-677 administration from the eight-hour GH profiles at initiation of treatment and at 2 and 8 weeks were analysed with the GHEA for estimation of non-22K GH isoforms. In addition, 12 selected non-peak total GH samples after the initial MK-677 administration, one from each subject that received MK-677, were analysed with the GHEA. In each subject, the non-peak total GH sample after the initial MK-677 administration was selected in order to have a similar total GH concentration as that in the peak total GH samples at 2 and 8 weeks of treatment. The initial MK-677 administration induced a rapid increase in total GH concentrations with mean peak GH concentration observed 0.96 (range 0.5–1.5) hours after administra-
tion of study drug. All the non-peak total GH samples were therefore from the decreasing phase of GH release [mean time after administration of MK-677 was 3.8 (range 1.5–8.0) hours].

After the first MK-677 administration, all GHEA measurements were above this detection limit of the GHEA of 0.25 µg/L. However, after MK-677 administration at 2 and 8 weeks, 3 and 4 of the GHEA measurements were below the detection limit of the assay. In the selected non-peak GH samples after the initial MK-677 administration, 2 of the GHEA measurements were below the detection limit of the assay. All measurements below 0.25 µg/L were given a value of half the detection limit (0.125 µg/L).

The 20K GH concentration was determined in samples obtained 2 h after MK-677 administration with a specific ELISA. The 2-h timepoint was chosen in order to have samples with reasonably high, but not peak, total GH concentrations. Furthermore, 20 K GH has a longer half-life than 22 K GH [9]. The mean time from MK-677 administration to peak GH concentration was 0.96 (range 0.5–1.5) hours after the initial MK-677 administration, and 1.58 (range 1–4) hours and 1.21 (range 0.5–2) hours, respectively, after MK-677 administration at 2 and 8 weeks. The measurements of 20K GH were therefore performed in samples drawn after the mean time for peak GH concentration.

2.4. Ethical consideration

The study was approved by the Ethics Committee at the University of Göteborg and by the Swedish Medical Products Agency, Uppsala, Sweden. Informed consent was obtained from each subject prior to study start.

2.5. Biochemical assays

The serum concentrations of non-22K GH isoforms were measured with the GHEA [36], which is based upon the extraction of monomeric and dimeric 22K GH from serum using an anti-22K GH monoclonal antibody (MCB, Genentech, San Francisco, CA, USA) and magnetic beads coated with rat antimouse IgG (Dynal, Oslo, Norway). After the 22K GH extraction, levels of the remaining non-22K GH isoforms are measured by the polyclonal IRMA from Pharmacia & Upjohn (Uppsala, Sweden). The GH standards provided in the IRMA were calibrated against the 1st International Reference Preparation from the WHO (IRP 80/505) in which 1 mg = 2.61 U of GH [37]. In the GHEA, total GH levels were also determined for each sample [one portion of each sample was only incubated with assay buffer, (without addition of MCB)]. The GHEA assay has a detection limit of 0.25 µg/L, and a between assay coefficient of variation for 22K extraction below 5%, as determined in GH-free serum spiked with monomeric or dimeric 22K GH, as well as in two different serum pools with known GH concentrations.

The serum concentrations of 20K GH were determined using ELISA as described previously [34]. The detection limit of the assay was 5 ng/L. The intra-assay CVs were 3.7%, 3.0%, and 2.5% at serum concentrations of 13.4, 237, and 766 ng/L, respectively. The inter-assay CVs were 5.2%, 2.8%, and 4.9% at serum concentrations of 44.7, 99.8, and 282 ng/L, respectively.

2.6. Statistical analysis

The descriptive statistical results are presented as the mean and standard error of the mean (SEM). Where appropriate, a logarithmic transformation was performed before statistical analysis. Logarithmic transformed data (total GH, non-22K and 20K GH values) are presented as the geometric mean (SEM). Between-group differences were analysed with unpaired t tests. Within-group differences were analysed with paired t tests. Correlations were calculated by using Pearson's linear regression coefficient. A two-tailed probability of <0.05 was considered significant.

3. Results

3.1. Serum total GH concentrations

The geometric mean serum peak total GH concentration, analysed concomitantly with the GHEA, was 23.5 (4.6) µg/L after the first MK-677 administration, 6.2 (1.5) µg/L at 2 weeks, and 5.1 (1.4) µg/L at 8 weeks. The mean time from MK-677 administration to peak total GH concentration was 0.96 (range 0.5–1.5) hours after the initial MK-677 administration, and 1.58 (range 1–4) hours and 1.21 (range 0.5–2) hours, respectively, after MK-677 administration at 2 and 8 weeks. In the selected non-peak GH samples after the initial MK-677 administration, the geometric mean serum total GH concentration was 4.4 (0.4) µg/L.

The geometric mean serum total GH concentrations in 2-h samples after MK-677 administration was 11.1 (4.7) µg/L after the first M-677 administration, 2.2 (0.6) µg/L at 2 weeks, and 2.0 (0.9) µg/L at 8 weeks.

3.2. Non-22K GH isoforms after MK-677 administration

Serum non-22K GH concentrations (absolute values) in peak total GH samples were significantly higher after the first MK-677 administration than after MK-677 administration at 2 and 8 weeks (p < 0.01 and p < 0.001, respectively; Fig. 1A). The proportion of non-22K GH isoforms (non-22K GH/total GH ratio) in peak total GH samples was greater after the first MK-677 administration than that after MK-677
administration at 2 and 8 weeks ($p < 0.01$ and $p < 0.05$, respectively; Fig. 1B). The proportion of non-22K GH isoforms in the selected non-peak GH samples after the initial MK-677 administration was similar to that in the peak total GH samples after MK-677 administration at 2 and 8 weeks (Fig. 1B).

Fig. 1. (A) Serum non-22K GH isoform concentrations (absolute values) in peak total GH samples after the initial MK-677 administration and after MK-677 administration at 2 and 8 weeks. (B) The proportion of non-22K GH isoforms (non-22K GH isoforms/total GH ratio) in peak total GH samples after MK-677 administration in 12 healthy obese males. The proportion of non-22K GH isoforms in selected non-peak GH samples after the initial MK-677 administration is indicated at the right side of B. The vertical bars indicate the SE for the mean values shown. $^a p < 0.01$ vs. corresponding value at 2 weeks, $^b p < 0.001$ vs. corresponding value at 8 weeks, and $^c p < 0.05$ vs. corresponding value at 8 weeks.

Fig. 2. (A) Serum 20K GH concentrations (absolute values) in 2-h samples after the initial MK-677 administration and after MK-677 administration at 2 and 8 weeks. (B) The proportion of 20K GH (20K GH/total GH ratio) in 2-h samples after MK-677 administration in 12 healthy obese males. The vertical bars indicate the SE for the mean values shown. $^a p < 0.001$ vs. corresponding value at 2 weeks, $^b p < 0.01$ vs. corresponding value at 8 weeks, and $^c p < 0.05$ vs. corresponding value at 8 weeks.
3.3. 20K GH after MK-677 administration

Serum 20K GH concentrations (absolute values) in 2-h samples after the initial MK-677 administration were higher than that after MK-677 administration at 2 weeks \( (p < 0.001; \text{Fig. 2A}) \), whereas it was similar to that after MK-677 administration at 8 weeks \( (p = 0.08; \text{Fig. 2A}) \). The proportion of 20K GH \( (20K\ GH/\text{total GH ratio}) \) in 2-h samples after the first MK-677 administration was lower than that after MK-677 administration at 2 and 8 weeks \( (p < 0.01 \) and \( p < 0.05 \), respectively; \text{Fig. 2B}) \).

3.4. Correlation analysis

In peak total GH samples after MK-677 administration, serum non-22K GH concentration was positively correlated with serum total GH concentration at all timepoints \( (\text{initial administration}: r = 0.99; \ p < 0.001; \text{2 weeks}: r = 0.90, p < 0.001; \text{8 weeks}: r = 0.98, p < 0.001) \). Serum concentrations of non-22K GH and total GH were also positively correlated in the selected non-peak GH samples after the initial MK-677 administration \( (r = 0.86; p < 0.001) \).

In 2-h samples after MK-677 administration, serum 20K GH concentration was positively correlated with serum total GH concentration at initiation of treatment and at 2 weeks \( (r = 0.90, p < 0.001 \) and \( r = 0.68, p < 0.05 \), respectively), but not at 8 weeks \( (r = 0.25) \).

The proportion of 20K GH in 2-h samples was not correlated with time from peak GH concentration \( (2 \text{~h—time for peak total GH concentration}) \) at any timepoint \( (\text{initial MK-677 administration}: \ r = 0.52; \text{2 weeks}: r = -0.51; \text{and 8 weeks}: r = -0.16) \).

4. Discussion

This study revealed that the proportion of non-22K GH isoforms in peak, but not in non-peak, total GH samples was higher after the initial MK-677 administration than that after MK-677 administration at 2 and 8 weeks of treatment. The proportion of 20K GH in 2-h samples was lower after the initial MK-677 administration than that after MK-677 administration at 2 and 8 weeks. Serum non-22K GH concentration was correlated with serum total GH concentration at all timepoints. Serum concentrations of 20K GH and total GH were positively correlated at initiation of treatment and at 2 weeks, but not at 8 weeks.

It is unlikely that the higher proportion of non-22K GH in peak GH samples after the initial MK-677 administration is due to an incomplete extraction of 22K by the GHEA at the high total GH concentrations found after the initial MK-677 administration. Previous validation of the GHEA has shown an extraction of 22K GH between 96.3% and 100% in serum spiked with monomeric or dimeric 22K at concentrations up to 100 μg/L [36]. Furthermore, after MK-677 administration at 2 and 8 weeks, 3 and 4 of the GHEA measurements were below the detection limit of the assay. In the selected non-peak GH samples after the initial MK-677 administration, 2 of the GHEA measurements were below the detection limit. All these measurements below 0.25 μg/L were given a value of half the detection limit \( (0.125 \mu g/L) \) in the statistical analyses. The results of the statistical analyses were, however, similar also if patients with measurements below the detection limit of the assay were excluded. It is therefore unlikely that measurements below the detection limit of the GHEA had a major effect on the results.

The proportion of non-22K GH isoforms, as measured with the GHEA, was increased in peak total GH samples after the initial MK-677 administration. Other studies have shown an unchanged proportion of non-22K GH isoforms after administration of GHRH or L-dopa in healthy subjects [9,29]. Single-dose administration of a GHS elicits, however, a higher total GH response than that observed after GHRH [5,38]. A MK-677 induced, massive initial release of stored pituitary GH may possibly represent several different isoforms. This hypothesis may be supported by the increased proportion of various non-22K GH isoforms found in several studies in acromegalic patients [30–33]. Furthermore, 22K GH is peripherally degraded to recirculating GH fragments [9]. It could be speculated that very high GH concentrations could increase this peripheral degradation of 22K GH.

The proportion of 20K GH was lower in 2-h samples after the initial MK-677 administration than that after MK-677 administration at 2 and 8 weeks. It may be argued that 20K has a longer half-life in the circulation than 22K [9], and therefore, an increase in 20K GH could appear later than an increase in total GH. In the present study measurements of 20K GH were therefore performed at 2 h, which was after the mean time for peak total GH concentration. Furthermore, no relation was found between the proportion of 20K GH and the time from peak total GH concentration \( (2 \text{~h—time for peak total GH concentration}) \) at any timepoint. In the previous study by Tsushima et al. [34], 20K/22K ratio was unchanged as compared with baseline 30 and 60 min after the administration of insulin or GHRH [34]. It is therefore less likely that our finding of a lower proportion of 20K GH in 2-h samples after the initial MK-677 administration is explained by differences in half-life between 20K and total GH.

Serum 20K GH concentrations (absolute values) in 2-h samples were similar at initiation of MK-677 treatment and at study end. The proportion of 20K GH was, as discussed above, lower after the initial MK-677 administration than that observed after multiple doses.
These findings suggest that the 20K GH is less affected by single-dose MK-677 administration than total GH and other non-22K GH isoforms, whereas no major desensitisation occurs of the 20K response during prolonged MK-677 treatment. The reason for this is, however, unknown.

We have previously reported differences in the treatment responses in energy expenditure [35], bone markers [39], serum leptin concentration [40], and circulating lipid concentrations [41] after 2 and 8 weeks of MK-677 treatment. The results of the present study suggest that these differences between 2 and 8 weeks are mainly due to the downregulation of the total GH response after MK-677 administration during the study (mean peak total GH concentration was approximately 4.6 times higher after the initial MK-677 administration than that after 8 weeks). Changes in the proportion of non-22K GH isoforms were fairly small (the mean proportion of non-22K GH isoforms in peak total GH samples after the initial MK-677 administration was approximately 1.3 times higher than that after 8 weeks). The changes in the proportion of non-22K GH isoforms were therefore probably of small importance for the clinical response to MK-677 treatment.

We concluded that the proportion of non-22K GH isoforms was higher in peak, but not in non-peak, total GH samples after the initial MK-677 administration than that observed after multiple doses. The proportion of 20K GH in 2-h samples after the initial MK-677 administration was lower than that after 2 and 8 weeks. These moderate changes in the proportion non-22 K GH isoforms are likely of small importance for the clinical response to MK-677 treatment.

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