CLINICAL STUDY

Pituitary-independent effect of octreotide on IGF1 generation

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Abstract

Background: Somatostatin analogues are frequently used for medical treatment of acromegaly. The rationale for their use is based on the inhibition of pituitary GH secretion; however, there is *in vitro* evidence that octreotide also acts to inhibit hepatic IGF1 generation.

Aim & design: We studied the pituitary-independent effects of octreotide on IGF1 generation in 11 severely GH-deficient (GHD) humans (age 38, range 23–52; seven males; body mass index 24.7 ± 3 kg/m²; peak-stimulated GH < 3 µg/l; 3 ± 1 pituitary hormone deficiencies) on a stable dose of GH replacement (0.4 ± 0.1 mg) for at least 6 months. Patients were studied before and after 50 µg of s.c. octreotide three times a day for 7 days.

Results: At study entry, all patients had total IGF1 within age- and gender-related reference range (SDS 0.4 ± 1.0). Octreotide treatment resulted in a significant decrease in total IGF1 (by 18%, 208 ± 89 vs 173 ± 62 µg/l, $P=0.04$), free IGF1 (by 13%, 0.83 ± 0.36 vs 0.70 ± 0.33 µg/l, $P=0.01$) and IGFBP3 (6%, 4475 ± 745 vs 4209 ± 912 µg/l, $P=0.02$). Octreotide suppressed fasting insulin from 8.1 ± 3.4 to 6.3 ± 4.1 mU/l ($P=0.01$) and was associated with an increase in fasting glucose from 5.2 ± 0.9 to 5.8 ± 0.9 mmol/l ($P<0.01$). IGFBP1 increased by 84% from 42 ± 26 to 95 ± 52 µg/l ($P=0.04$).

Conclusion: Our study demonstrates that octreotide induces a significant decrease in IGF1 in severely GHD adults on a fixed dose of GH replacement. This is the evidence for a non-pituitary action of octreotide on the GH/IGF1 axis, most likely by antagonising the action of GH on hepatic IGF1 generation and indirectly, by suppressing insulin secretion.

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Introduction

Somatostatin (SST) is a widely expressed, multifunctional neuropeptide, which is the primary physiological inhibitor of GH secretion from the pituitary gland. Its two most biologically active isoforms comprise 14 and 28 amino acids and have a short half-life of approximately 2–3 min. SST acts through a family of five G protein-coupled somatostatin receptors (SSTRs) 1–5 (1, 2) distributed in a tissue-specific pattern.

For therapeutic purposes, synthetic somatostatin analogues (SSAs) with longer half-life and different receptor subtype preferences have been developed. Octreotide, the first synthetic SSA proven to suppress GH in patients with acromegaly (3), is an octapeptide, which acts as a ligand with very high affinity for SSTR2, intermediate affinity for SSTR3 and a moderately high affinity for SSTR5 (1, 2). GH-secreting pituitary adenomas most frequently express SSTR2 and SSTR5 (4–8), and the efficacy of octreotide to decrease GH in patients with acromegaly correlates with the SSTR2 and SSTR5 expression in pituitary adenomas (4–11).

IGF1 is the main mediator of GH action, with the liver being the source of approximately 80% of circulating

IGF1 (12). In plasma, IGF1 is bound to a family of specific binding proteins (IGFBP1 to 6) that prolong its half-life to about 16 h (13) and assist in delivering IGF1 to target tissues (14, 15). As GH has a half-life of 15 min and is released in pulses mainly at night, serum IGF1 offers a more convenient biochemical marker of the GH action than circulating GH levels.

There is a log-linear correlation between mean circulating GH levels and IGF1, although various factors that alter the relationship have been described (16, 17). Intrinsic and extrinsic oestrogens antagonise the effect of GH on IGF1 generation in healthy subjects (18), patients with GH deficiency (19) and acromegaly (20, 21). Conversely, obesity increases GH sensitivity (22–24), a finding that may be at least partially explained by elevated insulin levels (25).

It has been suggested that, in patients with acromegaly, octreotide treatment reduces circulating IGF1 more than anticipated from the suppression of the endogenous GH secretion, and several groups have commented on this phenomenon (10, 26–28). These findings suggest that octreotide action is not confined to the suppression of GH secretion, but that it may also have a direct effect on IGF1 generation. This notion is

supported by studies in animals and *in vitro*. In hypophysectomised, GH-deficient (GHD) rats, octreotide reduced recombinant GH-stimulated mean serum IGF1 concentration by 26–60% (29, 30). No consistent changes were found in serum insulin, excluding it as a potential mediator of the octreotide-induced IGF1 reduction. *In vitro* studies in cultured rat hepatocytes demonstrated that octreotide reduces GH-induced IGF1 mRNA expression and peptide (30, 31).

We designed this study to test the hypothesis that in humans, octreotide lowers serum IGF1 not only by inhibiting GH secretion, but also by antagonising the ability of GH to stimulate IGF1 synthesis.

Patients and methods

Patients

Eleven GHD patients (median age 38 years, range 23–52; seven males, body mass index $24.7 \pm 3 \text{ kg/m}^2$; 3 ± 1 pituitary hormone deficiencies) fulfilling the Port Stevens consensus criteria (32) for severe adult GHD (peak-stimulated GH response $3 \text{ }\mu\text{g/l}$) were enrolled in the study according to IH GCP (International Harmonization Good Clinical Practice). Patients’ characteristics are given in Table 1. All patients were on an optimised, stable dose of hormone replacement therapy including GH for at least 6 months. Medications remained unaltered throughout the study.

Methods

After giving written informed consent, patients were asked to take their regular hormone replacement at fixed times. In particular, GH injections were given at the bed time. Patients attended on day 1 fasted from midnight for the baseline assessment. Blood samples were taken for total IGF1, free IGF1, IGFBP1, -2 and -3, insulin and glucose.

The samples were left for 20 min to clot before being centrifuged and aliquotted in duplicates. Serum was frozen at $-80 \text{ }^\circ\text{C}$ until assaying. All the assays were performed in a single batch.

Participants were taught to self-inject octreotide subcutaneously (their first injection of $50 \text{ }\mu\text{g}$ of octreotide was taken under supervision), and were provided with a 7-day drug supply and an injection diary and went on to inject themselves thrice daily at 0800, 1400 and 2200 h. On day 7, they had their morning octreotide injection at 0800 h and fasted for the reassessment at 0900 h. The blood tests from the baseline were repeated. At the end of the study, patients returned the diaries and remaining injection equipment for evaluation of compliance.

Assays

Total IGF1 was measured by IRMA using a commercial analyzer (Nichols Institute Diagnostics San Clemente, USA) with intra- and interassay coefficient of variation (CV) $< 7.4\%$ with age- and gender-related reference ranges established by Brabant *et al.* (33).

Free IGF1 was assessed using centrifuge-forced ultrafiltration followed by immunoassay as described previously (34). The lower detection limit was $0.045 \text{ }\mu\text{g/l}$. Intra- and interassay CV averaged at 15–20%.

IGFBP1 and IGFBP2 were assessed by in-house immunoassays as described previously (35). Intra- and interassay CV averaged < 5 and $< 16\%$ respectively, for the IGFBP1 assay, and 5 and 12% respectively, for the IGFBP2 assay.

IGFBP3 was measured by a commercial IRMA (DSL Inc. Webster, TX, USA; interassay CV $< 5\%$ and intra-assay CV $< 10\%$).

Insulin was assessed using a commercial electrochemiluminescence immunoassay by Roche Diagnostics GMBH (inter- and intra-assay CV $< 2.6\%$), while glucose was measured using an oxidative method Advia 1650/1800 by Bayer Healthcare (CV $< 6\%$).

Table 1 Patients characteristics.

Sex	Age (years)	BMI (kg/m ²)	Diagnosis	Surgery	RT	# PHD	GH dose (mg)
M	52	25.9	NFPA	Y	Y	4	0.4
M	43	22.6	CD	Y	N	4	0.4
M	36	25.9	Astro	Y	Y	2	0.5
F	35	26.7	Sheehan's	N	N	2	0.3
M	50	23.3	NPC	N	Y	3	0.4
F	39	20.7	CD	Y	Y	5	0.4
M	31	20.7	Lymph	N	Y	3	0.4
M	39	24.4	CD	Y	N	4	0.2
M	23	29.7	Pinealoma	N	Y	1	0.3
M	40	29.9	PRL	Y	Y	4	0.3
F	29	22.5	IGHD + GnD	N	N	2	0.5

RT, radiotherapy; # PHD, number of pituitary hormone deficiencies; NFPA, non-functioning pituitary adenoma; CD, Cushing's disease (cured); Astro, astrocytoma; NPC, nasopharyngeal carcinoma (cured); Lymph, lymphoma; PRL, prolactinoma (cured); IGHD + GnD, idiopathic GH deficiency and gonadotrophin deficiency.

Statistical analysis

The data had Gaussian distribution at baseline when assessed by Kolmogorov–Smirnov test ($P > 0.01$) and consequently results are presented as means $\pm$ s.d. The paired t -test was used for comparison of pre- and post-intervention results with statistical significance being defined as $P < 0.05$.

Results

All patients were on a stable, fixed dose of GH (mean dose 0.4 ± 0.1 mg) and had a total IGF1 within age- and gender-related reference range, as defined by Brabant *et al.* (33). With octreotide, total IGF1 fell by an average of 18%, from 208 ± 89 to 173 ± 62 $\mu\text{g/l}$ ($P = 0.04$), resulting in total IGF1 levels below the reference ranges in 2 out of 11 patients (Fig. 1).

At baseline, there was a sevenfold variation in individual free IGF1 results (0.189 – 1.149 $\mu\text{g/l}$). Octreotide decreased free IGF1 by an average of 13% (0.83 ± 0.36 vs 0.70 ± 0.33 $\mu\text{g/l}$, $P = 0.01$; Fig. 2).

At the study entry, IGFBP3 was within the normal range in all patients (4475 ± 745 $\mu\text{g/l}$). Octreotide treatment reduced IGFBP3 by 6% to 4209 ± 917 $\mu\text{g/l}$ ($P = 0.02$, Fig. 3), which remained within reference range.

Octreotide induced a decrease in fasting insulin of 26% from 8.1 ± 3.2 to 6.3 ± 4.1 mU/l, $P = 0.01$). The decrease in fasting insulin was reflected by an increase in fasting glucose of 11%, from 5.2 ± 0.9 to 5.8 ± 0.9 mmol/l ($P < 0.01$; Fig. 4).

IGFBP1 increased significantly ($+84\%$) with octreotide from 42 ± 26 to 95 ± 52 $\mu\text{g/l}$ ($P = 0.04$; Fig. 5), while IGFBP2 did not change (120 ± 44 vs 133 ± 90 $\mu\text{g/l}$, $P = 0.09$).

Discussion

Our study demonstrates that the addition of octreotide to a fixed dose of GH replacement therapy in adults with severe GHD causes a decrease in total and free IGF1 as well as IGFBP3, but an increase in IGFBP1. These

Figure 1 Octreotide reduced total IGF1 by 18% from 208 ± 89 $\mu\text{g/l}$, SDS 0.5 ± 1 to 173 ± 62 $\mu\text{g/l}$ ($P = 0.04$), resulting in IGF1 levels below the reference ranges in 2 out of 11 patients.

Figure 2 Octreotide decreased free IGF1 by an average of 13% (0.830 ± 0.359 vs 0.699 ± 0.332 $\mu\text{g/l}$, $P = 0.01$).

Figure 3 Octreotide reduced IGFBP3 by 6%, from 4475 ± 745 to 4209 ± 917 $\mu\text{g/l}$ ($P = 0.02$).

findings support the hypothesis that octreotide acts to inhibit GH action, as well as GH secretion.

Our findings are consistent with and extend those of Laursen *et al.* (36), who studied six GHD patients on recombinant human GH with and without the addition of octreotide and found a significant decrease in total IGF1 and an increase in IGFBP1 following combination therapy. The study by Laursen *et al.* failed to document a significant change in free IGF1, while we showed a significant reduction in free IGF1 with octreotide, supporting the total IGF1 results.

Figure 4 Octreotide reduced fasting insulin by 26% from 8.1 ± 3.2 to 6.3 ± 4.1 mU/l, $P = 0.01$.

Figure 5 As a consequence of insulin reduction caused by octreotide, IGFBP1 increased significantly ($+84\%$) from 42 ± 26 to 95 ± 52 $\mu\text{g/l}$ ($P=0.04$).

A decrease in total IGF1 may be partially explained by the decrease in IGFBP3, as 70–80% of circulating IGF1 is bound in a ternary complex with IGFBP3 and the acid-labile subunit. IGFBP3 is produced in hepatic Kupffer cells rather than hepatocytes, under the control of circulating GH (Rajaram *et al.* 1997), and although it is not known which, if any, SSTRs are expressed by Kupffer cells, the decrease in IGFBP3 indicates that the octreotide inhibition of the GH action is not confined to hepatocytes.

Alternatively, unbound IGFBP3 is known to be rapidly cleared from plasma and, therefore, the octreotide-induced decrease in IGF1 and parallel reduction in ternary complex formation could leave a greater proportion of IGFBP3 unbound and liable to rapid clearance (37, 38).

The decrease in free IGF1 in our study suggests that the effect of octreotide on total IGF1 cannot be explained solely by a reduction in circulating levels of its most abundant binding protein IGFBP3. Free IGF1 is believed to reflect the biologically relevant, unbound fraction of IGF1, but it is difficult to measure. The method used here is specifically designed to avoid inducing dissociation of loosely bound IGF1, particularly those bound to IGFBP1 (39). The decrease in free IGF1 implies a true reduction in IGF1, not an artefact secondary to IGFBP changes.

There are two, not mutually exclusive, possible mechanisms to explain the reduction in IGF1.

As described above (30, 31), *in vitro* studies have shown a direct, octreotide-induced inhibition of hepatic IGF1 generation. Murray *et al.* (40) demonstrated the expression of SSTR2 and SSTR3 on rat hepatocytes and a cross-talk between octreotide-activated SSTR2 and 3 and GH-activated GH receptor (GHR) pathways, by dephosphorylating the GHR-linked second messenger STAT5b. To date, the expression of SSTRs on the human liver has to be documented, but it is presumed to be similar to that in rodents. Another example of antagonism of GH action induced by interruption of intracellular second messenger pathway has been described with oestrogen. Leung *et al.* demonstrated that oestradiol via the oestrogen receptor α increased the expression of SOCS2, which is a negative regulator of a second messenger STAT5b involved in IGF1 gene transcription (41).

A second possible mechanism to account for the decrease in total and free IGF1 could be an indirect effect related to the inhibition of insulin secretion by octreotide.

In our study, octreotide suppressed mean fasting serum insulin levels by 26%. Plasma insulin and IGFBP1 are inversely correlated (42, 43); thus, in our study, the decrease in mean fasting insulin was associated with an increase in circulating IGFBP1 of 84%. Furthermore, there appears to be a direct stimulatory effect of SSTAs on IGFBP1 generation (44).

Under physiological conditions, approximately 10% of circulating IGF1 is bound to IGFBP1 (45); thus, an increase in IGFBP1 could contribute to the decrease in free IGF1, but does not explain the decrease in total plasma IGF1.

Leung *et al.* used a human hepatoma cell model to study the interaction between GH and insulin receptor pathways and found that insulin, at concentrations comparable with those in our study increased the number of GHR on the cell surface and enhanced the GH-induced intracellular signalling via JAK2 pathway.

This notion of insulin levels and positive regulation of hepatic GHR number and function may explain the increase in GH sensitivity seen in obesity (24).

The portal blood supply of the liver means that it is particularly sensitive to changes in the insulin secretion, and the changes measured in peripheral plasma insulin may not fully reflect the changes in the portal circulation. Pancreatic insulin secretion in humans is predominately regulated by SST through SSTR2 and, to a lesser extent, SSTR1 and 5 (46). SSTAs such as octreotide have low affinity for SR5 in comparison with the new SSTA SOM230 (47, 48); hence, any insulin-related reduction in IGF1 levels may be greater with such analogues, but, if accompanied by deterioration in the glucose tolerance, would be metabolically unacceptable.

An alternative interpretation of the data generated by this study is that octreotide inhibited residual endogenous GH secretion in our patients. This seems unlikely, as all our patients had severe GHD and were on long-term (>6 months) GH replacement that should have suppressed any residual intrinsic GH secretion.

This study provides evidence for an extra-pituitary effect of octreotide on IGF1 generation in humans, which is most likely a result of a direct GH-antagonising effect of octreotide on the liver of other organs, and an indirect effect via suppression of the insulin secretion. It is intriguing to speculate that this peripheral action of octreotide may be contributing to the successful use of octreotide with GHR antagonist in patients with acromegaly (49).

These findings are relevant to the treatment of acromegaly and its monitoring, and particularly interesting in the context of newer SSTAs with a different affinity for the various SRs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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