In vivo responsiveness of morphological variants of growth hormone-producing pituitary adenomas to octreotide

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The somatostatin analog, octreotide, is an inhibitor of growth hormone (GH) secretion that has been used to treat patients with GH-producing pituitary tumors. In this study we investigated the in vivo responsiveness to treatment with this analog in patients harboring different morphological types of GH-producing pituitary adenomas. Both GH and insulin-like growth factor I (IGF-I) plasma levels in 30 patients treated with octreotide (300 μg/day) for 4 months preoperatively were compared with those from 30 patients who did not receive treatment preoperatively. Tissue samples were studied using ultrastructural and immunohistochemical techniques. Amongst patients harboring densely granulated (DG) adenomas, mean GH levels were reduced to 32 ± 9% by octreotide, to 30 ± 7% by surgery and to 26 ± 9% of baseline by both interventions. Surgery was equally as effective in lowering GH levels in patients with sparsely granulated (SG) adenomas as it was in those with DG adenomas; in patients with SG adenomas, GH levels were reduced by surgery alone to 37 ± 16% and to 24 ± 15% when performed following octreotide pretreatment. In contrast, treatment with octreotide alone in patients harbouring SG adenomas reduced GH levels to only 70 ± 13% of baseline (p < 0.02 compared to surgery alone, or surgery and octreotide). We conclude that the GH inhibitory effects of octreotide are significantly better in patients harboring DG somatotroph adenomas compared with those harboring SG adenomas.

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Growth hormone (GH) is under dual hypothalamic influence by GH-releasing hormone (GHRH), which stimulates, and somatostatin (SRIH), which inhibits, GH secretion. Specific receptors for SRIH are expressed on somatotroph adenomas. Expression of this hormone in GH tumors appears to be reduced compared to that in the normal pituitary (1). Furthermore, nearly one-third of somatotroph adenomas are associated with a somatic mutation in the membrane coupling G protein (2). This defect results in constitutive activation of adenylate cyclase activity and hence increased intracellular cAMP levels. The inhibitory effects of SRIH on GH and cAMP production (3), therefore, support the rationale for the use of SRIH in the management of GH-producing pituitary adenomas. Indeed, SRIH lowers GH secretion in normal and acromegalic patients (4, 5), but use of this peptide is limited owing to its short half-life.

Current treatment options for patients with GH-secreting pituitary adenomas include surgical resection (6), external radiotherapy (7) or medical therapy (8). None of these approaches independently provides complete correction of the tumor mass and GH hypersecretory effects associated with these adenomas. The SRIH analog octreotide is an effective GH-lowering agent that has been used to control GH hypersecretion in patients with acromegaly (9, 10). We have reported previously the cellular effects induced by octreotide on somatotroph adenomas (11). The aim of the current study was to establish the relationships between hormonal response to octreotide and morphological subtype of somatotroph adenomas.

Methods

Patients and treatment protocol

Sixty-nine acromegalic patients from 16 centers in the USA, Canada and Europe were included in this study. Entry criteria included a serum GH concentration of >2 μg/l following oral glucose administration and a pituitary mass of >10 mm in diameter on computed tomography or magnetic resonance imaging. Patients who had undergone previous pituitary surgery or irradiation and/or those who received bromocriptine treatment were excluded.
Patients were randomized for transsphenoidal adeno-
mectomy with and without pretreatment with this
analog for 4 months prior to surgery. The study group
included 34 patients treated by combined octreotide
therapy and surgery and 35 patients treated by surgery
alone; the latter formed the control group. Treatment
was initiated with 50 µg of octreotide acetate (Sandosta-
tin, Sandoz Pharmaceuticals, Basel, Switzerland) sc every
8 h for the first week and this was subsequently increased
to 100 µg sc every 8 h for the duration of the 4 months.

Plasma GH determinations were made at time zero and
then hourly for the subsequent 4 h. The GH and
IGF-I measurements were performed at baseline, 4
months after octreotide treatment (where GH levels
were measured immediately before and hourly for 4 h
after 100 µg of octreotide sc where applicable) and 1
month following transsphenoidal surgery. Growth
hormone was measured by standard RIA techniques
at each individual center. Plasma IGF-I was measured
by direct RIA using a serum standard (Immuno Nuclear
Corp.). Although this method may not effectively exclude
IGF binding proteins, this does not affect interpretation
of relative IGF-I changes. Patients gave informed
consent after approval by each respective Institutional Review
Board for the protection of human subjects.

Morphological techniques

Morphological studies were performed without know-
ledge of the treatment administered. For light micro-
scopy, semithin sections of 4–6 µm from 4% buffered,
formalin-fixed and paraffin-embedded tissues were
stained by hematoxylin and eosin, periodic acid–Schiff
(PAS) and the Gordon–Sweet stain for demonstration of
reticulin fibers. The avidin–biotin–peroxidase complex
(ABC) technique was applied for immunohistochemical
localization of pituitary hormones GH, PRL, ACTH(1–39),
β-TSH, β-FSH, β-LH and the α-subunit of glycoprotein
hormones. The extent of immunoreactivity for GH was
classified with a four-step grade scale according to a
semiquantitative proportional estimate of immunopositive
adenoma cell numbers for each of the three hormones:
I. <25%; II, 26–50%; III, 51–75%; IV, 76–100%.

For ultrastructural analysis, samples of surgically
removed adenoma tissue were fixed in 2.5% phosphate-
buffered glutaraldehyde, postfixed in 1% osmium tetroxide
and embedded in an Apon–Araldite epoxy mixture.
Semithin sections were stained with toluidine blue to
select areas for ultrastructural analysis. Ultrathin sections
were stained with uranyl acetate and lead citrate and
examined with a Phillips 410 LS electron microscope.
Specimens not containing somatotroph adenoma tissue
suitable for diagnosis were excluded from the analysis.

Statistical analyses

Data are shown as means ± SEM. The control and
treatment groups were analyzed collectively and after
subdivision on the basis of the morphological phenotype
densely granulated (DG) vs sparsely granulated (SG)) of
the GH-producing adenoma. Differences between groups
were tested using the t-test and all reported p-values are
two-tailed. Tests based on non-parametric statistics
revealed similar findings and are not reported.

Results

Ultrastructural findings

There were no statistically significant differences between
treatment groups with respect to age, sex, race, height,
weight and days since diagnosis at baseline. The mean
age of the octreotide group was 46.5 years (21–72
years) and that for the surgical group was 45.0 years
(23–74 years).

Thirty-three patients were diagnosed as harboring a
DG somatotroph adenoma (18 received octreotide and
surgery; 15 had surgery alone) and 27 had the SG type
(12 received octreotide and surgery; 15 had surgery
alone). Eight other patients harbored tumors that were
composed primarily of a mammosomatotroph component
admixed with DG and/or SG somatotrophic elements.
One patient had an adenoma that could be classified
only as an acidophil cell adenoma.

Immunohistochemical findings

All tumors were immunopositive for GH, 90% were
immunopositive for PRL and 90% were immunopositive
for α-subunit. The DG adenomas exhibited mostly even
and extensive immunoreactivity compared with the SG
adenomas, which showed variable staining intensity.

Growth hormone and IGF-I findings

Mean pretreatment GH and IGF-I levels did not differ
between patients harboring DG adenomas (49 ± 15 µg/l
and 7443 ± 1117 U/l, respectively) and SG adenomas
(43 ± 10 µg/l and 8979 ± 1555 U/l, respectively). Mean
GH concentrations in the octreotide-treated group decreased
from 49 ± 9 µg/l at baseline to 17 ± 4 and 11 ± 6 µg/l
(p < 0.01) prior to and following
surgery, respectively. Amongst the patients who
were treated by surgery alone, GH concentrations were
reduced from 42 ± 14 to 22 ± 11 µg/l (p = 0.017).
Figure 1 depicts further analysis of the GH response in
the two treatment groups based on tumor morphology.
Amongst the patients harboring DG adenomas, mean
GH levels were reduced to 32 ± 9% by octreotide, to
30 ± 7% by surgery and to 26 ± 9% of baseline by both
interventions. In comparison, treatment with octreotide
in patients harboring SG adenomas reduced GH levels
to only 70 ± 13% of baseline (p = 0.017 compared to
surgery alone, or surgery and octreotide). Surgery,
however, was equally as effective in lowering GH levels
as in patients with DG adenomas. In patients with SG

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adenomas, GH levels were reduced by surgery to $37 \pm 16\%$ and $24 \pm 15\%$ when performed alone or following octreotide pretreatment, respectively (Fig. 1). The “other cell” category comprised four patients with morphological somatotroph adenomas who were treated with octreotide/surgery and five patients who were treated by surgery alone, four of whom harbored morphological somatotroph adenomas and one with an unusual acidophil cell adenoma. In the latter patient, mean GH levels actually increased from 45.6 to 323.4 µg/l following surgery, contributing to the wide variation shown in Fig. 1.

For the octreotide-treated group, mean IGF-I levels decreased from $6894 \pm 971$ U/l at baseline to $2442 \pm 406$ U/l ($p < 0.001$) and $1801 \pm 318$ U/l ($p < 0.001$) prior to and following surgery, respectively. For the surgical group, mean IGF-I values decreased from $8813 \pm 1221$ to $5188 \pm 1649$ U/l ($p = 0.007$). Figure 2 shows further analysis of the IGF-I response in patients harboring the different tumor types. Unlike the GH reduction, there was no statistically significant difference between the extent of IGF-I reduction achieved by the two treatment approaches in patients harboring either types of tumors or between the different tumor types.

The degree of GH and IGF-I reduction was not different between patients when stratified according to the degree of GH and/or α-subunit tumor immunoreactivity (data not shown).

Discussion

This is the first randomized controlled study to examine the relationship between the response to octreotide treatment and GH tumor morphology. The preoperative administration of octreotide resulted in enhanced reductions of GH levels in patients with DG adenomas compared with those harboring SG adenomas. These in vivo findings contrast with earlier in vitro observations on the effects of octreotide on cultured somatotroph adenomas where the release of GH was not significantly different under basal and octreotide-treated conditions between the two types of somatotroph adenomas (12). These differences may be attributed to overlap between the two groups of adenomas, which engenders a need for a large number of cases to determine statistical significance.

Our study indicates that the variable morphological appearance of GH-producing pituitary adenomas correlates with the response of these lesions to octreotide treatment. Approximately 30–40% of GH-producing pituitary adenomas are associated with G mutations (2). The presence of this mutation has been associated with variable plasma GH levels (2, 13). In the current study, we found no difference in basal GH or IGF-I levels between patients harboring DG adenomas and those with SG adenomas. As GH and IGF-I levels usually correlate with pituitary tumor size (10), the lack of such difference in our study may reflect the inclusion of only patients with macroadenomas. While the therapeutic response to octreotide has not previously been examined specifically with respect to the presence of this mutation, GH-producing adenomas with high basal adenylyl cyclase levels display greater inhibition of adenylyl cyclase in vitro and GH secretion in vivo by octreotide (3). Our data agree with these earlier findings inasmuch as greater inhibition of GH by octreotide was
noted in DG adenomas, which have been described to be associated with G<sub>1</sub> mutations (2).

The mechanism of action of octreotide on somatotroph adenomas appears to involve inhibition of hormone release without alteration of GH gene transcription (14). This is consistent with our previous data indicating that the diameters and cytoplasmic volume densities of secretory granules are increased in somatotroph adenomas resected from patients who were treated with octreotide, thus indicating retention of and inhibition of release of GH (11, 12). Lysosomal accumulation and mild crinophagy were modest findings restricted only to a few adenomas. The secretory apparatus of adenoma cells was preserved with a slight increase in GH immunoreactivity. Vascular changes resulting in cell necrosis were not apparent in any of the adenomas examined. The only prevalent finding was the presence of varying degrees of perivascular and interstitial fibrosis. The most striking cellular feature, however, was the lack of uniformity in the morphological response. These findings indicate that tumor morphology was not likely to have been altered by octreotide pretreatment. Furthermore, the lack of correlation between the degree of GH immunohistochemical staining intensity and the response to octreotide highlights the role of ultrastructural assessment in the characterization of pituitary adenomas.

The effects of octreotide are mediated through specific high-affinity membrane receptors. Earlier studies suggested a relationship between the density of SRIH receptors on GH tumors and the in vivo secretory response to this analog (15). Binding sites for SRIH, however, have been documented by autoradiography in tumors resistant to the GH-lowering effects of octreotide (3). These findings are consistent with differential adenyl cyclase coupling by the five subtypes of SRIH receptors and their heterogenous mRNA expression in pituitary adenomas (16). Collectively, these data indicate that the presence and function of SRIH receptors is only one of several determinants of responsiveness to the octreotide analog.

From a clinical perspective, the response to octreotide treatment of acromegaly is influenced by a number of variables. The magnitude of GH and IGF-I suppression appears to correlate with tumor size and hence baseline GH levels (10). Levels of IGF-I are normalized in nearly 80% of patients with GH-secreting microadenomas but in only 40% of patients harboring GH macroadenomas (10). Additionally, visualization of the adenoma by radiolabeled octreotide scintigraphy may correlate with a positive therapeutic response to SRIH analog treatment (17). Our current data indicate that ultrastructural tumor morphology should also be considered as an additional prognostic variable in predicting the response to octreotide treatment.

In agreement with other studies (18), we did not find a strict correlation between the decline in circulating GH and IGF-I levels. Acromegaly is characterized by GH excess, which is associated with enhanced hepatic production of IGF-I, GH's target growth factor (19). Inhibition of GH secretion would, therefore, be expected to result in parallel reductions in IGF-I levels. However, IGF-I circulates in the blood bound to several IGF binding proteins (IGFBPs). Of these, we have shown previously that octreotide stimulates IGFBP-1 levels in patients with acromegaly (20) as well as normal subjects (21). This may partly explain the discrepancy between the favorable clinical improvement and GH response with the absence of early normalization of IGF-I levels in some octreotide-treated patients. Long-term follow-up will be necessary to determine if this discrepant relationship between GH and IGF-I levels persists in patients who undergo pituitary surgery following octreotide treatment.

For acromegalic patients with both GH tumor subtypes, surgery represents the optimal first-line modality of treatment. The long-term surgical outcome of octreotide pretreatment in these subjects remains to be established. Patients in whom the analog achieves minimal hormone inhibitory effects should be suspected of harboring lesions with predominantly SG components. In the current study, these patients did not respond in a comparable way to patients whose tumors were predominantly DG in ultrastructural appearance. The biological significance of ultrastructural variants of GH-producing pituitary adenomas remains to be elucidated fully. Nevertheless, our data indicate that tumor morphological parameters correlate with the GH-reducing effects of octreotide treatment.

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