Lack of Somatostatin Analogs Effectiveness in Gonadotropin-Secreting Pituitary Adenomas
Report of a Case and Review of the Literature

Lorenzo Curtò, MD,* Rosaria M. Ruggeri, MD,* Diego Ferone, MD,§ Rosario Pivonello, MD,¶ Stefano Squadrito, MD,† Alfredo Campenni, MD,¶ Maria Trovato, MD,†† Sergio Baldari, MD,** Leo J. Hofland, MD,‡‡ Francesco Trimarchi, MD,* and Salvatore Cannavo, MD‡

Abstract: Gonadotropin-secreting pituitary adenomas are rare. Surgery remains the treatment of choice, whereas medical therapy is used when surgery has failed, is contraindicated, or is refused. Recently, data suggest a possible inhibitory effect of somatostatin analogs (SSAs) in these adenomas and their overall effectiveness is controversial. Moreover, although a subset of gonadotropinomas is partially responsive to SSAs in terms of hormone inhibition, SSA efficacy on tumor shrinkage is less clear. We report the case of a 55-year-old patient, with a pituitary mass of 55.47 mm, with high levels of alpha-subunit (7.2 U/L) and follicle-stimulating hormone (FSH) (67.7 U/L). Before surgery, octreotide LAR (20 mg intramuscularly every 28 days) was administered for 3 months. An OctreoScan revealed significant tumor uptake, but serum FSH, alpha-subunit levels, and tumor size were not decreased. Tumor specimens were studied by immunohistochemistry and by reverse transcriptase–polymerase chain reaction (RT-PCR). The adenomatous cells expressed SSTR subtype sst2A, agreeing with the OctreoScan result. RT-PCR analysis confirmed selective expression of sst2A as well as sst1 mRNAs. This report shows that in patients with a gonadotropinoma, responsiveness to an SSA is not always predicted by scintigraphy, and in vitro expression of SSTRs may be dissociated from the in vivo response to SSAs in terms of hormone secretion and tumor growth. On the basis of this case, we reviewed the literature on this subject and analyzed it in this report.

Key Words: gonadotropin-secreting pituitary adenoma, somatostatin analogs, OctreoScan, somatostatin receptors subtypes

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Learning Objectives
• Summarize what is known about gonadotropin-secreting pituitary adenomas, or Gn-omas, and their response to medical and surgical treatments.
• Recall the clinical, hormonal, scintigraphic, and immunohistochemical findings in this patient who harbored a Gn-oma, and how they may have related to the outcome of medical treatment with octreotide LAR, a long-acting release form of a somatostatin analog.
• Appraise competing (or complementary) explanations for this patient’s apparent resistance to octreotide therapy.

Gonadotropin-secreting pituitary adenomas (Gn-omas) are rare. Surgery is still the treatment of choice, and radiotherapy has been used for the management of residual tumor after surgery as well as recurrences. Medical therapy has given disappointing and unsatisfactory results but can be tried in cases in which surgery has failed, is contraindicated, or is refused.¹⁻³ In the last few years, data suggesting an inhibitory effect of somatostatin analogs (SSAs) in this type of pituitary adenomas have been published.⁴⁻⁷ SSAs are currently used for treating growth hormone (GH)-secreting pituitary adenomas and neuroendocrine tumors expressing somatostatin receptors (SSTRs) a family of
G protein-coupled, membrane receptors. In these tumors, SSAs inhibit hormone hypersecretion and can reduce tumor mass through binding to specific SSTR subtypes. Five different SSTR subtypes (sst1-5) are characterized. Currently available analogs, octreotide and lanreotide, bind with high affinity to sst2 and with lower affinity to sst3 and sst5. The variable response of pituitary adenomas to SSA treatment depends on the receptor subtype expression in the tumor cells. Different receptor subtypes can mediate the effects of SSAs on hormonal hypersecretion and/or tumor growth. Imaging techniques with \(^{111}\text{In-DTPA-octreotide}\) can visualize SSRs in various neoplasms in vivo. A positive scan can predict a good response to SSA treatment.

Various SSTR subtypes are found on the cell membranes of Gnomas by in vivo and in vitro studies. Some studies show that SSAs decrease serum gonadotropin levels and, in a minority of cases, shrink the tumor. The ultimate effectiveness of these drugs, however, remains doubtful.

We report the case of a patient with a follicle-stimulating hormone (FSH)-secreting pituitary macroadenoma in whom \(^{111}\text{In-DTPA-octreotide}\) scintigraphy showed a significant tumor uptake and the adenomatous cells expressed specific SSTR subtypes. Octreotide LAR administration, however, failed to decrease FSH secretion or reduce tumor mass during a short period of observation. On the basis of this case, we review what is known about the efficacy of SSA therapy in gonadotropinomas.

CASE REPORT

A 55-year-old man was referred to our Endocrine Unit with a severe headache and decreased libido for the last 5 years. Endocrine evaluation revealed increased serum levels of FSH (67.7 U/L; normal value, 0.7–11.1 U/L) and free-alpha-subunit (7.2 U/L; normal value, <1.8 U/L), associated with normal serum luteinizing hormone (LH) (2.1 U/L) and prolactin (PRL) (13.5 μg/L) values. FSH response to LHRH (100 μg intravenously [IV]) was impaired (FSH baseline: 67.7 U/L, peak: 72.8 U/L). Stimulation with 200 μg TRH IV had no effect on serum FSH and LH levels (FSH baseline: 68.6 U/L, peak: 76.7 U/L; LH baseline: 2.0 U/L, peak: 2.9 U/L). Serum total testosterone was within normal limits. Serum FT4 (14.7 pmol/L) and thyroid-stimulating hormone (TSH) (1.1 mU/L) were normal, as were serum cortisol (183 ng/mL), corticotropin (ACTH) (41.3 pg/mL), and 24-hour urinary free cortisol values (170 μg/24 hours; normal value, 35–270 μg/24 hours). Baseline serum GH levels were 0.1 ng/mL (normal value, <2.0 ng/mL) and serum IGF-1 levels (115 ng/mL; normal value, 149–245 ng/mL) were low.

Magnetic resonance imaging (MRI) of the sella turcica showed an isointense, homogeneously enhancing mass within the pituitary (55 × 47 mm in diameter) extending superiorly and involving both cavernous sinuses and carotid arteries. Planar and tomographic (SPECT) imaging revealed abnormal uptake in the hypothalamic and pituitary regions, corresponding with the intra- and extrasellar mass observed on the MRI.
4 and 24 hours after $^{[111]}$In-DTPA$^0$-octreotide (OctreoScan) injection (Fig. 1).

On the basis of this evidence, in the context of the patient’s refusal to undergo surgery, octreotide administration was begun (20 mg octreotide LAR intramuscularly every 28 days). The patient gave informed consent for this medical treatment. Serum levels of FSH and alpha-subunit were evaluated at 8 AM 28 days after each octreotide administration. Serum FSH and alpha-subunits levels, however, were unchanged (mean ± standard deviation: 56.1 ± 2.7 U/L and 6.8 ± 0.4 U/L, respectively). Also, FSH and the alpha-subunit did not change over a 3-month follow-up period: FSH (baseline value: 58.6 U/L; first month: 53.3 U/L; second month: 56.7 U/L; third month: 58.6 U/L) and alpha-subunit levels (baseline value: 7.2 U/L; first month: 6.2 U/L; second month: 7.2 U/L; third month: 6.4 U/L). Tumor size, performed by MRI at the end of the third month of therapy, showed no change. A higher dose of octreotide (30 mg every 28 days) was proposed, but the patient refused to continue the medical treatment, and, a few months later, underwent surgical removal of the pituitary adenoma.

MATERIALS AND METHODS

Immunohistochemistry

A part of the tumor was fixed in 4% formalin, paraffin-embbeded, and used for immunohistochemical studies. Sections of 5 μm were deparaffinized, rehydrated, and heated to 100°C for 15 minutes in citric acid buffer pH 6.0 as described by Gown et al.16 After antigen retrieval, endogenous peroxidase activity was blocked by 3% H2O2 in methanol, and the sections were incubated with specific antibodies overnight at 4°C.

Immunohistochemistry for anterior pituitary hormones and alpha-subunit was performed, separately, with mouse monoclonal antibodies against human FSH, LH, TSH, PRL, GH, and ACTH (Dako, Carpinteria, CA) and monoclonal antibody against alpha-subunit (Abcam, U.K.). Binding was demonstrated with the biotin–streptavidin–peroxidase method (LSAB kit; Dako). The reaction was developed with 3,3’-diaminobenzidine (DAB; Dako). Negative controls included: 1) omission of the primary antiserum, and 2) replacement of the primary antiserum with normal mouse or goat serum. In each of these conditions, no staining was evident. Immunohistochemistry for SSTR subtypes was performed with rabbit polyclonal antibodies against sst2A, sst3A, and sst5 (Biotrend, Cologne, Germany). A standard streptavidin–biotinylated–alkaline phosphatase complex (ABC kit; Biogenex, San Ramon, CA) was used to visualize the bound antibodies and the reaction was developed with NewFuscine/ Naphtol AS-MX. Negative controls included: 1) omission of the primary antibody, and 2) preabsorption of the antibody with the respective immunizing receptor peptide (at a concentration of 100 μM). Tissue was considered positive when immunostaining was abolished by preabsorption of the antibody with the respective peptide antigen. Specimens from thymomas that we had previously tested for specific SSTR subtypes were used as positive controls.17

Reverse Transcriptase–Polymerase Chain Reaction Studies

A part of the tumor was obtained at the operation, frozen on dry ice, and stored at −80°C for reverse transcriptase–polymerase chain reaction (RT-PCR) studies. The details of these experimental procedures are presented elsewhere.18

Briefly, poly A + mRNA was isolated from the tissue sample using Dynabeads Oligo (dT)25 (Dynal AS, Oslo, Norway). Complementary DNA (cDNA) was synthesized using the poly A + mRNA captured on the Dynabeads Oligo (dT)25. One tenth of the cDNA was used for each amplification by PCR using primer sets specific for human sst1–5 and hypoxanthine–guanine phosphoribosyltransferase (HPRT) as a control. The PCR reaction was carried out in a DNA thermal cycler with heated lid (Perkin Elmer Cetus Instruments, Gouda, The Netherlands). Several controls were included in the RT-PCR experiments as previously described.18 To show that no detectable genomic DNA was present in the poly A + mRNA preparation, cDNA reactions were also performed without reserve transcriptase and amplified with each primer pair. To exclude contamination of the PCR reaction mixtures, reactions were performed in the absence of cDNA template in parallel with cDNA samples.

RESULTS

Histology was consistent with a benign chromophobe macroadenoma (Fig. 2A). Immunohistochemical studies for anterior pituitary hormones revealed adenomatous cells immunoreacting with antibodies against common alpha-subunit and FSH (Fig. 2B, C, respectively), but not with those against LH, TSH, PRL, GH, and ACTH (“pure FSHoma”). Positive immunoreaction for FSH and alpha-subunit occurred in approximately 40% of the adenomatous cells, which were isolated or grouped in islets of variable size and dispersed in the tumor tissue. Immunostaining for both proteins was located in the cell cytoplasm and was moderate. These immunohistochemical findings were consistent with the endocrine abnormalities seen in our patient (elevated baseline concentration of FSH, but not of LH, and of alpha-subunit, and absent response of FSH after LHRH). These findings support the evidence that Gn-omas are heterogeneous tumors containing nonsecreting cells dispersed among secreting cells.

On the basis of the positive result of the $^{[111]}$In-DTPA$^0$-octreotide scintigraphy, we evaluated SSTR subtype expression in the tumor cells by immunohistochemistry using specific polyclonal antibodies. Sst2A expression was clearly detected in the adenomatous tissue (Fig. 2E). Sst2A immunoreactivity was diffuse, staining approximately 80% of the adenomatous cells in the plasma membrane and the cytoplasm of the cells. This immunostaining was completely abolished by preabsorption of the antibody with 100 μM of the respective peptide antigen (Fig. 2F). No immunoreactivity for sst3A and sst5 subtypes was detected on the tumor cells.

Immunohistochemical results were confirmed by RT-PCR analysis, which demonstrated a selective expression of the sst2A subtype. Positive signals for sst2A, as well as sst1, mRNA in adenomatous cells were found, whereas sst3, sst4, and sst5 mRNA was undetectable (Fig. 3).
DISCUSSION

Somatostatin receptors have been demonstrated in gonadotroph pituitary adenomas in vitro by various methods including RT-PCR, in situ hybridization, and immunohistochemistry.19–23 A significant proportion of these adenomas also expresses multiple SSTR subtypes (preferentially sst2 and/or sst3 and/or sst5) on the cell membrane. The presence of SSTRs in gonadotroph adenomas was confirmed in vivo by scintigraphic techniques using radiolabeled octreotide.8,13,24 These findings suggest that Gn-omas are potential candidates for therapy with somatostatin analogs. Nevertheless, from the review of literature, the effectiveness of SSAs is still controversial in patients with this type of pituitary tumor. The efficacy of SSAs in inhibiting hormone secretion was evaluated in previous studies both in vivo and in vitro. Vos et al described a significant reduction of serum LH values after acute or chronic octreotide administration in one patient with an LH-secreting adenoma.4 Another patient with a TSH + LH-secreting adenoma showed a decrease of serum hormone levels during octreotide treatment.5 In line with these reports, de Bruin et al used octreotide therapy in some patients with nonfunctioning, gonadotroph adenomas.25 A small but significant reduction in hormone levels (7–17%) was achieved in only 2 of 4 patients treated with a high dose of octreotide, despite the fact that specific SSTR subtypes were demonstrated in all the tumors both in vivo by using111In-octreotide scintigraphy and in vitro by autoradiography of surgical samples. In vitro incubation of the adenomatous cells with octreotide resulted in mild inhibition of gonadotrophin and alpha-subunit release, confirming the in vivo observation of a partial suppressive effect of octreotide on hormonal secretion.25 In another study of 6 patients with Gn-omas, Blanco et al showed a partial inhibition of gonadotrophin secretion by octreotide in 5.6 After acute administration of octreotide, FSH decreased in 2 of the 5 cases (by 38% and 76%, respectively), LH in 3 of them (by 30–56%), and alpha-subunit only in one by 20%. On the other hand, Evrard et al showed that a 3-month octreotide treatment did not reduce FSH concentration in a patient with a silent Gn-oma.26 In 2 series, cell cultures from 26 clinically nonfunctioning, gonadotropin-secreting adenomas were studied.27,28 Somatostatin induced, in vitro, a partial (28–34%) inhibition of FSH, LH, and/or alpha-subunit secretion in less than 50% of these tumors. Moreover, in a small subgroup of adenomas (3 of 10), hormone release was inhibited by native SS but not by the octapeptide analog octreotide.28 More recently, Saveanu et al described a patient with a mixed PRL, LH, and alpha-subunit-secreting adenoma in whom a single administration of octreotide reduced PRL, LH, and alpha-subunit levels by 65%, 65%, and 33%, respectively. Long-term treatment with slow-
release lanreotide achieved only a partial hormone inhibition. This latter case, evaluated in vitro, displayed a hyper-expression of sst2 and sst5 in adenomatous cells as well as a significant suppression of both LH and alpha-subunit secretion after native SS and octreotide administration in culture medium. The effectiveness of SSA appears even less clear when data on tumor shrinkage are considered. Although Sy et al described both the decrease of hormone levels and tumor size of the adenoma, tumor shrinkage (>20%) was reported in only one of 3 adenomas studied by De Bruin et al and in 2 of the 6 adenomas described by Katznelson et al. None of the 4 silent gonadotroph adenomas studied by Plokinger et al became smaller during a 3-month period of octreotide treatment, and both Saveanu and Evrad failed to demonstrate reduction of the tumor mass in their patients. In all cases, surgery remained the main therapeutic approach.

In our patient with an FSH-secreting adenoma, the administration of octreotide LAR, a SS analog that binds with high affinity to sst2, had no effect on hormonal secretion and tumor growth even when the presence of specific SSTRs was demonstrated in vivo by \(^{111}\)In-octreotide scintigraphy. A 3-month treatment did not decrease FSH and/or alpha-subunit plasma levels or reduce the tumor mass, and surgical removal of the adenoma was performed. In vitro studies confirmed the presence of SSTRs on adenomatous cells. Immunohistochemical analysis revealed positive immunostaining for sst2A on numerous tumor cells, whereas no immunoreaction for other SSTR subtypes was demonstrated. The presence of mRNAs encoding for sst2A was demonstrated by RT-PCR in adenomatous tissue, whereas sst3 and sst5 mRNAs were undetectable. Our observation of a selective expression of sst2 subtype differs from most of the gonadotroph adenomas, which express multiple SSTR subtypes on tumor cells. In previous qualitative analyses, Gn-omas were found to coexpress mainly sst2 and sst3 subtypes and, less frequently, sst5. Cell culture studies demonstrated that the administration of native SS achieved higher hormone suppression than that obtained with sst2 or sst3 preferential analogs, suggesting cooperation between these 2 receptor subtypes. This view is also supported by the observation that these receptors may form heterodimers with enhanced functional activity, and the synergistic activation of both receptors could achieve better control of GH secretion in a larger number of acromegalic patients. It is possible that SSTR subtypes other than sst2 may contribute to the antihormonal and antiproliferative effects of SSAs, and the interaction between different SSTR subtypes may enhance the biologic effects of these drugs on tumor tissue. In the light of these considerations, the absence of sst5 and sst3 mRNAs may explain the poor sensitivity to SSA observed in our patient. Another...
possible explanation for this finding is dissociation between receptor binding and postreceptor events. Postreceptor abnormalities, which impair the intracellular transmission of the signal, also might be responsible for the resistance to octreotide. Finally, scintigraphy with $^{111}$In-octreotide appears to be a poor predictor of response. We found a high uptake of $^{111}$In-DTPA$^\text{0}$-octreotide, which is known to be determined predominantly by sst$_2$ expression, but treatment with an sst$_2$ preferential analog was ineffective. This lack of correlation between in vivo receptor visualization and tumor response to SSAs also was observed in other studies, which demonstrate that somatostatin receptor scintigraphy is not always helpful in identifying octreotide-responsive adenomas.

CONCLUSIONS

Data from the literature show that the overall effectiveness of somatostatin and its analogs is unpredictable in gonadotroph adenomas; only a subset of Gn-omas appears to be responsive to SSAs in terms of hormone inhibition and even less in terms of tumor shrinkage. Medical therapy is usually unsatisfactory and surgery remains the main therapeutic option. Our results in an FSH-secreting adenoma confirm that significant changes in hormone levels and/or tumor size may not occur during octreotide therapy in Gn-oma patients. Moreover, a positive In-DTPA-D-Phe$_1$ octreotide scintigram does not predict the therapeutic effectiveness of SSAs in these patients and the in vitro demonstration of somatostatin receptor expression does not correlate with the in vivo response to SSAs.

REFERENCES


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