CLINICAL STUDY

A luteinizing hormone-, alpha-subunit- and prolactin-secreting pituitary adenoma responsive to somatostatin analogs: in vivo and in vitro studies

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Abstract


Design: A 30-year-old woman, with amenorrhea-galactorrhea, presented with a pituitary macroadenoma. The endocrine evaluation showed high plasma levels of PRL, LH, and alpha-subunit inhibited by 65%, 65% and 33% respectively under octreotide test (200 μg, s.c.). Long-term treatment with slow release (SR) lanreotide (30 mg/10 days, i.m.) restored menstrual cycles and normalized PRL values. Due to persisting supranormal levels of LH and alpha-subunit, and to the absence of tumoral shrinkage, the adenoma was resected by the transsphenoidal route.

Methods: In vitro characterization of the somatostatin receptor subtypes (SSTR) expression and functionality. Real-time polymerase chain reaction was performed to quantify the expression of SSTR mRNAs and functionality of the SSTRs was assessed in cell culture studies with various concentrations of native somatostatin (SRIF-14) and of analogues preferential for SSTR2 or SSTR5.

Results: This adenoma presented with high levels of SSTR2, SSTR3 and SSTR5 mRNAs, as compared with a series of gonadotroph adenomas. In cell culture studies, PRL, LH and alpha-subunit were inhibited by 60%, 47% and 33% respectively by SRIF-14 at a concentration of 10 nmol/l. The SSTR2 (BIM-23197, lanreotide) and SSTR5 (BIM-23268) preferential analogues both produced a partial 21–38% inhibition of PRL, LH, and alpha-subunit release.

Discussion: In this plurihormonal-secreting adenoma, the high efficacy of somatostatin analogues to inhibit PRL, LH and alpha-subunit secretion in vivo may be explained by the unusually high level of expression and by the functionality of both SSTR2 and SSTR5 receptor subtypes.

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Introduction

In addition to its known suppressive effects upon growth hormone (GH) and thyrotrophin (TSH) secretion, there is some evidence that somatostatin may regulate gonadotrophin release. Such an inhibitory effect is only partial as a 30% luteinizing hormone (LH) suppression by somatostatin was found in normal men (1). In gonadotroph pituitary tumours, somatostatin receptors were identified on the tumour cell membranes (2, 3). Indeed, such findings led to the study of the effect of long-term octreotide therapy in patients with pituitary tumours which released luteinizing (LH) and follicle-stimulating (FSH) hormones and/or free alpha-subunit. If octreotide was poorly effective upon tumour shrinkage, a significant reduction in serum FSH concentrations could be found in two out of four patients under long-term octreotide administration (4). More recently, it was shown by cell culture studies that somatostatin and its analogue, lanreotide, inhibited the proliferation of dispersed human non-functioning pituitary cells (5), which, in their majority, do synthesize LH, FSH or the alpha subunit (6–8). Such data, even if limited, indicated a possible inhibitory effect of somatostatin or its analogues in some gonadotroph tumours.

We report, in a women presenting with a pituitary macroadenoma co-secreting LH, alpha-subunit and PRL, the efficacy of the somatostatin agonist lanreotide in the control of hormonal hypersecretion. Subsequently, the patient underwent a selective surgical removal of the tumour. In this tumour, the quantitative analysis
of the different somatostatin receptor subtypes (SSTRs) as well as the functional responses obtained in cell culture with somatostatin analogues preferential for SSTR2 or SSTR5, may explain why only some, among the gonadotroph adenomas, may be responsive to somatostatin agonists.

Patient and Methods

Case report

A 30-year-old woman presented with amenorrhea and galactorrhea of 6 months duration. Prior to these symptoms, she presented with regular menses but with couple infertility for 3 years. A pituitary magnetic resonance imaging (MRI) scan found an intrasellar macroadenoma (maximal diameter 19 × 21 mm). Visual fields assessed by Goldman perimetry were normal. The basal plasma PRL value was 60 μg/l associated with supranormal plasma values of LH (30 U/l) and of α-subunit (7.2 U/l), contrasting with normal FSH plasma levels (7 U/l). Such an unusual pattern of PRL, LH, and α-subunit hypersecretion was not associated with TSH or GH hypersecretion. Estradiol and free testosterone plasma values were respectively 26 ng/l and 37 pmol/l. Insulin-like growth factor-I (IGF-I) levels were normal (173 μg/l) as were the free triiodothyronine (6 pmol/l), free thyroxine (15 pmol/l) and the 24-h urinary free cortisol (249 nmol/24 h) values.

The pharmacological evaluation was performed after stimulation by thyrotrophin releasing hormone (TRH; 200 μg, i.v.) or by gonadotrophin releasing hormone (GnRH; 100 μg, i.v). The plasma LH, FSH, α-subunit, and PRL values were measured before each test and every 30 min for 2 h after TRH or GnRH. Similarly, the effects of the somatostatin analogue, octreotide (Sandostatin, Novartis, Basel, Switzerland; 200 μg, s.c.) and of the dopamine agonist, quinagolide (Norprior, Novartis; 150 μg, oral) were tested on the inhibition of plasma levels of LH, FSH, α-subunit and PRL. Each test was performed on separate days. Under GnRH acute testing, the FSH, LH, and α-subunit plasma levels rose by 266, 279 and 42% respectively. A 112, 73 and 71% rise of PRL, LH and α-subunit values was also observed under acute TRH testing, while FSH values were unchanged. The dopamine agonist, quinagolide, produced only a slight (26% and 17%) maximal inhibition of plasma PRL and α-subunit and did not modify the LH or FSH plasma levels (Fig. 1, FSH not shown). In contrast, 2 to 4 h after octreotide injection, the plasma levels of PRL and LH decreased by 65% and those of α-subunit by 33% (Fig. 1). Such results, showing an overall much better inhibition of hormonal hypersecretion by octreotide than by quinagolide, led to the proposal of long-term treatment with a somatostatin analogue in order to further assess the efficacy of such a drug upon the control of hormone hypersecretion and upon the tumoral mass.

Long-term treatment with somatostatin analogue

The patient received an intramuscular injection of SR-Lanreotide 30 mg (Ipsen, Paris, France) repeated every 10 days for 3 months. The plasma levels of PRL, LH and α-subunit were measured hourly during the 6 h following the first injection and similarly each month before the next injection. The tumour mass was evaluated by MRI performed after 1 and 3 months of treatment. As shown in Fig. 2, during the 6 h following
the first SR-Lanreotide injection, the plasma values of LH and PRL decreased by 80 and 84% respectively, while the α-subunit levels were only reduced by 54%.

At the third month of treatment, the mean PRL value was in the normal range (15 ± 2 μg/l), in accordance with the resumption of ovulatory cycles 48 days after the start of the treatment. Meanwhile, the LH and α-subunit plasma levels (10 ± 1 U/l and 3.3 ± 0.4 U/l) remained above the normal range. The MRI examination, at 3 months, did not find any significant tumour shrinkage (−13% decrease of the maximal diameter). Subsequently, the patient was operated on by the transphenoidal approach. On day 8 post surgery, plasma PRL, LH, and α-subunit values were 5 mg/l, 1.9 U/l and 0.4 U/l respectively, and remained in these normal ranges in accord with the persistence of ovulatory cycles and with the occurrence of pregnancy 5 months after surgery.

**In vitro studies**

**Quantification of somatostatin receptor subtypes**

Part of the tumour was used for RNA extraction and purification using the guanidium isothiocyanate/phenol method followed by deoxyribonuclease I treatment, as previously published (9). The 5′ exonuclease (Taq Man; Perkin Elmer Biosystems, Courtaboeuf, France) assay, which allows a direct proportional readout for the progression of PCR reactions, was used to quantify the 5 SSTRs mRNAs. The details of these experimental procedures have been presented elsewhere (10). For quantification of data, SSTR mRNA levels were, in the same reaction, normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and expressed as picograms SSTR per picogram GAPDH. Such an SSTR mRNA quantification was performed on 30–60 mg tissues from the mixed PRL–LH–α-subunit adenoma as well as on tissue from another 8 gonadotroph non-secreting pituitary macroadenomas previously collected and stored in liquid nitrogen. In all these tumour fragments, RT-PCR with specific hormonal probes (9) confirmed the presence of the transcripts for β-LH, β-FSH or α-subunit as well as the absence of pro-opiomelanocortin (POMC), and GH mRNA expression. These data, confirming the gonadotroph origin of such tumours, are summarized in Table 1. In patients G1 and G2, PRL hypersecretion was of tumoral origin (PRL mRNA found in the tumour), while for G4, G7 and G8 PRL hypersecretion was interpreted as resulting from stalk compression.

**Cell culture studies**

A part of the tumour, obtained after surgery, was dissociated by mechanical and enzymatic methods. The 4 × 10⁶ dissociated cells obtained were plated in 24-multiwell culture dishes (3 × 10⁴ cells/well) coated with extracellular matrix from bovine endothelial corneal cells, as previously described (11). Hormone release was assessed after a 24-h incubation period on day 1. On day 4 of culture, the effects of various concentrations of quinagolide, native somatostatin (SRIF-14; Sigma Chemical Company, Saint-Quentin Fallavier, France), lanreotide and the previously shown (12) preferential analogues to SSTR2, BIM-23197, and SSTR5, BIM-23268 (Biomeasure Inc., Milford, MA, USA), on PRL, LH and α-subunit secretion were measured after an 8-h period of incubation with these drugs. Each concentration of the drugs was tested in 4 different culture wells.

**Results**

**SSTRs mRNA quantification**

The quantification of the 5 SSTR mRNAs in the patient’s tumour (G1, Fig. 3A) showed high expression of 3 SSTR subtypes: SSTR2 mRNA (327 pg/pg GAPDH), SSTR3 mRNA (950 pg/pg GAPDH) and SSTR5 mRNA (1396 pg/pg GAPDH). When the same analysis was performed in a series of 8 gonadotroph adenomas, a constant pattern of SSTR2 and SSTR3 mRNA expression was observed. The mean SSTR3

<table>
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<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>LH (U/l)</th>
<th>FSH (U/l)</th>
<th>αSU (U/l)</th>
<th>PRL (μg/l)</th>
<th>LH</th>
<th>FSH</th>
<th>αSU</th>
<th>PRL</th>
<th>GH</th>
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<tr>
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<td>22</td>
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<td>6</td>
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<td>+</td>
<td>+</td>
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αSU, alpha subunit.

**Table 1** Clinical characterization and hormonal status in vivo and in vitro (mRNA) of G1 and of eight other gonadotroph adenomas (G2-G9).
subtype expression (1413 ± 334 pg/pg GAPDH) was 12 times higher than that of SSTR2 mRNA (112 ± 35 pg/pg GAPDH). In most of these gonadotroph tumours (Fig. 3B), the SSTR5 subtype was not found. Only one other tumour (G2), which also coexpressed PRL mRNA with LH, FSH and α-subunit mRNAs (Table 1), presented with a high level of SSTR5 mRNA expression. Our mixed gonadotroph–PRL secreting adenoma (G1), as compared with the other gonadotroph tumours, presented with the highest expression of both SSTR2 and SSTR5 mRNAs.

**Cell culture studies**

The basal level of hormone release into the culture medium by 1×10⁶ cells during 24 h (day 1) was 660 ng for PRL, 165 mUI for LH, and 100 mUI for α-subunit. FSH, TSH and GH were not detected in the culture medium. These data confirmed the multi-secreting features of such a tumour as observed in vivo.

**Effects of SRIF-14 and somatostatin analogues**

In this series of experiments, the dose–response inhibition of LH, α-subunit and PRL release was examined with 10⁻¹² to 10⁻⁸ mol/l concentrations of SRIF-14, lanreotide, BIM-23197 and BIM-23268. As shown in Fig. 4, SRIF-14 produced a 60 ± 2%, 47 ± 3% and 33 ± 2% maximal suppression of PRL, LH and α-subunit respectively, at a concentration of 10 nmol/l. The half-inhibitory effects (EC₅₀) were achieved at 100 pmol/l for the three hormones. The SRIF-14-induced suppression of PRL and LH was greater than that of α-subunit. Such a partial suppressive effect upon α-subunit secretion had already been observed in vivo, following acute testing with octreotide. As compared with the results obtained in the presence of various concentrations of SRIF-14, the dose–response inhibition of PRL, LH and α-subunit was only partial in the presence of lanreotide, BIM-23197 and BIM-23268. These drugs produced, at nanomolar concentrations, a maximal LH, α-subunit and PRL suppression ranging from 21 to 38%. The EC₅₀ varied from 30 to 100 pmol/l, according to each compound. These data indicated that both SSTR2- (Lanreotide, BIM-23197) and SSTR5 (BIM-23268)-preferential analogues were able to suppress the hormone release. Nevertheless these SSTR2- or SSTR5-preferential analogues produced only a partial hormonal suppression as compared with that achieved by SRIF-14.
Effects of the dopamine agonist, quinagolide
Quinagolide, at the concentrations usually effective in inhibiting PRL release in prolactinomas (EC50 = 30 pmol/l (10)), was ineffective in the inhibition of α-subunit, LH and PRL release. Only at high quinagolide concentrations (1 to 10 nmol/l) was a 17–30% suppression of α-subunit, LH and PRL achieved (data not shown). Such results confirmed the poor sensitivity to the dopamine agonist in this tumour, as already observed in vivo.

Discussion
This report presents an unusual case of a multi-hormonal gonado-lactotroph secreting adenoma resistant to dopamine agonist and sensitive to somatostatin analogues in terms of inhibition of hormonal release. At least two individual cases of pituitary adenomas also cosecreting PRL and gonadotrophins have been reported in the literature (13, 14). One of these cases (14) presented as precocious puberty in a seven-year-old boy with high plasma levels of LH, FSH and PRL, which were not modified by octreotide. Another patient with an LH+α-subunit secreting adenoma (15) showed a 57 to 70% inhibition of high plasma levels of LH and α-subunit under octreotide treatment. This patient, as in our observation, was resistant to acute and long-term bromocriptine administration. Our observation differs from most of the gonadotroph adenomas, characterized by immunohistochemical methods, which behave, in vivo, as non-functioning pituitary adenomas in the majority of cases. In fact, cell culture studies of gonadotroph adenomas have found, in most of the cases, the secretion of small amounts of FSH, LH and/or α-subunit (6–8). In these cell culture studies, an associated gonadotrophin and PRL co-secretion has also been reported in 8/99 cases (7).

The overall efficacy of somatostatin and its analogues is very partial when evaluated in gonadotroph adenomas. In three previous series, 29 gonadotroph adenomas were studied in cell culture (4, 16, 17). Somatostatin produced, in vitro, a partial (28–34%) inhibition of FSH, LH or α-subunit in less than 28% of these tumours. Such a score parallels the in vivo observations of a partial suppressive effect of octreotide upon gonadotrophin plasma levels in such patients. In one study concerning patients with α-subunit adenomas, a 27–35% reduction in α-subunit plasma levels was achieved in 2/6 patients treated with octreotide s.c. (200 µg/day) for 2 months (18). Increasing daily doses of octreotide up to 1200 µg/day suppressed FSH plasma levels by only 7–17% in 2/4 patients with gonadotroph adenomas (4). The high suppressive effect of octreotide or lanreotide in our observation can be discussed in the light of our quantitative analysis of the somatostatin receptors in the tumour. In previous qualitative analyses, the non-functioning and gonado-troph adenomas were found to express mainly SSTR2 (63%) and SSTR3 (69%) mRNAs (5, 19–22). This is confirmed in all the tumours from our series, which constantly co-expressed SSTR2 and SSTR3 mRNA. The much higher relative expression of SSTR3 mRNA has already been stressed (22), but its functional significance is not yet explained. In our series, the level of SSTR2 mRNA expression was highly variable according to the tumours. The highest SSTR2 mRNA expression was found in tumour G1 and can explain the efficacy of octreotide and lanreotide in our observation, while both analogues are preferential mainly for SSTR2. In this adenoma, the high level of SSTR5 mRNA is also an unusual feature. The SSTR5 subtype has been found in only 10/35 of the non-functioning pituitary adenomas in previous studies (5, 22). In our mixed tumour, its presence is not surprising, while SSTR5 has been found highly expressed in PRL-secreting adenomas (10, 12). Such a SSTR5 receptor is functional while, in our culture study, BIM-23268, a highly preferential agonist for SSTR5, was able to produce a dose-dependent inhibition of PRL, as well as of LH and of α-subunit secretion. Interestingly, the native peptide, SRIF-14, achieved, in our case, significantly higher hormone suppression than that obtained with analogues highly preferential for SSTR5 or SSTR2. These data suggest cooperation between these two receptor subtypes, possibly by heterodimerization, as has been demonstrated using cloned human receptors (23), and already suggested in some GH-secreting pituitary adenomas (24).

If, from the data in the literature, a subset of gonadotroph adenomas is partially sensitive to somatostatin analogues in terms of hormone inhibition, the octreotide efficacy upon tumour shrinkage is less clear. Among 48 patients with non-functioning adenomas and gonadotrophinomas (25, 26), a significant (>20%) tumour shrinkage was documented in only 7 cases (15%). The SSTR scintigraphy using In111-pentreotide was of poor predictive value in one study (25). A possible explanation of this latter finding is that In111-pentreotide presents with binding affinities towards the SSTR subtypes different from those of octreotide. In particular, the binding affinity ratio SSTR3/SSTR2 was markedly higher for In111-pentreotide than for octreotide (27).

In conclusion, even if unusual, some gonadotroph tumours can be sensitive to the somatostatin analogues, which inhibit the plasma levels of LH or α-subunit, as illustrated in our observation. Indeed, in the first instance, surgery remains the main therapeutic option. Nevertheless, in such macroadenomas, surgery is often partial and a subsequent therapeutic option has to be proposed in the presence of tumour remnants. In such a situation, the hormonal suppressive effects of somatostatin (or of dopamine) analogues can be checked. If such tumours prove sensitive to such drugs (28), long-term treatment could be proposed for...
the suppression of hormonal release and could eventually prevent further tumoral growth.

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