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CLINICAL MANAGEMENT OF ENDOCRINE DISEASES

Treatment of multiresistant prolactinomas with a combination of cabergoline and octreotide LAR

Ernesto Sosa-Eroza[1] · Etual Espinosa[1] · Claudia Ramírez-Rentería[1] · Victoria Mendoza[1] · Rocío Arreola[2] · Moises Mercado[1]

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Abstract

Background and objectives Dopamine agonist (DA)-resistant prolactinomas are rare but they constitute a real challenge, since there are few therapeutic alternatives left for these patients.

Design and setting Proof-of-concept study at a tertiary care, referral center.

Patients and methods The studied population consisted of five patients (one female and four males, mean age at diagnosis 23.5 ± 19) with macroprolactinomas with persistent hyperprolactinemia and/or tumor mass despite high doses of cabergoline (CBG) and pituitary surgery, to whom 20 mg monthly of octreotide LAR was added for 6–13 months. Response was evaluated by measuring prolactin (PRL) levels and by magnetic resonance imaging. Immunohistochemistry (IHC) for pituitary hormones, Ki-67, and somatostatin receptor subtypes 2 and 5 was (SSTR2 and 5) was available in two of the subjects.

Results The addition of octreotide LAR to ongoing CBG treatment had no effect on either PRL levels or tumor size in three patients. In two of the five patients, combination treatment resulted in a significant reduction in PRL concentrations (from 7643 to 200 ng/mL and from 2587 to 470 ng/mL) as well as in adenoma size (93% reduction). IHC evaluation of tumor samples from two patients (a responder and a non-responder) revealed positive immunostaining for PRL and SSTR5 but not for other pituitary hormones or for SSTR2.

Conclusions The addition of a somatostatin analog to ongoing CBG treatment may be effective in some patients with DA-resistant macroprolactinomas, independently of the adenoma's SSTR expression profile.

Keywords Macroprolactinoma · dopamine agonist · cabergoline · octreotide · somatostatin receptors

Introduction

Since the recognition over 30 years ago that hypothalamic dopamine is the main negative regulator of prolactin (PRL) secretion by the pituitary lactotroph, the treatment paradigm for these tumors shifted from surgical to pharmacological [1, 2]. Thus, nowadays dopamine agonists (DA) are the mainstay of treatment for prolactinomas [1]. The efficacy of ergot-derived compounds such as bromocriptine was proved early on not only in terms of their ability to normalize serum PRL concentrations but also in terms of their tumor-shrinking capacity [2–4]. The DA most frequently used worldwide is cabergoline (CBG), which is very well tolerated and reasonably priced and can be administered from 1 to 3 times a week [1, 5]. CBG normalizes serum PRL levels and significantly reduces tumor size in over 85% of patients with microprolactinomas and intraselar macroadenomas [6, 7]. Response rates for macroadenomas vary between 50 and 75%, depending on the size and invasiveness of the tumor [6, 7].

Resistance to DA occurs in approximately 20–30% of macroprolactinomas and is thought to be due to a reduced expression of type 2 dopamine receptors (D2R) [8, 9]. In these cases, other treatment alternatives such as surgical debulking and radiation therapy are indicated, and although

✉ Moises Mercado
moises.mercado@endocrinologia.org.mx
mmercadoa@yahoo.com

[1] Endocrinology Service and the Experimental Endocrinology Unit, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, MX, USA

[2] Pathology Department, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, MX, USA

they frequently succeed in eliminating compressive symptoms, they seldom normalize PRL or result in restoration of gonadal function [1, 8, 10–12]. In some of these invasive lesions the alkylating agent temozolomide has been tried, again, without much success [12, 13]. PRL-secreting tumors frequently express somatostatin receptors, particularly SSTR5 and SSTR1 and to a lesser extent SSTR2 [14, 15], yet somatostatin analogs have not been formally used in the treatment of DA-resistant prolactinomas. The present report constitutes a proof-of-concept study regarding the potential beneficial effect of octreotide LAR in the treatment of multiresistant macroprolactinomas.

Patients and methods

The studied population consisted of five patients with macroprolactinomas with persistent hyperprolactinemia and/or tumor mass despite high doses of CBG and pituitary surgery, to whom 20 mg monthly of octreotide LAR was added for 6–13 months. Treatment outcome was assessed by both PRL measurements and magnetic resonance imaging (MRI) at baseline and 6–13 months after combined treatment with CBG and octreotide. All patients are followed at the Prolactinoma Clinic of Hospital de Especialidades CMN SXXI, IMSS, whereby, upon enrollment, they sign an informed consent. The study protocol was approved by our local scientific and ethics committees.

Hormonal assays

PRL levels were measured by an electrochemiluminescence immunoassay "ECLIA" (ROCHE Diagnostics, IN, USA), calibrated with the IRP WHO 3rd 84/500, with a detection limit of 0.047 ng/mL, and intra- and inter-assay coefficients of variation of 4% and 5%, respectively. When suspected, the hook effect was ruled out by performing the assay in 1:100 diluted serum samples. Macroprolactinemia was investigated and ruled out in all cases by means of polyethylene-glycol precipitation, as previously described [16]. The remaining hormones (cortisol, free T4, tyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, total testosterone, and insulin-like growth factor-1) were measured by a myriad of commercially available immunoassays.

Magnetic resonance imaging

All patients underwent MRI immediately before and 6–13 months after the addition of octreotide LAR. Tumor volume was calculated by means of the OsiriX DICOM viewer software (Geneva University Hospital) [17].

Immunohistochemistry

Immunohistochemistry was carried out on formalin-fixed, paraffin-embedded tumor sections from patients 1 and 3. After the samples were deparaffnized and rehydrated, heat-induced epitope retrieval was performed in sodium-citrate buffer (0.01 M, pH 6.0). Tumor samples were then incubated for variable periods with primary antibodies against GH (GeneTex GTX34768, Irvin, CA, USA); PRL (GeneTex GTX83800, Irvin, CA, USA); LH (GeneTex GTX34807, Irvin, CA, USA); FSH (BioCare Medical, Pacheco, CA, USA); TSH (BioCare Medical, Pacheco, CA, USA); Ki-67 (Dako, Carpinteria, CA, USA); adrenocorticotropic hormone (GeneTex GTX 41003, Irvin, CA, USA); SSTR2 [UMB1] (Abcam, Cambridge, UK); and SSTR5 [UMB4] (Abcam, Cambridge, UK). The detection system used was MACH 1 Universal HRP-Polymer kit (BioCare Medical, M1U539L10, Pacheco, CA, USA). The samples were revealed with diaminobenzidine. Appropriate positive and negative controls were used in each case; all samples were interpreted by the same pathologist (R.A.).

Results

Table 1 depicts the patient's baseline characteristics. All except one were males and the mean age at diagnosis and

Table 1 Clinical, biochemical, and imaging characteristics upon diagnosis

Case/sex	Age at Dx/current age	Prolactin, ng/mL	Tumor diameter Dx (AP × CC × T)[a]	Cabergoline dose, mg/week	Other treatments
(1) M	10/27	11 024	64 × 94 × 56	4.5	TCS × 2, RT TMZ, Tamox
(2) F	16/24	1007	12 × 14 × 11	3	—
(3) M	26/28	23 683	77 × 47 × 64	3	TSS
(4) M	26/36	4896	48 × 39 × 35	4.5	TSS
(5) M	37/53	1326	20 × 20 × 42	7.5	TSS

Dx diagnosis, *TCS* transcranial surgery, *TMZ* temozolomide, *Tamox* tamoxifen, *RT* radiation therapy, *AP* anteroposterior, *CC* cephalocaudal, *T* transverse

[a]Diameter expressed in mm

upon their last visit to clinic was 23.5 ± 19 and 40 ± 18.3 years, respectively. They all harbored macroprolactinomas with a median maximum tumor diameter of 61 mm (range 14–94) and a median serum PRL at diagnosis of 6174.5 ng/mL (1007–11 024). The median maximum weekly CBG dose was 6 mg (range 3–7.5).

Except for case 2, all patients had undergone one or more pituitary surgeries in an attempt to reduce both, tumor mass and serum PRL levels. Case 1, a patient with a giant prolactinoma, received a cycle of temozolomide after two unsuccessful transcranial surgeries; he was also treated with tamoxifen and was subjected to external radiotherapy, again, without success. Cases 3, 4, and 5 underwent transsphenoidal surgery without any significant reduction in tumor mass or PRL levels. Case 2, with an intrasellar macroprolactinoma and no compressive symptoms, had persistently elevated serum PRL levels despite several months of treatment with CBG, 3 mg weekly; she did not tolerate further increments in the DA dose and declined surgical treatment.

Whereas in cases 1, 2, and 5 combination treatment with CBG and octreotide LAR for 6–12 months had no effect on either tumor size or PRL levels, remarkable responses were found in cases 3 and 4 (Table 2; Fig. 1). In patient 3, serum PRL decreased by 97% (from 7643 to 300 ng/mL) and tumor volume was reduced by 93% (from 23.222 to 1.604 cm^3), whereas in case 4 serum PRL dropped by 81% (from 2587 to 470 ng/mL) and tumor mass decreased by 93.6% (from 25.363 to 1.616 cm^3; Table 2; Fig. 1).

Immunohistochemical analysis could be carried out in patients 3 (a responder) and 1 (a non-responder); tumor tissue was not available in the other three as they were operated at a different institution. Both tumors strongly immunostained for PRL but were negative for all other pituitary hormones. The Ki-67 index was 2% and 15% in adenoma tissue from the responder and non-responder patient, respectively. SSTR2 immunostaining was negative in both tumors. SSTR5 immunostaining was positive in 50% of the cells of the tumor from the responding patient and in 90% of the cells of the non-responding patient (Fig. 2).

Discussion

DA are the mainstay of treatment for both micro- and macroprolactinomas [1]. Even giant prolactinomas (defined by a PRL level above 1000 ng/mL and maximal tumor diameter >4 cm) respond quite well to CBG treatment, usually at doses below 2.50 ng/week [18, 19]. However, some patients with macroprolactinomas are either resistant or intolerant to treatment with DA [8, 9]. Surgical debulking of the pituitary adenoma, in most instances via the transsphenoidal approach, is capable of restoring DA sensitivity in approximately two-thirds of these patients [12]. Thus, a third of these patients persist with significantly elevated PRL levels and considerable tumor remnants, despite high doses of CBG and surgery. Few therapeutic options remain for these patients, including the palliative use of tamoxifen and aromatase inhibitors such as anastrazole, or even treatment with the alkylating agent temozolomide, without neither being particularly effective [13]. The present study demonstrates that adding a depot somatostatin analog such as octreotide LAR to ongoing treatment with CBG in these patients has a reasonable chance of achieving significant reductions in both, tumor mass and PRL levels.

That two out of our five patients with DA-resistant prolactinomas, responded so dramatically to combination therapy with CBG and octreotide, with significant reductions in both PRL levels and tumor volume, underscores the complex biology of somatostatin receptors. Few studies have evaluated the expression of SSTRs in prolactinomas precisely because very few of these patients end up requiring surgical treatment and thus, there is usually no tumor available for analysis. We were able to evaluate the expression of SSTR2 and SSTR5 by immunohistochemistry in two patients, a responder and a non-responder to combination treatment with CBG and octreotide, and found that neither of them expressed SSTR2, whereas both expressed SSTR5. This SSTR expression profile is consistent with the findings of a large-scale study that evaluated the expression by immunohistochemistry of all somatostatin receptor subtypes in a tissue microarray of 90 pituitary adenomas, 30 of which were prolactinomas [14]. Fusco et al. [20] reported the case of a

Table 2 Prolactin and tumor diameters before and after the addition of octreotide LAR to ongoing CBG treatment

Case	Duration of treatment, months	Prolactin, ng/mL		Tumor volume, mm^{3} [17]		
		Pre-octreotide	Post-octreotide	Pre-octreotide	Post-octreotide	% Change
(1) M	12	9564	9858	64.862	59.025	−9
(2) F	13	391	395	5.329	5.021	−5
(3) M	**10**	**7643**	**200**	**23.222**	**1.604**	**−93**
(4) M	**10**	**2587**	**470**	**25.365**	**1.616**	**−93.6**
(5) M	6	581	610	3.489	3.121	−10

Values are depicted in bold to highlight to distinguish these two responding cases from the rest, nonresponding cases

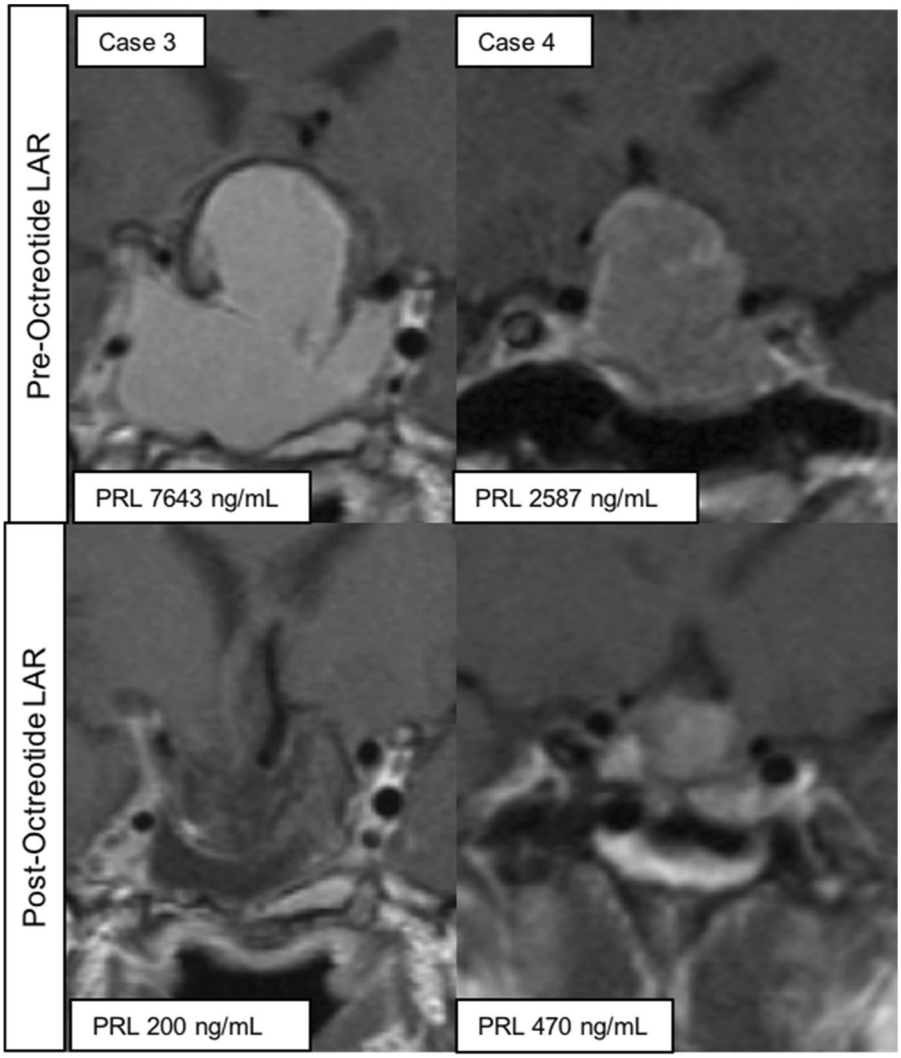

Fig. 1 T1-weighted, coronal MRI before and after the addition of octreotide LAR to ongoing CBG treatment in the two responding patients

15-year-old with an invasive macroptolactinoma resistant to CBG monotherapy in whom, similar to our responding cases, the addition of octreotide LAR resulted in the normalization of PRL levels and stabilization of tumor mass. Also like in our responding cases, tumor tissue from this patient positively immunostained for SSTR5 but not for SSTR2 [20]. Octreotide is a first-generation somatostatin analog with significantly greater affinity for SSTR2 than for SSTR5 [21]. Although in most GH-secreting adenomas long-term response to octreotide or lanreotide depends on the expression of SSTR2 [22], there are cases of responding patients whose tumors do not express this receptor subtype as well as of non-responding patients whose tumors express vast quantities [23]. Thus, it appears that response to somatostatin analogs not only depends on the mere expression of somatostatin receptors but on other, less well-understood aspects of their biology, such as receptor homo- and heterodimerization and recycling.

In a landmark study published almost 20 years ago, Jaquet and his collaborators evaluated the SSTR mRNA expression of 10 prolactinomas, including 3 that were considered resistant to DA [24]. SSTR5 was the dominant receptor subtype expressed in 7 of these 10 tumors, followed by SSTR1. Two of their 3 DA-resistant tumors did express SSTR5 and SSTR2 mRNA, albeit at lower levels than the DA-sensitive tumors, whereas in 1 of these lesions that showed a very aggressive biological behavior virtually no SSTR transcripts could be found [24]. PRL release inhibition was evaluated in primary cell cultures obtained from these 10 prolactinomas and whereas co-incubation with native somatostatin resulted in a mean PRL release inhibition of 52%, co-incubation with octreotide produced a mean inhibition of only 12% [24]. Thus, it seems that SSTR5-preferential somatostatin analogs are more capable of inhibiting PRL release by prolactinomas in vitro than the currently in use SSTR2 preferential analogs such as octreotide or lanreotide. We can speculate that heterodimerization of SSTR5 with D2R may result, under certain circumstances, in the inhibition of PRL secretion pathways. Alternatively, octreotide signaling through SSTR5 in the

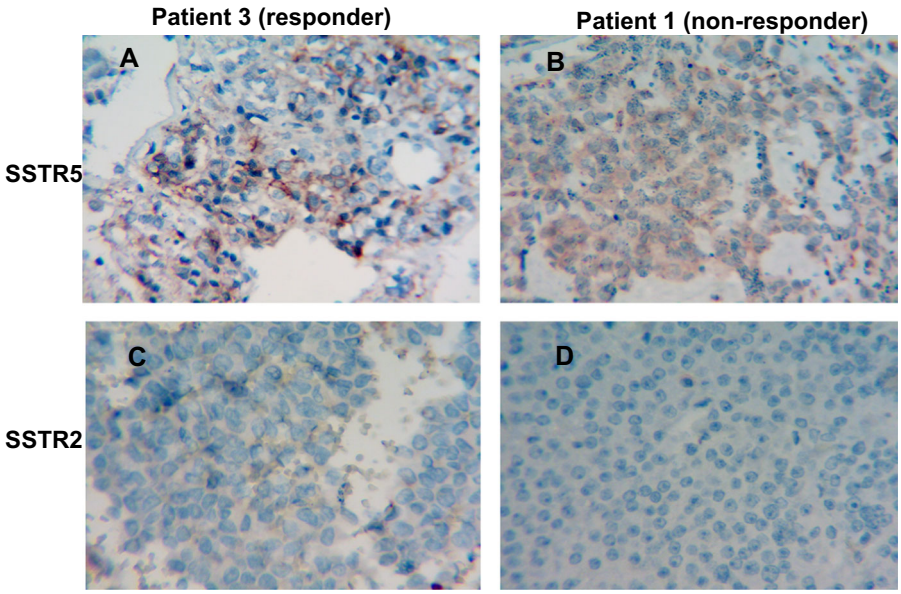

Fig. 2 Immunohistochemistry for SSTR2 and SSTR5 (×40). **a** Positive SSTR5 immunostaining in tumor from responding patient 3 in 50% of cells. **b** Positive SSTR5 immunostaining in tumor from non-responding patient 1 in 90% of cells. **c**, **d** Negative SSTR2 immunostaining in tumors from patients 4 and 1

tumoral lactotrope may restore sensitivity to DA and/or result by itself in PRL secretion inhibition. Native somatostatin administered by intravenous infusion has been shown to inhibit PRL secretion in vivo in patients with prolactinomas, however, there are no equivalent studies using octreotide [25]. Pasireotide, a new-generation somatostatin analog with high affinity for SSTR5 [21] has been shown to inhibit PRL release from prolactinomas in vitro [15], however it has not been tried clinically in patients with DA-resistant lesions.

We conclude that in some patients with DA-resistant macroprolactinomas, the addition of a long-acting somatostatin analog to ongoing CBG therapy may result in significant reductions in PRL levels and tumor volume. Since SSTR expression profiling of these tumors by immunohistochemistry is of little help in identifying potentially responding cases, we believe a therapeutic trial is worth trying in these patients, who otherwise have very few treatment alternatives.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All five patients signed the corresponding informed consent.

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