CLINICAL STUDY

Somatostatinergic ligands in dopamine-sensitive and -resistant prolactinomas

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Abstract

Objective: Ten percent of patients with prolactinoma fail to respond with normalization of prolactin (PRL) and tumor shrinkage under dopamine agonist (DA) therapy. The resistance to treatment is linked to a loss of dopamine receptor 2 (D2DR). Prolactinomas express somatostatin (SST) receptor subtypes, SSTR1, 2, and 5. The aim of this study was to determine whether different SST compounds could overcome the resistance to DA in prolactinomas.

Design and methods: The efficacy of SSTR1, SSTR2, and SSTR5 ligands; the universal SST ligand, SOM230; and the chimeric SST-DA compound, BIM-23A760, was compared with cabergoline in suppressing PRL secretion from primary cultures of ten prolactinomas (six DA responders and four DA resistant). Receptor mRNAs were assessed by quantitative PCR.

Results: The mean mRNA levels for D2DR, SSTR1, SSTR2, and SSTR5 were 92.3 ± 47.3 , 2.2 ± 1.4 , 1.1 ± 0.7 , and 1.6 ± 0.6 copy/copy β -glucuronidase (β -Gus) respectively. The SSTR1 agonist, BIM-23926, did not suppress PRL in prolactinomas. In a DA-resistant prolactinoma, it did not inhibit [³H]thymidine incorporation. The SSTR5 compound, BIM-23206, produced a dose-dependent inhibition of PRL release similar to that of cabergoline in three DA-sensitive prolactinomas. BIM-23A760 produced a maximal PRL inhibition superimposable to that obtained with cabergoline with a lower EC₅₀ (0.5 ± 0.1 vs 2.5 ± 1.5 pmol/l). In DA-resistant prolactinomas, BIM-23206 and SOM230 were ineffective. Cabergoline and BIM-23A760 produced a partial inhibition of PRL secretion (19 ± 6 and $21 \pm 3\%$ respectively).

Conclusion: Although the SSTRs are expressed in prolactinomas, the somatostatinergic ligands analyzed do not appear to be highly effective in suppressing PRL. D2DR remains the primary target for effective treatment of prolactinomas.

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Introduction

Dopamine agonists represent the treatment of choice in prolactin (PRL)-secreting pituitary adenomas (1). They control PRL secretion and cell proliferation by interacting with the dopamine D2 receptor that subsequently activates various transduction pathways (2). D2DR agonists are efficient in the majority of cases, but 10–15% of patients with prolactinomas fail to obtain PRL normalization and reduction in tumor size even with the most potent dopamine agonist, cabergoline (3, 4). These prolactinomas, resistant to dopamine agonists, are usually large and/or invasive, thus surgery cannot be a complete curative treatment. In such tumors poorly or partially responsive to dopamine agonists, an alternative medical treatment should be of real importance. Experimental data have demonstrated that different somatostatin receptor (SSTR)

subtypes are expressed at various levels in prolactinomas, SSTR5 being the most important in the regulation of PRL secretion (5, 6). We have previously shown a partial synergistic effect between D2DR and SSTR5 in suppressing PRL secretion (5). Such an additivity could be useful for hormonal inhibition in prolactinomas resistant to dopamine agonists.

Recently, hybrid molecules with high binding affinity for SSTRs and D2DR have been developed. These molecules, when tested in culture studies from mixed GH–PRL-secreting tumors partially responsive to SST analog therapy, showed greater efficacy than octreotide or a dopamine D2DR analog in suppressing GH or PRL secretion from mixed somatotroph adenomas in culture studies (7).

In the present study, we analyzed the PRL suppression produced by the ligands directed toward SSTR1, SSTR2, and SSTR5 used alone or in combination

in prolactinomas sensitive or resistant to dopamine agonists. We also evaluated the efficacy of more complex compounds such as the universal SSTRs ligand, SOM230 (8), and of a dopastatin molecule, BIM-23A760 (9), in suppressing PRL secretion in dopamine-sensitive and -resistant prolactinomas *in vitro*. The dose–response for suppression of PRL secretion by these compounds was compared with that of cabergoline in order to know whether SST analogs could be of interest in the treatment of prolactinomas resistant to cabergoline.

Design and methods

Patients

The present study was approved by the Ethics Committee of the University of Aix-Marseille (Aix-Marseille, France) and informed consent was obtained from each patient. Ten patients with PRL-secreting pituitary macroadenomas were included in the study (seven men, three women; mean age 42.2 ± 8.3 years). The endocrine and neuroradiological characterization of tumors were documented before any treatment. Before surgery, all patients were submitted to medical treatment with the dopamine agonist, cabergoline. Taking into consideration the hormonal response therapy, four patients were considered resistant to medical treatment (P1, P5, P7, and P10), as their mean ($\pm$ S.E.M.) basal plasma PRL value was 2720 ± 1360 μ g/l before treatment and their nadir mean value was 554 ± 129 μ g/l during chronic therapy with 0.5 mg cabergoline daily. The remaining six

patients were considered as sensitive to dopamine agonist treatments. Despite normalization of their plasma PRL levels under cabergoline, they were operated on due to intolerance to the drug. Four patients with mixed growth hormone prolactin (GH–PRL)-secreting pituitary adenoma were also enrolled in the study. All patients underwent transphenoidal surgery. A tissue specimen obtained from the surgical resection of the adenomas was used to quantify mRNA expression of D2DR and SSTR1–5. Another tumoral fragment was used for cell culture studies. Immunohistochemistry confirmed the PRL or GH–PRL character of the selected pituitary tumors. Clinical and immunohistochemical characteristics of prolactinomas and mixed tumors are presented in Table 1.

Quantification of D2DR and SSTR subtypes mRNA expression

Depending on the tumor, 1–1.5 μ g RNA were obtained. One microgram of total RNA was used for cDNA synthesis, as described previously (5). The detection of D2DR and SSTR subtypes mRNA expression was made by performing a real-time quantitative PCR using primer and probes as described previously (5, 10). TaqMan Gold nuclease assay was used (Perkin–Elmer, Foster City, CA, USA). The amplification reactions were realized on ABI PRISM 7700 sequence Detection System (Perkin–Elmer), according to the manufacturer's protocol. The D2DR and SSTR subtype mRNA levels were normalized in the same reaction to the β -Gus mRNA levels for quantification of the results. For each measurement, two independent RT-PCR analyses were performed.

Table 1 Clinical and immunohistochemical characteristics of ten pure prolactinomas (a) and four mixed growth hormone-prolactin (GH–PRL) adenomas (b).

Tumor	Age	Sex	Basal PRL (ng/ml) ^a	Tumor size (mm) ^b	IHC	
(a)						
P1	78	M	4000	30	PRL 90%	
P2	26	M	6500	36	PRL 90%	
P3	34	F	80	35	PRL 100%	
P4	37	M	800	20	PRL 100%	
P5	46	M	5000	55	PRL 40%	
P6	32	M	4000	80	PRL 100%	
P7	57	M	880	28	PRL 90%	
P8	22	F	146	15	PRL 100%	
P9	38	F	83	18	PRL 100%	
P10	52	M	1000	35	PRL 100%	
Tumor	Age	Sex	Basal GH (ng/ml) ^a	Basal PRL (ng/ml) ^a	Tumor size (mm) ^b	IHC
(b)						
A1	26	F	126	58	48	GH 30%, PRL 5–10%
A2	31	M	59	66	35	GH 60%, PRL 15%
A3	53	M	10	26	11	GH 75%, PRL 5–10%
A4	26	M	109	83	48	GH 30%, PRL 5–10%

IHC, immunohistochemistry; P, prolactinomas; A, mixed GH+PRL tumor.

^aSerum PRL or GH levels measured before medical therapy.

^bMaximal tumor diameter evaluated by MRI.

Tumor cell culture

Tissue specimens obtained during transphenoidal surgery were submitted to mechanical and enzymatic dissociation with collagenase at 37 °C for 30–45 min. The total cell amount was 4×10⁶ to 65×10⁶ depending on the tumor. Tumor cells were plated at a density of 2×10⁴ in 24-well culture dishes coated with extracellular matrix from bovine endothelial corneal cells, as described previously (11). The cells were cultured for 4 days at 37 °C in a CO₂ incubator with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal serum. At the start of the pharmacological studies, the medium was changed to DMEM supplemented with 1% fetal serum, 1% transferin, 1% selenium and 1% insulin, and effectors were added at various concentrations (10^{−13}–10^{−7} mol/l). The cells were incubated with different drugs for 16 h. Each drug concentration was tested in quadruplicate. At the end of the experiments, the medium was removed and stored at −20 °C for hormonal assays. All results were expressed as percent (%) inhibition of PRL release versus control (medium alone).

Pharmacological compounds

The receptor subtype specificity of each compound is shown in Table 2. The BIM compounds have been provided by Ipsen Inc. (Milford, MA, USA). BIM-23926 is a selective SSTR1 ligand (IC₅₀: 3.6 nmol/l). BIM-23197 is a preferential SSTR2 agonist with IC₅₀ 0.19 nM/l. BIM-23206 is a SSTR5-selective agonist with IC₅₀ of 2.4 nmol/l. The compound BIM-23A760 is a chimeric molecule capable of interacting with SSTR2, SSTR5, and D2DR. It shows very high affinity to SSTR2 (EC₅₀: 0.03 nmol/l) and low affinity to SSTR5 (EC₅₀: 3.7 nmol/l) when compared with SST 14 (SRIF14). Its dopaminergic pharmacophore presents with lower affinity than cabergoline for D2DR. SOM230 was supplied by Novartis Pharmaceuticals (Basel, Switzerland). This molecule is a multispecific ligand that, when compared with SRIF14, presents a higher affinity to SSTR5 (EC₅₀: 0.2 nmol/l) but lower affinities to SSTR2 (EC₅₀: 1 nmol/l)

and SSTR1 (EC₅₀: 9.3 nmol/l). The dopamine agonist cabergoline has been provided by Pfizer (Pharmacia & Upjohn, Kalamazoo, MI, USA). The SSTR agonists were dissolved as 10^{−3} mol/l solutions in 0.01 mol/l acetic acid containing 0.1% purified BSA (Life Technologies Inc). Cabergoline was prepared as 10^{−3} mol/l solution in 0.01 mol/l acetic acid and 70% ethanol. All drugs were stored at −80 °C as 10^{−3} mol/l solutions. For each experiment, a fresh aliquot was used in PBS supplemented with 1% BSA.

[3H]thymidine incorporation proliferation assay

DNA synthesis was measured in one resistant prolactinoma (P1) that expressed high levels of SSTR1 mRNA using the [3H]thymidine incorporation test (12). Cells were cultured at a density of 5×10⁴ in DMEM supplemented with 10% fetal serum. At day 3, the medium was replaced by DMEM with 1% fetal serum, 1% transferrin, 1% selenium and 1% insulin, and the cells were incubated with the selective SSTR1 ligand, BIM-23926, at different concentrations and cabergoline at 10^{−9} mol/l. At the end of a 72-h incubation, the medium was collected to perform hormonal measurement. Pharmacological effectors, 12-phorbol-13-myristate acetate (PMA; 10^{−7} mol/l) and 5 μCi/ml [3H]thymidine, were subsequently added to cell culture for a further 16-hour incubation. At the end of this period, the cells were washed with PBS and extracted with 10% trichloroacetic acid (TCA) and 10 M NaOH. Radioactivity incorporation was measured using a scintillation counter.

Hormonal assays

PRL and GH levels were measured in culture medium using a commercial IRMA kit. The anti-PRL and anti-GH monoclonal antibodies were furnished by Immuno-tech (Beckman Coulter, Marseilles, France). Regarding PRL, the normal values ranged from 1 to 18 ng/ml in men and 1 to 27 ng/ml in pre-menopausal women;

Table 2 Human dopamine receptor 2 (D2DR) and somatostatin receptor (SSTR) binding affinities of dopamine agonist (DA) and SST analogs.

Compound	Dopamine receptor (IC50: nmol/l) D2DR	Human somatostatin receptor subtype (IC50: nmol/l)				
		SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Somatostatin-14		1.9	0.2	1.2	1.8	1.4
BIM-23926		3.6	> 1000	> 1000	833	788
BIM-23197		6016	0.19	26.8	3897	9.8
BIM-23206		1152	166	1000	1618	2.4
BIM-23A760	15	622	0.03	160	1000	3.7
SOM230		9.3	1	1.5	> 100	0.2
Cabergoline	0.3					

Data for SOM230 are from Bruns et al. (8). The values for the BIM compounds are from Ipsen as previously published (9).

the coefficient of intra-assay variation was below or equal to 2.8%; and the coefficient of inter-assay variation was below or equal to 8%. For the GH assay, the intra- and inter-assay coefficients of variation were below or equal to 1.5 and 14% respectively.

Statistical analysis

The results are presented as the mean ± S.E.M. statistical significance between two unpaired groups was determined by the Mann–Whitney *U* test. To measure the strength of association between the pairs of variables without specifying dependencies, Spearman rank correlations were used. *P* < 0.05 was considered significant for all tests.

Results

D2DR and SSTR subtypes mRNA expression in prolactinomas and GH–PRL-secreting adenomas

D2DR, SSTR1, SSTR2, and SSTR5 mRNAs were found in all prolactinomas, but at very different levels of expression (Table 3). The mean mRNA levels of D2DR, SSTR1, SSTR2, and SSTR5 were respectively 92.3 ± 47.3, 2.2 ± 1.4, 1.1 ± 0.7, and 1.6 ± 0.6 copy/copy β-Gus. In all cases, the expression of D2DR was predominant over the SSTR mRNAs. In tumors from DA-sensitive patients, the mean level of D2DR expression was 139 ± 80 copy/copy β-Gus, higher than that measured in tumors from DA-resistant patients (18 ± 4 copy/copy β-Gus) (*P* < 0.05). SSTR1 was mainly found

in tumors from DA-resistant patients. SSTR2 levels were low with the exception of two DA-sensitive tumors (P3 and P4). SSTR5 expression was variable both in DA-sensitive and -resistant prolactinomas, with two tumors showing high levels of SSTR5 mRNA in each group (P4, P6, P1, and P7). SSTR3 mRNAs were detected in only three out of ten prolactinomas at very low levels of expression (not shown).

SSTR subtypes expression was also measured in four mixed GH–PRL-secreting adenomas (Table 3). In this small series, the mean mRNA levels of SSTR1, SSTR2, SSTR3, and SSTR5 were 0.5 ± 0.2, 2.3 ± 0.8, 0.4 ± 0.1, and 4.9 ± 2.2 copy/copy β-Gus. SSTR2 and SSTR5 mRNA were always coexpressed at a higher mean level than that in prolactinomas, while SSTR1 was present in three out of four adenomas. In two mixed GH–PRL adenomas, the levels of D2DR expression (8.3 ± 3.1 copy/copy β-Gus) were lower than those measured in prolactinomas.

Effect of SSTR1 agonist, BIM-23926, in prolactinomas

In order to investigate the putative role of SSTR1 in prolactinomas, we selected three DA-sensitive (P6, P8, and P9) and two DA-resistant (P1 and P7) tumors, expressing SSTR1 mRNA. As shown in Fig. 1A, the selective SSTR1 ligand, BIM-23926, was unable to inhibit PRL release at any concentration even in tumors expressing a high level of SSTR1 mRNA (P1 and P7). The SSTR5 ligand, BIM-23206, produced a dose-dependent inhibition of PRL release in two DA-sensitive tumors (P6 and P8). The addition of 10^{−8} mol/l of the SSTR1 ligand, BIM-23926, did not significantly modify the dose–response inhibition of PRL secretion

Table 3 Quantitative RT-PCR of dopamine receptor 2 (D2DR), somatostatin receptor (SSTR1), SSTR2, SSTR3, and SSTR5 mRNA expression in ten prolactinomas (a) and four mixed growth hormone and prolactin (GH and PRL) adenomas (b).

Tumor	D2DR and SSTR mRNAs (copy/copy β-Gus)				
	D2DR	SSTR1	SSTR2	SSTR3	SSTR5
(a)					
DA sensitive					
P2	65	0.1	0.1	0.1	0.1
P3	144	0.1	6.5	0	0.1
P4	445	0.1	1.0	0	2.1
P6	26	0.4	0.1	0	4.3
P8	13	0.2	0.5	0.1	0.5
P9	ND	0.9	0.1	0	0.1
DA resistant					
P1	15	12.4	0.1	0	1.3
P5	17	0.3	0.6	0	0.1
P7	12	3.9	0.1	0.1	4.3
P10	29	13.6	0.1	0	0.1
(b)					
A1	ND	0	0.4	0.3	15.2
A2	ND	1.4	0.8	0	0.5
A3	4	0.4	2.2	0	2.5
A4	13	0.4	6	1.1	1.5

D2DR and SSTR subtype levels are expressed as copy/copy β-Gus. P, prolactinomas; A, mixed GH+PRL tumors.

Figure 1 (A) Mean dose-response PRL suppression in cell culture from prolactinomas using the SSTR1 analog, BIM-23926 (in three DA-sensitive and two DA-resistant prolactinomas), and the SSTR5 analog, BIM-23206, alone or in combination with 10 nmol/l BIM-23926 (in two out of the three DA-sensitive prolactinomas). Results are expressed as the mean $\pm$ S.E.M. percent PRL suppression versus controls (medium alone). (B) Mean dose-response of [3 H]thymidine incorporation in cell culture from a DA-resistant prolactinoma (P1) in the presence of the SSTR1 analog, BIM-23926 (black bars) versus cabergoline 1 nmol/l (white bar). Results are expressed as mean $\pm$ S.E.M. percent of [3 H]thymidine incorporation compared with control. Each bar represents the mean of four wells.

produced by the SSTR5 analog ($44 \pm 8\%$ vs 48 ± 9 at 10 nM/l; Fig. 1A).

In order to evaluate the possible role of the SSTR1 compound on proliferation *in vitro*, we measured DNA synthesis through thymidine incorporation test in tumor P1. BIM-23926 was tested at 10^{-10} – 10^{-7} mol/l concentrations and compared with cabergoline at 10^{-9} mol/l. As shown in Fig. 1B, BIM-23926 was unable to inhibit the [3 H]thymidine incorporation at any concentration. In the same tumor culture, a $31 \pm 5\%$ reduction of [3 H]thymidine incorporation was obtained after incubation with cabergoline at 10^{-9} mol/l.

SSTR1 agonist versus SSTR2 and SSTR5 agonists in mixed GH–PRL adenomas

Four mixed GH–PRL-secreting adenomas were incubated with BIM-23926, BIM-23197, and BIM-23206 at a concentration ranging from 10^{-13} to 10^{-8} mol/l. In two tumors (A1 and A3), BIM-23926 did not significantly reduce PRL levels, while a superimposable 42–50% maximal PRL suppression was obtained with both the SSTR2 and SSTR5 compounds (data not shown). As shown in Fig. 2, in the other two tumors (A2 and A4), maximal PRL inhibition with the SSTR1 agonist was significant ($18 \pm 1\%$ and $30 \pm 5\%$ respectively, $P < 0.05$ versus control). Nevertheless, in both cases, PRL suppression was obtained at higher concentration than that with the SSTR2 and SSTR5 analogs. In tumor A2, BIM-23926 produced a maximal PRL

Figure 2 Individual dose-response PRL suppression in two mixed GH-PRL adenomas (A2 and A4) cultured in the presence of BIM-23926 (SSTR1 analog), BIM-23197 (SSTR2 analog) and BIM-23206 (SSTR5 analog). Results are expressed as the mean $\pm$ S.E.M. percent PRL suppression versus controls (medium alone). Each point represents the mean of four wells.

suppression of $18 \pm 1\%$ vs $50 \pm 2\%$ with BIM-23197 and $54 \pm 1\%$ with BIM-23206 ($P < 0.05$). In tumor A4, the SSTR5 analog was more effective in PRL suppression than either the SSTR2 or SSTR1 analog ($65 \pm 2\%$ vs $35 \pm 1\%$ and $30 \pm 5\%$ respectively, $P < 0.05$). When GH data were analyzed, the results superimposable to those obtained for PRL were evidenced (data not shown).

Effects of the SSTR2 and SSTR5 compounds alone or in combination with cabergoline in prolactinomas

Four DA-sensitive prolactinomas (P2, P3, P4, and P6) and three DA-resistant tumors were analyzed (P1, P7, and P10). The SSTR2 compound, BIM-23197, at nanomolar concentrations, was ineffective on PRL secretion, in two dopamine-sensitive prolactinomas (P2 and P3) even if P3 expressed high SSTR2 mRNA levels. In tumors P4 and P6, a partial lowering of PRL secretion was measured in the presence of BIM-23197 ($40 \pm 2\%$ vs $70 \pm 3\%$ for cabergoline, $P < 0.05$) (data not shown). The SSTR5 compound, BIM-23206, produced a dose-dependent inhibition of PRL release in two out of four DA-sensitive prolactinomas. As shown in Fig. 3A, in these two tumors (P4 and P6), the maximal PRL suppression by BIM-23206 ($68 \pm 4\%$) was similar to that produced by cabergoline ($70 \pm 3\%$). The combination of BIM-23206 and cabergoline at equimolar concentrations showed a dose-response inhibition of PRL secretion similar to that of cabergoline alone (maximal PRL suppression: $71 \pm 3\%$).

In the three DA-resistant prolactinomas, cabergoline (10^{-9} mol/l) produced a $26 \pm 5\%$ mean maximal inhibition of PRL secretion. In these tumors, the SSTR5 compound, BIM-23206 (Fig. 3B), or the SSTR2 compound, BIM-23197 (data not shown), partially suppressed PRL secretion at nanomolar concentrations. The coincubation of cabergoline and BIM-23206

Figure 3 (A) Mean dose–response for PRL suppression in cell culture from four DA-sensitive prolactinomas by the SSTR5 analog, BIM-23206 (open squares, continuous line for 2/4 SSTR5 responders, and dashed line for 2/4 SSTR5 non-responders) and cabergoline (open circles) alone or in combination (grey triangles). (B) Mean dose–response PRL suppression in cell culture from three DA-resistant prolactinomas by the SSTR5 analog, BIM-23206 (open squares) and cabergoline (open circles) alone or in combination (grey triangles). (C) Mean dose–response PRL suppression in cell culture from four DA-sensitive prolactinomas by the dopastatin molecule BIM-23A760 (black triangles) versus cabergoline (open circles). (D) Mean dose–response PRL suppression in cell culture from three DA-resistant prolactinomas by cabergoline (open circles), SOM230 (open stars), and BIM-23A760 (black triangles). Results are expressed as the percent PRL suppression (mean of four wells) versus control value (medium alone).

produced a dose–response inhibition of PRL secretion superimposable to that produced by cabergoline alone (Fig. 3B).

Effects of multiple receptor ligands, BIM-23A760 and SOM230, in prolactinomas

In DA-sensitive tumors (Fig. 3C), the maximal PRL inhibition by the chimeric compound BIM-23A760 ($72 \pm 4.3\%$) was similar to that obtained by cabergoline. Even if it did not achieve statistical significance, its mean EC_{50} (0.5 ± 0.02 pmol/l) was five times lower than that obtained with cabergoline (2.5 ± 1.5 pmol/l). In DA-resistant tumors, BIM-23A760 showed the same trend of higher potency when compared with cabergoline (EC_{50} : 4 ± 3 and 28 ± 24 pmol/l respectively). Despite such a sevenfold lower EC_{50} , the maximal suppression of PRL secretion with cabergoline and BIM-23A760 were identical (Fig. 3D).

The effectiveness of the SSTR multi-receptor ligand, SOM230 (10^{-13} – 10^{-9} mol/l), on PRL secretion was tested in three resistant tumors (P1, P5, and P10). In these three resistant prolactinomas, in which SSTR1-, SSTR2-, or SSTR5-selective agonists alone were poorly effective (less than 5% PRL inhibition), maximal PRL suppression achieved with SOM230 was not significant

($7 \pm 2.8\%$ at nanomolar concentration) and again less effective than that of cabergoline ($19 \pm 5.6\%$; Fig. 3D).

Discussion

Patients with prolactinomas who fail to obtain PRL levels normalization and tumor size reduction are considered resistant to dopamine agonists treatment (3). Pharmacological resistance to dopamine agonists has become less frequent with the use of cabergoline and represents about 10% of patients (3). In DA-resistant prolactinomas, a reduction in D2DR receptor levels has been demonstrated and accounts for the partial response to dopamine agonists (13). In our series of ten macroprolactinomas, we confirmed at a quantitative level that mean D2DR mRNA levels in dopamine-resistant tumors were significantly lower than those in dopamine-sensitive prolactinomas. However, a great variability in terms of D2DR mRNA levels has been found among sensitive and resistant adenomas and a clear-cut threshold cannot be established between the two categories. Defects in transduction pathways of D2DR in resistant prolactinomas (13) may explain for this overlapping.

In DA-resistant prolactinomas, SST analogs may be of therapeutic interest as different SSTR subtypes were identified in such tumors (14–19). More recently, quantitative mRNA (5) or immunohistochemical studies (20) confirmed the coexpression of three SSTR subtypes (SSTR1, SSTR5, and SSTR2 at a lower level). In the present study, SSTR1 and SSTR5, associated with SSTR2, were expressed in all prolactinomas, but with important quantitative variations among the tumors. SSTR1 or SSTR5 were the predominant SSTR subtypes of mRNA in nine out of the ten analyzed prolactinomas including all DA-resistant macroprolactinomas. SSTR1 and SSTR5 coexpression was characterized by the differences between high levels of one of such mRNA receptors level and low levels of the others. SSTR1 was predominant in DA-resistant prolactinomas. Since high SSTR1 expression seems a feature of lactotroph cells (20) and especially in resistant prolactinomas (our data), we investigated the role of this receptor in hormonal regulation and cell proliferation of human prolactinomas using the SSTR1 preferential analog, BIM-23926. The SSTR1 subtype differs from the other SSTRs as it does not internalize but upregulates after prolonged activation in transfected cells (21–23). After ligand binding to the receptor, it does not form homodimers (24). Nevertheless, SSTR1 shares with the other SSTRs the capacity to trigger different signaling transduction pathways (25, 26), resulting in an antiproliferative effect (27–29). In human endocrine tumors, SSTR1 modulates hormonal secretion and cell viability in non-secreting pituitary adenomas, human medullary thyroid carcinoma, and mixed GH–PRL-secreting adenomas (30–33). In the present study,

a suppressive effect of BIM-23926 on hormones release was also evidenced in two out of four mixed GH–PRL adenomas. Nevertheless, in these tumors, the SSTR1 compound proved less potent than the SSTR2 and SSTR5 compounds. Unexpectedly, BIM-23926, at any concentrations used, could not suppress PRL secretion from pure prolactinomas, whether sensitive or resistant to DA. Similarly, when tested on cell proliferation, BIM-23926 did not inhibit DNA synthesis in a pure prolactinoma expressing a high level of SSTR1. Taken together, such different responses of pure lactotrophs versus mixed GH–PRL cells, both expressing the SSTR1 receptor, indicate that the only level of expression of a given receptor cannot predict the responses of the whole cell phenotypes. Presently, the role of SSTR1 in prolactinoma remains unknown.

If SSTR1 by itself has no effect on prolactinoma cells, it may interact with the SSTR5-mediated suppression of PRL release. Indeed, in transfected cells, SSTR5–SSTR1 heterodimers formation is preferred to SSTR5 homodimers and results in the modification of ligands binding and the cAMP activation (34). Such a crosstalk between the two SSTR subtypes could not be induced by the ligands in our experiments of cocubation of BIM-23926 and BIM-23206 in prolactinoma cells. Similarly, Hofland *et al.* (35) using the multiple receptor ligand, SOM230, which recognized both SSTR1 and SSTR5, found that only the SSTR5 mRNA levels were correlated with the SOM230-mediated prolactin suppression in three prolactinomas. In contrast to our previous findings (5), such a correlation was not found in our present limited series. If the SSTR5 compound, BIM-23206, inhibited PRL secretion in the three DA-sensitive tumors (P4, P6, and P8) expressing the highest SSTR5 mRNA levels, it was ineffective on DA-resistant tumors P1 and P7 also expressing a high amount of SSTR5 mRNA. Such a combined absence of responsiveness to the SST and dopamine agonists suggests post-receptors defect(s) in the transduction pathways (13). In our limited experience, the three DA-resistant tumors non-responsive to the SSTR5 analog, BIM-23206, were equally non-responsive to SOM230.

We have previously shown that a SSTR5 analog, BIM-23268, combined with a D2DR analog, quinagolide, produced a partial additive effect on PRL suppression of prolactinomas (5). The chimeric compound, BIM-23A760, binds SSTR2, SSTR5, and also D2DR. In culture studies of mixed GH–PRL adenomas, BIM-23A760 suppressed PRL levels with a better efficacy than either octreotide or SSTR2–D2DR drug (9), with putative contribution of SSTR2, SSTR5, and D2DR affinities. Moreover, in acromegaly, this dopamine–SST ligand was more potent and more long lasting to inhibit GH secretion than its single components used alone or in combination (9). Such a synergistic effect was not found in prolactinomas in the present study. BIM-23A760 showed a better but non-statistically significant EC₅₀ in suppressing PRL secretion when compared with

cabergoline in both DA-sensitive and -resistant prolactinomas, without maximal PRL suppression improvement. These findings in prolactinomas are far away from the synergistic cooperation of these two receptors in cAMP suppression demonstrated in experimentally transfected cells (36). Recently, BIM-23A760 was found to suppress PRL in a similar manner to D2DR agonists in primary rat pituitary cell cultures and MMQ prolactin-secreting rat cell line (37). These results seem roughly similar to our prolactinoma data and confirm the crucial role of D2DR affinity for the PRL suppression by BIM-23A760.

In conclusion, we demonstrated that SSTR1 analogs have no effect on PRL suppression and cell proliferation in prolactinomas. The SSTR1 analog does not modify SSTR5-mediated PRL suppression. The SSTR5 preferential analogs suppress PRL secretion in a majority of dopamine-sensitive prolactinomas never exceeding cabergoline maximal suppressive effects, but proved ineffective on DA-resistant tumors. In this limited series, the universal SSTR ligand, SOM230 was also ineffective on PRL suppression in these tumors. Finally, the chimeric dopastatin compound, BIM-23A760, suppressed PRL at a slightly lower concentration when compared with cabergoline. Whether or not such a partial acute improvement will be of therapeutical interest remains to be demonstrated by long term, *in vivo*, studies.

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