0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(11):4482–4488

doi: 10.1210/jc.2006-1245

The Effects of SOM230 on Cell Proliferation and Adrenocorticotropin Secretion in Human Corticotroph Pituitary Adenomas

Dalia L. Batista, Xun Zhang, Roger Gejman, Peter J. Ansell, Yunli Zhou, Sarah A. Johnson, Brooke Swearingen, E. Tessa Hedley-Whyte, Constantine A. Stratakis, and Anne Klibanski

Neuroendocrine Unit (D.L.B., X.Z., P.J.A., Y.Z., S.A.J., A.K.), Neuropathology Unit (R.G., E.T.H.-W.), and Division of Neurosurgery (B.S.), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114; and Section on Endocrinology and Genetics (D.L.B., C.A.S.), Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 02892

Context: There is no tumor-directed medical therapy available for Cushing's disease.

Objective: The objective was to determine the *in vitro* effect of the somatostatin analog pasireotide (SOM230) on cell proliferation in human corticotroph tumors.

Design/Methods: Expression of somatostatin receptors (SSTR 1–5) was determined by quantitative RT-PCR in 13 human corticotroph tumors and by immunohistochemistry (IHC) in 12 of the 13 tumors. SOM230 effects on cell proliferation and ACTH release were evaluated *in vitro* using primary cultures of six of the 13 human corticotroph adenomas.

Results: In our series, we found expression of SSTR subtypes 1, 2, 4, and 5 in human corticotroph tumors by quantitative RT-PCR. All receptor subtypes were detected by IHC, with SSTR subtype 5 having the highest IHC score in 83% (10 of 12) of the cases. Significant suppression of cell proliferation was observed in all tumors cultured (percent suppression range: 10–70%; $P = 0.045$–0.001). SOM230 inhibited ACTH secretion in five of the six tumors cultured (percent suppression range: 23–56%; $P = 0.042$–0.001).

Conclusion: Corticotroph tumors express multiple SSTR subtypes. SOM230 significantly suppressed cell proliferation and ACTH secretion in primary cultures of human corticotroph tumors. These *in vitro* results support the hypothesis that SOM230 may have a role in the medical therapy of corticotroph tumors. **(*J Clin Endocrinol Metab* 91: 4482–4488, 2006)**

CUSHING'S DISEASE (CD) is caused by an ACTH-producing pituitary adenoma (1). Some of the clinical manifestations and complications include weight gain and truncal obesity, proximal muscle weakness, glucose intolerance, hypertension, mood disorders, and osteoporosis (1–3). Significant morbidity and mortality are associated with this disease (4). After confirmation of CD, transsphenoidal surgery (TSS) for selective corticotroph adenomectomy is the first line of treatment (5). Although the reported surgical cure rate for microadenomas, which represent the majority of these tumors, is 70–90%, these results depend largely on the anatomic location of the tumor and the experience of the neurosurgeon. In addition, recurrence rates as high as 25% have been reported (5, 6). In contrast, the reported cure rate for patients with corticotroph macroadenomas, even with an experienced pituitary surgeon, is much lower at 53–67% (7). For patients in whom TSS is unsuccessful, there are limited therapeutic options. Therefore, effective tumor-targeted medical therapy would be an important advance.

First Published Online August 29, 2006

Abbreviations: CD, Cushing's disease; IHC, immunohistochemistry; qRT-PCR, quantitative RT-PCR; SOM230, pasireotide; SST, somatostatin; SSTR, SST receptors; TSS, transsphenoidal surgery

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Somatostatin (SST) is a neuropeptide whose actions are mediated through five different membrane-bound receptors (SSTR 1–5) (8, 9). SSTRs are members of the G protein-coupled receptor superfamily. Binding of SST to its receptors causes inhibition of hormone secretion, inhibition of cell proliferation, or apoptosis, depending on the receptor subtype (8, 9). The development of SST analogs has been a major advance in the therapy of somatotroph adenomas. SST analogs inhibit GH secretion and cause growth suppression with tumor shrinkage in a subset of patients with a somatotroph adenoma (10). In contrast to the experience in patients with acromegaly, SST and its analogs have had a very limited therapeutic role in patients with CD, although human corticotroph adenomas express SSTRs (11–15). Previous reports have shown that SST and its available analog, octreotide, inhibit basal and stimulated ACTH secretion by many animal-derived cell lines, including the murine corticotroph tumor cell line AtT-20 as well as in human corticotroph adenomas *in vitro* (15–19). However, in patients with CD, SST and its available analog octreotide have not been shown to inhibit ACTH secretion significantly(20, 21).

SOM230 (pasireotide) is a SST analog that binds with high affinity to SSTR subtype 5 and also has binding affinity to SSTR subtype 2, 3, and 1, in this order (22). Recent studies show that SOM230 inhibits basal and stimulated ACTH release by human ACTH-secreting pituitary adenomas and the

murine corticotroph tumor cell line AtT-20, the only pituitary cell line available to study ACTH release. Furthermore, SOM230 was shown to suppress ACTH secretion in cultures of human ACTH-secreting adenomas more than octreotide (15, 19). In addition, preincubation of the AtT-20 cell line (which has specific binding sites for SSTR subtype 2 and 5) with glucocorticoids did not affect the ability of SOM230 to suppress ACTH release by this cell line, whereas the ability of octreotide to suppress ACTH release was decreased (15, 19). These observations suggest that SOM230 might be effective clinically in human corticotroph adenomas *in vivo*.

In addition to effects on ACTH secretion, the ability of a SST analog to suppress the growth of corticotroph tumor cells would be of importance in patients with CD, particularly those with unresectable macroadenomas and patients with Nelson's tumors. The antiproliferative effect of SOM230 in corticotroph adenomas is not established. We therefore determined the expression of SSTRs in human ACTH-secreting pituitary adenomas and investigated whether SOM230 suppresses cell proliferation and ACTH secretion in primary cell cultures of human corticotroph adenomas.

Subjects and Methods

Subjects

The sample consisted of 13 patients with the diagnosis of CD. There were nine females and four males with an age range of 12–52 yr (Table 1). Cushing's syndrome was diagnosed by standard methods including urinary free cortisol, high-dose dexamethasone suppression test, and bilateral inferior petrosal sinus sampling with CRH stimulation test, when indicated. TSS was performed for an excision of a pituitary adenoma in all cases.

Tumor specimens

Tumor specimens were obtained from these patients during TSS as approved by the Institutional Review Boards (IRBs) of Partners Health Care (Boston, MA) and the National Institute of Child Health and Human Development (NICHD) (Bethesda, MD). Histopathological examination of the surgical specimens confirmed the diagnosis of an ACTH-secreting adenoma in all cases.

Primary cell cultures and cell proliferation assay

Fresh tumor tissue was available from six of the 13 human corticotroph adenomas for primary cell culture. Mechanical and enzymatic dispersion of the tumor fragments was performed, and they were cultured as previously described (23–25). Each experiment was performed in duplicate or triplicate, depending on the sample availability. The cells were plated at 20,000–70,000 per well in 24-well plates (Becton Dickinson Labware, Bedford, MA). Tumor cells were incubated at 37 C in 5% CO_2 with 1 ml DMEM media. After 24 h incubation, cells were treated with SOM230 (provided by Novartis, Basel, Switzerland) at concentrations of 10^{-7}, 10^{-8}, 10^{-9} M for 24, 48, 72, and 96 h. The number of viable cells after treatment with SOM230 was assessed at 48, 72, and 96 h of treatment using the Cell Titer 96Aqueous One Solution Proliferation Assay (MTS assay; Promega, Madison, WI) as previously described (24). In addition, before each proliferation assay was performed, 200 μl of conditioned media was removed and saved for ACTH measurement.

Hormone measurements

ACTH levels were determined in conditioned medium collected before the proliferation assay by using an enzyme immunoassay kit (MD Biosciences, St. Paul, MN), according to the manufacturer's instruction. Each assay was performed in triplicate after dilution.

RNA extraction and cDNA preparation

Total RNA was extracted from primary cultures using the cells that were plated in the control untreated wells and directly from tumor tissue samples. TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD) was used for RNA isolation. RNase-free deoxyribonuclease digestion was also performed to exclude genomic DNA amplification using the DNA-free Kit (Ambion, Austin, TX) according to the manufacturer's instructions. cDNA was synthesized from 1 μg deoxyribonuclease-treated RNA with an oligo-dT primer, using the RT kit from Promega, following the manufacturer's protocol.

Quantitative (real time) PCR (qRT-PCR)

qRT-PCR for SSTR expression was performed using a 25-μl working master mix containing: 1.5 μl of the cDNA template in 1× TaqMan universal Master Mix (Applied Biosystems, Foster City, CA) and 200 nM final concentration of the primers and the probe (SSTR 1 catalog no. Hs00265617_s1, SSTR 2 catalog no. Hs00990356_m1, SSTR 3 catalog no. Hs01066399_m1, SSTR 4 catalog no. Hs01547826_m1, SSTR 5 catalog no. Hs00265647_s1, FAM labeled; Applied Biosystems). A housekeeping gene, Cyclophilin A (catalog no. Hs99999904_m1, Applied Biosystems),

TABLE 1. Demographic data and SSTR (1–5) mRNA levels

CT	Age	Sex	UFC	SSTR 1	SSTR 2	SSTR 3	SSTR 4$^+$	SSTR 5
1	47	M	1040 ± 10	1.3				2.6
2	40	F	60 ± 9[a]	2.2	0.5			5.5
3	30	F	184 ± 5	1.0	0.97			1.3
4	32	F	73 ± 5[a]					9.0
5	34	F	697 ± 27	6.0				13.0
6	52	F	1069	7.0				4.0
7	18	F	379 ± 173	1.7	0.75			2.2
8	12	F	486 ± 146	0.70	0.05		+	0.9
9	12	M	380 ± 150	1.0	1.0		+	2.0
10	14	M	246 ± 38	0.40	0.005		+	1.3
11	13	M	787 ± 56	2.0	0.02		+	1.0
12	12	F	337 ± 34	3.0	0.27		+	2.0
13	14	F	506 ± 70	4.0	0.38			2.5

CT, Cushing's tumor number; UFC, urine free cortisol (μg/24 h; in children, UFC = μg/BSA/24 h) (normal cortisol excretion, <70 μg/24 h). To convert values of UFC to nanomoles per meter squared of body surface area per day, multiply by 2.759. Values are expressed as mean ± SD. Normal pituitary samples expressed receptors 1, 2, 3, and 5 mRNA, and their levels were designated as 1. The receptor mRNA levels in tumors were compared with and presented as fold of the level of the normal pituitary. None of the tumor samples expressed SSTR subtype 3. Normal pituitary did not express SSTR subtype 4 mRNA, and relative quantitation for SSTR subtype 4 was not calculated for the experimental samples. +, Presence of SSTR4.

[a] For these patients, normal UFC < 35 μg/24 h.

was amplified in parallel with experimental samples and used to normalize the results to allow relative quantitative analysis of SSTR expression. The reaction was run in a SmartCycler II thermal cycler (Cephid, Sunnyvale, CA), using the following cycling parameters: 50 C for 2 min, 95 C for 10 min, 40 cycles of 95 C (denature) for 15 sec, with 60 C for 1 min (annealing extension). For controls, postmortem human normal pituitaries were obtained from the Harvard Brain Tissue Resource Center (Belmont, MA). cDNA from three normal human pituitary glands was used as the control sample for relative mRNA determination of SSTR by qRT-PCR. In addition, positive controls included the MCF-7 breast cancer cell line and the pituitary tumor-derived folliculostellate cell line PDFS. These cell lines express SSTRs. Each qRT-PCR was processed in duplicate experiments. PCR products were sequenced, and bands were confirmatory for the SSTRs.

SSTR protein expression by immunohistochemistry (IHC)

Immunostaining was performed in 12 of 13 corticotroph tumors. Sections were cut at 5 μm from representative formalin-fixed, paraffin wax-embedded blocks and floated onto positively charged slides (SuperFrost Plus; Menzel-Glaser, Portsmouth, NH). The slides were dewaxed in xylene and rehydrated through graded ethanols. Histological sections were incubated with primary antibodies against goat, SSTR 1–5 diluted 1:200 in TnB blocking buffer (0.1 M Tris-HCl; 0.15 M NaCl, pH 7.5; 0.5% Blocking Buffer; PerkinElmer, Shelton, CT). (SSTR 1 catalog no. sc-11604, SSTR 2 catalog no. sc-11606, SSTR 3 catalog no. sc-11610, SSTR 4 catalog no. sc-11619, and SSTR 5 catalog no. sc-11623 from Santa Cruz Biotechnology., Santa Cruz, CA). Antibodies were specific for each SSTR subtype (26). Specificity for these antibodies is 100% for SSTR 1, 2, 3, 5, and 94% for SSTR 4, and they do not cross-react with each other. After sections were hybridized overnight with primary antibodies, they were incubated with biotinylated secondary antibody (rabbit anti-goat, catalog no. BA-5000, Vector Laboratories, Burlingame, CA) at a 1:500 dilution for 1 h at room temperature. To enhance the immunohistochemical signal, this was followed by an additional amplification step with biotinylated tyramide (27) using a commercially available kit (catalog no. NEL700A from PerkinElmer Life and Analytical Sciences, Shelton, CT). Negative controls included omission of the primary antibody. Pancreatic tissue was used as a positive control. Qualitative and quantitative analyses were performed. The intensity of IHC staining for various SSTR subtypes was scored 0 (negative), 1+ (mild positive staining), 2+ (mild-moderate positive), 3+ (moderate positive staining), 4+ (moderate-strong positive staining), and 5+ (strong positive staining) (Table 2).

Statistical analysis

Results are expressed as percent of the untreated wells (control), mean ± SE of the mean (SEM). All statistical analyses were performed using a Student's *t* test; a *P* value of less than 0.05 was considered significant.

Results

SSTR mRNA expression in human corticotroph adenomas

We examined the expression of SSTR subtypes 1–5 by quantitative real-time PCR. As shown in Table 1, SSTR subtype 5 mRNA was detected in all tumors examined. SSTR subtype 1 was detected in 92% (12 of 13) of cases, subtype 2 in 69% (9 of 13), subtype 4 in 38% (5 of 13), and subtype 5 in 100% (13 of 13) of cases. SSTR 3 was not detected in the tumor samples examined.

SSTR protein expression in human corticotroph adenomas by IHC

Protein expression of SSTR subtypes 1–5 was detected by IHC in all 12 corticotroph tumors examined (Table 2). There were differences in the intensity of the immunostaining as well in the number of positive cells. Receptor subtype 5 showed the highest IHC score in 10 of 12 cases. Cytoplasmic as well as complete or incomplete membranous immunoreactivity was noted in all tumors but not in all cells (Fig. 1).

Effect of SOM230 on tumor cell proliferation

Cell proliferation was measured as the number of viable cells compared with untreated control. Corticotroph adenomas responded to different concentrations of SOM230. Tumor samples 1, 2, 5, and 6 showed maximal response to concentration of 10^{-8} M. For tumor samples 3 and 4, the maximal response was achieved with concentration of 10^{-9} M. There was a significant *in vitro* suppression of cell proliferation with SOM230 in all tumors (Fig. 2). The percent suppression was 52%, $P = 0.001$ (tumor 1); 22%, $P = 0.001$ (tumor 2); 10%, $P = 0.001$ (tumor 3); 70%, $P = 0.001$ (tumor 4); 43%, $P = 0.001$ (tumor 5); and 61%, $P = 0.045$ (tumor 6), respectively.

Effect of SOM230 in ACTH secretion in vitro

ACTH was measured in media from the primary cell cultures of human corticotroph adenomas. All collection times were available for tumor samples 1, 2, 3, and 6 to carry out this experiment. For tumor samples 4 and 5, only 48- and 72-h collection time points were available. SOM230 suppressed ACTH secretion in five of six tumors. The maximal effect was observed at concentrations of 10^{-8} or 10^{-9} M. Maximum

TABLE 2. IHC staining of SSTR subtypes in human corticotroph pituitary adenomas

CT no.	SSTR 1		SSTR 2		SSTR 3		SSTR 4		SSTR 5	
	Staining	(+) Cells (%)	Staining	(+) Cells (%)	Staining	(+) Cells (%)	Staining	(+) Cells (%)	Staining	(+) Cells (%)
1	2+	60	2+	60	3+	95	1+	40	3+	95
2	5+	70	3+	100	3+	95	4+	100	5+	100
3	2+	100	3+	100	2+	80	4+	100	5+	100
4	1+	95	2+	95	2+	95	4+	100	5+	100
5	3+	100	3+	100	3+	100	3+	100	4+	100
7	5+	100	4+	100	3+	100	3+	100	4+	100
8	2+	55	2+	44	3+	30	3+	95	2+	40
9	4+	70	3+	85	4+	85	4+	75	5+	95
10	3+	60	3+	40	3+	75	3+	90	4+	90
11	3+	98	2+	98	2+	90	4+	100	5+	100
12	2+	100	2+	100	2+	100	1+	100	5+	100
13	1+	99	3+	99	3+	99	3+	99	5+	100

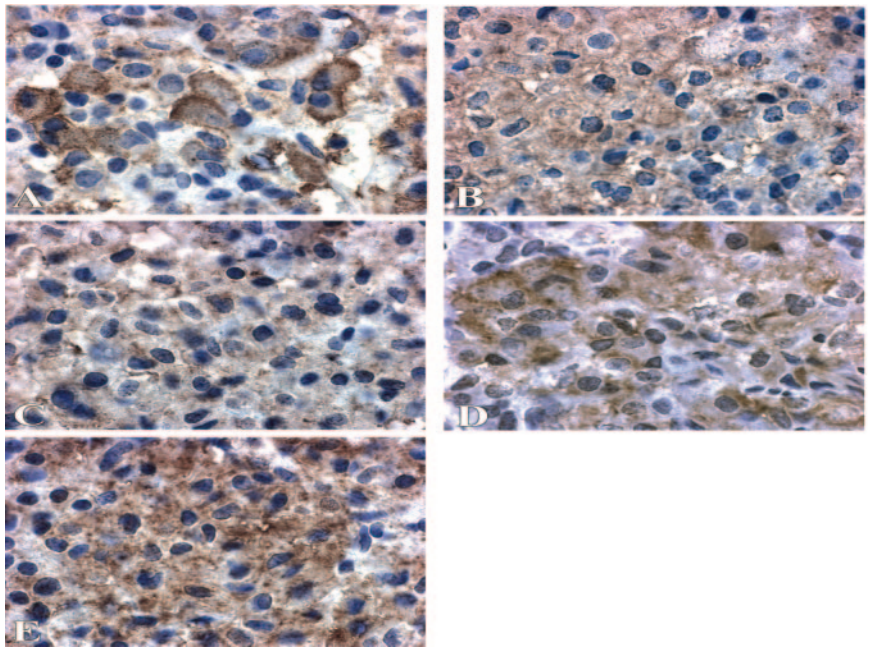

FIG. 1. A–E, Immunoperoxidase staining for SSTR subtypes 1, 2, 3, 4, and 5, counterstained with hematoxylin and eosin, magnification ×1000. A, SSTR 1; B, SSTR 2; C, SSTR 3; D, SSTR 4; E, SSTR 5. Immunostaining was noted for all SSTR subtypes. Membranous as well as cytoplasmic staining was noted. Negative controls included omission of the primary antibody.

ACTH suppression was seen at 96 h for tumors 1, 2, and 3 (Table 3 and Fig. 2): suppression was 31%; $P = 0.001$ (tumor 1), 47%; $P = 0.042$ (tumor 2), and 47%; $P = 0.010$ (tumor 3), respectively. For tumor samples 4 and 5 maximum ACTH suppression was observed at 72 h. ACTH suppression was 29%; $P = 0.044$ for sample 4 and for sample 5 was 23%; $P = 0.083$. Significant ACTH suppression was observed in tumor sample 6 at 48 h. Percent ACTH suppression was 56%, $P = 0.044$ (tumor 6).

Dissociation between suppression of cell proliferation and ACTH secretion by SOM230 was observed. For tumor 3, although SOM230 significantly inhibited ACTH release by 47% ($P = 0.010$), a minimal but statistically significant effect (10%, $P = 0.001$) on cell proliferation was observed. For tumor 5, the percent of ACTH suppression was 23% ($P = 0.083$), whereas suppression of cell proliferation was 43% ($P = 0.001$).

Discussion

Current medical therapy for CD includes compounds that inhibit steroidogenesis, modulate ACTH release, or act as glucocorticoid receptor antagonists (28, 29). All of these therapeutic agents act by decreasing adrenal steroid secretion, except for glucocorticoid receptor antagonists (28, 29). In general, inhibitors of steroidogenesis are the most commonly used agents, but they are only employed as a temporary measure until a definitive therapy is given. SOM230 is a new SST analog that has both antisecretory and potential antiproliferative properties (15, 19, 30). Although the antisecretory effect of SOM230 in corticotroph tumors has been reported, its ability to suppress tumor growth has not been previously studied. However, in other tumor phenotypes, the use of SST analogs has been associated with significant suppression of tumor growth (10, 31).

The results of our study show that SOM230 significantly suppresses cell proliferation in all human corticotroph tumors studied, with the percent suppression ranging from 10–70%. An antiproliferative property of SOM230, as an SST analog, would be of value in patients with ACTH-dependent macroadenomas where control of tumor mass is an important clinical end point. Macroadenomas causing CD are refractory to therapy, and the reported cure rate after TSS for a macroadenoma is between 53–67% (7, 32). Our *in vitro* cultured sample included two invasive macroadenomas and four microadenomas. Suppression of cell proliferation by SOM230 was observed in all tumors, including the microadenomas studied.

The role of SSTRs in the control of corticotroph adenomas cell proliferation is unclear. Previous studies have shown that SOM230 has a high affinity for SSTR subtypes 5, 2, 3, 1 and that all SSTRs might have antiproliferative effects. SSTR subtypes 1, 2, 4, and 5 are cytostatic. They inhibit cell proliferation through a phosphotyrosine-phosphatase-dependent pathway and by interaction with the mitogen-activated protein kinase pathway as well (8, 9). This pathway is associated with inhibition of cell cycle progression and an increase in the expression of the tumor suppressor retinoblastoma (Rb) protein and p21 protein (cyclin-dependent kinase inhibitor) (8, 9). In contrast, SSTR subtype 3 is cytotoxic and causes cell death or apoptosis through a phosphotyrosine-phosphatase-dependent mechanism and activation of p53 and Bax protein (9). Furthermore, it has been shown that SSTR subtypes form dimers that can influence their binding affinities as well as other receptor subtypes (22, 33). SOM230 can also indirectly influence growth by decreasing secretion of pituitary hormones and growth factors (30). Further studies are needed to clarify the mechanism of SOM230 antiproliferative effect on corticotroph adenomas. As in other tumor phenotypes, differences in receptor concentration analyzed quantitatively and the presence of specific SSTRs subtypes contribute to tumor responsiveness (34).

In agreement with previous observations, the results of

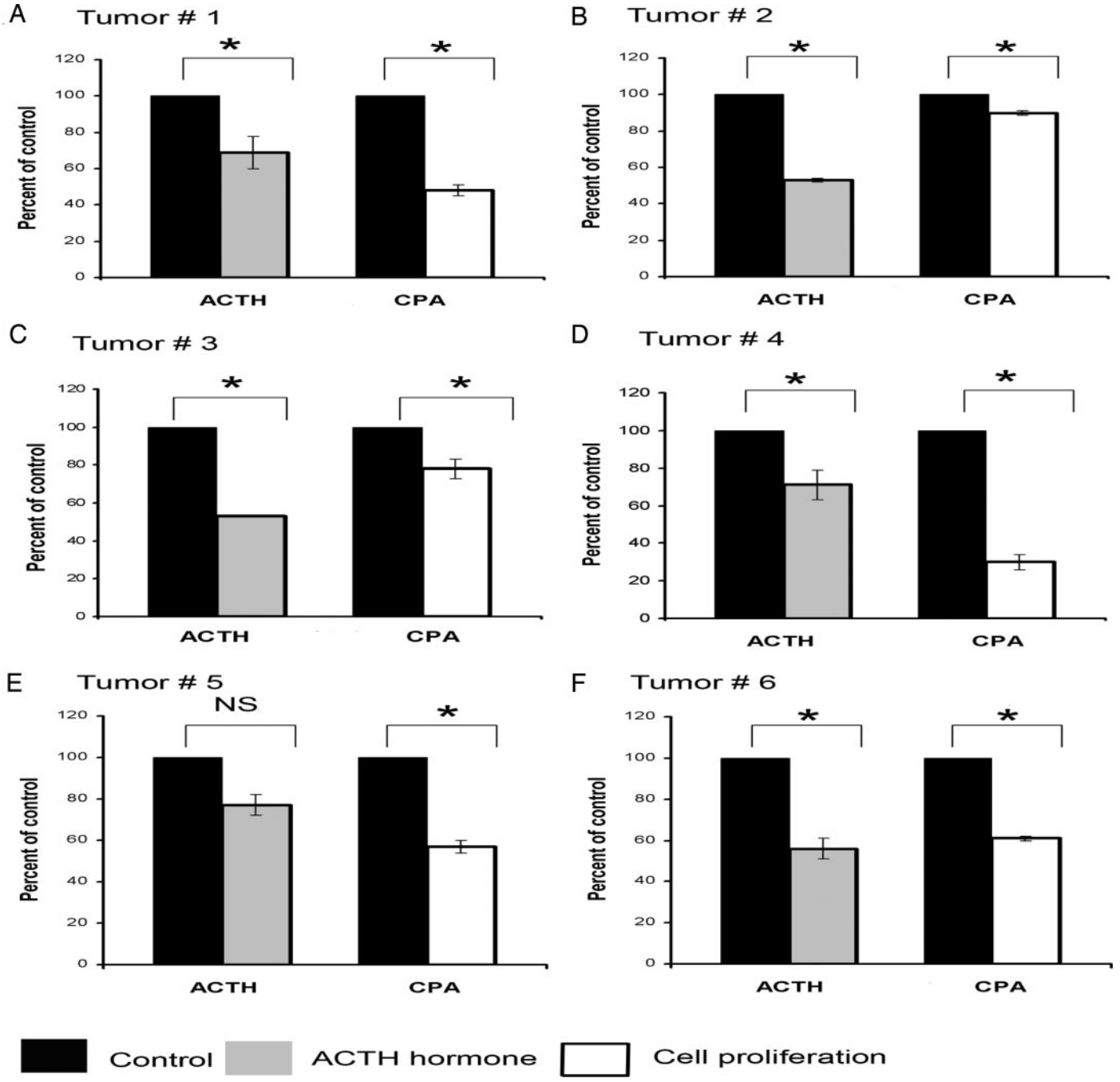

FIG. 2. ACTH, SOM230 effects on ACTH secretion and CPA, cell proliferation (cell proliferation assay) in six human corticotroph pituitary adenomas. Tumors were cultured for 48–96 h in the absence or in the presence of SOM230 (concentration 10^{-7}, 10^{-8}, 10^{-9} M). Panels A–F represent Cushing tumor no. 1–6, respectively. The y-axis represents percent of control (untreated as 100%). The x-axis represents samples: *filled bars,* control without treatment; *gray bars,* relative ACTH levels in the culture media after treatment; *open bars*, relative viable cell populations after treatment. Results are presented as percentage of control (mean ± SEM). *, $P < 0.05$, compared with control. NS, not significant.

our study showed that human corticotroph adenomas express multiple SSTR subtypes (11–15). We did not detect SSTR subtype 3, in agreement with Miller's data (13) and in disagreement with other studies (12, 14, 15). Overall, SSTR subtypes 1, 2, and 5 are the most frequently expressed receptors at the mRNA level in corticotroph tumors (11–15). The difference in the detection of receptor expression from one study to another may represent the heterogeneity of receptor expression in these tumors at the mRNA level and differences in the efficiency of the techniques and methodology used to detect these receptors.

All SSTR subtypes were detected by IHC. However, there were differences in the intensity of the immunostaining as well as in the number of positive cells. Although IHC does not provide information about the binding ability of the tumor receptor subtype, in the majority of the cases in our

TABLE 3. SOM230 effect on ACTH secretion by human corticotroph adenomas

Tumor no.	Tumor size (mm)	Time response (h)	Analog concentration (M)	ACTH levels (pg/ml)		% Reduction
				Control	Treatment	
1	15 mm (invasive)	96	SOM^{-8}	33,324 ± 800	22,887 ± 870	31[a]
2	7 mm	96	SOM^{-8}	85,087 ± 2,303	44,777 ± 430	47[a]
3	5 mm	96	SOM^{-9}	4,050 ± 15	2,150 ± 5	47[a]
4	8 mm	72	SOM^{-9}	5,993 ± 146	4,283 ± 210	29[a]
5	6.5 mm	72	SOM^{-8}	36,585 ± 5	28,347 ± 1,093	23
6	>10 mm (invasive)	48	SOM^{-8}	23,888 ± 1,567	10,626 ± 1,864	56[a]

Values are expressed as mean ± SEM. Time represents maximal inhibition on ACTH (corticotropin) secretion. To convert values for corticotropin (pg/ml) to picomoles per liter, multiply by 0.2202.

[a] Statistically significant difference ($P < 0.05$) when compared with control values.

series, SSTR subtype 5 had the highest receptor protein expression by IHC. Complete or incomplete membranous as well as cytoplasmic immunostaining was observed by IHC in all tumors. This staining pattern was also observed in GH-secreting adenomas as well (27). The significance of an intracellular location of some of these receptors by IHC is not well understood, and further studies using electron microscopy might be helpful in assessing the exact location of SSTR subtypes in these tumor cells.

In our study, receptor expression by IHC did not always correlate with expression at the mRNA level. This interesting dissociation can be to due to several reasons. First, *in vivo* and *in vitro* studies with rodent pituitary cell cultures showed that glucocorticoid suppression of SSTR mRNA expression levels can be transcriptionally mediated (by decreased gene expression) or posttranscriptionally mediated (by decreased mRNA stability) (19, 35). Changes in mRNA expression levels may not necessarily correlate with altered protein levels due to changes in mRNA stability, translational efficiency, and protein clearance. Although these studies were performed in rodent pituitary cell lines, they may explain the heterogeneity of SSTR expression at the mRNA level in corticotroph tumors as well. Second, in our series, to enhance the detection of these receptors at the protein level, several amplification steps were performed during the IHC. It is likely that our IHC method was sensitive enough to detect SSTR protein expression at a very low level. To our knowledge, there is only one previously published study that evaluated the protein expression of SSTRs in corticotroph tumors by IHC. In agreement with our IHC results, in this study by Unger *et al.* (36), all five SSTRs were detected by IHC in the majority of the tumors examined.

In our series, SOM230 significantly suppressed ACTH secretion in five out of six tumors, with the percent suppression ranging from 23–56%. This percent suppression is similar to the *in vitro* effects of SST analogs in suppressing GH secretion in human somatotroph, in which suppression ranged from 22–73% (24, 37). Of interest, in a previously published study where normalization of IGF-I levels was observed in four out of seven patients with acromegaly treated with an SST analog, tumors from these patients were cultured *in vitro*, and a SST analog suppressed GH release by 23–43% (24). Thus, it is possible that patients with CD may have a clinically significant response to SOM 230 based on these *in vitro* results, although of course only the results of clinical studies can prove treatment efficacy.

In vitro studies and animal models implicate SSTR subtype 2 and 5 in the regulation of ACTH release, and SSTR subtypes 2 and 5 knockout mice resulted in increased ACTH levels (15–19, 38, 39). The mechanism by which SOM230 inhibits ACTH release in human corticotroph tumors might require further study. SOM230 has binding affinity for SSTR subtype 2 and 5. However, considering that SOM230 has a higher binding affinity for SSTR subtype 5 and that this receptor is abundant in corticotroph tumors, SSTR subtype 5 may play a more important role than subtype 2 in the suppression of ACTH release in these tumors, although the overall effects of SOM230 are likely due to the combination of all SSTR subtypes.

We observed dissociation between suppression of cell proliferation and ACTH secretion by SOM230. Our *in vitro* study of SOM230 effects on tumor cell proliferation showed no correlation with effects of SOM230 on ACTH secretion in all cases. These *in vitro* data suggest that SOM230 suppression of cell proliferation and ACTH secretion may occur through independent mechanisms, similar to the dissociation between the effects of SST analogs on GH secretion and tumor growth suppression in human somatotroph adenomas (24).

In conclusion, the results of our experiments show that SOM230 has antiproliferative effects on human corticotroph tumors in addition to antisecretory effects. These *in vitro* data support the hypothesis that SOM230 may be a potential candidate for the medical therapy of a subset of patients with CD, particularly those patients with unresectable adenomas and Nelson's syndrome. Studies in patients with CD will be needed to assess any potential therapeutic role of SOM230 in this disease. The molecular mechanism by which SOM230 induces suppression of cell growth and ACTH release and the role of specific SSTRs in transducing growth inhibitory signals in corticotroph tumors require further study.

Acknowledgments

Received June 12, 2006. Accepted August 18, 2006.

Address all correspondence and requests for reprints to: Anne Klibanski, M.D. Neuroendocrine Unit, Massachusetts General Hospital, 55 Fruit Street, BUL457, Boston, Massachusetts 02114. E-mail: aklibanski@partners.org.

This work was supported by Novartis Pharma, Basel, Switzerland (to A.K.), and by the Intramural Research Program of the National Institutes of Health, National Institute of Child Health and Human Development Grant HD-000642-04 (to C.A.S.).

Disclosure statement: D.L.B., X.Z., R.G., P.J.A., Y.Z., S.A.J., E.T.H-.W., and C.A.S. have nothing to declare. B.S. has equity interests and has received lecture fees from Novartis. A.K. has received lecture fees and consults for Novartis.

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