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Effects of selective somatostatin analogs and cortistatin on cell viability in cultured human non-functioning pituitary adenomas

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Abstract

Clinically "non-functioning" human pituitary adenomas (NFPA) constitute about 35% of pituitary adenomas. Somatostatin receptors (SSTR) expression in these adenomas has previously been described both *in vitro* and *in vivo*, without evidence for a correlation with tumor volume or the therapeutic efficacy of somatostatin analogs.

This study was performed on 13 surgically removed pituitary macroadenomas, diagnosed before surgery as "non-functioning". In addition, 3 growth hormone (GH)-secreting adenomas served as controls. A specimen from each tumor was dispersed and digested to isolate and culture the tumor cells, and the *in vitro* effects of SSTR2 and SSTR5 selective analogs and Cortistatin (CST) (100 nM) on cell viability were studied. The quantity of viable cells was estimated using the XTT method. RNA purification of tumor samples and subsequent RT-PCR studies for SSTR2 and SSTR5 expression were performed.

Somatostatin analog with high affinity for SSTR2 reduced cell viability by 20–80% in 8 of 13 NFPAs studied, all expressing the SSTR2. The inhibitory effect on cell viability of SSTR5-selective analog was 15–80% in 10 of 13 NFPAs studied, all but three expressing the SSTR5. CST, however, effectively reduced cell viability in only 6 NFPAs. Cell viability was inhibited by all peptides studied in 2 out of 3 GH-secreting adenomas, expressing both receptors. The third adenoma responded to SSTR2 analog and expressed only SSTR2.

These results suggest the involvement of SSTR2 and SSTR5 in the anti-proliferative effects of somatostatin; however, CST is less potent in reducing cell viability in these tumors.

Keywords: Non-functioning adenomas; Somatostatin; Cortistatin; Cell viability

1. Introduction

Somatostatin, known as somatostatin release-inhibiting factor (SRIF), is a cyclic tetradecapeptide secreted from the hypothalamus and peripheral tissues (Brazeau et al., 1973), and is a potent inhibitor of endocrine and exocrine tissue secretion, an important regulator of cell differentiation, growth and proliferation, and functions as a neuromodulator in the central nervous system. In the pituitary, somatostatin is a known inhibitor of growth hormone (GH) and thyroid-stimulating hormone (TSH) secretion (Lamberts, 1988). Somatostatin occurs naturally in two major forms: a tetradecapeptide (SRIF-14), and a 28 amino acid form (SRIF-28).

Somatostatin exerts its biologic effects through at least five different high affinity G protein-related receptors (SSTRs), which are widely expressed in human tissues (Reisine and Bell, 1995; Patel, 1997). SSTRs have been identified in all somatostatin target tissues, including the anterior pituitary gland. In humans, the fetal pituitary shows mRNA expression of SSTR1, 2, and 5 (Shimon et al., 1997). In the adult normal human pituitary, SSTR1, 2, and 5 are usually expressed (Miller et al., 1995; Panetta and Patel, 1995), whereas SSTR4 is absent. Miller et al.

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showed expression of SSTR1, 2, and 5 in non-functioning pituitary adenomas and also in hormone-secreting adenomas (Miller et al., 1995). Panetta and Patel also studied mRNA expression of SSTRs in secreting and non-functioning adenomas and demonstrated SSTR1 and SSTR2 in most adenomas while SSTR3, 4 and 5 were expressed in about half of the adenomas (Panetta and Patel, 1995). Jaquet et al. (2000) showed a large predominance of SSTR5 over SSTR2 mRNA in GH-secreting adenomas using RT-PCR quantitative analysis. Thus, SSTR2 and SSTR5 are expressed in almost all GH-secreting tumors. These receptors are also identified in many non-functioning pituitary adenomas.

Cortistatin (CST) is a neuropeptide expressed mainly in the brain cortex, with high homology to somatostatin (de Lecea et al., 1996). It shares many functional and pharmacological properties with somatostatin, although they are encoded by different genes (de Lecea et al., 1996, 1997). In addition to the brain cortex, its precursor prepro-CST mRNA is also expressed in the hippocampus, the adenohypophysis, and in peripheral tissues. CST-14, the rat homolog, binds to SSTRs on GH4 pituitary cells (de Lecea et al., 1996), and CST-17, the human homolog, binds to SSTRs overexpressed in Chinese hamster ovary cells (Fukusumi et al., 1997). We have recently shown the *in vitro* effects of CST on human GH and PRL release. CST suppressed both GH and PRL secretion in cultured pituitary tissues (Rubinfeld et al., 2006). The regulation of PRL release from cultured adenomas by CST appeared to be primarily mediated by SSTR5.

Clinically "non-functioning" human pituitary adenomas (NFPA) constitute about 35% of pituitary adenomas. Usually, these are large and invasive macroadenomas with considerable growth potential and surgical treatment of these tumors is not always satisfactory. Unlike for GH-, TSH- and PRL-secreting adenomas, no effective medical therapy is available for these common adenomas. The clinically available somatostatin analogs, octreotide and lanreotide, are poorly effective for the non-functioning adenomas. We thus studied the *in vitro* effects of SSTR2- and SSTR5-selective analogs and CST on cell viability of 13 surgically removed non-functioning pituitary macroadenomas. In addition 3 GH-secreting adenomas served as controls.

2. Material and methods

2.1. Peptides

Cortistatin-17 (human; IC_{50}, 0.4–0.6 nM for all SSTRs (Fukusumi et al., 1997)) was available from Bachem AG (Bubendorf, Swizerland). SST-14 was available from Sigma (St. Louis, MO). BIM-23120 and BIM-23206, somatostatin analogs selective for SSTR2 and SSTR5, respectively, were obtained from Biomeasure, Inc/IPSEN Group (Milford, MA). The specific binding affinities of BIM-23120 (for human SSTR2; $IC_{50,}$ 0.34 nM) and BIM-23206 (for human SSTR5; $IC_{50,}$ 2.4 nM) were determined by radioligand membrane receptor binding assays, as previously described (Ren et al., 2003).

2.2. Patients

Specimens of pituitary adenomas, diagnosed before surgery as "clinically non-functioning" and GH-secreting adenomas, were obtained during transsphenoidal surgical resections, after informed consent was provided by the patients. The clinical characteristics of the patients are presented in Table 1. All tissues were placed in culture medium for cell culture studies, and were also snap-frozen for RNA assays. Another part of each specimen was immunostained for pituitary hormones (Table 1). Noteworthy, most specimens expressed β-FSH and/or β-LH. However, NFPA12 expressed also GH, PRL and TSH and may be considered also as silent hormone expressing adenoma.

2.3. Cell viability

The specimens were mechanically dispersed and enzymatically dissociated using 0.35% collagenase and 0.1% hyaluronidase as previously described (Rubinfeld et al., 2006). For measuring cell viability, $\sim 5 \times 10^4$ cells were seeded in 96-well tissue culture plates and incubated for 48 h in low-glucose DMEM supplemented with 10% FCS supplemented with either SSTR-analogs

Table 1
Clinical characteristics, immunohistochemistry and SSTR-mRNA expression of patients with pituitary adenomas

Tissue	Size (mm)	Gender	Age (year)	Immunohistochemistry						SSTR2	SSTR5
				β-FSH	β-LH	ACTH	PRL	GH	TSH		
NF1	22	f	70	+	+	–	–	–	–	+	–
NF2	20	m	46	+	+	–	–	–	–	+	+
NF3	20	m	58	+	–	–	–	–	–	+	–
NF4	15	f	83	+	–	–	–	–	–	NA	NA
NF5	Macroadenoma	f	65	–	–	–	–	–	–	+	+
NF6	Macroadenoma	f	59	+	+	–	–	–	–	+	–
NF7	Macroadenoma	f	77	–	–	–	–	–	–	+	–
NF8	40	m	37	–	–	–	–	–	–	+	+
NF9	16	m	48	+	–	–	–	–	–	+	–
NF10	Macroadenoma	f	71	+	+	–	–	–	–	+	–
NF11	Macroadenoma	f	44	+	+	–	–	–	–	+	+
NF12	16	f	53	–	–	–	+	+	+	+	+
NF13	35	m	74	+	+	–	–	–	–	+	+
GH1	8	m	37	–	–	–	–	+	–	+	–
GH2	13	f	49	–	–	–	–	+	–	+	+
GH3	7	f	36	–	–	–	+	+	–	+	+

NA, not available.