EDITORIAL

Antiproliferative effect of somatostatin analogs in gastroenteropancreatic neuroendocrine tumors

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Abstract

Somatostatin analogs were initially developed for the control of hormonal syndromes associated with neuroendocrine tumors (NETs). In recent years, accumulating data has supported their role as antiproliferative agents, capable of stabilizing tumor growth in patients with metastatic neuroendocrine malignancies, including carcinoid and pancreatic endocrine tumors. A phase Ⅲ, randomized, placebo-controlled trial has now demonstrated that octreotide long-acting repeatable (LAR) 30 mg can significantly prolong time to tumor progression among patients with metastatic midgut NETs regardless of functional status, chromogranin A level or age. In addition to significantly lengthening time to tumor progression in the overall study population, subset analysis suggests that patients with low tumor burden are most likely to experience disease stabilization with octreotide LAR 30 mg, supporting the early use of octreotide LAR in patients with metastatic disease. Further research efforts are underway to evaluate the use of somatostatin analogs as antiproliferative agents in other types of gastroenteropancreatic-NETs. Ongoing studies are also evaluating novel somatostatin analogs and somatostatin analogs in combination with other anti-tumor therapies.

INTRODUCTION

The human hormone somatostatin was first isolated in 1973 and identified as a hypothalamic inhibitor of growth hormone[1-4]. It was subsequently discovered in multiple tissues, including the central nervous system, endocrine system and gastrointestinal tract[3]. Somatostatin has been characterized as a universal endocrine "off-switch" due to its exocrine, endocrine, paracrine and autocrine inhibitory effects[5-7]. In the digestive tract, it reduces secretion and motility, decreases portal blood flow, inhibits gallbladder contraction and reduces the secretion of other gastrointestinal hormones[8]. The effects of somatostatin are mediated through interaction with five somatostatin receptors (sst$_{1-5}$)[9], belonging to a family of G-protein coupled receptors with seven transmembrane domains.

The clinical utility of native human somatostatin is limited by its short half life of approximately two minutes. Both bioactive forms of the hormone, the fourteen-peptide somatostatin-14 and a C-terminally extended form, somatostatin-28, contain multiple enzymatic cleavage sites resulting in rapid circulatory degradation[6]. In order to improve the pharmacokinetic profile, synthetic somatostatin

analogs (SSAs) have been developed by shortening the polypeptide chain while retaining binding affinity to somatostatin receptors (Figure 1)[10]. The two commercially available analogs, octreotide and lanreotide, are octapeptides that bind with high affinity to somatostatin receptor subtype 2 (sst_2) and with moderate affinity to sst_5 (Table 1).

Octreotide has been used in clinical practice since data emerged in the 1980s confirming its ability to palliate carcinoid syndrome[14], as well as other hormonal syndromes caused by metastatic gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Octreotide was initially available in an immediate-release formulation suitable for deep subcutaneous or intravenous administration[15]. Octreotide subcutaneous (sc) has been tested primarily at doses ranging from 100 to 500 μg two to three times daily. During the past decade, a long-acting repeatable (LAR) depot formulation of octreotide (Sandostatin LAR®) has been available, which allows monthly intramuscular dosing. Octreotide LAR has demonstrated similar efficacy to octreotide sc in the control of flushing and diarrhea associated with carcinoid syndrome[16]. The dose of octreotide sc and octreotide LAR should be titrated per symptom control for optimal patient care[17]. A second somatostatin analog, lanreotide, was licensed in Europe in 1998 for the treatment of symptoms associated with neuroendocrine (particularly carcinoid) tumors. A long-acting formulation of lanreotide (Somatuline Autogel®)[18] has also been developed as a deep subcutaneous injection.

Early on, clinical trials of SSAs tested their ability to inhibit the release of neuroendocrine hormones such as serotonin, glucagon, insulin, gastrin and vasoactive intestinal peptide (VIP)[14,19-22]. These trials formed the basis for the approval of octreotide and lanreotide as antisecretory agents indicated for treatment of hormonally active GEP-NETs. It was not until several years after the approval of octreotide that evidence of antineoplastic activity emerged. Although objective radiographic responses associated with SSAs were rare, many cases of prolonged disease stability were documented in the literature, leading to the hypothesis that SSAs exert an inhibitory effect on tumor growth. Recently, this hypothesis was tested in a Phase III, randomized, placebo-controlled clinical trial evaluating octreotide LAR 30 mg. This review summarizes the preclinical and clinical evidence supporting the role of SSAs as antiproliferative agents in the treatment of patients with GEP-NETs. To date, most data (including the results from the only phase III randomized, placebo-controlled trial) have been generated in studies evaluating octreotide sc and LAR.

BIOLOGICAL BASIS FOR THE ANTIPROLIFERATIVE EFFECTS OF SSAS

Over the past two decades there has been significant progress in our understanding of the molecular basis for the antiproliferative effects of somatostatin and its analogs. Antitumoral activity appears to be mediated *via* direct and indirect mechanisms. Direct mechanisms involve the
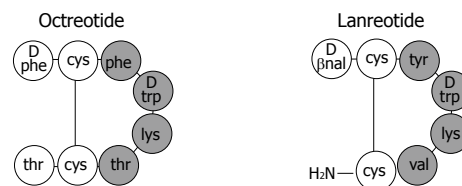

Figure 1 Chemical structure of the synthetic somatostatin analogs octreotide and lanreotide (adapted from[11]).

Table 1 Receptor binding affinities of somatostatin, octreotide and lanreotide

	sst_1	sst_2	sst_3	sst_4	sst_5
Receptor binding affinity (IC_{50} nmol/L)					
Somatostatin[12]	0.93	0.15	0.56	1.50	0.29
Octreotide[12]	280.00	0.38	7.10	> 1000	6.30
Lanreotide[13]	> 1000	0.80	107	> 1000	5.20

Table 2 Receptor mediation of cell proliferation

	sst_1	sst_2	sst_3	sst_4	sst_5
Induction of G1 cell cycle arrest	+	+		+	+
Induction of apoptosis		+	+		

activation of somatostatin receptors on tumor cells leading to modulation of intracellular signaling transduction pathways. Multiple *in vitro* studies using cell lines transfected with somatostatin receptors indicate that all receptor subtypes (sst_{1-5}) may mediate inhibition of cell proliferation[23], whereas specific receptor subtypes ($sst_{2,3}$) may mediate apoptosis (Table 2)[24-26]. These actions appear to be regulated primarily *via* the MAP-kinase signaling pathway and through activation of phosphotyrosine phosphatases (Figure 2)[27-29]. Indirect antiproliferative mechanisms include inhibition of mitogenic growth factors such as insulin-like growth factor (IGF), as well as inhibition of tumor angiogenesis through interaction with somatostatin receptors on endothelial cells and monocytes[30].

Activation of phosphotyrosine phosphatases
Several phosphotyrosine phosphatases (PTPs), including SHP-1 and SHP-2, have emerged as important regulators of intracellular signaling pathways[27]. Somatostatin receptor-mediated activation of SHP-1 results in arrest of cell proliferation in various cell lines, including cells derived from pancreatic, breast and prostate carcinomas[31,32]. In pituitary adenoma cells, activation of sst_2 inhibits PI3 kinase activity and causes cell growth arrest *via* stimulation of SHP-1[33]. The enzymatic activity of SHP-1 has also been implicated in sst_3-dependent apoptosis in transfected Chinese Hamster Ovary (CHO) cells[34]. Stimulation of SHP-1 in sst_2-expressing CHO cells has led to G1 cell cycle arrest *via* induction of the cyclin-dependent kinase inhibitor p27[35]. SHP-2 has also been identified as a mediator of the antiproliferative effects of somatostatin receptors,
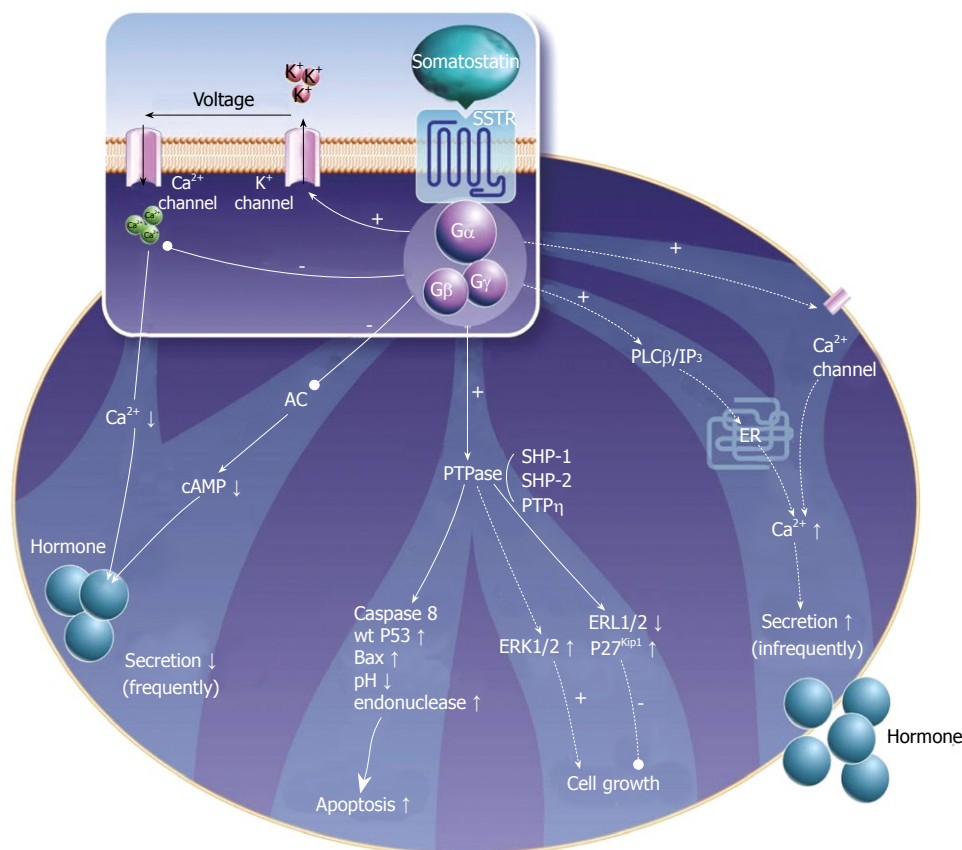
Figure 2 Somatostatin receptor-mediated effects on neuroendocrine cells (adapted from[23]).

primarily through inactivation of tyrosine kinase receptors for insulin and epidermal growth factors[36]. Moreover, activation of PTPs has been shown to down-regulate Raf-1[37] and block the MAP-kinase pathway[38].

Modulation of the mitogen activated protein-kinase pathway

Both inhibition and stimulation of the mitogen activated protein (MAP)-kinase pathway have been linked to the antiproliferative effects of somatostatin and its analogs. In a glioma cell line, the receptor-like PTP, PTPeta, mediated the antiproliferative effects of somatostatin through inhibition of ERK1/2[39]. Conversely, another study of sst1-expressing CHO cells demonstrated that somatostatin robustly activated MAP-kinase, which in turn enhanced the expression of the cyclin-dependent kinase inhibitor p21, thereby inhibiting cell proliferation[40]. Another study in CHO cells demonstrated that activation of p38 MAP-kinase *via* sst2 and sst4 mediated the inhibitory effects of somatostatin on fibroblast growth factor induced proliferation[41].

Indirect antiproliferative mechanisms

Suppression of tumor growth may occur *via* inhibition of various circulating growth factors, including insulin-like growth factor (IGF), epidermal growth factor (EGF) and growth hormone (GH). Inhibition of GH is thought to be mediated primarily *via* sst2 and sst5, which are strongly expressed in the anterior pituitary[42-44]. Octreotide has been shown to suppress circulating levels of IGF-1, both

via suppression of pituitary secretion of GH as well as through direct inhibition of IGF-1 production in the liver[45,46].

The antiangiogenic effects of octreotide have been demonstrated in multiple *in vitro* tumor models[47,48]. Octreotide has been shown to inhibit proliferating endothelial cells that over-express sst2 and sst5[49]. The primary mechanism of angiogenesis inhibition may be suppression of endothelial nitric oxide release[50]. Inhibition of circulating vascular-endothelial growth factor (VEGF) appears to also play a role in suppression of peritumoral vessel growth[51,52].

EARLY CLINICAL EVIDENCE FOR THE ANTIPROLIFERATIVE EFFECTS OF SSAS

Since the introduction of SSAs, multiple phase II trials and retrospective series have demonstrated that SSA treatment is associated with prolonged survival and disease stabilization in a large proportion of patients. For example, a single-institution retrospective study of 146 patients with metastatic mid-gut NETs, 91% of whom received long term octreotide treatment, demonstrated a 5-year survival rate of 75% (compared to 19% historically)[53]. Additionally, an analysis of the US-based Surveillance, Epidemiology and End Results (SEER) database found a significant increase in survival from 1988 to 2004 compared with 1973 to 1987, coinciding with the introduction of octreotide[54].

In general, early clinical studies evaluating the dis-

ease-stabilizing effect of SSAs in patients with GEP-NETs are characterized by their lack of randomized design and enrollment of heterogeneous populations of patients with GEP-NETs. Although objective radiographic response rates have been rare (generally < 5%), the rate of tumor stabilization observed in most studies has ranged from 40%-60%, with higher rates observed in patients without documented disease progression at onset of treatment[55].

Among the first prospective studies documenting the antiproliferative effects of SSAs in GEP-NETs was one conducted by the German Sandostatin Study Group[56]. In this study, 103 patients with metastatic carcinoid and pancreatic endocrine tumors were treated with octreotide 200 μg thrice daily until evidence of radiographic progression. Among patients who had disease progression documented at treatment outset, the rate of disease stability lasting at least 3 mo was 37%, whereas among patients with documented stable disease at treatment outset, disease stability lasting at least 12 mo was documented in 54% of patients[57]. No objective tumor responses were observed. Another phase II clinical trial testing octreotide as an antiproliferative agent in 34 patients with progressive metastatic NETs demonstrated a disease stabilization rate of 50% lasting a median of 5 mo[58].

The antiproliferative effect of intramuscular lanreotide SR 30 mg every 10 or 14 d was evaluated in a phase II trial of 46 patients with carcinoid and pancreatic endocrine tumors. Two patients (4%) achieved an objective radiographic response while 19 patients (41%) experienced stable disease for a mean duration of 9.5 mo[59]. In another phase II study of lanreotide SR 30 mg in 55 patients with GEP-NETs (48 with carcinoid tumors, six with gastrinomas and one with a VIPoma), 7% of 31 assessable patients achieved a partial response and 81% experienced disease stability[60]. In one study of patients with progressive tumors, participants received either octreotide LAR 30 mg or lanreotide SR 60 mg (this study considered all patients as a single cohort). Among 31 assessable patients, 14 (45%) achieved disease stability *vs* 55% who continued to progress radiographically[61]. Overall survival was considerably prolonged among patients with stable *vs* progressive disease. In multivariate analysis, pancreatic endocrine tumors appeared significantly less likely to achieve disease stabilization compared to intestinal carcinoid tumors. Extra-hepatic metastases were also associated with a poor prognosis. Table 3 summarizes the results of multiple non-randomized clinical trials evaluating the antineoplastic effects of octreotide and lanreotide in GEP-NETs.

THE PROMID TRIAL

Although providing initial evidence for the antitumor effects of SSAs, studies described in the previous section have a number of features that prevent them from providing conclusive evidence. Examples of these features include relatively small patient cohorts, lack of a randomized placebo control group, and analysis of heterogeneous populations. As such, to prove or to disprove an antipro-

Table 3 Summary of non-randomized clinical trials evaluating the antiproliferative effect of somatostatin analogs *n* (%)

Analog	Author	*n*	CR/PR	SD	PD
Patients with documented tumor progression					
Lanreotide	Faiss *et al*[62], 2003	22	1 (4)	7 (32)	14 (64)
Lanreotide	Aparicio *et al*[63], 2001	35	1 (3)	20 (57)	14 (40)
Octreotide	Arnold *et al*[64], 1993	52	0 (0)	19 (36)	33 (63)
Octreotide	Saltz *et al*[58], 1993	34	0 (0)	17 (50)	17 (50)
Octreotide	di Bartolomeo *et al*[19], 1996	58	2 (3)	27 (46)	29 (50)
		201	4 (1)	90 (45)	107 (53)
Patients without documented tumor progression					
Lanreotide	Wymenga *et al*[60], 1999	31	2 (6)	25 (80)	4 (13)
Lanreotide	Ducreux *et al*[59], 2000	39	2 (5)	21 (54)	16 (41)
Lanreotide	Eriksson *et al*[65], 1997	19	1 (5)	12 (63)	6 (32)
Lanreotide	Tomasetti *et al*[66], 1998	18	0 (0)	14 (77)	4 (22)
Octreotide	Tomasetti *et al*[67], 2000	16	0 (0)	14 (87)	2 (12)
Octreotide	Ricci *et al*[68], 2000	15	1 (6)	6 (40)	8 (53)
		138	6 (4)	92 (67)	40 (29)

CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease.

liferative effect of octreotide LAR 30 mg, the PROMID (Placebo-controlled, Prospective, Randomized study in patients with metastatic neuroendocrine midgut tumors) study was initiated. This randomized, double-blind, placebo-controlled, phase III trial, was among the very few randomized trials performed in patients with this rare tumor type. To avoid confounding variables, only patients with well-differentiated inoperable or metastatic midgut tumors were included. Additionally, octreotide LAR 30 mg was the only dose of octreotide LAR evaluated.

High-level evidence of the antiproliferative effects of octreotide emerged after publication of the PROMID trial[69]. Eighty-five participants with well-differentiated carcinoid tumors originating in the distal intestine and proximal colon were randomized to receive either octreotide LAR 30 mg or placebo until radiographic evidence of progression or death. The primary endpoint was time to tumor progression. Most patients (75%) had evidence of somatostatin receptor expression as evidenced by radiotracer uptake on Octreoscan. Nearly half of patients (38%) manifested the carcinoid syndrome (flushing and/or diarrhea associated with elevation in urine 5-HIAA). Only patients with mild carcinoid syndrome who tolerated flushing without intervention or responded to treatment with loperamide and/or cholestyramine in cases of diarrhea were included.

Median time to tumor progression was 14.3 mo in the octreotide LAR 30 mg group *vs* 6.0 mo in the placebo group (*P* = 0.000072, Figure 3). This significantly lengthened time-to-tumor progression was seen in the overall study population, regardless of tumor functionality, chromogranin A level or age. At 6 mo, tumor progression was observed in 24% of patients on the octreotide LAR 30 mg arm *vs* 66% of patients receiving placebo (*P* = 0.0079). Serious adverse events were nearly evenly balanced (11 patients in the octreotide LAR 30 mg arm and 10 patients in the placebo arm). On multivariate analysis, the highest rates of disease stabilization were observed in patients
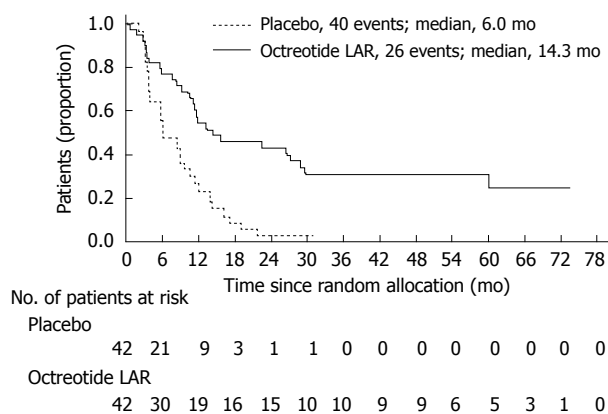
Figure 3 Kaplan Meier curve demonstrating time to tumor progression in patients treated with octreotide long-acting repeatable (LAR) *vs* placebo[69]. Log-rank test stratified by functional activity: *P* = 0.000072, HR = 0.34 (95% CI: 0.20-0.59).

with low hepatic tumor load (< 10%) and resected primary tumor, however both of these subgroups contained the majority of study patients. Even patients with higher hepatic tumor burden (> 10%) experienced a near doubling in time to progression on the octreotide LAR arm of the study. The small number of deaths in both treatment arms (seven in the octreotide LAR 30 mg arm; nine in the placebo arm) precluded any analysis of differences in survival.

FUTURE DIRECTIONS

Novel somatostatin analogs

NETs generally express multiple somatostatin receptors[13,70], all of which may mediate the antiproliferative effects of SSAs. These receptor subtypes can undergo heterodimerization with each other and with other receptor families (such as the dopamine receptor family), enhancing their binding affinities and internalization[71,72]. Thus, novel SSAs that bind to multiple receptor subtypes as well as analogs capable of binding to different families of receptors may prove to be effective antisecretory and antiproliferative agents in patients refractory to octreotide or lanreotide.

Pasireotide is one such novel somatostatin analog; it binds avidly to four of the five somatostatin receptors ($sst_{1,2,3}$ and sst_5). Compared with octreotide, pasireotide has a 40-, 30- and 5-fold higher binding affinity for sst_5, sst_1 and sst_3, and a slightly lower affinity for sst_2[73]. Pasireotide also has a 2-times higher binding affinity for sst_5 than endogenous somatostatin[73]. In an *in vitro* study evaluating the use of octreotide and pasireotide on HEK293 cells expressing somatostatin receptor subtype sst_2 on the cell membrane, treatment with octreotide resulted in an internalization of sst_2 receptors at 30 min whereas treatment with pasireotide did not lead to sst_2 internalization. Such findings may suggest that a persistent and more durable efficacy could be obtained with pasireotide[74].

An open-label trial evaluated the activity of pasireotide sc in patients with carcinoid syndrome whose symptoms (flushing and diarrhea) were inadequately controlled with octreotide LAR[75]. Preliminary data indicated activity in this refractory population. Future clinical trials are being designed to test the antiproliferative effects of pasireotide in neuroendocrine carcinomas. Other compounds capable of interacting with sst_2 as well as with the dopamine D2 receptor (DAD2) are in clinical development[76].

Radioactive labeling of SSAs is another promising approach to treatment of neuroendocrine malignancies which express high levels of somatostatin receptors. Early clinical trials employing ^{111}In-pentetreotide produced limited objective responses, probably due to the small particle range and short tissue penetration of Auger electrons emitted by the ^{111}In isotope[77]. The next generation of radiolabled SSAs used ^{90}Y-DOTATOC, a β-particle emitter with a tissue range of approximately 12 mm[78-80]. Objective response rates of 10%-30% were reported in phase Ⅰ and Ⅱ clinical trials. Dose-limiting side effects included bone marrow and renal toxicity.

The latest research efforts in radiolabeled SSAs have focused on ^{177}Lu octreotate, a β-and γ-emitting radionuclide with a shorter range of tissue penetration (2 mm) than [90]Y. A recent phase Ⅱ clinical trial reported an objective radiographic response rate of 30% among 310 patients with GEP-NETs, and a median progression-free survival duration of 40 mo[81].

CONCLUSION

SSAs were initially developed as antisecretory agents used primarily for the control of hormonal syndromes associated with NETs. In recent years, accumulating laboratory and clinical data has supported their role as antiproliferative agents, capable of stabilizing tumor growth in a large proportion of patients with metastatic carcinoid and pancreatic endocrine tumors. The recently-published PROMID study provides high-level evidence validating the role of octreotide LAR 30 mg as an antiproliferative agent in patients with metastatic carcinoid tumors of the midgut. Subset analysis suggests that patients with low tumor burden are most likely to experience disease stabilization, supporting the early use of octreotide LAR 30 mg in patients with metastatic disease. Further research efforts are underway to evaluate the use of novel SSAs, SSAs as antiproliferative agents in other types of GEP-NETs, and SSAs in combination with other anti-tumor agents.

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