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Somatostatin receptor ligands in acromegaly: clinical response and factors predicting resistance

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Abstract
Introduction Somatostatin (SST) receptor ligands (SRL), in particular those of first generation (Octreotide and Lanreotide), are widely used in medical treatment of acromegaly, but they assure biochemical control of disease (and the possibility of an improvement of clinical symptoms and tumor shrinkage), only in a subset of patients.
Discussion The mechanisms underlying the so called "SRL resistance" are various and involve in particular SST receptor expression and molecular pathways of signal transduction. Different predictors of SRL response have been reported, including clinical and biochemical features (gender, age, growth hormone and insulin-like growth factor-I levels at diagnosis), and tumor characteristic (both at preoperative magnetic resonance imaging study and histopathology) as well as expression of SST receptors. In some cases, only a "partial resistance" to SST can be detected, probably due to the presence of other impaired molecular mechanisms involved in signal transduction, which compromise specific pathways and not others. This may explain some cases of dissociated response between biochemical control and tumor shrinkage.

Keywords Somatostatin receptors ligands · Acromegaly · Resistance · Octreotide · Lanreotide · Pasireotide

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Introduction

With the discovery of somatostatin (SST) in hypothalamic extracts in the early 1970s, the understanding of the regulation of growth hormone (GH) secretion and consequently the opportunity to develop drugs mimicking SST action, the scenario of medical treatment of acromegaly has changed extraordinarily.

First-generation somatostatin receptor ligands (SST ligands, SRL) Octreotide and Lanreotide represent for most endocrinologists the first-line medical treatment for patients affected by acromegaly in whom surgery fails to control the disease or cannot be considered. The response rate of medical treatment, in terms of control of GH hypersecretion and restoring normal serum insulin-like growth factor-1 (IGF-1), varies in the different studies. While initial evaluations reported a biochemical response rate as high as 70–80 % of patients [1], more recently prospective randomized trials showed much lower success rate (20–30 %). These discrepancies probably derive from different criteria used in patient selection, as heterogeneous patient populations, analyses that exclude treatment non-responders, contribute to obtain higher success rates [1]. Recently, Pasireotide, a new SRL with broader receptor ligand binding profile, has been shown to be superior in normalizing IGF-1 in treatment-naïve patients (38.6 vs. 23.6 %), at the expenses of worsening glucose control [2]. However, although SRL can effectively control hormonal hypersecretion in GH-secreting pituitary adenomas, significant differences in the efficacy of treatment are observed among patients. This may be due both to a differential expression of SST receptor (SSTR) subtypes among tumors, and to the development of mechanisms of resistance to SST.

In this review, the factors predicting clinical response and resistance to SRL in acromegaly will be discussed.

Use of SRL in acromegaly

The biological anti-proliferative effects of SST and, consequently, of SRL are mediated by their interaction with five SSTRs, called SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5, cloned between 1992 and 1994 [3]. SSTR2 exhibits alternate mRNA splicing at the 3′ end of the coding segment to produce two isoforms, SSTR2A and SSTR2B [4]. All five SSTRs have been identified in the central nervous system, gastrointestinal tract, endocrine glans, exocrine glands, and inflammatory and immune cells [5]. These receptors, belonging to the family of G-protein coupled membrane receptors, are encoded by genes localized on different chromosomes [6], and consist of a single polypeptide chain with seven transmembrane spanning domains: the extracellular domain contains the ligand binding sites, while the intracellular domain provides linkage to second messenger activation [7].

By coupling with G proteins, SSTRs inhibit adenylyl cyclase activity and some subtypes reduce calcium entry by modulating Ca^{2+} and K^{+} channels, thus reducing hormone secretion [8]. Both SSTR2 and SSTR5 mediate the anti-proliferative effects of SRL by tyrosine phosphatase activation and ERK1/2 phosphorylation inhibition, respectively, while SSTR2 and SSTR3 subtypes mediate apoptotic effects [9–11].

SRL have different affinity for each SSTRs subtype: Octreotide and Lanreotide bind preferentially to SSTR2 and SSTR5, with moderate affinity for SSTR3 and low affinities for SSTR1 and SSTR4 [12]; on the contrary, the more recent multireceptor ligand Pasireotide (SOM230), recently approved also for the treatment of acromegaly [13], binds with high affinity to subtypes SSTR1, SSTR2, SSTR3, and SSTR5 [14].

GH-secreting human pituitary tumors more commonly express SSTR2 (more than 95 %), followed by SSTR5 (more than 85 %), SSTR3 and SSTR1 (both expressed in more than 40 %). SSTR4 has rarely been found in GH-secreting pituitary tumors [15]. The predominant expression of SSTR2 and SSTR5 represents the basis for the successful clinical application of Octreotide and Lanreotide, which act by suppressing abnormal GH secretion by the pituitary adenoma, by inhibiting hepatic IGF-1 synthesis, and by controlling tumor growth [16].

The percentage control of acromegaly by SRL varies greatly in different clinical studies: the conventional doses of Octreotide LAR (20–30 mg/month intramuscular) normalize IGF-1 in 38–85 % and reduces GH $<$ 2.5 mcg/L in 33–75 % of patients [17]. Similar results are obtained with Lanreotide Autogel, at conventional doses of 60, 90 or 120 mg (38–80 % for GH and in 39–80 % for IGF-1) [18]. Tumor shrinkage has been documented in about 63 % of patients treated with Lanreotide Autogel [19] and in about 66 % of patients treated with Octreotide LAR [20].

The efficacy of Octreotide LAR appears to be generally similar to that of Lanreotide Autogel and slightly better than that of Lanreotide Slow Release [21]. Dose increases can offer a better control in patients who were inadequately controlled with conventional starting SRL doses, without significantly changing safety and adverse events.

Clinical predictors of "response" to SRL and resistance to SRL

Criteria of remission in acromegaly

Resistance to SRL treatment in acromegaly can be explained considering the notions of "biochemical resistance" and "tumor resistance". Criteria of biochemical remission in acromegaly include normalization of age- and gender-adjusted IGF-1 levels and random GH $<$ 1 mcg/L [22]. The measurement of GH can greatly vary depending in the assay: in fact, comparing GH values during a standard 75 g oral glucose tolerance test both in acromegaly and in healthy subjects using three different commercially available assays that were calibrated according to recommended GH standards, GH values may vary greatly [23]. Similar differences have been reported for IGF-1 measurements [24]. Moreover, discrepancies may occur between GH and IGF-1 levels. In particular, "high GH phenotype" (high GH and normal IGF-1) is more frequently reported in younger estrogen-sufficient females (suggesting a possible role of estrogen in this biochemical mismatch), while "high IGF-1 phenotype" is associated with worse acromegaly-related metabolic complications, suggesting that high IGF-1 is more indicative than high GH of persistently active disease [25].

Tumor resistance is defined as lack of hormonal normalization, and increase in tumor size or a tumor shrinkage less than 20 % compared with baseline volume [25]. While most studies report that biochemical control is significantly associated with tumor shrinkage [26], in some patients biochemical and tumor responses are dissociated [27]. A reduction of IGF-1 levels after 12 months and tumor shrinkage after 3 months [28, 29] are the major predictors of a further tumor shrinkage after 12 months of continuous SRL therapy.

A duration of therapy of least 12 months and dose maximization should be completed before considering SRL resistance [17].

Clinical predictors of responsiveness to SRL and SRL resistance

Several predictors of response to SRL in acromegaly have been identified, related both to patient and tumor characteristics.

Clinical factors predicting a better response to SRL are female gender, older age, and lower circulating IGF-1 and GH levels at diagnosis [21]. Interestingly, macroadenomas seem to respond, in terms of volume reduction, better than microadenomas in first line SRL treatment [25]. This is may be due in part to a methodological bias. Indeed, it is more difficult to evaluate tumor volume reduction for a microadenoma. Moreover, study designs are highly heterogeneous and employ different criteria to define tumor volume before and after therapy. Patients treated with first-line therapy with SRL achieve better shrinkage than those treated after unsuccessful surgery. If surgery is chosen as first line approach, the cure rate depends on the tumor size at presentation and is higher for microadenomas (about 80 %) than non-invasive or invasive macroadenomas (about 70 and 40 % respectively). Tumor invasiveness, which compromises the radicality of surgery, reduces remission rate both after surgery and in response to SRL [30]. Previous radiotherapy improves the chance of IGF-1 reduction on SRL while the effects on GH values are not significant [31].

Some histopathological predictors of SRL therapy have been reported. In particular, "densely granulated adenomas" are typically highly responsive to SRL, while "sparsely granulated" adenomas show a lower grade of response [32]. Interestingly, these histopathological patterns have been found to be correlated to a specific pre-operative radiological pattern at magnetic resonance imaging (MRI), thereby allowing pre-operative prediction of SRL responsiveness. Adenomas that are hypointense on T2-weighted images are more likely to be densely granulated and less invasive (and respond better to SRL), while T2 hyperintensivity suggests a sparse granulation pattern and a more invasive tumor [33]. Conversely, the use of somatostatin receptor scintigraphyis not helpful in predicting SRL responsiveness [34].

The main explanation for the occurrence of resistance to SRL in acromegaly is the absence or the reduced tumor density of SSTR, especially SSTR2 which mainly mediate Lanreotide and Octreotide activity [35]. Densely granulated tumors which, as mentioned before, show a better response to SRL, have higher SSTR2A expression than sparsely granulated adenomas [32]. Accordingly, GH suppression and tumor shrinkage induced by SRL correlates with SSTR2 mRNA levels, and Octreotide resistance in GH-secreting adenomas occurs due to a selective loss of SSTR2A [36]. However, other studies have found that the density of SSTR is poorly correlated with the in vitro effect of Octreotide on GH suppression [37]. Furthermore, some GH secreting tumors are resistant to SRLs despite high SSTR2 expression. In these cases, other factors acting on SSTR2 function may be involved. Recently, the activity of the beta-arrestins has been emphasized as important regulator on SSTR2 function: arrestins are proteins that bind to G-protein-coupled receptors, blocking further signaling and targeting receptors for internalization. Accordingly, low beta-arrestin expression and high SSTR2/beta-arrestin ratio are associated with responsiveness to long-term treatment with SRL [38]. Interestingly, compared with Octreotide, the multiligand SRL Pasireotide in vitro results in lower beta-arrestin recruitment and therefore the SSTR2 recycles rapidly to the plasma membrane after endocytosis in treated cells [14].

Recently, a role for cytoskeleton protein filamin A (FLNA) in SSTR expression in acromegaly has been demonstrated, suggesting a correlation between FLNA expression and responsiveness of pituitary adenomas to SRL. Low levels of FLNA, which cause loss of coupling of SSTR2 with downstream signal transduction molecules, were hypothesized to explain the resistance to SRL analogs in GH-secreting pituitary tumors even if in the presence of SSTR2 expression [39]. However, this mechanisms, and in particular the role of FLNA in regulating SSTR2 expression, has not yet been demonstrated in human somatotropinomas, and there are no studies to date demonstrating that FLNA expression could represent a predictor of SRL response.

Although SSTR2 expression seems to have the main role in regulating tumor sensitivity to SRL, other studies have underlined the role of SSTR5. SSTR5 activation has a synergistic effect with SSTR2 on GH release. As demonstrated for several different G protein-coupled receptors, activation by ligand induces SSTR dimerization (both homo- and hetero-dimerization), which results in modification of ligand binding affinity [40]. As a consequence, an absent or reduced expression of SSTR5 may explain the reduced sensitivity or resistance of a group of GH-secreting pituitary tumors to SRL even when SSTR2 is expressed. However, other studies have found that patients bearing tumors with high SSTR5 mRNA levels tend to respond less to SRL therapy [41]. The reason for this observation remains to be explained. An interesting hypothesis has been proposed by Taboada et al., who speculate that the high levels of SSTR5 expression may result into its association with other G-protein-coupled receptors, which may in turn modify the response to the ligand.

The resistance to SRL is not an all-or nothing phenomenon. Reduction, but failure to achieve complete normalization of GH and IGF-1 plasma levels despite at least 3–6 months of treatment with high doses of SRLs is

defined as biochemical "partial resistance" to SRLs [42]. Variable SSTR expression or reduced receptor density can be at the basis of this phenomenon, which also appears to be linked to a selective loss of SSTR2 expression. Moreover, high SSTR5 mRNA expression can be found in association with loss of SSTR2 mRNA expression in cases of partial resistance. However, exact mechanisms leading to "partial resistance" have still to be fully elucidated. In this context the role of both multireceptor ligand Pasireotide and dopamine-agonist Cabergoline, which can resolve some cases of partial resistance to SRLs independently of prolactin status, should be considered. Along this line, the possible efficacy of chimeric molecules directed towards multiple somatostatin and dopamine receptors is presently being investigated.

In some cases, SSTR1 expression could play a role in predicting GH response to SRL treatment. SSTR1 mRNA directly correlated with in vitro GH inhibition, and is associated to normalization of GH and IGF-1 levels on SRL [43]. However, the role of SSTR1 in the development of resistance to SRL remains to be clarified. SSTR desensitization has a less relevant role in SRL resistance, while reduced SSTR density can explain cases of partial resistance to SRLs [21].

Mutations in the coding sequence of SRL's and/or loss of heterozigosity (LOH) are very rare event. A germline missense mutation (R240 W) in the SSTR5 gene, located in a critical region for G protein coupling and causing failure of inhibition of GH release and cell proliferation, has been found in one acromegaly patient resistant to Octreotide [44]. Polymorphic variants in SSTR2 gene seem to have no role in determining SRL resistance of GH-secreting tumors, while T allele of rs34037914 single nucleotide polymorphism of SSTR5 predisposes to resistance to the antiproliferative effects of SRL, increasing both biological aggressiveness and the risk of post-surgical reoccurrence of pituitary tumors [45]. LOH at the SSTR5 gene locus occurs in about 10 % of pituitary tumors but is associated with a normal responsiveness to Octreotide [46]. Conversely, the expression of a truncated variant of SSTR5 (SST5TMD4) has been found to reduce the response to Octreotide, and to be associated with aggressive features and tumor invasiveness, even in the presence of a high expression of SSTR2 [47], suggesting its dominant-negative effect on SSTR2-mediated signaling. These molecular features are not routinely available to the clinician.

Beside alterations in SSTRs, defects in signaling pathways activated by these receptors might be involved in the pharmacological resistance. This hypothesis is supported by the occasional dissociation between the antisecretory and antiproliferative effects of SRL. It is possible that alterations in the coupling with a specific G protein could determine the lack of activation of a specific pathway without affecting the other signaling cascades. Indeed, there is a well-known association between activation of the GSP oncogene (through gain-of-function mutation of the GNAS1 gene) in about 40 % of somatotroph adenomas. These mutations result in increased resistance of guanosine triphosphate (GTP)-bound $G_{s\alpha}$ to hydrolysis, and a concomitant increase in adenylyl cyclase A activity, with constitutional activation of cyclic AMP pathways. Patients harboring these mutations have been reported to be more sensitive or SRL [48, 49].

Despite this association few data are available about a possible role of other G proteins in resistance to SRL, and even if a low expression of G_{i1-3} proteins has been reported in GH-secreting adenomas [50], a possible correlation with SRL responsiveness has not been evaluated.

Alterations in SSTRs signal transduction are at the basis of Octreotide-resistance (in terms both of biochemical control and tumor shrinkage) documented in presence of the germline mutations in aryl hydrocarbon receptor interacting protein (AIP) gene (*AIP*) (in familial isolated pituitary adenoma syndrome) [51], or in sporadic adenomas expressing low levels of AIP [52]. Patients harboring germline *AIP* mutations generally are male presenting young-onset, sparsely granulated GH-secreting pituitary tumors or GH/PRL mixed adenomas [53]. Inactivation of AIP is thought to determine tumorigenesis by defective G_i signaling [54] and, interestingly, its low expression is not associated with resistance to Pasireotide [55], suggesting that AIP is not involved in the signal transduction of SSTR5 subtypes. ZAC1 is a tumor suppressor gene which mediates AIP tumor suppressor activity. In a group of patients treated preoperatively with SRL, a positive correlation between treatment response (both IGF-1 normalization and tumor reduction) and ZAC1 immunoreactivity has been found [56].

Tumors with high Ki-67 expression appear to be more resistant to SRL [21]. Another molecular predictor of response to SRL action is the expression of the adhesion protein E-cadherin. Since adequate cell-to-cell adhesion is crucial for the epithelial phenotype of pituitary cells, loss of E-cadherin has been found to be associated with invasiveness and dedifferentiated phenotype in pituitary GH secreting adenoma, while E-cadherin protein expression correlates negatively to tumor size and positively to acute SRL response [57]. Finally, the MAPK signaling pathway is activated after binding to the somatostatin receptor. The phosphorylated Raf kinase inhibitory protein (RKIP) inhibits the internalization of the receptor and its degradation, increasing SRL responsiveness. Accordingly, RKIP levels inversely correlate to both the acute and the long-term SRL response in GH secreting adenomas, as low levels are associated to poor clinical response to SRL [58].

Regrettably, most of the above-mentioned molecular predictors are not routinely available in clinical practice.

Conclusions

First generation SRL Octreotide and Lanreotide still represent the mainstay of medical therapy for acromegaly patients, both after surgery in case of persistent disease, or as first choice therapeutic option if surgery is contraindicated. However, biochemical control and/or tumor shrinkage is not obtained in the totality of patients who, if non responders to adjusted SRL dose after a period of at least 12 months, can be defined as "resistant" to first generation SRL. Clinical predictors of response in patients with acromegaly include gender, age, initial GH and IGF-1 levels, tumor size, MRI characteristics and histopathology. Several molecular mechanisms, and not all fully understood, may contribute to resistance. Certainly, an important role is played by SSTR expression, but the role of other molecules which mediate or regulate SSTR activity and intracellular signaling is increasingly appreciated. The understanding of these physio-pathological mechanisms and the knowledge of factors predicting the response to SRL would be very useful in the clinical practice. In presence of SRL resistance, the new SRL Pasireotide should be considered, paying particular attention to worsening of glucose control. Recent immunohistochemical study showed that expression of SSTR5 might be predictive of responsiveness to Pasireotide. AIP deficient, and sparsely granulated-adenomas may benefit from this treatment [55].

Recent guidelines [22] have advocated the addition of a dopamine agonist (Cabergoline) or a GH receptor antagonist (Pegvisomant) to somatostatin analogue treatment to obtain a better clinical and biochemical control when partial resistance occurs. In the ever growing appreciation of the need of "personalized medicine", the ability of predicting SRL responsiveness will greatly help appropriate therapeutic planning for acromegaly patients [55].

Compliance with ethical standards

Conflict of interest Rosa Maria Paragliola and Salvatore Maria Corsello have declares that they have no conflict of interest. Roberto Salvatori serves in advisory board for Pfizer, Novo Nordisk, and Ionis Pharmaceutical, receives research support from Novartis, Pfizer, and Chiasma, and has received in the past research support from Ipsen.

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