### Title

Revealing and overcoming resistance to somatostatin analogues in real clinical practice

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### Abstract

Resistance to somatostatin analogues (SSA) can be defined as lack of the biochemical and tumoral response to the treatment which is carried out within 12 months. An achievement of target hormonal criteria of acromegaly treatment or at least depression of the GH and/or IGF-1 levels by >50% is considered to be the adequate biochemical response. Decrease of somatotropinoma volume by ≥20% (when using SSA as the first line of treatment) is considered as the tumoral response to treatment. According to the effectiveness of treatment patients can be classified as not resistant (biochemical control of acromegaly and tumoral response), partially resistant (some degree of biochemical and/or tumoral response) or fully resistant (neither biochemical nor tumoral response) to SSA therapy. The majority of patients (up to 60-70%) exhibits partial resistance when the first generation of SSA is used. Clinical and biochemical predictors of resistance to SSA include young age, male gender, high levels of GH/IGF-1, a large invasive sparsely granulated somatotropinoma with high Ki-67 and hyperintense T2-weighed MR-signal. In recent years various molecular and genetic predictors of resistance to SSA have been found which were also discussed in this review. The need to introduce a panel of tumor biomarkers in broad clinical practice for the personalized choice of drug treatment was emphasised. Treatment options for patients who are not controlled with first-generation SSA are dose escalation, combined treatment with SSA and cabergoline, switch to pasireotide or pegvisomant (not available in Russia yet); non-drug options include tumor debulking with the subsequent resumption of SSA treatment and radiosurgery/radiotherapy.

### Keywords

### Acromegaly, somatostatin analogues, resistance

Somatostatin analogues (SSA) appeared in an arsenal of opportunities of acromegaly treatment more than 30 years ago and significantly expanded the possibilities of achievement of control over this disease. Somatostatin analogues of the first generation – octreotide and lanreotide – proved to be an effective and safe method of treatment of acromegaly which can be used both for the reduction of the hormonal activity of a somatotropinoma (i.e. for the achievement of biochemical control) and for the reduction of the volume of a tumor [1-3].

Regulatory effects of native somatostatin are implemented through the somatostatin receptors (SSTR) interfaced to heterodimeric G-proteins and divided into 5 subtypes (SSTR1 – SSTR5). All SSTR subtypes are similar in terms of the structural and functional organization and are rather homologous in primary structure (39-57%) [4].

Interaction of somatostatin or its analogues with somatostatin receptors launches several intracellular signal cascades associated with different classes of heterotrimeric G-proteins:   
adenylyl cyclase inhibition, activation of K+-dependent channels and suppression of Са2+-dependent channels, stimulation of phosphotyrosine phosphatase activity and inhibition of activity of Na+/H+- metabolizable protein NHE1, stimulation of activity of phospholipase С and increase in concentration of intracellular calcium [4-6].

The combination of quantity and types of SSTR, as well as the type of G-protein associated with SSTR can vary in different tissues. In case of activation of SSTR of the 1st, 2nd and 4th subtypes Gβγ-dimers of G-proteins are released that leads to the activation of tyrosine kinases of Src-family and launches a cascade of the mitogen - activated protein kinases (MAPK). G-proteins also influence the intracellular mechanisms by way of close interactions between different signal cascades that are not affected directly – for example, those associated with MAPK, JAK-tyrosine kinases and different forms NO-synthases [4-6]. All these mechanisms provide the fundamental cellular processes such as growth, differentiation and apoptosis [4-6].

Native somatostatin binds to various subtypes of SSTR and has an influence on the hormonal secretion to various extents, blocking the cellular cycle of neuroendocrine cells and accelerating their apoptosis (see Tab. 1).

Table 1. Some physiological functions of somatostatin receptors

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | SSTR1 | SSTR2 | SSTR3 | SSTR4 | SSRT5 |
| Effects on hormones secretion | | | | | |
| Growth hormone |  |  |  |  |  |
| Insulin |  |  |  |  |  |
| Effects on the cellular growth of somatotroths | | | | | |
| Cellular cycle arrest |  |  |  |  |  |
| Apoptosis |  |  |  |  |  |

There is a slight difference in structure between SSA and native hormone, that allows prolonging their half-life. However it slightly changes the profile of binding of SSTR: SSA of the first generation, octreotide and lanreotide, are mainly bound with SSTR2 and to a lesser extent to SSTR5; the analogue of a somatostatin of the latest generation, pasireotide, is actively bound to SSTR2, SSTR3, SSTR5 and to a lesser extent – with SSTR1 (see Tab. 2.). Nevertheless when binding to the corresponding subtypes of SSTR they cause the same effects as somatostatin. Clinically it is shown by depression of synthesis and secretion of the growth hormone (GH) with the corresponding decrease of production of the 1 type insulin-like factor and the reduction of the volume of a somatotropinoma.

Table 2. Relative ability of somatostatin and its analogues of binding with SSTR

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | SSTR 1 | SSTR 2 | SSTR 3 | SSTR 4 | SSTR 5 |
| Somatostatin |  |  |  |  |  |
| Octreotide |  |  |  |  |  |
| Lanreotide |  |  |  |  |  |
| Pasireotide |  |  |  |  |  |

SSA are strongly recommended as an adjuvant therapy in patients with a persistent disease after a neurosurgical intervention [1-3]. However they are also successfully used as the first line of treatment of acromegaly, especially in patients with low probability of radical neurosurgical removal of somatotropinoma [7-9]. A vast clinical experience of use of SSA of the first generation, octreotide and lanreotide, has been accumulated. Octreotide and lanreotide have a crucial importance both in the world and Russian medicated treatment of acromegaly. The presented data imply the use of these particular SSA.

The response to the SSA therapy is estimated according to the GH and IGF-1 levels, reached during the treatment as well as according to the reduction of the size of somatotropinoma. The target hormonal indicators during the treatment of acromegaly have to be the GH and IGF-1 levels at which the chance of disability for the patients is minimized and the population life expectancy is restored. Besides, another important factor is the reduction of the sizes of somatotropinoma during the treatment. According to the data presented in various registers, 74-82% of patients with acromegaly suffer from pituitary macroadenomas [10-18], which is caused not only by the delayed diagnostics of a disease, but also by the properties of pituitary tumor itself. Therefore elimination and prevention of effects of the tumor are an important component of treatment of patients with an acromegaly.

In real clinical practice these objectives are achieved not for all the patients. During the treatment various possible responses to the treatment are observed, which consist of 2 main components: biochemical and tumoral response. According to the response to the treatment with analogues of somatostatin, the patients with an acromegaly can be classified into the following groups:

* not resistant – i.e. those who reached the target hormonal criteria of treatment and in whom significant reduction of the somatotropinoma volume was observed,
* partially resistant (or partially sensitive) – i.e. those who did not reached the target hormonal criteria of treatment, but demonstrated a significant decrease in GH / IGF-1 levels AND/OR in whom significant reduction of the somatotropinoma volume was observed
* fully resistant – there was no significant decrease in GH / IGF-1 levels AND the reduction of the somatotropinoma volume

*Full biochemical resistance* can be defined as an absence of a significant decrease in hormonal levels and an impossibility to reach biochemical control of acromegaly. Respectively the *tumoral resistance* can be defined as an absence of significant reduction of the volume of the tumor during the medicated treatment.

An important aspect of the assessment the treatment effectiveness is the determination of the target hormonal criteria of acromegaly treatment. The GH target level varies from <1 to <5 ng/ml in different research. Recently it has been shown that in order to restore the life expectancy of the patients with acromegaly the GH random level has to be ≤1 ng/ml since the GH level over this threshold is a reliable predictor of the increased mortality among the patients with acromegaly [19]. However this criterion can be applied only on condition of the calculation of the GH level with highly sensitive devices [3]. When using standard devices for the measurement of GH in many papers the target criterion of the biochemical control was the GH level ≤ 2-2.5 ng/ml [8-11, 15, 17, 18, 22, 24-26].

IGF-1 level is less important as a mortality predictor in comparison with the concentration of GH, but many clinical manifestations of acromegaly are associated to excess concentrations of IGF-1. Therefore in order to improve of quality of life and to reduce the symptoms the IGF-1 level has to be within gender and age values [1-3].

The characteristics of the laboratory methods and sensitivity of the devices used in diagnostics for the measurement of the hormonal levels play a very important role, since the hormonal levels can vary significantly [20]. The absence of standardization of the used laboratory techniques and test kits can also significantly influence the results of the effectiveness of the treatment.

The definition of "significant" reduction of the GH / IGF-1 levels and the tumor size a is a topical issue. The cut-off levels are factitious and empiric, nevertheless, most of experts highlight that a significant reduction for the hormonal levels is a decrease in their levels by >50% from the initial level, whereas for the tumor volume a significant reduction is by ≥20% [21, 22]. These criteria are used for determination of resistance to SSA (see Table 3).

Table 3. Criteria of measurement of sensitivity/resistance to SSA as the first-line treatment

|  |  |  |  |
| --- | --- | --- | --- |
| Answers | Biochemical control | | Tumor shrinkage by ≤20% |
| Achievement of target levels of GH and IGF-1 | GH and/or IGF-1 reduction by  >50% |
| not resistant | YES | Not applicable\* | YES |
| partially resistant | YES | Not applicable\* | NO |
| NO | YES | YES |
| NO | YES | NO |
| fully resistant | NO | NO | NO |

\* - the degree of GH and IGF-1 reduction does not matter if the target hormonal levels of the treatment has been achieved

The analysis of the results of the application of somatostatin analogues of the first generation – octreotide and lanreotide – shows a considerable variability of the biochemical response: the target hormonal levels were reached with the frequency from 20 to 75% of cases in different studies [22, 23]. Why the difference is so great? The reasonable explanation is the distinctions in many parameters of study design among which it is possible to mention the features of the patients selection, different target hormonal levels (the GH level, or the IGF-1 level, or the GH+IGF-1 levels); retrospective or prospective study; analysis of intention-to-treat versus per protocol population; fixed time point versus response at last follow-up; treatment duration; previous experience of SSA use before including into the study; the highest possible doses of SSA or lower ones without a possibility of a titration were used; if standard or highly sensitive assays were employed for hormonal diagnostics; SSA were applied as primary or secondary line of therapy, etc.

Prospective studies with the inclusion in the analysis of all the patients who earlier did not use SSA, and the evaluating final levels of both GH and IGF-1 are associated with lower frequency of the biochemical response [24]. The use only one hormonal criterion as a final point, the exclusion from the analysis of the patients who did not respond to the treatment and the previous use of SSA, on the contrary, can lead to higher rates of effectiveness [24].

The review of foreign studies of the recent years shows that during the treatment octreotide of long-acting release the GH level ≤ 2.5 ng/ml was reached in 60% of patients and normalization of the IGF-1 level was noticed in 59% of participants, during the treatment with lanreotide autogel – 62% and 49%, respectively [24]. In a number of patients a dissociation of the biochemical response to the treatment with SSA is noticed: achievement of the GH target level with the sustained increase in IGF-1 levels, or normalization of the IGF-1 level with concentration of GH > 2.5 ng/ml.

It explains why when using a combination of two target hormonal criteria the frequency of the biochemical response is slightly lower and on average is equal to 50% (for the GH level ≤ 2,5 ng/ml). These data correspond with the results of the Russian the prospective studies of the effectiveness of octreotide depot in the treatment of patients with acromegaly, according to which the treatment within 12 months led to a complete biochemical control in 50-55% of patients (the target GH level was considered ≤ 2.5 ng/ml). The partial biochemical response was observed in 20-23% of patients, and 21-22% of patients showed biochemical resistance [25, 26].

To understand which factors can influence the biochemical response during the treatment with SSA, a special meta-analysis devoted to the influence of design of studies on the biochemical effectiveness of the treatment with SSA was conducted. It included various research with participation of not less than 10 patients who received the treatment for not less than 3 months, the results of which were published from 1974 to 2012 in English in the journals indexed by the PubMed system (the total number of the participants was 4464) [27]. In general, according to this meta-analysis, the GH target levels and normalization of the IGF-1 level were reached in 56% and 55% of patients respectively.

No statistically significant influence was caused by the following factors: the number of the centers conducting the research; retrospective or prospective study; octreotide or lanreotide were used; prior use of SSA; whether there was a switch from one type of SSA to another; what doses of the medicine were used. At the same time the treatment duration significantly influenced the achievement of target levels for both GH (р<0.001) and IGF-1 (р =0.02); the previous investigation of SSA and the year of the publication were significant factors for the biochemical monitoring of GH (р =0.01 and р =0.03 respectively), but did not matter for the IGF-1 level [27]. These factors must be considered in case of interpretation of data on the frequency of biochemical resistance to SSA in the treatment of an acromegaly.

Tumor shrinking is observed more often than the achievement of a complete biochemical control. The foreign reviews devoted to the influence of SSA therapy on tumor volume in patients with acromegaly showed that considerable (≥ 20% of initial) reduction of volume is noticed in 55-75% of the patients who received octreotide of long-term release as the first line of treatment [28, 29]. According to one of the latest prospective of multicenter studies of effectiveness of lanreotide autogel as the first line of therapy, tumor shrinking by more than 20% in 54,1% of the patients was noticed in less than 12 weeks of treatment [30]. In the Russian research similar results were received: in 12 months of treatment with octreotide depot the volume of a tumor decreased by more than 20% from the initial in 63% of the patients who received primary medicated therapy [31].

There are various predictors of resistance to the somatostatin analogues that can be provisionally divided into clinical, histopathomorphological and molecular-genetic (see Table 4) [22, 32-34].

Table 4. Possible predictors of resistance to the SSA of the first generation

|  |  |
| --- | --- |
| Clinical and biochemical | young age,  male gender,  gigantism  high hormonal activity of somatotropinoma  hyperintense T2-weighed MR-signal  Negative acute octreotide test (?) |
| Histopathomorphological | Sparsely granulated somatotropinoma with high Ki-67 |
| Molecular-genetic | Mutations of somatostatin receptors (rarely)  Low expression of sst2/sst5  Geterogeneous sst2 expression  Impairment of intracellar transmission of the signal  Low expression or mutation of AIP |

Many studies showed that the young age and the male gender of the patients are associated with greater resistance to all the types of received treatment including to SSA [16, 32-36]. Acrogigantism can be also considered a clinical predictor of resistance to SSA since among the patients with an acrogigantism male patients with large invasive somatotropinomas prevail [37]. One more factor is the hormonal activity of a tumor: the higher concentrations of GH and IGF-1 at the moment of diagnostics of the disease increase the probability of a partial or full resistance to SSA [22, 34, 35-36].

Due to the development and modernization of visualization techniques lately the interrelation between the MR-characteristics of pituitary tumors and their proliferative activity attract a lot of attention: it has been shown that somatotropinomas with a hyperintense signal on the T2-weighed MR-images are associated with a higher hormonal production and large volume of a tumor as well as with the resistance to the received treatment [38]. In spite of the fact that the predictive value of the test with a short-term octreotide is still debatable, the absence of decrease in the GH and IGF-1 levels during the test can also be considered as a predictor of the lower sensitivity of a somatotropinoma to SSA [28, 34, 36, 39]. Among the histopathomorphological predictors of the resistance to SSA can be mentioned sparsely granulated somatotropinoma and high Ki-67 expression[22, 28, 34].

In recent years molecular and genetic predictors of the resistance to SSA have been revealed. First of all, among them can be mentioned low and/or heterogeneous expression of SSTR2 (revealed by immunohistochemical investigation), since octreotide and lanreotide are bound mostly to this SSTR subtype [34, 40]. However even in case of sufficient amount of SSTR2 the impairment of an intracellular signal pathway can be found. For the adequate biological effect during the binding of SSA to SSTR2 a sufficient amount of aryl hydrocarbon protein (AIP) is required. The latter, in turn, stimulates a tumor-suppressive gene *ZAC1* [4-6, 41]. The reduction of AIP expression or its structural change in case of *AIP* gene mutations and also the decrease of ZAC1 activity after a long-term use of SSA are predictors of resistance to SSA [5, 22, 28, 34, 41]. Besides, filamin A is required for the optimal expression of SSTR2, their proportionate distribution and implementation of inhibitory effects on the hormonal secretion and cellular proliferation. The reduction of expression and density of SSTR2 is observed with long-term use of SSA in case of absence of filamin A that leads to the development of resistance to drug treatment [41]. Low expression of E-cadherin and inhibitory protein of Raf-kinase as well as the high expression of β-arrestin also lead to the impairment of the signal cascades [21-23, 28, 34]. Mutations of SSTR are rare, however the polymorphism of receptors matters: for example, high expression of SSTR5 with the transmembrane domain of type 4 or decrease of the ratio of expression SSTR2/SSTR5 [21-23].

Summarizing the data on the biochemical and tumoral resistance, it is possible to conclude that after 12 months of the treatment with SSA as the first line of therapy adequate biochemical and tumorsupressive effects were achieved in 35-50% of patients, and 21-25% of the patients are fully resistant to the treatment. The rest of the patients show partial biochemical and/or tumoral resistance to the medicated treatment [22, 23, 33].

There are several options of overcoming partial of full resistance to SSA of the first generations such as:

* high-dose therapy with octreotide / lanreotide
* combination of octreotide / lanreotide with the dopamine receptor agonists,
* switch to the analogue of somatostatin of the latest generation—pasireotide,
* switch to the antagonist of the GH receptors pegvisomant or a combined treatment with SSA and pegvisomant,
* tumor debulking surgery (preferably neurosurgical removal more than 75% of tumor tissue)
* radiotherapy or radiosurgery.

In literature several studies are present in which the titration of dose of long-term release octreotide up to 40 mg a month [42-47] and even up to 60 mg a month was carried out for the patients [48]. According to their results, the increase in a dose of long-term release octreotide up to 30-40 mg a month and more provoked the growth in frequency of reduction of the level of GH ≤2.5 ng/ml and normalization of the IGF-1 level in 25-30% of other patients who did not reach the target biochemical criteria on a dose of 20 mg. At the same time high-dose therapy of SSA is well-tolerated without any change of the safety profile.

Another possible method of treatment is the combined therapy of SSA and cabergoline [1-3, 50, 51]. According to various authors, monotherapy with cabergoline allows reaching normalization of the IGF-1 level in 10-30% of patients [50-51]. However it is necessary to use doses 2-4 times higher than are used in the treatment of hyperprolactinemia, and the medicine is effective only in patients with moderate activity of an acromegaly [50]. In the patients who did not reached the biochemical control with monotherapy of SSA, the addition of cabergoline allows to normalize the IGF-1 level in 40-50% of patients [50-51]. Moreover, cabergoline is rather cheap and well-tolerated medicine. However it is necessary to consider that it is "off-label" treatment for the patients with acromegaly and normal prolactin level.

The analogue of a somatostatin of the latest generation pasireotide shows high effectiveness of treatment of acromegaly even when there is a resistance to octreotide and lanreotide [22, 23, 28, 52]. Pegvisomant, which blocks the action of excess concentrations of GH in periphery tissues, leads to normalization of the IGF-1 level in 75-90% of patients, but does not influence the GH level and pituitary tumor volume [53]. However these drugs are not registered in the Russian Federation yet. Therefore, unfortunately, these options are inaccessible in Russian clinical practice.

Another possibility to overcome the resistance to SSA that is applied as the first line of treatment is tumor debulking [22, 29, 54, 55]. In cases when the radical neurosurgical removal of a tumor is technically impossible, and at the same time when there is a resistance to the SSA therapy, it is possible to overcoming the biochemical resistance after removal of a considerable part of a tumor (preferably removal of more than 75% of tumor tissue). Therefore even in cases when the resistance to SSA was noted preoperatively, it is recommended to prescribe SSA after the neurosurgical intervention [22, 28, 34, 54, 55].

Stereotactic radiosurgery and fractional stereotactic radiotherapy are also treatment methods for the patients with persistent acromegaly after neurosurgical operation and drug treatment [1-3, 22, 28, 34, 56]. Stabilizing or reduction of volume of tumoral tissue is observed in 93-100% of patients within 5-10 years after radiotherapy, and target hormonal levels are reached in 40-60% of patients within 5 years [56]. The frequency of development of hypopituitarism and risk of radiological lesion of craniocereberal nerves is relatively high.

**СONCLUSION**

Resistance to somatostatin analogues (SSA) can be defined as lack of the biochemical and tumoral response to the treatment that is carried out within 12 months.An achievement of target hormonal criteria of acromegaly treatment or at least depression of the GH and/or IGF-1 levels by >50% is considered to be the adequate biochemical response. Decrease of somatotropinoma volume by ≥20% (when using SSA as the first line of treatment) is considered as the tumoral response to treatment. The majority of patients (up to 60-70%) show partial resistance when the first generation of SSA is used. Clinical and biochemical predictors of resistance to SSA include young age, male gender, high levels of GH/IGF-1, a large invasive sparsely granulated somatotropinoma with high Ki-67 expression and hyperintensive T2-weighed MR-signal. In recent years various molecular and genetic predictors of resistance to SSA have been found which must be included in broad clinical practice for the personalized choice of drug treatment. Among the treatment options of overcoming the SSA resistance for patients in Russian clinical practice high dose SSA therapy and combined therapy with the maximum doses of SSA and cabergoline can be mentioned as drug methods; non-drug options include tumor debulking surgery with the subsequent resumption of SSA treatment and radiotherapy.

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