OCTREOTIDE TREATMENT RESULTS IN THE INHIBITION OF GH GENE EXPRESSION IN THE ADENOMA OF THE PATIENTS WITH ACROMEGALY

NOBUHIRO TSUKAMOTO***, TAKASHI NAGAYA***, AKIO KUWAYAMA**, KAZUE TAKANO***, KAZUO SHIZUME****, KENICHIRO SUGITA**, AND HISAO SEO*

*Department of Endocrinology and Metabolism, The Research Institute of Environmental Medicine, Nagoya University, Nagoya 464,
**Department of Neurosurgery, Nagoya University School of Medicine, Nagoya 466,
***Department of Medicine, Tokyo Women's Medical College, Tokyo 162, and
****Foundation for Growth Science in Japan, Tokyo 151, Japan

Abstract. Seven patients with growth hormone (GH)-secreting pituitary adenoma were treated preoperatively with octreotide (Sandostatin or SMS 201-995; a somatostatin analogue), and were compared with 18 non-treated patients in their clinical courses and adenoma analyses. Octreotide treatment improved the endocrinological data in all 7 cases. The octreotide-treated adenomas were soft and easily removed by suction and curettage. The postoperative normalization of endocrinological data was encountered more often in the octreotide-treated cases than in the non-treated, although the statistical significance was not observed by the limited number of cases. The adenoma tissues were examined with conventional histology and immunohistochemistry, and the amount of GH messenger ribonucleic acid (mRNA) was quantitatively assessed. The studies demonstrated: 1) No fibrosis nor necrosis was observed in the adenomas from the octreotide-treated patients. 2) Immunohistochemistry for human GH revealed no remarkable differences between the octreotide-treated and the non-treated adenomas. 3) The amounts of GH mRNA in the adenoma from the octreotide-treated patients were 4.2 ± 1.8 (mean ± SEM; expressed in an arbitrary unit) and were significantly less than those from the non-treated (33.6 ± 9.1). These data suggest that octreotide inhibits not only GH release from the adenoma but also its biosynthesis.

Key words: Acromegaly, Somatostatin, Growth hormone, mRNA, Pituitary adenoma.

(Endocrine Journal 41: 437–444, 1994)

SOMATOSTATIN is a hypothalamic peptide hormone consisting of 14 amino acid residues. It was isolated and purified in 1973 by Brazeau et al. [1] as an inhibitory factor of growth hormone (GH) secretion. It was also demonstrated to strongly inhibit GH secretion from somatotroph cell adenomas [1, 2]. However, its clinical application has been limited because of its short half-life in plasma (a few minutes) [3].

Octreotide acetate (Sandostatin, SMS 201–995), a somatostatin analogue consisting of 8 amino acid residues [4], was introduced for the treatment of GH-secreting adenoma because of its longer half-life [around 110 min. [5]]. When it was administered by repeated or continuous subcutaneous injection, marked improvement of clinical symptoms [6–8], normalization of circulating GH and somatomedin-C levels [9], and disappearance of paradoxical responses of GH [10] were reported. Shrinking of the adenomas was also demonstrated by magnetic resonance imaging (MRI) [11, 12].
Thus, octreotide administration could be a good choice for the medical treatment of GH-secreting adenoma. However, the influence of octreotide pretreatment of the pituitary surgery is not well evaluated and the mechanism of its inhibitory action on GH-secreting adenoma has not been fully understood.

In this report, we studied the efficacy of pretreatment with octreotide and analyzed adenoma samples resected from the patients pretreated with octreotide. Histological findings and the amounts of GH messenger ribonucleic acid (mRNA) in the adenoma tissues were compared with those from the untreated. Our results suggest octreotide treatment brings about inhibition of GH synthesis of the somatotroph adenoma cell without cell necrosis or fibrosis.

**Subjects**

Twenty-five patients with GH-secreting pituitary adenoma were included in the present study. They were treated by transsphenoidal adenomectomy consecutively at the Department of Neurosurgery, Nagoya University School of Medicine. The clinical diagnosis was made on the following criteria: typical clinical signs and symptoms, elevated serum levels of GH and/or somatomedin-C, non-suppressible GH level in glucose tolerance test, and the presence of pituitary adenoma demonstrated by CT and/or MRI. The size of the adenoma was graded according to the classification of Hardy [13]. Seven patients among these 25 were treated with octreotide before surgery. Informed consent was obtained from each patient prior to octreotide treatment.

**Materials and Methods**

**Octreotide treatment**

Seven patients with GH-secreting adenoma were treated by octreotide at the Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women’s Medical College. Octreotide was administered by the multiple subcutaneous injection method with a portable infusion pump. The protocol for octreotide treatment was previously described [12]. The duration of the octreotide treatment at the maintenance dose varied from 20 to 74 days among the 7 patients (Table 1). The administration of octreotide was terminated in the evening of the previous day of the transsphenoidal surgery.

**Endocrinological evaluation**

Serum levels of GH and somatomedin-C were determined by commercially available radioimmunoassay kits (hGH RIA kit 2, Dainabot Co. Ltd., Tokyo, Japan and Somatomedin C Ehken, Ehken Chemical Co. Ltd., Tokyo, Japan) from the blood samples obtained at 0800 h before the octreotide treatment, on the day of transsphenoidal surgery and 1 week after the operation. The provocative tests were carried out before the octreotide treatment and after surgery as follows. In one test, TRH (thyrotropin-releasing hormone: 500 µg), GnRH (gonadotropin-releasing hormone: 100 µg) and insulin (regular insulin: 0.1 U/kg) were intravenously administered and blood samples were obtained before as well as 15, 30, 60, and 90 min after the administration. Another test was the 75 g oral glucose tolerance test (OGTT). The paradoxical response of GH to TRH or GnRH was regarded as positive when the peak level of GH exceeded 5.0 ng/ml and the ratio of GH levels (peak/base) was more than 2.0.

**Adenoma tissues**

A part of the resected adenoma was fixed with 10% formaldehyde, embedded in paraffin, sliced into 5-µm thick sections and subjected to light microscopic examinations, including conventional hematoxylin-eosin stain, azan stain and immunohistochemistry. The remaining specimen was frozen and stored at -70°C for RNA extraction.

**Immunohistochemistry**

An indirect immunoperoxidase staining for human GH (hGH) was performed as described by Nakane and Pierce [14]. The primary antibody against hGH was provided by National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK). The second antibody conjugated with peroxidase was obtained from Medical Biological Laboratory (MBL, Tokyo, Japan). Before the incubation with the first antibody, the tissue sections were treated with 0.3% H₂O₂ to block endogenous peroxidase.
activity. After the incubation with the first and the second antibodies, the section was incubated with diaminobenzidine followed by counterstaining with hematoxylin.

**RNA Dot hybridization**

Total RNA was extracted from adenoma samples as described by Chomczynski and Sacchi [15]. Hybridization of RNA was performed as described by Thomas [16]. After precipitation with ethanol, the RNA samples were dissolved in water to a concentration of 1 µg/µl H2O. The RNA was denatured with 50% deionized formamide and 6% formaldehyde at 50°C for 1 h, serially diluted with 15× standard saline citrate (SSC: 0.15 M NaCl, 0.015 M trisodium citrate) and applied to GeneScreen Plus membrane (NEN Products, Boston, MA, U.S.A.) employing Minifold apparatus (Schleicher and Schuell, Keene, NH, U.S.A.). The membrane was prehybridized at 42°C for 2.5 h in a solution consisting of 50% deionized formamide (Merck, Darmstadt, Germany), 5×Denhardt's solution [0.1% Ficoll, 0.1% polyvinyl-pyrrolidone, 0.1% BSA, 5×SSPE (SSPE: 0.15 M NaCl, 0.01M NaH2PO4 and 0.02 M EDTA), 1% sodium dodecyl sulfate (SDS), and 0.01% denatured herring sperm DNA]. It was then hybridized overnight with 32P-labelled human GH complementary deoxynucleic acid (cDNA) [17] probe (10⁶ cpm/ml buffer). The preparation of GH cDNA probes was described previously [18]. After washing twice in 2×SSC at room temperature for 5 min, twice in 2×SSC containing 1% SDS at 60°C for 30 min, and twice in 0.1×SSC at room temperature for 30 min, the sheet was exposed for a few days to Kodak X-Omat AR film in a cassette at −70°C.

After washing the membrane in 0.01×SSC and 0.01% SDS at 95°C for 20 min, it was rehybridized with 32P-labelled β-actin cDNA.

Relative amounts of mRNA were determined by densitometry (Image Analyzer TIB 100, Immunomedica, Shizuoka, Japan) and expressed in an arbitrary unit.

**Statistical analysis**

The data were expressed as the mean ± standard error. All the statistical significance was analyzed by unpaired Student’s t test.

**Results**

**Clinical data in the octreotide-treated patients**

Table 1 summarizes the clinical data of the 7 octreotide-treated patients. In five cases the pituitary adenomas were enclosed in the sella turcica (Hardy’s grade 1 & 2). In the other two cases, the adenomas were the invasive-type (grade 3 & 4). In all cases, serum levels of GH and somatomedin-C were above the normal range. Paradoxical response of GH to TRH was observed. The paradoxical response to LH-RH was observed in 2 cases. As shown in Table 1, the serum levels of GH and somatomedin-C were 70.8 ± 18.5 ng/ml and 6.1 ± 0.7

| Table 1. Clinical data of the 7 patients treated with octreotide |
|------------------|-------------------|---------------------|---------------------|-------------------|
|                  | adenoma size*     | pre-treatment       | octreotide-treatment | post-operation     |
|                  | (Hardy’s grade)   | GH (ng/ml)          | Sm-C (U/ml)         | Sm-C (U/ml)       |
|                  |                   | response to         | amount (µg/day)     | duration (day)    | GH (ng/ml)       | Sm-C (U/ml)       | response to TRH     |
|                  |                   | TRH-LH-RH           | (U/ml)              | (U/ml)            | GH (ng/ml)       | Sm-C (U/ml)       | LH-RH               |
| 1 M              | 25                | 12                  | 7.2                 | +                 | -                | 600              | 45                 | 11.0                | 2.1                  | 0.9                 | 0.7                 | -                   | -                   |
| 2 M              | 47                | 80                  | 5.0                 | +                 | -                | 200              | 24                 | 8.2                 | 3.5                  | 1.2                 | 0.7                 | -                   | -                   |
| 3 F              | 41                | 86                  | 6.3                 | +                 | -                | 800              | 45                 | 4.3                 | 3.2                  | 3.8                 | 1.1                 | +                   | -                   |
| 4 F              | 50                | 22                  | 8.6                 | +                 | -                | 300              | 30                 | 2.8                 | 0.9                  | 4.9                 | 1.1                 | +                   | -                   |
| 5 M              | 32                | 56                  | 3.9                 | +                 | -                | 480              | 74                 | 6.0                 | 3.6                  | 1.8                 | 1.6                 | -                   | -                   |
| 6 M              | 38                | 88                  | 3.7                 | +                 | -                | 600              | 30                 | 15.0                | 2.8                  | 7.3                 | 2.9                 | -                   | -                   |
| 7 M              | 36                | 160                 | 7.9                 | +                 | +                | 600              | 20                 | 45.0                | 3.8                  | 2.0                 | 0.9                 | -                   | -                   |
| mean ±SEM        | 38.4 ± 3.2        | 2.3 ± 0.2           | 70.8 ± 18.5         | 6.1 ± 0.7         | 551 ± 129        | 38 ± 3            | 13.1 ± 5.5         | 3.0 ± 0.4           | 3.1 ± 0.9           | 1.3 ± 0.3           |
| normal range     | < 5.0             | < 2.0               | -                   | -                  | < 5.0            | < 2.0             | < 5.0              | < 2.0               |

*The size of adenoma was graded according to the classification of Hardy [13].
U/ml, respectively, before the octreotide treatment. GH levels decreased after the treatment to 13.1 ± 5.5 ng/ml and somatomedin-C to 3.0 ± 0.4 U/ml. However, both GH and somatomedin-C levels became normal in only 1 patient (case 4). Further improvement of both GH and somatomedin-C was observed after the transsphenoidal surgery. Namely, GH levels decreased to 3.1 ± 0.9 ng/ml and somatomedin-C to 1.3 ± 0.3 U/ml. Both GH and somatomedin-C levels were normalized post-operatively in 6 out of 7 cases (86%). Neither severe side effects nor remarkable complications were encountered during the treatment and surgery.

Macroscopic findings during surgery

Under the microscope at surgery, neither fibrotic change nor necrosis could be seen in the adenomas of the octreotide-treated patients. The adenomas were soft and easily dissected out from normal pituitary gland by suction and curettage. Bleeding from the tumor bed was not severe.

Comparison of postoperative findings of the octreotide-treated patients with those of 18 non-treated patients

As shown in Table 2, there were no significant differences in age and pretreatment serum levels of GH and somatomedin-C between the octreotide-treated and non-treated groups. Of the octreotide-treated group, two patients had invasive adenomas (grade 3 & 4), while no invasive adenomas were found in the non-treated. The postoperative endocrinological findings of these two groups were summarized in Table 3. The rate of normalization of GH and somatomedin C was higher in the octreotide-treated group than in the non-treated group. The disappearance of paradoxical response after surgery was also observed at a higher rate in the octreotide-treated group. There was, however, no statistical significance in the surgical outcome between these two groups as the case number was small.

Morphological study

Conventional histological studies by hematoxylin-eosin staining showed no significant necrosis or hemorrhage in the sections of either group (Fig. 1a & d). No significant fibrosis was detected by azan stain (Fig. 1, b & e). The size of each adenoma cell of the octreotide-treated group seemed to be smaller than that of the non-treated.

The immunohistochemical studies revealed no significant difference between the two groups; GH immunoreactivity was detected in the cytoplasm of many adenoma cells, with some cells lacking GH immunoreactivity (Fig. 1, c & f).

Determination of GH mRNA level in the adenoma tissues

Since case 1 had a microadenoma and sufficient amount of adenoma tissue could not be obtained, the analysis of RNA levels was performed with 6 octreotide-treated cases and 18 non-treated ones. Both GH mRNA and β-actin mRNA were detected in all the adenoma samples by RNA dot hybridization. The amounts of GH and β-actin mRNA were determined by densitometry and expressed in an arbitrary unit. Since β-actin is a cyto-skeletal pro-

| Table 2. Preoperative data of the octreotide-treated and non-treated patients |
|-------------------------------|-----------------|----------------|-------------|
|                              | octreotide-treated | non-treated | significance |
| total number of cases        | 7                | 18            |             |
| Grade 1 & 2*                 | 5                | 18            |             |
| Grade 3 & 4*                 | 2                | 0             |             |
| paradoxical response**       | 7                | 10            |             |
| age (yr.)                    | 38.4 ± 3.2       | 44.4 ± 2.1    | n.s.        |
| untreated GH (ng/ml)         | 70.8 ± 18.5      | 94.1 ± 21.4   | n.s.        |
| untreated SmC (U/ml)         | 6.1 ± 0.7        | 5.4 ± 1.0     | n.s.        |

The data were expressed as mean ± SEM. The duration and the dose of octreotide treatment were depicted in Table 1. *The size of adenoma was graded according to the classification of Hardy [13]. **Number of the cases with the paradoxical response of GH to TRH and/or LH-RH.
tein regarded to be stably expressed in any viable cells, GH mRNA/β-actin mRNA ratio was used as an index of GH mRNA abundance in the adenoma. The ratio of GH/β-actin mRNA in the octreotide treated group was 4.2 ± 1.8, whereas the ratio in the non-treated group was 33.6 ± 9.1 (Fig. 2). The GH/β-actin mRNA ratio was significantly less in the octreotide treated group as shown in Fig. 2.

Table 3. Postoperative endocrinological findings of the octreotide-treated and non-treated patients

<table>
<thead>
<tr>
<th></th>
<th>octreotide-treated</th>
<th>non-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH &lt; 5.0 ng/ml*</td>
<td>86 %</td>
<td>61 %</td>
</tr>
<tr>
<td>SmC &lt; 2.0 U/ml**</td>
<td>86 %</td>
<td>70 %</td>
</tr>
<tr>
<td>disappearance of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>paradoxical response(s)***</td>
<td>71 %</td>
<td>40 %</td>
</tr>
</tbody>
</table>

*The percentage of the cases with postoperative GH levels less than 5.0 ng/ml. ** The percentage of the cases with postoperative somatomedin-C level less than 2.0 U/ml. *** The percentage of cases with paradoxical response(s) of GH to TRH or LH-RH postoperatively disappeared.

Fig. 1. Histological findings of the adenomas obtained from the patients treated (Case 6) (a-c) and not treated (d-f) with octreotide. a and d, Hematoxylin-eosin stain (×400); b and e, Azan stain (×200); c and f, Immunohistochemistry using anti-human GH antiserum and counterstained with hematoxylin (×400).
Seven patients with GH-secreting pituitary adenoma were operated on after the pre-treatment with octreotide. The treatment brought about an effective decrease in the serum GH and somatomedin-C levels [6-10, 12]. At the surgery, the adenomas were soft and easily removed. The postoperative outcomes were better than those of the 18 patients who were not treated with octreotide, as indicated by the higher incidence of disappearance of paradoxical response and the normalization of the circulating levels of GH and somatomedin-C. Barkan et al. [19] reported similar results when they compared the postoperative findings of the patients with invasive GH-secreting adenomas treated or non-treated with octreotide before surgery. However, Stevenaert et al. [20] described that the higher remission rate was observed in the enclosed adenomas with octreotide pretreatment rather than in the invasive adenomas.

Histological analyses revealed that the treatment with octreotide caused no necrosis nor fibrosis in the adenoma tissues, which is consistent with the macroscopic findings during surgery. This indicates that the pretreatment with octreotide is beneficial for the surgical dissection of the adenomas. In limited cases, perivascular fibrosis due to the octreotide treatment was reported [19-21], but octreotide-induced necrosis was quite rare [19]. It is contrary to the effects of long-term treatment of prolactinoma with bromocriptine, which results in fibrosis of the adenoma tissue and difficulty in surgical removal [22].

Octreotide treatment is often associated with the shrinking of the pituitary adenoma [11, 12, 19], but this effect is reversible [23]. The electron microscopical study [21] revealed that the nuclear and cytoplasmic areas of the adenoma cells from octreotide-treated patients were smaller than those of the controls. This finding supports the idea that the shrinking of the adenoma could be due to the size reduction of adenoma cells, but not to the decrease in their number. These findings are compatible with the notion that the final treatment of GH secreting adenoma is surgical removal.

The present study revealed that the treatment with octreotide decreases GH mRNA levels in the adenoma tissue without affecting GH immunoreactivity. This is not consistent with previous in vitro studies in the rat pituitary [24-31]. In those studies, somatostatin was found to suppress GH release without affecting the GH gene transcription. However, the results were obtained by using shorter-acting, native somatostatin, and the amount of applied somatostatin may have been insufficient to suppress GH gene expression. The in vivo effect of octreotide (a long-acting somatostatin analogue) on the GH gene expression with a sufficient dose has not been well evaluated. It could be postulated that octreotide at a sufficient concentration inhibits both secretion and synthesis of GH in somatotroph cell adenoma in vivo.

The action of somatostatin is mediated by at least 5 types of somatostatin receptors (SSTR1-5) [28, 29], and reduces intracellular cAMP generation and Ca$^{2+}$ entry. Somatostatin inhibits GH synthesis by reducing the intracellular cAMP levels via Gi protein, which results in the inhibition of hormone gene transcription via the cAMP-responsive element, since it has been demonstrated that GH gene is increased via the cAMP responsive element.
Acknowledgements

The authors are indebted to Drs. R. Horikawa, and T. Tsushima of Tokyo Women’s Medical College for referring the patients. We are indebted to National Institute of Diabetes, Digestive and Kidney Disease for the provision of antibody against GH. We thank Dr. J. A. Martial (University of Liege, Belgium) and Dr. R. H. Singer (University of Massachusetts Medical School) for the gift of human GH and β-actin cDNA clones. We also thank Dr. L. F. Alexander (University of Mississippi Medical Center) for his critical review of the manuscript.

References


