CLINICAL STUDY

DG3173 (somatoprim), a unique somatostatin receptor subtypes 2-, 4- and 5-selective analogue, effectively reduces GH secretion in human GH-secreting pituitary adenomas even in Octreotide non-responsive tumours

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Abstract

Objective: Somatostatin analogues (SSA) reduce autonomous GH secretion by activating somatostatin receptors (sst) 2 and 5 in 50–60% of acromegalic patients. However, by inhibiting insulin secretion these SSA reduce glucose tolerance. DG3173 is a novel SSA with additional binding to sst4 and low insulin-suppressing activity. We investigated the effect of DG3173, including its relation to specific tumour characteristics, on GH secretion in human somatotroph adenoma cell cultures (hSA) in comparison with Octreotide.

Methods: Twenty-seven hSA were characterised immunohistochemically for their hormone- and sst-expression, granularity and pre-surgical therapy with SSA. GH was determined in supernatants of hSA treated with DG3173 or Octreotide in time- ($n=6$) and dose-response ($n=21$) experiments. A positive response was defined as GH suppression to below 80% of baseline.

Results: In the dose-response experiments DG3173 suppressed GH secretion in more adenomas than Octreotide (10/21 vs 5/21), including 38% (6/16) of Octreotide non-responders. In responders the extent of GH suppression and IC_{50} were comparable for both SSA. The response-rate of both SSA was higher in monohormonal vs bihormonal adenomas, yet GH declined similarly in both groups. Neither pre-surgical SSA ($n=6$) nor tumour morphology was related to the GH response. However, semi-quantitative analysis indicated a small but significant negative correlation between the GH response to Octreotide and the immunoreactivity scores of sst2 expression.

Conclusions: DG3173 equalled Octreotide in suppressing GH secretion in hSA. Since DG3173 suppressed GH in some Octreotide-non-responsive adenomas, its clinical effectiveness will be worth testing. Moreover, its reduced insulin-suppressive potency would make it a valuable alternative to Octreotide.

European Journal of Endocrinology 166 223–234

Introduction

Transsphenoidal surgery reduces autonomous GH secretion in up to 60% of acromegalic patients (1). When autonomous GH secretion persists post-operatively, somatostatin analogues (SSA) are the first treatment option (2). The clinically available SSA, Octreotide and Lanreotide respectively, significantly suppress GH secretion in up to 50–70% of acromegalic patients (2, 3, 4) while strict biochemical control (GH <1 µg/l) is achieved in up to 33% (3). However, these SSA also suppress insulin secretion and thus negatively

affect glucose homeostasis in a significant number of patients (3, 5, 6, 7, 8).

Somatostatin receptors (sst) belong to two subfamilies, SRIF-1 (sst2, sst3 and sst5) and SRIF-2 (sst1 and sst4), with different internalisation upon SSA exposure (9, 10). The distinct affinity profile of each SSA translates into specific pharmacological properties (10, 11, 12). Octreotide and Lanreotide bind with a high affinity at sub-nanomolar concentration to sst2. They have a moderate affinity to sst3 and sst5, and very low or absent binding to sst1 and sst4 (11). In one-third of acromegalic patients, who do not respond to Octreotide,

there is diminished expression of sst2A, but persistent sst5 expression (13). SOM230 (Pasireotide), a new multiligand SSA with binding to all sst except to sst4, is currently investigated in clinical trials (14, 15, 16, 17, 18, 19). Nevertheless, its negative effects on glucose tolerance are even more pronounced than with Octreotide (14, 20). On the other hand, BIM-23A760 (Dopastatin), a chimeric molecule directed towards somatostatin (mainly sst2 and sst5) and dopamine D2 receptors (21, 22, 23), suppresses GH *in vitro* but was ineffective when applied to acromegalic patients in clinical trials (press release 15 Dec 2010, Ipsen Pharma).

DG3173 is a heptapeptide SSA with a novel amino acid sequence and a unique cyclic backbone (24). In addition to its affinities to sst2 and sst5, DG3173, previously named PTR-3173, is the only SSA binding with nanomolar affinity to sst4 (24). mRNA for sst4 is rarely detected in human pituitary adenomas (25, 26) and immunohistochemical staining for sst4 protein has given contradictory results. While no staining was reported by some investigators (27, 28), others detected sst4 protein in many somatotroph adenomas (29). So far the role of sst4 in the suppression of GH secretion by SSA with high affinity to sst4 has not been investigated in detail.

Furthermore, the potency of DG 3173 to inhibit GH secretion *in vitro* is 10,000-fold higher than its inhibition of insulin secretion (24). This low insulin-suppressing activity was also demonstrated *in vivo* (A Steuernagel, C Meyer, R Wehr and C Dohrmann, unpublished results). For the treatment of acromegalic patients this would be a valuable advantage, if confirmed by clinical trials.

In an explorative *in vitro* investigation we reported that DG3173 suppresses GH in human pituitary adenomas non-responsive to Octreotide (30). In this study, we aim to verify in a larger set of human GH-secreting pituitary adenomas whether DG3173 results in significant control of GH secretion *in vitro* comparable to that achieved by Octreotide. In addition, we analysed factors that might influence the effects of SSA on GH secretion *in vitro*, such as pre-operative SSA treatment, co-expression of other hormones by the adenomas, vesicular granularity of the tumour tissue or the expression of sst1, sst2A, sst3, sst4 and sst5 respectively.

Materials and methods

Patients

We investigated 27 adenomas from acromegalic patients (female: $n=12$, median age 47, range 32–72 years; male: $n=15$, median age 43, range 27–69 years; Table 1). Acromegaly had been diagnosed by failure of the GH concentration to be suppressed to below 1 µg/l during an oral glucose load and/or an increased IGF1 concentration according to current consensus (2, 31).

The study was approved by the ethics committee of the Charité-Universitätsmedizin Berlin. Upon written informed consent from each patient, adenoma tissue samples were collected during surgery from three clinical sites (Neurochirurgische Klinik mit Poliklinik, Universitätsklinikum Erlangen-Nürnberg; Interdisziplinäre Endokrinologie/Hypophysenchirurgie, Universitätsklinikum Hamburg Eppendorf; Klinik für Neurochirurgie, Charité-Universitätsmedizin Berlin).

Somatostatin analogues

DG3173 acetate and Octreotide acetate were purchased from Bachem AG (Bubendorf, Switzerland). Stock solutions of 1 mM DG3173 or 1 mM Octreotide (net peptide, respectively) were prepared by dilution of lyophilised peptide in 37 mM lactic acid/250 mM mannitol buffer (pH 4.3). Stock solutions were stored at 2–5 °C and protected from light until use.

Adenoma tissue preparation

A portion of each adenoma obtained at surgery was dissociated by mechanical and enzymatic methods. Depending on tumour size, 6×10^5 – 3×10^7 tumour cells were initially cultured in DMEM supplemented with 10% FCS, pH 7.3 and plated at a density of 1×10^5 or 5×10^4 cells/well for 60 h. On day 4, the cells were washed with PBS and used for the time- or dose-response study. Collagenase type 1 was purchased from Worthington (#CLS-1 Lakewood, NJ, USA); DNase (#D4138), hyaluronidase (#H2126), Soybean Trypsin Inhibitor (#T6522), 3 nM 3,3',5-triiodo-L-thyronine sodium salt (#T6397), 5.5 mM NaHCO₃ and BSA fat free (#A6003) from Sigma; DMEM–low glucose, 1% ITX and 1% glutamax from Gibco–Invitrogen; 10% FCS, 1% HEPES, PBS (#L1820) and 1% penicillin–streptomycin from Biochrom (Berlin, Germany).

SSA treatment

For the time-series experiments isolated cells from six different adenomas (nos 65–70, Table 1) were treated in parallel with 1 µM Octreotide or 1 µM DG3173 for 2, 4, 6, 8 and 24 h respectively. For the dose-response experiments 21 tumours were used. Of these, six out of 21 patients had pre-operative SSA therapy (Table 1). During the dose-response experiments 300 µl DG3173 and Octreotide, respectively, were added in concentrations ranging from 10^{-6} to 10^{-12} M and incubated for 6 h. At the time points indicated the supernatant was carefully removed and stored at 300 µl aliquots at –20 °C until GH determination. All experiments were performed at least in quadruplicate. The investigators were blinded for the type of SSA. For some adenomas cell numbers were insufficient to setup and analyse all time points or SSA concentrations. Response was

Table 1 Patient characteristics, immunohistochemical characteristics of the adenomas, GH concentration at baseline and percentage decline after treatment with either DG3173 or Octreotide.

Patient characteristics			Sst protein expression ^a					GH baseline ($\mu\text{g/l}$) ^f	Post-tx DG3173		Post-tx Octreotide	
No	Sex/ age	Pre-op. SSA-Tx ^b	GH ^c	PRL ^d	Granularity ^e	Sst1	Sst2A	Sst3	Sst4	Sst5	% of baseline	R/NR ^g
(A) Time-series experiments ($n=6$)												
65 ^h	M/27	NA	Neg ⁱ	Neg	Sparse	NA	NA	NA	NA	NA	49.8	R ^j
66	M/69	NA	Pos	Neg	Dense	NA	NA	NA	NA	NA	111.2	NR
67	F/48	NA	Pos	Neg	Sparse	NA	NA	NA	NA	NA	103.9	NR
68	M/35	Oct LAR ⁱ	Pos	Neg	Dense	NA	NA	NA	NA	NA	52.3	R ^j
69	M/55	NA	Pos	Neg	Dense	NA	NA	NA	NA	NA	99.0	NR
70	M/49	NA	Pos	Neg	Sparse	NA	NA	NA	NA	NA	64.1	R ^j
(B) Dose-response experiments ($n=21$)												
71	F/32	Oct LAR	Pos	Neg	Sparse	+	+	+	+	+	195.9 $\pm$ 7.63	R ^j
72	F/45	Oct LAR	Neg ⁱ	Pos	Sparse	+	+	+	+	+	6.5 $\pm$ 1.3	R ^j
73	F/44	Oct LAR	Pos	Neg	Dense	+	+	+	+	+	363.4 $\pm$ 2.65	R ^j
74	F/49	None	Pos	Neg	Dense	+	+	+	+	+	641.9 $\pm$ 0.83	NR
75	F/72	None	Pos	Pos	Dense	NA	NA	NA	NA	NA	320.3 $\pm$ 2.64	NR
76	F/52	None	Pos	Pos	Dense	+	+	+	+	+	114.6 $\pm$ 8.87	R ^j
77	F/46	None	Pos	Neg	Sparse	+	+	+	+	+	106.8 $\pm$ 8.33	NR
78	F/44	None	Pos	Neg	Dense	+	+	+	+	+	849.3 $\pm$ 1.031	NR
79	M/48	None	Pos	Neg	Sparse	+	+	+	+	+	17.2 $\pm$ 7.0	R ^j
80	M/50	None	Pos	Neg	Sparse	+	+	+	+	+	103.3 $\pm$ 0.9	R ^j
81	M/60	Lanreotide	Pos	Pos	Sparse	+	+	+	+	+	156.0 $\pm$ 7.4	NR
82	M/29	Pasireotide	Pos	Neg	Dense	+	+	+	+	+	163.1 $\pm$ 5.31	NR
83	M/37	None	Pos	Neg	Sparse	+	+	+	+	+	322.2 $\pm$ 3.51	R ^j
84	M/39	None	Pos	Neg	Sparse	+	+	+	+	+	69.1 $\pm$ 1.8	R ^j
85	M/57	None	Pos	Neg	Dense	NA	NA	NA	NA	NA	111.6	NR
87	F/60	Oct LAR	Pos	Neg	Dense	NA	NA	NA	NA	NA	369.3 $\pm$ 7.51	NR
89	M/31	None	Pos	Pos	Dense	+	+	+	+	+	45.4 $\pm$ 4.5	NR
90	M/43	None	Pos	Neg	Dense	+	+	+	+	+	835.7 $\pm$ 1.09	NR
91	M/34	None	Pos	Neg	Sparse	+	+	+	+	+	44.1 $\pm$ 7.4	NR
92	F/49	None	Pos	Neg	Dense	+	+	+	+	+	36.4 $\pm$ 3.1	R ^j
93	F/37	None	Pos	Neg	Dense	+	+	+	+	+	132.7 $\pm$ 4.6	NR
Summary of dose-response experiments ($n=21$)												
11F/10M	6 Pre-tx; 15 tx naive	16 Mono-; 5 bihormonal	9 Sparse; 12 dense	11 Pos; 7 neg	15 Pos; 3 neg	18 Pos; 0 neg	10 Pos; 0 neg	8 Pos; 10 neg	18 Pos; 0 neg	156.0 (6.5–849.3) ^k	66.4 (R (DG3173))	64.9 (R (Oct))
											10/21 (R/total)	5/21 (R/total)

^aSst protein expression scores were classified as negative (–), weak (+), moderate (++) or strong (+++) (see Methods); detailed results regarding percentage of positive cells and intensity of staining is available in Supplementary Table 1: sst expression data from adenomas used to establish the experimental setup in time-course series were not obtained.

^bSSA-Tx, pre-surgical treatment with SSA, the type of SSA used is indicated.

^cImmunoreactivity for GH given as the percentage of cells below 5% (neg, negative), below 30% of cells (low), >30% – >70% (medium) and >70% (high) positivity for GH.

^dImmunoreactivity for PRL negative, <5% of cells with positive immunoreactivity for PRL, positive >5% of cells with positive immunoreactivity for PRL.

^eIndicates whether the adenoma was densely or sparsely granulated with hormone storage vesicles.

^fMean $\pm$ S.E.M.

^gResponse was defined as GH suppression to at least 80% of baseline or less (SSA doses ranging from 10^{-12} to 10^{-6} M).

^hSix hours time point not available; 8 h shown.

ⁱDespite the lack of GH immunopositivity but due to the number of fibrous bodies these tumours was classified as an atypical sparsely granulated GH-secreting adenoma.

^jResponders with significant GH decline, $P<0.05$; NA, not available; Oct, Octreotide long-acting release formulation; R, responder (numbers in bold); NR, non-responder.

^kMedian (range).

defined as GH suppression to at least 80% of baseline or less. This is in accordance with overall *in vitro* results reported by others (32). Furthermore, the 20% margin covers most of the variability of GH suppression within individual tumours (mean coefficient of variation of 20.2%, including variability of the GH assay (see below)).

Human GH assay

Human GH in pituitary adenoma cell culture supernatant was analysed by a commercial ELISA according to the manufacturer's instructions (for assay validation see Diagnostic Systems Laboratories Deutschland GmbH, Sinsheim, Germany, active ultra-sensitive human GH ELISA #DSL-100-19100 with a LLOQ of 0.66 pg/ml). Additional GH standards were prepared with recombinant human GH (Sigma–Aldrich). The intra-assay coefficient of variation of duplicates was 4.23 ± 5.11 (mean ± S.D.).

Structural and hormonal expression analysis

All adenomas underwent histological analysis on paraffin and in part Epon sections for tumour tissue pattern and cellular details (pleomorphy, mitoses, granularity). Immunohistochemistry was performed for GH (monoclonal anti-GH, Sigma Immunochem, Zytomed, Berlin, Germany dilution 1:300), PRL (monoclonal anti-prolactin (PRL), Immunotech, Marseille, France 1:400), ACTH (polyclonal anti-ACTH, Zytomed, 1:30), TSH (monoclonal anti-TSH, Immunotech, 1:5000), FSH (monoclonal anti-FSH, Immunotech, 1:80 000), LH (monoclonal anti-LH, Immunotech, 1:60 000) and α-subunit (monoclonal anti-α-subunit, Immunotech, 1:1500). A positive immunohistochemical hormone expression was defined as more than 5% of the tumour cells demonstrating positive staining in any of the three grades (low, medium and high). Normal pituitaries from autopsies were used as positive control.

Sst subtype expression analysis

The rabbit monoclonal anti-sst2A, -sst3 and -sst5 antibodies UMB-1, UMB-5 and UMB-4, respectively, as well as the polyclonal anti-sst1 and -sst4 antibodies (E4317) and (4802) were generated against the carboxyl-terminal tail of the respective human sst. The identity of the peptides used for immunisations of the rabbits is given in Table 2. The MABs, as well as the polyclonal antibody (E4317), were obtained from Epitomics (Burlingame, CA, USA), the polyclonal antibody (4802) from Gramsch Laboratories (Schwabhausen, Germany). The polyclonal antibodies were purified against their immunising peptide as described by Schulz *et al.* (33, 34, 35, 36). Receptor-binding profiles for sst, as well as pharmacodynamic constants

Table 2 Amino acid sequences of the COOH-terminal regions of human sst receptors used for generation of the respective antibodies.

Human sst (residues)	Sequence
Sst1 (377–391)	ENLESGGVFRNGTCTSRITTL
Sst2A (355–369)	ETQRTLLNGDLQTSI
Sst3 (398–418)	QLLPQEASTGEKSSTMRISYL
Sst4 (366–388)	CQQEALQPEPGRKRIPLTRTTTF
Sst5 (344–364)	QEATPPAHRAAANGLMQTSKL

for both DG3173 and Octreotide, are given in Supplementary Table 1, see section on supplementary data given at the end of this article.

Data were only obtained from adenomas used for the dose–response experiments. Tissue specimens were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections (5 µm) were prepared from the paraffin blocks and floated onto positively charged slides. Immunostaining of paraffin sections was performed by an indirect peroxidase labelling method. Briefly, sections were dewaxed, microwaved in 10 mM citric acid (pH 6.0) for 16 min at 600 W and then incubated with the respective anti-sst antibody overnight at 4 °C. Detection of the primary antibody was performed by a biotinylated anti-rabbit IgG followed by incubation with peroxidase-conjugated avidin (Vector ABC ‘Elite’ kit, Vector, Burlingame, CA, USA). Binding of the primary antibody was visualised using 3-amino-9-ethylcarbazole in acetate buffer (BioGenex, San Ramon, CA, USA). Sections were then rinsed, lightly counterstained with Mayer’s hematoxylin and mounted in Vectamount mounting medium (Vector Laboratories). Pancreatic islets were used as positive control for staining with antibodies to sst1, sst2A, sst3 and sst5. Sst4 staining in islets was not detectable. For immunohistochemical controls, the primary antibody was either omitted or adsorbed for 2 h at room temperature with 10 µg/ml of the peptide used for immunisations.

Two independent investigators evaluated all immunohistochemical stainings. All sections were scored by means of the immunoreactivity score (IRS) according to Remmele & Stegner (37), noting the intensity of the colour as well as the percentage of cells showing a positive cytoplasmic staining or staining of the cell membranes. The percentage of positive cells was calculated as follows: no positive cells (0); <10% positive cells (1); 10–50% positive cells (2); 51–80% positive cells (3); and >80% positive cells (4). For the intensity of staining: no staining (0); mild staining (1); moderate staining (2); and strong staining (3). The overall IRS was calculated as (percentage of positive cells) × (intensity of staining), and classified as negative (‘–’; IRS 0 and 1), weak expression (positive ‘+’; IRS 2 and 3), moderate expression (positive ‘++’; IRS 4–8) or strong expression (positive ‘+++’; IRS 9–12).

Statistical analysis

Results are expressed as mean $\pm$ s.e.m. or median (min–max) as appropriate. Statistical differences between the groups were determined by Mann–Whitney *U* test. Correlations were calculated by χ^2 -test for dichotomous variables by Fisher's exact *P* two-sided if numbers were below 7, while Spearman's correlation coefficient was used for interval variables (semi-quantitative analysis). All calculations were performed by Statistica V6 (StatSoft, Hamburg, Germany). A *P* value <0.05 was considered significant. The analysis of the GH dose–response was performed by GraphPad Prism software (La Jolla, CA, USA) with a sigmoidal dose–response curve fitting model where $Y = \text{Bottom} + ((\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogIC}_{50} - X))))$ and where *X* is the logarithm of the concentration and *Y* is the response concentration; *Y* starts at Bottom and goes to Top with a sigmoid shape.

Results

Adenoma characterisation

Twenty-five of the 27 adenomas (93%) were positive for GH expression, while two (nos 65 and 72) were classified as GH-secreting adenomas due to the presence of fibrous bodies, characteristic morphology and GH secretion *in vitro* (Table 1). The majority of the adenomas were negative for ACTH, LH, FSH, TSH and PRL and were classified accordingly as monohormonal. Five adenomas demonstrated additional positivity for PRL and hence were defined as bihormonal for GH and PRL (nos 72, 75, 76, 81 and 89). Granularity was characterised as either dense (*n* = 15) or sparse (*n* = 12) and was almost equally distributed (Table 1).

Sst protein expression

Sst expression was investigated in 18 of the 21 (86%) adenomas of the dose–response experiments (Table 1). Eleven of the eighteen (61%) were positive for sst1 and 15 of the 18 (83%) were positive for sst2A. Sst3 and sst5 were expressed in all adenomas while sst4 immunoreactivity was positive in eight of the 18 (44%) adenomas. Individual IRSs are indicated in Table 1. Examples of positive immunoreactivity to sst are given in Fig. 1. For further details on intensity of staining and percentage of positive cells see Supplementary Table 2, see section on supplementary data given at the end of this article.

Basal GH secretion

The basal GH secretion varied considerably between tumours (Table 1). In the dose–response study it was 156 $\mu\text{g/l}$ (6.5–849.3; median, range). The basal GH secretion was independent of the pre-operative treatment strategy (SSA-pre-treated vs therapy-naïve, $r = -0.22$, *P* = NS), the adenomas' hormonal expression pattern (mono- vs bihormonal, $r = -0.1049$, *P* = NS), granularity of the tumour tissue (densely vs sparsely, $r = -0.4191$, *P* = NS), as well as the IRS of sst1–sst5. In addition, the individual cell cultures' cell numbers, counted immediately before the addition of SSA (data not shown), were unrelated to the basal GH concentration of each adenoma, indicating the high variability of GH secretion by the individual tumour cells.

Suppression of GH by SSA is time- and dose-dependent

Six adenomas were used for the time-series experiments. Suppression of GH secretion increased with time in three of the six adenomas responsive to either SSA

Figure 1 Immunohistochemical expression of sst1, sst2A, sst3, sst4 and sst5. Examples (adenoma nos 73, 82 and 90) of specific staining for each sst are shown. Pancreatic islets were used as positive control for staining with antibodies to sst1, sst2A, sst3 and sst5. Sst4 staining in islets was not detectable.

Figure 2 Time-course experiments. Time-dependent decline of the GH concentration in the three responders (% of baseline; adenoma nos 65, 68 and 70) and one non-responder (adenoma no. 69) after incubation with DG3173 (1 μ M, grey line) or Octreotide (1 μ M, black line). Results are expressed as the mean ($\pm$ S.E.M.; three wells per time point) GH suppression relative to baseline. The dashed line indicates the baseline, set as 100%, the crossing with the y-axis representing the start of the experiment.

(nos 65, 68 and 70, Table 1 and Fig. 2) and was significantly suppressed to levels below 80% of baseline at 6 h. Hence, this time interval was used for the consecutive dose-response study. A dose-dependent (range 10^{-12} to 10^{-6} M for both SSA) suppression of GH could be demonstrated in responsive adenomas (examples are given in Fig. 3). Dose-response curves could not be generated from the other responsive adenomas due to insufficient cell material. The individual IC_{50} values varied by up to 2 log magnitudes, reflecting the heterogeneity of these adenomas (Table 3). Although the median IC_{50} value for DG3173 was about 1 log magnitude lower than for Octreotide, the range for both SSA was similar. Maximum GH suppression was achieved with either 100 nM or 1 μ M of DG3173 or Octreotide respectively (Fig. 4).

DG3173 suppresses GH secretion in a larger number of adenomas compared with Octreotide

Treatment with DG3173 achieved GH suppression to at least 80% of baseline in a larger number of adenomas than Octreotide (DG3173 vs Octreotide: 10/21, 48% vs 5/21, 24%; $P=NS$, Table 1). Whereas the difference failed to be significant, the distribution of GH suppression was found to be interesting, since six non-responders to Octreotide (nos 72, 80, 81, 83, 90 and 91) demonstrated a positive response to DG3173, and only one adenoma that was responsive to Octreotide was non-responsive to DG3173 (no. 76). In responding adenomas the extent of the GH-lowering effect was similar for DG3173 and Octreotide respectively (per cent of basal GH conc., mean $\pm$ S.E.M., DG3173: $66.6\% \pm 4.4$, Octreotide: $64.9\% \pm 5.4$, $P=NS$, Table 1).

Analysis of factors that might influence tumour response to SSA

Pre-operative therapy with SSA Pre-operative Octreotide therapy did not affect GH suppression by either SSA. Of six out of 21 adenomas (dose-response experiment) with pre-operative SSA therapy, four of the six (67%) and two of the six (33%) responded to DG3173 and Octreotide respectively. Similarly, of 15 out of 21 therapy-naïve adenomas, six of the 15 (40%) and three of the 15 (20%) responded to DG3173 and Octreotide respectively. Thus, neither the number of responding adenomas, nor the extent of the GH suppression (Table 1), was influenced by pre-operative SSA treatment (Table 4) and this result was confirmed if only those tumours with a significant decline of the GH concentration were considered.

Hormone expression and morphology of the adenoma In both, mono- and bihormonal adenomas, a response to SSA was observed more often for DG3173 than for Octreotide (monohormonal: 8/16, 50% vs

Figure 3 Dose-response experiments. Individual dose-response curves for the inhibition of GH secretion by SSA titrated from 10^{-12} to 10^{-6} M in six adenoma cell cultures. A dose-dependent suppression of GH secretion (% of baseline) by both SSA (DG3173, triangles and black line; Octreotide, squares and grey line) was observed in four adenomas (A–D; adenoma nos 71, 73, 79 and 91). GH secretion was dose-dependently suppressed in one additional adenoma, respectively, by either Octreotide (E; adenoma no. 84) or DG3173 (F; adenoma no. 81; bihormonal). Results are expressed as the mean ($\pm$ S.E.M.) GH suppression relative to baseline (C, Control=baseline; medium alone). The corresponding IC_{50} values are shown in Table 3.

Table 3 Summary of adenomas with dose-dependent suppression of GH.

Adenoma	IC ₅₀	
	DG3173	Octreotide
Number		
71	4.9×10^{-10}	5.3×10^{-9}
73	3.9×10^{-9}	2.9×10^{-10}
79	1.7×10^{-9}	1.7×10^{-9}
81	3.3×10^{-10}	— ^a
84	— ^a	5.0×10^{-11}
91	1.6×10^{-11}	2.8×10^{-9}
Summary		
n	5	5
Mean	1.28×10^{-9}	2.03×10^{-9}
s.d.	1.59×10^{-9}	2.14×10^{-9}
Median	4.90×10^{-10}	1.70×10^{-9}
Range ^b	1.6×10^{-11} – 3.9×10^{-10}	5.0×10^{-11} – 5.3×10^{-10}

^aNo dose-dependency could be established.
^b5th/95th percentile.

4/16, 25%; bihormonal 2/5, 40% vs 1/5, 20%, respectively), however this was not significant. Overall, hormonal expression (mono- vs bihormonal) did not influence the response to either SSA (Table 4) and this result was confirmed if only those tumours with a significant decline of the GH concentration were considered.

In addition to hormonal expression, cell granularity was assessed as a morphological parameter that might influence the response of tumour tissue to SSA. DG3173 induced a positive response significantly more often in sparsely than densely granulated adenomas ($\chi^2=10.75$, $P=0.002$), while no such correlation

occurred with Octreotide (Table 4). Overall, sparsely granulated adenomas demonstrated a larger number of positive responses to SSA compared with densely granulated adenomas ($\chi^2=5.84$, $P=0.023$) and again these results were confirmed if only those tumours with a significant decline of the GH concentration were considered ($\chi^2=7.988$, $P=0.009$).

Sst status Eighteen adenomas were available for the evaluation of sst expression. Here DG3173 achieved a positive response in a larger number of adenomas than Octreotide in each of the sst-subtype positive adenomas. Analysing sst subtypes for their correlation to the GH response for each of the SSA indicated a correlation between the presence of sst1 and the GH response to DG3173 ($\chi^2=7.9$, $P=0.009$) and sst2 and the GH response to Octreotide ($\chi^2=9.36$, $P=0.012$; Table 4). This relationship failed to be significant if only adenomas with a significant decline of the GH concentration were considered. Due to the dichotomous nature of the variables these statistical results do not indicate the direction of the relationship (positive or negative correlation).

A semi-quantitative approach using the calculated IRSs (ranging from 0 to 12) and the per cent decline of the GH concentration was then used (Spearman's correlation coefficient). This showed a negative correlation between the IRS for sst2 and the per cent decline of the GH concentration induced by Octreotide (Spearman's $r=-0.565$, $P<0.02$), while no such correlation was found for DG3173. No other significant correlations were observed between the extent of GH inhibition by either SSA and the IRS of sst expression.

Figure 4 GH suppression by either DG3173 or Octreotide. GH secretion (% of baseline) in adenoma cell cultures ($n=21$). Maximal suppression of GH in adenomas responsive to DG3173 (black bars) or to Octreotide (grey bars) at 100 nM or 1 μ M incubated for 6 h. Results are expressed as the mean ($\pm$ S.E.M.; 3–6 wells per concentration) GH suppression relative to control. Response was defined as GH suppression to at least 80% of baseline or less (dashed line). ¹Patients pre-treated with SSA (see Table 1); ²bihormonal adenomas (see Table 1).

Table 4 Summary of variables affecting response to SSA treatment in pituitary adenoma cultures *in vitro*.

Variable/subgroup	Response ^a		P ^b	
	DG3173 (n=10)	Octreotide (n=5)	DG3173	Oct.
Pre-operative treatment				
SSA-treated (n=6)	4 (66.7)	2 (33.3)	NS	NS
SSA-naïve (n=15)	6 (40.0)	3 (20.0)		
Hormonal expression				
Monohormonal (n=16)	8 (50.0)	4 (25.0)	NS	NS
Bihormonal (n=5)	2 (40.0)	1 (20.0)		
Morphology				
Sparse (n=9)	8 (88.8)	3 (33.3)	$\chi^2=10.75$	NS
Dense (n=12)	2 (16.7)	2 (16.7)	$P=0.002$	
Sst protein expression ^c				
Sst1 negative (n=7)	1 (14.3)	1 (14.3)	$\chi^2=7.9$	NS
Sst1 positive (n=11)	9 (81.8)	4 (36.4)	$P=0.009$	
Sst2A negative (n=3)	2 (66.6)	3 (100.0)	NS	$\chi^2=9.36$
Sst2A positive (n=15)	8 (53.3)	2 (13.3)		$P=0.012$
Sst3 negative (n=0)	0	0	NA	NA
Sst3 positive (n=18)	10 (55.6)	5 (27.8)		
Sst4 negative (n=10)	5 (50.0)	2 (20.0)	NS	NS
Sst4 positive (n=8)	5 (62.5)	3 (37.5)		
Sst5 negative (n=0)	0	0	NA	NA
Sst5 positive (n=18)	10 (55.6)	5 (27.8)		

^aResponse was defined as GH suppression to at least 80% of baseline or less (SSA doses ranging from 10^{-12} to 10^{-6} M, where available).

^b χ^2 by Fisher's two-sided test (NS, not significant; NA, not applicable).

^cFor classification of sst expression see Methods section.

Discussion

The clinically available SSA Octreotide and Lanreotide are the first-line medical therapy in acromegalic patients not cured by surgery (2). However, SSA therapy results in suppression of the GH concentration to <2.5 $\mu\text{g/l}$ in only 48–58% of the patients (4) with suppression of insulin as an unwanted side effect (5, 6).

Sst distribution in somatotroph adenomas is heterogeneous and even varies in individual tumours, and thus SSA with a broad range of sst binding may prove clinically useful. BIM-23244, an SSA with high-affinity binding to sst2 and sst5 receptors, has higher efficacy in GH suppression *in vitro* than Octreotide (38). DG3173 is a new SSA that binds to sst2 and sst5, and also binds with nanomolar affinity to sst4. Importantly, it has only low insulin-suppressing activity (24). Thus, DG3173 may prove to be at least as efficient, as well as even more specific as already existing SSA. In contrast, another new SSA SOM230 with a broad range of sst binding has been shown to substantially inhibit insulin secretion *in vivo* (14, 20, 39, 40).

The GH-antagonist pegvisomant normalised the IGF1 concentration in up to 97% of patients under study conditions (41, 42). In contrast, long-term observational data describe a significantly lower percentage of IGF1 normalisation (72 and 58%) after 4 and 5 years of therapy respectively (43, 44). However, the risk of tumour regrowth is still of concern in patients with tumour remnants (45). Additional possible side effects are the abolition of any GH effect, with lipohypertrophy at injection sites and an increase in intra-abdominal fat mass (46, 47, 48).

In conclusion, there is still need for an effective treatment of acromegaly without the side effects occurring with current therapies.

We herein report the *in vitro* effect of DG3173 in a large number of somatotroph adenomas, extending a previous report (24). In addition, we investigated possible correlations of GH suppression with pre-surgical SSA therapy, hormonal expression of the tumour, tumour morphology and sst expression.

Overall DG3173 suppressed GH secretion with a similar efficacy, yet in a larger number of tumours, than Octreotide. Interestingly, it proved to be effective in some tumours that did not respond to Octreotide at all, suppressing GH in 38% (6/16) of Octreotide non-responders. Similar effects have already been described for SOM230 which has a 30, 5 and 40 times higher binding affinity to sst1, sst3 and sst5 receptors, respectively, and 2.5 times lower affinity to sst2 (20). By comparing reports from the literature on the efficacy of SOM230 with our results, a similar number of tumours responded to both Octreotide and SOM230, with GH suppression in seven of the nine (Octreotide) and eight of the nine (SOM230), rendering SOM230 only slightly more effective than Octreotide (49). In contrast DG3173 suppressed GH in six adenomas, non-responsive to Octreotide, indicating a broader range of responsive adenomas compared with Octreotide.

Overall, in this experimental setting, DG3173 was slightly more efficacious than Octreotide in suppressing GH secretion in terms of IC_{50} (IC_{50} : 0.49 vs 1.7 nM, DG3173 vs Octreotide), with a broad range in individual adenomas for both SSA. A similar efficacy

has been reported, with IC_{50} in nanomolar concentrations for both Octreotide and SOM230, where the IC_{50} of SOM230 was slightly above that of Octreotide (50). Comparing these data it seems possible that DG3173 might effectively reduce GH secretion in a larger number of somatotroph adenomas at even lower drug concentrations than both Octreotide and SOM230 respectively. However, a direct comparison of DG3173 and SOM230 has not yet been reported. Nevertheless, if our results are confirmed DG3173 may be a clinical alternative to the available SSA, as more patients might demonstrate GH suppression than with Octreotide.

In an attempt to identify possible predictors of DG3173 efficacy in suppressing GH secretion we investigated several parameters that might influence SSA effects *in vitro*.

Pre-operative therapy with SSA

Pre-operative SSA treatment had no effect on the number of responding tumours, or the degree of GH suppression, by either of the analogues. In seven patients that underwent pre-operative SSA therapy, long-acting SSA were used in all but one patient. However, a persistent drug effect on *in vitro* GH secretion is highly unlikely, due to washing and diluting effects during the preparation of the cell cultures.

Hormone expression and morphology of the adenoma

Tumour responsiveness to either SSA was independent of histo-morphological parameters, i.e. hormone expression (only GH or GH plus PRL expression). Saveanu *et al.* (51) investigated eleven GH-secreting adenomas including seven somatolactotroph tumours. In their investigations the GH-suppressing effect of Octreotide was independent of the hormonal status of the adenoma, but related to the expression of sst2 mRNA. In a mixed gonadotroph adenoma Lanreotide was effective in reducing LH, α -subunit and PRL secretion due to a high expression of sst2 and sst5 mRNA (52). Casarini *et al.* (53) found a differential expression of sst mRNA in mono- vs bihormonal GH-secreting adenomas. However, no analysis was performed with respect to the response to SSA therapy for mono- vs bihormonal tumours. There are only few data available relating the immunohistochemical expression of both, pituitary hormones and sst, indicating a similar pattern of sst distribution for both mono- and bihormonal adenomas (29). No reports are available that correlate these findings to the tumour response to SSA therapy. The pattern of hormone expression is a specific characteristic of any given pituitary tumour and may thus well influence the distribution of sst receptors. Since we could not demonstrate a significant correlation between the GH decline and hormone expression, its possible influence

on the immunohistochemical presence of sst and remains to be further elucidated.

GH-secreting adenomas are sub-classified into either densely or sparsely granulated tumours, with a disputed tendency for more aggressive behaviour, including increased hormone secretion, proliferation and recurrence after surgery in sparsely granulated adenomas (54). In our series both types of adenomas were well represented. The significant positive correlation of a positive response for DG3173 with sparsely granulated tumours was not evident for Octreotide. However, lack of significance may be due to the small numbers in this subgroup. Our data are in contrast to reports from the literature. Stefanescu *et al.* (55) demonstrated a mild decrease of the GH and sst2 mRNA signals in the densely granulated adenomas of 14 somatotropinomas with pre-operative Octreotide therapy. The authors concluded that densely granulated adenomas may have a more favourable response to Octreotide therapy than sparsely granulated adenomas. Thodou *et al.* (56) described higher GH secretion in densely ($n=5$) compared with sparsely ($n=5$) granulated somatotroph adenomas. In a semi-quantitative *in vitro*-approach, using reverse haemolytic plaque assays (rHPA) for the determination of GH secretion, in those six tumours treated with Octreotide the GH secretion was preferentially reduced in the densely granulated adenomas. Bhayana *et al.* (57) investigated the correlation of adenoma morphology to subsequent *in vivo* Octreotide therapy in 40 acromegalic patients, including 23% with irradiation before SSA therapy. The presence of a dense granulation was a significant predictor of complete remission. However, using Kaplan–Meier analysis, with the time interval to complete remission as an endpoint and densely vs sparsely granulated adenomas as variables, this relationship failed to be significant. These different approaches using either GH mRNA, rHPA as an indicator of GH secretion or *in vivo* GH secretion are not strictly comparable to our investigation as we compare *in vitro* morphological data to quantitative determination of the *in vitro* GH secretion. In our investigation the overall distribution of sst was not significantly different between sparsely and densely granulated adenomas. Thus, it is conceivable that the granularity of the adenoma is related to specific tumour characteristics that either support or reduce the GH-suppressing effect of SSA in these adenomas. However, larger series of GH-secreting adenomas will have to be analysed to confirm this hypothesis.

Somatostatin receptors

The qualitative analysis (presence/absence of sst vs presence/absence of a positive response to each of the SSA) confirmed a correlation between sst2 receptor expression at the protein level and the GH-lowering effect of Octreotide, as has also been demonstrated by others (26, 58). A semi-quantitative (expression of sst

quantified by IRS vs per cent decline of the GH concentration for each SSA) analysis revealed a negative correlation of the IRS for sst2 and the per cent GH decline induced by Octreotide. No further correlations were evident for the other ssts and Octreotide or any sst and DG3173. This may in part be due to the small number of adenomas in each of the relevant ssts subgroups. However, the negative correlation of the IRS with the GH decline induced by Octreotide is unexpected. This may indicate that either the IRS may not fully represent the functional activity of the ssts or that the GH-lowering effect is transferred via functional interaction with more than one sst. Furthermore, sst may form homo- and heterodimers, and this may modify the functional properties of the receptors and result in higher efficacy (9). In addition, heterodimerisation of the different ssts upon binding would preclude any clear-cut correlation between receptor expression and signal transduction after ligand binding in multiligand SSA (9, 10). We have previously demonstrated a lack of correlation between the results of sst scintigraphy (reflecting sst2-status of the adenoma) and the effect of Octreotide on GH secretion or tumour shrinking in acromegaly (59).

In conclusion DG3173, a new SSA with a unique binding pattern to sst, suppresses GH in most Octreotide-responsive somatotroph adenomas as well as in a large number of tumours not responding to Octreotide, thus expanding the range of tumours that can be possibly treated with SSA. In addition, others (24, 30) demonstrated the weak effect of DG3173 on insulin secretion. Should our *in vitro* results on GH secretion be confirmed in clinical studies, then the combination of these two effects would be a strong argument for the use of DG3173 in acromegalic patients not cured by surgery.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-11-0737>.

Declaration of interest

U Plöckinger, U Hoffmann, M Geese, A Lupp, J Flitsch, M Buchfelder, P Vajkoczy, W Jakob, W Saeger, S Schulz and C Dohrmann have nothing to declare. U Hoffmann, M Geese and C Dohrmann are employees of Develogen.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement

U Plöckinger and U Hoffmann designed, performed and supervised the *in vitro* investigation, calculated the data and wrote the manuscript. M Geese supervised the GH assays and prepared all figures. W Saeger, A Lupp and S Schulz performed the immunohistochemical analysis on

pituitary hormones (S Schulz) and somatostatin receptors (A Lupp and S Schulz). M Buchfelder, J Flitsch and P Vajkoczy provided the tumour tissue. W Jakob helped with patient identification, recruitment and clinical data collection of the Berlin patients. All authors provided critical comments and a final approval of the manuscript.

Acknowledgements

We thank Monique Erling and Christina Kothe for GH ELISA analyses (DeveloGen AG), and Ines Eichhorn for adenoma tissue preparation and cell culture experiments (Stoffwechsel-Centrum, Charité). The following neuropathologists have contributed to the publication by allowing us to use part of the adenoma tissue for these experiments: Prof. Dr Frank Heppner (Institut für Neuropathologie, Charité-Universitätsmedizin Berlin) and Prof. Dr med. Rolf Buslei (Institut für Neuropathologie, Universität Erlangen-Nürnberg).

References

- 1 Buchfelder M & Schläpfer S. Surgical treatment of pituitary tumours. *Best Practice & Research. Clinical Endocrinology & Metabolism* 2009 **23** 677–692. (doi:10.1016/j.beem.2009.05.002)
- 2 Cook DM, Ezzat S, Katznelson L, Kleinberg DL, Laws ER Jr, Nippoldt TB, Swearingen B & Vance ML. AACE Medical Guidelines for Clinical Practice for the diagnosis and treatment of acromegaly. *Endocrine Practice* 2011 **Suppl 4** 1–44. (doi:10.4158/11215.OR)
- 3 Ben-Shlomo A & Melmed S. Somatostatin agonists for treatment of acromegaly. *Molecular and Cellular Endocrinology* 2008 **286** 192–198. (doi:10.1016/j.mce.2007.11.024)
- 4 Freda PU, Katznelson L, van der Lely AJ, Reyes CM, Zhao S & Rabinowitz D. Long-acting somatostatin analog therapy of acromegaly: a meta-analysis. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4465–4473. (doi:10.1210/jc.2005-0260)
- 5 Plockinger U, Holst JJ, Messerschmidt D, Hopfenmuller W & Quabbe HJ. Octreotide suppresses the incretin glucagon-like peptide (7–36) amide in patients with acromegaly or clinically nonfunctioning pituitary tumors and in healthy subjects. *European Journal of Endocrinology* 1999 **140** 538–544. (doi:10.1530/eje.0.1400538)
- 6 Mazziotti G, Floriani I, Bonadonna S, Torri V, Chanson P & Giustina A. Effects of somatostatin analogs on glucose homeostasis: a metaanalysis of acromegaly studies. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 1500–1508. (doi:10.1210/jc.2008-2332)
- 7 Singh V, Brendel MD, Zacharias S, Mergler S, Jahr H, Wiedenmann B, Bretzel RG, Plockinger U & Strowski MZ. Characterization of somatostatin receptor subtype-specific regulation of insulin and glucagon secretion: an *in vitro* study on isolated human pancreatic islets. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 673–680. (doi:10.1210/jc.2006-1578)
- 8 Strowski MZ & Blake AD. Function and expression of somatostatin receptors of the endocrine pancreas. *Molecular and Cellular Endocrinology* 2008 **286** 169–179. (doi:10.1016/j.mce.2008.02.007)
- 9 Duran-Prado M, Malagon MM, Gracia-Navarro F & Castano JP. Dimerization of G protein-coupled receptors: new avenues for somatostatin receptor signalling, control and functioning. *Molecular and Cellular Endocrinology* 2008 **286** 63–68. (doi:10.1016/j.mce.2007.12.006)
- 10 Olias G, Viollet C, Kusserow H, Epelbaum J & Meyerhof W. Regulation and function of somatostatin receptors. *Journal of Neurochemistry* 2004 **89** 1057–1091. (doi:10.1111/j.1471-4159.2004.02402.x)
- 11 Poll F, Lehmann D, Illing S, Gjinj M, Jacobs S, Lupp A, Stumm R & Schulz S. Pasireotide and octreotide stimulate distinct patterns of sst2A somatostatin receptor phosphorylation. *Molecular Endocrinology* 2010 **24** 436–446. (doi:10.1210/me.2009-0315)

- 12 Ma P, Wang Y, van der Hoek J, Nedelman J, Schran H, Tran LL & Lamberts SW. Pharmacokinetic–pharmacodynamic comparison of a novel multiligand somatostatin analog, SOM230, with octreotide in patients with acromegaly. *Clinical Pharmacology and Therapeutics* 2005 **78** 69–80. (doi:10.1016/j.clpt.2005.04.003)
- 13 Plockinger U, Albrecht S, Mawrin C, Saeger W, Buchfelder M, Petersenn S & Schulz S. Selective loss of somatostatin receptor 2 in octreotide-resistant growth hormone-secreting adenomas. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1203–1210. (doi:10.1210/jc.2007-1986)
- 14 Petersenn S, Schopohl J, Barkan A, Mohideen P, Colao A, Abs R, Buchelt A, Ho YY, Hu K, Farrall AJ, Melmed S & Biller BM. Pasireotide (SOM230) demonstrates efficacy and safety in patients with acromegaly: a randomized, multicenter, phase II trial. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2781–2789. (doi:10.1210/jc.2009-2272)
- 15 Petersenn S, Unger N, Hu K, Weisshaar B, Zhang Y, Bouillaud E, Hermosillo Resendiz K, Wang Y & Mann K. Pasireotide (SOM230), a novel multi-receptor-targeted somatostatin analogue, is well tolerated when administered as a continuous 7-day subcutaneous infusion in healthy male volunteers. *Journal of Clinical Pharmacology* 2012. In press. (doi:10.1177/0091270011408727)
- 16 van der Hoek J, de Herder WW, Feelders RA, van der Lely AJ, Uitterlinden P, Boerlin V, Bruns C, Poon KW, Lewis I, Weckbecker G, Krahne T, Hofland LJ & Lamberts SW. A single-dose comparison of the acute effects between the new somatostatin analog SOM230 and octreotide in acromegalic patients. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 638–645. (doi:10.1210/jc.2003-031052)
- 17 Boerlin V, van der Hoek J, Beglinger C, Poon KW, Hartmann S, Dutreix C, Kovarik JM, Bruns C, Weckbecker G, Lewis I, Schnieper P, Hofland LJ & Lamberts SW. New insights on SOM230, a universal somatostatin receptor ligand. *Journal of Endocrinological Investigation* 2003 **26** 14–16.
- 18 Ben-Shlomo A & Melmed S. Pasireotide – a somatostatin analog for the potential treatment of acromegaly, neuroendocrine tumors and Cushing's disease. *IDrugs: the Investigational Drugs Journal* 2007 **10** 885–895.
- 19 Schmid HA. Pasireotide (SOM230): development, mechanism of action and potential applications. *Molecular and Cellular Endocrinology* 2008 **286** 69–74. (doi:10.1016/j.mce.2007.09.006)
- 20 Bruns C, Lewis I, Briner U, Meno-Tetang G & Weckbecker G. SOM230: a novel somatostatin peptidomimetic with broad somatotropin release inhibiting factor (SRIF) receptor binding and a unique antisecretory profile. *European Journal of Endocrinology* 2002 **146** 707–716. (doi:10.1530/eje.0.1460707)
- 21 Ferone D, Gatto F, Arvigo M, Resmini E, Boschetti M, Teti C, Esposito D & Minuto F. The clinical-molecular interface of somatostatin, dopamine and their receptors in pituitary pathophysiology. *Journal of Molecular Endocrinology* 2009 **42** 361–370. (doi:10.1677/JME-08-0162)
- 22 Jaquet P, Gunz G, Saveanu A, Barlier A, Dufour H, Taylor J, Dong J, Kim S, Moreau JP & Culler MD. BIM-23A760, a chimeric molecule directed towards somatostatin and dopamine receptors, vs universal somatostatin receptors ligands in GH-secreting pituitary adenomas partial responders to octreotide. *Journal of Endocrinological Investigation* 2005 **28** 21–27.
- 23 Jaquet P, Gunz G, Saveanu A, Dufour H, Taylor J, Dong J, Kim S, Moreau JP, Enjalbert A & Culler MD. Efficacy of chimeric molecules directed towards multiple somatostatin and dopamine receptors on inhibition of GH and prolactin secretion from GH-secreting pituitary adenomas classified as partially responsive to somatostatin analog therapy. *Journal of Endocrinology* 2005 **153** 135–141. (doi:10.1530/eje.1.01950)
- 24 Afargan M, Janson ET, Geleman G, Rosenfeld R, Ziv O, Karpov O, Wolf A, Bracha M, Shohat D, Liapakis G, Gilon C, Hoffman A, Stephensky D & Oberg K. Novel long-acting somatostatin analog with endocrine selectivity: potent suppression of growth hormone but not of insulin. *Endocrinology* 2001 **142** 477–486. (doi:10.1210/en.142.1.477)
- 25 Taboada GF, Luque RM, Bastos W, Guimaraes RF, Marcondes JB, Chimelli LM, Fontes R, Mata PJ, Filho PN, Carvalho DP, Kineman RD & Gadelha MR. Quantitative analysis of somatostatin receptor subtype (SSTR1–5) gene expression levels in somatotropinomas and non-functioning pituitary adenomas. *European Journal of Endocrinology* 2007 **156** 65–74. (doi:10.1530/eje.1.02313)
- 26 Taboada GF, Luque RM, Neto LV, Machado Ede O, Sbaffi BC, Domingues RC, Marcondes JB, Chimelli LM, Fontes R, Niemeyer P, de Carvalho DP, Kineman RD & Gadelha MR. Quantitative analysis of somatostatin receptor subtypes (1–5) gene expression levels in somatotropinomas and correlation to *in vivo* hormonal and tumor volume responses to treatment with octreotide LAR. *European Journal of Endocrinology* 2008 **158** 295–303. (doi:10.1530/EJE-07-0562)
- 27 Pawlikowski M, Pisarek H, Kunert-Radek J & Radek M. Somatostatin receptors in GH-secreting pituitary adenomas – their relationship to the response to octreotide. *Endokrynologia Polska* 2008 **59** 196–199.
- 28 Pisarek H, Pawlikowski M, Kunert-Radek J & Radek M. Expression of somatostatin receptor subtypes in human pituitary adenomas – immunohistochemical studies. *Endokrynologia Polska* 2009 **60** 240–251.
- 29 Thodou E, Kontogeorgos G, Theodossiou D & Pateraki M. Mapping of somatostatin receptor types in GH or/and PRL producing pituitary adenomas. *Journal of Clinical Pathology* 2006 **59** 274–279. (doi:10.1136/jcp.2005.026914)
- 30 Shimon I, Rubinek T, Hadani M & Alhadeef N. PTR-3173 (somatoprim), a novel somatostatin analog with affinity for somatostatin receptors 2, 4 and 5 is a potent inhibitor of human GH secretion. *Journal of Endocrinological Investigation* 2004 **27** 721–727.
- 31 Giustina A, Barkan A, Chanson P, Grossman A, Hoffman A, Ghigo E, Casanueva F, Colao A, Lamberts S, Sheppard M & Melmed S. Guidelines for the treatment of growth hormone excess and growth hormone deficiency in adults. *Journal of Endocrinological Investigation* 2008 **31** 820–838.
- 32 Tulipano G, Bonfanti C, Milani G, Billici B, Bollati A, Cozzi R, Maira G, Murphy WA, Poiesi C, Turazzi S & Giustina A. Differential inhibition of growth hormone secretion by analogs selective for somatostatin receptor subtypes 2 and 5 in human growth-hormone-secreting adenoma cells *in vitro*. *Neuroendocrinology* 2001 **73** 344–351. (doi:10.1159/000054651)
- 33 Schulz S, Schmitt J, Wiborny D, Schmidt H, Olbricht S, Weise W, Roessner A, Gramsch C & Holtt V. Immunocytochemical detection of somatostatin receptors sst1, sst2A, sst2B, and sst3 in paraffin-embedded breast cancer tissue using subtype-specific antibodies. *Clinical Cancer Research* 1998 **4** 2047–2052.
- 34 Mawrin C, Schulz S, Hellwig-Patyk A, Kirches E, Roessner A, Lendeckel U, Firsching R, Vorwerk CK, Keilhoff G, Dietzmann K, Grimm K, Lindberg G, Gutmann DH, Scheithauer BW & Perry A. Expression and function of somatostatin receptors in peripheral nerve sheath tumors. *Journal of Neuropathology and Experimental Neurology* 2005 **64** 1080–1088. (doi:10.1097/01.jnen.0000190065.36182.25)
- 35 Fischer T, Doll C, Jacobs S, Kolodziej A, Stumm R & Schulz S. Reassessment of sst2 somatostatin receptor expression in human normal and neoplastic tissues using the novel rabbit monoclonal antibody UMB-1. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4519–4524. (doi:10.1210/jc.2008-1063)
- 36 Lupp A, Hunder A, Petrich A, Nagel F, Doll C & Schulz S. Reassessment of sst5 somatostatin receptor expression in normal and neoplastic human tissues using the novel rabbit monoclonal antibody UMB-4. *Neuroendocrinology* 2011 **94** 255–264. (doi:10.1159/000329876)
- 37 Remmele W & Stegner H. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. *Der Pathologe* 1987 **8** 138–140.
- 38 Saveanu A, Gunz G, Dufour H, Caron P, Fina F, Ouafik L, Culler MD, Moreau JP, Enjalbert A & Jaquet P. Bim-23244, a

- somatostatin receptor subtype 2- and 5-selective analog with enhanced efficacy in suppressing growth hormone (GH) from octreotide-resistant human GH-secreting adenomas. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 140–145. (doi:10.1210/jc.86.1.140)
- 39 Hofland LJ, van der Hoek J, Feelders R, van der Lely AJ, de Herder W & Lamberts SW. Pre-clinical and clinical experiences with novel somatostatin ligands: advantages, disadvantages and new prospects. *Journal of Endocrinological Investigation* 2005 **28** 36–42.
 - 40 Weckbecker G, Briner U, Lewis I & Bruns C. SOM230: a new somatostatin peptidomimetic with potent inhibitory effects on the growth hormone/insulin-like growth factor-I axis in rats, primates, and dogs. *Endocrinology* 2002 **143** 4123–4130. (doi:10.1210/en.2002-220219)
 - 41 van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA & Thorner MO. Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. *Lancet* 2001 **358** 1754–1759. (doi:10.1016/S0140-6736(01)06844-1)
 - 42 Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF & Davis RJ. Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *New England Journal of Medicine* 2000 **342** 1171–1177. (doi:10.1056/NEJM200004203421604)
 - 43 Buchfelder M, Schlaffer S, Droste M, Mann K, Saller B, Brubach K, Stalla GK & Strasburger CJ. The German ACROSTUDY: past and present. *European Journal of Endocrinology* 2009 **161** (Suppl 1) S3–S10. (doi:10.1530/EJE-09-0350)
 - 44 Brue T. ACROSTUDY: status update on 469 patients. *Hormone Research* 2009 **71** (Suppl 1) 34–38. (doi:10.1159/000178035)
 - 45 Marazuela M, Paniagua AE, Gahete MD, Lucas T, Alvarez-Escola C, Manzanares R, Cameselle-Teijeiro J, Luque-Ramirez M, Luque RM, Fernandez-Rodriguez E, Castano JP & Bernabeu I. Somatotroph tumor progression during pegvisomant therapy: a clinical and molecular study. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E251–E259. (doi:10.1210/jc.2010-1742)
 - 46 Plöckinger U & Reuter T. Pegvisomant increases intra-abdominal fat in patients with acromegaly: a pilot study. *European Journal of Endocrinology* 2008 **158** 467–471. (doi:10.1530/EJE-07-0637)
 - 47 Maffei P, Martini C, Pagano C, Siculo N & Corbetti F. Lipohypertrophy in acromegaly induced by the new growth hormone receptor antagonist pegvisomant. *Annals of Internal Medicine* 2006 **145** 310–312.
 - 48 Marazuela M, Dauden E, Ocon E, Moure D & Nattero L. Pegvisomant-induced lipohypertrophy: report of a case with histopathology. *Annals of Internal Medicine* 2007 **147** 741–743.
 - 49 Feelders RA, Hofland LJ, van Aken MO, Neggers SJ, Lamberts SW, de Herder WW & van der Lely AJ. Medical therapy of acromegaly: efficacy and safety of somatostatin analogues. *Drugs* 2009 **69** 2207–2226. (doi:10.2165/11318510-000000000-00000)
 - 50 Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, Bruns C, Weckbecker G, Feelders R, van der Lely AJ, Beckers A & Lamberts SW. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas *in vitro*. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1577–1585. (doi:10.1210/jc.2003-031344)
 - 51 Saveanu A, Lavaque E, Gunz G, Barlier A, Kim S, Taylor JE, Culler MD, Enjalbert A & Jaquet P. Demonstration of enhanced potency of a chimeric somatostatin-dopamine molecule, BIM-23A387, in suppressing growth hormone and prolactin secretion from human pituitary somatotroph adenoma cells. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 5545–5552. (doi:10.1210/jc.2002-020934)
 - 52 Saveanu A, Morange-Ramos I, Gunz G, Dufour H, Enjalbert A & Jaquet P. A luteinizing hormone-, alpha-subunit- and prolactin-secreting pituitary adenoma responsive to somatostatin analogs: *in vivo* and *in vitro* studies. *European Journal of Endocrinology* 2001 **145** 35–41. (doi:10.1530/eje.0.1450035)
 - 53 Casarini AP, Jallad RS, Pinto EM, Soares IC, Nonogaki S, Giannella-Neto D, Musolino NR, Alves VA & Bronstein MD. Acromegaly: correlation between expression of somatostatin receptor subtypes and response to octreotide-lar treatment. *Pituitary* 2009 **12** 297–303. (doi:10.1007/s11102-009-0175-1)
 - 54 Saeger W, Ludecke DK, Buchfelder M, Fahlbusch R, Quabbe HJ & Petersenn S. Pathohistological classification of pituitary tumors: 10 years of experience with the German Pituitary Tumor Registry. *European Journal of Endocrinology* 2007 **156** 203–216. (doi:10.1530/eje.1.02326)
 - 55 Stefanescu L, Kovacs K, Thapar K, Horvath E, Melmed S & Greenman Y. Octreotide effect on growth hormone and somatostatin subtype 2 receptor mRNAs of the human pituitary somatotroph adenomas. *Endocrine Pathology* 2000 **11** 41–48. (doi:10.1385/EP:11:1:41)
 - 56 Thodou E, Kontogeorgos G, Kyrodinou E, Salla H, Ramyar L, Vamvassakis E, Piaditis G, Agnastopoulos N, Tzanis S, Levedis A, Rologis D & Asa SL. Structure–function correlations of growth hormone or/and prolactin-producing pituitary adenomas: an *in vitro* study with the reverse hemolytic plaque assay. *Journal of Endocrinological Investigation* 1999 **22** 671–680.
 - 57 Bhayana S, Booth GL, Asa SL, Kovacs K & Ezzat S. The implication of somatotroph adenoma phenotype to somatostatin analog responsiveness in acromegaly. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6290–6295. (doi:10.1210/jc.2005-0998)
 - 58 Acunzo J, Thirion S, Roche C, Saveanu A, Gunz G, Germanetti AL, Couderc B, Cohen R, Figarella-Branger D, Dufour H, Brue T, Enjalbert A & Barlier A. Somatostatin receptor sst2 decreases cell viability and hormonal hypersecretion and reverses octreotide resistance of human pituitary adenomas. *Cancer Research* 2008 **68** 10163–10170. (doi:10.1158/0008-5472.CAN-08-1857)
 - 59 Plöckinger U, Bäder M, Hopfenmüller W, Saeger W & Quabbe HJ. Results of somatostatin receptor scintigraphy do not predict pituitary tumor volume- and hormone-response to octreotide therapy and do not correlate with tumor histology. *European Journal of Endocrinology* 1997 **136** 369–376. (doi:10.1530/eje.0.1360369)

Received 22 August 2011

Revised version received 16 October 2011

Accepted 7 November 2011