CLINICAL STUDY

Octreotide in insulinoma patients: efficacy on hypoglycemia, relationships with Octreoscan scintigraphy and immunostaining with anti-sst2A and anti-sst5 antibodies

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Abstract

Objective: We studied the efficacy of octreotide treatment on hypoglycaemia in patients with insulinoma and its relationships with Octreoscan scintigraphy and the presence of tumoral somatostatin receptors sst2A and sst5.

Design and methods: 17 patients with insulinoma were evaluated using (i) evaluation of blood glucose, insulin and C-peptide during a short 100 µg octreotide test in fasting patients and/or treatment over 8 days–8 months with octreotide, (ii) Octreoscan scintigraphy and (iii) immunostaining of the tumor with anti-sst2A and anti-sst5.

Results: Octreotide was effective on hypoglycaemia in 10/17 patients. Octreoscan scintigraphy detected 4/17 insulinomas. sst2A receptor was detected in 7/17 insulinomas and sst5 in 15/17 insulinomas. Octreotide was effective on hypoglycaemia in those seven patients with sst2A receptor-expressing insulinoma, and in three patients with undetectable sst2A receptor and detectable sst5; it was ineffective in six patients whose tumor expressed the sst5 receptor with undetectable sst2A and in one patient with undetectable sst2A and sst5 receptor.

Conclusions: Octreotide is an effective treatment of hypoglycaemia in more than 50% of patients with insulinoma. Detection of responsive patients was better based on a positive short test with subcutaneous octreotide than on the results of Octreoscan scintigraphy. Positive anti-sst2 receptor immunostaining is associated with efficacy of octreotide treatment, but does not account for all cases of responsiveness to octreotide. Expression of sst5 receptor does not appear to explain *per se* the efficacy of octreotide on sst2A-negative insulinomas.

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Introduction

Insulinomas are tumors developed from islet β -cells that are liable to induce symptomatic and often severe hypoglycemia. Insulinomas are treated by surgery. However, a medical treatment to normalize blood glucose levels is useful in insulinoma patients with symptomatic hypoglycemia before performing surgery, or when surgical treatment is not possible. The reference medication remains diazoxide, but it is not effective in all patients, and can lead to several adverse effects (1).

Somatostatin is an ubiquitous polypeptide with numerous inhibitory functions. In the pancreas, somatostatin inhibits the secretion of insulin and glucagon (2). The effects of somatostatin are mediated through specific G protein-coupled transmembrane

receptors. To date, five receptors (sst1–sst5) have been cloned (3). The gene of sst2 gives rise to splice variants sst2A and sst2B which differ only in the length of the cytoplasmic C-terminal tail. Modifications in the amino acid sequence at the N- and C-terminal ends of endogenous human somatostatin led to synthetic somatostatin analogs, octreotide and lanreotide, that are by now widely employed for clinical practice. Such somatostatin analogs display preferential affinity for sst2A and sst5 receptors, and bind to a lesser extent to sst3 receptors (4). In addition, a somatostatin analog can be labelled by γ -emitting radioisotopes (Octreoscan) leading to visualization of somatostatin receptor-expressing tissues (4).

Since the presence of somatostatin receptors was observed in insulinomas, treatment with somatostatin analogs has also been performed successfully in

insulinoma patients (5). However, the usefulness of somatostatin analogs in the treatment of insulinoma patients remains controversial (6). In addition, scintigraphic imaging with Octreoscan has been introduced in an attempt to improve topographic assessment of insulinomas. The results were disappointing, since Octreoscan scintigraphy with planar imaging led to detection of only 20–50% of insulinomas (7–9). The use of single-photon emission computerized tomography (SPECT) was reported recently to improve detection of insulinomas by Octreoscan scintigraphy (9).

We have previous personal experience in our department of successful long-term treatment with octreotide in old patients with biological and radiological evidence for benign insulinomas, or in patients in whom curative surgery could not be performed. This prompted us to do the present clinical study in order to determine, in 17 new patients with insulinoma in whom surgery could be performed, and thereby providing histopathological confirmation of the diagnosis, (1) the efficacy of octreotide treatment in the control of hypoglycemia in insulinoma patients, (2) the role of imaging with Octreoscan in the management of insulinomas and its possible relationship with the efficacy of octreotide on hypoglycemia and (3) the relationships between the efficacy of octreotide or detection of the tumor with Octreoscan and the presence of sst2A and sst5 receptors on tumoral cells.

Patients and methods

Patients

The patients were 17 subjects (three male, 14 female) aged 53 ± 15 years (mean $\pm$ S.D.; range 18–78 years; Table 1). The diagnosis of insulinoma was made based on hyperinsulinemic hypoglycemia during a fast test (10, 11) and confirmed by histopathological examination of the tumor. Sixteen insulinomas were benign and one insulinoma was malignant with liver metastases (patient 12). The insulinoma was located in the pancreas in 16 patients (patients 1–8 and 10–17) and it was ectopic (in the peritoneal tissue under the pancreas) in one patient (patient 9). The mean tumor size of insulinoma was 19 ± 10 mm (range 6–40 mm). Abdominal computed tomography (CT) scan, ultrasonography and echoendoscopy were used to localize insulinomas. The present study was in agreement with the Helsinki Declaration of 1975, as revised in 2000.

Evaluation of the efficacy of octreotide on hypoglycemia

The efficacy of octreotide on hypoglycemia was studied in all patients using a short octreotide test and/or treatment with octreotide.

Short octreotide test

13 patients (patients 1, 2, 6–11, 13–17) with a benign insulinoma and one patient with a malignant insulinoma (patient 12) underwent the short octreotide test. Basal blood glucose (glucose oxydase), serum insulin (IRMA Pasteur Bi-Insulin Biorad kit; Pasteur-Diagnostics, Marnes La Coquette, France) and C-peptide (RIA C-peptid CTK kit; Sorin Biomedica, Saluggia, Italy) were measured after an overnight fast (last dietary intake at 00:00 h, basal samples being taken at 07:00 h). Then 100 μ g octreotide (Sandostatine; Novartis, Rueil-Malmaison, France) were injected subcutaneously, and blood samples were collected from an antecubital vein at hourly intervals over 6 h in order to measure blood glucose, serum insulin and C-peptide concentrations. The patient remained fasting throughout the test. The test was stopped in the case of symptomatic hypoglycaemia with blood glucose below 45 mg/dl (2.5 mM). In such cases, the patient was considered to be not responsive to the short octreotide test. Conversely, the patient was considered to be a responder if blood glucose increased to at least 100 mg/dl (5.5 mM) during the 6 h following the octreotide injection despite the lack of food intake.

Treatment with somatostatin agonists

Ten patients (patients 1, 3–7, 10, 12, 13, 15) underwent this treatment. It was instituted in order to normalize blood glucose during the period of time between the diagnosis of insulinoma and surgical removal of the tumor. The somatostatin agonist employed for treatment was subcutaneous octreotide (Sandostatine). The treatment was performed over 2 ± 3 months (8 days–8 months). All patients were treated initially with subcutaneous administration of 100 μ g octreotide twice a day, then the dose and the treatment protocol were modified rapidly, if necessary, according to blood glucose monitoring. Seven patients (patients 3–5, 7, 10, 13, 15) were treated with subcutaneous injections twice or three times a day while three patients (patients 1, 6, 12) were treated with a continuous subcutaneous administration of octreotide using an automated pump. The dose of octreotide was 280 ± 160 μ g/day (150–500 μ g) in the patients treated with multiple subcutaneous injections and 850 ± 350 μ g (500–1200 μ g) in the patients treated with continuous subcutaneous administration of octreotide.

Responders were defined as those patients whose clinical symptoms of hypoglycemia subsided during octreotide treatment. Blood glucose levels were measured during treatment with octreotide by self-monitoring (paper strip) in all the patients and by collecting blood samples from an antecubital vein during a stay in our department at 4-h intervals during 24 h in five patients (patients 1, 3, 5–7). Blood glucose

Table 1 Results of Octreoscan scintigraphy, response to octreotide and somatostatin sst2A and sst5 receptor expression in 17 insulinomas. Octreoscan scintigraphy: 0, no Octreoscan uptake; 1, uptake less than the physiological uptake of the liver; 2, uptake similar to that of the liver; 3, uptake greater than that of the liver. Response to octreotide: +, efficacy of octreotide on blood glucose levels; –, no response to octreotide administration. Immunostaining with anti-sst2A and anti-sst5 antibodies: M, cell membranes; C, cytoplasm; –, negative immunostaining; +, ++, +++, positive immunostaining, less than, similar to or greater than that of normal islets, respectively. ND = not done.

Patient no.	Age (years)	Sex	Tumor size (mm)	Location of tumor	Histopathology	Octreoscan scintigraphy	Response to short octreotide test	Efficacy of octreotide treatment	Immunostaining	
									sst2A	sst5
1	45	F	30	Pancreatic	Benign	0	+	+	C+++ M–	C+++
2	38	F	10	Pancreatic	Benign	0	–	ND	–	C+
3	52	F	10	Pancreatic	Benign	0	ND	+	C++ M–	C++
4	56	M	6	Pancreatic	Benign	0	ND	–	–	C++
5	78	F	15	Pancreatic	Benign	0	ND	+	–	C+
6	68	F	19	Pancreatic	Benign	3	+	+	C++ M++	C++
7	70	F	22	Pancreatic	Benign	0	+	+	C+ M+	C++
8	57	F	15	Pancreatic	Benign	0	–	ND	–	C++
9	58	F	20	Ectopic	Benign	3	+	ND	C+ M+	–
10	28	M	35	Pancreatic	Benign	1	+	+	–	C+
11	55	F	30	Pancreatic	Benign	0	–	ND	–	C+
12	54	F	40	Pancreatic	Malignant	3 (primitive tumor and metastasis)	+	–	Primitive tumor: C++ M– Metastasis: C+++ M+++	Metastasis: C+
13	18	F	12	Pancreatic	Benign	0	+	+	C++ M–	C++
14	65	F	8	Pancreatic	Benign	0	–	ND	–	–
15	56	F	15	Pancreatic	Benign	0	+	+	–	C+
16	56	M	13	Pancreatic	Benign	0	–	ND	–	C+
17	44	F	36	Pancreatic	Benign	0	–	ND	–	C+

levels were found to be more than 60 mg/dl in all these subjects.

Scintigraphy with labeled octreotide (Octreoscan)

All 17 patients had Octreoscan scintigraphy. The tracer used was ^{111}In -DTPA-D-Phe1-octreotide (Octreoscan; Mallinckrodt Medical, Petten, The Netherlands). To be injected, the radiochemical purity of ^{111}In -pentetreotide had to be higher than 90%. Images were acquired at 4 h (planar and whole-body images) and 24 h (planar and whole-body images, and SPECT) post-injection, using a large-field-of-view, double-head gamma camera fitted with a medium-energy collimator. The crystal thickness of this camera was 15.8 mm. Symmetrical 20% energy windows were centered over both photo peaks of ^{111}In and the data from both windows were added. Two intrinsic sensitivity maps (one for each ^{111}In photo peak; 173 and 247 KeV) were determined using a point source of ^{111}In . The procedure used was as follows: (i) 150 MBq Octreoscan were injected intravenously; (ii) patients were asked to void before image acquisitions and (iii) anterior and posterior planar localized images of the head, chest, abdomen and pelvis were acquired, using a 256×256 word matrix. The acquisition was stopped at 500 000 counts/frame (250 000 for the head). Whole-body images were acquired in anterior and posterior view into a 1024×256 word matrix from the head to the feet (bed speed of 10 cm/min). (iv) SPECT imaging was performed systematically on the abdomen and pelvis but might also concern other regions depending on the clinical history or pathological or suspicious pattern on planar images. SPECT acquisition parameters were as follows: 6° angular sampling, 128×128 matrix, 360° rotation (for each detector), 40 s/stop. Bowel cleansing was performed before scintigraphy, using a 3-day diet (low-fiber) and administration of a laxative preparation before imaging. All parameters were selected to yield the best detection with the camera employed. These procedures do not differ significantly from those recommended by the Society of Nuclear Medicine procedure guidelines for Octreoscan scintigraphy (12).

Eight of our 17 patients (patients 1, 2, 4–7, 10, 12) had been treated with octreotide before the time when scintigraphy was performed. In three of these patients (patients 1, 2, 4) the treatment was stopped 5.7 ± 1.2 days (4–7 days) before the day of the Octreoscan injection. Octreotide treatment was carried on at the time when scintigraphy was performed in the other five patients (patients 5–7, 10, 12). These five patients were treated with octreotide during the 13 ± 8 days preceding scintigraphy (5–25 days) with a dose of $390 \pm 236 \mu\text{g}/24 \text{ h}$ ($200\text{--}750 \mu\text{g}/24 \text{ h}$).

The intensity of Octreoscan uptake was quantified in contrast with the intensity of uptake of the liver (1, intensity less than that in the liver; 2, intensity equal to that in the liver; 3, intensity more than that in the liver).

SPECT was also used to rule out false-positive results due to a normal tissue uptake.

Immunostaining with anti-sst2A and anti-sst5 antibodies

Sections of $4 \mu\text{m}$ were cut into tumors embedded in paraffin wax and floated on to positively charged slides for immunohistochemical staining. Sections were de-waxed three times in xylene and rehydrated in a graded series of ethanol (from 100 to 50%).

Prior antigen retrieval based on microwave oven heating ($3 \times 5 \text{ min}$, 750 W) in 10 mM citrate buffer, pH 6, was performed. Specimens were then allowed to cool at room temperature.

Immunostaining with anti-sst2A antibody

After blocking of non-specific binding sites with DAKO protein-block serum-free fluid (DAKO, Glostrup, Denmark), sections were incubated during 36 h at 4°C with the anti-sst2A antibody (6291; a gift from Dr S. Schulz, Department of Pharmacology and Toxicology, Otto-von-Guericke University, Magdeburg, Germany) diluted at 1/2000. This polyclonal rabbit antibody is directed specifically towards the ETQRTLLN-GDLQTSI peptide, which corresponds to residues 355–369 of the C-terminal region of human sst2A receptor (13). Endogenous peroxidases were then inhibited in a preparation of BSA, H_2O_2 and methanol. Staining of primary antibody was detected using a secondary antibody (porcine anti-rabbit; code 70 196; DAKO) and a tertiary antibody (rabbit anti-porcine; code P0164; DAKO) diluted at 1/50. Tissues were then rinsed and stained with the DAKO AEC + high-sensitivity substrate-chromogen system. Slides were counterstained with hematoxylin. Positive controls were performed using murine cerebellum, which is known to contain abundant sst2A receptors, and normal pancreatic islets adjacent to the tumors. The immunostaining was performed several times in different areas of four tumors (patients 1, 3, 5, 10). The specificity of the sst2A antibody was demonstrated previously (13–15). Negative controls were performed by incubating the tissue with BSA instead of the primary antibody.

Immunostaining with anti-sst5 antibody

Specimens were washed three times in Tris-buffered saline. The slides were processed using a Techmate Horizon (DAKO) slide processor. Affinity-purified polyclonal antibody against sst5 receptor was generated in rabbits immunized with EPRPDR peptide corresponding to

amino acids 334–339 of the human sst5 C-terminus. This peptide was a kind gift of Dr L Moroder (Max-Planck Institute of Biochemistry, Martinsried, Germany). This antibody specifically recognizes human sst5 recombinant receptor expressed in Chinese hamster ovary cells (immunocytochemistry and Western blot assay in the presence or absence of recombinant peptide) (16) (P Cordelier *et al.*, unpublished results). The antibody was used at 1 µg/ml, incubated for 1 h and revealed using a two-step peroxidase-conjugated polymer backbone-visualization system (EnVision), according to the manufacturer's protocol. Chromogenic substrate was 3,3'-diaminobenzidine (DAB; DAKO). Slides were counterstained with hematoxylin. Positive controls were performed using normal pancreatic islets adjacent to the tumors and mouse cerebellum. Negative controls were performed by incubating the tissue with BSA instead of the primary antibody.

sst2 and sst5 immunostaining in the tumors was compared with that obtained in the normal adjacent pancreatic tissue. The immunostaining was described as +++ if its intensity was greater than that observed in the normal pancreas, ++ if it was similar, and + if it was less than that of the normal pancreas and – when no immunostaining was found in the tumor.

Statistical analysis

Data are expressed as mean ± S.D. Statistical analysis was performed using the Statview 5 program. Non-parametric Mann–Whitney U test was used to compare groups of patients when data were not paired. Wilcoxon's signed-rank test for paired data was used to compare the results observed basally and after administration of 100 µg octreotide in responders. Fisher's exact test was used to compare the proportions of patients observed in two groups when evaluating the effect of pre-treatment with octreotide on the results

of Octreoscan scintigraphy and immunostaining. The results were considered to be significant if $P < 0.05$.

Results

The main results of this clinical study are summarized in Table 1 and Fig. 1.

Evaluation of the efficacy of octreotide on hypoglycemia

Short octreotide test Before injection of octreotide, basal blood glucose levels were 55 ± 15 mg/dl (range 35–84 mg/dl). Regarding the results of the short octreotide test, 8/14 patients (57%) were responders (Table 1). In these eight patients, blood glucose levels reached at least 100 mg/dl within the 6 h following a subcutaneous injection of 100 µg octreotide. Among the six non-responders, five tests (patients 2, 8, 11, 14, 17) were stopped before 6 h because of symptomatic hypoglycemia. One test was not stopped before 6 h despite hypoglycemia because the patient was clinically asymptomatic (patient 16). During the short octreotide test, maximal blood glucose levels of the responders were significantly greater than those of the non-responders (140 ± 60 versus 30 ± 7 mg/dl; $P = 0.005$; Fig. 1). In the responders blood glucose levels became higher than the basal value 2 h after the octreotide injection in all but one patient (patient 15, in whom blood glucose increased only 1 h after the injection); maximal blood glucose levels were reached 4.1 ± 1.2 h (2–6 h) after the subcutaneous injection of octreotide. In the non-responders the test was interrupted 2–6 h (3.3 ± 1.4 h) after the subcutaneous injection of octreotide and blood glucose levels were below 45 mg/dl at the time of cessation of the test. None of our patients had blood glucose levels

Figure 1 Response to short octreotide test in 17 patients with insulinoma.

remaining between 45 and 100 mg/dl throughout the short octreotide test.

The mean insulin and C-peptide concentrations at the beginning of the test were 11.2 ± 14.3 mIU/l (range 1–29 mIU/l) and 3.8 ± 2.5 ng/ml (range 1–9 ng/ml) respectively. The responders' insulin and C-peptide concentrations at the beginning of the test were not significantly different from those of the non-responders ($P = 0.07$ for insulin and $P = 0.4$ for C-peptide). In all responders insulin and C-peptide levels were lower than their basal values at the time of maximal blood glucose levels: insulin levels, 4.4 ± 2.5 mIU/l (1.7 – 9 mIU/l) at the time of maximal blood glucose levels versus 17.3 ± 7.3 mIU/l (10 – 28 mIU/l) in the basal serum samples, $P = 0.028$; C-peptide levels, 1.0 ± 0.4 ng/ml (0.3 – 1.4 ng/ml) at the time of maximal blood glucose levels versus 3.6 ± 1.3 ng/ml (1.8 – 5.7 ng/ml) in the basal serum samples, $P = 0.027$. The decrease of the insulin and C-peptide concentrations during the short octreotide test reached 31–84% ($65.1 \pm 23.4\%$) and 22–92% ($64.1 \pm 27.1\%$) of the basal values for insulin and for C-peptide, respectively, at the time when maximal blood glucose levels were observed. In the six patients who were found to be unresponsive to the short octreotide test on the basis of blood glucose levels, insulin and/or C-peptide levels were unchanged at the time of cessation of the test in three patients (patients 2, 16, 17), reduced by less than 20% in two patients (patients 8 and 14), and significantly decreased in only one patient (patient 11, who had a 58% decrease of insulin level and a 39% decrease of C-peptide level).

Treatment with somatostatin agonists Octreotide treatment was performed over 2 ± 3 months (8 days–8 months) in 10 patients (patients 1, 3–7, 10, 12, 13, 15). Clinical symptoms of hypoglycemia subsided in 8/10 patients (80%; i.e. all treated patients except patients 4 and 12; Table 1). In responders, hypoglycemia subsided as soon as the treatment was started. Blood glucose was found to be above 60 mg/dl during treatment when measured by daily self-monitoring, and/or by repeated venous sampling at 4-h intervals during 24 h performed 2 weeks–7 months after the onset of treatment. However, two of the five patients who were treated during several months experienced recurrence of hypoglycaemia 20–30 days after octreotide treatment had been started (patients 1 and 5). This tachyphylaxis to octreotide therapy was overcome by increasing the dose of octreotide and continuous administration of the medication with an automated pump (1200 μ g/day instead of 900 μ g/day in patient 1 and 500 μ g/day instead of 200 μ g/day in patient 5). After increasing the dose of octreotide, blood glucose levels remained normal until surgery, which was performed 2 months later, and no escape to therapy was observed.

Among the seven responders to the short octreotide test who also underwent octreotide treatment (patients 1, 6, 7, 10, 12, 13, 15), 6/7 (87.5%) presented with normal blood glucose levels during the treatment (Table 1). The only patient (patient 12) who was a responder to the short octreotide test despite the lack of efficacy of octreotide treatment was the patient who presented with a malignant insulinoma and liver metastases. In this patient treatment with octreotide at the dose of 750 μ g/day became totally ineffective within less than 48 h.

Scintigraphy with labeled octreotide (Octreoscan)

Scintigraphy with Octreoscan led to localization of the insulinoma in 4/17 (24%) patients (patients 6, 9, 10, 12; Table 1). The Octreoscan uptake scores were 3 for patients 6, 9, 12 and 1 for patient 10. The ectopic insulinoma and the malignant insulinoma included in our study were among the tumors detected by scintigraphy. All the insulinomas detected with Octreoscan scintigraphy except the ectopic insulinoma had also been detected by transabdominal ultrasound examination and/or abdominal CT scan. Only Octreoscan scintigraphy enabled us to localize the ectopic insulinoma and then conventional CT scan directed by the result of Octreoscan scintigraphy visualized the insulinoma in the peritoneal tissue under the pancreas. The size of the insulinomas detected by Octreoscan scintigraphy (28 ± 10 mm, range 19–40 mm) tends to be greater than that of the other insulinomas (17 ± 9 mm, range 6–36 mm; $P = 0.06$, not significant).

Octreotide treatment prior to Octreoscan injection does not appear to hamper significantly tumor detection by scintigraphy in our series. The five patients (5–7, 10, 12) who were treated with octreotide at the time of scintigraphy comprised three patients with positive scintigraphy, while among the 12 patients who were untreated at the time of scintigraphy, only one (patient 9) had a positive scintigraphy ($P = 0.27$ versus treated patients, not significant).

Octreoscan scintigraphy leads to underestimation of the number of patients with insulinoma who are responsive to octreotide treatment. Among the 10 patients who were responders to octreotide, 6/10 (patients 1, 3, 5, 7, 10, 13) had no detectable Octreoscan uptake. On the other hand all the benign insulinomas that were detected with Octreoscan were responsive to octreotide treatment. The malignant insulinoma was visualized by scintigraphy, and responsive to the short octreotide test, but then hypoglycaemia was not controlled by continuous subcutaneous administration.

Immunostaining with anti-sst2A and anti-sst5 antibodies

The sst2A receptor was expressed in 7/17 insulinomas (41%; patients 1, 3, 6, 7, 9, 12, 1; Table 1 and Fig. 2). The receptor was located only in

Figure 2 sst2 and sst5 immunostaining of normal pancreas and insulinomas. (A) sst5 immunostaining of normal pancreatic endocrine islet (original magnification, $\times 400$). (B) sst5 immunostaining of insulinoma ($\times 300$). Note the residual islets of Langerhans (asterisks) used as positive controls. (C) sst2A immunostaining of normal pancreatic endocrine islet ($\times 400$). (D) sst2A cytoplasm immunostaining of insulinoma. (E) Cytoplasm and cell-membrane sst2A immunostaining of liver metastasis ($\times 400$).

the cytoplasm in four tumors (patients 1, 3 and 13, and patient 12's primitive pancreatic tumor), whereas it was located both in the cytoplasm and the cellular membranes in four cases (patients 6, 7 and 9, and patient 12's metastasis). In patient 12, immunostaining observed in the liver metastasis was greater than that of the primitive tumor. sst2A was not detected in 10/17 patients (patients 2, 4, 5, 8, 10, 11, 14–17). The sst5 receptor subtype was expressed in 15/17 insulinomas (88%) (all patients except patients 9 and 14; Table 1 and Fig. 2). All the seven sst2A-positive tumors also expressed the sst5 receptor, except the ectopic insulinoma (patient 9).

Octreotide treatment and its duration before surgery does not appear to affect significantly the immunohistochemical detection of the sst2A or sst5 receptors in our series. Indeed among the seven patients with positive anti-sst2A immunostaining, 6/7 had been treated with octreotide before surgery for 63 ± 93 days (8–248 days) whereas 1/7 patient with sst2A (patient 9) had remained untreated with octreotide. Conversely, among the 10 sst2A-negative patients, 3/10 had been treated with octreotide over 70 ± 31 days (45–105 days) whereas octreotide treatment had not been performed in the other 7/10 patients ($P = 0.33$ versus sst2A-positive cases, not significant).

Regarding the expression of sst5, the two patients with negative anti-sst5 immunostaining had not been treated with octreotide before surgery whereas those patients with positive anti-sst5 immunostaining had either been untreated ($n = 6$) or treated for 65 ± 75 days (8–248 days; $n = 9$).

All seven patients presenting with sst2A-positive tumors (patients 1, 3, 6, 7, 9, 12, 13) were responsive to octreotide either during the short octreotide test or during long-term administration. On the other hand, among the 10 patients with sst2A-negative tumors, seven (patients 2, 4, 8, 11, 14, 16, 17) were unresponsive to octreotide, but three (patients 5, 10, 15) were responders (Table 1). These three patients expressed the sst5 receptor. However, in the other six patients who also had positive anti-sst5 immunostaining and undetectable sst2A receptor (patients 2, 4, 8, 11, 16, 17) octreotide administration had no effect on blood glucose levels.

Among the four insulinomas with tumoral uptake of Octreoscan, two (patients 6 and 12) expressed both the sst2A and sst5 receptors, one (patient 9, ectopic insulinoma) expressed only the sst2A receptor, and one (patient 10) expressed only the sst5 receptor. Most of the sst2A-positive and/or sst5-positive insulinomas did not present with detectable Octreoscan uptake. In

addition, one can notice that among the three insulinomas with positive anti-sst2A immunostaining on the cell membranes (patients 6, 7, 9), two were detected by Octreoscan scintigraphy.

Discussion

In this group of patients with insulinomas, 57% of them are responsive to treatment with octreotide. This is in agreement with the conclusions of several case reports and studies on small series of insulinoma patients (5, 17–19). This is also in agreement with the percentage of responders to octreotide that we had observed in 21 other patients not included in the present study, who had been diagnosed to have insulinoma in our department within the previous 15 years (11/21, i.e. 52%). In the present study there was a very rapid escape to the effect of octreotide on hypoglycaemia in the patient with malignant insulinoma and liver metastasis and no change in the protocol of administration or the dose of octreotide could overcome this resistance to treatment. According to some reports, octreotide can control hypoglycaemia in some patients with malignant insulinoma (20). In our personal experience with the other 21 patients not included in the present study, including four patients with malignant insulinoma, octreotide was ineffective at controlling hypoglycaemia in all the patients who had liver metastases.

Two of the five patients with benign insulinomas who were treated with octreotide over several months presented with an escape of the response to the treatment, which was quickly overcome once the dose of octreotide had been increased or once a continuous administration of octreotide with an automated pump had been used. This tachyphylaxis has also been observed during the treatment of thyrotroph adenomas (21, 22) and other digestive endocrine tumors (23) whereas it had not been noted during somatostatin analog treatment of somatotroph adenomas (24). There are several hypotheses to explain the escape to the effects of therapy with somatostatin agonists (23). It could be explained in benign insulinomas by internalization of membrane somatostatin receptors during treatment with somatostatin agonists (25, 26). Desensitization to somatostatin agonist at a post-receptor level, related to altered coupling with second messengers, could also be involved (26, 27).

One should try to select those of the patients who can benefit from octreotide therapy before surgery or when surgery is not possible. According to our results, and as observed with carcinoid tumors (28) or thyrotroph adenomas (29), a positive response to the short octreotide test appears to be predictive of the efficacy of octreotide treatment in our patients with benign insulinomas. Unlike what had been reported previously (6, 30), we did not observe worsening of hypoglycaemia

in patients treated with octreotide. Only one of the patients who were unresponsive to the short octreotide test displayed a significant decrease in insulin and C-peptide levels concomitant with a lack of increase in blood glucose levels, suggesting that in this patient the effect of octreotide on glucagon secretion by normal islets could overcome that on tumoral insulin secretion, which might have led to worsening of symptoms if this patient had been treated with octreotide.

Among the five somatostatin receptor subtypes, sst2A and sst5 display the higher affinity for the clinically used analogue octreotide (4). For this reason, the expression of these two receptors was preferentially studied in the present work. Our results confirm that sst5 receptor is more often expressed than the sst2A receptor in insulinomas (13). In our data, this expression was mainly cytoplasmic, maybe as a consequence of the easier internalization of sst5 receptor subtype in comparison with that of sst2 (25). Most of the insulinomas that express the sst2A receptor also express the sst5 receptor, as already found by Bertherat *et al.* (31), who used other methods (an autoradiographic study with competition experiments using selective ligands).

It has been shown that the efficacy of somatostatin agonists in endocrine pancreatic tumors is closely dependent on the presence of sst2A receptors (13). Our study is in agreement with this view, since all patients with positive anti-sst2A immunostaining are responsive to octreotide. However, three of our patients without detectable sst2A receptor had normal blood glucose levels during treatment with octreotide. It has been shown that *in vitro* inhibition of insulin secretion could depend on the sst5 receptor subtype, without sst2A (32, 33). However, in our patients taken together, expression of sst5 *per se* does not appear to account for the efficacy of octreotide on hypoglycaemia in all the insulinomas that lack the sst2A receptor. Despite the fact we used several positive and negative controls, we cannot totally rule out the hypothesis that, in our sst2A-negative cases with a positive response to octreotide, immunoreactivity for sst2A receptors might be confined to a few undetected cell clusters (13, 34). Alternatively, mechanisms different from those involving sst2A and sst5 receptors could account for the clinical efficacy of octreotide on hypoglycaemia at least in some patients.

In our study, Octreoscan scintigraphy with planar views followed by SPECT resulted in localization of only 24% of insulinomas. Octreoscan scintigraphy with planar views was reported to lead to detection of 70–90% of all endocrine tumors (35) whereas only 20–50% of insulinomas can be detected using this method (7–9). The use of tomography (SPECT) was reported to improve the detection of insulinomas: 80% were detected in a study about 14 patients with insulinoma (9). Pretreatment with octreotide might lead to saturation of somatostatin receptors (36).

However, such treatment within the week preceding scintigraphy was also reported to improve contrast and tumor detection (37–39). Three of our four Octreoscan-positive insulinomas were found among the five patients treated at the time of scintigraphy. In our study, the size of the tumors that we detected by scintigraphy tended to be larger, as already suggested by others (15). However, others (40) had reported no effect of the tumor size. It cannot be ruled out that some differences in the imaging techniques unrelated to the use of SPECT *per se* might be responsible for the poor sensitivity that we observed. For instance, in the study that reported a 80% sensitivity, 250 MBq Octreoscan were injected instead of 150 MBq, and SPECT was also performed at 4 h. Using the same methods as in our insulinoma patients, we detected 409/459 (89.1%) non-insulinoma, well-differentiated digestive endocrine tumors (16). Since most studies report the results of Octreoscan scintigraphy in less than 20 insulinoma patients, random differences in the groups of patients studied could play a role in the differences between the sensitivity reported for this scintigraphy in such patients, most studies finding, like us, a greater sensitivity in other digestive endocrine tumors (12, 35).

Octreoscan scintigraphy was useful for the detection of the ectopic insulinoma. Planar views of the whole body are obtained with scintigraphy, while conventional radiological techniques tend to focus only on the pancreas when attempting to localize an insulinoma.

Since the biochemical configurations of octreotide and Octreoscan are very similar, one could have expected that the results of Octreoscan scintigraphy might be predictive of the efficacy of octreotide treatment on hypoglycemia (41), as observed in a study on various endocrine tumors (28). In our study, as observed in somatotroph adenomas (42–44), evaluation of Octreoscan uptake by insulinomas did not predict accurately the efficacy of octreotide treatment. Octreoscan displays a lower affinity for somatostatin receptors (especially sst2) than octreotide (4). Considerations related to the internalization and storage of the radioisotopes and specific problems of imaging techniques, not involved in the efficacy of octreotide, might also explain our results.

Three of our four patients with tumoral Octreoscan uptake expressed the sst2A receptor, and one insulinoma with negative anti-sst2A immunostaining was detected by Octreoscan scintigraphy. In addition, among the only three insulinomas with positive anti-sst2A immunostaining on the cell membranes, two were detected by Octreoscan scintigraphy. Octreoscan uptake by carcinoid tumors and neuroblastomas was found to be dependent on sst2A receptor (45, 46). On the other hand, a few studies had already reported that visualization of the tumor with Octreoscan scintigraphy was possible despite the lack of sst2A receptors in some thyroid tumors (47, 48), in endocrine tumors

(49, 50), in a thymoma (32) and in pheochromocytomas with membrane-associated sst3 immunoreactivity (15). Coexpression of sst2 and sst5 receptors in insulinomas does not seem sufficient to result in Octreoscan uptake by all the tumors (31).

Conclusion

Octreotide is an effective treatment of hypoglycemia in more than 50% of patients with insulinoma. Detection of responsive patients was better based on a positive short test with subcutaneous octreotide than on the results of Octreoscan scintigraphy. The efficacy of octreotide was observed in all patients with sst2A-positive benign insulinomas, and in a few sst2A-negative patients; it was unrelated to expression of sst5 receptor *per se*. Further investigations are necessary to determine all the mechanisms underlying the clinical efficacy of somatostatin analogs in all patients with insulinoma.

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