High-dose treatment with a long-acting somatostatin analogue in patients with advanced midgut carcinoid tumours

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Abstract

Objective: High-dose somatostatin analogue treatment has shown an antiproliferative effect in one study including patients with neuroendocrine tumours. To explore this therapeutic strategy further, we have studied the effect of a high-dose formula of octreotide, octreotide pamoate, in midgut carcinoid patients.

Design and methods: Twelve patients with advanced midgut carcinoid tumours with a median duration of disease of more than 5 years were included. All were in a progressive state despite several previous treatment modalities. Octreotide pamoate (160 mg) was given as an intramuscular injection every 2 weeks for 2 months and then monthly. Radiological and biochemical responses were monitored.

Results: Tumour size and biochemical markers were stabilised for a median of 12 months in 75% of the patients. Ten patients had symptomatic improvement of flush and diarrhoea.

Conclusion: In this group of patients with advanced midgut carcinoid tumours and progressive disease, octreotide pamoate managed to improve symptoms, and stabilise hormone production and tumour growth in 75% of the patients. We believe that high-dose treatment with somatostatin analogues can be an important addition to the therapeutic arsenal for patients with advanced progressive midgut carcinoid tumours.

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Introduction

Midgut carcinoid tumours are rare malignant tumours with an incidence of 0.7–2.1/100 000; they can produce several hormones and neuropeptides and induce the carcinoid syndrome consisting of diarrhoea, flush, carcinoid heart disease and bronchial obstruction (1).

Surgery is the primary treatment for midgut carcinoid patients, but since the tumour has often metastasised when diagnosed, few patients can be cured and medical treatment is needed. The drugs most frequently used are α-interferon and somatostatin analogues. Radiological response rates reported for α-interferon treatment vary between 10 and 20% in midgut carcinoid patients and biochemical and symptomatic responses are observed in 40–50% (2).

Somatostatin is a hypothalamic hormone known to inhibit secretion of other hormones from endocrine cells (3). However, several other mechanisms of action are also mediated by somatostatin. Five somatostatin receptors (ssts) have been characterised, sst1–5 (4–6). sst2 and sst5 mediate inhibition of hormone secretion (7) while sst1–2 and sst5 induce cell growth inhibition in vitro (8, 9), and sst2 and sst3 may induce apoptosis (10, 11).

The expression of different ssts has been investigated in midgut carcinoid tumours and most express sst1–2 and sst5 (12, 13). The two commercially available somatostatin analogues, octreotide and lanreotide, have high affinity for sst2 and sst5 and intermediate affinity for sst3 (14). Only 5–10% of the midgut carcinoid patients treated with ordinary doses (up to 600 μg/day octreotide) of these somatostatin analogues show a significant decrease in tumour size while a biochemical and/or symptomatic response is found in 50–70% (15).

Part of the antiproliferative effect induced by somatostatin analogues in vitro might be due to an increased apoptosis (16). A hypothesis that higher doses of somatostatin analogues could have a stronger antiproliferative effect formed the basis for high-dose studies, and indeed radiological responses could be observed in up to 30% of patients receiving high-dose treatment (up to 12 000 μg lanreotide/day) (17, 18). In tumour
specimens from patients treated with high doses of lanreotide, an increased apoptosis was seen, supporting this hypothesis (19).

This study was performed to investigate whether even higher doses, 160 mg octreotide injected every 2–4 weeks, could induce an antiproliferative and symptomatic response in midgut carcinoid patients progressing on regular treatment. To elucidate the possible mechanisms behind such a response, cell-cycle proteins, angiogenic and proliferation markers and markers for apoptosis were examined in tumour specimens before and during treatment.

Materials and methods

Patients

Patient characteristics are summarised in Tables 1 and 2. Twelve patients, seven men and five women, with histopathologically confirmed malignant midgut carcinoid tumours were included. The median age at diagnosis was 50 years (40–64 years) and the median duration of disease from diagnosis until the study was started was 64.5 months (27–165 months).

All patients were previously heavily treated, including debulking surgery (n = 11), α-interferon treatment (n = 11) and somatostatin analogue treatment (n = 11). Other therapies included liver embolisation (n = 4), 111In-octreotide treatment (n = 2) and external radiation (n = 1) against painful bone metastases. To be included in the study all patients had to have pathological tracer uptake in tumour lesions at sst scintigraphy, and to have progressive disease despite ongoing treatment. At the start of the study, ten out of twelve had progressive disease on radiological investigations. Patient numbers 2 and 5 showed biochemical progress.

Treatment

α-Interferon treatment was withdrawn at least 1 month prior to the start of the study and long-acting octreotide was discontinued 2 months before the study. If a patient was unable to manage without somatostatin analogue treatment such treatment was allowed to continue with a short-acting analogue until 4 days prior to the study. All patients gave written informed consent to their participation in the study; the study protocol was reviewed and approved by the local ethics committee of the hospital.

The somatostatin analogue octreotide pamoate was given as an intramuscular injection at a dose of 160 mg every second week for 2 months followed by monthly injections. Treatment was continued for 12 months. Patients who were stable at 12 months were allowed to continue with octreotide pamoate treatment for another 12 months or until octreotide pamoate became unavailable. Octreotide pamoate was provided by Novartis Pharma AG, Basel, Switzerland.

Pharmacokinetics

A 10 ml plasma sample was collected before the first injection and then before every injection until day...
155. The plasma concentration of octreotide was measured using an RIA method with a polyclonal rabbit antiserum and $^{125}$I-D-Tyr-(3)-octreotide (Novartis Pharma AG).

**Biochemistry**

Biochemical markers, chromogranin A and urinary 5-hydroxyindoleacetic acid (U-5HIAA) were measured before treatment was initiated and during treatment. Plasma chromogranin A levels were analysed monthly and U-5HIAA was calculated as the mean of two 24-h urine collections and measured every second month. In addition, haematology, liver enzymes, kidney function tests and electrolytes were checked before every injection.

**Radiology**

Radiological evaluation was performed at baseline and every third month with contrast-enhanced computerised tomography by a senior consultant in radiology (A S) who reviewed all examinations. Ultrasonography examinations were performed before the study started and every sixth month to monitor development of gallstones.

**Biopsies**

Tumour tissue for histopathological diagnosis and immunohistochemical staining was collected before treatment was initiated and every sixth month using a 1.2 mm needle with ultrasound guidance. Tumour tissues were immediately frozen in liquid nitrogen and kept at $-70^\circ$C until further use or fixed in formalin and embedded in paraffin wax.

**Immunohistochemistry**

Immunostaining was performed using the streptavidin–biotin complex technique (Vector Laboratories, Burlingame, CA, USA). Polyclonal antibodies against sst1–5 (20) were used as primary antibodies in the following dilutions: 1:1200 for sst1, 1:5000 for sst2–3 and sst5, and 1:1000 for sst4. Monoclonal antibodies against vascular endothelial growth factor (VEGF), p27 and p16 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were applied at titres of 1:50, 1:100 and 1:40 respectively. A polyclonal antibody against FMS related tyrosine kinase 1 (FLT1) (Santa Cruz Biotechnology Inc.) was diluted 1:600, a monoclonal antibody against Ki-67 antigen (Dako, Copenhagen, Denmark) was applied at a titre of 1:100. The immune reaction was visualised using a Vectastain Elite ABC kit (Vector Laboratories) with 3-amino-9-ethylcarbazole in dimethylsulphoxide as chromogen and 0.02% hydrogen peroxide as substrate. The sections were counterstained with Mayer’s haematoxylin (Apoteksbolaget, Stockholm, Sweden).

**Examination of apoptosis**

The presence of apoptosis in tumour specimens was examined by the TUNEL method, which was performed as described previously (21), and which is based on the specific binding of terminal deoxynucleotidyl transferase to the 3'-OH ends of DNA breakage. Immunostaining as described above was also carried out with a polyclonal antibody against cleaved caspase 3 (Cell Signaling Technology Inc., Beverley, MA, USA) which was diluted 1:100. Tumour specimens were obtained before treatment was initiated and every third month during treatment.

**Ki-67**

Cell nuclei immunostained with Ki-67 were counted in hot spot areas of the tumour tissue. One hundred cells were counted and the result is presented as percentage of positive cells.

**Definition of response**

Radiological tumour response was defined as $>50\%$ reduction in tumour size, calculated as the sum of the products of the largest perpendicular diameters of measurable lesions. Stable disease was defined as $<50\%$ reduction or $<25\%$ increase in tumour size. Progressive disease was hence defined as $>25\%$ increase in tumour size. Biochemical response was defined as $>50\%$ reduction in one tumour marker, while stable disease was defined as $<50\%$ reduction or $<25\%$ increase of a tumour marker. Progressive disease was defined as $>25\%$ increase in a tumour marker.

**Results**

**Radiological response (Table 2)**

Tumour size remained stable in nine of the twelve patients with a median duration of 12 months ($6–24$). Although no significant radiological response was detected, minor tumour shrinkage could be demonstrated in five patients. Three patients showed progressive radiological disease at the first evaluation.

**Biochemical response (Table 3)**

A biochemical response in plasma chromogranin A or U-5HIAA was obtained in four and two patients respectively, with a median duration of 5 months ($3–24$) and 13.5 months ($3–24$). Stabilisation was seen in seven and nine patients respectively, with a median duration of 6 months ($2–14$) and 9 months ($3–24$). Only two patients showed biochemical progression when octreotide pamoate treatment begun.
Pharmacokinetic data

The plasma concentration of octreotide was measured several times in all patients. The mean plasma level at day 43 immediately before injection of octreotide pamoate was 82.5 ng/ml (range 49.7–127 ng/ml). At day 155, the mean plasma concentration was 40 ng/ml (range 15.7–60.8 ng/ml).

Survival

The median survival in the group of patients continuing with the treatment (n = 6) together with those who stopped for reasons other than progressive disease (n = 3) was 116 months (68–194) after diagnosis and 37 months (8–63) after inclusion in this study (Table 2). Three of them are still alive. In the group that continued to have progressive disease, the median survival from diagnosis was 66 months (39–72) and from inclusion in the study 12 months (8–18).

Reasons for discontinuing the study

Three patients discontinued the study because of progressive disease after 3 months (patient nos 1, 4 and 5). Another three patients were withdrawn from the study for other reasons, patient no. 12 due to post-operative adherences resulting in intestinal obstruction (6 months), patient no. 6 needed heart surgery due to a severe carcinoid heart disease (6 months) and patient no. 7 because of the patient’s choice (6 months) (Table 2). The remaining six responding patients continued with octreotide pamoate for 12–24 months.

Immunohistochemistry

All tumour specimens showed immunoreactivity for chromogranin A. All patients expressed sst1–3 and sst5 while sst4 was found in nine out of twelve patients (data not shown). The expression did not change during treatment. Apoptosis investigated by TUNEL and cleaved caspase 3 remained unchanged during treatment, as did the expression of p16, VEGF and FLT1 (data not shown). The marker for cell cycle inhibition, p27, seemed to decrease in expression during treatment (Table 4). The proliferation marker Ki-67 indicated that most tumours had a low proliferation rate despite the fact that all patients were in a progressive state. However, there were exceptions and five patients had a proliferation index of 15% or more. Of these, two continued to progress at 3 months and a third patient who was referred for heart surgery after 6 months increased to a proliferation rate of 50%. Three months after discontinuing the study the patient showed progression with development of new bone metastases. However, at the time when the patient was withdrawn from the study the size of all tumour lesions was stable. Thus, the progression appeared during a time when the patient did

Table 3 Clinical results.

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<th>Patient number</th>
<th>Best response to radiology</th>
<th>Baseline chromogranin A (nmol/l)</th>
<th>Best response to chromogranin A (nmol/l)</th>
<th>Baseline U-5HIAA (μmol/h)</th>
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Partial response (PR): a decrease in tumour area or biochemical marker of > 50% from baseline. Stable disease (SD): a decrease of less than 50% or an increase of less than 25% from baseline. Progressive disease (PD): an increase of more than 25% from baseline values.
Table 4 Immunohistochemical data for Ki-67 and p27.

<table>
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<td>2</td>
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<td>++</td>
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</table>

ND, not done; neg, negative.

not receive any treatment for his carcinoid tumour. Two of the patients with a high proliferation rate showed a decrease in proliferation index after 6 months of treatment (Table 4).

Side-effects

Few side-effects regarded as caused by the treatment were observed. One patient developed new asymptomatic gallstones and six patients had unexplained episodes of fever and were hospitalised but no infection could be verified.

Discussion

In this study, the effect of a high-dose treatment with the somatostatin analogue octreotide was evaluated in heavily pretreated patients with advanced malignant midgut carcinoid tumours. All had liver metastases, all but one had lymph node metastases and all were in a progressive state. Tumour tissue from all patients expressed sst’s visualised by both sst scintigraphy and immunostaining, and the immunohistochemical expression was unchanged during octreotide pamoate treatment.

Several reports have shown a growth-inhibiting effect of somatostatin analogues on tumour cells in vitro (8, 9). Therefore much interest has been directed towards finding treatment regimes that would induce such an effect in vivo. High doses of the somatostatin analogue lanreotide have induced tumour reduction in about 30% of patients with midgut carcinoid tumours (17, 18) injected four times daily. Today many patients are treated with the long-acting analogue octreotide-LAR injected at a dose of 10–30 mg once every fourth week. The mean peak concentration reached after injection of 100 μg octreotide was 2.8 ng/ml and even lower after injection of up to 30 mg octreotide acetate long-acting release (22). In our patients the mean plasma concentration after injections every second week was 82.5 ng/ml and after monthly injections 40 ng/ml.

In the present study, 75% of the patients showed stabilisation of tumour growth and/or biochemical markers for a median of 12 months (ranging from 6 to 24 months). Only three patients continued to progress. We also found an improvement of symptoms of the carcinoid syndrome in ten out of twelve patients. Unfortunately, the treatment with octreotide pamoate had to stop since the drug became unavailable. An extensive tumour biology programme was performed in order to try to identify tumour biology markers affected by the high-dose somatostatin analogue treatment. We were not able to detect any change in apoptosis or expression of angiogenic markers. The expression of the cell cycle inhibitor p24 seemed to decrease during treatment, but the significance of this observation remains unclear.

We were not able to identify any significant tumour shrinkage in the study. However, we have used a long-acting formula providing continuous activation of the sst’s. In previous studies on tumour size response, others have used short-acting preparations with multiple injections every day (17, 18). There are no studies with long-acting preparations showing an effect on tumour size in more than 10% of the patients. However, we found that at least three patients showed a decrease in proliferation index during the treatment. In the future, new recently developed somatostatin analogues that bind with high affinity to sst1–3 and sst5 might prove more effective since some antiproliferative effects are mediated through sst1 (8, 9).

Only one patient developed asymptomatic gallstones, and other side-effects were mild. The only adverse events that were unexpected and for which the reason is still unclear were episodes of fever occurring in half of the patients. No bacterial infection could be verified and systemic inflammation could not be confirmed. One possible explanation is that fever is an indicator of tumour necrosis, but this still remains to be proven.

In conclusion, we have treated patients with advanced midgut carcinoid tumours with octreotide pamoate, a long-acting somatostatin analogue, in very high doses inducing high plasma concentrations of octreotide. Seventy-five percent of the patients showed stabilisation of tumour growth for a median of 12 months. Biochemical markers remained stable in 58% of the patients and were significantly reduced in another 25%. Most patients experienced an amelioration of their symptoms. Thus we believe that the patients had a true benefit from this treatment. Therefore high-dose treatment with somatostatin analogues might be an important addition to the therapeutic arsenal for patients with advanced progressive midgut carcinoid tumours. Future studies will have to show if other ways of administration or new somatostatin...
analogue with a broader receptor-binding profile might be superior in this respect.

Acknowledgements

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