Clinical Endocrinology (1993) 39, 239–243

*Case of the Month*

# A case of antibody formation against octreotide visualized with $^{111}$In-octreotide scintigraphy

**D. J. Kwekkeboom*, J. Assies†, L. J. Hofland‡, J. C. Reubi§, S. W. J. Lamberts‡ and E. P. Krenning*‡**

*Departments of *Nuclear Medicine and ‡Internal Medicine, University Hospital Dijkzigt, Rotterdam, The Netherlands, and †Department of Medicine, Academic Medical Centre, Amsterdam, The Netherlands, §Institute of Pathology, University of Berne, Switzerland*

*(Received 18 January 1993; returned for revision 15 February 1993; finally revised 11 March 1993; accepted 30 March 1993)*

## Summary

**A case of antibody formation in a patient with carcinoid syndrome is described. The patient was treated with octreotide in dosages up to 1·5 mg/day. Serum samples were analysed for the presence of octreotide antibodies before and after 20 months of octreotide treatment. In-vivo $^{111}$In-octreotide scintigraphy was performed before and during therapy, and after antibodies had developed.**

**Before treatment, no serum antibodies against octreotide were detected. After 20 months of treatment, they were detectable up to a 1:115 serum dilution. The serum binding of $^{125}$I-Tyr$^3$-octreotide was blocked by adding excess unlabelled Tyr$^3$-octreotide, indicating the presence of specific octreotide antibodies. Before treatment, a normal distribution of radioactivity in the spleen and kidneys, irregular uptake in the liver due to metastases, and a hot spot in the lower abdomen were found during $^{111}$In-octreotide scintigraphy. After antibodies had developed, increased radioactivity over the heart and high background radioactivity in the abdomen with only faint visualization of the spleen, liver, and kidneys were found, indicating a prolonged presence of $^{111}$In-octreotide in the blood resulting from its being bound to antibodies. Increased radioactivity was also seen at the injection sites of the drug in the upper legs. In-vitro incubation of biopsy tissue from this site with $^{125}$I-Tyr$^3$-octreotide revealed diffuse guanosine triphosphate (GTP) independent specific binding, indicating non-G-protein linked binding of labelled octreotide.**

**This report describes the characteristic abnormalities during in-vivo $^{111}$In-octreotide scintigraphy in a patient with octreotide antibodies. These consisted of high background radioactivity due to prolonged circulation of antibody coupled $^{111}$In-octreotide together with visualization of the injection sites, which most probably results from local accumulation of antibodies.**

Correspondence: D. J. Kwekkeboom, University Hospital Dijkzigt, Room V 220, 40 Dr. Molewaterplein, 3015 GD Rotterdam, The Netherlands, Fax: 31-10-4635997.

Antibody formation in patients treated with the somatostatin analogue octreotide is extremely rare, and has until now been described in only three cases (Kendall-Taylor *et al.*, 1989; Ørskov *et al.*, 1991) although hundreds of patients with acromegaly or gastrointestinal APUDomas have been treated for several years.

We here report a fourth patient in whom antibodies against octreotide were detected because of characteristic abnormalities during in-vivo $^{111}$In-octreotide scintigraphy.

## Materials and methods

### *Serum antibody assessment*

The patient's serum samples (10 $\mu$l) were diluted in 20 $\mu$l phosphate buffered saline containing 10 g/l bovine serum albumin with or without excess unlabelled octreotide (final concentration 1 $\mu$M), and were then incubated with $^{125}$I-Tyr$^3$-octreotide (SDZ 204-090, Sandoz, Basel, Switzerland) (200 $\mu$l) for 24 and 48 hours. The final sample dilution was thus 1:23. All incubations were carried out in duplicate. After addition of polyethylene glycol (PEG), the samples were centrifuged, the supernatants decanted, and the precipitates counted. For comparison, sera from two control patients not treated with octreotide were handled in the same way.

### *In-vivo octreotide scintigraphy*

The somatostatin analogue DTPA-D-Phe$^1$-octreotide (215-811) was obtained from Sandoz, Basel, Switzerland. It was coupled to $^{111}$In as described previously (Bakker *et al.*, 1991a).

The in-vivo visualization of somatostatin receptor positive tumours after injection of $^{111}$In-DTPA-D-Phe$^1$-octreotide, a radiolabelled somatostatin analogue, has been described previously (Bakker *et al.*, 1991b; Krenning *et al.*, 1992). In brief, static images were obtained 24 and 48 hours after injection of the $^{111}$In-coupled somatostatin analogue. Preset counts for images obtained 24 hours after injection of the $^{111}$In-coupled somatostatin analogue were $3 \times 10^5$ for the

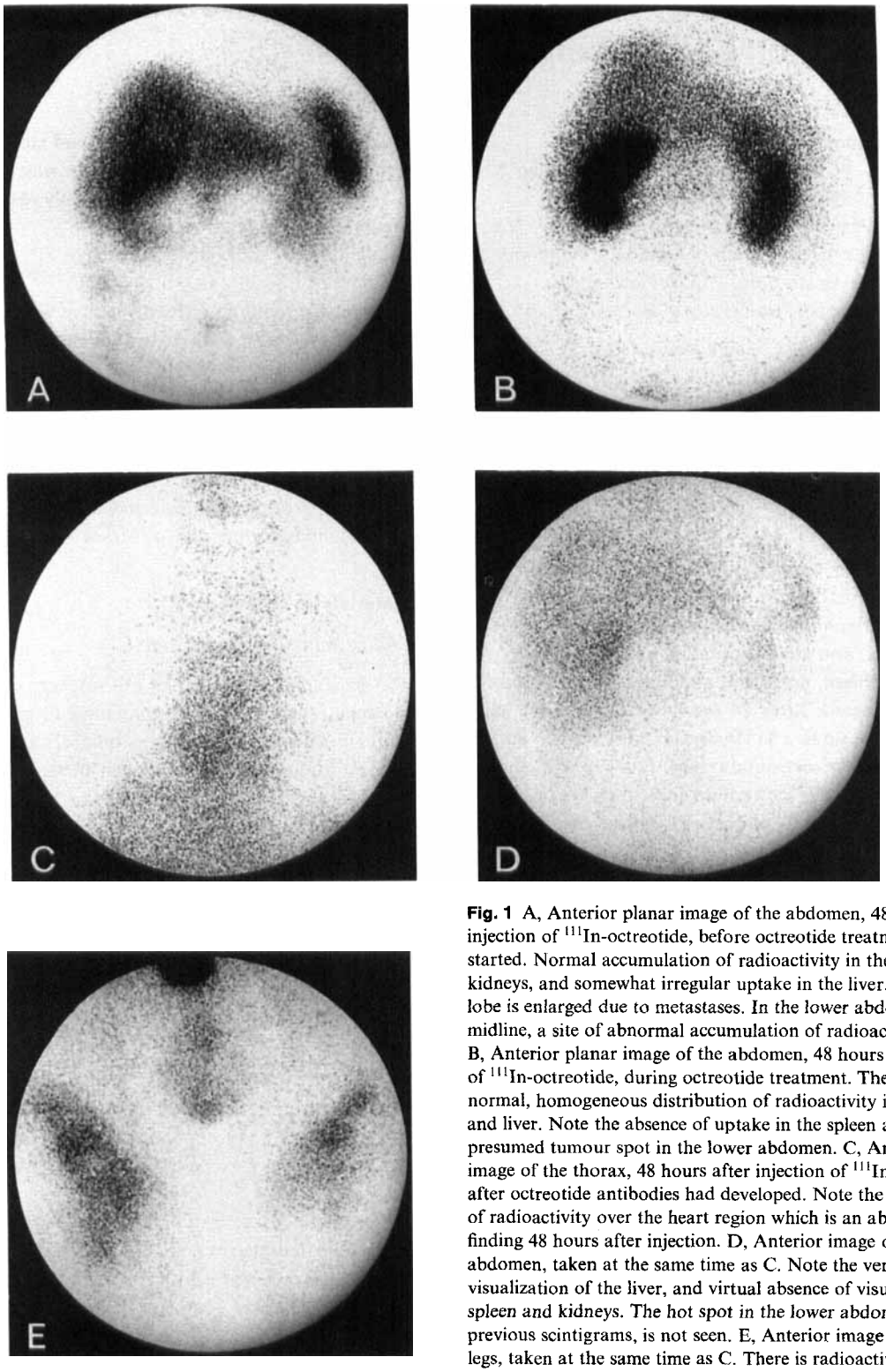


**Fig. 1** A, Anterior planar image of the abdomen, 48 hours after injection of $^{111}$In-octreotide, before octreotide treatment was started. Normal accumulation of radioactivity in the spleen and kidneys, and somewhat irregular uptake in the liver. The left liver lobe is enlarged due to metastases. In the lower abdomen in the midline, a site of abnormal accumulation of radioactivity is seen. B, Anterior planar image of the abdomen, 48 hours after injection of $^{111}$In-octreotide, during octreotide treatment. There is a normal, homogeneous distribution of radioactivity in the kidneys and liver. Note the absence of uptake in the spleen and in the presumed tumour spot in the lower abdomen. C, Anterior planar image of the thorax, 48 hours after injection of $^{111}$In-octreotide, after octreotide antibodies had developed. Note the accumulation of radioactivity over the heart region which is an abnormal finding 48 hours after injection. D, Anterior image of the abdomen, taken at the same time as C. Note the very faint visualization of the liver, and virtual absence of visualization of spleen and kidneys. The hot spot in the lower abdomen seen on previous scintigrams, is not seen. E, Anterior image of the upper legs, taken at the same time as C. There is radioactivity in the urethra and penis (upper edge of the image), and clearly increased radioactivity at the sites of daily injections of octreotide in both upper legs.

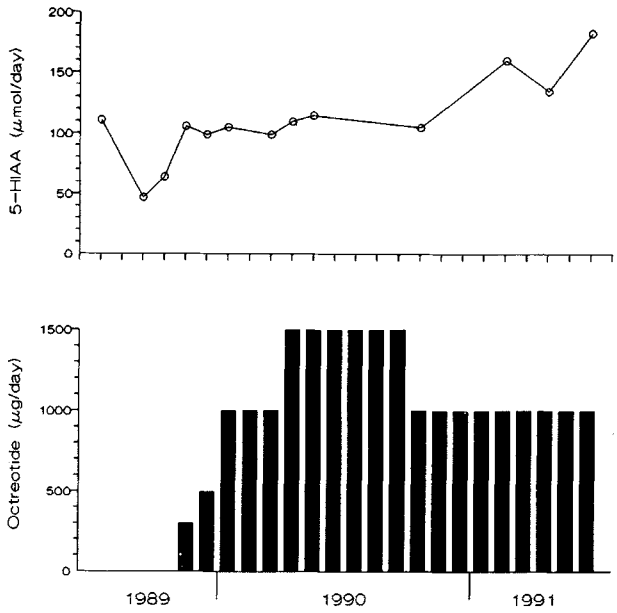


**Fig. 2** Urinary 5-HIAA levels before and during octreotide treatment.

head and neck and $5 \times 10^5$ for the remainder of the body; preset time for images obtained 48 hours after injection was 15 minutes (Krenning *et al.*, 1992). For chest images, the liver and spleen were almost entirely excluded from the field of view. To define the tumours as visualized during this scanning procedure, we used a simple yes/no system.

### *In-vitro antibody assessment on tissue*

A biopsy from the injection site was frozen at −80°C and processed as described previously for somatostatin receptor autoradiography (Reubi *et al.*, 1987). The iodinated somatostatin analogue, $^{125}$I-Tyr$^3$-octreotide was used as radioligand. Non-specific binding was determined by adding unlabelled Tyr$^3$-octreotide at a concentration of 1 μM. Binding of $^{125}$I-Tyr$^3$-octreotide was also evaluated after co-incubation with guanosine triphosphate (GTP; 100 μM). Lastly, autoradiography was performed using a radioiodinated somatostatin-28 analogue, Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$-SS-28.

## Case report

The 69-year-old patient was referred because of flushes in July 1989. Eight years before, he had had a pulmonary lobectomy because of a bronchial carcinoid with mediastinal lymph node involvement. His urinary 5-hydroxyindoleacetic acid (5-HIAA) at presentation varied from 44 to 111 μmol/24 h on five different sampling days in 3 months (normal values

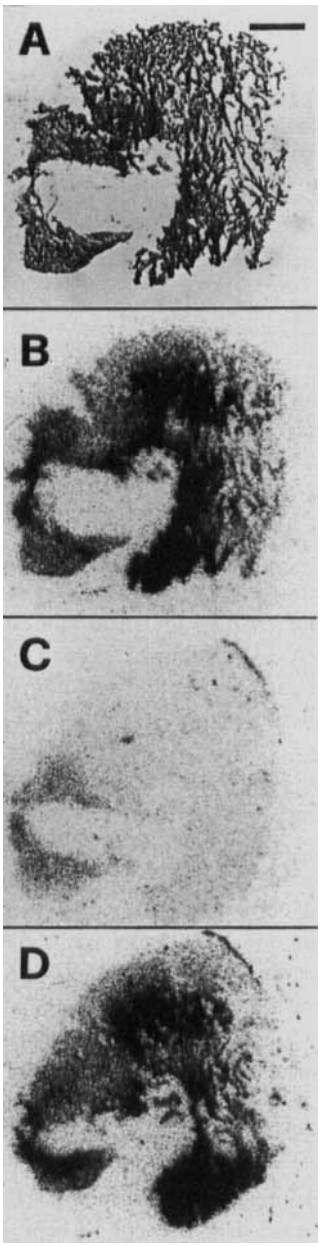


**Fig. 3** A, Haematoxylin–eosin stained section from the skin biopsy from the induration in the upper leg. Bar 1 mm. B, Autoradiogram showing total binding of $^{125}$I-Tyr$^3$-octreotide. The whole tissue is labelled. C, Autoradiogram in the presence of 1 μM Tyr$^3$-octreotide, showing non-specific binding of $^{125}$I-Tyr$^3$-octreotide. D, Autoradiogram in the presence of 100 μM GTP, showing no effect of GTP on $^{125}$I-Tyr$^3$-octreotide binding.

< 50 μmol/24 h). Computed tomography and ultrasound of the abdomen showed multiple liver metastases up to 6 cm in largest diameter, but no extrahepatic tumour localizations. $^{111}$In-octreotide scintigraphy at that time showed irregular uptake in the liver, most notably on SPECT images, which is

**Table 1** Binding of $^{125}$I-Tyr$^3$-octreotide to patient serum samples

| Serum | Percentage binding of $^{125}$I-Tyr$^3$-octreotide | |
|---|---|---|
| | Without octreotide | With 1 $\mu$M octreotide |
| Patient, before octreotide treatment | 2·3 | 1·4 |
| Patient, after octreotide treatment | 22·5 | 1·8 |
| Control 1 | 0·6 | 0·8 |
| Control 2 | 1·3 | 1·4 |

Samples were diluted to a final incubation volume of 230 $\mu$l (1:23). Binding is expressed as a percentage of total counts added.

compatible with the presence of somatostatin receptor positive metastases. Also, abnormal accumulation of radioactivity was seen at one spot in the abdomen (Fig. 1A). Octreotide therapy was started in November 1989, at an increasing dose up to 1 mg/day in January 1990. Although urinary 5-HIAA levels remained at about 100 $\mu$mol/day during this treatment (Fig. 2), the flushes initially decreased in duration and frequency. Ultrasound of the liver in December 1989 revealed no further growth of the metastases. Four months later however the symptoms worsened and physical examination and ultrasound of the heart indicated tricuspid and aortic valve insufficiency. Also, the number and diameter of liver metastases had increased. It was therefore decided to increase the dose to 1·5 mg (0·5 mg t.d.s.) octreotide/day. In July 1990, octreotide scintigraphy was repeated, on and off octreotide treatment. The image of the abdomen during octreotide therapy is shown in Fig. 1B. As the known abdominal site was not visualized during therapy, but again became apparent when treatment was stopped for 3 days (not shown), it was concluded that somatostatin receptors at this stage were still present on the tumour. An only partially successful attempt at embolization of part of the liver metastases was made in August 1990. Because of the persistence of clinical symptoms, octreotide therapy was continued.

From the beginning of 1991, urinary 5-HIAA levels rose and symptoms worsened. Because a receptor down-regulation of the tumour was considered, octreotide scintigraphy was repeated after 3 days of discontinuation of medication in June 1991. At this time, subcutaneous indurations at both upper legs, the sites of injection, were noticed. During octreotide scintigraphy an unusual distribution of radioactivity was seen, both 24 and 48 hours after injection. A relatively high background radioactivity was noticed. In the thorax, increased radioactivity was found over the heart region, whereas in the abdomen liver and spleen were only faintly visualized and the abdominal hot spot seen on previous scintigrams was absent (Fig. 1C and D). An image of the upper legs showed accumulation of labelled octreotide at the site of the subcutaneous indurations found at physical examinations (Fig. 1E).

A biopsy from one of these indurations was shown to consist of normal subcutaneous tissue without signs of inflammation or granuloma formation. Immunostaining for IgG and IgA was diffusely positive, but negative for IgM. Subsequent in-vitro autoradiography of this tissue revealed diffuse specific binding of $^{125}$I-Tyr$^3$-octreotide (Fig. 3). Co-incubation of GTP did not influence this binding, indicating non-G-protein linked binding of the substrate. No specific binding was found when autoradiography was performed with radioiodinated somatostatin-28 (data not shown).

Sera taken before and after 20 months of treatment were then analysed for the presence of octreotide-antibodies. A tenfold higher binding of labelled octreotide was found in the sample after treatment, as compared to before. This binding was blocked by adding excess unlabelled octreotide (Table 1), indicating the presence of specific antibodies. Sera from control patients showed non-displaceable binding of labelled octreotide comparable to the patient's serum sample taken before treatment had started (Table 1). In the sample taken after treatment, increased binding of iodinated octreotide was still found after a fivefold sample dilution (resulting in a final incubation dilution of 1:115), but not after further dilutions (data not shown).

## Discussion

This report presents the fourth case of antibody formation in a patient treated with octreotide. It is striking that in all four cases described so far, octreotide was administered in high dosages of 1·5 mg/day or more. The low antigenicity of this small peptide might explain why so few cases of antibody induction seem to occur and why in all the cases described high dosages of the drug were used.

Ørskov *et al.* (1991) reported a prolonged plasma half-life of octreotide in their two patients with antibodies against the peptide. The scintigraphic images in our patient indicate the

same: visualization of the heart 48 hours after injection of $^{111}$In-octreotide and increased background radioactivity indicate a prolonged presence of $^{111}$In-octreotide in the blood resulting from its being bound to antibodies.

In our patient a decreased uptake in liver and spleen, and a supposed tumour localization in the abdomen which had not unequivocally been confirmed by conventional imaging, were found during the patient's last octreotide scintigraphy when antibodies were present. This seems to indicate that labelled octreotide is less available to somatostatin receptor positive tissues, like tumour and spleen, and would also suggest that octreotide treatment would be less effective. This is in agreement with a previous report from Kendall-Taylor *et al.* (1989) but contrasts with the continued efficacy of octreotide treatment in two acromegalic patients who had antibodies, as reported by Ørskov *et al.* (1991). As we did not find a clear-cut response to octreotide in terms of a significant suppression of urinary 5-HIAA levels or an improvement in clinical symptoms in our patients we cannot address the question whether octreotide remains therapeutically effective once antibodies are present. Kendall-Taylor *et al.* (1989) reported a skin rash and an induration at the site of injection in their patient who developed octreotide antibodies. In our patient, who had no rash, the induration was shown to be non-inflammatory. Positive immunostaining for IgG and IgA was found, indicating the accumulation of these immunoglobulins at the sites of injection. The combination of the visualization of these lesions during octreotide scintigraphy and the high, specific and GTP independent binding of labelled octreotide found with in-vitro techniques in the same tissue provide circumstantial evidence that the tissue where the highest antigen concentrations occur are the sites where highest antibody concentrations are present.

In conclusion, this report describes the characteristic abnormalities during in-vivo $^{111}$In-octreotide scintigraphy in a patient with octreotide antibodies. These consisted of high background radioactivity due to prolonged circulation of antibody coupled $^{111}$In-octreotide, and visualization of the injection sites which most probably results from local accumulation of antibodies.

**Acknowledgements**

We are indebted to P. M. van Koetsveld for his enthusiastic and expert technical assistance.

This work was supported by a grant from the Dutch National Health Service Board (Ziekenfondsraad).

**References**

Bakker, W.H., Albert, R., Bruns, C., Breeman, W.A.P., Hofland, L.J., Marbach, P., Pless, J., Koper, J.W., Lamberts, S.W.J., Visser, T.J. & Krenning, E.P. (1991a) [$^{111}$In-DTPA-D-Phe$^1$]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabelling and in-vitro validation. *Life Sciences*, **49**, 1583–1591.

Bakker, W.H., Krenning, E.P., Reubi, J.C., Breeman, W.A.P., Setyono-Han, B., de Jong, M., Kooij, P.P.M., Bruns, C., Van Hagen, P.M., Marbach, P., Visser, T.J. & Lamberts, S.W.J. (1991b) In-vivo application of [$^{111}$In-DTPA-D-Phe$^1$]-octreotide for detection of somatostatin receptor-positive tumors in rats. *Life Sciences*, **49**, 1593–1601.

Kendall-Taylor, P., Chatterjee, S., White, M.C., Harris, M.M., Davidson, K., Besser, G.M. & Wass, J.A.H. (1989) Octreotide. *Lancet*, **ii**, 859–860.

Krenning, E.P., Bakker, W.H., Kooij, P.P.M., Breeman, W.A.P., Oei, H.Y., de Jong, M., Reubi, J.C., Visser, T.J., Kwekkeboom, D.J., Reijs, A.E.M., Van Hagen, P.M., Koper, J.W. & Lamberts, S.W.J. (1992) Somatostatin receptor scintigraphy with [$^{111}$In-DTPA-D-Phe$^1$]-octreotide in man: metabolism, dosimetry and comparison with [$^{123}$I-Tyr$^3$]-octreotide. *Journal of Nuclear Medicine*, **33**, 652–658.

Ørskov, H., Christensen, S.E., Weeke, J., Kaal, A. & Harris, A.G. (1991) Effects of antibodies against octreotide in two patients with acromegaly. *Clinical Endocrinology*, **34**, 395–398.

Reubi, J.C., Heitz, P.U. & Landolt, A.M. (1987) Visualization of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: evidence for different tumor subclasses. *Journal of Clinical Endocrinology and Metabolism*, **65**, 65–73.