Therapy of neuroendocrine tumors with radiolabeled somatostatin-analogues

M. DE JONG, W. A. P. BREEMAN, H. F. BERNARD, P. P. M. KOOIJ, G. D. SLOOTER*, C. H. J. VAN EIJCK*
D. J. KWEKKEBOOM, R. VALKEMA, H. R. MÄCKE**, E. P. KRENNING**

Peptide receptor scintigraphy with the radioactive somatostatin-analogue [^{111}In-DTPA^0]octreotide (DTPA = diethylenetriaminepentaacetic acid) is a sensitive and specific technique to show in vivo the presence and abundance of somatostatin receptors on various tumors. With this technique primary tumors and metastases of neuroendocrine cancers as well as of many other cancer types can be localised. A new application is the use of peptide receptor radionuclide therapy, administrating high doses of ^{111}In- or ^{90}Y-labeled octreotide-analogues.

***Preclinical*. We investigated the radiotherapeutic effect of ^{90}Y- and ^{111}In-labeled [DOTA^0,Tyr^3]octreotide (DOTA= tetraazacyclododecanetetraacetic acid) or [^{111}In-DTPA^0]octreotide in Lewis rats bearing the somatostatin receptor-positive rat pancreatic tumor CA20948 in A) the flank or B) in the liver.**

***Patients*. Thirty end-stage patients with mostly neuroendocrine progressing tumors were treated with [^{111}In-DTPA^0]octreotide, up to a maximal cumulative patient dose of about 74 GBq, in a phase 1 trial.**

***Preclinical results*. A) *Flank model*: at least two 111MBq injections of [^{111}In-DOTA^0,Tyr^3]octreotide were needed to reach tumor response, in 40% of the animals complete tumor remission was found after a follow-up period of 10 months. One or two injections of [^{90}Y-DOTA^0,Tyr^3] octreotide yielded transient stable disease.**
B) *Liver model*: we found that peptide receptor radionuclide therapy is only effective if somatostatin receptors are present on the tumors, and is therefore receptor-mediated. High radioactive doses of 370 MBq [^{111}In-DTPA^0]octreotide or 93 MBq [^{90}Y-DOTA^0,Tyr^3]octreotide can inhibit the growth of somatostatin receptor-positive metastases.

***Clinical results*. There were no major clinical side effects after up to 2 years treatment, except that a transient decline in platelet counts and lymphocyte subsets can occur. Promising beneficial effects on clinical symptoms, hormone production and tumor proliferation were found. Of the 21 patients with progressive disease at baseline and who received a cumulative dose of more than 20 GBq [^{111}In-DTPA^0]octreotide, 8 patients showed stabilisation of disease and 6 other patients a reduction in size of tumors. There is a tendency towards better results in patients whose tumors have a higher accumulation of the radioligand.**

***Conclusion*. Radionuclide therapy with octreotide-derivatives is feasible, both with ^{111}In and ^{90}Y as radionuclides.**

KEY WORDS: Neuroendocrine tumors - Somatostatin analogues and derivates - Radioisotopes, therapeutic use.

From the Department of Nuclear Medicine
**Surgery, **Kantonspital Basel, Switzerland*
*and ***Internal Medicine III*
University Hospital and Erasmus University
Rotterdam, The Netherlands

Address reprint requests to: E. P. Krenning, Department of Nuclear Medicine, University Hospital Rotterdam, 3015 GD Rotterdam.
E-mail: krenning@nuge.azr.nl

The inhibitory effect of somatostatin (SS) on hormone secretion led to the concept of beneficial effects of somatostatin in the treatment of diseases based on gland hyperfunction or overproduction of hormones by endocrine-active tumors. The tetradecapeptide SS_{14} itself is unsuitable for treatment, because of its very short half-life of ≈3 minutes in man after intravenous injection and because of its diversity of action, such as lowering insulin levels. Successful efforts have been undertaken to synthesise more

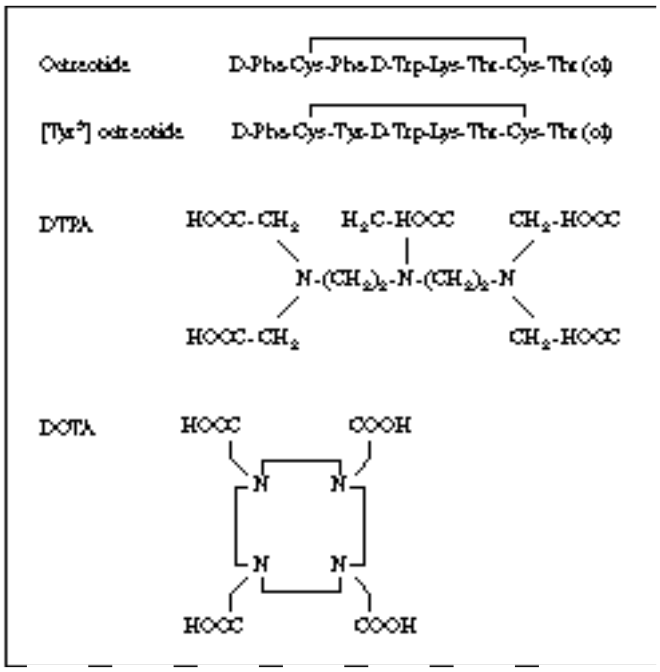

Fig. 1.—Structures of octreotide, [Tyr^3]octreotide, DTPA and DOTA.

stable somatostatin-analogues, resulting in octreotide (Fig. 1).

Somatostatin receptors are integral membrane glycoproteins. Five different human somatostatin receptor types have been cloned. All subtypes bind SS_{14} with high affinity, while the affinity of numerous somatostatin-analogues differ considerably.[1-3] Octreotide binds with high affinity to the somatostatin receptor subtype 2 (sst_2), while this analogue has a lower affinity for sst_3 and sst_5 and shows no binding to sst_1 and sst_4.[1-4] Octreotide scintigraphy is, therefore, based on the visualisation of (an) octreotide-binding somatostatin receptor(s), most probably the sst_2.

Peptide receptor scintigraphy with the radioactive somatostatin-analogue, [^{111}In-DTPA^0]octreotide (Fig. 1), is a sensitive and specific technique to show *in vivo* the presence and abundance of somatostatin receptors on various tumors. In general, mapping of the presence of various peptide receptors on the cell membrane by peptide receptor scintigraphy may become an attractive, non-invasive, harmless, easy-to-perform tool for an individual therapeutic approach of the cancer patient.[5-8] A new and fascinating application is the use of radiolabeled peptides for peptide receptor radionuclide-therapy, further referred to as radionuclide-therapy. The success of the therapeutic strategy relies upon the amount of radioligand, which can be concentrated within tumor cells and the rates of internalisation, degradation and recycling of both ligand and receptor will among other things determine this. Binding of several peptide hormones to specific surface receptors is generally followed by internalisation of the ligand receptor complex via invagination of the plasma membrane.[9,10] The resulting intracellular vesicles, termed endosomes, rapidly acidify, which causes the ligand to dissociate from the receptor. The ligand may be delivered to the lysosome [11] and the receptor may recycle back to the plasma membrane. The whole process takes approximately 15-min,[9] and a single receptor can deliver numerous ligand molecules to the lysosomes. We have studied internalisation and degradation of radiolabeled [DTPA^0]octreotide and we detected internalisation of the radiopharmaceutical in tumor cells, in accordance with the findings of Andersson *et al.*,[12] this process was receptor-specific and temperature dependent.[13] Receptor-mediated internalisation of [^{111}In-DTPA^0]octreotide will most probably result in degradation to ^{111}In-DTPA-D-Phe, this metabolite is not capable passing the lysosomal membrane.[11]

Since ^{111}In emits not only gamma-rays, which are visualised during scintigraphy, but also short-ranged Auger-electrons, an effect on tumor cell proliferation could be expected, as the radiotoxicity of Auger-electrons is very high if the DNA of the cell is within the particle range.[14-16] ^{111}In emits Auger- and conversion-electrons having a tissue penetration of 0.02 to 10 µm and 200 to 500 µm, respectively, and can therefore be used to investigate its antiproliferative effect in cancer. We reported a biological half-life for ^{111}In of >700 hours in tumor tissue.[6] Therefore, ^{111}In-labeled [DTPA^0]octreotide has an appropriate distribution profile in humans for scintigraphy and radionuclide therapy.[17,18] Earlier we reported [19] that high radioactive doses with [^{111}In-DTPA^0]octreotide for radionuclide-therapy could inhibit the growth of somatostatin receptor-positive liver metastases in an animal model, and that radionuclide-therapy was only effective if somatostatin receptors were present on the tumors. The effect of radionuclide-therapy with [^{111}In-DTPA^0]octreotide could be reduced by pretreatment with excess unlabeled octreotide, which indicates that receptor binding is essential for radionuclide-therapy.

For radiotherapeutic applications, other radionuclides have also been proposed and investigated for

coupling to octreotide-analogues. ^{90}Y is a β-particle emitter, the maximum energy of the electrons is 2.3 MeV, their mean range is a few millimeters in tissue. ^{90}Y shows dissociation from DTPA-conjugated peptides in serum, resulting in hematopoietic toxicity *in vivo*, therefore, Tyr^3-octreotide (Fig. 1), which has a higher binding affinity for sst_2 than octreotide itself, has been derivatized with the chelator DOTA enabling stable radiolabeling with both ^{90}Y and ^{111}In. Preclinical and clinical studies with [DOTA^0,Tyr^3]octreotide showed favourable biodistribution and tumor uptake characteristics.[20 21]

In this paper preclinical and clinical studies on the radiotherapeutic effect of radiolabeled octreotide-derivatives are described:

Preclinical studies.—A) *Flank model:* experiments using ^{90}Y- and ^{111}In-labeled [DOTA^0,Tyr^3]octreotide in Lewis rats bearing the somatostatin receptor-positive rat pancreatic tumor CA20948 in their flank.

B) *Liver model:* timing and dosage characteristics of radionuclide therapy using [^{111}In-DTPA^0]octreotide and [^{90}Y-DOTA^0,Tyr^3]octreotide in rats inoculated with CA20948 tumor cells in the portal vein of the liver.

Clinical study.—The side-effects and antiproliferative effect of high, multiple radiotherapeutic doses of [^{111}In-DTPA^0] were investigated in a phase 1 study. We included end-stage patients with mainly a high tumor load of progressing somatostatin receptor-positive neuroendocrine tumors.

Materials and methods

Labeled peptides

[DTPA^0]octreotide (DTPA = diethylenetriaminepentaacetic acid) and $^{111}InCl_3$ (DRN 4901, 370 MBq/ml in HCl, pH = 1.5 - 1.9) were obtained from Mallinckrodt Medical BV (Petten, The Netherlands). [DTPA^0]octreotide was labeled with $^{111}InCl^3$ as has been described previously.[22] [DOTA^0,Tyr^3]octreotide (DOTA= tetraazacyclododecanetetraacetic acid) was synthesised by Prof. H. Mäcke (Basel, Switzerland). ^{90}Y- and ^{111}In-labeling of [DOTA^0,Tyr^3]octreotide was performed as described.[20]

Preclinical in vivo radionuclide therapy experiments using radiolabeled somatostatin-analogues

A) *Flank model.*—The CA20948 pancreatic tumors were grown at the flank of Lewis rats. Male Lewis rat (Harlan, The Netherlands; 250-300 g) were injected subcutaneously into both flanks, each with 500 µl of a cell suspension of the CA20948 tumor, prepared from 5 g crude tumor tissue in 100 ml saline. At the start of therapy, rats were anaesthetised, and the peptides were injected into the dorsal vein of the penis. Five groups of 6 rats were formed. The four therapeutic groups received either a single injection or two injections (one week apart) of either 111 MBq [^{111}In-DOTA^0,Tyr^3]octreotide or 111 MBq [^{90}Y-DOTA^0,Tyr^3] octreotide. Specific activity of the peptides was 37 MBq/1.2 µg peptide. The control group received a concordant amount of unlabeled [DOTA^0,Tyr^3]octreotide (3.6 µg).

In an earlier study we investigated the effects of variation in injected peptide amount on tumor uptake in CA20948 pancreatic tumor-bearing rats for ^{111}In-labeled [DOTA^0,Tyr^3]octreotide, in order to find the peptide amount at which the highest uptake in the target was achieved. We found that uptake in the tumor as a function of injected amount peptide showed a bell shape with the highest uptake at 0.5 µg peptide. At higher peptide amounts tumor uptake of radioactivity was decreased to about 50% of the maximum tumor uptake after injection of 4 µg peptide [Eur J Nucl Med, July 1999:26(7):693-8]. In the present study we had to inject 3.6 µg peptide, this was administered in 2 injections of 1.8 µg each, 1 hour apart.

The tumors were measured on the day of radioligand administration and every 3-4 days thereafter. Tumor growth, animal condition and body weight were determined independently by two investigators. At progressed stage of the CA20948 tumor necrosis may occur. Besides loss of more than 10% of original body weight, tumor necrosis was an indication to sacrifice the rats.

B) *Liver model.*—The tumor was transplanted and maintained in the liver after direct injection of tumor cells into the vena porta to produce artificial liver metastases. Therefore, tumors were excised from donor livers, cleaned from normal liver tissue and pressed through sieves with decreasing mess size. The suspension was washed twice in phosphate-buffered saline. Viability was measured with trypan-blue exclusion. A suspension of 2.5×10^6 living cells/mL was used for injection into the vena porta. All rats were sacrificed 20-21 days after inoculation of tumor. Tumor growth was determined by two investigators

counting and ranking the number of metastases (from 0 to 5+), while blinded for treatment modality. Experiment 1 was designed to determine the minimal effective dosage of radionuclide-therapy: the rats were injected with 3.7, or 37 or 370 MBq (0.5 µg) [^{111}In-DTPA^{0}]octreotide on day 1. In experiment 2 rats were treated with 370 MBq (0.5 µg) [^{111}In-DTPA^{0}]octreotide on day 6 or 12. In experiment 3 rats received radionuclide-therapy on day 7 or 14. In experiment 4 the rats were injected at day 1 with saline (A), or with vehicle (2 µg [DOTA^{0},Tyr^{3}]octreotide) (B), or with 93 MBq (2 µg) [^{90}Y-DOTA^{0}, Tyr^{3}]octreotide (C). Experi-ments 1-4 were performed twice. Rats were weighed 3 times/week, and their body weight was expressed as the percentage of their body weight at day 3.

Statistical analysis

Statistical analysis was performed using Mann-Whitney "U"-test on categorised outcomes, Fisher's exact test on proportions and analysis of variance. Statistical significance was defined as $p<0.05$.

Clinical in vivo *radionuclide therapy experiments using radiolabeled somatostatin-analogues*

The typical dose per administration is 6000-7000 MBq ^{111}In incorporated in 40-50 µg [DTPA^{0}]octreotide. Doses were given with at least 2 week intervals between administrations and a total of 8 administrations is aimed at with extensions in a few patients to about 20 administrations. Radionuclide therapy with [^{111}In-DTPA^{0}]octreotide was applied after witnessed informed consent by the patient and approval by the medical ethics committee of our hospital. The following measurements, carried out prior to and between all administrations, served as parameters of possible side-effects: the usual haematological and chemical analyses of bone-marrow, liver, kidney and endocrine pancreatic (glucose or Hb A_{1c}) function. Pituitary function (Free T_4, post-menopausal women: LH and FSH, men: testosterone) was assessed prior to and 4 weeks after the 4th and 8th administration of [^{111}In-DTPA^{0}]octreotide; also possible effects on: 1) the endocrine activity of the tumors and/or their production of specific serum markers, and 2) tumor-size (CT or MRI) were investigated. Pituitary-adrenal-axis function testing (metyrapone test) prior to and after 8 administrations as well as long-term follow up with 3-4 month intervals was investigated if feasible.

Dosimetry of [^{111}In-DTPA^{0}]octreotide in humans

Scoring of tumor radioactivity uptake in patients prior to the start of treatment with [^{111}In-DTPA^{0}]octreotide was done visually by using scintigrams obtained 24 hr after injection of a diagnostic dose (220 MBq) of [^{111}In-DTPA^{0}]octreotide. The scoring grades used were: 4 = intense, 3 = clear (higher than liver uptake), 2 = clear but faint (lower than or equal to liver uptake), 1 = equivocal and 0 = no accumulation. Patients were also scanned 3 and 7 days after each administration of the radiotherapeutic dose. Percentage uptake of the administered dose in total body and in the most prominent tumor was calculated (data not shown). Uptakes decreased slowly or remained the same if the interval between the successive administrations was less than one month. In patients who had 6 or more administrations of 6000 to 7000 MBq of [^{111}In-DTPA^{0}]octreotide with intervals of maximally one-month between administrations, uptake in the tumor was still clearly visible after the last administration. Typical radiation doses to tissues with doses of 6000-7000 MBq [^{111}In-DTPA^{0}]octreotide are: kidneys 300-1400 (depending on the relative biological effectiveness [RBE: 1- 20] for Auger electrons) cGy, spleen 200 cGy, liver 50 cGy, bone marrow 13 cGy (target organ for gamma photons), thyroid gland 25 cGy and pituitary 70 cGy, the critical organs are kidneys and spleen. With these doses the estimated tumor radiation doses for a 10 g tumor (assumptions: 1% uptake; effective half-life is the physical half-life) 1700 and 6700 cGy (RBE for Auger electrons 1 and 20, respectively) and for a 100 gram tumor (1 % uptake) 250 and 750 cGy, respectively.

Results and discussion

Preclinical in vivo *radionuclide therapy experiments using radiolabeled somatostatin-analogues*

A) *Flank model.*—Figure 2 shows that the tumors of the rats in the control group grew excessively. Those rats had to be sacrificed on average 12 days post unlabeled peptide injection. The treatment with a single dose of 111 MBq [^{111}In-DOTA^{0},Tyr^{3}]octreotide did not induce tumor response, whereas two injections of

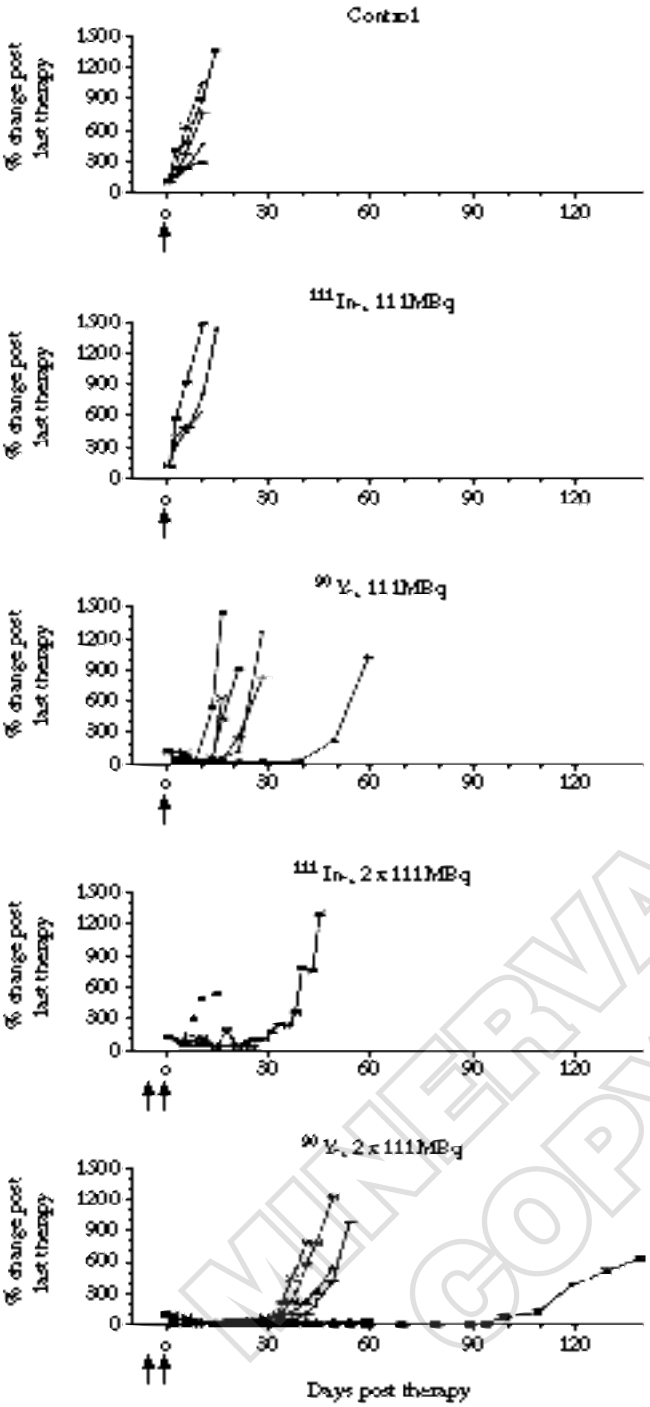

Fig. 2.—Effect of ^{111}In- or ^{90}Y-labeled [DOTA^0,Tyr^3]octreotide (one or two injections of 111 MBq/rat, indicated by the arrows at the horizontal axis) on tumour growth in Lewis rats bearing CA20948 tumors in the flanks. Control animals received unlabeled peptide in the same amount as given in the radiolabeled administrations (3.6 µg).

TABLE I.—*Effects of therapy with [^{111}In-DTPA^0]octreotide on count of tumor colonies in liver.*

Exp 1	Tumor colonies count						Rats (n)
	0	1+	2+	3+	4+	5+	
3.7 MBq				2	5	1	8
37 MBq					4	4	8
370 MBq			4	4			8*, **, §, ¶, ¶¶
Control						4	4

*: p<0.05 *versus* Control, **: p<0.05 *versus* 37 MBq, §: p<0.05 *versus* 3.7 MBq, ¶: p<0.05 *versus* 37 MBq, based on liver weight, ¶¶: p<0.05 *versus* 3.7 MBq, based on liver weight.

111 MBq resulted in at least partial tumor remission. Of the animals in this group 40% reached complete tumor remission. Mean survival time in the latter group was 85 days post therapy (p<0.001 *versus* control), determined 140 days post therapy. After a single injection with [^{90}Y-DOTA^0,Tyr^3]octreotide transient regression of the tumors was found and the rats lived 28 days post therapy (p<0.001 *versus* control). Two injections with 111 MBq [^{90}Y-DOTA^0,Tyr^3]octreotide, one week apart, resulted in a mean survival of 65 days post therapy (p<0.001 *versus* control), no complete remission was reached.

We and others have also studied several parameters like histology of various organs, blood cell count and endocrine parameters (manuscript in preparation), no obvious toxicology of the given radiotherapy has been found so far in rats.

Liver model.—In experiment 1 we investigated radionuclide-therapy at day 1 after injection of 370, 37 or 3.7 MBq [^{111}In-DTPA^0]octreotide (Table I). Figure 3 shows examples of livers from control (left) and 37 MBq-treated (right) rats. Twenty days after the direct injection of CA20948 tumor cells into the portal vein the rats were sacrificed. In all groups tumor colonies were counted. The 370 MBq dosage had significantly more effect on tumor score and inhibited the increase of liver weight due to tumor growth more than the 37 or 3.7 MBq dose. Starting from day 14 after therapy we also found a significant relation between the decrease of body weight (100% at day 3) and the liver weight and tumor score at sacrifice at day 20 (data not shown). A significant predictive value for tumor growth was found at a loss of ≥9% of body weight (data not shown).

Since a dose response was found, we continued our studies with 370 MBq for experiments 2 and 3. In

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