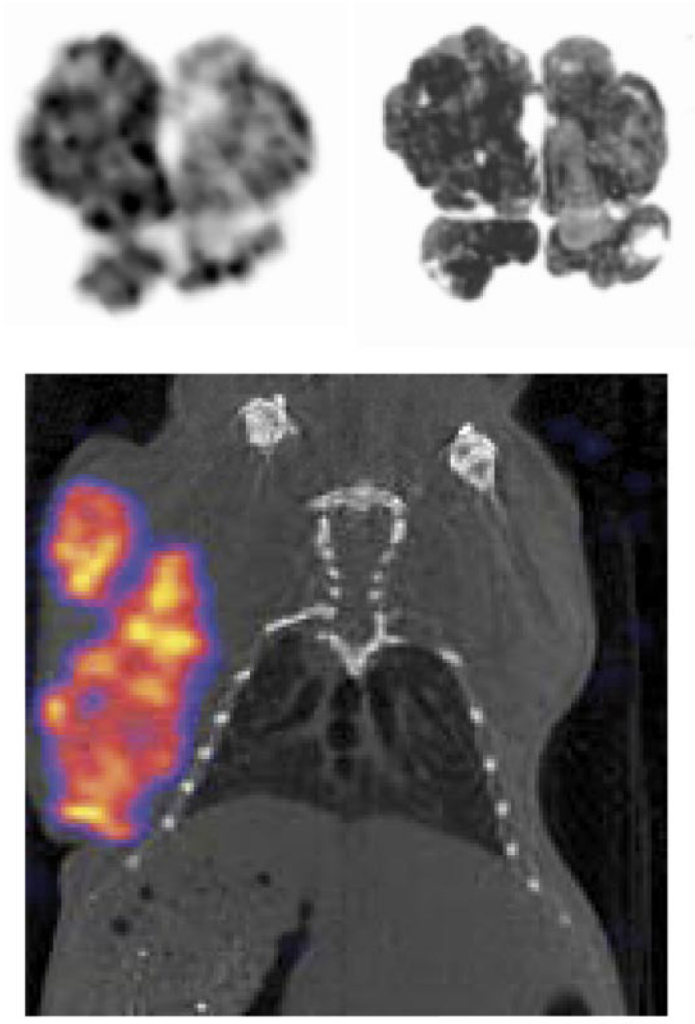
Targeted Radionuclide Therapy:
Current status and potentials for future improvements



Flavio Forrer

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Targeted Radionuclide Therapy:

Current status and potentials for future improvements

Receptor Radionuclidentherapie:

Huidige status en mogelijkheden voor verbetering in de toekomst

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Imagination is more important than knowledge.
For knowledge is limited to all we now know and understand,
while imagination embraces the entire world,
and all there ever will be to know and understand.
(Albert Einstein)

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CHAPTER 1

INTRODUCTION

Adapted from:
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Best Practice & Research Clinical Endocrinology & Metabolism
2007;21:111-129

In targeted radionuclide therapy the goal is to deliver the highest radioactivity possible to the target cell while the absorption of the radioactivity in non-target tissue should be as low as achievable. Usually, this goal is reached by coupling the radionuclide to a vector which recognises a structure, e.g. receptor, on the target cell. By far the most established combination is the somatostatin receptor (sst) and radiolabeled somatostatin analogues.

The majority of neuroendocrine tumours feature a strong over-expression of the somatostatin receptors (sst), mainly subtype 2 (sst_2). Somatostatin receptors are attractive targets for radiolabelled peptides since the density of sst on tumours is vastly higher than on non tumour tissue [1,2]. In addition to the favourable receptor distribution, sst_2 internalises into the cell after a ligand bound to the receptor. Consequently, radioactivity delivered by the vector is captured in the target cell after binding [3].

Development of Peptide Receptor Radionuclide Therapy
Somatostatin receptor scintigraphy was introduced in the late 1980s and after the development of [Indium-111-DTPA^0]-octreotide ([^{111}In-DTPA^0]-octreotide) this radiolabelled hormone analogue became the gold standard for staging sst-positive neuroendocrine tumours [4,5]. Since then many improvements concerning the peptide and the radiolabelling were made. Nowadays, somatostatin analogues labelled with positron emitters are available. The use of these compounds with an integrated PET/CT camera provides a highly valuable combination of physiological and anatomical information [6-8].

The high tumour to non-tumour ratio that can be achieved with radiolabelled somatostatin analogues resulted in attempts to treat patients with metastatic, sst-positive, neuroendocrine tumours with these drugs. In turn, diagnostic scans with radiolabelled somatostatin analogues are not only used for staging of patients, but also to identify suitable candidates for peptide receptor radionuclide therapy (PRRT) and for the monitoring of the therapy.

The first therapy studies using radiolabelled somatostatin analogues were performed with high dosages of ^{111}In-octreotide which was available for diagnostic purposes at that time [9-12]. Later on peptides with higher receptor affinity were developed and conjugated with the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator, which allowed stable labelling with the pure, high energy beta-emitter Yttrium-90 (^{90}Y). A number of studies using [^{90}Y-DOTA^0,Tyr^3]octreotide (^{90}Y-DOTATOC), [^{90}Y-DOTA]lanreotide and [^{90}Y-DOTA^0,Tyr^3]octreotate have been published [13-19]. In a next step, studies using the intermediate energy beta emitter Lutetium-177 (^{177}Lu) were presented [20-23]. Currently several studies using different radionuclides, peptides and treatment protocols are performed in different centres. The detailed results of the various studies are reported below.

Radionuclides

Over the past decade, the most frequently used radionuclides in PRRT with somatostatin analogues were Indium-111 (^{111}In), Yttrium-90 (^{90}Y), and Lutetium-177 (^{177}Lu). These radionuclides have different physical characteristics which will influence the effects of the therapy. I.e. different particles are emitted at different energies resulting in various tissue penetration ranges. The peptide is conjugated with a chelator which forms a stable complex with these three radionuclides. Beside the gamma-radiation, which makes ^{111}In suitable for imaging with a gamma-camera, ^{111}In emits Auger electrons. Auger electrons are low energy electrons with a short tissue penetration range of 0.02 – 10 μm. The first clinical therapy trials

were performed with [^{111}In-DTPA^{0}]-octreotide [9,10,24]. In contrast, ^{90}Y is a pure beta-emitter. The electrons are emitted with a relatively high energy (E_{max} = 2.28 MeV) resulting in a tissue penetration range of up to 12 mm. Therefore, a pronounced "cross fire effect" is found when using ^{90}Y. On the one hand the cross fire effect is beneficial since it allows to irradiate tumour cells which are not directly targeted by the radiopharmaceutical. On the other hand, the long range of the ^{90}Y beta-particles appears to be less favourable concerning kidney toxicity [25]. The third radionuclide used frequently for PRRT, ^{177}Lu, emits intermediate energy beta-particles with an E_{max} = 0.5 MeV resulting in tissue penetration range of up to 2 mm. In addition, ^{177}Lu has two gamma peaks at 113 and 208 keV which makes it suitable for imaging with a gamma camera as well. Imaging can be used for posttherapeutic dosimetry [20-23].

Another difference between these radionuclides is their physical half-life. Although the influence of the physical half-life is not fully understood yet, it is very likely that it influences the therapeutic as well as the secondary effects. For ^{111}In and ^{90}Y it is almost identical with 2.8 and 2.7 days, respectively, whereas the physical half-life of ^{177}Lu is more than double (6.7 days).

Somatostatin analogues used for PRRT

The two known natural somatostatins consist of 14 or 28 amino acids, respectively. As neurotransmitter with endocrine and paracrine functions *in vivo* and are rapidly degraded by peptidases. The serum half life of these peptides in blood is approximately 2 minutes which is too short to qualify natural somatostatin as a radiopharmaceutical [26].

The breakthrough in somatostatin receptor imaging and consecutively in therapy was made when the octapeptide octreotide was radiolabelled [4]. This small peptide is metabolically more stable. It has a plasma half-life of approx. 1.7 hours. Initially the non-radiolabelled octreotide was developed to be used as a drug inhibiting the secretion of growth hormone, which is one of the physiological actions of somatostatin [27].

Five different subtypes of sst are known (sst_1 to sst_5). Not all subtypes are equally important for PRRT [28]. For neuroendocrine tumours sst_2 appears to be the most important subtype [29]. Octreotide has a high affinity for sst_2, a lower affinity for sst_3 and sst_5 and no affinity for sst_1 and sst_4 (Table 1) [29-31]. Modifications of octreotide, like the conjugation with a chelator can provoke a change in the affinity profile. Remarkably, the same holds true when identical conjugated peptides are labelled with different radionuclides [29].

Table 1

Affinity profiles (IC 50) for human sst1–sst5 receptors of a series of somatostatin analogues

Peptide	sst_1	sst_2	sst_3	sst_4	sst_5
Somatostatin-28	5.2±0.3 (19)	2.7±0.3 (19)	7.7±0.9 (15)	5.6±0.4 (19	4.0±0.3 (19)
Octreotide	>10,000 (5)	2.0±0.7 (5)	187±55 (3)	>1,000 (4)	22±6 (5)
DTPA-octreotide	>10,000 (6)	12±2 (5)	376±84 (5)	>1,000 (5)	299±50 (6)
In-DTPA-octreotide	>10,000 (5)	22±3.6 (5)	182±13 (5)	>1,000 (5)	237±52 (5)
DOTA-TOC	>10,000 (7)	14±2.6 (6)	880±324 (4)	>1,000 (6)	393±84 (6)
Y-DOTA-TOC	>10,000 (4)	11±1.7 (6)	389±135 (5)	>10,000 (5)	114±29(5)
DOTA-LAN	>10,000 (7)	26±3.4 (6)	771±229 (6)	>10,000 (4)	73±12 (6)
Y-DOTA-LAN	>10,000 (3)	23±5 (4)	290±105 (4)	>10,000 (4)	16±3.4 (4)
DOTA-OC	>10,000 (3)	14±3 (4)	27±9 (4)	>1,000 (4)	103±39 (3)
Y-DOTA-OC	>10,000 (5)	20±2 (5)	27±8 (5)	>10,000 (4)	57±22 (4)
Ga-DOTA-TOC	>10,000 (6)	2.5±0.5 (7)	613 ±140 (7)	>1,000 (6)	73±21 (6)
Ga-DOTA-OC	>10,000 (3)	7.3±1.9 (4)	120±45 (4)	>1,000 (3)	60±14 (4)
DTPA-[Tyr^3]-octreotate	>10,000 (4)	3.9±1 (4)	>10,000 (4)	>1,000 (4)	>1,000 (4)
DOTA-[Tyr^3]-octreotate	>10,000 (3)	1.5±0.4 (3)	>1,000 (3)	453±176 (3)	547±160 (3)
In-DTPA-[Tyr^3]-octreotate	>10,000 (3)	1.3±0.2 (3)	>10,000 (3)	433±16 (3)	>1,000 (3)
Y-DOTA-[Tyr^3]-octreotate	>10,000 (3)	1.6±0.4 (3)	>1,000 (3)	523±239 (3)	187±50 (3)
Ga-DOTA-[Tyr^3]-octreotate	>10,000 (3)	0.2±0.04 (3)	>1,000 (3)	300±140 (3)	377±18 (3)

All values are IC 50 ± SEM in nM. The number of experiments is in parentheses.
Reported after Reubi et al. [31]

The introduction of small changes in amino acids of octreotide created a batch of peptides with different affinity profiles for the different receptor subtypes (Table 1) [29]. The peptides used most frequently in PRRT are discussed in more detail in the next paragraphs.

Clinical studies

A number of Phase I and II therapy studies using different somatostatin analogues, different radionuclides, and different treatment protocols have been published to date. The numerous variables, including different patient characteristics, make it nearly impossible to compare the results of these studies properly. However, it became evident that the kidneys and / or the bone marrow are the major dose limiting organs for this treatment.

Studies using [^{111}In-DTPA^0]octreotide

[^{111}In-DTPA^0]octreotide, developed initially for diagnosis [4], was the first radiolabelled somatostatin analogue used for PRRT. In several studies, the total cumulative dose ranged from 3.1 to 160.0 GBq [9-12]. The number of objective responses according to WHO or SWOG criteria was low. Valkema et al. reported the outcome in 50 patients with sst-positive tumours, including 26 patients with gastroenteropancreatic (GEP) tumours [8]. All patients had documented progressive disease (PD) at the time of inclusion. From the 26 patients with GEP tumours, 15 (58%) achieved a stabilisation of their disease (SD) and 2 (8%) achieved a minor remission (MR), defined as a reduction of tumour mass between 25% and 50%. These 17 patients (65%) were considered to have benefited from the therapy.

Anthony and colleagues reported a trial including 26 evaluable patients with GEP tumours [11]. A partial remission (PR) was found in 2 patients (8%) and 21 patients (81%) achieved stabilisation (SD) of their disease. However, this study did not use WHO or SWOG criteria to define the outcome. In a smaller study, including 12 patients with GEP tumours, Buscombe et al. reported results with a follow up of at least 6 months after the last therapy cycle [12]. In 7 patients (58%) SD was found, 2 patients (17%) achieved a PR and 3 patients (25%) remained progressive despite therapy according to RECIST criteria.

Although the number of objective responses was rather small, these results were encouraging, especially when seen in the context of the results that can be achieved with other therapy modalities like chemotherapy [32]. Nevertheless it appeared that the anti-tumour effect of [^{111}In-DTPA^{0}]octreotide is not ideal for macroscopic tumours.

Experimental data collected in rats, suggested that high doses of [^{111}In-DTPA^{0}]octreotide can inhibit the growth of sst 2 positive liver metastases after the injection of tumour cells into the portal vein [33]. These results indicated that [^{111}In-DTPA^{0}]octreotide might be particularly effective in micro-metastases. However, no clinical studies that confirmed these findings are available.

Studies using [^{90}Y-DOTA^{0},Tyr^{3}]octreotide (^{90}Y-DOTATOC), [^{90}Y-DOTA]lanreotide and [^{90}Y-DOTA^{0},Tyr^{3}]octreotate

In order to improve the anti-tumour effect, subsequent studies were performed with ^{90}Y labelled somatostatin analogues. With the introduction of ^{90}Y the need of a new chelator arose since it cannot be bound in a sufficient stable way by DTPA [34]. ^{90}Y as well as ^{177}Lu (see below) is a “bone seekers”, i.e. free radionuclides would accumulate in the bone which consecutively would lead to a high absorbed dose to the bone marrow. DOTA is the most frequently used chelator in PRRT. DOTA has the ability to bind ^{90}Y as well as ^{177}Lu stably under various conditions [35]. An overview over the most important PRRT studies using ^{90}Y is given in Table 2.

Table 2

Peptide receptor radionuclide therapy with ^{90}Y- and ^{177}Lu-labelled somatostatin analogues in patients with neuroendocrine tumours.

Authors	n	PD at time of inclusion	Response [a]					
			CR	PR	MR [b]	SD	PD	CR+PR
[^{90}Y-DOTA^0,Tyr^3]octreotide (^{90}Y-DOTATOC)								
Otte et al. [13]	16	N/I	0	1 (6%)	N/I	14 (88%)	1 (6%)	1/16 (6%)
Waldherr et al. [14]	37	34/37 (84%)	1 (3%)	9 (24%)	N/I	23 (62%)	4 (11%)	10/37 (27%)
Waldherr et al. [15]	37	37/37 (100%)	1 (3%)	7 (19%)	N/I	6 (70%)	3 (8%)	8/37 (22%)
Bodei et al. [17]	21	N/I	0	6 (29%)	N/I	11 (52%)	4 (19%)	6/21 (29%)
Valkema et al. [41]	54	41/54 (76%) [c]	0	4 (7%)	7 (13%)	33 (61%)	10 (19%)	4/54 (7%)
[^{90}Y-DOTA]-lanreotide								
Virgolini et al. [18]	39	39/39 (100%)	0	0	8 (20%)	17 (44%)	14 (36%)	0/39 (0%)
[^{90}Y-DOTA^0,Tyr^3]octreatate								
Baum[d] et al. [19,44]	75	67/75 (89%)	0	28[d] (37%)	N/I	39[d] (52%)	8[d] (11%)	28/75[d] (37%)
[^{177}Lu-DOTA^0,Tyr^3]octreotate (^{177}Lu-DOTATATE)								
Kwekkeboom et al. [22]	129	55/129 (43%)	3 (2%)	32 (25%)	24 (19%)	44 (34%)	22 (17%)	35/131 (27%)

N/I, not indicated.

[a] Criteria of tumour response (SWOG / WHO): CR (complete remission), no evidence of disease; PR (partial remission), >50%reduction in tumour size; SD (stable disease), ±25% reduction or increase in tumour size; PD (progressive disease), >25% increase in tumour size.

[b] Modification of SWOG criteria including MR (minor remission), between 25 and 50% reduction in tumour size.

[c] R. Valkema, personal communication, 2004.

[d] Criteria for tumor response are not published in this study

The research group at Basel University reported the first clinical results in 1997 [36]. In this study 10 patients with sst-positive tumours were included. Two (20%) achieved a PR after treatment with [^{90}Y-DOTA^0,Tyr^3]octreotide (^{90}Y-DOTATOC). In the following studies patients were treated with either 6.0 or 7.4 GBq/m^2 ^{90}Y-DOTATOC. The objective response rates (OR) (defined as CR + PR) were 27% (10 out of 37 patients) and 22% (8 out of 37 patients) respectively [14,15]. In another study from the same group, including 116 patients, who were treated with 6.0 to 7.4 GBq/m^2 ^{90}Y-DOTATOC an OR of 27% was found [16]. This study is reported in **chapter 2a**.

The research group from the European Cancer Institute in Milan also published several studies using ^{90}Y-DOTATOC [17,37-40]. In the most recent study [40] Bodei et al. reported the results of 141 patients with various sst-positive tumours. An OR was found in 26 out of 113 patients with progressive disease before therapy (23%) and in 9 out of 28 patients (32%) with stable disease before therapy. However, the results were not subdivided for different tumour types. In a study reported in more detail [17], 40 patients with sst- positive tumours were included. The patients were treated in 2 cycles with a cumulative doses ranging from 5.9

to 11.1 GBq. In the group of patients with GEP tumours the OR rate was 29% (6 out of 21 patients). Eleven out of 21 patients (52%) achieved a stabilisation of their disease and 4 (19%) remained progressive.

The goal of a multicentre phase I study, performed in Rotterdam, Brussels, and Tampa, was to determine the maximum tolerated injected activity in a single or in four cycles [41-43]. Escalating doses of ^{90}Y-DOTATOC up to 9.3 GBq/m^2 as a single injection and up to 14.8 GBq/m^2 in four cycles were administered in 60 patients. Fifty-four patients could be treated with their maximum allowed activity. From these patients 4 (7%) achieved a PR, in 7 patients (13%) a minor response was found, and 33 (61%) had SD. The median time to progression was not reached at 26 months after the last treatment cycle. However, the maximum tolerated injected activity could not be determined since, based on ^{86}Y-DOTATOC dosimetry, the dose to the red marrow would be too high.

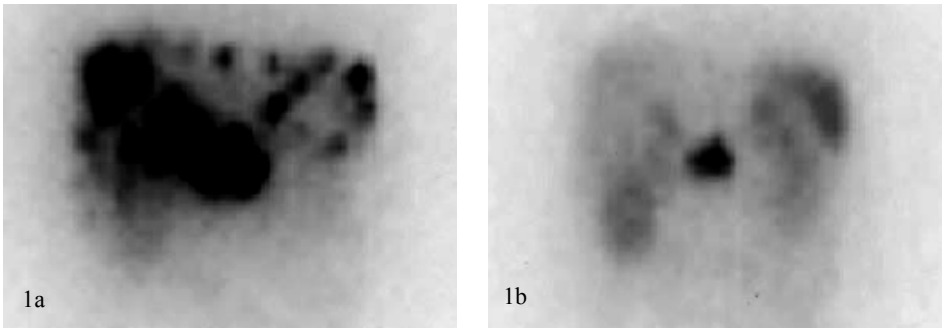
Another ^{90}Y labelled peptide, ^{90}Y-DOTA-lanreotide, was investigated in a European multicentre trial (MAURITIUS). In total 39 patients with GEP tumours were treated with a cumulative dose ranging from 1.9 to 8.6 GBq [18]. Minor remissions were found in 8 out of these 39 patients (20%) and 17 patients had SD (44%).

Recently data have been published of a study using the ^{90}Y labelled [DOTA^0,Tyr^3]octreotate [19,44]. However, the treatment schemes are very inconsistent and the evaluation of benefit is not defined. The results reported are an objective response rate (PR) of 37% (28 out of 75) and a stabilisation of the disease in 39 out of 75 patients (52%). In the same study the intraarterial use of [^{90}Y-DOTA^0,Tyr^3]octreotate in 5 patients is described. However, no detailed results for this application are available and all data have not been confirmed by another research group.

The first long term follow up and survival data for ^{90}Y-DOTATOC were published by Valkema et al. [45]. In this study 58 patients were treated in a dose escalating study with 1.7 to 32.8 GBq of ^{90}Y-DOTATOC. The response rates were comparable to other studies using ^{90}Y labelled somatostatin analogues, but in addition to the encouraging response rates a significant longer overall survival (36.7 months) was shown compared to a group treated with [^{111}In-DTPA^0]octreotide (median survival 12.0 months) [9]. Although the relevant patient characteristics did not show significant differences, the patients treated with [^{111}In-DTPA^0]octreotide had a somewhat lower Karnofsky Performance Status, which might have slightly influenced the results.

The use of different protocols, peptides and the difficulties in the comparison of the patients included makes it virtually impossible to compare the results of these therapy-studies with ^{90}Y labelled peptides. Nevertheless, the results with ORs rates up to 37% and the suggested prolonged overall survival represent an improvement in therapeutic effectiveness compared to the studies with [^{111}In-DTPA^0]octreotide.

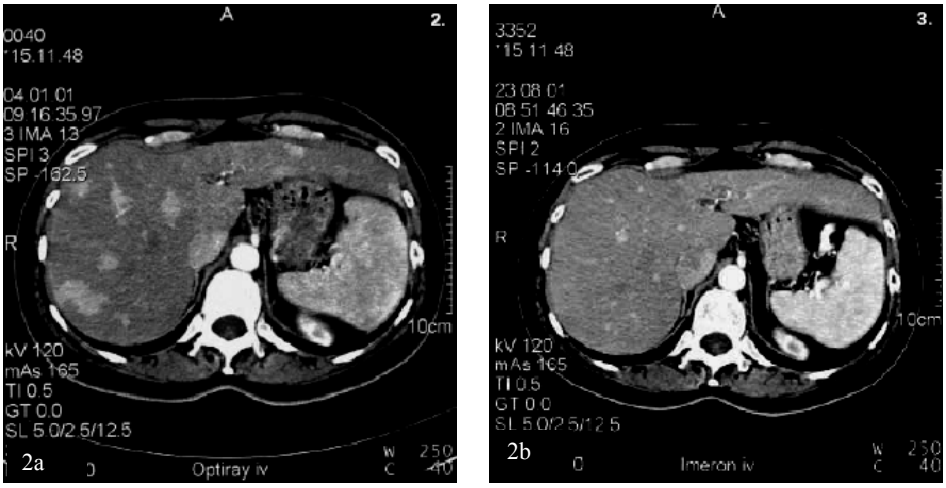
Figure 1



1a *Planar scintigraphic scan of the abdomen 46 hours after the injection of 7.4 GBq ^{90}Y-DOTATOC and 111 MBq ^{111}In-DOTATOC in a patient with a neuroendocrine tumor of the pancreas with liver metastases. The scan was performed after the first treatment.*

1b *Planar scintigraphic scan of the abdomen 46 hours after the injection of 7.4 GBq ^{90}Y-DOTATOC and 111 MBq ^{111}In-DOTATOC in the same patient shown in figure 1a. The scan was performed after the second treatment. Scintigraphically a clear reduction of the primary tumor and the liver metastases can be seen.*

Figure 2



2a Pretherapeutic CT-scan of the patient shown in figure 1. Multiple liver metastases can be seen.

2b CT-scan 3 month after the second treatment of the same patient. Liver metastases are not demonstrated anymore.

Studies using [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-DOTATATE) and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotide (^{177}Lu-DOTATOC)

In 2003 the first results from a study using a ^{177}Lu labelled peptide were published [20]. In this study 35 patients with neuroendocrine GEP tumours were treated with escalating dosages of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-DOTATATE) up to a final cumulative dose of 22.2 – 29.6 GBq. The effects of the therapy could be evaluated in 34 patients. Three months after the last treatment cycle 1 CR, 12 PR, 14 SD and 7 PD (including 3 patients who died during therapy) were found. This equals an objective response rate, i.e. PR or CR, of 38%. A later update of this study in 76 patients essentially confirmed these results [21]. In the follow-up the median time to progression in the patients that had at least SD after the treatment was not reached at 25 months from the beginning of the therapy. In a more recent evaluation of 131 GEP tumour patients these outcomes were confirmed again and a median time to progression of more than 36 months was found [22]. The latest evaluation of these patients with focus on long term outcome revealed an overall survival which appears to be even longer than the results published for ^{90}Y-DOTATOC (D.J. Kwekkeboom, personal communication 2006). However, influences resulting from different patient characteristics can currently not be ruled out.

So far only one study was published using ^{177}Lu-DOTATOC [23]. One of the inclusion criteria was relapse after ^{90}Y-DOTATOC treatment and only one therapy cycle was administrated because of the pretreatment. Yet effectiveness of the treatment could be demonstrated, but because of the different setting the results of the outcome can not be compared. The detailed results are shown in **chapter 2b**.

Side Effects and Toxicity

Generally PRRT can be regarded as a relatively safe treatment and severe side effects are rare, especially when compared with side effects in studies using chemotherapy [46-48]. The side effects in PRRT can be divided into acute side-effects and more delayed effects caused by radiation toxicity.

The acute effects occurring at the time of injection up to a few days after therapy include nausea, vomiting and increased pain at tumour sites (approximately 30% of the patients), symptoms that were reported after treatments with all radionuclides [5,11,14]. These side effects are generally mild, can be controlled by symptomatic treatment. In patients treated with ^{177}Lu-DOTATATE mild hair loss was reported [5], however hair growth had normalised at follow up 3 to 6 months after the treatment.

Beside these minor side effects severe toxicity may occur as a result of the radiation absorbed dose in healthy organs. The organs at risk are mainly the kidneys, the bone marrow and to a lower extend the liver.

Haematological toxicity

Essentially all studies investigating PRRT report haematological toxicity. It appears that the absorbed radiation dose to the bone marrow is mainly caused by the circulation of the radioactivity in the blood [49], which limits the options to reduce the absorbed dose. Severe haematological toxicity (> grade 2 for haemoglobin, white blood cells and platelets) was reported in a maximum of 15% of the patients treated [11,13,14,17,21,41]. In general the decrease in blood counts was transient. Blood transfusions were needed only occasionally and

patients recovered fully. More serious side effects were reported from a study where 3 out of 50 patients developed a myelodysplastic syndrome (MDS) after treatment with total cumulative doses higher than 100 GBq ^{111}In-octreotide [9]. In a dose escalating phase I study with ^{90}Y-DOTATOC to determine the maximum tolerated dose (MTD), one patient developed a MDS two years after PRRT [41]. In a recently updated record of roughly 500 patients treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, 3 patients developed a MDS (D.J. Kwekkeboom, personal communication) and a recent update of roughly 700 patients treated with ^{90}Y-DOTATOC showed that 2 patients developed a MDS (J. Mueller-Brand, personal communication). Two out of these 5 cases (one in each group) were most probable related to prior chemotherapy. Generally, the definition of the cause for these MDS cases is difficult because most of the patents that were included into PRRT trials are pretreated, many of them with chemotherapy and external beam radiation. Currently a maximum absorbed radiation dose to the bone marrow of 2Gy is assumed to be safe [20]. However, most studies lack long term follow up data which makes them ineffective to estimate long term risks.

Haematological toxicity following PRRT is frequent but generally mild and transient. MDS may occur, but the limited data on long term follow up does not allow a reliable, precise estimation of the risk to date.

Renal Toxicity

Conjugated peptides are predominantly cleared by the kidneys. Although the major part of the radiopharmaceutical is excreted into the urine, partial reabsorption in the tubular cells can lead to a considerable radiation dose to the kidneys [25,49,50]. It was shown recently that the localization of the radiopeptide in the kidney is not homogeneous, but predominantly in the cortex where it follows a striped pattern, with most of the radioactivity centred in the inner cortical zone [51]. This pattern of up-take results in different dose distributions for different radionuclides [25]. The reabsorption of radiolablled somatostatin is mediated by the multiligand scavenger receptor megalin [52]. The high capacity of megalin challenges the reduction or blockade of renal reabsorption of the radiolabelled somatostatin analogues. However, it was proven that the co-administration of amino acids, especially arginine and lysine, significantly reduces the renal uptake of radiopeptides [53,54].

Gelatine based plasma expander were recently shown to reduce renal uptake of diagnostic [^{111}In-DTPA^{0}]octreotide efficiently in animals and patients [55,56]. However, the benefit in patients during PRRT remains to be proven. Another promising approach might be the use of amifostine [57,58]. Amifostine is the first drug investigated for PRRT that does not aim at reducing the renal uptake but which acts as a radical scavenger to reduce systemically the toxic effects of the radiation on normal tissue. Because amifostine is acting by a different mechanism, a combination with drugs that reduce the renal uptake appears most promising. Combinations of different drugs to reduce renal uptake are worth being tested as well. Preliminary results of a combination of gelatine based plasma expander and amino acids showed very promising results in rats (unpublished data).

In a phase I study to define the MTD of ^{90}Y-DOTATOC that was performed without amino acids co-administration 2 out of 16 patients developed renal toxicity grade IV [13]. Renal biopsies of patients treated with ^{90}Y-DOTATOC that developed renal toxicity revealed mainly thrombotic microangiopathy and abnormalities in the tubules, histological changes comparable to the changes that occur after external beam radiation [59]. Despite the co-administration of amino acids a number of later studies using ^{90}Y-labelled peptides reported renal toxicity [60-63]. The MTD with amino acid co-infusion for ^{90}Y-DOTATOC was defined

as 7.4 GBq/m^2 body surface in this study. Nevertheless a case of late onset renal toxicity after less than 7.4 GBq/m^2 was reported [60]. It was shown that individual dosimetry can be helpful to avoid kidney failure [62]. An absorbed dose of 23 Gy to the whole kidney is generally accepted to be safe. However, this value is derived from external beam radiation (fractions of 2 Gy) [64] and is therefore not indisputable. In contrast to external beam radiation the physical characteristics of the radiation in PRRT is different, applying radiation in a very low dose rate over a long period of several days.

In contrast to the use of ^{90}Y labelled somatostatin analogues, no renal toxicity was reported after the therapeutic use of very high doses of [^{111}In-DTPA^0]octreotide [9]. For ^{177}Lu-DOTATATE one patient out of a group of 201 patients was reported who developed renal insufficiency [21].

This is an indication that the physical characteristics of the radionuclide have a significant impact on renal toxicity. While the Auger electrons emitted by ^{111}In have a range of approximately 5 μm in tissue, the maximum range of the ^{90}Y electrons can be up to 12 mm. Auger electrons emitted within the tubular cells do not reach the radiosensitive glomeruli [65]. Several studies investigated kidney toxicity after PRRT more detailed [62,65,66]. It was shown that together with the total absorbed dose to the kidney, the dose volume, fractionation rate and clinical parameters like hypertension, diabetes and age play an important role for the development of kidney failure. Especially the fractionation influences the specific biologic efficacy of internally deposited radiation strongly [66].
If renal toxicity occurs it can not be regarded as fixed kidney damage. It appears rather that the loss of function is a continuous process with a defined pace of progression that can be expressed as loss of clearance per year [65].

Liver Toxicity
Beside the fact that most patients who are treated with PRRT suffer from liver metastases, physiological uptake in normal liver tissue also occurs after administration of radiolabelled somatostatin analogues. The sum of this physiological uptake and the dose to the normal liver from the specific uptake in liver metastases can result in a considerable radiation absorbed dose to the liver [49]. However, since the tumour load in the liver shows a high interpatient variability, it is difficult to generalise radiation absorbed doses to the liver.

In a study using [^{111}In-DTPA^0]octreotide three out of 27 patients showed a temporary increase in liver enzymes corresponding to a grade 3 liver toxicity (WHO) [11]. All three patients had a liver tissue replacement of more than 75% of their hepatic parenchyma by metastases and treatment associated necrosis on the computed tomography scans was suggested.

A significant increase in liver enzymes after the administration of ^{90}Y-DOTATOC was reported in two studies [41,67]. Valkema et al. reported one transient grade 3 toxicity in a group of 60 patients treated with ^{90}Y-DOTATOC in a phase I study [41]. In another study, 15 patients with known liver metastases (of whom 12 had extensive liver involvement, defined as 25% or more) from neuroendocrine tumours were treated with three cycles of 120 mCi (4.4 GBq) each [67]. In four of these 15 patients, one or more of the three liver enzymes that were measured (serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) increased. Increase was defined as at least one grade, according to the WHO criteria, from baseline to final follow-up measurement (4-6 weeks post cycle 3). It was concluded that patients with diffuse sst-positive hepatic metastases could be treated with a

cumulative administered activity of 360 mCi (13.2 GBq) of ^{90}Y-DOTATOC with only a small chance of developing mild acute or subacute hepatic injury.

In the group of patients treated with ^{177}Lu-DOTATATE, significantly increased liver function parameters (grade 4 liver toxicity) was evident in two patients after the first cycle of treatment (D.J. Kwekkeboom, personal communication, 2004).

In summary, liver toxicity is very rare and if it occurs it is mostly mild and reversible. However, extensive liver metastases seem to be a risk factor for liver impairment after PRRT. Especially in these patients though, it is difficult to distinguish between real toxicity caused by radiation from effects by the metastases themselves.

Dosimetry

In order to improve the efficacy of PRRT and to limit toxicity, appropriate dosimetry helps to choose an injected activity that delivers an optimal radiation absorbed dose to the tumor while the dose to normal organs does not exceed defined limits. Additionally, dosimetry is mandatory to characterize a new compound properly in patients, especially when it is foreseen for therapy. The basic principles of dosimtery in Nuclear Medicine are explained in **chapter 3a** on the basis of a comparison in patients of the two most frequently used peptides for PRRT (DOTA-TOC and DOTAT-[Tyr^3]-octreotate.

The dose limiting organs in PRRT are usually the kidneys and / or the bone marrow. Especially dosimetry of the bone marrow is very challenging since the bone marrow is not a solid organ. The definition of the volume and mass is associated with many sources of error. For radiolabeled somatostatin analogues, there are several models which are used to calculate the absorbed radiation dose to the bone marrow. Most often the residence time of the radiopharmaceutical in the bone marrow - a value that is mandatory for dosimetry - is calculated from the residence time in the blood. A correction factor is added depending on the vector used for treatment [49]. This method was validated by bone marrow aspirations for antibodies but not for radiopeptides [68,69].

Reliable dose estimation for the bone marrow is mandatory for several reasons. In order to achieve a maximum anti-tumor effect, patients should be treated with the highest justifiable dose of the radiopharmaceutical that does not cause serious toxicity. Many studies with radiolabeled somatostatin analogues showed that the toxicity is generally mild and transient [13,15,20,22]. It should however not be neglected that in a phase 1 study with [^{111}In-DTPA^0]octreotide 3 out of 50 patient developed a myelodysplastic syndrome (MDS) which was probably related to the therapy [9]. Calculations from these data resulted in an estimated radiation absorbed dose for the bone marrow of approximately 3 Gy. In another study with [^{177}Lu-DOTA^0,Tyr^3]octreotate, one MDS was observed in a patient who had had chemotherapy with alkylating agents 2 years before study entry [21]. In the latest update of our own records of roughly 500 patients treated with [^{177}Lu-DOTA^0,Tyr^3]octreotate, 3 patients (including the patient mentioned before) developed a MDS (unpublished data). To avoid MDS, a maximum absorbed dose of 2 Gy to the bone marrow is generally accepted [20]. Nevertheless even if this limit is not exceeded the risk for the patient to develop a MDS can not be excluded completely, but an accurate estimation of the absorbed dose to the bone marrow will help to find an adequate dosage.

For daily practise the method to estimate the absorbed dose to the bone marrow has to be easily applicable and should not cause a lot of discomfort to the patient. This is given with the method to calculate the residence time in the bone marrow from the blood. However, taking a bone marrow sample is probably the most reliable method for bone marrow dosimetry. A detailed comparison of different methods to calculate the absorbed radiation dose to the bone marrow compared with a bone marrow aspiration is made in **chapter 3b**.

Current Clinical Practice

In symptomatic patients at the time of diagnosis metastatic disease is present in 90% and surgical cure is not possible [70]. nevertheless it remains an important cornerstone in the management of these tumours. Beside surgery, radiofrequency ablation (RFA) or chemo-embolisation is a minimal invasive treatment option when the disease is limited to the liver or when the tumour load in the liver is very high. Several small series have shown good responses [71-73]. However, RFA and chemo-embolisation are not systemic approaches and will not treat extrahepatic (occult) disease.

In patients with metastasised neuroendocrine tumours in whom surgery is no longer an option, PRRT appears to be the most effective therapeutic option with limited side-effects. Conventional chemotherapy and external radiotherapy either alone or in a variety of permutations are of minimal efficacy and should be balanced against the decrease in quality of life often caused by such agents [73]. Non-radiolabelled somatostatin analogues, particularly in a subcutaneous depot formulation are effective in symptom alleviation and improvement of quality of life but their effect on tumour burden is very limited [73]. A randomised controlled study of PRRT with other treatment modalities is lacking though. The 'wait-and-see' approach often still remains the mainstay of initial management in patients with unresectable disease. The rationale for this approach is found in the natural course of well-differentiated GEP tumours. Tumours can be indolent for many years and the well-being of patients, even with metastasised tumours, can be unchanged for a long period. However, the reported studies on PRRT clearly indicate that patients with documented progressive disease or a substantial increase in symptoms benefit in a high percentage from this therapy. The recognition of the possible benefit of PRRT for patients with GEP tumours is increasing, but its implementation within the whole therapeutic array is rather poor. The fact that PRRT is a relatively new therapeutic modality may be one of the contributing factors. Another factor is the lack of approved radiopharmaceuticals. Several reasons account for this: beside increased governmental demands, and therapy-related costs, it has to be kept in mind that neuroendocrine tumours are rather rare which limits the interest of the pharmaceutical industry to invest specifically into these tumours.

Indications for Peptide Receptor Radionuclide Therapy

The approach of PRRT with radiolabelled somatostain analogues allows theoretically treating all sst-positive tumours. However, due to the potential morbidity of PRRT patients should be selected carefully. Incurability by surgery is an absolute prerequisite for the inclusion of a patient. In addition, the presence of a sufficient high density of sst has to be proven by means of scintigraphy. Usually this will be an ^{111}In-octreotide scintigraphy with sufficient tumour uptake. Recently PET with radiolabelled somatostatin analogues became available and might be used alternatively However, these PET methods are available only at a few centres, the radiopharmaceuticals are not FDA approved yet and the methods have yet to be formally validated. Inclusion criteria for most studies were tumour uptake equal or higher than liver

uptake on the ^{111}In-octreotide scintigraphy [74]. High uptake on ^{111}In-octreotide scintigraphy has been shown to correlate with tumour regression after PRRT [20].

Because of the potential, renal and haematological toxicity of PRRT patients need to fulfil certain minimal criteria in addition. Blood and kidney function parameters have to be checked before therapy. Details are given in table 3. Bone metastases, present only in a minority of the patients, are not an exclusion criteria. However it seems that bone and cystic lesions respond in a more protracted way than the common solid liver metastases although no formal analysis to this end are available.

Table 3

Criteria for peptide receptor radionuclide therapy in patients with neuroendocrine tumours

Inclusion
Sufficient tumour uptake on ^{111}In-octreotide scintigrams (tumour uptake ≥ liver uptake)
Haematology: • Haemoglobin ≥ 5.0 mmol/l • White blood cell count ≥ 2–3.5×10^9/l • Platelet count ≥ 75–100×10^9/l
Kidney function: • Creatinin (serum) ≤150 μmol/l or creatinine clearance ≥ 40 ml/min
Karnofsky Performance Status ≥ 50
Life expectancy > 3 months
Written informed consent
Exclusion
Chemotherapy within 6 weeks prior to the start of treatment
Pregnancy/lactation
Distinct restricted liver function

Timing of Therapy

The best time point to initiate PRRT in patients with malignant neuroendocrine tumours remains uncertain up to now. The stage of disease at the time of diagnosis is highly variable. It ranges from a small localised primary tumour to advanced or even end-stage disease with limited liver function and ascites. In addition, the variation in tumour-differentiation results in

highly variable rates of progression. In a study in which the relationship between delay of diagnosis, extent of disease and survival in 115 patients with carcinoid was studied, a mean delay in the diagnosis of 66 months was found [75]. It was concluded that the diagnosis of carcinoid is difficult, and therefore a delay of diagnosis by physicians is common. Strikingly, the delay of the diagnosis did not correlate with the extent of the disease. However, the extent of the disease did correlate with survival. Patients with primary tumours and lymph node metastases were less likely to die of carcinoid disease than patients with hepatic metastases, carcinomatosis or extra-abdominal metastases.
Although there are no guidelines yet for the initiation of PRRT there are certain hints that the treatment is more effective when given in an earlier stage. The degree of liver involvement is inversely related to the chance of remission [22]. In a trial with [^{111}In-DTPA^{0}]octreotide it was reported that a beneficial effect of PRRT is less likely in end-stage patients than in patients with less tumour burden and in better general conditions [9,45]. Furthermore, it was clearly shown that patients benefit in quality of life after PRRT [15,76]. This justifies treating all symptomatic patients that fulfil the inclusion criteria and are not responding to treatment with non-radiolabelled somatostatin analogues (anymore).

Another argument for an earlier treatment is the fact that neuroendocrine tumour can dedifferentiate over time. Dedifferentiation is commonly associated with a decrease in sst density. In turn PRRT using radiolabelled somatostatin analogues will be less effective or even impossible. The administration of PRRT in an early stage does not exclude patients from a later repetition of the treatment. It was shown recently that patients can be retreated. A good response after the first treatment cycles was found to be a positive predictor for the effectiveness of the retreatment [23].

A randomised study comparing the long term survival of patients with malignant, unresectable neuroendocrine tumours that undergo PRRT compared to a “wait and see” strategy is lacking. Keeping in mind the latest follow up data of patients treated with ^{177}Lu-DOTATATE (median time to progression > 36 months) [20] makes however such a study disputable from an ethical point of view.

Future Developments

Future research to improve PRRT with radiolabelled somatostatin analogues consists of 5 main directions. Improving the vehicle, i.e. the peptide, is highly interesting. Many new somatostatin analogues with a higher affinity for sst_2 or with a wider affinity for several sst subtypes were already introduced into the preclinic [77].

Simultaneously investigations have been made to improve the delivery of the radiopharmaceutical to the target. This includes the way of application, e.g. intra-arterially, at a slower rate or fractionated, as well as the improvement of the availability of the target by different peptide concentrations or by modulation of the receptor with drugs [78,79].

Furthermore the most suitable radionuclide will have to be defined. In preclinical studies, comparing ^{90}Y and ^{177}Lu it appeared that ^{90}Y was more effective for bigger tumours while with ^{177}Lu less relapses occurred when treating smaller lesions [42,80]. Beside the effects on the tumour, the different physical properties cause differences in microdosimetry which in turn will influence the toxicity profile of a compound [25]. Beside the commonly used ^{90}Y

and ^{177}Lu a number of other radionuclides with different physical characteristics including alfa-emitters are under investigation [81,82].

In external beam radiation the application of radio-sensitizers to improve the anti-tumour effect of the radiation is established [83]. In PRRT, the introduction of combination therapies will open a whole new field of research to improve the treatment. A multi-centre trial with ^{177}Lu-DOTATATE and Capecitabine was initiated recently (E.P. Krenning, personal communication, 2006).

Finally, the improvement of the toxicity-profile of the current radiopeptides is an important issue. Especially with respect to the reduction of the kidney uptake, several studies were recently published and also other strategies to reduce radiation toxicity in general are under investigation [55-58]. An important step towards an improved toxicity profile will be a better understanding of the low dose rate irradiation. Currently, the generally accepted maximum tolerated absorbed doses to normal organs are still derived from external beam radiation [64]. There is emerging evidence in the literature of a low dose hypersensitivity phenomenon where at low doses and at very low dose rates, a significantly increased cell kill is found compared with high dose rates and compared with what would be predicted from the classical linear quadratic model [84,85].

Other Peptides for new Peptide Receptor Radionuclide Therapies

Beside radiolabelled somatostatin analogues a number of newly developed peptides were introduced lately, targeting different receptors [86]. Bombesin is just one example of these new peptides. Bombesin is a well characterized 14 amino acid neuropeptide binding (among others) to the gastrin-releasing-peptide (GRP) receptor. Several bombesin derivatives with high affinity for the GRP receptor have been developed, analogous to somatostatin, labelled with an array of radionuclides [87]. Overexpression of GRP receptors was found on many neoplasms, especially on prostate and breast cancer. Remarkably the GRP receptor is not expressed on healthy prostate tissue. This might give the chance to distinguish *in vivo* between benign and malignant prostate nodules by means of receptor imaging and in a second step to apply PRRT to GRP receptor-positive tumours.

Gastrin analogues have a high affinity for the Cholecystokinin (CCK) B-receptor which is overexpressed e.g. on medullary thyroid carcinomas. To date eight patients with advanced metastatic disease were injected in a dose-escalation study with potentially therapeutic activities of a ^{90}Y-labelled minigastrin derivative at 4-6-weekly intervals with 1.1-1.8 GBq/m^2 per injection for a maximum of four injections. Hematologic and renal were identified as the dose-limiting toxicities. Two patients experienced partial remissions, 4 stabilization of their previously rapidly progressing disease [88].

Clinical Summary

PRRT has been proven to be an effective and safe treatment alternative for sst-positive, unresectable neuroendocrine tumours. Currently the maximum tolerated dose is defined by the dose to the critical organs, kidney and bone marrow. It is likely that the dose can be increased in future by the introduction of new protective agents, different treatment schemes and radionuclides.

The present data in the literature do not allow defining the most suitable peptide and radionuclide for the treatment of neuroendocrine tumours. Especially concerning the radionuclide there is emerging evidence that a combination of nuclides with different physical characteristics might be more effective.

The principle of targeted treatment has a number of obvious advantages over unspecific systemic treatments. Therefore PRRT holds great promise for the future.

Preclinical Studies

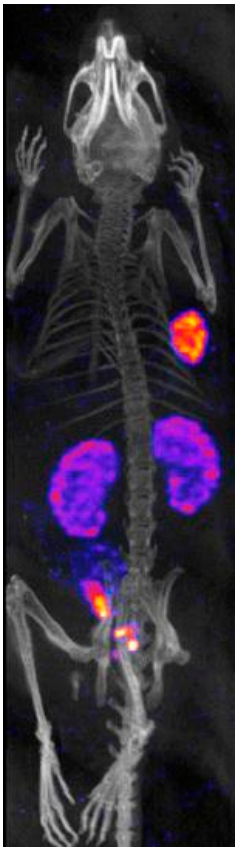
As mentioned previously, new approaches could potentially improve PRRT further but a number of questions still need to be answered.

Although results from preclinical studies can not always be translated easily to the clinics, preclinical evaluation and test of new compounds or new strategies will remain a corner stone to improve PRRT. Before new drugs or treatment strategies are evaluated in patients, they are usually tested first in preclinical studies. Animal experiments are of high relevance in this stage of the development. To date mainly biodistribution studies were performed. This is associated with a large number of animals that are needed to investigate different processes and function at different time points.

Over the last years dedicated small animal imaging devices gained increasing influence on preclinical research. Particularly single photon emission computed tomography (SPECT) and positron emission tomography (PET) as tools for molecular imaging were proven to be valuable e.g. to follow physiological processes in an animal over time.

Small animal SPECT/CT
For our research we had a dedicated small animal SPECT/CT at our disposal [89]. The camera was a four headed multiplexing multi-pinhole camera. Each head is fitted with an application-specific tungsten collimator with nine pinholes. The rat apertures, e.g., comprise a total of 36 2-mm-diameter pinholes imaging a cylindrical field of view that is 60 mm in diameter by 24 mm in length. These rat apertures provide a reconstructed resolution below 1.6 mm at 140 keV, with an average sensitivity of 1,100 cps/MBq across the field of view (FOV). The axial FOV is extended using a step-and-shoot helical scan of the animal, with the user defining a range from 24 to 270 mm according to the region to be imaged. Accordingly the mouse high resolution apertures comprise of 1-mm-diameter pinholes resulting in a resolution in the sub millimeter range.

Figure 3



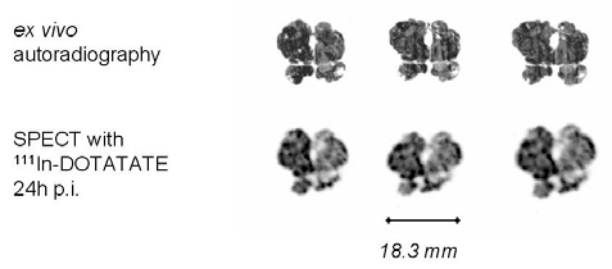
Whole body SPECT/CT of a PC3-tumor-bearing mouse 24 hours after the injection of 50 MBq ^{111}In-Bombesin.

After the acquisition, the data are reconstructed iteratively with the HiSPECT (Bioscan Inc., Washington D.C., USA) software, a dedicated ordered subsets-expectation maximization (OSEM) software package for multiplexing multi-pinhole reconstruction. The camera is calibrated with a phantom, approximately of the size of the animals, filled with a known activity of ^{99m}Tc such that voxel values in the reconstruction provide a proper estimate of the activity level without further calculation. Regions of interest (ROI) can be drawn manually around the object of interest; the 3D activity distribution within the ROI is then summed to determine the radioactivity. No correction for scatter or attenuation is performed because the quantification factor also corrects for attenuation within the animal. Quantification is performed with the INTERVIEW XP (Mediso Ltd., Budapest, Hungary) software. The absolute *in vivo* quantification is probably the most important tool to evaluate new tracers. The detailed evaluation of the accuracy of this function is described in **chapter 4a**.

In various studies the capabilities of the system were evaluated (unpublished data). Depending on the injected activity and the imaging time a very high resolution for static images can be achieved. An example of a "standard" SPECT/CT of a tumor bearing mouse is shown in Figure 3.

Using long scanning times and high resolution apertures allow even to resolve inhomogeneities of tracer uptake within the tumor caused by inhomogeneous distribution of receptors. These images can be correlated very well with *ex vivo* autoradiograms. An example is shown in figure 4.

Figure 4

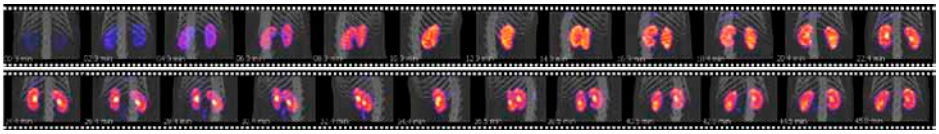


Correlation between ex vivo autoradiograms and in vivo SPECT slices of a somatostatin receptor positive rat pancreatic tumor (CA20948) after the injection ^{111}In-Octreoscan.

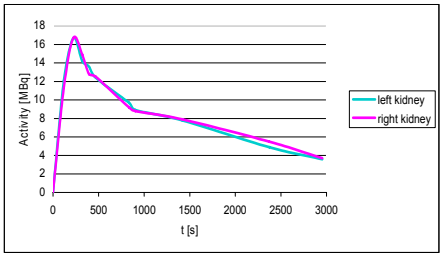
Beside this high resolution that is provided by pinhole SPECT, the multi-pinhole technique results in sensitivity comparable to parallel-hole collimators. On the one hand this allows injecting relatively small amounts of radioactivity and on the other hand it allows keeping the acquisition times or the time per projection respectively low. In turn this gives a very good temporal resolution. We investigated dynamically rat kidney function with ^{99m}Tc-MAG3 and tumor uptake of ^{111}In-Octreoscan in a somatostatin receptor positive tumor in a rat. The time per scan could be kept as low as 60 seconds per scan. Up to 45 scans were acquired per study. The resulting time activity curves as well as an example of subsequent MIP images of a ^{99m}Tc-MAG3 study are shown in figure 3.

Figure 5

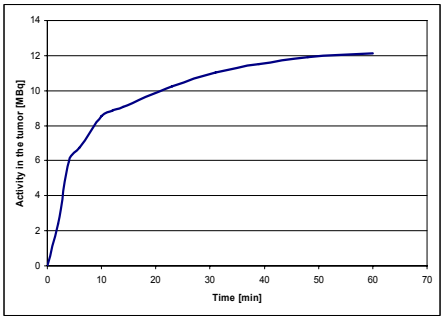
5a



5b



5c



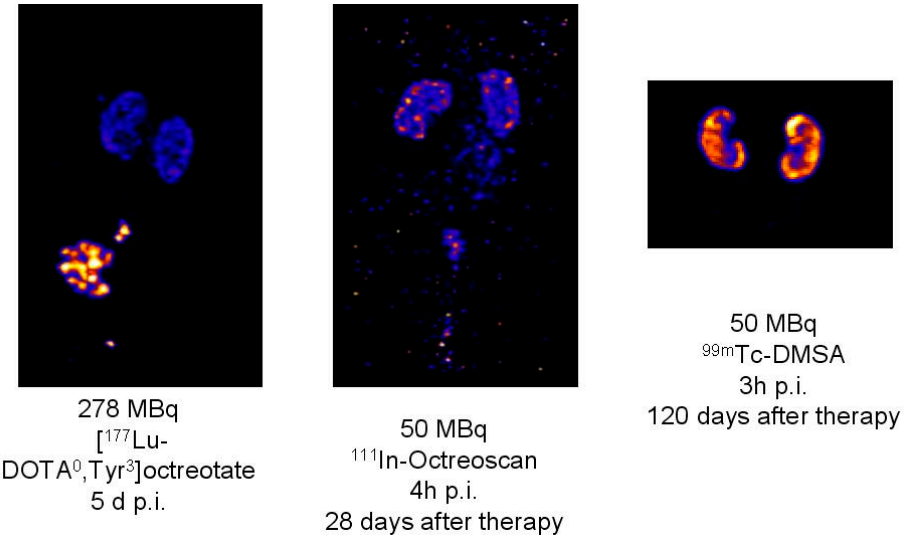
5a. Dynamic in vivo SPECT images (MIP data) of rat kidneys after the injection of 40 MBq ^{99m}Tc-MAG3.

5b. In vivo renograms of rat kidneys generated with the NanoSPECT/CT after the injection of 40 MBq ^{99m}Tc-MAG3.

5c. Dynamic measurement of ^{111}In-Octreoscan tumor uptake in a somatostatin receptor positive rat pancreatic tumor (CA20948). The data are generated with the NanoSPECT/CT in vivo.

The variety of tracers available allows to look at different physiological functions in one animal or to follow a process over time. In a therapy study with tumor bearing rats we were able to monitor the animals over time. Five days after injection the biodistribution of the therapeutic [^{177}Lu-DOTA^{0},Tyr^{3}]-octreotide was documented. Approximately 4 weeks after the treatment the response to the therapy was assessed by an ^{111}In-Octreoscan scintigraphy and finally before sacrificing the animals the kidney function was monitored with ^{99m}Tc-DMSA. An example of these three scans in one animal is shown in figure 6.

Figure 6



SPECT images of the identical rat with different tracers at different time points as indicated.

In a first study, taking advantage of the NanoSPECT/CT we investigated mechanism of kidney damage in rats during high dose PRRT [90]. Besides small animal imaging a number of other molecular imaging methods along with histology using different staining were applied. The results of this study are shown in **chapter 4b**.

In conclusion we believe that small animal imaging will strongly influence further preclinical research. It allows to follow processes over time or to monitor different functions in a single animal at the same time which will reduces the number of animals required and in turn it will save costs as well. Additionally, the situation as it is given in a patient is reflected better when different functions can be monitored in one animal simultaneously and as true *in vivo* investigation.

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CHAPTER 2

A. TARGETED RADIONUCLIDE THERAPY WITH ^{90}Y-DOTATOC IN PATIENTS WITH NEUROENDOCRINE TUMORS

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Targeted Radionuclide Therapy with ^{90}Y-DOTATOC in Patients with Neuroendocrine Tumors

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Abstract. *Background: The aim of this study was to assess the efficacy and safety of targeted radionuclide therapy with [^{90}Y-DOTA^{0},Tyr^{3}]-octreotide (^{90}Y-DOTATOC) in patients with metastatic neuroendocrine tumors. Patients and Methods: One hundred and sixteen patients with metastatic neuroendocrine tumors were included. All patients were pre-therapeutically staged with morphological imaging procedures and with somatostatin receptor scintigraphy. The scintigraphy was positive in all cases. The patients were treated with 162-200 mCi/m^2 body surface. In 57 patients, the quality of life was assessed with the National Cancer Institute grading criteria (NCI-CTC). Restaging was performed 8 - 12 weeks after the last treatment cycle. Blood samples were drawn every 2 weeks after the treatment to evaluate toxicity. Results: Complete remissions were found in 4%, partial remissions in 23%, stabilization in 62% and progressive disease in 11%. A significant reduction of symptoms was found in 83%. No serious adverse event occurred and the toxicity was acceptable. Conclusion: ^{90}Y-DOTATOC is a safe and effective treatment for patients with metastatic neuroendocrine tumors.*

Neuroendocrine tumors (NET) are a large, inhomogeneous group of malignancies considered to be derived from the diffuse neuroendocrine system (1). Most of these tumors show an overexpression of somatostatin receptors, mainly of subtype 2 (2). Malignant NET have a poor prognosis (1) and surgery is curative in less than 5% of all patients (3, 4), although it remains an important cornerstone in the management of these tumors. Until the 1980s, chemotherapy was the standard treatment for NETs, although single-agent chemotherapy should be considered ineffective (5-7). Combination chemotherapy showed somewhat better results, but considerable toxicity was found. The objective response rates ranged from 0 to 33% (5, 7-9). Later, therapies with α-Interferon and somatostatin analogs significantly improved clinical management. However, in these treatments as well, the objective response rate was rather disappointing. In trials with somatostatin analogs, the response rates ranged from 0 to 9% (5, 10, 11), while in trials with Interferon it ranged from 7 to 20% (12, 13).

In recent years, radionuclide therapy with radiolabelled somatostatin analogs have become an important tool in the management of NETs (14-20). Convincing results were found for both objective tumor response and quality of life (20-22). Nevertheless controversial debates about the most suitable radionuclides and somatostatin analogs are ongoing (23).

In 1999, a phase I study with [^{90}Y-DOTA^{0},Tyr^{3}]-octreotide (^{90}Y-DOTATOC) identified renal toxicity as dose-limiting. The maximum tolerated dose was defined as 162 mCi/m^2 body surface ^{90}Y-DOTATOC without co-infusion of an amino acid solution for kidney protection (24). A following phase II study increased the injected activity to 200 mCi/m^2 body surface ^{90}Y-DOTATOC with amino acid co-infusion, achieving safe administration with tolerable toxicity (21).

Several studies with different radionuclides and different somatostatin analogs have been subsequently published. However, most of these studies deal with rather small groups of patients (14-20). This prospective study reports on the tumor response and palliative effect in a large group of patients with metastatic NET treated with ^{90}Y-DOTATOC.

Patients and Methods

This study was approved by the local ethical committee and the Swiss authorities. All patients gave written informed consent.

Patients. One hundred and sixteen patients with metastatic NET were included. One hundred and nine of these were progressive at the time of inclusion, while 7 suffered from symptomatic, stable disease. Pretherapeutically, all patients underwent staging with

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CT, ^{111}In-pentetreotide-scintigraphy (OctreoScan®; Mallinckrodt, Inc., St. Louis, MO, USA), control of blood counts and blood chemistry. All patients had uptake on the ^{111}In-octreotide-scintigraphy preceding the therapy that was at least as high as the uptake in normal liver tissue. No patient was under treatment with long-acting somatostatin analogs (Octreotide LAR, Novartis Pharma; Lanreotide, Ipsen Ltd.) for at least within the 6 weeks prior to treatment, or with short-acting somatostatin analogs (Octreotide s/c, Novartis Pharma) within the last 3 days before treatment. None of the patients had prior treatment with other radiolabelled somatostatin analogs. The prerequsites for treatment were: Hb ≥100 g/l, WBC ≥2x10^9/l, platelets ≥100x10^9/l, serum creatinine ≤150 µmol/l and a Karnofsky Performance Score ≥50.

Methods. The somatostatin analog DOTATOC was synthesized in-house according to a previously published procedure and radiolabelled with the pure β-emitter ^{90}Y, as published previously (24-26). Yttrium-90 was purchased from Perkin Elmer Inc. (Wellesley, MA, USA). The labelling yield and the radiopharmaceutical purity were checked using C_{18}-RP-HPLC (labelling yield > 99.5%). For imaging procedures, 111 MBq of ^{111}In-DOTATOC, prepared similarly, were added for each injection. Indium-111 was purchased from Tyco Healthcare (Petten, The Netherlands).

An infusion of amino acids (Hartmann-HEPA 8% amino acid solution; B. Braun Medical AG, Sempach, Switzerland) was started each time 30 minutes before the administration of the radiopharmaceutical and lasted up to 3 hours afterwards. The total treatment dose was 162 mCi/m^2 body surface for 41 patients and 200 mCi/m^2 for 75 patients. Eighty patients were treated in 4 sessions every 6 weeks and 36 were treated twice with an interval of 8 weeks.

Routine hematology, liver and kidney parameters were checked before every treatment cycle and every 2 weeks after the last treatment up to 8 weeks.

Fifty-seven patients filled out a detailed questionnaire using the National Cancer Institute grading criteria (NCI-CTC) before and after each cycle of treatment. For the other patients, the questionnaire was not available at the time.

Four weeks before the first and 8-12 weeks after the last treatment cycle, tumor growth and tumor response were monitored by either CT, MRI or sonography. Tumor response was defined according to the WHO standard criteria and was evaluated again 3 months later. The side-effects of ^{90}Y-DOTATOC treatment were investigated and scored according to the NCI-CTC.

Results

The study population comprised 116 patients (mean age 53.3 years) with metastatic NET. Forty-five patients had a neuroendocrine pancreatic tumor, 28 had a NET of unknown primary, 24 had an intestinal NET, 10 had a bronchial NET and 9 patients had other NET. One hundred and nine patients were progressive at the time of inclusion and 7 patients had a symptomatic stable disease (carcinoid syndrome and/or tumor-related pain). All the patients had been pretreated with other modalities (surgery and/or chemotherapy and/or octretide and/or external beam radiation). Eighty patients were treated with 4 treatment cycles and 36 patients with 2 treatment cycles. Of the 80 patients treated with 4 cycles, 41 received a total injected activity of 162 mCi/m^2 body surface ^{90}Y-DOTATOC. The other 39 were injected with a total activity of 200 mCi/m^2 body surface. Thirty-six patients received 2 treatment cycles with a total injected activity of 200 mCi/m^2 body surface ^{90}Y-DOTATOC.

Nausea and vomiting within the first 24 hours after the injection of the radiopharmaceutical occurred in 23% of the patients. No serious adverse events occurred during or after the treatment.

A WHO toxicity lymphopenia grade 3 or pancytopenia grade 3 occurred in 9 (8%) and 3 (3%) of the patients, respectively. One renal toxicity grade 4 with need for hemodialysis occurred. No other toxicity > grade 2 was found.

The effects of the therapy on the tumor size were evaluated in all patients. Eight to 12 weeks after the final administration, complete remissions were found in 5 patients (4%), a partial remission in 26 patients (22%) and stabilization of the disease (including minor responses) in 72 patients (62%). Thirteen (11%) of the patients remained progressive. An example of a patient who achieved a partial remission is shown in Figures 1 and 2.

Fifty-seven consecutive patients completed a detailed clinical benefit questionnaire about their disease history and their clinical features. They scored all symptoms according to the NCI-CTC before and after each treatment cycle. The symptoms of malignant carcinoid syndrome decreased significantly. A significant reduction of clinical features was found in 83% of patients with diarrhea, in 46% of patients with flushes, in 63% of patients with wheezing and in 75% of patients with pellagra. Those patients suffering from tumor-related pain achieved a significant reduction. All patients (5/57) with morphine-dependent tumor-associated pain were able to change to NSAID or to stop all pain relief medication completely.

Discussion

The results of this clinical study, on the antitumor effects and quality of life benefits in patients with NET after targeted radionuclide treatment with ^{90}Y-DOTATOC, are most encouraging.

Several studies have shown the effectiveness of treatment with radiolabelled somatostatin analogs, however the number of patients in these studies was relatively low. Here, the use and effectiveness is shown on a collective of 116 patients. A problem faced in these studies of patients suffering from metastatic NET is the inhomogeneity of the patient cohort. There is no simple classification system available for grading the aggressiveness and the extension

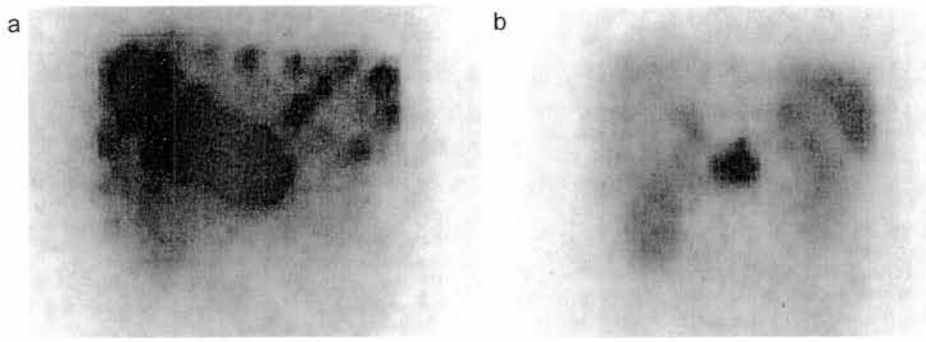

Figure 1. *a. Planar scintigraphic scan of the abdomen 46 hours after the injection of 200 mCi ^{90}Y-DOTATOC and 3 mCi ^{111}In-DOTATOC in a patient with NET of the pancreas with liver metastases. The scan was performed after the first treatment. b. Planar scintigraphic scan of the abdomen 46 hours after the injection of 200 mCi ^{90}Y-DOTATOC and 3 mCi ^{111}In-DOTATOC in the same patient shown in Figure 1a. The scan was performed after the second treatment. Scintigraphically, a clear reduction of the primary tumor and the liver metastases can be seen.*

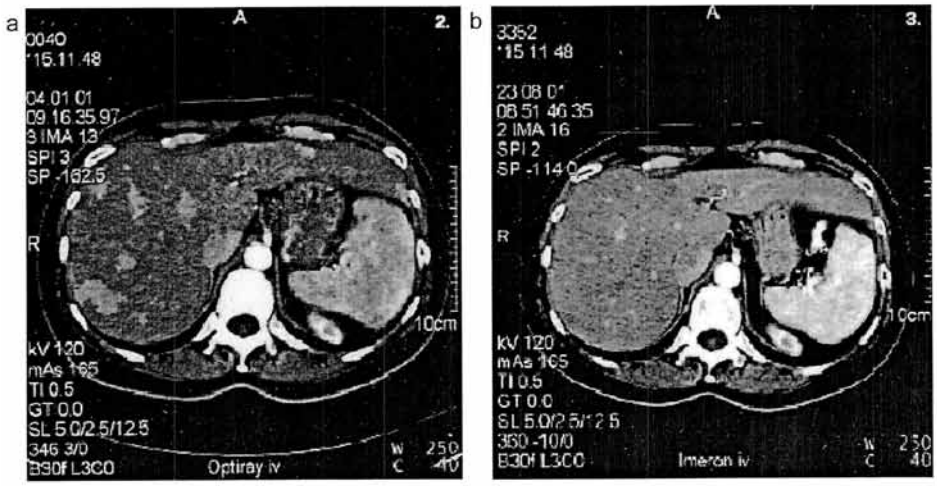

Figure 2. *a. Pre-therapeutic CT-scan of the patient shown in Figure 1. Multiple liver metastases can be seen. b. CT-scan 3 months after the second treatment of the same patient. Liver metastases are no longer demonstrated.*

of these tumors; therefore, comparison with other treatment modalities and other studies is very difficult.

We found a objective response rate of 26% in our patients. The treatment was generally well-tolerated and the toxicity was acceptable. Using an amino acid co-infusion, the renal toxicity was tolerable. The side-effects of the treatment with ^{90}Y-DOTATOC were few and mostly transient.

The objective response rates for other modalities reported in the literature are clearly lower. For non-radiolabelled somatostatin, the objective response rate

ranged from 0 to 9% (5, 10, 11) and Interferon showed objective response rates from 7 to 20% (12, 13). Single-agent chemotherapy should be considered inactive. Studies with combinations of chemotherapies reported objective response rates from 0 to 33%. In these trials, in which high response rates were achieved, considerable side-effects were shown (5).

In this study, the results of previous studies with ^{90}Y-DOTATOC and with other radiolabelled somatostatin analogs were confirmed.

In our opinion, targeted radionuclide therapy with radiolabelled somatostatin analogs is the most suitable treatment currently available for metastatic, progressive or symptomatic NET. The treatment achieves high objective response rates and improvements in the quality of life.

It has been shown previously, in animal experiments, that ^{90}Y-labelled somatostatin analogs are more effective for larger tumors and that ^{177}Lu-labelled somatostatin analogs are more effective for smaller tumors (27-29). A combination of the two radionuclides could potentially improve the results. In external beam radiation, the use of radio-sensitizers is very common (30, 31). In the near future, the use of such substances in combination with targeted radionuclide therapy should be evaluated.

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CHAPTER 2

B. TREATMENT WITH ^{177}LU-DOTATOC OF PATIENTS WITH RELAPSE OF NEUROENDOCRINE TUMORS AFTER TREATMENT WITH ^{90}Y-DOTATOC

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Treatment with ^{177}Lu-DOTATOC of Patients with Relapse of Neuroendocrine Tumors After Treatment with ^{90}Y-DOTATOC

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Therapy with [^{90}Y-DOTA^{0}, Tyr^{3}]-octreotide (DOTATOC, where DOTA = tetraazacyclododecane tetraacetic acid and TOC = D-Phe-c(Cys-Tyr-D-Trp-Lys-Thr-Cys)-Thr(ol)) is established for the treatment of metastatic neuroendocrine tumors. Nevertheless, many patients experience disease relapse, and further treatment may cause renal failure. Trials with ^{177}Lu-labeled somatostatin analogs showed less nephrotoxicity. We initiated a prospective study with ^{177}Lu-DOTATOC in patients with relapsed neuroendocrine tumors after ^{90}Y-DOTATOC treatment. **Methods:** Twenty-seven patients, pretreated with ^{90}Y-DOTATOC, were included. The mean time between the last treatment with ^{90}Y-DOTATOC and ^{177}Lu-DOTATOC was 15.4 ± 7.8 mo (SD). All patients were injected with 7,400 MBq of ^{177}Lu-DOTATOC. Restaging was performed after 8–12 wk. Hematotoxicity or renal toxicity of World Health Organization grade 1 or 2 was not an exclusion criterion. **Results:** Creatinine levels increased significantly, from 66 ± 14 μmol/L to 100 ± 44 μmol/L ($P < 0.0001$), after ^{90}Y-DOTATOC therapy. The mean hemoglobin level dropped from 131 ± 14 to 117 ± 13 g/L ($P < 0.0001$) after ^{90}Y-DOTATOC therapy. ^{177}Lu-DOTATOC therapy was well tolerated. No serious adverse events occurred. The mean absorbed doses were 413 ± 159 mGy for the whole body, 3.1 ± 1.5 Gy for the kidneys, and 61 ± 5 mGy for the red marrow. After restaging, we found a partial remission in 2 patients, a minor response in 5 patients, stable disease in 12 patients, and progressive disease in 8 patients. Mean hemoglobin and creatinine levels did not change significantly. **Conclusion:** ^{177}Lu-DOTATOC therapy in patients with relapse after ^{90}Y-DOTATOC treatment is feasible, safe, and efficacious. No serious adverse events occurred.

Key Words: ^{177}Lu-DOTATOC; ^{90}Y-DOTATOC; radionuclide therapy; somatostatin; neuroendocrine tumors

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Treatment options for metastatic neuroendocrine tumors are limited. Trials with long-acting somatostatin analogs (octreotide or lanreotide), interferon-α, or chemotherapy, mostly 5-fluorouracil based, have shown rather low response rates with regard to cytoreduction (*1–3*). However, somatostatin analogs inhibit flushing, diarrhea, and other symptoms of the carcinoid syndrome (*4,5*). A retrospective case series in 1996 suggested that survival has increased since the introduction of somatostatin analogs (*6*). In the last few years, treatment strategies with radiolabeled somatostatin analogs have shown more convincing results (*7–13*). The 3 most investigated radiopharmaceuticals in clinical trials are [^{111}In-diethylenetriaminepentaacetic acid (DTPA)^{0}]-octreotide, [^{90}Y-DOTA^{0}, Tyr^{3}]-octreotide (DOTATOC, where DOTA = tetraazacyclododecane tetraacetic acid and TOC = D-Phe-c(Cys-Tyr-D-Trp-Lys-Thr-Cys)-Thr(ol)), and [^{177}Lu-DOTA^{0}, Tyr^{3}, Thr^{8}]-octreotide (DOTATATE) (*7–13*).

Initial studies with high activities of [^{111}In-DTPA^{0}]-octreotide were encouraging. Although partial remissions were not found, favorable effects on symptoms were reported. Many patients in poor clinical condition were included (*12,13*). For the other 2 radiopeptides, a high overall response rate and distinct improvement in quality of life could be demonstrated (*10,14*). Although the results with these radiolabeled somatostatin analogs seem promising, relapses occur after a certain time in many patients (*15*), and further treatment with ^{90}Y-DOTATOC can cause renal failure (*16*). According to data in the literature, the median time to progression after treatment with ^{90}Y-DOTATOC is 30 mo (*17,18*). For ^{177}Lu-DOTATATE, the median time to progression had not been reached at 25 mo after the start of therapy (*19*).

In comparison to ^{90}Y, which is a high-energy, pure β-emitter (E_{max}, 2.25 MeV), ^{177}Lu is a low-energy β-emitter (maximum electron energy [E_{max}], 0.497 MeV) with a small γ-component that is suitable for scintigraphic imaging (133 keV [6.5%]; 208 keV [11%]) without using a radionuclide surrogate. Small peptides such as DOTATOC are reabsorbed by the proximal tubules of the kidneys (*20*). The

damage that can occur after treatment with ^{90}Y-DOTATOC is in the glomeruli. It is conceivable that the length of the β-particles influences kidney toxicity. This hypothesis is supported by animal experiments (*21*).

Renal toxicity has been identified as the dose-limiting factor of ^{90}Y-DOTATOC therapy (*9*). In a study with [^{177}Lu-DOTA^0, Tyr^3, Thr^8]-octreotide, no nephrotoxicity was found (*11*). Although no long-term outcome data concerning nephrotoxicity after treatment with ^{90}Y-DOTATOC or ^{177}Lu-DOTATATE are available, we assumed that ^{177}Lu might be less nephrotoxic than ^{90}Y.

In vitro, a higher affinity to the somatostatin receptor subtype 2 was demonstrated for Y$^{(III)}$-DOTATATE than for Y$^{(III)}$-DOTATOC (*22*). However, because in humans a better tumor-to-kidney-ratio was found for ^{111}In-DOTATOC than for ^{111}In-DOTATATE (*23*), we decided to use DOTATOC as a DOTA-peptide conjugate labeled to ^{177}Lu in patients with relapse.

We initiated a prospective feasibility study with ^{177}Lu-DOTATOC in patients with relapse of neuroendocrine tumors after successful treatment with ^{90}Y-DOTATOC. Because of the assumption that ^{177}Lu-DOTATOC is less nephrotoxic than ^{90}Y-DOTATOC, we did not consider World Health Organization (WHO) grade 1 or 2 renal toxicity, based on creatinine levels, to be an exclusion criterion, nor were patients with WHO grade 1 or 2 hematotoxicity excluded. Human data for ^{177}Lu-DOTATATE show promising results and a tolerable toxicity for injected activities of around 22.2–29.6 GBq (600–800 mCi) in patients who are not pretreated with peptide receptor–mediated radionuclide therapy (*11*). But for ^{177}Lu-DOTATOC, we could find no human data in the literature. Because our patients were pretreated with peptide receptor–mediated radionuclide therapy, and because no dosimetric data were available, we started with a relatively low injected activity. We treated all patients with a fixed activity of 7,400 MBq (200 mCi).

MATERIALS AND METHODS

The study was approved by the local ethical committee and the Swiss authorities. All patients gave written informed consent.

Patients

Twenty-seven patients (17 men and 10 women) were included. The mean age (± SD) was 58 ± 9 y. All patients had a histologically confirmed metastatic neuroendocrine tumor, which was progressive at the time of treatment. The progression was demonstrated by CT or ultrasound in all patients. All patients were pretreated with ^{90}Y-DOTATOC and benefited from this treatment. Benefit was defined as complete remission, partial remission, minor response, or stable disease according to the WHO standard criteria. For the partial remissions in our collective, the mean time to progression was 15.4 ± 6.9 mo. Many patients were pretreated with surgery, chemotherapy, octreotide, or interferon as well. Details are listed in Table 1.

Pretherapeutically, all patients underwent staging with CT, ^{111}In-pentetreotide scintigraphy (OctreoScan; Mallinckrodt, Inc.), complete blood counts, and blood chemistry. The findings of ^{111}In-octreotide scintigraphy were strongly positive in all patients. None of the patients had been treated with the long-acting somatostatin analogs octreotide (Sandostatin LAR; Novartis) or lanreotide (Somatuline; Ipsen) during at least the last 6 wk before receiving ^{177}Lu-DOTATOC or with short-acting octreotide (Sandostatin s.c.; Novartis) during the last 3 d before receiving ^{177}Lu-DOTATOC.

Radiotracer

DOTATOC was synthesized as previously described (*24*). For radiolabeling DOTATOC, we used lyophilized kits containing DOTATOC, gentisic acid, inositol, and sodium ascorbate (pH 5.0).

We added 7,400 MBq of $^{177}LuCl_3$ (IDB Holland BV) to the lyophilized DOTATOC kits and heated them for 30 min at 95°C. After they had been cooled to room temperature, a quality control check was performed using an analytic high-performance liquid chromatograph (model 1050; Hewlett Packard) with a radiometric detector (model LB 506 C1; Berthold). Additionally, the labeling yield was determined by separation of bound and free $^{177}Lu^{3+}$ using Sep-Pak C18 cartridges (Waters). After ^{177}Lu-DOTATOC had been loaded onto the cartridge, the free ^{177}Lu was eluted with sodium acetate buffer (0.4 mol/L, pH 5.0), and bound ^{177}Lu-DOTATOC was then eluted with methanol. Each fraction was measured on a γ-counter.

Treatment

The patients were hospitalized for 3 d in accordance with the legal requirements for radioactivity control. A single, fixed-activity treatment protocol was used. The injected activity was 7,400 MBq of ^{177}Lu-DOTATOC. An infusion of 2,000 mL of an amino acid solution (Ringer's lactated Hartmann solution, Proteinsteril [B. Braun Medical AG] HEPA 8%, Mg 5-Sulfat [B. Braun Medical AG]) to inhibit tubular reabsorption of the radiopeptide was started 30 min before administration of the radiopharmaceutical and was continued until up to 3 h after administration of the radiopharmaceutical (*20,25,26*).

Imaging and Dosimetry

Imaging was performed with a dual-head Prism 2000 XP camera (Picker) using parallel-hole, medium-energy, general-purpose collimators. The windows were centered over both ^{177}Lu photon peaks (113 and 208 keV) with a window width of 20%. In 4 patients, whole-body scans for dosimetry were obtained immediately and at 4, 24, and 28 h after injection. The acquisition time for the whole-body scans was 15 min. In all other patients, whole-body scans and spot images were obtained after 24 and 28 h for control of biodistribution.

To determine blood clearance, we drew blood samples from 4 patients at 5, 10, 30, and 60 min and at 12, 4, 24, and 28 h after injection. Radioactivity in blood was measured with a γ-counter (Cobra II; Canberra-Packard).

For dosimetric calculations, regions of interest were drawn manually on the whole-body scans from anterior and posterior projections. Those parts of the kidneys showing tumor infiltration or superimposition were excluded from the evaluation of organ uptake. The Odyssey XP program (Philips Electronics N.V.) was used. Background regions were placed close to the regions of interest for background correction. The geometric mean value between anterior and posterior was taken and corrected for attenuation and physical decay. Whole-body activity acquired immediately after injection was defined as 100% of the injected activity.

TABLE 1
Patient Characteristics

Patient no.	Sex	Age (y)	Diagnosis	Date of diagnosis	Classification 3 mo after ^{90}Y-DOTATOC	Pretreatments (except ^{90}Y-DOTATOC)	Number of ^{90}Y-DOTATOC treatments	Total dose of ^{90}Y-DOTATOC/m^2	Months since last treatment with ^{90}Y-DOTATOC
1	F	71	Neuroendocrine tumor of small bowel	Nov 01	Stable disease	—	2	200	16
2	M	55	Neuroendocrine tumor of pancreas	Jun 97	Partial remission	S, Oct, Ch	3	300	11
3	M	63	Neuroendocrine tumor of pancreas	Dec 00	Stable disease	Ch, INF	2	200	18
4	F	74	Neuroendocrine tumor of appendix	Jan 00	Stable disease	S	3	200	8
5	F	55	Neuroendocrine tumor of pancreas	Feb 01	Partial remission	S	2	200	22
6	F	60	Neuroendocrine tumor with unknown primary	Dec 99	Partial remission	Oct	2	200	16
7	F	59	Neuroendocrine tumor of stomach	Nov 00	Minor response	S	3	300	10
8	F	51	Neuroendocrine tumor of rectum	Aug 95	Partial remission	S	2	200	22
9	M	56	Neuroendocrine tumor of small bowel	June 02	Partial remission	Oct	2	200	9
10	M	60	Neuroendocrine tumor of small bowel	Mar 99	Partial remission	S	2	200	11
11	F	65	Neuroendocrine tumor of pancreas	Dec 98	Partial remission	Oct, INF	2	200	10
12	M	38	Neuroendocrine tumor with unknown primary	Mar 98	Stable disease	Oct, Ch, ^{186}Re-HEDP	3	300	18
13	M	58	Neuroendocrine tumor with unknown primary	May 01	Minor response	S	2	200	17
14	M	54	Neuroendocrine tumor of small bowel	Oct 00	Minor response	S	2	200	24
15	M	63	Neuroendocrine tumor of pancreas (insulinoma)	Sep 01	Partial remission	Oct	2	200	13
16	M	76	Neuroendocrine tumor of pancreas	Nov 98	Partial remission	S	2	200	32
17	M	49	Neuroendocrine tumor of pancreas	Dec 01	Partial remission	Oct	2	200	9
18	M	43	Neuroendocrine tumor of pancreas	Feb 01	Partial remission	S, Ch	3	300	14
19	F	66	Neuroendocrine tumor of pancreas	Aug 96	Partial remission	S	2	200	22
20	M	49	Neuroendocrine tumor of rectum	Jun 00	Partial remission	S, Oct, INF	2	200	11
21	F	46	Neuroendocrine tumor of unknown origin, most likely insulinoma	Jan 97	Stable disease	Oct	3	300	32
22	M	51	Neuroendocrine tumor of small bowel	Apr 00	Stable disease	S	3	300	5
23	M	65	Neuroendocrine tumor of small bowel	Feb 02	Stable disease	S	2	200	4
24	F	60	Neuroendocrine tumor of small bowel	Oct 99	Stable disease	S	3	300	22
25	M	65	Neuroendocrine tumor of bronchus	Mar 99	Partial remission	Ch	3	300	6
26	M	50	Neuroendocrine tumor of pancreas	May 98	Stable disease	S, Oct, INF, Ch	2	200	27
27	M	51	Neuroendocrine tumor of pancreas (gastrinoma)	Feb 00	Stable disease	S	2	200	8

S = surgery; Oct = octreotide (long- or short-acting) or lanreotide; Ch = chemotherapy; INF = interferon.

Data were expressed as percentage injected activity as a function of time. The resulting time–activity data were fitted to a monoexponential curve for the whole-body clearance and to a biexponential curve for the kidneys to calculate residence time. Published radiation dose factors were used to calculate the absorbed doses (*27*).

The activity in blood was fitted to a biexponential curve to determine the residence time in blood. The dose to the red marrow was calculated from the residence time in blood, assuming no specific uptake, a uniform distribution of activity, and clearance from red marrow equal to that from blood. A correction factor of 1 was used as described by Cremonesi et al. (*28*).

Evaluation of Results and Assessment of Clinical Benefit

Pretherapeutically, patients underwent disease staging. Eight to 12 wk after peptide receptor–mediated radionuclide therapy, tumor growth and tumor response were monitored by CT or ultrasound. Tumor response was defined according to the WHO standard criteria. In addition, complete blood cell and platelet counts were obtained every 2 wk for at least 8 wk or until resolution of nadir. Side effects were scored according to the WHO criteria.

Statistics

Paired *t* testing was used to determine statistical significance. Differences at the 95% confidence level ($P < 0.05$) were considered significant.

RESULTS

The study included 27 patients with metastasized tumors, 11 of whom had neuroendocrine pancreatic tumors and 16, neuroendocrine tumors of other sites (7 of the small bowel, 4 of unknown primary, 2 of the rectum, 1 of the stomach, 1 of the bronchus, and 1 of the appendix). Detailed patient characteristics are listed in Table 1.

Evaluation of Long-Term Outcome After ^{90}Y-DOTATOC Therapy

All patients had progressive disease before ^{90}Y-DOTATOC therapy and before ^{177}Lu-DOTATOC therapy. One criterion for inclusion into this study was benefit from ^{90}Y-DOTATOC therapy. Of the 27 patients studied, we found a partial remission in 14, a minor response in 3, and stable disease in 10 at 3 mo after the last treatment with ^{90}Y-DOTATOC.

The mean time between the last treatment with ^{90}Y-DOTATOC and the treatment with ^{177}Lu-DOTATOC was 15.4 ± 7.8 mo (range, 4–32 mo).

Before therapy with ^{90}Y-DOTATOC, the mean hemoglobin level was 131 ± 14 g/L, the mean thrombocyte level was 306 ± 123 × 10^9/L, and the mean creatinine level was 66 ± 14 μmol/L. Before treatment with ^{177}Lu-DOTATOC, the level of hemoglobin was significantly lower: 117 ± 13 g/L ($P < 0.0001$). The thrombocyte counts (263 ± 83 × 10^9/L) were lower as well but did not show significant changes. Creatinine levels increased to 100 ± 44 μmol/L. The difference was significant ($P < 0.0001$), although a high SD was seen. Details are listed in Table 2.

Labeling of ^{177}Lu-DOTATOC

The quality control testing of ^{177}Lu-DOTATOC was done using 2 independent systems; the labeling efficiency was determined by analytic high-performance liquid chromatography and ranged from 99% to 100%. When the labeling yield was less than 99.5%, DTPA (1 mmol/L, pH 7.4) was added.

Dosimetry

Dosimetric calculations were performed on 4 patients and resulted in a mean whole-body absorbed dose of 413 ± 159 mGy. The mean absorbed dose to the kidney was 3.1 ± 1.5 Gy, and that to the red marrow was 61 ± 5 mGy.

Treatment with ^{177}Lu-DOTATOC

The treatment was well tolerated. No severe adverse events occurred. Nausea and vomiting within the first 24 h after treatment occurred in 8 patients (30%). All cases of nausea and vomiting could be treated successfully with domperidone and ondansetron. Some increase of pain at the site of the tumor was experienced by 5 patients (19%) within the first 48 h after treatment. All cases could be controlled with analgesics. No other nonhematologic toxicity was found.

As expected. ^{177}Lu-DOTATOC showed a high specific uptake in somatostatin receptor–positive tumors. The γ-component of ^{177}Lu allowed acquisition of scintigraphic images of a high level of quality (Fig. 1A).

At the time of restaging, we found no change in creatinine levels. With these findings, late nephrotoxicity cannot be excluded definitely. But if nephrotoxicity arises, an increase in creatinine levels has usually been found 3 mo after treatment (*16*). Before treatment, 9 patients had grade 1 anemia and 1 had grade 2. Eight to 12 wk after treatment, 8 patients had grade 1 anemia, 1 had grade 2, and 1 had grade 3. The mean level of thrombocytes decreased significantly, from 263 ± 82 to 197 ± 70 × 10^9/L ($P < 0.01$). Details are listed in Table 2.

Eight to 12 wk after treatment, 8 patients did not show a benefit from peptide receptor–mediated radionuclide therapy and continued to have progressive disease. Nineteen patients (70%) showed a benefit: 12 with stabilization of the disease, 5 with a minor response, and 2 with partial remission. Scans of patient 9, with a minor response, are shown in Figure 1, and corresponding anatomic images are shown in Figure 2. According to the referring physicians, the general condition of the patients improved for 15 (56%), remained the same for 11 (41%), and decreased for only 1 (4%).

The subgroup of patients who achieved partial remission after ^{90}Y-DOTATOC ($n = 14$) included 2 with partial remission, 5 with a minor response, and 7 with stable disease after ^{177}Lu-DOTATOC treatment. In no patient of this subgroup did the disease remain progressive.

The overall time of follow-up was 4–17 mo (mean, 11.0 ± 4.0 mo). The time of remission (stable disease, minor response, or partial remission) ranged from 4 to 13

TABLE 2
Blood Values and Clinical Results

Patient no.	Hemoglobin (g/L)			Thrombocytes ($\times 10^9$/L)			Creatinine (μmol/L)			Clinical result after treatment with ^{177}Lu-DOTATOC
	Before ^{90}Y-DOTATOC treatment	Before ^{177}Lu-DOTATOC treatment	After ^{177}Lu-DOTATOC treatment	Before ^{90}Y-DOTATOC treatment	Before ^{177}Lu-DOTATOC treatment	After ^{177}Lu-DOTATOC treatment	Before ^{90}Y-DOTATOC treatment	Before ^{177}Lu-DOTATOC treatment	After ^{177}Lu-DOTATOC treatment	
1	104	87	72	406	372	206	83	133	118	Stable disease
2	116	102	105	176	188	143	77	117	124	Stable disease
3	133	115	115	302	251	122	68	94	62	Progressive disease
4	116	115	108	285	188	200	60	111	75	Progressive disease
5	127	119	116	129	173	95	52	65	70	Partial remission
6	107	107	109	311	152	133	54	59	62	Stable disease
7	115	100	102	268	239	108	33	60	63	Progressive disease
8	133	116	115	218	303	190	45	58	43	Minor response
9	145	137	116	226	182	132	74	96	97	Minor response
10	135	134	115	218	214	329	61	58	68	Minor response
11	111	110	112	679	299	127	57	62	61	Minor response
12	136	123	128	227	462	252	61	84	82	Stable disease
13	126	127	112	275	234	208	71	88	84	Progressive disease
14	160	140	135	243	244	219	71	96	114	Progressive disease
15	110	105	80	348	187	109	65	197	167	Partial remission
16	141	109	104	241	282	241	70	137	112	Stable disease
17	146	131	130	279	245	239	88	90	102	Minor response
18	149	102	114	455	366	215	69	120	104	Stable disease
19	124	129	129	521	334	243	49	51	64	Stable disease
20	140	128	126	404	158	138	77	128	111	Stable disease
21	134	100	101	237	329	283	57	248	269	Stable disease
22	144	114	123	339	409	342	81	88	120	Progressive disease
23	146	123	118	231	162	157	65	73	71	Stable disease
24	137	103	118	218	225	186	78	101	97	Progressive disease
25	134	137	136	301	300	267	91	145	147	Stable disease
26	138	125	127	181	222	138	77	67	70	Progressive disease
27	134	114	105	544	378	303	49	79	49	Stable disease
Mean ± SD	131 ± 14	117 ± 13	114 ± 14	306 ± 123	263 ± 82	197 ± 70	66 ± 14	100 ± 44	97 ± 45	

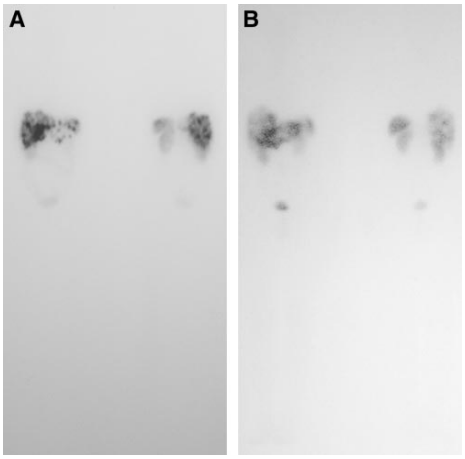

FIGURE 1. Anterior whole-body scans of patient 9. (A) Scan obtained 24 h after injection of 7,400 MBq of ^{177}Lu-DOTATOC shows several abdominal metastases (liver, spleen, and lymph nodes). (B) Scan obtained 6 h after injection of 185 MBq ^{111}In-Octreoscan 6 mo after treatment with 7,400 MBq of ^{177}Lu-DOTATOC shows a decrease in tumor load. Especially, a reduction of liver metastases can be seen.

mo (mean, 8.3 ± 3.4 mo). Presently, 8 patients are still without disease progression; therefore, the overall time to progression will increase further.

DISCUSSION

The labeling of ^{177}Lu-DOTATOC was straightforward, and its application was safe. No serious adverse events occurred.

The group of 27 patients was selected from patients treated earlier with ^{90}Y-DOTATOC; all showed stable disease, a minor response, or partial remission after treatment but experienced relapse rather early and a short time to progression (15 ± 7.8 mo) The time to progression after treatment with ^{90}Y-DOTATOC in these patients was shorter than has been reported in the literature (*18*).

The absorbed doses to normal organs, especially to the kidneys, were low. In previous clinical trials, a cumulative absorbed dose to the kidneys of 23 or 27 Gy was taken as the maximum tolerated dose (*11,26,29*). But these values are controversial (*30*) because they are derived from external-beam radiation (*31*) with a potentially different mechanism. The low absorbed doses are compatible with the fact that no increase of creatinine levels was found.

When the clinical results after ^{177}Lu-DOTATOC are correlated with the clinical results after ^{90}Y-DOTATOC, a good response after ^{90}Y-DOTATOC (partial remission in our patients) is obviously a positive prognostic factor for further radionuclide treatment. Some tumors seem to be especially suited for peptide receptor–mediated radionuclide therapy. Two reasons are possible: There could be a high density of somatostatin receptors leading to a high radiation-absorbed dose, or there could be some tumors that are more radiosensitive than others.

The general condition of the patients was not scaled before treatment with ^{177}Lu-DOTATOC but was worse than before the first treatment with ^{90}Y-DOTATOC because all patients had a longer history of illness and experienced progression after remission or stabilization after ^{90}Y-DOTATOC therapy. The total amount of injected activity (fixed activity, 7,400 MBq of ^{177}Lu-DOTATOC) was rather low because we included patients with an increased serum creatinine level or with a diminished hemoglobin level.

The toxicity in patients with increased creatinine or diminished hemoglobin levels was not different from that in patients with normal values. We found no severe toxicity and, especially, no increase of creatinine levels. Therefore, we conclude that the treatment with ^{177}Lu-DOTATOC in cases of relapse after treatment with ^{90}Y-DOTATOC is feasible and safe. Clinical improvement could be observed, and most patients benefited from the treatment.

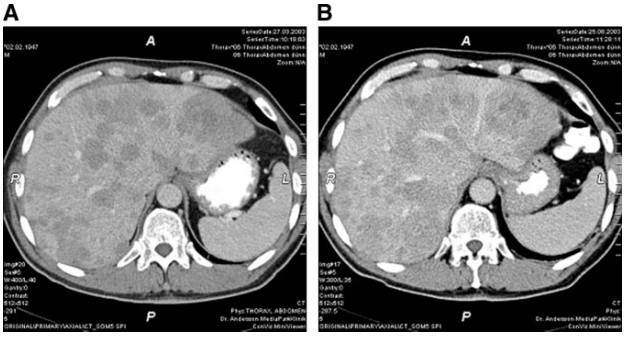

FIGURE 2. CT scans of patient 9. (A) Nine months after treatment with ^{90}Y-DOTATOC and 4 wk before treatment with ^{177}Lu-DOTATOC, CT scan shows multiple liver metastases. (B) Corresponding CT scan 4 mo after treatment with ^{177}Lu-DOTATOC shows minor response.

With regard to the radiobiologic mechanisms of ^{177}Lu and ^{90}Y, the combination of the 2 radionuclides could improve the biologic efficiency. The high-energy β-emitter ^{90}Y deposits high doses to tumors and also to areas with low target protein expression and to heterogeneous tumor tissue. Because of the strong crossfire effect, parts of the tumor that either are poorly differentiated and therefore have a low density of somatostatin receptors or are poorly vascularized can be reached. ^{177}Lu, on the other hand, seems to have more favorable physical characteristics for the treatment of small tumors (*32–34*).

Another mechanism that is not well defined is the so-called low-dose hypersensitivity-inducible radioresistance hypothesis as described by Joiner et al. (*35*). The administration of only a low absorbed dose at a low dose rate might be more effective in inducing tumor cell death than are higher absorbed doses.

CONCLUSION

Treatment with ^{177}Lu-DOTATOC of patients who were pretreated with ^{90}Y-DOTATOC is feasible and appears to be safe even when patients present with grade 1 or 2 hematotoxicity or nephrotoxicity. Clinical response at a low injected activity is promising. A good response after treatment with ^{90}Y-DOTATOC is a positive predictor for successful treatment with ^{177}Lu-DOTATOC.

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CHAPTER 3

A. A COMPARISON OF ^{111}IN-DOTATOC AND ^{111}IN-DOTATATE: BIODISTRIBUTION AND DOSIMETRY IN THE SAME PATIENTS WITH METASTATIC NEUROENDOCRINE TUMOURS

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Peter Bernhardt, Jan Mueller-Brand, Helmut R. Maecke
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A comparison of ^{111}In-DOTATOC and ^{111}In-DOTATATE: biodistribution and dosimetry in the same patients with metastatic neuroendocrine tumours

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Abstract. [Yttrium-90-DOTA-Tyr^3]-octreotide (DOTATOC) and [^{177}Lu-DOTA-Tyr^3-Thr^8]-octreotide (DOTATATE) are used for peptide receptor-mediated radionuclide therapy (PRMRT) in neuroendocrine tumours. No human data comparing these two compounds are available so far. We used ^{111}In as a surrogate for ^{90}Y and ^{177}Lu and examined whether one of the ^{111}In-labelled peptides had a more favourable biodistribution in patients with neuroendocrine tumours. Special emphasis was given to kidney uptake and tumour-to-kidney ratio since kidney toxicity is usually the dose-limiting factor. Five patients with metastatic neuroendocrine tumours were injected with 222 MBq ^{111}In-DOTATOC and ^{111}In-DOTATATE within 2 weeks. Up to 48 h after injection, whole-body scans were performed and blood and urine samples were collected. The mean absorbed dose was calculated for tumours, kidney, liver, spleen and bone marrow. In all cases ^{111}In-DOTATATE showed a higher uptake (%IA) in kidney and liver. The amount of ^{111}In-DOTATOC excreted into the urine was significantly higher than for ^{111}In-DOTATATE. The mean absorbed dose to the red marrow was nearly identical. ^{111}In-DOTATOC showed a higher tumour-to-kidney absorbed dose ratio in seven of nine evaluated tumours. The variability of the tumour-to-kidney ratio was high and the significance level in favour of ^{111}In-DOTATOC was P=0.065. In five patients the pharmacokinetics of ^{111}In-DOTATOC and ^{111}In-DOTATATE was found to be comparable. The two peptides appear to be nearly equivalent for PRMRT in neuroendocrine tumours, with minor advantages for ^{111}In/^{90}Y-DOTATOC; on this basis, we shall continue to use ^{90}Y-DOTATOC for PRMRT in patients with metastatic neuroendocrine tumours.

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Introduction

Somatostatin receptors have been identified in high density on neuroendocrine tumours as well as on tumours of the central nervous system, the breast, the lung and the lymphatic tissue [1]. To demonstrate the presence of somatostatin receptors in vivo, scintigraphy with radiolabelled somatostatin analogues such as [^{111}In-DTPA-D-Phe^1]-octreotide (Octreoscan) has become the gold standard [2]. Peptide receptor-mediated radionuclide therapy (PRMRT) has been used for several years in the treatment of progressive, metastasised, somatostatin receptor-positive tumours. Both somatostatin analogues, [^{90}Y-DOTA-Tyr^3]-octreotide (DOTATOC) and [^{177}Lu-DOTA-Tyr^3-Thr^8]-octreotide (DOTATATE), have been used for this purpose and have shown encouraging results [3–6]. Recently, Kwekkeboom et al. showed that ^{177}Lu-DOTATATE had an up to fourfold higher tumour uptake than [^{111}In-DTPA]-octreotide in six patients [7]. Moreover, Reubi et al. have shown that Y$^{(III)}$-DOTATATE has an approximately sevenfold higher binding affinity to the somatostatin receptor subtype 2 (hsst2) compared with Y$^{(III)}$-DOTATOC [8], which suggests that ^{111}In/^{90}Y-DOTATATE would show a higher tumour uptake in patients. However, no patient data comparing these two compounds are available so far.

Therefore, the aim of this study was to compare the pharmacokinetics of ^{111}In-DOTATOC and ^{111}In-DOTATATE in the same patients. Special emphasis was placed on the mean absorbed doses for tumour, kidney

and bone marrow since the dose-limiting organs for PRMRT with radiolabelled somatostatin analogues are usually the kidneys and the bone marrow [3, 4, 9]. In addition, ^{111}In-DOTATOC/^{111}In-DOTATATE was taken as a surrogate for ^{90}Y-DOTATOC/^{90}Y-DOTATATE and the calculated mean absorbed doses of ^{90}Y-DOTATOC were compared with the doses of this radiopeptide known from the literature [10–13].

Materials and methods

Patients. Five male patients (age 49–73, mean 62 years) (Table 1) with known metastatic neuroendocrine tumours were injected with 222 MBq ^{111}In-DOTATOC and 222 MBq ^{111}In-DOTATATE, with an interval of 2 weeks between the administrations. In three patients, ^{111}In-DOTATATE was injected first, while in two, ^{111}In-DOTATOC was injected first. In two patients the primary tumour was in the pancreas. In three patients the origin of the disease was unknown. None of the patients had received medication with somatostatin analogues (Octreotide s/c or LAR, Novartis Pharma; Lanreotide, Ipsen Ltd.) within the 8 weeks before the examinations. All patients had a histologically confirmed neuroendocrine tumour and had been treated with ^{90}Y-DOTATOC before. There were at least 14 months between the last therapy and the beginning of the study (14–25 months, mean 20.25 months). Metastatic disease had been confirmed in all cases by recent morphological imaging with magnetic resonance imaging (MRI), computed tomography (CT) or ultrasonography. Based on these examinations, the tumour volumes were calculated. The study was approved by the Swiss authorities and by the local ethical committee (Ethikkommission beider Basel). All patients gave informed consent.

Radiopharmaceuticals. Both somatostatin analogues, DOTATOC and DOTATATE, were synthesised in house according to a previously published procedure [8, 14] and radiolabelled with ^{111}In as published previously [15]. ^{111}In was purchased from Tyco Healthcare (Petten, The Netherlands). The labelling yield and the radiopharmaceutical purity were checked using C_{18} reversed-phase high-performance liquid chromatography.

Imaging. All images were acquired with a dual-head gamma camera Picker Prism 2000 XP (Philips, Eindhoven, The Netherlands). The windows were centred over both ^{111}In photon peaks (245 and 172 keV) with a width of 20%. Parallel-hole, medium-energy general-purpose collimators were used. For both compounds, the same protocol was followed: dynamic imaging up to 20 min post injection with a field of view over the kidneys and liver from the posterior projection (80 images, 15 s per image). Whole-body scans were obtained 1, 2, 4, 24 and 48 h after injection. The acquisition time for all whole-body scans was 15 min.

Measurement of radioactivity in blood and urine. Blood samples were drawn 10, 20, 30 and 60 min and 2, 4, 24 and 48 h after injection. Urine was collected in four intervals: 0–2, 2–4, 4–24 and 24–48 h after injection. Radioactivity in blood and urine was measured with a gamma counter (Cobra II Autogamma, Packard, A Canberra Company).

Pharmacokinetics and dosimetry. Regions of interest (ROIs) were drawn manually on the whole-body scans from the anterior and posterior projections for the whole body, the kidneys, the spleen, the liver, the bladder and tumour lesions. The Odyssey XP program was used. Background regions were placed close to the ROIs for background correction. Parts of the organs showing tumour infiltration or superimposition were excluded from the evaluation of organ uptake. The geometric mean value, between anterior and posterior, was taken and corrected for attenuation and physical decay. Whole-body activity acquired 1 h after injection was defined as 100% of the injected activity (IA). The patients did not empty the bladder during this period. All data for the whole body, organs and tumour lesions were expressed in %IA. A compartment model as described previously was used to calculate the residence time from the time-activity data resulting from the scans [10]. The activity in blood was fitted by three exponential curves. The residence times were determined using these data and the respective half-lives of ^{111}In and ^{90}Y. Assuming no specific uptake in the red marrow, a uniform distribution of the activity, and that the red marrow clearance was the same as in blood, the dose to the red marrow was calculated with a correction factor of 1 from the residence time in blood as published previously [10].

Table 1. Patient details

Patient	Age (yrs)	Histology and primary tumour
1	69	Neuroendocrine tumour of the pancreas
2	65	Neuroendocrine tumour of unknown origin
3	73	Neuroendocrine tumour of unknown origin
4	53	Neuroendocrine tumour of unknown origin
5	49	Neuroendocrine tumour of the pancreas

Statistics. Paired *t* test was used to determine statistical significance. Differences at the 95% confidence level (P<0.05) were considered significant.

Results

Patients showed no clinical adverse reactions and no side-effects after the intravenous injection of ^{111}In-DOTATOC or ^{111}In-DOTATATE.

Pharmacokinetic studies

Figure 1 displays the mean plasma radioactivity (and standard deviation) expressed as %IA. The clearance of both peptides was fast. The time-activity in blood could be fitted by three exponential curves. In all patients and for both radiopeptides, the activity in blood decreased to less than 10% within the first 4 h. ^{111}In-DOTATOC showed a somewhat slower clearance initially. After 24 and 48 h, a slightly higher amount of ^{111}In-DOTATATE was found in the blood of all patients. The mean residence time (τ) was 1.178 h (SD±0.19 h) for ^{111}In-DOTATOC and 1.156 h (SD±0.32 h) for ^{111}In-DOTATATE. Therefore, the mean absorbed dose to the red marrow was not significantly different between the two compounds (Table 2). Only a small interpatient variability was found (Table 3).

Table 2. Mean absorbed doses (mGy/MBq) for ^{90}Y-DOTATOC (TOC) and ^{90}Y-DOTATATE (TATE) derived from biodistribution data in five patients using the ^{111}In-labelled peptides

Patient	Kidneys		Liver		Spleen		Red Marrow	
	TOC	TATE	TOC	TATE	TOC	TATE	TOC	TATE
1	2.95	3.53	0.88	1.31	15.5	13.4	0.15	0.15
2	3.15	3.62	0.82	2.39	1.71	3.3	0.17	0.16
3	3.60	3.62	1.33	2.16	4.8	7.74	0.17	0.20
4	2.59	3.58	1.15	1.34	4.71	7.98	0.19	0.16
5	1.91	5.17	0.41	1.18	6.12	18.5	0.15	0.13
Mean ± SD	2.84±0.64	3.90±0.71	0.92±0.35	1.68±0.56	6.57±5.25	10.18±5.87	0.17±0.02	0.16±0.03
P value	0.135		0.031		0.205		0.591	

Table 3. Comparison of mean absorbed doses (±SD) (mGy/MBq) for ^{90}Y-DOTATOC derived from ^{111}In-DOTATOC and ^{86}Y-DOTATOC

	Forrer et al. this study	Cremonesi et al. [10]	Förster et al. [11]	Krenning et al. [12]
Derived from	^{111}In-DOTATOC	^{111}In-DOTATOC	^{86}Y-DOTATOC	^{86}Y-DOTATOC
Kidney	2.84 (±0.64)	3.31 (±2.22)	2.73 (±1.41)	2.1 (±0.78)
Liver	0.92 (±0.35)	0.72 (±0.57)	0.66 (±0.15)	–
Spleen	6.57 (±5.25)	7.62 (±6.30)	2.32 (±1.97)	1.83 (±1.45)
Red marrow	0.17 (±0.02)	0.03 (±0.01)	0.049 (±0.002)	0.11 (±0.06)

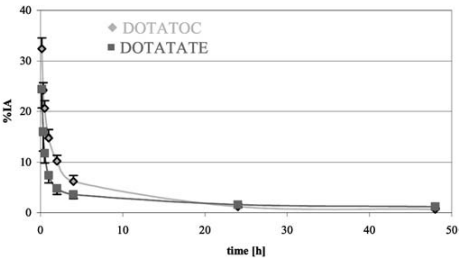

Fig. 1. Blood clearance expressed as percentage of the injected activity (%IA) in the blood (mean ± SD)

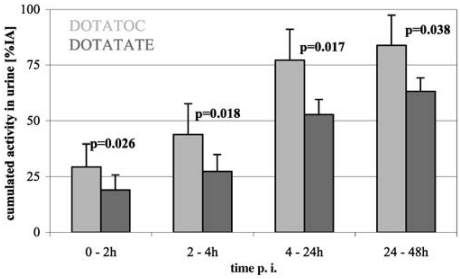

Fig. 2. Cumulative activity excreted into the urine expressed as %IA (mean ± SD)

Cumulative activity excreted into the urine was higher for ^{111}In-DOTATOC in all samples and all patients (Fig. 2). For all periods (0–2, 2–4, 4–24 and 24–48 h) the difference was significant (P<0.05).

Biodistribution and dosimetry

The distribution pattern of ^{111}In-DOTATATE was initially comparable to the pattern using ^{111}In-DOTATOC. In four of the five patients a distinct specific uptake in tumour sites was seen after approximately 2 min. Also, there was fast visualisation of the liver, kidneys and spleen. The fifth patient showed no tumour uptake with either compound due to an impressive decrease in tumour load after ^{90}Y-DOTATOC therapy. In this patient, only two small liver metastases <1 cm were found on a recent CT scan.

We found higher mean absorbed doses to the kidneys and liver for ^{111}In-DOTATATE. The calculated difference for ^{90}Y, when taking ^{111}In as a surrogate, was significant in the liver (P<0.05) but not in the kidneys (P=0.135). The dose to the spleen showed a high interpatient variability. Although in three patients the mean absorbed dose to the spleen for ^{111}In-DOTATATE was higher (Table 2), the difference did not reach significance (P=0.205). The calculated absorbed doses for ^{90}Y-DOTATOC (taking ^{111}In-DOTATOC as the surrogate) for the various organs are comparable with the doses known from the literature [10–12]. Our values, along with literature data, are shown in Table 3.

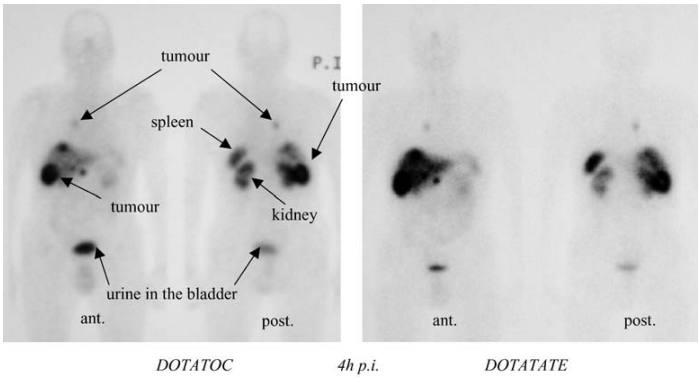

Fig. 3. Whole-body scans of patient no. 2, 4 h after injection of 222 MBq ^{111}In-DOTATOC (*left*) and ^{111}In-DOTATATE (*right*). Better visualisation of the tumours in the liver can be seen in ^{111}In-DOTATOC

Table 4. Mean absorbed tumour doses (mGy/MBq) for ^{90}Y-DOTATOC and ^{90}Y-DOTATATE derived from biodistribution data in nine tumours using the ^{111}In-labelled peptides and the respective tumour-to-kidney ratio

Patient	Lesion (localisation)	Tumour doses (mGy/MBq)		Tumour-to-kidney ratio	
		DOTATOC	DOTATATE	DOTATOC	DOTATATE
1	1 (ln)	12.23	12.12	4.15	3.43
	2 (li)	33.34	24.26	11.30	6.87
2	3 (li)	2.88	4.60	0.91	1.27
	4 (li)	6.31	8.19	2.00	2.26
4	5 (b)	2.80	1.73	1.08	0.48
	6 (li)	41.66	55.54	16.08	15.51
	7 (li)	26.72	15.93	10.32	4.45
	8 (li)	15.77	12.13	6.09	3.39
5	9 (b)	2.37	6.15	1.24	1.18
Mean ± SD		16.01±14.64	15.63±16.40	5.91±5.48	4.32±4.36
P value		0.879		0.065	

(ln), Abdominal lymph node; (li), liver; (b), bone

Overall, nine metastases could be evaluated in scintigraphic images and correlated with a lesion on CT, MRI or sonography. In five of the nine lesions, a somewhat higher mean absorbed dose (mGy/MBq) was found for ^{111}In-DOTATOC; however, high variability between the lesions was observed (Table 4).

Since most often the dose-limiting organ in therapy with radiopeptides is the kidneys, the absorbed dose ratio of lesion to kidneys will determine the therapeutic window. Due to the high variability in mean absorbed doses in the tumours, we found a high variability in the dose ratios as well. However, in seven out of nine lesions, ^{111}In-DOTATOC showed a higher ratio and the difference in mean values almost reached significance (P=0.065) (Table 4).

In two patients, whole-body scans 4, 24 and 48 h after injection showed better visualisation of liver metastases with ^{111}In-DOTATOC. In the other three patients, the scans were visually identical. The better demarcation was due to the lower uptake in the normal liver. Findings in one of the patients with better demarcation of the liver metastases with ^{111}In-DOTATOC are shown in Fig. 3.

Discussion

In this study, both radiopeptides, ^{111}In-DOTATOC and ^{111}In-DOTATATE, showed the expected high specific uptake in somatostatin receptor-positive tissue. Visually, the results obtained with the two compounds were com-

parable, although better visualisation of some liver metastases was found with ^{111}In-DOTATOC. The dosimetric analyses showed small differences between the radiopeptides, but a significantly higher mean absorbed dose to the liver was found for ^{111}In-DOTATATE, and a favourable tumour-to-kidney ratio (P=0.065) was calculated for ^{111}In-DOTATOC. These findings were unexpected since data from animal models have shown a more favourable biodistribution for DOTATATE-derived radiopeptides [16]. Because the total administered therapeutic dose with radiolabelled somatostatin analogues is determined by tumour-to-kidney mean absorbed dose ratios (and/or tumour-to-red marrow absorbed dose ratios), these ratios are the most important parameters for therapeutic success. Slightly better results were found for ^{111}In-DOTATOC in three of the four patients with well-defined uptake in metastases.

In this study, we used ^{111}In as a surrogate for ^{90}Y, as we assume high similarity between the tracers although differences in pharmacological parameters have been shown if DOTATOC is labelled with ^{67}Ga/^{68}Ga instead of ^{111}In [15]. In addition, de Jong et al. [17] showed differences in the biodistribution of ^{111}In-DOTATOC and ^{90}Y-DOTATOC in rats bearing the CA 20948 tumour. On the other hand, Froidevaux et al. [18] showed an essentially similar performance of ^{111}In-DOTATOC and ^{90}Y-DOTATOC when using the AR4-2J bearing mouse model.

The mean absorbed doses calculated for ^{90}Y-DOTATOC are comparable with the absorbed doses published in the literature so far [9–13] (Table 3), confirming the accuracy of our methodology. Although the absorbed doses calculated for the red marrow were higher than the doses reported by Cremonesi et al. [10] and Förster et al. [11], they are comparable with the doses published by Krenning et al. [12] (Table 3).

A high interpatient absorbed tumour dose variability was found, which is not unexpected as receptor densities vary markedly among patients and tumours. This fact is well known from the literature [10–13].

A better tumour-to-kidney absorbed dose ratio can be achieved by co-infusion of amino acids, especially lysine and arginine [13, 19–21]. It is unclear whether the results obtained in comparing ^{111}In-DOTATOC and ^{111}In-DOTATATE would be the same if the measurements were to be performed with co-infusion of amino acids. The difference in charge (positive overall charge of ^{111}In-DOTATOC and neutral charge of ^{111}In-DOTATATE) might lead to different results with regard to the kidney absorbed dose after amino acid co-infusion.

The accuracy of the absolute values obtained by organ dosimetry using gamma-scintigraphy may still be limited owing to many potential sources of error. However, since the main aim of this study was to compare two compounds in the same patients with the same methods, this would not have affected the reliability of the findings.

We could not confirm the assumption, based on animal experiments, that ^{90}Y-DOTATATE may have more favourable characteristics for PRMRT compared with ^{90}Y-DOTATOC. Therefore, we will continue treatment with ^{90}Y-DOTATOC.

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CHAPTER 3

B. BONE MARROW DOSIMETRY IN PEPTIDE RECEPTOR RADIONUCLIDE THERAPY WITH [^{177}LU-DOTA^{0},TYR^{3}]OCTREOTATE

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Abstract

Purpose: Adequate dosimetry is mandatory for effective and safe peptide receptor radionuclide therapy. The radiation-dose to the bone marrow can be calculated from the residence time of the radiopeptide in the blood, but it might be underestimated since stem cells express somatostatin-receptors. We verified the blood-method by comparing the results with bone marrow aspirations. Also, we compared other models, taking into account the radioactivity in source organs and the remainder of the body. **Methods:** Bone marrow aspirates were drawn in 15 patients after treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Radioactivity in the bone marrow was compared with radioactivity in the blood drawn simultaneously. The nucleated cell fraction was isolated from the bone marrow aspirate and radioactivity was measured. Furthermore, the absorbed dose to the bone marrow was calculated from the remainder of the body, with and without the additional radioactivity from source organs. All results were correlated to the change in platelet counts 6 weeks after treatment. **Results:** Strong linear correlation and high agreement in measured radioactivities between bone marrow aspirates and blood was found ($r=0.914$, $p<0.001$). No correlation between any of the calculated absorbed doses and the change in platelets was found. The best relation was found for the radioactivity in the nucleated cells of the bone marrow aspirate. **Conclusions:** There is a high agreement between the radioactivities in the bone marrow aspirate and blood. Neither the models had a significant correlation with the change in platelet counts. For the prediction of haematological toxicity many other factors may be crucial as well.

Keywords:
Dosimetry, bone marrow, [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, therapy, somatostatin receptor

Introduction

Peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogues, such as [^{90}Y-DOTA^{0},Tyr^{3}]octreotide (^{90}Y-DOTATOC) or [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, is an effective treatment in patients with metastatic neuroendocrine tumours [1-6]. The dose-limiting organs with this treatment are usually either the kidneys or the bone marrow [7-10].

Haematological toxicity due to the radiation absorbed dose to the bone marrow occurs with most radionuclide therapies, like for instance therapy with ^{186}Re-hydroxyethylidene diphosphonate (HEDP) for bone metastases of prostate carcinoma, therapy with radiolabeled antibodies for Non-Hodgkin Lymphoma, and ^{131}I-metaiodobenzylguanidine (MIBG) therapy for neuroblastoma. The bone marrow toxicity with ^{186}Re-HEDP is mainly due to the bone seeking nature of this radiopharmaceutical, whereas bone marrow toxicity with radiolabeled antibodies or ^{131}I-MIBG results from the circulation of the radioactivity in the blood and its retention in organs, tumours and in the remainder of the body [11-14].

For radiolabeled somatostatin analogues, the absorbed dose to the bone marrow is estimated with the aid of the MIRD scheme [15, 16]. Contributions to the red marrow dose arise from the activity in the marrow tissue itself (self-dose), from activity in source organs, and/or activity in the whole body (cross dose) :

$$D_{rm} = \tilde{A}_{rm} S_{rm \leftarrow rm} + \sum_{h} \tilde{A}_{h} S_{rm \leftarrow h} + \tilde{A}_{rb} S_{rm \leftarrow rb}$$

with S(RM←RM) the self-dose S-factor and S(RM←RB) the cross-dose S-factor for the radiation from the remainder of the body. The residence time of the radiopharmaceutical in the bone marrow τ_{RM} is calculated from the residence time in the blood. Taking into consideration the red marrow to blood activity concentration ratio (RMBLR), a correction factor can be added depending on the vector used for treatment [17]. This method was validated by bone marrow aspirations for antibodies but not for radiopeptides [18, 19]. In addition, especially for radiolabeled antibodies several more sophisticated methods have been developed recently, taking into account the patient-specific bone marrow concentration and contribution of other organs [14, 16, 19-21].

Relevant results were shown using ^{86}Y as a surrogate for ^{90}Y to calculate the residence time in the red marrow by PET before treatment with ^{90}Y-DOTATOC. A region of interest (ROI) was drawn around a segment of the thoracic spine and rescaled for the whole red marrow mass using the standard fraction of active red marrow present in the thoracic spine [22].

Recently a comparable method showing relevant results as well was presented for iodinated antibodies. Instead of a PET scan patients underwent a SPECT/CT during therapy [23]. Acquiring a CT and using an integrated SPECT/CT camera allows placing the ROIs anatomically more accurately than using a SPECT alone. This is especially important in radiopharmaceuticals, e.g. most radiopeptides, which do not present sufficiently high bone marrow uptake to be indisputably identified on the scans. In addition the CT provides an attenuation map that can be used to apply an attenuation and scatter correction. With three SPECT scans at different time points after therapy we investigated the feasibility of this method in a therapeutic setting.

The method calculating the absorbed radiation dose in the bone marrow from the residence time in the blood is the easiest to apply in daily clinical practice. It deals with the assumption

of no specific uptake of the radiolabel in the bone marrow. Since certain haematological cells (lymphocytes, monocytes) and haematopoietic progenitor cells express somatostatin receptors, mainly subtype 2 [24-26], this assumption may result in an underestimation of the absorbed radiation dose to the bone marrow. It is not known whether in patients a significantly higher radioactivity in the bone marrow, compared to the blood, may be present [17]. Also, the method using the whole body retention, with or without taking into account the contribution from the blood and/or source organs to calculate the bone marrow residence time could be more accurate. An overview of the parameters required and the advantages and disadvantages of the most commonly used methods for bone marrow dosimetry is given in Table 1.

Reliable dose estimation for the bone marrow is mandatory for several reasons. In order to achieve a maximum anti-tumour effect, patients should be treated with the highest justifiable dose of the radiopharmaceutical that does not cause serious toxicity. Many studies with radiolabeled somatostatin analogues showed that the toxicity is generally mild and transient [1-3, 6]. It should however not be neglected that in a phase 1 study with [^{111}In-DTPA^{0}]octreotide 3 out of 50 patient developed a myelodysplastic syndrome (MDS) which was probably related to the therapy [27]. Calculations from these data resulted in an estimated radiation absorbed dose for the bone marrow of approximately 3 Gy. In another study with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, one MDS was observed in a patient who had had chemotherapy with alkylating agents 2 years before study entry [28]. In the latest update of our own records of roughly 500 patients treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, 3 patients (including the patient mentioned before) developed MDS (unpublished data). To avoid hypoplasia, a maximum absorbed dose of 2 Gy to the bone marrow is generally accepted [29, 30]. At a total body dose of 2 Gy the probability for developing leukaemia is approximately 2% [31]. In radioiodine therapy of metastatic thyroid cancer a threshold of 2 Gy to the blood as surrogate for bone marrow has been maintained since the pioneering work of Benua and co-workers in 1962 [32]. More recent work has set this limit to even 3 Gy, by using more patient-specific dosimetry techniques [33]. Nevertheless even if this limit is not exceeded the risk for the patient to develop a MDS can not be excluded completely, but an accurate estimation of the absorbed dose to the bone marrow will help to find an adequate dosage.

To perform bone marrow dosimetry in the clinical routine, the method has to be easily applicable. We compared the radioactivity in the bone marrow aspirate with the radioactivity in the blood. In addition we determined the difference of radioactivity in the nucleated cell fraction of the bone marrow, including the stem cells, versus that in the blood to demonstrate a potential difference which could be attributed to the specific binding of the radiopeptide to somatostatin receptor-positive cells of the bone marrow. Finally the calculated absorbed doses to the bone marrow calculated from the blood, the remainder of the body, with or without taking into account the contribution from the blood and/or source organs, and from the SPECT scans were correlated with the change in the platelets counts 6 weeks after the treatment.

Table 1

Calculation method for the absorbed dose to the red marrow	Experimental data to be collected / measured	Advantages	Disadvantages / uncertainties
From the blood	Approx. 5 blood samples	Easy and cheap to perform; low discomfort level for patients; no inter-observer variability	Use of standard volumes for blood
From the remainder of the body	Urine; Images at approx. 3 time points	Non invasive	Urine collection is a source of errors; time consuming; inter-observer variability; difficult quantitative determination
From a bone marrow aspiration	One bone marrow aspiration	Real information from the bone marrow; no inter-observer variability	The time dependency is not known; highly invasive; discomfort for the patients; time consuming; costly
From PET-scans with a ROI around a segment of the thoracic spine	PET scans at approx. 3 time points	Non invasive	PET surrogate necessary; can only be done pretherapeutically; additional examination; not quantitative concerning partial volume effect, scattering
From SPECT/CT-scans with a ROI around a segment of the thoracic spine	SPECT/CT scans at approx. 3 time points	Non invasive	SPECT/CT necessary; ; not quantitative concerning partial volume effect, scattering; validated only for radiolabeled antibodies

Table 1 gives an overview over the parameters that need to be collected / measured and the advantages and disadvantages for the two most common methods to calculate the absorbed dose to the red marrow in peptide receptor radionuclide therapy. In addition the same information is given for the calculation of the absorbed dose by a bone marrow aspiration. For antibodies a combination of the first and second method have been performed (13).

Material and Methods

Patients
Fifteen patients with somatostatin receptor-positive neuroendocrine tumours were studied. All patients were admitted to our clinic for PRRT with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and fulfilled the inclusion criteria as previously described [6]. None of the patients had known bone metastases.
All patients gave written informed consent to participate in the study, which was approved by the medical ethical committee of the hospital.

Comparison of the radioactivity in the bone marrow and nucleated cell fraction with the radioactivity in the blood
In addition to the examinations, the treatment, and the scans that are performed routinely after the first treatment cycle, patients underwent a bone marrow aspiration from their iliac crest 4 days (7 patients), 7 days (7 patients) or 8 days (1 patient) after the treatment. A rough differential count of cells was performed on the bone marrow samples to prove the presence of a sufficient number of bone marrow cells and the samples were analyzed for haematological abnormalities. The volume of the aspirate was recorded and the radioactivity was measured in a gamma counter (Perkin Elmer, Groningen, the Netherlands). One patient (aspiration 4 days after treatment) was excluded because no bone marrow could be aspirated. In all patients, a blood sample was drawn simultaneously to determine the radioactivity in the blood.

Part of the blood samples and of the bone marrow samples were purified for the mononuclear cells including stem cells (bone marrow only). The samples (blood: 8 - 48 mg; 26 ± 12.2 mg (mean ± SD); bone marrow: 2 - 49 mg; 22 ± 16.6 mg) were diluted with 5 ml Dispase (Roche Diagnostics, Almere, the Netherlands) and mixed for 20 minutes to suspend the cells. Then the samples were diluted with 50 ml phosphate buffered saline (PBS) (pH = 7.4) and in total 3 times centrifuged for 10 minutes at 2500 rpm (≡ 60xg) each time. In between, the samples were washed with 50 ml PBS after every step. The mononuclear cells, including the stem cells in the samples from the bone marrow, were isolated by Ficoll Paque gradient sedimentation (density = 1.077 g/ml) (GE Healthcare Europe GmbH, Diegem, the Netherlands). Afterwards the weight of the cell pellets was determined and the radioactivity was measured in a gamma counter.

Bone marrow dosimetry
For all patients the cumulated activity in the bone marrow was estimated with different methods:
The distribution in the remaining tissue of the body besides organ and tumour uptake is assumed to be homogeneously distributed in the remainder of the body [34]. In addition, cross radiation from organs and tumours as well as blood can be taken into account. Lastly, the activity concentration in the red marrow can be assumed to be equal to the activity concentration measured in the plasma [35].
Also, quantitative imaging of the radioactivity uptake in the thoracic spine at several time-points can give an estimate of the total bone marrow kinetics by rescaling these values according to the average fraction of active marrow in the observed regions [36].

Bone marrow dosimetry using different models
At three different time points between 24 and 168 hours after the administration of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, scans of tumour deposits, liver, kidney, spleen and bladder were acquired using a dual head camera (Picker Prism 2000 XP, Philips, Eindhoven, The

Netherlands). For all quantitative analyses of planar scans and SPECT, only the higher energy peak of ^{177}Lu was considered, i.e. the energy window was set at 208 keV ± 10%. Regions of interest were drawn manually with the Odysse XP software around visible tumours, the liver, the kidneys, the spleen and the bladder. The radioactivity in these organs and in the tumours was determined as described previously [37]. Attenuation correction was applied using the data from the pretherapeutical CT scan. Urine was collected up to 24 hours after treatment and the radioactivity was measured in the gamma counter. From these data the radioactivity in the remainder of the body (i.e. total body minus source organs and minus excreted radioactivity) was calculated, taking into account the fact that the radioactivity was overestimated since the urine was collected for 24 hours only. In 2 patients the determination of radioactivity in the remainder was not possible. Assuming the bone marrow being part of the remainder of the body, the absorbed dose to the bone marrow was calculated. Also, the contribution of source organ irradiation and/or blood radioactivity was additionally investigated.
The bone marrow dosimetry using the residence time of the radiopeptide in the blood was based on at least 5 blood samples per patient drawn between 0 and 168 hours post injection. Assuming no specific uptake in the red marrow, a uniform distribution of the activity, and that the red marrow clearance was the same as in blood, the dose to the red marrow was calculated. Additionally the residence time of the radiopharmaceutical in tumour and organs (liver, kidneys and spleen) was calculated from planar scans at three different time points between 24 and 168 hours after the administration of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Due to overlap of these tumours and organs in all patients, this was regarded as one single source. Using OLINDA, the cross-dose from this source was added to the absorbed dose to the red marrow calculated from the blood. The results were compared with the dose calculated from the blood alone and it was correlated with the change in platelets as well.

Bone marrow dosimetry using SPECT scans
In addition, at three different time points between 24 and 168 hours after treatment, SPECT scans of the thorax were acquired (energy window 208 keV ± 10%, Matrix 128 x 128, 120 projections, 20 sec/Projection) .

Correlation with the haematological response
Six weeks after the treatment, blood was drawn from 13 patients to determine haematological toxicity after the treatment. For one patient no blood results were available. All values were correlated with the decrease in platelet counts expressed as percentage of the pretherapeutic value. The results of the bone marrow (full bone marrow and nucleated cell fraction) radioactivity were corrected for physical decay in order to compare the inter-individual values of the samples drawn at different time points. Similarly, calculated absorbed dose to the bone marrow from the remainder of the body, and from the residence time in the blood (with and without the dose from the source organs) were correlated with the decrease of platelet counts.

Statistics
To correlate the results, Pearson's correlation coefficient was calculated. A p-value ≤ 0.05 was considered significant.

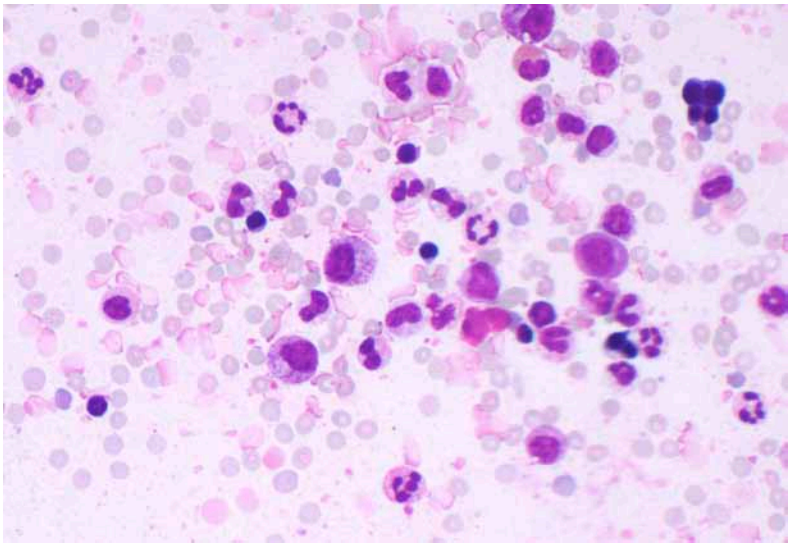
Results

The treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate was well tolerated by all patients and no serious adverse events occurred. The injected radioactivity ranged from 7.26 to 7.75 GBq (7.47 ± 0.10 GBq; mean ± SD). On the post-therapeutic scans all patients showed the

expected distribution of the radiopharmaceutical with specific uptake in all known tumours. No patient had known or visible bone metastases.

The bone marrow aspiration was uneventful. One patient had a dry tap. The volume of bone marrow that was aspirated in the other patients ranged from 1 to 9.2 g (5.3 ± 2.9 g). Simultaneously a tube of blood of 2.8 to 7.3 g (5.8 ± 1.3 g) was drawn. Smears were made from the bone marrow aspirate. A differential count was performed to establish the numbers of immature (bone marrow) and mature nucleated cells. The fraction of immature cells ranged from 25 to 80% (51 ± 15%) indicating that the purified aspirations consisted of a considerable amount of bone marrow and that the contamination with blood was moderate. A typical example of a purified bone marrow smear is shown in Figure 1.

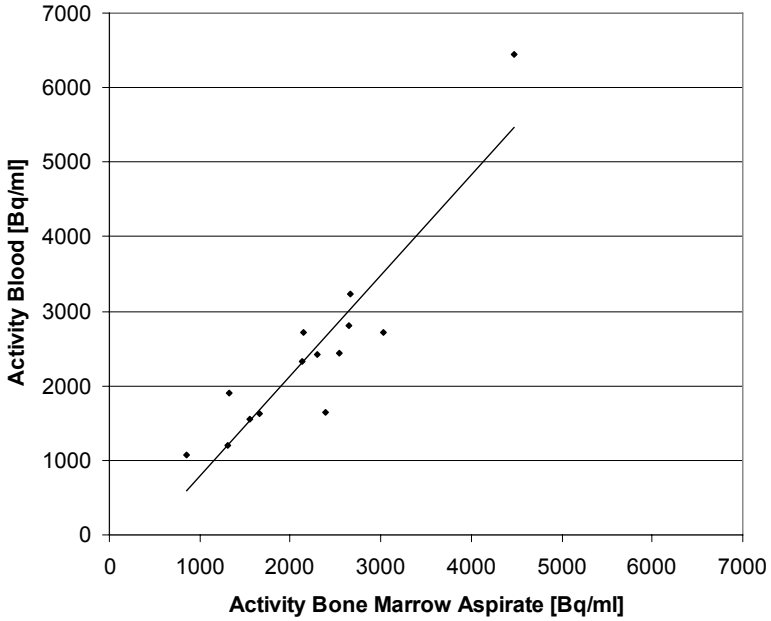
Figure 1



Typical example of a smear of one of the bone marrow aspirations. It shows a mixture of immature nucleated cells originating from the bone marrow as well as red blood cells and mature nucleated cells originating from peripheral blood.

The radioactivity in the full bone marrow samples ranged from 850 to 4473 Bq/ml (2216 ± 899 Bq/ml). The radioactivity in the blood ranged from 1077 to 6451 Bq/ml (2437 ± 1324 Bq/ml). Fitting the correlation line through the origine (0,0) as it seems right from a theoretical point of view, a strong, significant, linear correlation between the radioactivity determined in the blood and in the bone marrow aspirate was found ($y=1.13x$, $r = 0.90$, $p < 0.001$) (Figure 2). This results in a mean Red Marrow over Blood Ratio of 0.88 (value not significantly different from 1). Both qualitatively and quantitatively the results showed strong agreement over a whole range of activities at the three different time points.

Figure 2



Correlation between the activities measured in the bone marrow aspirate and the activities measured in the blood at the same time points in Bq/ml. The straight line is the linear regression line: $r = 0.914$, $p < 0.001$. The slope of the regression line is 1.35 indicating that also the absolute values of the measured activities are comparable.

The absorbed dose to the bone marrow calculated from the residence time in the blood, from the residence time in the remainder of the body, from both and from both together with the cross radiation from source organs as well as the change in platelets counts after 6 weeks are listed in table 2. The contribution of the remainder of the body to the red marrow dose was substantial in relation to the contribution of the blood alone, whereas the additional contribution from source organs was relatively insignificant.

Table 2

Patient	A	B	C	D	Drop in platelet counts 6 weeks after therapy [%]
1	17	12	29	39	40
2	24	21	44	58	8
3	43	123	165	173	40
4	17	83	100	107	16
5	21	22	41	53	4
6	36	79	114	127	-
7	29	0	29	68	-9
8	38	29	65	71	-9
9	29	128	155	-	47
10	29	429	455	466	8
11	41	69	109	114	27
12	19	31	50	61	32
13	30	31	61	74	28
14	17	39	56	64	21

A: Absorbed dose to the red marrow [mGy] after the injection of 7400 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, calculated from the residence time in the blood

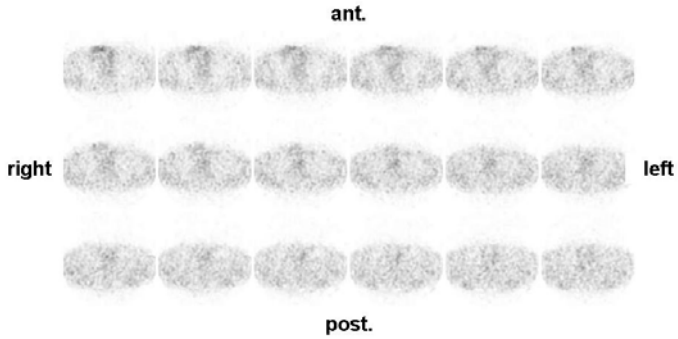
B: Absorbed dose to the red marrow [mGy] after the injection of 7400 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, calculated from the residence time in remainder of the body

C: Absorbed dose to the red marrow [mGy] after the injection of 7400 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, calculated as a combination of the dose to the red marrow from the blood and the remainder of the body

D: Absorbed dose to the red marrow [mGy] after the injection of 7400 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, calculated as a combination of the dose to the red marrow from the blood, the remainder of the body and taking into account the cross radiation from source organs (tumours, liver, kidneys and spleen)

No or merely very faint uptake in the bone marrow could be seen on the SPECT scans of the thorax. Bone marrow to background (ROI placed into the lungs) ratios between 1 and 1.8 were found. Therefore it was not possible to place a ROI reliably and consequently no absorbed dose to the bone marrow was calculated from SPECT scans. An example of transaxial SPECT slices of one patient is shown in Figure 3.

Figure 3

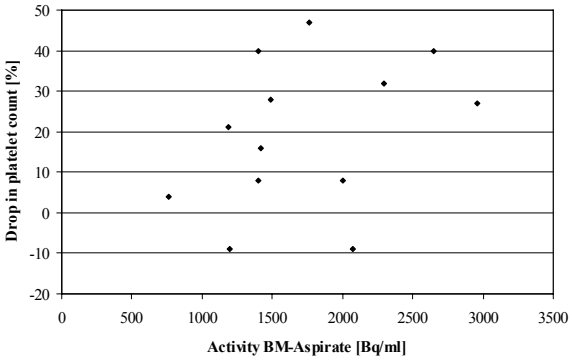


Typical example of transverse SPECT slices through the thorax, obtained 24h after therapy with 7.4 GBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Note that no clear uptake over the spine area is found.

No correlation was found between any of the calculated absorbed doses to the bone marrow and the decrease in platelets, expressed as percentage of the pre-treatment value.

The relation between the activity in the full bone marrow aspirate and the drop in platelet counts was poor (r = 0.35, p = 0.24) (Figure 4). However, the relation between the absorbed doses calculated with the different methods and the decrease in platelet counts was less significant. The correlations were r=0.07 for the absorbed dose calculated from the blood and r=0.12 for the absorbed dose calculated from the remainder of the body. Taking into account both doses resulted in r=0.07 and including the cross radiation from source organs resulted in r=0.06.

Figure 4

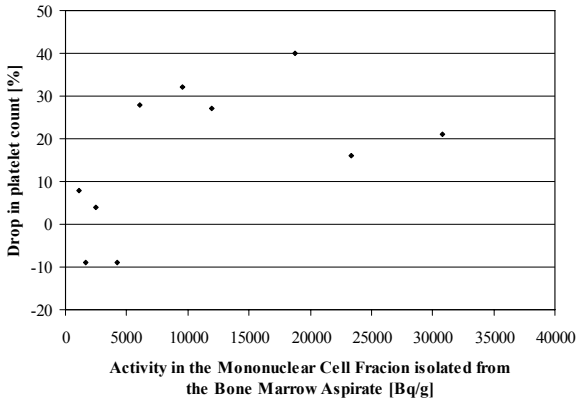


Correlation between the radioactivity per ml in the bone marrow aspirate with the drop of thrombocytes expressed as % of drop from the value measured before the treatment. The activities measured in the bone marrow were decay corrected to the theoretical value at day 8. The straight line is the linear regression line: r = 0.35, p = 0.24.

The ratio of radioactivity in the isolated mononuclear cell fraction of the bone marrow to the isolated mononuclear cell fraction of the blood, obtained at the same day , yielded highly variable results. We found ratios ranging from 0.29 to 536 (59.3 ± 143.6). In three patients a higher radioactivity could be found in the mononuclear cells isolated from the blood whereas 11 patients showed a higher radioactivity in the mononuclear cells isolated from the bone marrow.

For 11 patients the radioactivity in the isolated mononuclear cell fraction, microscopically containing a mixture of immature and mature cells, could be weight-corrected. In the other 3 patients, the isolated fraction could not be weighed reliably, because of the small number of isolated cells. Weight-corrected ratios of the radioactivity in the isolated cell fraction of the bone marrow to the isolated cell fraction of the blood ranged from 0.54 to 133.41 (19.4 ±38.6). Only one patient showed a higher radioactivity in the isolated cell fraction per gram of the blood. The correlation coefficient between the radioactivity per gram in the nucleated cell fraction of the bone marrow aspirate and the drop in platelet counts after the treatment was r = 0.51 (p = 0.13) (Fig 5). From our study this was the best relation that could be obtained.

Figure 5



Correlation between the radioactivity per gram measured in the isolated mononuclear cell fraction of the bone marrow aspirate with the drop of platelet counts after the treatment. The straight line is the linear regression line: r = 0.51, p = 0.13.

Discussion

Accurate bone marrow dosimetry is mandatory for safe PRRT. All methods available have certain disadvantages: aspiration of bone marrow is costly, time consuming and it is an invasive procedure associated with a high level of discomfort for the patients. The diverse models also all have their theoretical limitations: Calculating the dose to the red marrow from the residence time in the blood deals with the assumption that no specific binding of the radiolabeled somatostatin analogue occurs in the bone marrow. Also, the determination of the radioactivity in the remainder of the body depends on accuracy in urine collection, which is subject to errors. Besides these considerations, it should be considered that at present none of

the diverse models to estimate the dose to the bone marrow has been actually confirmed with toxicity data, be it thrombocytopenia or the occurrence of MDS. Apart from the risk for the patients to develop MDS the most essential issue of bone marrow dosimetry is to predict or better to avoid severe haematological toxicity caused by PRRT.
However, the dose to the red marrow is only one of several factors influencing the haematological toxicity after PRRT. A high inter patient variability in the haematological response after PRRT has been found and also previous therapies can highly influence the results [22, 29]. Using different radiopharmaceuticals, some investigators have found a correlation between haematological toxicity and injected dose of radioactivity [13, 14], whereas others did not find such a correlation [20].

It is possible that additional factors like age and sex of the patients might influence the haematological toxicity as well although in a trial with radiolabeled antibodies there was only a minor influence of these factors [38]. In 2000, Blumenthal and colleagues already reported that plasma levels of FLT3-L help to predict haematological toxicity after radioimmunotherapy [39]. In 2003 this was confirmed by Siegel et al. for radioimmunotherapy with iodinated anti-CEA antibodies [40]. However, no studies taking this into account are published for PRRT. Nevertheless, the importance of introducing biological parameters into treatment planning is indisputable [41].

Promising results for predicting the haematological response were obtained by using ROI surrounding a section of the thoracic spine for determining the absorbed dose to the red marrow. In these studies the absorbed dose was calculated pretherapeutically with the positron emitter ^{86}Y before treatment with [^{90}Y-DOTA^{0}-Tyr^{3}]octreotide [22, 42]. Yttrium-86 can be regarded as the ideal surrogate for ^{90}Y and offers a high resolution when using a PET-scanner. However, imaging with ^{86}Y is currently only available in specialized centres because despite the need of a cyclotron to produce ^{86}Y, the reconstruction of the PET data requires sophisticated correction algorithms. There is certain evidence in literature that imaging with ^{86}Y might overestimate doses, particularly if close to dense tissue as is the spine [43, 44]. Besides, dosimetry using ^{86}Y will be a gold standard only for treatments with ^{90}Y because in PRRT the radionuclide used might influence the receptor affinity and consequently the biodistribution of the compound [45]. Moreover, the relatively short physical half life of ^{86}Y (14.7 h) does not allow to follow the activity over several days which is important for the planning of the treatment with ^{177}Lu. Despite these drawbacks of ^{86}Y it should be emphasized that a very good dose response curve was found for predicting the haematological toxicity after [^{90}Y-DOTA^{0},Tyr^{3}]octreotide treatment [22, 42].

So far one group found interesting results when calculating the absorbed radiation dose to the bone marrow from scans for radiolabeled antibodies using an integrated SPECT/CT camera [23]. The results are still lacking of confirmation by other groups and no results using this method with radiopeptides have been published so far. A validation of this method for radiopeptides is needed. Remarkably no bone marrow dosimetry was feasible on SPECT scans in our setting because none or only very faintly visible uptake in the bone marrow was present on the scans (Fig. 3). On the one hand this is an indication that the biodistribution is not identical for [^{86}Y-DOTA^{0},Tyr^{3}]octreotide and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. On the other hand this underlines the need of a different, reliable method for [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate dosimetry. The lower uptake in the bone marrow of radiopeptides compared to radiolabeled antibodies might be a drawback for this method in PRRT in general. Nevertheless it is an interesting method that should be evaluated further using a SPECT/CT.

We found a high correlation between the radioactivity in the blood and in the bone marrow aspirate during PRRT with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. The most important explanation for the high congruence between the radioactivity measured in the blood and in the bone marrow aspirate is that the amount of stem cells in a bone marrow aspirate is low and that most of the aspirate consists of blood. On the other hand, the high volume of blood in the bone marrow aspiration reflects that the blood contributes most of the radiation absorbed dose to the bone marrow. Taking into consideration the path length of the common therapeutic radionuclides in the millimetre range and the structure of the bone marrow it is evident that the radiation from the blood will reach all bone marrow structures. Calculating the radioactivity in the remainder of the body on the other hand deals with the assumption of a homogeneous distribution of all the activity that is not excreted or absorbed in one of the source organs.

As stated in the introduction, a homogenous distribution of the activity in the bone marrow has to be assumed in order to apply the blood-method for dosimetry. Again the high agreement between the radioactivity in the blood, where the activity is distributed homogeneously, and in the bone marrow aspirates indicates that this assumption might count in the case of small peptides. However, it is not possible to prove this assumption with these data.

Measuring the radioactivity in the blood is simple, accurate and the discomfort for the patients is limited. Since the time radioactivity curve in blood usually is fitted by a bi-exponential or three exponential curve [17, 37], a sum of five blood samples appears to be reasonable [46] whereas the time points of drawing the blood should be chosen depending on the biokinetics of the vector and the half life of the radionuclide.

The relation between the calculated absorbed doses to red marrow using different methods and the decrease in platelet counts is disappointing. A number of reasons may account for this. The absorbed doses were compared with only one post therapeutic platelet count. This platelet count, six weeks after the treatment, does not reflect the nadir of each patient. Another explanation could be the relatively small number of patients that was studied. Moreover, probably the most important reason is that the response of an individual patient to PRRT is not only related to the radiation absorbed dose in the bone marrow but also to the pretherapeutical status of the bone marrow. Especially previous, potentially haematocytotoxic treatments can influence the response after the treatment.

At this point no conclusions can be drawn between the calculation of the radiation absorbed dose in the bone marrow and the risk of developing MDS. However, developing MDS is probably also related to the pretherapeutic status of the bone marrow and previous treatments.

The field of bone marrow dosimetry is a large and very difficult field. Beside all factors mentioned previously that may influence the dosimetry of an individual patient, many other problems have to be faced. The bone marrow is not a solid organ and simply the determination of the mass is virtually impossible. As for all internal radiotherapy treatments the dose rate in PRRT is low. Most values that deal with the maximum tolerated dose of healthy organs are derived fom external beam radiation with a much higher dose rate. The influence of such physical properties is not well understood and may as well highly influence the results of internal dosimetry and the biological response.

Calculating the absorbed dose to the bone marrow from both the blood and the remainder of the body appears to be a cautious, but feasible method. Adding the radiation dose derived

from organs and tumours seems relatively insignificant. However, since the correlation with the drop in platelets is poor, more attention should be paid to other factors that might influence the haematological response after PRRT in the future. Also, because of the lack of correlation between any of the calculations for the bone marrow dose and the haematological response, no conclusions can be drawn from this study as to which theoretical model is adequate. Such testing of models requires dose escalation studies for which approval of medical ethical committees may prove impossible.

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CHAPTER 4

A. IN VIVO RADIONUCLIDE UPTAKE QUANTIFICATION USING A MULTI-PINHOLE SPECT SYSTEM TO PREDICT RENAL FUNCTION IN SMALL ANIMALS

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In vivo radionuclide uptake quantification using a multi-pinhole SPECT system to predict renal function in small animals

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Abstract. *Purpose:* In vivo quantification of radiopharmaceuticals has great potential as a tool in developing new drugs. We investigated the accuracy of in vivo quantification with multi-pinhole single-photon emission computed tomography (SPECT) in rats.
Methods: Fifteen male Lewis rats with different stages of renal dysfunction were injected with 50 MBq ^{99m}Tc-dimercaptosuccinic acid. Four to six hours after injection, SPECT of the kidneys was acquired with a new four-headed multi-pinhole collimator camera. Immediately after imaging the rats were sacrificed and the kidneys were counted in a gamma-counter to determine the absorbed activity. SPECT data were reconstructed iteratively and regions of interest (ROIs) were drawn manually. The absolute activity in the ROIs was determined.
Results: Uptake values ranging from 0.71% to 21.87% of the injected activity were measured. A very strong linear correlation was found between the determined activity in vivo and ex vivo (r^2=0.946; slope m=1.059).
Conclusion: Quantification in vivo using this multi-pinhole SPECT system is highly accurate.

Keywords: Animal SPECT – In vivo quantification – ^{99m}Tc-DMSA – Renal uptake

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Introduction

In vivo quantification of radiopharmaceuticals has great potential as a tool in the development of new drugs [1]. Accurate in vivo quantification allows the user to determine the uptake of a radiopharmaceutical without sacrificing the animal. Another consequence of accurate in vivo quantification is that a physiological process can be followed in the same animal over time.

Treatment with radiolabelled somatostatin analogues has become the treatment of choice for patients with metastatic, somatostatin receptor-positive neuroendocrine tumours [2]. Such treatments, when repeated, result in a high absorbed radiation dose in the kidney, which in turn may cause renal failure [3]. To investigate the effect of different agents on kidney protection during peptide receptor radionuclide therapy (PRRT) in animals, a tool to quantify the renal damage after PRRT is needed. Renal damage after PRRT occurs mainly in the glomeruli and proximal tubules of the kidneys [4]. ^{99m}Tc-dimercaptosuccinic acid (DMSA) is a marker for tubular function [5]. After glomerular filtration, ^{99m}Tc-DMSA is taken up by functional tubular cells. In this preliminary study we investigated the accuracy of in vivo quantification of ^{99m}Tc-DMSA uptake in rat kidneys with the NanoSPECT, a new multi-pinhole four-headed camera, after induction of different levels of kidney damage by high-dose PRRT. In several small-animal SPECT systems a very high resolution has been demonstrated previously [6, 7]. The NanoSPECT is a small-animal SPECT system which has been shown to greatly improve sensitivity while achieving high, even submillimetre, resolution [8]. Some previous studies have used semi-quantitative quantification methods [9, 10], though to date only one small-animal SPECT study has shown accuracy of absolute quantification in vivo. This study, however, was performed with the Linoview system using a different acquisition technique, and only two animals were scanned [11]. Our aim was to investigate the accuracy of in vivo quantification over a broad range of activity concentrations in the animals.

Materials and methods

The animal experiments were performed in compliance with the regulations of the institution and with generally accepted guidelines governing such work.

Radiopharmaceuticals

[^{177}Lu-DOTA^{0},Tyr^{3}]octreotate was synthesised and labelled as described previously [12]. The ^{99m}Tc-DMSA kit was purchased from GE Healthcare (Roosendaal, the Netherlands) and labelled according to the indicated procedure.

Animal studies

Fifteen male Lewis rats were treated with different activities of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. The aim was to deliver renal irradiation with different activities in order to induce different levels of renal dysfunction. Two rats were not injected with the radiopeptide and served as a control group. The activity injected in the other 13 rats ranged from 278 to 555 MBq.

Between 105 and 146 days after [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate injection, the rats received 50 MBq ^{99m}Tc-DMSA i.v., and 4–6 h after ^{99m}Tc-DMSA injection, SPECT was acquired as described below. Immediately after the imaging procedure, the animals were sacrificed, the left kidney was removed and the activity in the kidney was determined in a gamma-counter (Perkin Elmer, Groningen, the Netherlands). Beforehand the gamma-counter had been calibrated with different volumes and activities to exclude errors caused by volume effects or dead time.

Animal SPECT (NanoSPECT) and software

SPECT imaging was performed with a four-headed multiplexing multi-pinhole NanoSPECT (Bioscan Inc., Washington D.C.) (Fig. 1). Each head is fitted with an application-specific tungsten collimator with nine pinholes. For this study we imaged with the rat apertures, which comprise a total of 36 2-mm-diameter pinholes imaging a cylindrical field of view that is 60 mm in diameter by 24 mm in length. These rat apertures provide a reconstructed resolution below 1.6 mm at 140 keV, with an average sensitivity of 1,100 cps/MBq across the field of view (FOV). The axial FOV is extended using a step-and-shoot helical scan of the animal, with the user defining a range from 24 to 270 mm according to the region to be imaged. The energy peak for the camera was set at 140 keV. The window width was ±10%. The rats were scanned 4–6 h after the injection of approximately 50 MBq ^{99m}Tc-DMSA. An acquisition time of 30 s per view was chosen, resulting in acquisition times ranging from 6 to 9 min per animal. After the acquisition, the data were reconstructed iteratively with the HiSPECT (Bioscan Inc., Washington D.C., USA) software, a dedicated ordered subsets-expectation maximisation (OSEM) software package for multiplexing multi-pinhole reconstruction. The NanoSPECT is calibrated with a phantom, approximately of the size of the animals, filled with a known activity of ^{99m}Tc such that voxel values in the reconstruction provide a proper estimate of the activity level without further calculation. A region of interest (ROI) was drawn manually around both kidneys; the 3D activity distribution within the ROI was then summed to determine the uptake. Because of the favourable biodistribution of ^{99m}Tc-DMSA, limited to the kidneys, the ROI could be drawn generously to prevent partial volume effects at the edges. No correction for scatter was performed. All measured activities were corrected for decay and expressed as % injected activity (%IA). The injected activity was determined by measuring the syringe in a dose calibrator before and after injection of the animal. The difference was defined as the injected activity. Quantification of the ROI is performed with the INTERVIEW XP (Mediso Ltd., Budapest, Hungary) software.

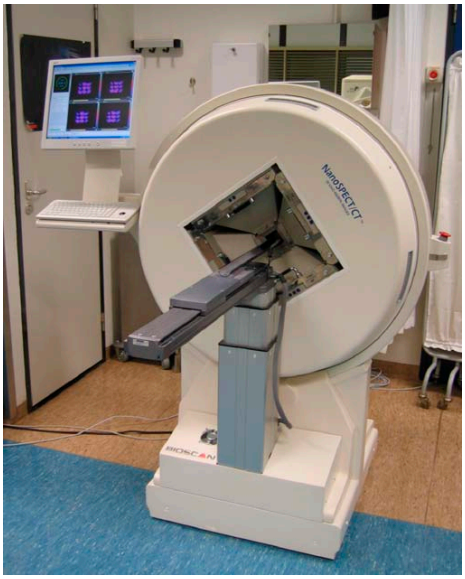

Fig. 1. The NanoSPECT (Bioscan Inc., Washington D.C., USA), a commercially available four-headed multiplexing multi-pinhole camera

Statistical analyses

Linear regression was performed with the values calculated with SPECT plotted against those collected with the gamma-counter. The square of the correlation factor (r^2) was then calculated to provide some measure of the results.

Results

In all rats, both kidneys could be visualised. The spatial resolution was very high. Differentiation between the parenchyma characterised by tracer accumulation and the cold regions indicative of the renal basin was easily possible over a broad range of activity concentrations. Scans of two animals with different activities in the kidneys are demonstrated in Figs. 2 and 3. As a consequence of the high contrast, the ROI around the kidney could be placed indisputably.

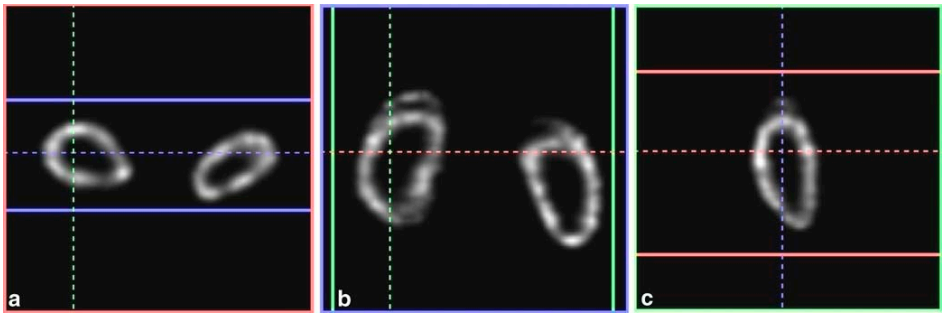

Fig. 2. Transaxial (**a**), coronal (**b**) and sagittal (**c**) slices of a rat kidney acquired by multi-pinhole SPECT, 6 h after the injection of 50 MBq ^{99m}Tc-DMSA. Of note is the very high resolution. Clear differentiation is possible between the parenchyma and the renal basin. In the left kidney, 21.87%IA was determined by SPECT

Over the kidneys of the healthy animals with the highest ^{99m}Tc-DMSA uptake, we achieved count rates of approx. 1,500 cps per detector. The maximum capacity of the detectors is specified by the manufacturer to be 50,000 cps. Therefore effects of dead time can be excluded.

Uptake values of 0.71% to 20.77%IA were determined in the gamma-counter. By comparison, SPECT values ranging from 0.74 to 21.87%IA in the left kidney were measured. We found a very good linear correlation between the values determined by the gamma-counter and the values determined by SPECT. The square of the correlation factor was r^2=0.946 and the slope of the correlation line was m=1.059 (Fig. 4). Both qualitatively and quantitatively, the results showed strong agreement over a whole range of activities.

The results determined by SPECT for the left and the right kidney in the same animal were nearly identical. No difference >1.5%IA was found (data not shown).

Discussion

We found a strong linear correlation between the two methods determining the absolute activity of ^{99m}Tc-DMSA in the kidney. The slope of the regression line and the correlation factor were close to 1, indicating that the two methods produce nearly identical results. We assumed the determination in the gamma-counter to be the gold standard, though both modalities used in this study have some inherent variance. With these results, we have shown that it is possible to perform absolute quantification of activity in vivo with the NanoSPECT.

These results will influence our future planning of animal studies. We have now demonstrated that this system is capable of quantifying radiopharmaceuticals in vivo. Thus, we can follow physiological processes in the same animal over time, i.e., we are able to perform longitudinal studies. In addition to saving animals, following a function

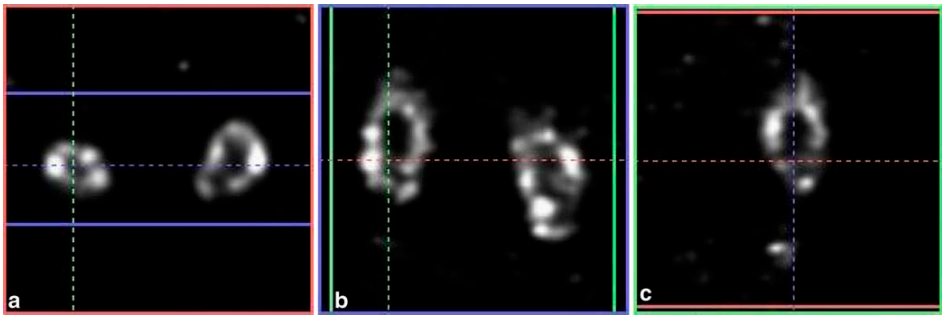

Fig. 3. Transaxial (**a**), coronal (**b**) and sagittal (**c**) slices of a rat kidney acquired by multi-pinhole SPECT. The experimental setting and acquisition mode were as in Fig. 2. This rat had severe renal damage after receiving 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate 125 days earlier. In the left kidney, 3.08%IA was determined by SPECT

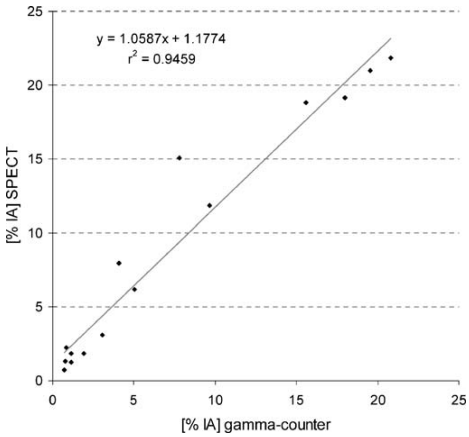

Fig. 4. Percentage injected activity of ^{99m}Tc-DMSA in the left kidney of rats with different levels of kidney damage. The *x-axis* indicates the activity determined in the left kidney by the gamma-counter and the *y-axis* indicates the activity determined in the left kidney by SPECT. The *straight line* is the linear regression line for these values

in one animal over time is closer to the situation encountered in clinical practice.

In our study we used ^{99m}Tc-DMSA, which is filtered by the glomeruli and actively reabsorbed by the functional tubule cells. This tracer is very suitable for quantification as the activity is strictly limited to the organ of interest. The volume of the ROI did not influence the results substantially, since nearly no activity was located outside the kidneys (data not shown). Further investigations will have to show how reliable the results are when more background activity is present, e.g. in tumours. When more background activity is present, the shape and size of the ROI will strongly influence the results, which in turn will increase inter-observer variability. In the next generation of the NanoSPECT, an X-ray CT will be implemented to provide anatomical information that in turn will enable the imager to better define ROIs.

We conclude that in our setting, in vivo quantification with the NanoSPECT is highly accurate, resulting in precise determination of the absolute activity in an ROI over a broad range of activities of ^{99m}Tc-DMSA in rat kidneys.

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CHAPTER 4

B. FROM OUTSIDE TO INSIDE? DOSE-DEPENDENT RENAL TUBULAR DAMAGE AFTER HIGH-DOSE PEPTIDE RECEPTOR RADIONUCLIDE THERAPY IN RATS MEASURED WITH IN VIVO ^{99M}TC-DMSA-SPECT AND MOLECULAR IMAGING

Flavio Forrer, Edgar Rolleman, Magda Bijster, Marleen Melis, Bert Bernard, Eric P. Krenning, Marion de Jong

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From Outside to Inside? Dose-Dependent Renal Tubular Damage After High-Dose Peptide Receptor Radionuclide Therapy in Rats Measured with *In Vivo* ^{99m}Tc-DMSA-SPECT and Molecular Imaging

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ABSTRACT

In peptide receptor radionuclide therapy (PRRT), the dose-limiting organ is, most often, the kidney. However, the precise mechanism as well as the exact localization of kidney damage during PRRT have not been fully elucidated. We studied renal damage in rats after therapy with different amounts of [^{177}Lu-DOTA^0,Tyr^3]octreotate and investigated ^{99m}Tc-DMSA (dimercaptosuccinic acid) as a tool to quantify renal damage after PRRT. ***Experimental Design:*** *Twenty-nine (29) rats were divided into 3 groups and injected with either 0, 278, or 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate, leading to approximately 0, 46, and 92 Gy to the renal cortex. More than 100 days after therapy, kidney damage was investigated using ^{99m}Tc-DMSA single-photon emission computed tomography (SPECT) autoradiography, histology, and blood analyses.* ***Results:*** In vivo *SPECT with ^{99m}Tc-DMSA resulted in high-resolution (<1.6-mm) images. The ^{99m}Tc-DMSA uptake in the rat kidneys was inversely related with the earlier injected activity of [^{177}Lu-DOTA^0,Tyr^3]octreotate and correlated inversely with serum creatinine values. Renal* ex vivo *autoradiograms showed a dose-dependent distribution pattern of ^{99m}Tc-DMSA. ^{99m}Tc-DMSA SPECT could distinguish between the rats that were injected with 278 or 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate, whereas histologic damage grading of the kidneys was nearly identical for these 2 groups. Histologic analyses indicated that lower amounts of injected radioactivity caused damage mainly in the proximal tubules, whereas as well the distal tubules were damaged after high-dose radioactivity.* ***Conclusions:*** *Renal damage in rats after PRRT appeared to start in a dose-dependent manner in the proximal tubules and continued to the more distal tubules with increasing amounts of injected activity.* In vivo *SPECT measurement of ^{99m}Tc-DMSA uptake was highly accurate to grade renal tubular damage after PRRT.*

Key words: PRRT, renal damage, [^{177}Lu-DOTA^0,Tyr^3]octreotate, ^{99m}Tc-DMSA, animal SPECT

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogs has become an important tool in the management of neuroendocrine tumors. Convincing results were found for both objective tumor response and quality of life.[1–4] During PRRT, using somatostatin analogs labeled with β-emitters, such as ^{90}Y and ^{177}Lu, usually the kidney is the dose-limiting organ.[5,6]

Although the major part of the radiopharmaceutical is excreted into the urine, the partial

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reabsorption in the tubular cells leads to a considerable radiation dose to the radiosensitive kidneys.[7,8] It was shown recently that the localization of the radiopeptide in the kidney is not homogeneous, but predominately in the cortex, where it forms a striped pattern, with most of the radioactivity centered in the inner cortical zone.[9] In the only study that included biopsies of human kidneys after PRRT, mainly thrombotic microangiopathy was found despite minor tubular atrophy and interstitial fibrosis.[10] In addition, it is known that PRRT can lead to radiation nephritis.[11,12] The precise mechanism of renal damage, however, is not fully known, nor has the localization of the most pronounced damage yet been identified.

The potential coherence of the inhomogeneous distribution of radioactivity in the kidney and the localization of damage is highly relevant, as a number of radionuclides potentially suitable for therapy are available. The beta-emitters, ^{90}Y, ^{177}Lu, and ^{131}I, are widely used for therapies with a number of vectors.[13,14] Several therapy studies were performed with the Auger-emitter, ^{111}In, and several new radionuclides, including alpha-emitters, are under investigation.[15,16] The different physical characteristics of these radionuclides result in a different tissue penetration range of the therapeutic particles and will, therefore, lead to a different distribution pattern of absorbed radiation dose in the kidney.[17]

The aim of this study was to investigate rat kidneys more than 100 days after injections of different amounts of [^{177}Lu-DOTA^0,Tyr^3]octreotate. The highest activity injected was intended to induce severe kidney damage. The kidneys were investigated with a number of methods: *in vivo* autoradiography, histologic analysis with different staining methods and measurement of serum creatinine to get a complete overview of function and morphology. As it is known that radiopeptides are absorbed partially in the tubular epithelial cells, *in vivo* single-photon emission computed tomography (SPECT) with ^{99m}Tc-DMSA (dimercaptosuccinic acid) as a marker for renal tubular damage,[18,19] were acquired with a dedicated animal SPECT camera (NanoSPECT Bioscan Inc.; Washington, DC., USA), that allows absolute *in vivo* quantification of renal ^{99m}Tc-DMSA-uptake.[20] The ^{99m}Tc-DMSA scintigrams were performed to evaluate the value of this tracer in the follow-up of renal function after PRRT in rats in order to develop a sensitive method to follow renal function over time.

MATERIALS AND METHODS

Animal experiments were performed in compliance with the regulations of the institution and with generally accepted guidelines governing such work.

Radiopharmaceuticals

[^{177}Lu-DOTA^0,Tyr^3]octreotate was synthesized and labeled as described previously.[21] The ^{99m}Tc-DMSA kit was purchased from GE Healthcare (Buckinghamshire, United Kingdom) and labeled according to the indicated procedure.

Animal Studies

Twenty-nine (29) young, male Lewis rats (Harlan; Horst, The Netherlands), with a body weight of 250–300 g, were divided into 3 groups. The control group consisted of 9 rats. Ten (10) rats were intravenously (i.v.) injected with 278 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate and 10 rats with 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate. In 20 rats (5 controls, 7 of the 278 MBq group and 8 of the 555 MBq group), SPECT scans with ^{99m}Tc-DMSA were acquired. Because renal damage is late toxicity, the scans were acquired between 109 and 146 days after the injection of ^{177}Lu-DOTA^0,Tyr^3]octreotate. The ^{99m}Tc-DMSA uptake in the kidneys was quantified, after which an autoradiogram of the ^{99m}Tc-DMSA uptake was performed in 6 rats (2 from each group). Kidneys from all animals were analyzed histologically.

Animal SPECT (NanoSPECT) and Software

SPECT imaging was performed with a four-headed multiplexing multipinhole NanoSPECT. Each head was outfitted with an application-specific tungsten collimator with 9 pinholes. For this study, we imaged with rat apertures that were comprised of a total of 36 2-mm diameter pinholes imaging a cylindrical field of view that was 60 mm in diameter by 24 mm in length. These rat apertures provided a reconstructed resolution below 1.6 mm at 140 keV, with an average sensitivity of 1100 cps/MBq across the field of view (FOV). The images were acquired in a step-and-shoot helical scan-mode, which allowed to image a defined range from 24 to 270 mm, according to the region to be imaged. The energy-peak for the camera was set at 140 keV. The window width was $\pm$10%. The

rats were scanned 4–6 hours after the injection of 50 MBq ^{99m}Tc-DMSA. An acquisition time of 30 seconds per projection was chosen, resulting in total acquisition times ranging from 6 to 9 minutes per animal. The data were reconstructed iteratively with the HiSPECT© software (Bioscan), a dedicated ordered subsets-expectation maximisation (OSEM) software package for multiplexing multipinhole reconstruction. The NanoSPECT was calibrated with a phantom, approximately of the size of the animals, filled with a known activity of ^{99m}Tc, such that voxel values in the reconstruction provided a proper estimate of the activity level without further calculation.

A volume of interest (VOI) was drawn manually around both kidneys; the three-dimensional (3D) activity distribution within the VOI was then summed to determine the uptake. Because of the favourable biodistribution of ^{99m}Tc-DMSA, limited to the kidneys, the VOI could be drawn generously to prevent partial-volume effects at the edges. All measured activities were corrected for decay and expressed as percent injected activity (%IA). The IA was determined by measuring the syringe in a dose-calibrator before and after injection of the animal. The difference was defined as the IA. The quantification of the VOI was performed with INTERVIEW XP© software (Mediso Ltd.; Budapest, Hungary). After imaging, the rats were sacrificed.

Autoradiography

In 6 animals (2 from each group), after euthanasia, 1 kidney was removed, quickly frozen on liquid nitrogen–cooled isopentane, and processed further for autoradiography. The tissue was embedded in TissueTek (Sakura; Zoeterwoude, The Netherlands) and processed for cryosectioning, as described previously.[22] Briefly, tissue sections (10-μm) were mounted on glass slides. The sections were exposed to SR phosphor imaging screens (Packard Instruments Co.; Canberra CT) for 1 day in radiographic cassettes. The screens were analyzed using a Cyclone phosphor imager

Table 1. Criteria for the Histological Kidney Damage Score

Grade	*Overview*	*Glomeruli*	*Tubules*
1	More or less normal aspect High cell count glomeruli	Apoptotic cells in the endothelium Inflammatory infiltrate	Apoptotic cells Rough protein staining Little dilated Normal BM No protein cylinders
2	Dilation of tubules Damaged tubule cells	Like grade 1	More apoptotic cells More pronounced dilation BM thickened Little protein cylinders in tubules Regenerating cells (mitotic activity)
3	Stronger dilated tubules Cell-rich infiltrate Regenerating tubules PAS: thickened BM PAS: protein cylinders	Vascular lumina smaller, few erythrocytes Sometimes shrinkage	Flat epithelium, partly total loss of epithelium Strong dilation Inflammatory infiltrate Regeneration present Protein cylinders More pronounced BM thickening
4	Heavily dilated tubules Heavily thickened BM Protein cylinders	Like Grade 3 More optical empty space owing to shrinkage of glomeruli	Like Grade 3, but more empty cylinders Periferal fibrosis

BM, basal membrane

and a computer-assisted OptiQuant 03.00 image processing system (Packard).

Histology

Immediately after removing them from the animal, the kidneys were fixed in 10% neutral buffered formalin, trimmed, and processed by standard techniques for embedding in paraffin. Four-micron (4-μm) sections were cut and stained with haematoxylin-eosin (HE) or periodic acid-Schiff reagent (PAS). The microscopic renal damage score (RDS) was graded, blinded to the treatment protocol ranging from 0 (no damage) to 4 (severe damage). The criteria for these grades are listed in Table 1. The PAS-stained sections were used for better differentiation between proximal and distal tubules.

Blood Analyses

At the day of sacrifice (134 $\pm$ 11 days after inclusion), blood samples were drawn by cardiac puncture from a total of 19 animals (6 controls, 6 of the 278 MBq group, and 7 of the 555 MBq group). Blood chemistry and hematological parameters were determined by standard hospital analysis procedures.

Statistics

To correlate the results, the Pearson's correlation coefficient was calculated. The Student's t test was used to test for significance of differences. A p-value ≤ 0.05 was considered significant.

RESULTS

The administration of [^{177}Lu-DOTA^0,Tyr^3]octreotate to the rats was straightforward. No acute discomfort was observed in the rats treated. After inclusion, the body weight of the rats from all the groups dropped slightly, by not more than 5%. After this initial decline, the body weight of the

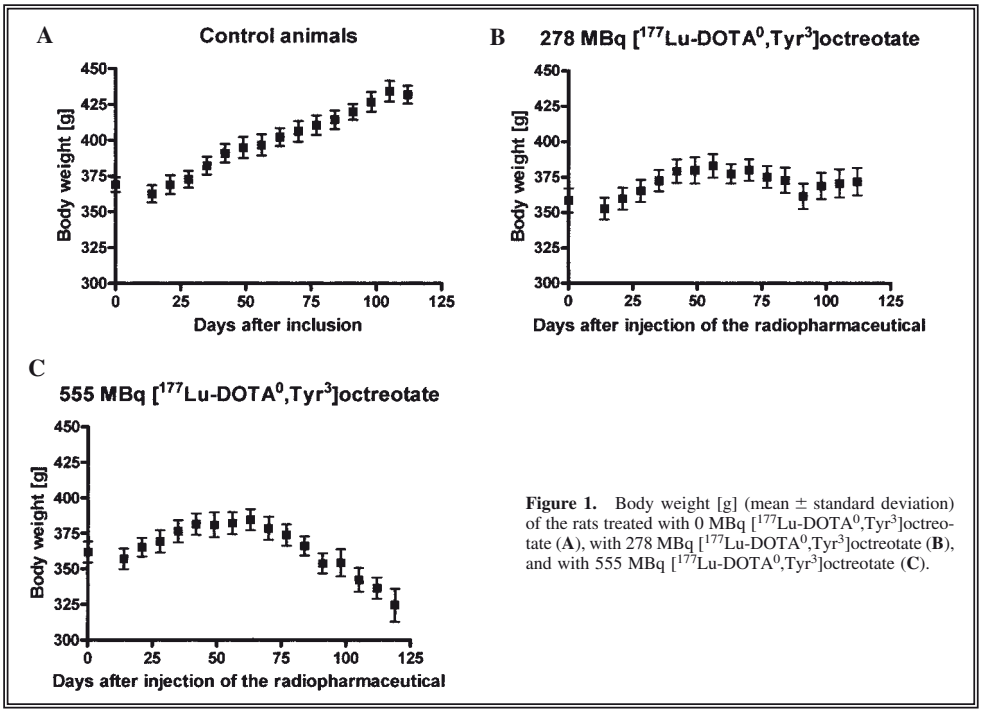

Figure 1. Body weight [g] (mean $\pm$ standard deviation) of the rats treated with 0 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate (**A**), with 278 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate (**B**), and with 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate (**C**).

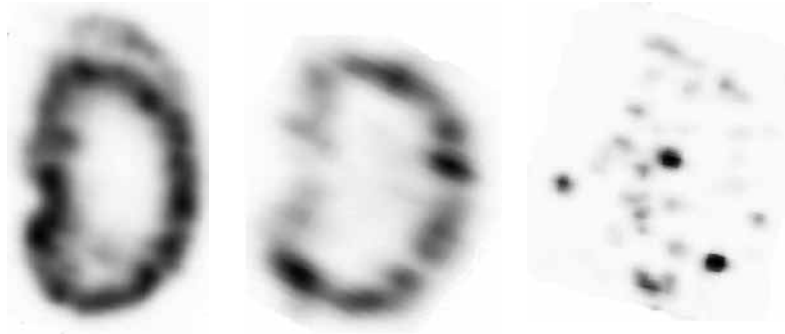

Figure 2. Coronal SPECT slices of rat kidneys acquired 4–6 hours after the injection of 50 MBq ^{99m}Tc-DMSA. The slices correspond to the kidneys in Figures 3 and 4. (**A**) is from a control animal, (**B**) is from an animal 115 days after therapy with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, and (**C**) is from an animal 109 days after therapy with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. SPECT, single-photon emission computed tomography; DMSA, dimercaptosuccinic acid.

control group rats increased continuously, as expected. In contrast, the body weight of the rats treated with 278 MBq initially increased slightly and then remained stable, whereas the body weight of the rats treated with 555 MBq initially increased and then dropped approximately 70 days after PRRT (Fig. 1A–1C).

By SPECT with ^{99m}Tc-DMSA, in all rats, both kidneys could be visualized, although kidneys in the group injected with 555 MBq were only faintly visible. The spatial resolution of the images was high, with a spatial resolution below 1.6 mm. Differentiation between functional parenchyma, characterized by tracer accumulation and the cold regions indicative of the renal pelvis, was easily determined (Fig. 2A–C). The renal uptake of ^{99m}Tc-DMSA was significantly different between the 3 groups (all $p < 0.01$). The mean values $\pm$ standard deviation (SD) were 23.2 $\pm$ 1.2 %IA for the control group, 9.9 $\pm$ 6.3 %IA for the

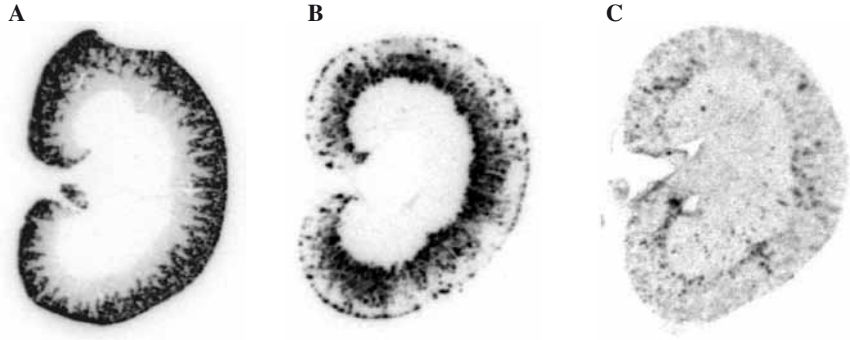

Figure 3. Renal autoradiograms after an *in vivo* injection of 50 MBq ^{99m}Tc-DMSA. The rats were sacrificed 6 hours after injection. (**A**) shows the autoradiogram of a control animal with a normal radioactivity distribution. (**B**) shows the autoradiogram of an animal 115 days after therapy with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. (**C**) shows the autoradiogram of an animal 109 days after therapy with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. The images correspond to the kidneys in Figures 2 and 4. DMSA, dimercaptosuccinic acid.

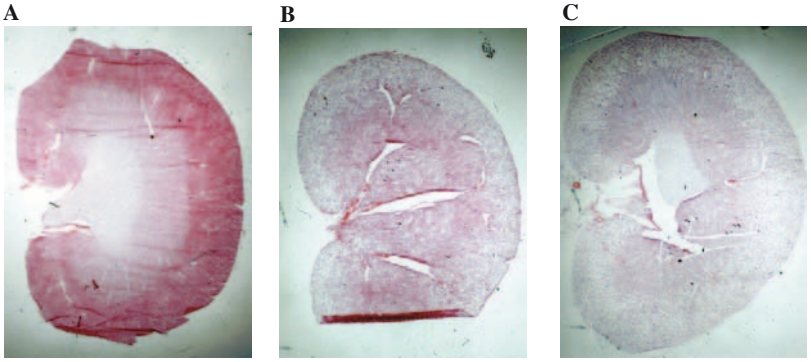

Figure 4 (A–C). Low-power overviews (3×) of coronal histologic slices of rat kidneys, stained with hematoxylin and eosin. The slices correspond to Figures 2 and 3.

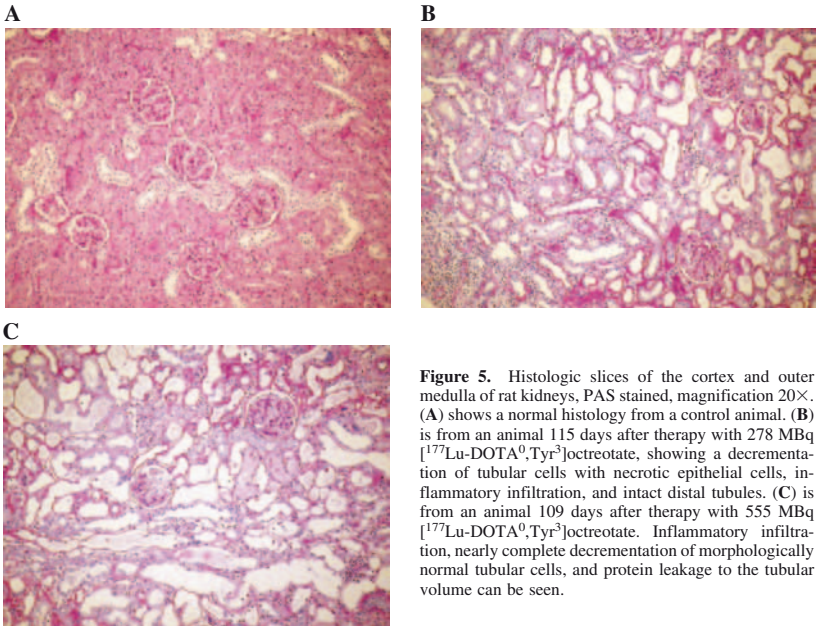

Figure 5. Histologic slices of the cortex and outer medulla of rat kidneys, PAS stained, magnification 20×. (**A**) shows a normal histology from a control animal. (**B**) is from an animal 115 days after therapy with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, showing a decrementation of tubular cells with necrotic epithelial cells, inflammatory infiltration, and intact distal tubules. (**C**) is from an animal 109 days after therapy with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Inflammatory infiltration, nearly complete decrementation of morphologically normal tubular cells, and protein leakage to the tubular volume can be seen.

group injected with 278 MBq, and 1.4 ± 0.5 %IA for the group injected with 555 MBq.

Figure 3A–C shows examples of the autoradiograms with ^{99m}Tc-DMSA from rat kidneys after treatment with 0, 278, and 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate. Figure 3A shows a normal distribution of ^{99m}Tc-DMSA in a control rat, with a high accumulation in the renal cortex. In contrast, in Figure 3C, showing an autoradiogram of a rat treated with 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate, hardly any ^{99m}Tc-DMSA uptake can be seen, indicating a severely damaged tubular function. Figure 3B shows an autoradiogram of a rat treated with 278 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate. Here, intermediate renal ^{99m}Tc-DMSA uptake was found. The distribution pattern of the radioactivity was obviously different, compared to that of the control rat. We found a "shift" of the radioactivity from the cortex to the outer medulla. The corresponding SPECT scans are displayed in Figure 2A–C. A very positive match between SPECT and autoradiograms was found, underlining the high accuracy of the SPECT images.

In the third row (Figure 4A–C), the corresponding histologic HE-stained overview images of adjacent slices of that providing the autoradiograms are shown. A dose-dependent loss of eosinophile cytoplasm can already be seen in the low-power (3×) overview.

The detailed histology showed, as expected, no significant abnormalities in the control rats. One (1) kidney was scored with a damage score of 2, whereas all other kidneys did not show any damage and were scored 0. An example of a histologic, PAS-stained slice of a control rat is shown in Figure 5A.

In the rats treated with 555 MBq, a detailed histology revealed intense changes in the proximal and distal tubules [^{177}Lu-DOTA^0,Tyr^3]octreotate. A mixed picture with inhomogeneous nuclei, apoptotic, and necrotic cells was found (Figure 5C). In all kidneys of this group, we found extensive protein leakage into the tubules and collecting tubes. Furthermore, interstitial nephritis with inflammation of the cells was found. The glomeruli, however, showed no, or only very mild, changes. Based on the criteria given in Table 1, all kidneys of the rats treated with 555 MBq were histologically scored as having Grade 4 damage.

The rats treated with 278 MBq showed severe histologic damage as well. The proximal tubules, especially, were heavily damaged with atrophy, dilatation, apoptotic nuclei, and necrosis. However, in contrast to the kidneys of the rats that were treated with 555 MBq, there was a notable number of tubules that did not show histologic damage. The PAS staining revealed that these were mainly distal tubules, located in the outer medulla. In addition to the tubular damage, signs of interstitial nephritis with inflammation of the cells were found as well (Figure 5B). The tubular damage was accentuated in the cortex, which reflects the uptake of ^{99m}Tc-DMSA very well. The histologic scoring resulted in one Grade 2 score, one Grade 3 score, and eight Grade 4 scores. Thus, regarding only the histologic score, no significant difference to the group treated with 555 MBq was found ($p = 0.18$).

At the day of sacrifice, a blood sample was drawn by cardiac puncture to measure the serum creatinine values. The results (mean ± SD) were 36.5 ± 17.5 μmol/L in the control group, 129.7 ± 79.9 μmol/L in the group injected with 278 MBq, and 425.3 ± 219.2 μmol/L in the group injected with 555 MBq. All differences between all groups were significant ($p < 0.05$) (Fig. 6). Furthermore, a significant ($p < 0.01$) correlation was found between the creatinine values and the %IA determined by SPECT.

DISCUSSION

High-dose PRRT could cause severe renal damage in rats as well as in humans because of the radiation-absorbed dose to the kidney during

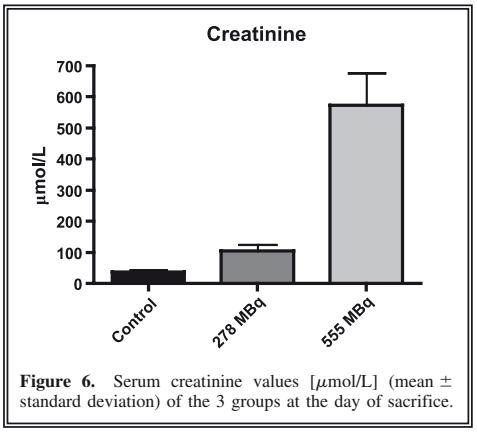

Figure 6. Serum creatinine values [μmol/L] (mean ± standard deviation) of the 3 groups at the day of sacrifice.

therapy.[5,8,10,12] Recently, a number of new drugs were introduced with the potential to reduce renal toxicity during PRRT, but the effectiveness of these drugs during PRRT remains to be proven in patients.[23–26] What is worthwhile to highlight are the studies with amifostine, because this was the first drug investigated for PRRT that did not aim at reducing the renal uptake, but which acted as a radical scavenger to systemically reduce the toxic effects of the radiation. Because amifostine acts by a completely different mechanism, a combination with drugs that reduce the renal uptake appears promising.[23]

To improve kidney protection during PRRT, it is important to understand the mechanism of renal damage. One step toward a better understanding could be close monitoring of kidney function after PRRT *in vivo* and over time. The newly available dedicated small-animal gamma cameras offer the possibility to investigate physiologic processes in the same animal over time. However, a tracer, one that is easily available for daily routine, needs to be defined. The relation that was found between ^{99m}Tc-DMSA uptake and serum creatinine values, as well as the relation between ^{99m}Tc-DMSA uptake and the injected activity of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, indicate that ^{99m}Tc-DMSA is an accurate marker for renal function after PRRT in these animals.

The only work containing histologic data from human kidneys after PRRT reports, despite minor fibrosis and tubular atrophy, is mainly thrombotic microangiopathy (involving glomeruli, arterioles, and small arteries). These pathologic changes were comparable to the changes found after external beam radiation, when the kidney is within the field of radiation. The data for this study were generated by investigating kidney biopsies of patients after treatment with DOTATOC labeled with ^{90}Y, a high-energy beta-emitter.[10]

Recently published data showed that the highest concentration of radiolabeled peptides in rat and in human kidneys was found in the proximal tubular cells.[9,22] The multiligand scavenger receptor, megalin, appeared to play a crucial role for the reabsorption of radiopeptides into the tubular cells.[27] Using the high-energy beta-emitter ^{90}Y, emitting beta particles with a maximum energy of 2.27 MeV, will result in a fairly homogenous energy distribution over the whole kidney, including a high radiation-absorbed dose to the glomeruli.[28] For this study ^{177}Lu was used, emitting beta particles with a maximum energy of 0.50 MeV, which results in a significant lower-tissue penetration range of these particles and a different energy distribution over the kidney. Taking into account the space between tubules and glomeruli as well as microdosimetric aspects, a lower radiation-absorbed dose to the glomeruli when using ^{177}Lu, compared to ^{90}Y, can be expected. It was calculated recently that other radiolanthanides with even lower energy beta particles could improve energy distribution further.[16] Recently, several articles were published using alpha-emitters for internal radiation therapy.[28–30] After the administration of ^{213}Bi-labeled DOTATOC to Lewis rats, no histologic changes were observed in kidney glomeruli and tubules. As a consequence of the treatment with 22.2 MBq of ^{213}Bi-DOTATOC, a merely mild interstitial nephritis was observed. It is very likely that the physical characteristics of the radionuclide that was used might have had a strong influence on kidney damage and might be one of the reasons why no histologic changes in the glomeruli were found in this study using ^{177}Lu.

The estimated radiation-absorbed dose to the kidney is high after a single-dose administration of 278 or 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in rats. Dosimetric calculations showed that injecting these activities into rats resulted in doses to the cortex of approximately 46 and 92 Gy, respectively, and approximately 35 and 70 Gy, respectively, to the whole kidney.[17] For the treatment of patients, a maximum tolerated dose to the kidneys of 23 Gy is generally accepted, although this value is derived from external beam radiation, dealing with different properties, especially concerning the dose rate and energy distribution within the kidney.[6] Taking into account the potential dose reduction by the coinfusion of amino acids, 278 MBq of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate would result in approximately 23 Gy.

The results of this rat study strongly suggest that late renal damage after high-dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate therapy is mainly tubular. This is supported by the results of the ^{99m}Tc-DMSA studies, being a marker for the renal tubular function. The dose-dependent reduction of ^{99m}Tc-DMSA uptake in the rat tubules suggests that [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate provokes a dose-dependent tubular damage.

CONCLUSIONS

In conclusion, ^{99m}Tc-DMSA appears to be a good marker to quantify the extent of damage after

PRRT. Using ^{99m}Tc-DMSA in combination with a dedicated small-animal gamma camera will allow for the following of renal function after PRRT in animals over time.

The localization and extent of damage, respectively, after PRRT was found to be dose dependent. Whereas in rats treated with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate kidney damage was found to be mainly in the proximal tubule, higher injected radioactivities resulted in a decline of morphologically undamaged tubules.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

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Peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogues was proven to be a well tolerated, effective treatment for patients with metastatic, somatostatin receptor positive neuroendocrine tumors [1-8]. An overview of the current status as well as some perspectives is given in **chapter 1.** In **chapter 2a** a clinical study including 116 patients treated with ^{90}Y-DOTATOC demonstrates exemplary the effectiveness as well as potential toxicity of PRRT with ^{90}Y-DOTATOC. In general, the treatment is well tolerated and side-effects are mostly rare and transient. The kidney is the dose-limiting organ in most studies, especially in all studies with ^{90}Y labeled somatostatin analogs. Recently the bone marrow was identified as the dose limiting organ in 70% of the patients that are treated with ^{177}Lu-DOTATATE (personal communication, D.J. Kwekkeboom). Although PRRT with radiolabeled somatostatin analogues can be regarded as the treatment of choice for patients with metastatic, somatostatin receptor positive neuroendocrine tumors most of the patients relapse after a certain time. For this situation we demonstrated that re-treatment is feasible and effective with tolerable toxicity in the case of relapse [9]. This is shown in a clinical study in **chapter 2b**. Interestingly a good response after the first treatment was identified as a positive predictive factor for a good response after the second treatment. Although the effectiveness of PRRT was proven with regard to tumor load, quality of life as well as overall survival, certain limitations still arise. For example it remains unclear which somatostatin analogue is most suitable for treatment. In **chapter 3a** five patients with somatostatin receptor positive tumors were studied with two different peptides. Three out of these five showed a better tumor-to-kidney-ratio with ^{111}In-DOTATOC whereas one showed a better tumor-to-kidney-ratio with ^{111}In-DOTATATE. Somewhat different results were found by Esser and colleagues who compared ^{177}Lu-DOTATOC with ^{177}Lu-DOTATATE [10]. In this study only one out of 7 patients had a more favorable tumor-to-kidney-ratio with DOTATOC. Beside other possible explanations, one reason for these differences might be that neuroendocrine tumors are a highly heterogeneous group of malignancies, showing different profiles of receptor expression. The slightly diverse affinity profiles of DOTATOC and DOTATATE might, depending on the receptor expression on the tumor, result in different tumor-to-kidney-ratios.

Currently a vast number of different somatostatin analogs with different affinity profiles are available [11]. Individualized treatment planning with patient specific dosimetry might help to improve PRRT. Currently this is not feasible since the time and effort needed for one patient are vastly to big. In addition, many difficulties have to be overcome in internal dosimetry. Some of the difficulties as well as possible solutions are shown in **chapter 3b** exemplary for bone marrow dosimetry. It is obvious that currently no fully accurate dosimetry can be performed. Using diverse methods, huge differences in the calculated absorbed doses were found [12]. Additionally, a number of other factors, like e.g. the dose rate which a lot lower in nuclear medicine therapies compared to external beam radiation, are not fully understood and might influence the effects of the radiation treatment. Another role of dosimetry could be to predict the hematological response after PRRT. However, no correlation was found between the calculated absorbed dose to the red marrow and the drop in platelets after therapy. It seems are that e.g. the influence of factors like cytokines or pre-treatments with other potentially toxic drugs are important, but not many studies in this field have been performed as yet. More studies are needed to improve internal dosimetry further in order to achieve more reliable results and to be able to predict benefit and toxicity more precisely. Nevertheless, dosimetry, in particular pre-therapeutic dosimetry would be desirable to improve PRRT as well as to improve the understanding of physiological processes after PRRT.
Especially with regard to radiation-biological processes animal studies can help to improve the understanding. In **chapter 4a** we showed that it is possible to determine accurately *in vivo* the absolute radioactivity with a dedicated small animal SPECT camera. This will allow to

perform accurate in vivo dosimetry and to follow the same animal to study the corresponding late toxicity [13].
In general, true *in vivo* investigations enabled by small animal imaging allows following one animal over time and to study different functions over time. This appears to be of particular interest in therapy studies since side effects are often late toxicity whereas e.g. dosimtery has to be performed during therapy. By investigating the same animal a bias between the different groups can be excluded. In addition, small animal imaging will help to reduce the number of animals that is needed to answer a particular question. In the long turn this will safe costs – notably when genetically engineered animals are used - as well as it will reduce disputes from an ethical point of view.
Currently, mainly the absorbed radiation dose to the kidneys is the dose limiting factor for PRRT with radiolabeled somatostatin analogues [1]. Co-infusion of cationic amino acids became a standard procedure during PRRT since it was shown that an amino acid co-infusion can reduce the kidney uptake by up to 50 % [14]. Lately, several new strategies to decrease renal toxicity further have been developed [15-17]. It remains unclear whether these new methods will replace the amino acid co-infusion or if they can be applied together with an amino-acid co-infusion in order to achieve an additional effect.
To improve the kidney protection further it is important to understand the mechanism of damage during PRRT better. In **chapter 4b** the localization of the damage was analyzed with different methods: *In vivo* ^{99m}Tc-DMSA SPECT, histology with different staining, and biochemical analyzes. A clear dose dependency of the damage could be demonstrated. Additionally we found indications that the damage starts in the proximal tubules when lower amounts of radioactivity are applied. During high dose treatments the damage appears to be more extensive, involving the distal tubules as well. Furthermore we could prove that ^{99m}Tc-DMSA is a highly valuable tracer to grade renal damage after PRRT in rats. E.g. we found a very good correlation between the ^{99m}Tc-DMSA-uptake and the 1/creatinine value. This finding of ^{99m}Tc-DMSA being a valuable tracer to quantify kidney damage after PRRT will have impact on further preclinical studies on kidney protection during PRRT. With all the studies we performed in rats with ^{99m}Tc-DMSA, we established rough normal values for ^{99m}Tc-DMSA in rats. In the future it will be possible to compare kidney function quantitatively in different rats and longitudinally or even in rats from different studies.
It will be interesting to investigate whether ^{99m}Tc-DMSA could be a valuable tracer in patients too. In comparison to patients [18] we did not find any glomerular damage in rats. However, since the patient study was performed with ^{90}Y and the rat study with ^{177}Lu the question arises if physical characteristics of the radionuclide might be responsible for the different localization of the damage. Recent studies in micro-dosimetry revealed significant differences in the dose distribution when different radionuclides were used [19]. It appears that the most suitable radionuclide for PRRT is not defined as yet.

Conclusions

Peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogues is the treatment of choice for patients with metastatic, neuroendocrine, somatostatin receptor positive tumors. The treatment is generally well tolerated and the toxicity is low. Re-treatment after a standard therapy is feasible; however, the dose limiting organ is usually the kidney that will make further therapies impossible at some point. Many different somatostatin analogues with somewhat diverse affinity profiles for the somatostatin receptor subtypes are available. The most suitable peptide for PRRT in neuroendocrine tumors still remains to be defined. It appears that in different patients different peptides might have more favorable characteristics.

Unfortunately, accurate dosimetry is difficult and an individual, pre-therapeutic dosimetry with different peptides is currently not feasible.
Many new approaches are currently investigated with the goal to improve PRRT, e.g. new agents to protect the kidneys or new peptides with improved characteristics. Newly developed, dedicated small animal SPECT/CT cameras with sub-millimeter resolution allow performing real *in vivo* studies on a pre-clinical level. This will help to design studies in a setting that is much closer to the situation as it is given in patients. The possibility to reliably quantify activity *in vivo* gives the opportunity to follow an animal over time and to investigate different physiological functions in the same animal. Among other methods the small animal SPECT/CT helped to localize the damage in the kidney after high dose PRRT more precisely. This might have consequences for the design of new peptides as well as for the planning of further studies.

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SAMENVATTING EN CONCLUSIES

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Peptide-receptor-radionuclidentherapie (PRRT) met radioactief gelabelde somatostatine analoga heeft bewezen een goed te verdragen, effectieve behandeling te zijn voor patiënten met gemetastaseerde somatostatinereceptor-positieve neuroendocriene tumoren [1-8]. Een overzicht van de huidige status alsmede enige toekomstperspectieven worden weergegeven in **hoofdstuk 1.** In **hoofdstuk 2a** wordt een klinische studie beschreven van 116 met ^{90}Y-DOTATOC behandelde patiënten die duidelijk zowel de effectiviteit als ook de mogelijke toxiciteit van PRRT met ^{90}Y-DOTATOC weergeeft. De behandeling is in het algemeen goed te verdragen en bijwerkingen zijn zeldzaam en van voorbijgaande aard. In de meeste studies is de nier het orgaan dat de dosislimiet bepaalt, met name in alle studies met ^{90}Y gelabelde somatostatine analoga. Recent is vastgesteld dat bij 70% van met ^{177}Lu-DOTATATE behandelde patiënten het beenmerg mede de dosislimiet bepaalde (persoonlijke mededeling van D.J. Kwekkeboom). Hoewel PRRT met radiogelabelde somatostatine analoga gezien wordt als voorkeursbehandeling voor patiënten met gemetastaseerde somatostatinereceptor-positieve neuroendocriene tumoren, krijgen de meeste patiënten na zekere tijd een terugval vanwege groei van de tumor(en). Voor dergelijke patienten geval tooonden wij aan dat een voortgaande behandeling mogelijk en effectief is met een toelaatbare toxiciteit [9]. Dit wordt aangetoond in een klinische studie beschreven in **hoofdstuk 2b**. Van belang is dat een goede reactie op de eerste behandeling gezien kan worden als een positief voorspellende factor voor een goede reactie na een tweede behandeling. Hoewel de effecten van PRRT gunstig zijn met betrekking tot tumorregressie, kwaliteit van leven en overleving, blijven er nog zekere beperkingen over. Het blijft bijvoorbeeld onduidelijk welk somatostatine analoog het meest geschikt is voor behandeling. In **hoofdstuk 3a** wordt de behandeling met twee verschillende peptiden bij vijf patiënten beschreven. In drie van deze vijf patiënten blijkt een betere tumor/nier ratio gevonden te worden met ^{111}In-DOTATOC terwijl ^{111}In-DOTATATE in één patiënt een betere tumor/nier ratio geeft . Iets andere resultaten werden gevonden door Esser en collega's die ^{177}Lu-DOTATOC met ^{177}Lu-DOTATATE vergeleken [10]. In deze studie gaf DOTATOC maar in één van de zeven patiënten een betere tumor/nier ratio. Deze verschillen kunnen veroorzaakt zijn door het feit dat neuroendocriene tumoren behoren tot een in hoge mate heterogene groep maligniteiten met verschillende profielen van receptorexpressie. De enigszins verschillende affiniteitsprofielen van DOTATOC en DOTATATE kunnen, afhankelijk van de receptorexpressie van de tumor, verschillen in tumor/nier ratio opleveren.

Op dit moment is er een groot aantal verschillende somatostatine analoga met onderscheidend affiniteitsprofiel beschikbaar [11]. Een op het individu gericht behandelplan met een patiëntgerichte dosimetrie kan helpen de PRRT te verbeteren. Momenteel is dit nog niet haalbaar vanwege de te investeren tijd per patiënt, bovendien moeten nog veel problemen bij het bepalen van de interne dosimetrie overwonnen worden. Sommige problemen alsmede mogelijke oplossingen typisch voor beenmergdosimetrie worden in **hoofdstuk 3b** beschreven. Het is onmiskenbaar dat op dit moment geen volledig nauwkeurige dosimetrie gedaan kan worden. Bij de verschillende gebruikte methoden zijn er grote verschillen in de berekende geabsorbeerde doses gevonden [12]. Bovendien is een aantal andere factoren die de effecten van een bestralingsbehandeling kunnen beinvloeden, zoals bijvoorbeeld het dosistempo van de ioniserende straling die bij therapieën in de nucleaire geneeskunde een stuk lager is dan bij uitwendige bestraling, niet volledig bekend. Een andere rol voor dosimetrie ligt in de voorspelling van de hematologische reactie na PRRT. Er is echter geen correlatie gevonden tussen de berekende geabsorbeerde dosis en de achteruitgang in trombocytenaantal na therapie. Het lijkt dat de invloed van bijvoorbeeld cytokines en voorbehandeling met andere beschikbare chemotherapeutica van belang zijn, maar er zijn nog niet veel studies gedaan op dit gebied. Er zijn meer studies nodig om goed gebruik te kunnen maken van interne dosimetrie teneinde meer betrouwbare resultaten te verkrijgen waardoor we in staat zijn om de voordelen en toxiciteit nauwkeuriger te voorspellen. Niettemin, dosimetrie, in het

bijzonder pre-therapeutische dosimetrie, zou een welkome aanvulling zijn om PRRT te verbeteren alsmede om meer inzicht te verkrijgen in het fysiologische proces na PRRT.
Speciaal met betrekking tot de stralingsbiologische processen kan onderzoek met proefdieren helpen om de inzichten te vergroten. In **hoofdstuk 4a** laten we zien dat het *in vivo* mogelijk is om zeer nauwkeurig de absolute hoeveelheid radioactiviteit vast te stellen met behulp van een specifieke SPECT camera, voor kleine dieren. Deze zorgt er voor dat in een dier nauwkeurig de *in vivo* dosimetrie bepaald kan worden en dat tevens hetzelfde dier gevolgd kan worden voor het vastleggen van toxiciteit na verloop van tijd [13].
In het algemeen worden waardevolle *in vivo* onderzoeken verkregen met "small animal imaging" door het volgen van een enkel dier en de verschillende functies in de tijd te bestuderen. Dit blijkt van bijzonder belang te zijn bij therapiestudies, omdat neveneffecten vaak op langere termijn toxiciteit opleveren. Dit betekent dat bijvoorbeeld dosimetrie bepaald moet worden tijdens de therapie. Door hetzelfde dier te onderzoeken kan een afwijking tussen de verschillende groepen worden uitgesloten. Daarbij zal de "small animal imaging" het aantal dieren, dat nodig is om een antwoord te verkrijgen op een specifieke vraag, helpen verminderen. Uiteindelijk zal dit kostenbesparend werken - met name wanneer er genetisch gemodificeerde dieren worden gebruikt - en ook zal het de discussies vanuit een ethisch standpunt beperken.
Op dit moment is hoofdzakelijk de geabsorbeerde stralingsdosis in de nieren de beperkende factor bij PRRT met radioactief gelabelde somatostatine analoga [1]. Een infuus met aminozuren is een standaardprocedure bij PRRT, omdat is aangetoond dat een gelijktijdig gegeven infuus met aminozuren de opname in de nieren met 50% kan reduceren [14]. Onlangs zijn er verscheidene nieuwe strategieën ontwikkeld om de toxiciteit in de nier te verminderen [15-17]. Het lijkt nog onduidelijk welke van deze nieuwe methoden de plaats zal innemen van het aminozuurinfuus of dat ze samen toegediend kunnen worden om een aanvullend effect te verkrijgen.
Om de nierbescherming verder te verbeteren is het belangrijk om het mechanisme van de schade door PRRT beter te begrijpen. In **hoofdstuk 4b** is de locatie van de schade geanalyseerd met verschillende methoden: *in vivo* ^{99m}Tc-DMSA SPECT, histologie met verschillende kleuringen en biochemische analyses. Een duidelijke dosisafhankelijkheid van de schade kon worden vastgesteld. Daarbij vonden we aanwijzingen dat de schade in de proximale tubuli al begint wanneer lagere hoeveelheden radioactiviteit worden toegediend. Schade bij behandelingen met hoge doses blijkt meer intensief te zijn; ook de distale tubuli lopen dan schade op. Verder konden we bewijzen dat ^{99m}Tc-DMSA een zeer waardevolle tracer is om de graad van nierschade in ratten te bepalen na PRRT. We vonden bijvoorbeeld ook een erg goede correlatie tussen de ^{99m}Tc-DMSA-opname en de 1/creatinine waarde. Deze bevinding van ^{99m}Tc-DMSA als een waardevolle tracer om nierschade te kwantificeren na PRRT, zal van betekenis zijn voor verdere preklinische studies op het gebied van nierbescherming tijdens PRRT. Met alle studies die we met ^{99m}Tc-DMSA in ratten hebben gedaan, hebben we grofweg de normale waarden voor ^{99m}Tc-DMSA nieropname in ratten vastgesteld. In de toekomst zal het mogelijk zijn om de nierfunctie kwantitatief in verschillende ratten te vergelijken of zelfs in ratten uit verschillende studies.
Het zal interessant zijn om te onderzoeken of ^{99m}Tc-DMSA ook een waardevolle tracer is voor patiënten. In vergelijking met patiënten [18] vonden we geen glomerulaire schade in ratten. Omdat de patiëntenstudie is uitgevoerd met ^{90}Y en de rattenstudie met ^{177}Lu doet zich echter de vraag voor of fysische eigenschappen van het radionuclide verantwoordelijk kunnen zijn voor de verschillen in locatie van de schade. Recente onderzoeken in micro-dosimetrie wezen uit dat er significante verschillen zijn in dosisverdeling wanneer er verschillende radionucliden worden gebruikt [19]. Het blijkt dat het meest geschikte radionuclide voor PRRT nog niet is gedefinieerd.

Conclusies

Peptide receptor radionuclide therapie (PRRT) met radioactief gelabelde somatostatine analoga is de voorkeursbehandeling voor patiënten met gemetastaseerde neuroendocriene somatostatinereceptor-positieve tumoren. De behandeling is in het algemeen goed te verdragen en de toxiciteit is laag. Opnieuw behandelen na de standaardtherapie is mogelijk; echter het orgaan dat de dosislimiet bepaalt, meestal de nier, maakt verdere therapie op een gegeven moment onmogelijk. Veel verschillende somatostatine analoga met enigszins diverse affiniteitsprofielen voor somatostatine-receptorsubtypen zijn beschikbaar. Welk peptide het meest geschikt is voor PRRT bij neuroendocriene tumoren moet nog verder bepaald worden. Het blijkt dat bij verschillende patiënten verschillende peptiden meer of minder gunstige eigenschappen kunnen hebben. Jammer genoeg is nauwkeurige dosimetrie moeilijk en een individuele dosimetrie voor therapiebehandeling met verschillende peptiden op dit moment niet mogelijk.
Vele nieuwe manieren van aanpak worden op dit moment onderzocht met als doel de PRRT te verbeteren, bijvoorbeeld nieuwe middelen om de nieren te beschermen of nieuwe peptiden met verbeterde eigenschappen. Een recente ontwikkeling is het gebruik van de “kleine dieren” SPECT/CT camera’s met submillimeterresolutie die de mogelijkheid geven echt *in vivo* studies op preklinisch niveau te doen. Dit zal helpen om onderzoek op te zetten in een situatie die veel meer op die van patiënten lijkt. De mogelijkheid om de radioactiviteit *in vivo* betrouwbaar te kwantificeren geeft de gelegenheid om een dier te volgen in de tijd en de verschillende fysiologische functies in hetzelfde dier te onderzoeken. Tezamen met andere methoden hielp de small animal SPECT/CT om de schade in de nier nauwkeuriger te lokaliseren na een hoge dosis PRRT. Dit gegeven kan gevolgen hebben voor zowel het ontwerpen van nieuwe peptiden als voor de planning van verdere onderzoeken.

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CHAPTER 7

ACKNOWLEDGEMENTS, CURRICULUM VITAE, LIST OF PUBLICATION

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Dozens of people have contributed with a lot of effort to this thesis. Without their help, this work would never have become a thesis. It is virtually impossible to mention everybody by name. The work was done over the last years and at two different institutes involving many people. It is often a small thing that somebody helps you with, but very often these small things are essential for the progress of the work. Therefore, first of all I would like to thank all the people from the departments of Nuclear Medicine in Rotterdam and Basel as well as all those who collaborated with us for their expert help.

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The thesis consists among other parts of several manuscripts that were published over the last years. My thanks go to all co-authors who contributed valuable work in order to publish all these manuscripts.

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Curriculum Vitae

The author of this thesis was born on November 28th, 1973 in Basel. He graduated (Matura) from high school (Mathematisch- Naurwissenschaftliches Gymnasium, Basel) in 1993. In the same year he started medical school at the University of Basel. Graduation from University was achieved in 1999. In 2000 the promotion to a Medical Doctor with the thesis "Schizophrenie in der Frankfurter Allgemeinen Zeitung" was obtained (Promotor: Prof. Dr. A. Finzen). From 1999 till the end of 2000 he worked in the Department of Surgery in the Hospital of Aarberg, Switzerland (Head: Dr. C. Kleiber). In 2001 the specialisation at the University Hospital Basel in Nuclear Medicine was started (Head: Prof. Dr. J. Müller-Brand). The specialisation (Nuclear Medicine FMH) was obtained in 2006.

From 2003 till 2005 several studies contributing to this thesis were performed in close collaboration with the Division of Radiological Chemistry at the University Hospital Basel. The head of Radiological Chemistry is Prof. Dr. H.R. Maecke who is co-promotor of this thesis.

From 2005 till 2006 the author worked as research fellow in the pre-clincal group of the Department of Nuclear Medicine at the Erasmus MC in Rotterdam (Head: Prof. Dr. E. P. Krenning). The PhD studies were performed under the supervision of the promoter of this thesis Prof. Dr. M. de Jong.

Since January 2007 he is working as a senior physician again at the Department of Nuclear Medicine of the University Hospital Basel in Switzerland.

List of Publications

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2007 Forrer F, Rolleman E, Bijster M, Melis M, Bernard B, Krenning EP, de Jong M. From Outside to Inside? Dose dependent Renal Tubular Damage after high-dose Peptide Receptor Radionuclide Therapy in Rats measured with ^{99m}Tc-DMSA. Cancer Biother Radiopharm. 2007;22:40-9.
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1998 Forrer F, Mannhart C, Held T, Marti B. Comparison of measurement of skinfolds and foot-to-foot-bioimpedance-device to estimate body fat content of variable trained men and women. Swiss Journal of sports medicine and sports traumatology 1998;46:103-108
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Book Contribution

2006 Flavio Forrer and Marion de Jong. Encyclopedic Reference of Imaging; Receptor studies, neoplasms. Springer-Verlag GmbH, Heidelberg, Germany

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2007	Rolleman EJ, Forrer F, Deckers J, de Groot H, Valkema R, de Jong M, Krenning EP. Anaphylactoid reaction from amifostine. Radiother Oncol. 2007;82:110-1. Impact Factor: 4.0
2005	Forrer F, Mueller-Brand J, Maecke H. Pre-therapeutic dosimetry with radiolabelled somatostatin analogues in patients with advanced neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2005 Apr;32(4):511-2 Impact Factor: 4.0

Awards

2006	Award for the Top Clinical Abstract Submission from a Young Investigator at the Annual Meeting of the Academy of Molecular Imaging 2006
2005	Winner of a poster price at the Life Beyond NHL: Expert Investigator Forum 2006
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