KIDNEY PROTECTION DURING PEPTIDE RECEPTOR RADIONUCLIDE THERAPY

Edgar Johannes Rolleman

The described research in this thesis was performed at the Department of Nuclear Medicine, Erasmus MC, Rotterdam, The Netherland (head prof.dr. E.P.Krenning).

ISBN: 978-90-9021846-5

Lay-out and translation: Thera Rolleman
Cover: Reclamestudio Adri Edenburg

Printed by: Haveka, Alblasserdam, The Netherlands.

KIDNEY PROTECTION DURING PEPTIDE RECEPTOR RADIONUCLIDE THERAPY

NIERBESCHERMING TIJDENS PEPTIDE RECEPTOR RADIONUCLIDE THERAPIE

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor
Promoties.

De openbare verdediging zal plaatsvinden op
14 juni 2007 om 11.00 uur.

door

Edgar Johannes Rolleman
geboren te Rotterdam

PROMOTIECOMMISSIE

PROMOTOREN:	Prof.dr. E.P. Krenning
	Prof.dr.ir. M. de Jong
OVERIGE LEDEN:	Prof.dr.ir. T.J. Visser
	Prof.dr. W. Weimar
	Prof.dr. S. Pauwels

Hem komt de eer toe, die door de kracht die in ons werkt bij machte is oneindig veel meer te doen dan wij vragen of denken (Ef. 3:20-21).

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Edgar J. Rolleman, Roelf Valkema, Eric P. Krenning and Marion de Jong

Cancer is a leading cause of morbidity and mortality in the modern world. Treatment modalities comprise radiation therapy, surgery, chemotherapy and hormonal therapy. In the recent past new treatment modalities have been explored using new knowledge of molecular characteristics and processes in the tumour. One of these methods is peptide receptor radionuclide therapy (PRRT) [1], that takes advantage of high expression of specific receptors on certain tumour cells [2-4]. This strategy has been explored using radiolabelled somatostatin analogues for treatment of somatostatin receptor-positive tumours, such as neuro-endocrine tumours. Promising anti-tumour results were obtained. However, soon after the start of clinical studies, concern has risen on the kidney absorbed radiation dose as kidney toxicity was reported. The aim of this chapter is to summarize the current status on kidney protection in PRRT with radiolabelled somatostatin analogues.

The use of somatostatin analogues in PRRT

In the early 1970's it has been found that the small cyclic peptide hormone somatostatin had inhibitory effects on the physiological secretion of various hormones. In addition, the hormonal overproduction in certain tumour types, e.g. neuro-endocrine tumours, could also be inhibited by infusion of somatostatin [5].

Somatostatin is present in the human body in two forms: somatostatin-14 (consisting of 14 amino acids), and somatostatin-28 (consisting of 28 amino acids). After injection, somatostatin is rapidly degraded (circulation half-life of less than three minutes) and this is followed by postinfusion rebound of hormonal hypersecretion [5], making somatostatin itself unsuitable for therapy. Different more stable somatostatin analogues have been synthesised of which octreotide was the first to be used in patients. Octreotide is a small cyclic molecule, consisting of eight amino acids (Figure 1), with a threoninol at the C-terminus [6]. This peptide inhibits hypersecretion of growth hormone, insulin and glucagon more powerfully than somatostatin [6], it has an elimination half-life after subcutaneous administration of two hours [6] and is not associated with rebound hypersecretion [7].

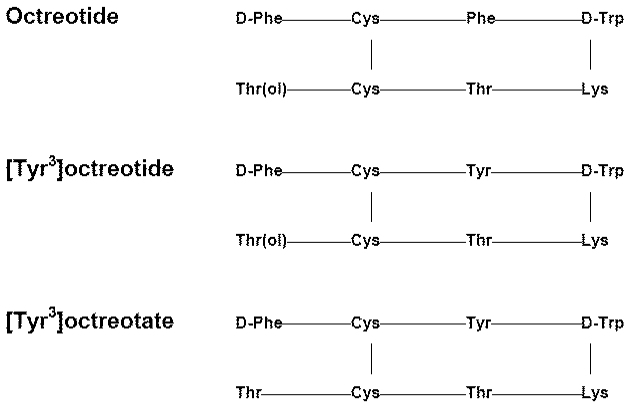


Figure 1. Amino acid sequence of different somatostatin analogues.

Octreotide and the slow-release depot intramuscular formulation of octreotide (Sandostatin LAR) are nowadays widely used in the treatment of symptoms from neuro-endocrine tumours, such as growth hormone-producing pituitary adenomas, carcinoids and gastroenteropancreatic tumours [5]. Although such treatment can improve quality of life, objective tumour regression is only seen in less than 10%, albeit in more than 80% of patients temporary stabilisation of tumour volume can be seen [5].

Many tumour types are not only characterized by hypersecretion of various hormones, they can also have a high expression of somatostatin receptors [8-10]. Somatostatin receptors are high affinity G-protein coupled membrane receptors and five different human subtypes (sst_1-sst_5) have been characterised and cloned [11-13]. Native somatostatin binds to all these receptors with high affinity (Table 1). Octreotide has high affinity for somatostatin receptor subtype 2 (sst_2) and lower affinities to sst_3 and sst_5.

Table 1. IC50 values of somatostatin analogues for somatostatin receptor subtypes (sst) 1-5 in nM. Adapted from [4].

	sst_1	**sst_2**	**sst_3**	**sst_4**	**sst_5**
SS28	5.2	2.7	7.7	5.6	4.0
Octreotide	>10000	2.0	187	>1000	22
[DTPA^0]octreotide	>10000	12	3790	>1000	299
[^{111}In-DTPA^0]octreotide	>10000	22	182	>1000	237
[DOTA^0,Tyr^3]octreotide	>10000	14	880	>1000	393
[^{90}Y-DOTA^0,Tyr^3]octreotide	>10000	11	389	>10000	114
[DOTA^0,Tyr^3]octreotate	>10000	1.5	>1000	453	547

The following step was the introduction of radiolabelled somatostatin analogues for *in vivo* visualisation of somatostatin receptor-positive tumours. The first radiolabelled somatostatin analogue was ^{123}I-labelled Tyr^3-octreotide [2]. This compound had limited power of abdominal neuro-endocrine tumours due to hepatic clearance and short retention in tumour cells [14]. Thereafter, octreotide was labelled with ^{111}In, via the attachment of diethylenetriamine-N,N,N',N',N-penta-acetic acid (DTPA) as chelator. [^{111}In-DTPA^0]-octreotide had superior pharmacokinetic characteristics: fast blood clearance, renal excretion, longer retention in tumour cells despite somewhat lower affinities for somatostatin receptors [14, 15] as compared to [^{123}I-Tyr^3]octreotide. Somatostatin receptor scintigraphy with [^{111}In-DTPA^0]-octreotide was developed in our institution [15, 16] and has proven to have great potential for the visualisation of somatostatin receptor-positive tumours [17]. It has become the initial imaging modality of choice in patients with gastroenteropancreatic tumours [18]. Scintigraphy with [^{111}In-DTPA^0]octreotide provides a more accurate staging of the disease by demonstrating tumour sites that were not shown by conventional imaging [19]. Furthermore, it can be used for prediction of benefit from therapy [5]. Recently, ^{99m}Tc-

labelled tetra-amine [Tyr^3]octreotate (Demotate) [20, 21] and the PET tracer [^{68}Ga-DOTA^0,Tyr^3]octreotide [22, 23] have been introduced for somatostatin receptor scintigraphy, but the clinical value of these two tracers remains to be established.

Extrapolating the experience that radiolabelled somatostatin analogues can be successfully used for the visualisation of somatostatin receptor-positive tumours combined with the limited number of effective therapeutic options, the aim was then to attach therapeutic radionuclides to somatostatin analogues to bring therapeutic radiation to somatostatin receptor-positive tumours.

The basis for the use of radiolabelled somatostatin analogues for visualisation and treatment of somatostatin receptor-positive tumours is binding of the radiolabelled compound to the receptor after injection. Subsequently receptor-mediated internalisation of the receptor-ligand complex can occur [24-26], resulting in degradation to metabolites in the lysozomes [27]. These metabolites are retained in the lysozomes resulting in a long retention of radioactivity in sst_2-positive tumour cells. After binding or internalisation, a therapeutic radionuclide can exert its radiation effects to tumour cell DNA and other cell structures.

The first radiolabelled somatostatin analogue that was used for PRRT of somatostatin receptor-positive tumours was [^{111}In-DTPA^0]octreotide [28]. The choice for this compound was made on the knowledge that the Auger electrons and internal conversion electrons that ^{111}In emits in addition to the γ-radiation could have therapeutic effects. Treatment of patients with metastasized neuro-endocrine tumours with high activities of [^{111}In-DTPA^0]octreotide resulted in encouraging effects, although objective responses were uncommon [29-31].

Although ^{111}In emits some therapeutic particles, it is not an optimal radionuclide for radiotherapy. A beta-particle emitting radionuclide like ^{90}Y (maximum beta energy 2.3 MeV, half-life 64 hours) is more suitable for this purpose. However, [DTPA^0]octreotide can not be stably labelled with ^{90}Y, which would result in haematopoietic toxicity caused by accumulation of free ^{90}Y in the skeleton after injection of this complex. In contrast, the chelator 1,4,7,10-tetraazacyclododecane-*N,N',N'',N''''*-tetraacetic acid (DOTA) can stably retain ^{90}Y, so DOTA-peptides were synthesized. [DOTA^0,Tyr^3]octreotide has an improved affinity for sst_2 as compared to [^{111}In-DTPA^0]octreotide and can be labelled in a stable way with ^{90}Y. Several groups have reported beneficial effects of [^{90}Y-DOTA^0,Tyr^3]octreotide in patients with somatostatin receptor-positive tumours [32-36] in terms of objective response, quality of life and survival [33]. Despite differences in the protocols used, complete remissions and partial remissions in most studies with [^{90}DOTA^0,Tyr^3]octreotide were in the range of 10-30%, higher than those obtained with [^{111}In-DTPA^0]octreotide [37].

A few years ago, another radiolabelled somatostatin analogue was introduced: [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. It differs from [DOTA^{0},Tyr^{3}]octreotide in that the C-terminal threoninol is replaced with threonine. Compared to [DOTA^{0},Tyr^{3}]octreotide, [DOTA^{0},Tyr^{3}]octreotate has a 9-fold higher affinity for the somatostatin receptor subtype 2 [4]. In phase 1 and 2 studies with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate partial and complete remissions were found in up to 30 percent of patients. Additionally, treatment resulted in a significant improvement of quality of life [38].

The efficacy of the different radiolabelled somatostatin analogues applied in the different trials mentioned can not easily be compared, as many factors play a role: different protocols, different criteria for evaluation and differences in patient selection [37]. Overall, treatment with radiolabelled somatostatin analogues is a promising new tool in the management of patients with otherwise untreatable, metastasized neuro-endocrine tumours. Tumour regression results are encouraging and these data compare favourably with those of the limited number of alternative treatment approaches [37].

The kidney in PRRT

The radiolabelled somatostatin analogues that are being used in PRRT are predominantly cleared by the kidneys. The small peptides are filtered by the glomerulus and most of the activity is excreted into the urine. However, part of the injected activity is taken up in the proximal tubular cells. After transport to the lysozomes, about 2% of the radioligand is retained there [27], resulting in prolonged kidney irradiation. This is supported by *ex vivo* autoradiography studies that have shown that most of the injected [^{111}In-DTPA^{0}]octreotide is located in the cortex of the kidney (Figure 2, [39]). In the human kidney the distribution of radioactivity is also inhomogeneously distributed [40]. Three patients that underwent nephrectomy because of renal cancer were injected with [^{111}In-DTPA^{0}]octreotide prior to the surgery. Ex vivo autoradiography of healthy kidney tissue showed that the radioactivity was localized predominantly in the cortex in a striped pattern, with the highest radioactivity in the inner cortical zone (Figure 2, [40]).

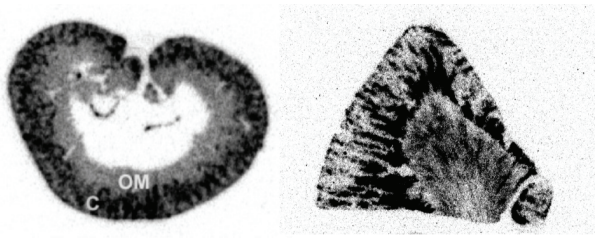


Figure 2. Radioactivity distribution in a rat kidney (left) and in a section of a human (right) kidney. Both autoradiography studies were performed 24 h after injection of [^{111}In-DTPA^{0}]octreotide. C=cortex, OM=outer medulla.

The megalin/cubulin system plays a significant role in the reabsorption of many low-molecular-weight proteins and peptides. This occurs via receptor-mediated endocystosis. In

studies with wild opossum kidney cells, Barone et al. showed that uptake of [^{111}In-DTPA^{0}]octreotide was inhibited by different megalin ligands [41]. Also, in kidney specific megalin-deficient mice it was shown that the uptake of [^{111}In-DTPA^{0}]octreotide was only 15 to 30 per cent of that in control mice [42]. These studies indicate that most of the kidney uptake of radiolabelled somatostatin analogues is megalin-dependent.

Stahl et al. reported a 20% reduction of renal uptake of [^{111}In-DOTA^{0},Tyr^{3}]octreotide by probenecid, which is a blocker of various organic anion transporters [43]. This suggests that also peritubular uptake of somatostatin analogues can occur. We were not able to confirm this finding (unpublished data) however.

Radiation nephropathy after PRRT

No renal toxicity has been reported after PRRT with [^{111}In-DTPA^{0}]octreotide, but soon after the introduction of [^{90}Y-DOTA^{0},Tyr^{3}]octreotide for PRRT severe kidney toxicity was reported. In an intra-patient dose-escalating study, Otte et al. [34] reported renal toxicity in 4 out of 29 treated patients after cumulative activities of 7611-8924 MBq/m^2 [^{90}Y-DOTA^{0},Tyr^{3}]octreotide. These four patients had not received co-infusion of amino acids and two of them needed haemodialysis treatment. Serum creatinine levels started to rise 2-4 months after the last treatment cycle in these patients. As the 25 patients who had no renal toxicity received cumulative activities up to 7400 MBq/m^2 (some with amino acids for kidney protection) the authors suggested this cumulative activity to be the threshold dose of [^{90}Y-DOTA^{0},Tyr^{3}]octreotide for renal toxicity.

However, Cybulla et al. [44] reported a case history of a 78 years old patient that was treated with 5659 MBq/m^2 [^{90}Y-DOTA^{0},Tyr^{3}]octreotide. This patient was initially included in the study by Otte et al. [34]. She developed progressive deterioration of renal function within 15 months after cessation of PRRT and developed end stage renal disease. This patient had been treated with ten chemotherapy cycles in the years preceding PRRT.

Moll et al. [45] reported the clinical course and histopathological changes in the five patients that had been included in the study of Otte et al. using [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34]. In these five patients the onset of renal failure varied from 0.5-6 months. All had normal blood pressure and serum creatinine before treatment, only one patient had chemotherapy treatment previously. At onset of renal failure all had hypertension, proteinuria and microhematuria. In one patient fragmentocytes were found. In three patients kidney biopsy examination was performed showing the picture of thrombotic microangiopathy (TMA). Capillary loop collapse, focal segmental thrombi of fibrine and other proteins in capillaries, mesangiolysis and double contouration of the basement membrane were seen. Arterioles and small arteries showed transmural hyaline deposits and were partially or completely occluded by fibrin and

protein thrombi. Tubular atrophy and interstitial fibrosis were prominent in all cases. Depositions of fibrin, IgM and IgA were observed in both the lumen and the wall of arterioles and arteries.

Paganelli et al. [46, 47] reported in a dose-escalating study one out of 30 patients having grade 2 renal toxicity. This 58 years old patient, who had suffered from hypertension for 6 years, received 3.3 GBq [^{90}Y-DOTA^{0},Tyr^{3}]octreotide in three cycles, and the reported estimated kidney absorbed radiation dose was 12 Gy. One year later serum creatinine returned to normal, but ^{99m}Tc-DTPA scintigraphy remained abnormal.

Stoffel et al. [48] reported on a patient who was treated with 6 cycles of [^{90}Y-DOTA^{0},Tyr^{3}]octreotide for metastasized medullary thyroid carcinoma. She was admitted for progressive weakness, anemia and arterial hypertension ten months after her last PRRT treatment. She had mild glomerular and tubular proteinuria, as well as a low creatinine clearance (14 mL/min). Eventually, she was in need for haemodialysis. Kidney biopsy was performed and showed segmental mesangiolysis in 6/15 glomeruli with disruption and vacuolar expansion of the mesangium. No deposits were present. There was marked tubular atrophy and compensatory interstitial fibrosis, as well as minimal arterial damage. No intra-arterial thrombosis or necrosis was observed. Unfortunately, Stoffel et al. did not include administered activity or dosimetry data.

Barone et al. [49] reported on two patients who suffered from hypertension, proteinuria, edema and anemia after [^{90}Y-DOTA^{0},Tyr^{3}]octreotide treatment. At biopsy, radiation-induced microangiopathy was proven in one of these patients. The conventionally calculated kidney absorbed dose were 27.5 and 27.1 Gy, whereas the biological equivalent doses were 54.1 and 56.1 Gy.

In our institution kidney biopsy from four patients has been obtained showing no histological damage after kidney absorbed doses of 29.3 and 32.3 Gy ([^{90}Y-DOTA^{0},Tyr^{3}]octreotide) and 35 and 49 Gy ([^{111}In-DTPA^{0}]octreotide). The intervals between first treatment and biopsy ranged between 12-17 months.

Animal data on PRRT related renal toxicity are scarce. Lewis et al. [50] found no functional or histological damage up to 38 days after 102-123 mCi/kg [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

In view of the clinical reports of renal toxicity in PRRT, methods to protect the kidneys are warranted. The goal for these applications should not only be to prevent radiation nephropathy, but also to allow higher activities to be administered to the patients in order to achieve higher tumour radiation doses. Elementary for development of these strategies is knowledge on the factors that influence radiation damage. Also, knowing the maximum safe kidney radiation dose is mandatory.

Radiation nephropathy in external beam radiation therapy

Most knowledge and experience with radiation nephropathy has been derived from external beam radiation therapy. The first description of radiation related nephropathy stems from the early 1900's. Later, Luxton and Kunkler et al. [51-54] described the clinical manifestations and classified 5 syndromes related to radiation exposure of both kidneys.

Acute radiation nephritis appears 6-12 months after exposure and is characterised by azotemia, anemia, proteinuria, hypertension and heart failure [52, 55]. Kidney biopsy examination shows mesangiolysis, as well as atrophy and tubulointerstitial scarring. Severe cases have features of thrombotic microangiopathy (TMA) [55]. Chronic radiation nephritis can appear 18 months to years following treatment, clinically ranging from only loss of energy and nocturia to a fatal chronic uremic state [52]. Benign or malignant hypertension was also noticed as a clinical syndrome after renal irradiation, occurring 18 months to years after treatment. The so-called benign hypertension coincides with proteinuria and may not be as benign as it seems. Death by congestive heart failure or progression to malignant hypertension is not uncommon [52]. Once malignant hypertension has started, progress is rapid and causes death within weeks. An additional clinical renal radiation syndrome has been recognized consisting of patients that have solely asymptomatic intermittent proteinuria of small amounts [52]. These patients do have impaired renal function though, as shown by rises of blood urea nitrogen (BUN) due to physical stress. Overlap of these syndromes is common as is progression of the acute to the chronic phase, often with hypertension [56].

Avioli et al. [57] described the acute changes in renal function parameters after irradiation. After renal irradiation doses of 20 Gy or more, the glomerular filtration rate (GFR) rose with 15-20% in the course of the fractionated treatment. In the months following irradiation, GFR decreased on average by 20-25% from baseline in 8 out of 10 patients. The renal plasma flow consistently decreased, but tubular function appeared to recover at 7-12 months after an initial decrease. Conventional renal function parameters, such as serum BUN levels and creatinine clearances rarely showed changes in the 6 months following irradiation.

Almost all knowledge on morphological changes that occur initially after radiation are based on animal studies, as data on human kidney are almost completely limited to late or end-stage renal disease [56]. These animal data allow different interpretations in terms of the site of the kidney that is initially involved in the onset of damage, but a multiple target explanation where glomeruli, tubuli, interstitium and blood vessels have their role seems the best explanation for the clinical picture in patients [56].

The most susceptible structure might be the glomerulus as changes in the glomeruli can be seen earlier than tubular changes. However, renal failure occurs mainly when glomerular,

tubular and tubulointerstitial damage are all present. The progressive reduction in renal function after kidney irradiation is associated with concomitant time- and dose-dependent alterations in all components of the nephron leading to glomerulosclerosis and/or tubulointerstitial fibrosis [58].

The initial reaction to irradiation of the glomerulus is increased permeability resulting in leakage of high molecular weight proteins to both extravascular space and tubules [59]. A small proportion of these proteins become insoluble in the extravascular space. The basement membrane is thickened and proliferation of endothelial cells results in irregular diameter and increased tortuosity. The irregular diameters may lead to thrombus formation and occlusion. If too many capillaries per glomerulus are injured the glomerulus becomes non-functioning. The blood supply of the tubular cells is mainly delivered by the efferent arteriole, that is formed by anastomoses of the glomerular capillaries, and ischemia of tubules may result from damaged vessels. Most clinical manifestations are caused by vascular damage, but late effects may be caused by loss of parenchymal cells. Tubule cells appear to be more radiosensitive than epithelial cells from other cells systems [60], probably their limited capacity to proliferate is further reduced by irradiation .

Radiation-induced DNA damage leading to cell death is only one aspect of overall cellular tissue response. Ionizing radiation, in common with other forms of stress, causes pronounced changes in cell phenotype [61]. These changes comprise increased gene expression of extracellular matrix (ECM) components and of regulatory factors of the ECM turnover.

Cohen and Robbins [55, 62] reviewed the *in vivo* mediators and mechanism of kidney radiation injury. Radiation-induced normal tissue injury involves complex and dynamic interactions between several cell types. These cells now are not viewed as passive bystanders, but as active participants in an orchestrated, yet limited response to injury. The development and progression of radiation nephropathy involves multiple cell types, including the glomerular endothelial cell, the mesangial cell and the tubular epithelial cell. Some important findings are listed below:

- attachment of neutrophils to the endothelium via chemotactic factors or up-regulation of cell adhesion molecules; some of these molecules are associated with attenuation of injury.
- development of vascular or glomerular thrombosis may be caused by changes in the cells that favour co-agulation, like elevated levels of plasminogen activator inhibitor-1 (PAI-1). The latter prevents fibrinolysis and may promote fibrosis through inhibition of matrix degradation.
- Fibrosis may be enhanced by radiation induced activation of fibroblasts to contractile myofibroblasts that are a major source of matrix deposition in fibrosis.

- Glomerular fibrin deposition is associated with tubular fibrin deposition, which may promote tubulointerstitial fibrosis via activation of interstitial fibroblasts.
- A marked time- and dose-dependent atrophy and lysis of tubular cells after irradiation is seen and found to be associated with enhanced collagen III, fibronectin and transformin growth factor β (TGFβ) staining [63].
- Ionizing radiation results in persistent oxidative stress [64], and this may play an important role in the pathogenesis of radiation nephropathy, just as in other chronic kidney diseases [65].
- Up-regulation of both PAI1 and TGFβ can be inhibited by angiotensine converting enzyme (ACE)-inhibitor treatment [66, 67], indicating that the renine-angiotensine-aldosterone-system (RAAS) plays a role in the pathogenesis of radiation nephropathy. Angiotensine II and has been recognized as a pro-inflammatory molecule in many renal disease settings, and it leads to up-regulation of different pro-inflammatory pathways [68].
- No change of intrarenal or plasma angiotensin II levels have been shown so far [69] in radiation nephropathy, albeit the number and affinity of angiotensine II receptors might be increased after irradiation [55].

Knowing the maximum safe renal radiation dose: dosimetry

In the recent decade, several data have been reported that can help to estimate the maximum radiation dose that can be safely delivered to the kidney in PRRT.

In fractionated external beam radiation therapy, the risk to develop radiation nephropathy within 5 years after renal absorbed doses of 23 Gy is 5%, rising to 50% after renal absorbed doses of 28 Gy [70]. However, it should be kept in mind that a radiation dose delivered during radionuclide therapy differs in many respects from a dose delivered by external beam irradiation. In radionuclide therapy: (a) the dose rate is lower, (b) the penetration range of the radiation is shorter and (c) the radionuclide has a specific intra-organ distribution, while in external beam radiation therapy the radiation is delivered homogeneously to the kidneys or the part of the kidney that is in the field of irradiation [71-73].

So, in PRRT dosimetry is needed for establishment of the maximum safe dose to the kidneys and for patient-tailored therapy planning. Several authors reported large interindividual variation in uptake and radiation doses [74-76] stressing the need for such an approach.

For optimal dosimetry, time-consuming pharmacokinetic methods are required, preferably with the same peptide and radionuclide used for therapy [74]. This is possible with radionuclides that have both beta- and gamma- emissions, but not with the pure beta-emitter ^{90}Y.

To determine absorbed doses of [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, strategies using [^{111}In-DTPA^{0}]octreotide [75], [^{86}Y-DOTA^{0},Tyr^{3}]octreotide [49, 77] and [^{111}In-DOTA^{0},Tyr^{3}]octreotide [76, 78-80] have been reported. It must be noted that minor changes in the peptide and differences in radionuclide can significantly influence sst_2-mediated uptake and therefore the reliability of dosimetry data [4]. In addition, uptake in liver and kidneys may be influenced by radionuclide-determined differences in lipophility and charge. ^{86}Y is chemically identical to ^{90}Y and it is therefore reasonable that the ^{86}Y-labelled [DOTA^{0},Tyr^{3}]octreotide reliably predicts the renal absorbed radiation dose to the kidney during treatment with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide. It is generally considered as the gold standard for mimicking therapy [76]. However, the use of the positron-emitter ^{86}Y is limited by its availability, the positron abundance of 33% and the relative short half-life of about 14 hours [74, 76].

As an alternative, dosimetry using [^{111}In-DTPA^{0}]octreotide or [^{111}In-DOTA^{0},Tyr^{3}]octreotide can be performed. Although an intra-patient comparison of [^{111}In-DOTA^{0},Tyr^{3}]octreotide versus [^{86}Y-DOTA^{0},Tyr^{3}]octreotide is desirable, the biokinetics of [^{111}In-DTPA^{0}]octreotide versus [^{86}Y-DOTA^{0},Tyr^{3}]octreotide have been compared instead. The results indicate that although [^{111}In-DTPA^{0}]octreotide is not the optimal surrogate [74, 75, 81, 82] for measuring dosimetry for [^{90}Y-DOTA^{0},Tyr^{3}]octreotide.

Dosimetry performed in PRRT is only then valuable when its outcome correlates with radiation effects and when it predicts toxicity. Ultimately, factors that may influence the dose-response of the kidney to radiation are: the type of radionuclide used, the nature of the peptide and subsequently its intrarenal distribution, fractionation and previous toxic insults like chemotherapy or pre-existent conditions with renal involvement like diabetes and hypertension.

Valkema et al. [29] showed that treatment of 21 patients with [^{111}In-DTPA^{0}]octreotide did not result in renal toxicity after renal absorbed doses up to 45 Gy, followed up for up to 3 years. ^{111}In emits 2 gamma rays and in addition Auger electrons with a tissue penetration of 0.02 - 10 μm and conversion electrons with a penetration of 200-500 μm. Using a microdosimetry model Konijnenberg et al. [83] showed that ^{111}In and ^{177}Lu labelled somatostatin analogues are likely to have higher renal toxicity thresholds than ^{90}Y labelled somatostatin analogues, as the beta-particles of ^{90}Y have a maximum path length of 12 mm. Consequently, the inhomogeneous, mostly cortical, intrarenal distribution of radiolabelled somatostatin analogues is only of importance for ^{177}Lu and ^{111}In labelled peptides.

In the studies with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in our institution, the maximum kidney absorbed renal dose is limited to 23 Gy. In over 500 patients treated so far only one patient has renal function reduction. This patient had a creatinine clearance of 41 mL/min at baseline

due to unexplained rises in serum creatinine before PRRT. Renal function further deteriorated during PRRT.

In another study, Valkema et al. [84] evaluated the renal function loss in 28 patients treated with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide and 37 patients treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate over a period of at least 18 months after start of therapy. All patients received amino acid solutions and the total renal absorbed dose, estimated by [^{86}Y-DOTA^{0},Tyr^{3}]octreotide or [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate imaging, was limited to 27 Gy. In the [^{90}Y-DOTA^{0},Tyr^{3}]octreotide group 9 patients had a creatinine clearance loss per year of more than 15%, and two patients in the [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate group. The mean yearly decline in creatinine clearance was not different between the two groups (about 3,8% per year) but the cumulative renal absorbed doses was significantly higher in the [^{90}Y-DOTA^{0},Tyr^{3}]octreotide group (26.9 vs 19.7 Gy). In patients with a yearly decline in creatinine clearance of more than 25 % per year end-stage renal disease could be expected within 5 years after treatment. Furthermore, five risk factors were identified for more rapid detoriation of kidney function loss: cumulative renal absorbed doses more than 25 Gy, diabetes, hypertension, age>60 years and a renal radiation dose >14 Gy per cycle.

Experimental studies show that the kidney has an extensive capacity for repair of sublethal radiation damage. The size per fraction markedly influenced the total tolerance dose [85, 86]. So, there is reason to implement the effects of fractionation in the current dosimetry protocols. Barone et al. [49] reported dosimetric data of 18 patients treated with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide and correlated these data with changes in creatinine clearance loss. The injected activities were individualized so that the renal absorbed dose did not exceed 27 Gy. Despite this limit, several patients developed renal toxicity as notified by significant creatinine clearance loss, proteinuria, hypertension, edema and anemia. The creatinine clearance loss did not correlate with the renal absorbed dose, even after correction for actual kidney volume using CT. In contrast, the biological equivalent dose (BED) (corrected for CT-assessed actual kidney volume) that was estimated according to the Linear Quadratic (LQ) model correlated well with the yearly loss of creatinine clearance. The LQ model allows evaluation of the effects of fractionation on biological effects of radiation. The effects are a linear and quadratic function of the dose per fraction and a function of the fraction number [55]. From the study of Barone et al. [49], it can be concluded that a calculated BED between 27 and 42 Gy correlates with creatinine clearance loss rates of up to 10% per year, whereas a BED higher than 45 Gy resulted in yearly creatinine clearance loss of 26-56%. Also, it was shown that co-existing factors, like hypertension, diabetes mellitus or previous chemotherapy negatively influenced the dose-response rate of kidney function to the doses applied, as also found by Valkema et al. [84].

For comparison: when the available data of Otte et al. [34] are put in the LQ model (assuming that the absorbed dose is 3.2 mGy/MBq [76], and the $T1/2_{EFF}$ is 31 as calculated from Barone et al. [49]) the BEDs uncorrected for actual kidney volume ranged from 56 to 64 Gy. The patient reported by Cybulla et al. [44] who had chemotherapy in the past would have had a BED of 39 Gy. These numbers underline the proposed safe BED by Valkema et al. [84] and Barone et al. [49] of about 37 Gy, as well as the importance of co-existing factors like previous chemotherapy treatment, diabetes and hypertension.

Classical dosimetry is performed with standard reference kidney volumes, based on planar scintigraphy and is not corrected for fractionation. New methods that include the actual kidney size and that take into account the intra-renal activity distribution and the effects of fractionation may provide better tools for clinical practice in PRRT. Additional studies need to be performed to evaluate the reliability of such methods.

Prevention of radiation nephropathy in PRRT

The following strategies can be applied in order to prevent radiation nephropathy and to make application of higher doses in PRRT possible within safe kidney radiation limits:

1. dose fractionation.
2. reduction of kidney uptake of radiolabelled peptides by uptake inhibitors.
3. mitigation of the radiation effects.
4. development of new peptides with a higher affinity for the receptor or lower renal uptake.

1. Dose fractionation

Many reports from the field of external beam radiation therapy indicate that fractionation of the total radiation dose results in less kidney damage as compared to the unfractionated total dose [72]. The kidney is capable of extensive repair of non-lethal damage during the interval between the frcactions as indicated by many authors [72, 86-90]. Although fractionation of the total dose may be favourable for the kidneys, it is not sure whether or not fractionation affects anti-tumour efficacy. It can be expected that tumours will have less protection by fractionation because their alpha/beta-values (an index of the sensitivity of an tumour/organ to changes in dose rate [49]) are high.

Clinical data indicate that fractionation, and correction for this by using the LQ-model for calculation of the kidney biological effective radiation dose, has significance in PRRT. In a rat model administering high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, we investigated the effect of fractionation on kidney dysfunction. Furthermore, we described the onset and characteristics of renal damage after high dose PRRT in this model (**Chapter 2**).

2. Reduction of uptake of radiolabelled somatostatin analogues

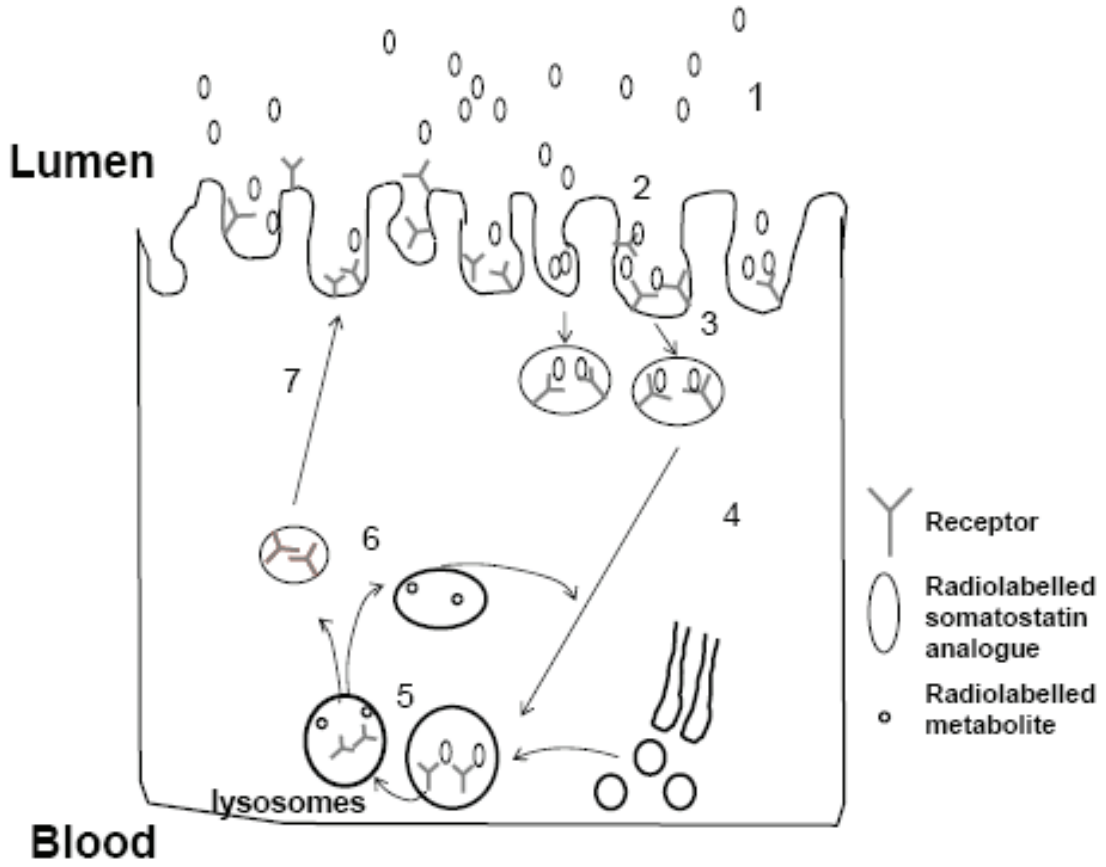


Figure 3. Schematic reproduction of the uptake of radiolabelled somatostatin analogues in a proximal tubule cell. Adapted from Behr et al. [91]. Radiolabelled peptides are freely filtered by the glomerulus (step 1) and enter the tubular fluid. The peptide binds to a membrane receptor (possibly megalin, step 2), and the complex is internalised in a vesicle of cell membrane parts (step 3). This vesicle is transported to the lysozomal apparatus (step 4) where the receptor and the radiolabelled peptide are separated and the latter is metabolised (step 5). The radiolabelled metabolites are retained in the lysozomes (step 6), but the receptor is transported back to the cell membrane (step 7). See [27, 92-94].

Figure 3 shows the different processes involved in kidney uptake of radiolabelled somatostatin analogues. Mogensen et al. [95] found that proximal tubular reabsorption of low-molecular-weight proteins (LMWP) and peptides can be reduced by positively charged basic amino acids. This finding led many to investigate whether these amino acids might reduce renal reabsorption of radiolabelled somatostatin analogues. Hammond et al. [96] were the first to publish a semi-quantitative study showing that the renal uptake of [^{111}In-DPTA^{0}]octreotide could be reduced by a commercially available mixture of amino acids. A total of 35.2 g arginine and 9.8 g lysine was infused in these 16 patients. Behr et al. [97] showed that the renal uptake of radiolabelled monoclonal antibody fragments could significantly be reduced by about 80% by injection of lysine.

Based on this knowledge, we performed experiments in rats exploring different potential blockers of kidney uptake of [^{111}In-DTPA^{0}]octreotide. Among others, maleic acid and the positivey charged amino acids lysine and arginine were tested, and these studies are described in **Chapter 3.1**.

Amino acid solutions have a high osmolarity requesting high volumes to be infused and often result in nausea and vomiting. In addition, amino acid infusion can result in metabolic changes of which a life-threatening hyperkalemia is the most important. Human studies were initiated in order to find an amino acid solution that results in optimal kidney uptake reduction of [^{111}In-DTPA^{0}]octreotide with minimal side effects. These studies are described in **Chapter 3.2**, and these have studies have led to standard use of a combined infusion of 25 grams of lysine plus 25 grams of arginine, dissolved in 1 liter, in our institution.

Jamar et al. [77] compared various amino acid solutions as well. They showed that a mixed amino acid solution (1800 L, containing 240 gram mixed amino acids amongst which 10.3 grams lysine and 16 grams of arginine) reduced the uptake of [^{86}Y-DOTA^{0},Tyr^{3}]octreotide by 21 % with concurrent reductions in estimated kidney radiation doses when applied to [^{90}Y-DOTA^{0},Tyr^{3}]octreotide therapy. Prolongation of the infusion further enhanced the reduction. The maximum allowed dose (MAD) of [^{90}Y-DOTA^{0},Tyr^{3}]octreotide that would result in a 23 Gy cut off dose to the kidneys was calculated. The MAD was 46% and 62% higher for mixed AA and the combination of 25 grams of lysine plus 25 grams of arginine, respectively.

Many reabsorption processes in the proximal tubule are dependent on microtubules. These microtubules are essential in the intracellular trafficking of all kinds of organelles, including endocytosed receptor-ligand complexes. Elkjaer et al. [98] and Gutmann et al. [99] reported that administration of colchicine, an anti-gout drug known to inhibit microtubule function, changed the intracellular distribution of megalin and other membrane molecules in proximal tubules. The localisation of these molecules was greatly moved from the brush membrane to other subcellular compartments, resulting in disabled endocytosis. In **Chapter 3.3** studies on the effects of colchicine on kidney uptake of [^{111}In-DTPA^{0}]octreotide are reported.

As the renal uptake of radiolabelled somatostatin analogues is mediated by the megalin/cubulin system, van Eerd et al. [100] tested the potential of the plasma expander Gelofusine to reduce kidney uptake of radiolabelled somatostatin analogues. Gelofusine infusion was reported to result in enhanced excretion of megalin ligands [101, 102]. Gelofusine consists of succinylated bovine gelatin molecules and is used in clinical emergencies as a plasma expander. Van Eerd et al. [100] found that co-injection of Gelofusine could reduce kidney uptake of [^{111}In-DTPA^{0}]octreotide by about 45 %, comparable to what is found for reductions by lysine co-injection. These preclinical findings were followed by a

study in five healthy subjects, showing comparable results [103]. In **Chapter 3.4** we report on our studies on the effects of the combination of Gelofusine plus lysine on the kidney uptake of radiolabelled somatostatin analogues.

Although most data point to proximal tubular reabsorption as the main site of uptake, other factors like sst_2-mediated or peritubular uptake may play a role as well. Glomeruli, tubule cells and vasa recta in the human kidney express sst_2 [104, 105]. We investigated the contribution of the human kidney sst_2-mediated uptake to the total kidney uptake of radiolabelled somatostatin analogues and these studies can be found in **Chapter 3.5**.

3. Mitigating radiation effects

Another renoprotective strategy is to reverse or interfere with the cascade of events after radiation. Two methods have been applied so far.

As radiation causes a chronic state of oxidative stress with continuous production of reactive oxygen species, the radioprotective drug amifostine (Ethyol, WR-2721) has successfully been used in the protection of healthy tissues in radiation therapy of throat tumours [106] and in chemotherapy with cisplatinum [107]. After injection, this drug is activated by alkaline phosphatases and subsequently taken up by healthy cells. Kidney concentrations of the active metabolite WR-1065 are about 100 times higher than tumour concentrations, which forms the basis for selective healthy tissue protection. In our rat model of radiation nephropathy, we studied the effects amifostine in PRRT, and these studies are described in **Chapter 4.1**.

As pointed out earlier, the RAAS system plays a key role in the development of radiation damage to the kidney. Different reports from external beam radiation therapy showed that treatment with ACE-inhibitors or angiotensin II receptor blockers can prevent the development of radiation damage and even established radiation nephropathy can successfully be treated [108-112]. Jaggi et al. [113] tested the effects of RAAS inhibition in a model of radioimmunotherapy with an alpha-emitter-labelled antibody. They showed that captopril treatment enhanced the functional and histological damage, whereas treatment with low-dose spironolactone, and to a lesser extent with an angiotensine II receptor blocker, could prevent the development of histopathological and functional changes caused by internal radiation. In **Chapter 4.2** our rat studies with inhibition of RAAS-activity can be found.

CHAPTER 2

LONG TERM TOXICITY OF [^{177}Lu-DOTA^{0},Tyr^{3}]OCTREOTATE IN RATS

Edgar J. Rolleman, Eric P. Krenning, Bert F. Bernard, Monique de Visser, Magda Bijster, Theo J. Visser, Marcel Vermeij, Jan Lindemans and Marion de Jong

Eur J Nucl Med Mol Imaging 2007; 34:219-227

ABSTRACT

Purpose and methods

Studies on peptide receptor radionuclide therapy (PRRT) using radiolabelled somatostatin analogues have shown promising results with regard to tumour control. The efficacy of PRRT is limited by uptake and retention in the proximal tubules of the kidney, which might lead to radiation nephropathy. We investigated the long-term renal toxicity after different doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and the effects of dose fractionation and lysine co-injection in two tumour-bearing rat models.

Results

Significant renal toxicity was detected beyond 100 days after start of treatment as shown by elevated serum creatinine and proteinuria. Microscopically, tubules were strongly dilated with flat epithelium, containing protein cylinders. Creatinine levels rose significantly after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, but were significantly lower after 278 MBq (single injection) or two weekly doses of 278 MBq. Renal damage scores were maximal after 555 MBq and significantly lower in the 278 and 2x278 MBq groups. Three doses of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with intervals of a day, a week or a month significantly influenced serum creatinine (469±18 umol/L, 134±70, 65±15, respectively; P<0.001). Renal histological damage scores were not significantly influenced by dose fractionation. Lysine co-administration with three weekly treatments of 185 MBq significantly lowered serum creatinine and proteinuria.

Conclusions

Injection of high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate resulted in severe renal damage in rats as indicated by proteinuria, elevated serum creatinine and histological damage. This damage was dose dependent and became overt between 100 and 200 days after treatment. Dose fractionation had significant beneficial effects on kidney function. Also, lysine co-injection successfully prevented functional damage.

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) using radiolabelled somatostatin analogues has been reported to yield convincing results in various somatostatin receptor-positive tumours [29, 32]. Partial remission rates of up to 30% have been reported, with sporadic complete remissions [32, 114], as well as significant improvements in quality of life [38] and survival [33]. This treatment modality takes advantage of overexpression of somatostatin receptors on tumours to which the radiolabelled molecules bind after injection. In addition to therapeutic radiation effects on tumours, healthy tissues can be irradiated and this might result in radiotoxicity. In PRRT, the kidneys and the bone marrow are the critical organs.

The peptides used in PRRT are small and easily filtered by the glomeruli in the kidneys with subsequent reabsorption by the proximal tubular (PT) cells. The renal reabsorption of the radiolabelled peptides is not quantitative, but the amount of radioactivity taken up and retained in PT cells might result in high renal radiation doses. The kidneys are the main dose-limiting organs during PRRT with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Currently, kidney radiation doses between 23-27 Gy are accepted, based on external beam radiation therapy data [70]. The exact maximum tolerated kidney dose in PRRT, however, is unknown. In a recent clinical PRRT trial using [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate [114] with maximum renal radiation doses of 23 Gy, no significant kidney toxicity was found except in one patient that developed end-stage renal disease, requiring haemodialysis [114]. Other authors have also reported clinically significant renal toxicity after PRRT [34, 44, 45, 48]. They did not report on kidney radiation dose data, so these studies cannot give a clear indication at what kidney radiation doses toxicity appears.

Several factors play a role in the development of renal radiation damage and these factors need to be addressed to study how the onset of renal damage can be avoided: the total dose administered, the organ radiation dose, dose fractionation, inhomogeneous distribution of the radiolabelled peptide, co-administration of positively charged amino acids and the characteristics of the radionuclide.

From external beam radiation therapy it is known that fractionation of the total dose results in better tolerance of the therapy, enabling higher total administered doses to be tolerated without toxicity. Although dose fractionation is common in PRRT, only one study has shown the beneficial effects of fractionation in PRRT. Barone et al. [49] showed that the biologically equivalent dose [based on the linear-quadratic (LQ) model] correlates well with the progression of detoriation in kidney function.

In both animal studies and the clinical setting positively charged amino acids have successfully been used to reduce renal uptake and subsequent retention of radiolabelled somatostatin analogues [35, 115-117]. This allows higher cumulative doses to be administered

to patients, and thus higher tumour radiation doses, without exceeding safe limits with regard to the kidneys. Although amino acid solutions are being widely used for kidney protection during PRRT, no validation studies have been published that their use results in larger administered doses being tolerated with regard to renal toxicity.

As noted previously, in PRRT the bone marrow is at risk as well. In several clinical studies, bone marrow toxicity has been reported that comprises anaemia, leucopenia and thrombocytaemia and is usually reversible [37].

The aim of the present study was to describe the long-term effects of PRRT with different doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate on kidneys and bone marrow in rats. Furthermore, we studied the effects of dose fractionation and we validated the effects of the amino acid lysine with regard to its kidney-protecting potential during PRRT with high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

MATERIALS AND METHODS

Radionuclides, peptides and cells

^{177}Lu was obtained from IDB (Baarle Nassau, The Netherlands). [DOTA^{0},Tyr^{3}]octreotate was supplied by BioSynthema (St Louis, Mo, USA). Labelling of [DOTA^{0},Tyr^{3}]octreotate with ^{177}Lu was performed as described earlier [118, 119].

Radionuclide therapy experiments using [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate

All animal experiments were approved by the governing Animal Welfare Committee and experiments were conducted according institutional regulations. Studies were performed in two different rat models of somatostatin receptor-positive tumours, i.e. the rat pancreatic CA20948 and AR42J tumour models, to investigate and compare the anti-tumour activity of the applied doses in relation to their effects in dose-limiting organs.

CA20948 tumour-bearing male Lewis rats (250-300 g; Harlan, Horst, The Netherlands; implantation of tumours in the flank as earlier described [120]) were injected with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate into the dorsal vein of the penis on day 1. The rats received 555 MBq, 278 MBq or two doses of 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with an interval of a week. Rats were monitored for up to 300 days post therapy. In accordance with animal welfare regulations, rats were sacrificed when tumour growth exceeded 15 cm^{2}, when the skin over the tumour was ruptured or when weight loss was more than 10% of initial body weight. Under anaesthesia, rats were sacrificed by opening the chest and subsequently transecting the aorta. Blood samples were drawn to determine kidney function and haematological

parameters. One kidney was taken out of every animal and processed for histological evaluation (see below).

The effects of dose fractionation and lysine co-injection on renal and bone marrow toxicity were evaluated in AR42J-tumour bearing male Lewis rats (250-300 g; Harlan, Horst, The Netherlands). Implantation of the AR42J tumour in the right flank was performed in a manner comparable to the implantation of the CA20948 tumour [120]. AR42J rat pancreatic cancer cells were purchased from ATCC. The rats were injected with three doses of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with intervals of a day, a week or a month. In addition, three rats received 3x185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate at intervals of 1 week plus 400 mg/kg D-lysine (Sigma, St. Louis, MO, USA) as a co-injection with each radioactivity dose to reduce kidney reabsorption of radioactivity. In addition, another group of rats was treated with three doses of 37 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate at intervals of a week. To determine proteinuria, rats were placed in metabolic cages to collect 24-h urine on a weekly basis. Rats were euthanised under anaesthesia on day 150 after the first injection of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate by opening the chest and subsequently transecting the aorta. Then a blood sample was taken by cardiac puncture for biochemical determination of kidney function and for haematological parameters. One kidney was excised for histological evaluation (see below).

Analytical and histological procedures

Kidneys were fixed in 10% buffered formalin, trimmed and processed by standard techniques for embedding in paraffin. Four-micrometre sections were stained with haematoxylin-eosin (HE) and periodic acid-Schiff reagent (PAS). The renal damage was microscopically graded from 0 (no damage) to 4 (severe damage). The criteria for the different scores were:

- **Grade 1:** an inflammatory infiltrate in the glomeruli and little dilatation of tubules; no basal membrane thickening or protein cyclinders
- **Grade 2:** criteria as for grade 1, but in addition rough protein staining, more pronounced tubule dilatation, basal membrane thickening and mitotic activity; a few protein cyclinders in the tubules
- **Grade 3:** shrinkage of a small number of glomeruli; flat or lost tubule epithelium, strong tubule dilatation and more pronounced basal membrane thickening; more protein cylinders
- **Grade 4:** increased shrinkage of glomeruli leading to optical emptiness; strongly dilated tubules with massive protein cylinders and signs of periferal fibrosis

The authors who scored the renal damage had no knowledge of the previous treatment of the rats. Urinary protein was measured in 24-h urine samples with a commercially available colorimetric method (BioRad, Veenendaal, The Netherlands). Blood chemistry and haematological parameters were determined by standard analysis procedures.

SDS-page

Sodium dodecyl sulfate-polyacrylamide gel electropheresis (SDS-PAGE) reagents were obtained from Bio-Rad (Richmond, IL, USA). To 90 µL urine solution, containing 0.2% of the total amount of protein in the rat urine samples (mg/24 h), 30 µL SDS-PAGE loading buffer was added containing 10 m*M* Dithiothreitol (DTT). Subsequently the proteins were separated by running the electrophoresis overnight in a 10-cm 12% polyacrylamide gel overlaid by a 2-cm 5% stacking gel. The gels were stained for 4 h with Coomassie Brilliant Bleu R-250 (Merck, Darmstadt, Germany) in 45% methanol/10% acetic acid and de-stained overnight with 45% methanol/10% acetic acid, both at room temperature. Finally, the gels were dried under vacuum at 80°C.

Statistical analysis

Data are expressed as mean ± SD. One- or two-way ANOVA followed by Tukey's test or student's *t*-test was used to test significance of differences. *P*-values less than 0.05 were considered significantly different.

RESULTS

Anti-tumour effects of applied doses [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate

As described previously [118], the applied doses of 278 MBq, 555 MBq or two weekly cycles of 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate resulted in excellent anti-tumour activity in the CA20948 tumour. Figure 4a shows the tumour response to the given treatment.

In the experiments in rats bearing the AR42J-tumour in their flank, all rats treated with either three daily, weekly or monthly cycles of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate survived 150 days without apparent tumour re-growth. Figure 4b shows the tumour response to treatment in these rats. In contrast, all control AR42J tumour-bearing rats that had not been treated with radioactivity had to be killed because of large tumour burden within 50 days after inoculation.

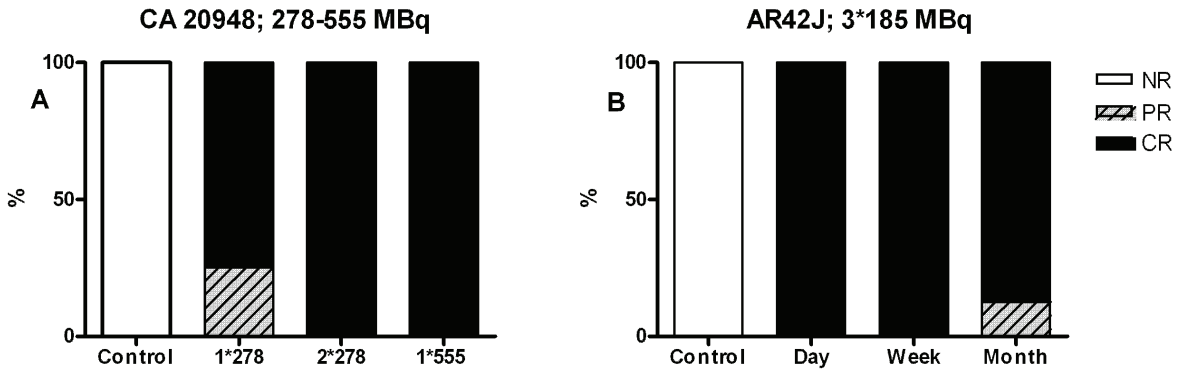


Figure 4. Tumour size responses found in groups of tumour-bearing rats. **a** Responses in the CA20948 tumour model after indicated doses of [^{177}Lu-DOTA^{0},Tyr^{3}]. **b** Responses in AR42J tumour bearing rats after three cycles of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with an interval of a day, a week or a month. Groups indicated with control were not treated with [^{177}Lu-DOTA^{0},Tyr^{3}]. Responses were defined according to the South-West Oncology Group criteria: partial response (*PR*) was defined as at least 50% reduction of the product of the two largest perpendicular tumour diameters vs pre-treatment values, whereas complete response (*CR*) was defined as 100% reduction at 150 days after start of treatment. *NR*: no response.

Renal damage after high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate

Figure 5 shows the serum creatinine concentrations in rats treated with single doses of 555 MBq or 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in the periods of 0-100 or 101-200 days post therapy. In rats sacrificed between 0 and 100 days after a single dose of 555 MBq, the serum

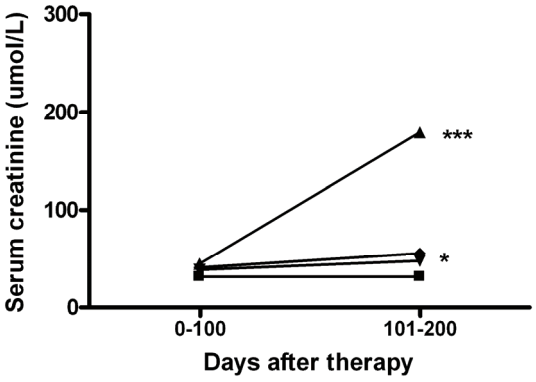


Figure 5. Serum creatinine in rats, 0-100 days and 101-200 days after single doses of 555 MBq (▲) or 278 MBq (▼), or two weekly cycles of 278 MBq (♦) [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Control rats are depicted with ■. (***) P<0.001 vs control, 278 and 2x278 MBq and 555 MBq at 0-100 days; (*) P<0.05 for both 2x278 and 278 MBq vs control. Each group consisted of at least 4 rats. For clarity, standard deviations are not shown.

creatinine was not significantly elevated as compared to the control situation, whereas it rose significantly at 101-200 days after radioactivity injection (P<0.001 vs control; P<0.001 vs 555 MBq 0-100 days). A single dose of 278 MBq resulted in a slightly elevated serum creatinine concentration at 101-200 days (P<0.05 vs control).

Histological damage was microscopically evaluated. Figure 6a shows HE sections of rat kidneys, depicting renal damage scores of 1 (minor damage) up to 4 (severe damage). Figure 6b shows the mean renal damage score after single doses of 555 MBq and 278 MBq over time.

Both groups of rats sacrificed before 100 days after therapy, had a mean histological damage grade of about 1.8. In rats treated with a single dose of 278 MBq the renal damage score remains unchanged for up to 300 days after treatment, whereas rats that were treated with 555 MBq reached an average score of 4 already at 100-200 days after start of therapy.

Effects of fractionation on renal damage after high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate

Fractionation of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate into two weekly doses of 278 MBq largely prevented the rise in serum creatinine concentrations. However, the serum creatinine concentration was still significantly higher as compared to control rats (P<0.05) at 101-200 days after start of therapy (Figure 5).

Figure 7 shows the serum creatinine concentrations at day 150 after start of the treatment. Three cycles of 185 MBq at an interval of a day caused severe renal insufficiency, but the rise in serum creatinine was less pronounced after three weekly doses (P<0.001 vs three cycles of 185 MBq with an interval of a day). Enlarging the interval of administration to a month significantly lowered serum creatinine values as compared with the same doses at intervals of a week or a day. Serum creatinine concentrations of rats treated with three monthly cycles of 185 MBq and of control rats were not significantly different.

Fractionation had a beneficial effect on the histology as well. Rats that had been treated with two weekly cycles of 278 MBq had slowly progressing renal damage scores that ultimately reached an average of 4 on day 200-300, whereas rats treated with a single dose of 555 MBq had a renal damage score of 4 at 101-200 days (Figure 6b).

Table 2 shows that all rats treated with three daily cycles of 185 MBq had a grade 4 renal damage score. In rats in which the interval between treatments was instead a week or a month, there was a trend towards less damage, but differences were not statistically significant.

Table 2. Effects of dose fractionation and lysine co-injection on rat kidney histological damage score 150 days after treatment with high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

Treatment	Histological Damage Score (Mean ± SD)	Number of rats
3x37 MBq, week interval	1.0 ± 0.0	4
3x185 MBq, day interval	4.0 ± 0.0	3
3x185 MBq, week interval	3.9 ± 0.5	20
3x185 MBq, week interval + lysine	3.0 ± 1.0	3
3x185 MBq, month interval	3.3 ± 0.8	7

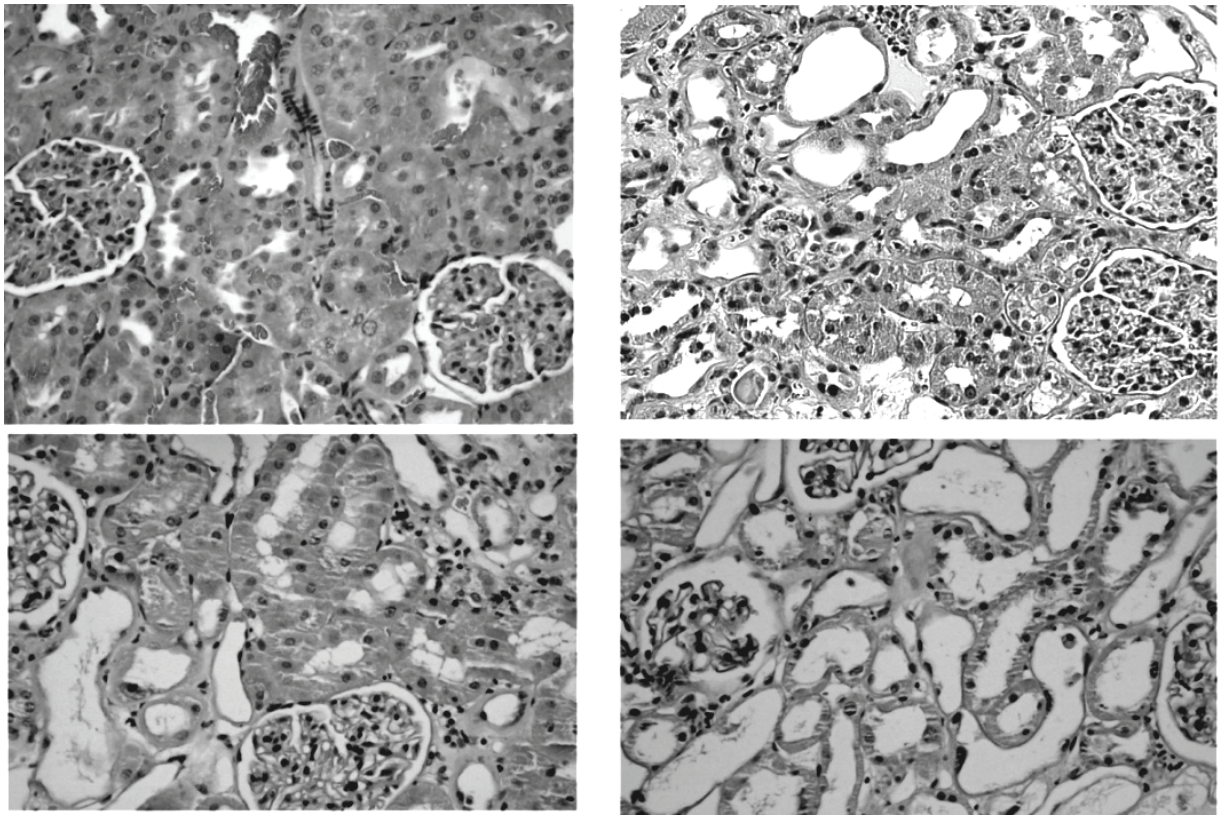


Figure 6a. Examples of renal damage scoring. HE sections (20x) of grade 1 (upper left), grade 2 (upper right), grade 3 (lower left) and grade 4 (lower right) are shown.

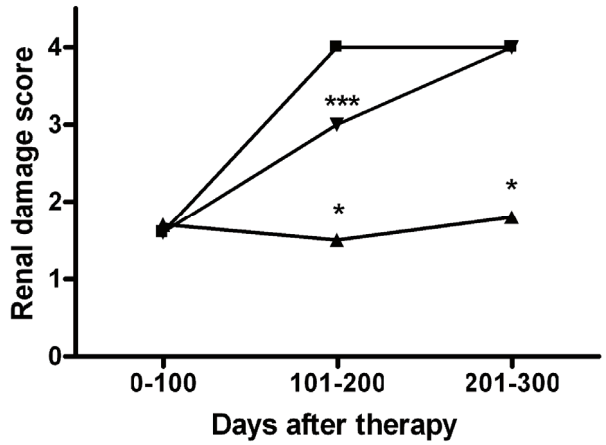


Figure 6b. Renal damage score in rats sacrificed between 0-100 days, 101-200 days and 201-300 after single doses of 555 MBq (■), 278 MBq (▲), or two weekly cycles of 278 MBq (▼) [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Each group consisted of at least 8 rats. (***) P<0.001 vs 555 MBq at same time point; (*) P<0.001 vs both 2x278 MBq and 555 MBq at same time points. For clarity, standard deviations are not shown.

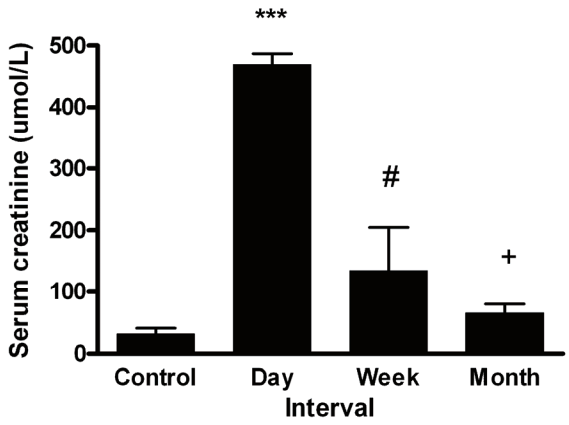


Figure 7. Serum creatinine of rats 150 days after three injections of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate at an interval of a day (*n*=3), a week (*n*=16) or a month (*n*=8). (***) *P*<0.001 vs control, weekly and monthly administration; (#) *P*<0.001 vs control and *P*<0.01 vs monthly administration; (+) NS vs control.

Administration of three cycles of 185 MBq of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate resulted in significant proteinuria, peaking between 70 and 140 days after the start of therapy, as shown in Figure 8. The peak of urinary protein excretion came earlier in the group of rats treated at an interval of a day (peak around 84 days after start therapy) than in animals treated with an interval of a week or a month (peaks around 126 days after start of treatment). The maximum 24-h protein excretion was not significantly different between groups.

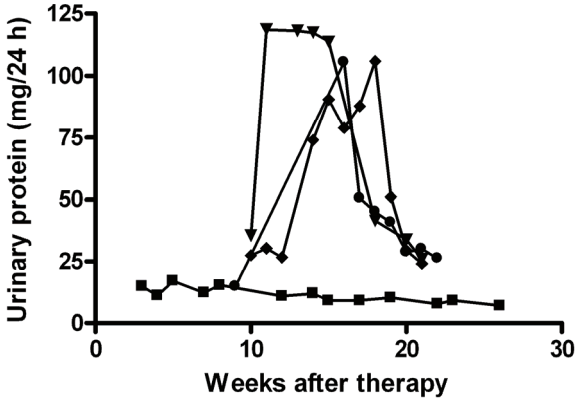


Figure 8. Urinary protein excretion (expressed as mg/24 h) in control rats (■; *n*=6) and in rats after 3 injections of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with intervals of a day (▼; *n*=3), a week (♦; *n*=8) or a month (●; *n*=7). For clarity standard deviations are not shown.

Figure 9 shows an SDS-PAGE from urine samples from rats after treatment with three weekly doses of 185 MBq or 37 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Rats treated with three weekly cycles of 185 MBq had significant proteinuria as compared with control rats and rats treated with three weekly low-dose cycles of 37 MBq. It is also clear that proteinuria increased from 98 to 112 days after treatment with 3x185 MBq.

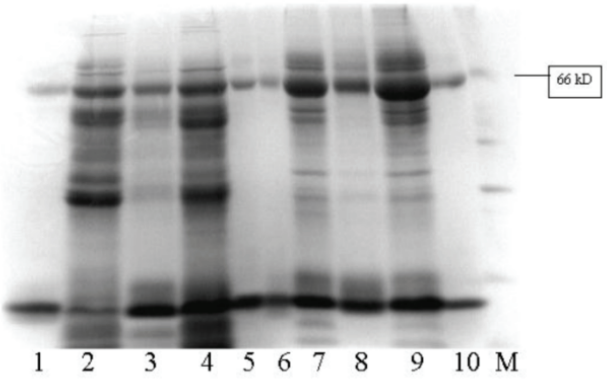


Figure 9. SDS-PAGE of rat urine samples 98 and 112 days after treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Rats received three weekly cycles of 37 MBq (*lanes 1 and 6*), three weekly cycles of 185 MBq (*lanes 2, 4, 7 and 9*), three weekly cycles of 185 MBq with co-injection of 400 mg/kg lysine (*lanes 3 and 8*) or no radioactivity (*lanes 5 and 10*). Urine samples collected at day 98 after first injection of radioactivity are shown in *lanes 1-5*, whereas lanes *6-10* depict the situation at day 112 after radioactivity injection.

Effects of lysine on [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate-mediated kidney damage

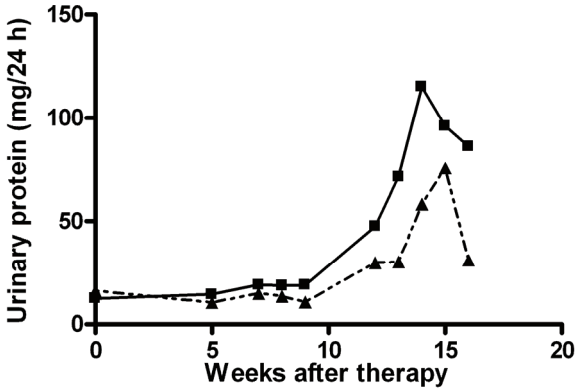


Figure 10. Urinary protein excretion in rats (expressed as mg/24 h) after three injections of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate at intervals of a week. ■ represents rats that were injected with only [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (n=8). ▲represents two rats that were co-injected with 400 mg/kg lysine (n=3). For clarity, standard deviations are not shown. Overall, proteinuria was significantly lower in lysine treated rats (P<0.01).

As shown in Figure 10, protein excretion was significantly lower in the rats that were treated with three weekly cycles of 185 MBq plus co-injection of 400 mg/kg lysine as compared with three weekly cycles of 185 MBq alone (P<0.01). Urine samples of lysine-treated rats had SDS-PAGE profiles that were comparable to those of control rats and rats treated with three weekly cycles of 37 MBq (Figure 9). Rats treated with three weekly cycles of 185 MBq plus lysine had creatinine values of 47 ± 1 umol/L, which were statistically different from the values in rats treated with three weekly cycles of 185 MBq without lysine (134 ± 70 umol/L, P<0.001), but not from those in control rats. Table 2 shows that lysine co-injection did not significantly ameliorate renal histological damage scores.

Effects of high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate on blood haematological values

Figure 11 shows the white blood cell counts and platelet counts from the rats that were treated with single doses of 555 MBq, 278 MBq or with two weekly cycles of 278 MBq. For the two highest doses, there was a significant time-dependent decline in white blood cells and platelets (ANOVA with linear trend: P<0.0077 for white blood count, P<0.0003 for platelets).

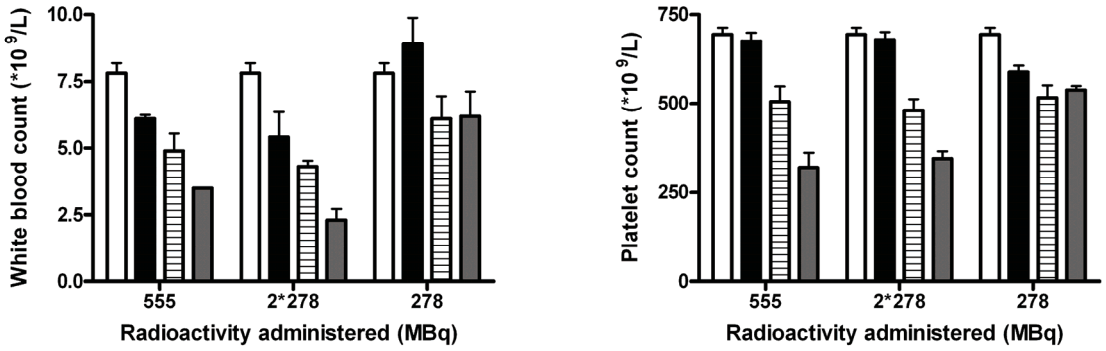


Figure 11. White blood cell (**a**) and platelet (**b**) counts in rats at different time points after injection with 555, 2x278 at an interval of a week or 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. For the two highest doses, there was a significant time-dependent decline of white blood cells and platelets (ANOVA with linear trend: $P<0.0077$ for white blood cells, $P<0.0003$ for platelets).
White bars, control values; *black bars*, 0-100 days; *lined bars,* 101-200 days; *grey bars,* 201-300 days. Each bar represents data of three to eight rats.

The group of rats that was treated with a single dose of 278 MBq had no lowered platelet or white blood cell counts in any period. As shown in Figure 12, platelet and white blood counts were significantly lowered in all groups treated with three cycles of 185 MBq as compared with control values. The three daily cycles of 185 MBq caused significantly lower white blood cell and platelet counts than both the weekly and monthly administrations. The counts in the three weekly cycles of 185 MBq were not significantly different from those in the rats treated with three monthly cycles of 185 MBq. For all groups of rats, no significant changes were noted in haemoglobin concentrations.

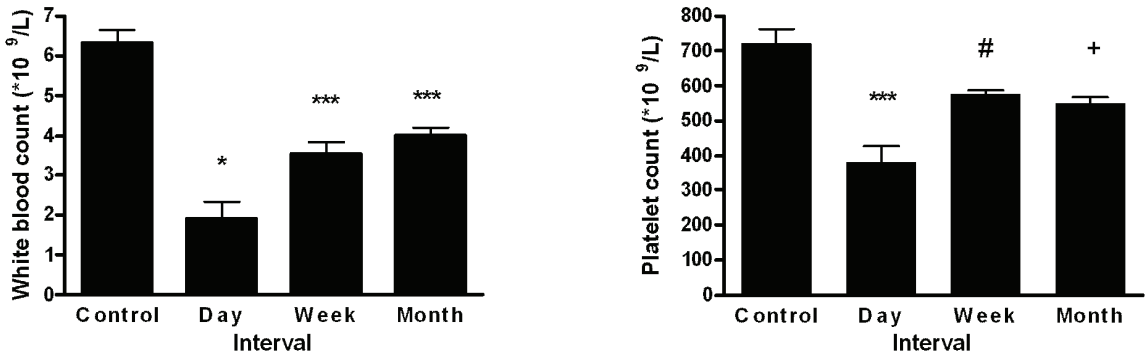


Figure 12. White blood cell (**a**) and platelets (**b**) counts in rats, 150 days after three injections of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate at intervals of a day, a week or a month. (*) $P<0.001$ vs control and $P<0.05$ vs weekly interval; (***) $P<0.001$ vs control; (**) $P<0.001$ vs control and $P<0.01$ vs weekly interval; (#) $P<0.05$ vs control; (+) $P<0.01$ vs control.

DISCUSSION

Most knowledge on radiation damage originates from external beam radiation therapy, but such data cannot easily be generalised to PRRT. For instance, in PRRT the radionuclide is heterogeneously spread, having a long residence in specific organs and specific decay characteristics, whereas external beam radiation is administered in a homogenous fashion and at a higher dose rate. In PRRT, little is known about the time course of renal damage or about maximum tolerated doses, dose dependence and effects of fractionation. In this study, we induced radiation nephropathy in rats by administration of high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. The doses used in this study showed marked anti-tumour activity to CA20948 and AR42J tumours, as also shown in our recent study [118]. Due to this excellent anti-tumour activity the rats lived long and could be followed up to 300 days after therapy for evaluation of normal organ toxicity.

The administration of high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate resulted eventually in marked proteinuria, elevated serum creatinine concentrations and histological damage. According to recently published methods and data, the kidney absorbed dose caused by a single injection of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (the highest dose used in this study) is about 70 Gy [83] when it is assumed that the radioactivity is homogenously distributed over the whole kidney. However, radioactivity uptake is largely located in the cortex and to a lesser extent in the outer medulla region of the kidney [40]. When this distribution pattern is taken into account, the cortex radiation dose after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate is about 95 Gy [83].

Radiation nephropathy due to external beam radiation therapy in humans can be divided into an acute syndrome, occurring within 6 to 12 months after high radiation doses, and a chronic state after lower radiation doses, becoming overt between 1 to 5 years after therapy [70]. Such a discrimination was also shown by Robbins and Bonsib for external beam radiation in mice [58] as well as by Behr et al. for radiolabelled antibody fragments in mice [121]. In our study, renal damage by high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate developed late after kidney irradiation. Until day 100 after the start of therapy, rat kidneys showed little or no histological damage and no significant alterations in serum creatinine were found. This is in accordance with the findings of Lewis et al., who did not find significant (acute) toxicity of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate 6 weeks after injection [50]. At later stages, the rats in our study developed severe functional and histological damage. Clinical data on renal toxicity indicate that in most cases the toxicity becomes overt one year or later after PRRT [34, 44, 45].

In this study, the histological damage was located mainly in the proximal tubules, which showed flat dilated tubular cells and thickened basal membranes. In accordance with previous

animal study reports using radiolabelled antibody fragments [121], little glomerular damage was seen. In patients, high-dose kidney radiation (either in external beam radiation therapy or in PRRT) resulted in tubular atrophy and interstitial fibrosis but most attention has been paid to the radiation-induced glomerular damage [45, 48, 51]. Also, the distinct histopathological feature of thrombotic microangiopathy (TMA) has been reported [45, 48, 51], which can exist in a variety of diseases [122]. In several experimental studies with rats and mice, no TMA like histological picture could be shown [55].

Our present study clearly shows the renal radiation damage to be dose dependent, as lower doses resulted in less toxicity. Also, we showed that development of radiation damage was strongly influenced by dose fractionation. The rationale behind dose fractionation is to spare normal tissues by repairing sublethal damage during the interval between doses. In our study, there was significantly more renal damage after one single dose as compared with the same dose fractionated over two equal doses. In the experiments using three doses of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, functional renal damage (elevation of serum creatinine and proteinuria) was significantly less with longer intervals between cycles. This is in accordance with data in humans [49] showing that a biologically equivalent dose (BED) based on fractionation and actual kidney volume correlates better with the occurrence of renal toxicity than the kidney absorbed dose estimated using standard calculations methods. The report of Barone et al. [49], showed also that applying the LQ model resulted in a higher critical renal dose than derived from external beam radiation therapy. The median BED was 37 Gy in their patients, and a BED of 45 Gy was proposed as a threshold for kidney function loss [49]. Although fractionation of the total dose may be favourable for the kidneys, not everything is known about effects of fractionation on anti-tumour efficacy. It can be expected that tumours will have less protection by fractionation because their alpha/beta-values (an index of the sensitivity of an tumour/organ to changes in dose rate [49]) are high. Furthermore, we have shown that fractionating a dose of 555 MBq into two or three cycles has an anti-tumour efficacy that is comparable to that of the unfractionated dose. In fact, the anti-tumour efficacy of fractionated PRRT may even be enhanced owing to higher expression of somatostatin receptors after radiation, as was previously reported [123, 124].

The use of amino acids, such as lysine, to inhibit kidney uptake of radiolabelled somatostatin analogues is generally accepted. At present, amino acid (lysine plus arginine) infusions are standard procedure in PRRT in our hospital [116]. The use of these amino acids will probably allow higher administered radioactivity doses, and thus higher tumour radiation doses, within safe limits for kidney radiation. However, no validation studies have been published so far on PRRT with radiolabelled somatostatin analogues in either animals or humans. Studies using radiolabelled antibody fragments in mice showed that lysine co-injection allowed for higher

doses to be administered with concomitantly less kidney damage [121]. Our present study shows that co-injection of lysine prevented functional kidney damage as both creatinine values and the extent of proteinuria were significantly reduced in comparison to the rats injected with the same dose of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate without lysine co-injection.

We found a discrepancy between the histological damage data and functional parameters. No clear correlation was found between histological score and serum creatinine concentrations. For instance, a maximum renal damage score of 4 could be accompanied by either very high or almost normal creatinine values. An explanation might be that kidney tissues at very advanced stages of microscopical damage have different levels of functional reserve.

Although amino acids are being used successfully, other strategies to protect the kidneys are needed, the ultimate goal being to deliver the highest possible dose to the tumour. As ionising radiation generates a persistent state of oxidative stress whereby reactive oxygen species (ROS) cause cellular and DNA damage and also modulate kidney cell phenotype [55], one might consider anti-oxidants as new kidney protectors in PRRT. Kidney damage by radiation or chemotherapy has successfully been prevented by the anti-oxidant amifostine [106], a pro-drug that is activated in normal tissues by alkaline phosphatases, and to a much lower extent in tumour tissue. It is rapidly taken up into the cells, where it would act as a creator of local hypoxia and as an ROS scavenger. At present, studies on the renoprotective effects of amifostine are being performed in our department.

In PRRT, the radiosensitive bone marrow is the second dose-limiting factor. In patients the bone marrow toxicity is limited and mostly reversible [37]. In this rat study, the platelet and white blood cell counts declined over the course of time. Dose fractionation with long intervals (a week or a month) resulted in significantly higher counts than the 1-day interval.

CONCLUSION

We showed that injection of high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate resulted in severe renal damage in rats as indicated by proteinuria, elevated serum creatinine and histological damage. This damage was dependent on the total dose administered and became overt between 100 and 200 days after treatment. Fractionation of doses with intervals of a week or more significantly ameliorated kidney function. Lysine co-injection successfully prevented functional kidney damage.

CHAPTER 3

REDUCTION OF RENAL UPTAKE OF RADIOLABELLED SOMATOSTATIN ANALOGUES

CHAPTER 3.1

INHIBITION OF RENAL UPTAKE OF INDIUM-111-DTPA-OCTREOTIDE IN VIVO

Marion de Jong, Edgar J. Rolleman, Bert F. Bernard, Theo J. Visser, Willem H. Bakker, Wout A.P. Breeman and Eric P. Krenning

J Nucl Med 1996; 37:1388-92

ABSTRACT

Background

[^{111}In-DTPA^{0}]octreotide has been successfully used for imaging of somatostatin receptor-positive lesions. However, significant renal uptake of [^{111}In-DTPA^{0}]octreotide exists, reducing the scintigrafic sensitivity for detection of small tumours in the perirenal region and the possibilities for radiotherapy. The aim of the present study was to determine whether renal uptake of [^{111}In-DTPA^{0}]octreotide could be reduced in vivo in rats.

Methods

Male wistar rats (200-250 g) were placed in metabolic cages and injected with [^{111}In-DTPA^{0}]octreotide (0.2 MBq and 0.5 μg octreotide), in the presence or absence of re-uptake blockers. Twenty hours after injection, rats were sacrificed and organs were isolated and counted for radioactivity.

Results

Adding NH_4Cl or $NaHCO_3$ to the food, resulting in the production of more acid or alkaline urine respectively, resulted in less radioactivity in the kidney 20 h after injection compared to controls. Lysine in a single dose of 400 mg/kg resulted in an inhibition of kidney uptake of 40%. When lysine was injected 30 min before [^{111}In-DTPA^{0}]octreotide, the inhibition was 25%. Arginine had less effect on tubular uptake of [^{111}In-DTPA^{0}]octreotide than lysine (20% inhibition). Sodium maleate inhibited kidney uptake of [^{111}In-DTPA^{0}]octreotide most successfully. Acetazolamide (100 mg/kg), succinylacetone (100 mg/kg), cystine dimethylesther (340 mg/kg) and increase in urinary flow did not influence [^{111}In-DTPA^{0}]octreotide retention to the kidneys.

Conclusion

It appeared possible to reduce re-uptake of [^{111}In-DTPA^{0}]octreotide in the rat kidney in vivo. The most pronounced effects were seen after administration of sodium maleate or lysine but, because of the described toxic effects of maleate, we will study further only the effects of lysine in a clinical setting.

INTRODUCTION

[^{111}In-DTPA^{0}]octreotide is a radiopharmaceutical that binds to somatostatin receptors (subtypes 2, 3 and 5) present in certain tissues. It is being used for scintigraphic imaging of somatostatin receptor-positive lesions, such as gastrointestinal pancreatic tumours, neuroblastoma, pheochromocytoma, breast cancer, Hodgkin's lymphoma and small-cell lung cancer [16, 125]. This peptide is cleared from the body mostly by the kidneys, 50% within the first four hours after injection. However, a significant amount of the dose accumulates in the renal parenchyma (in humans about 7% dose, 4 hr after injection), reducing the scintigraphic sensitivity for detection of small tumours in the perirenal region in the abdomen [126].

In this study we investigated if this renal accumulation could be reduced in vivo in rats. The infusion of certain amino acids, particularly lysine and arginine, has been shown to block renal tubular peptide reabsorption [95]. An infusion of synthetic amino acids containing lysine and arginine among other amino acids, significantly reduced parenchymal uptake (up to 50%) of [^{111}In-DTPA^{0}]octreotide 4 h after injection in eight patients, without an effect on glomerular filtration rate [96]. Also in mice, reduction of renal tubular reabsorption of ^{111}In-labelled Fab fragment was affected by systemic administration of lysine [127]. We have, therefore, tested the influence of a single dose of lysine or arginine on renal tubular uptake of [^{111}In-DTPA^{0}]octreotide in vivo in the rat. We also investigated the influences of: increased urine production; changes in the pH of the urine, the latter by addition of NaCl, $NaHCO_3$ or NH_4Cl to the food; and sodium maleate on the re-uptake process. Maleate is known to produce a generalized defect of renal tubular transport, causing an immediate and transient diuresis, natriuresis, glucosuria and proteinuria, probably by inhibition of renal cortical Na-K-ATPase and ATP production [128-130]. In addition, we tested the compounds succinylacetone, acetazolamide and cystine dimethylesther, because these compounds have been described to interfere with renal uptake processes [131-133].

MATERIALS AND METHODS

Radiolabeling and radiopharmaceutical quality control

[DTPA^{0}]octreotide and $^{111}InCl_3$ (DRN 4901, 370 MBq/mL in HCl, pH 1.5-1.9) were obtained from Mallinckrodt Medical (Petten, The Netherlands). The radiolabelling procedure was performed as described earlier [134].

Tissue distribution and specific binding of [^{111}In-DTPA^{0}]octreotide

Male Wistar rats (200-250 g) were placed in metabolic cages 24 h before the start of the experiment. Rats were fed either dry rat chow ad libitum or 35 g/day chow suspension (35 g=14 g chow in 21 mL water). Drinking water was always available ad libitum. At time t=0,

rats were anaesthetized with ether and injected with [^{111}In-DTPA^{0}]octreotide (0.2 MBq and 0.5 μg octreotide), preceded or not by injection of uptake-blocking compounds (listed below), into the dorsal vein of the penis (volume 200 μL). In order to study nonspecific binding, some rats were injected subcutaneously with 0.5 mg octreotide in 1mL 0.05 M acetic acid in 154 mM NaCl, 40 min before injection of [^{111}In-DTPA^{0}]octreotide.

Twenty hours after injection of the radiolabelled product, rats were sacrificed with ether and organs were isolated. Tissue distribution was studied by measuring radioactivity in isolated organs as well as in blood samples.

Statistical evaluation was performed using one-way analysis of variance followed by comparison among class means and Student's t-test, corrected for multiple pair wise comparisons between means. Results are expressed as mean ± SD.

Methods and compounds used to reduce renal uptake of [^{111}In-DTPA^{0}]octreotide

Rats were fed either dry rat chow or, to increase urine production, 35 g/day chow suspension (35 g=14 g chow in 21 mL water). Rats were given intravenous injections of: positively charged amino acids L-lysine (400 mg/kg) or L-arginine (460 mg/kg), that can bind to negatively charged sites on renal tubular membranes; and compounds that have been described to interfere with renal uptake processes-sodium maleate (200,400 and 600 mg/kg), acetazolamide (100 mg/kg), succinylacetone (100 mg/kg) and succinyl dimethylesther (340 mg/kg). Also, NaCl (control), $NaHCO_3$ or NH_4Cl was added to the food, during the 48 hours before the start of the experiment, in order to induce changes in the pH of the urine.

All compounds injected intravenously were administered at physiological pH in volumes of 200 μL immediately before [^{111}In-DTPA^{0}]octreotide injection, unless otherwise stated.

RESULTS

In Table 3, the distribution of radioactivity in organs of control rats is shown, expressed as percentage injected dose (%IA)/g tissue, 20 h after administration of [^{111}In-DTPA^{0}]octreotide. Under the conditions used in our experiments, excretion into the urine is ≥70% dose within 20 h. Radioactivity in the kidneys is 1.52% IA/g at this time.

In Figure 13, the influence of increase in urine production on tubular reabsorption of [^{111}In-DTPA^{0}]octreotide is depicted. Rats that eat dry rat chow (set of points in Figure 13) produce less urine (range 3.2-13.5 mL/20 h) than rats that eat food suspended in water (set of points further to the right in Figure 13; range 18.8-26.6 mL/20 h). However, the increase in urine production did not lower the amount of radioactivity in the kidneys (dry food: 1.49% ± 0.22% IA/g; food suspension: 1.53% ± 0.13% IA/g; not significantly different).

Organ	%IA/g
Blood	0.0019 ± 0.0003
Kidneys	1.52 ± 0.15
Liver	0.061 ± 0.012
Pancreas	0.52 ± 0.12
Spleen	0.030 ± 0.005
Adrenals	0.76 ± 0.10

Table 3. Distribution of [^{111}In-DTPA^{0}]octreotide in organs of control rats 20 hours after administration (0.2 MBq/0.5 µg). Mean ± SD, *n*=8-29.

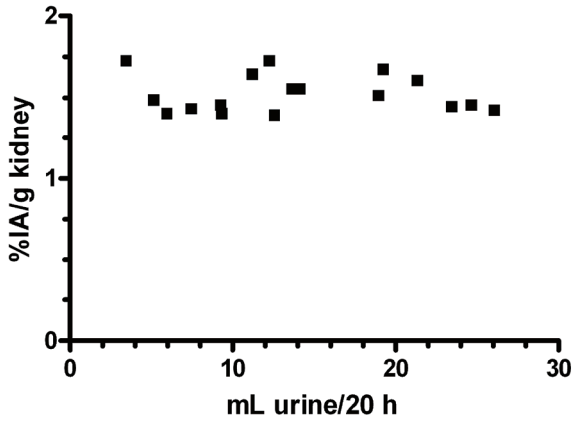


Figure 13. Influence of urine production during the experiment in kidney uptake of [^{111}In-DTPA^{0}]octreotide (*n*=17).

In Figure 14 the effect of changes in pH of the urine, induced by addition of electrolytes to the food, on tubular reabsorption is shown. Control rats received 1 mmol/day NaCl in their food suspension, resulting in a normal urinary pH (6.75 ± 0.22). To induce production of more acidic or alkaline urine, 1 mmol NH_4Cl or $NaHCO_3$ was added to the food. This resulted in urine pH values of 5.84 ± 0.18 and 7.35 ± 0.04. Figure 14 shows that both alkalinazation and acidification of the urine resulted in a lower dose in the kidney 20 h after administration of the radiolabelled compound.

Figure 15 shows the effects of several compounds, injected intravenously (just prior to the [^{111}In-DTPA^{0}]octreotide administration, unless otherwise stated), on the kidney dose 20 h after administration of [^{111}In-DTPA^{0}]octreotide. Administration of lysine in a single dose of 400 mg/kg resulted in an inhibition of the kidney uptake of 40% (P<0.001 vs control). When lysine was injected 30 min before [^{111}In-DTPA^{0}]octreotide, the inhibitory effect was less pronounced at 25% (P<0.001 vs control). Arginine, also a positively charged amino acid, had less effect on tubular re-uptake of [^{111}In-DTPA^{0}]octreotide than lysine (20% inhibition).

Sodium maleate was the most effective compound tested. As shown, the effects of sodium maleate were dose dependent. It appeared however, that rats given sodium maleate ate less of their food than control animals. They ate 81.6% of control when given 600 mg/kg maleate and 77.5% of control when given 400 mg/kg maleate.

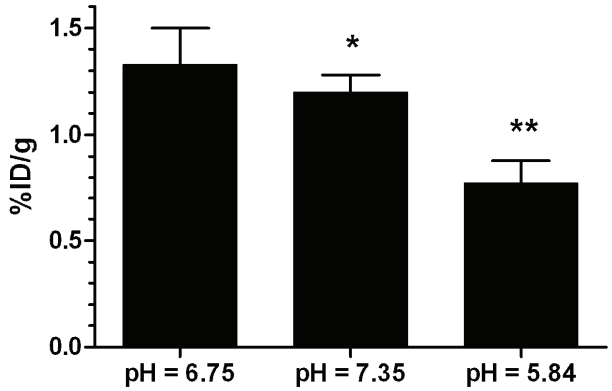


Figure 14. Influence of urinary pH on kidney uptake of [^{111}In-DTPA^{0}]octreotide. (*) P<0.05; (**) P<0.005 vs control (pH=6.75), all groups n=3.

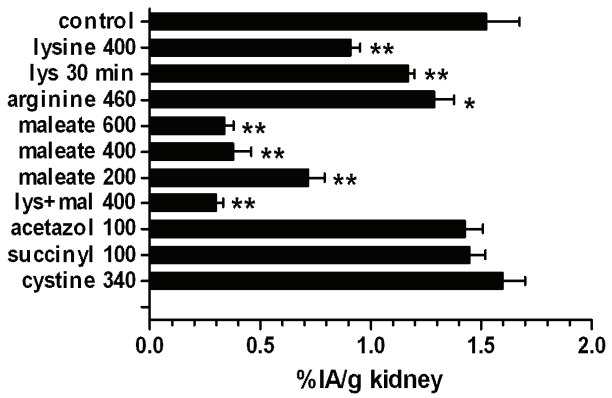


Figure 15. Influence of several compounds on kidney uptake of [^{111}In-DTPA^{0}]octreotide. Doses are given in mg/kg.
control= controls (n=29); lysine 400= lysine 400 mg/kg (n=8); lys 30 min=lysine injected 30 min before radiolabelled compound (n=3); lys+mal= 400 mg/kg lysine plus 400 mg/kg maleate (n=4); acetazol= acetazolamide 100 mg/kg (n=3); succinyl= succinyl acetone (n=3), cystine= cystine dimethylester (n=3). For groups arginine 460 mg/kg, maleate 600 mg/kg and maleate 200 mg/kg: n=3; maleate 400 mg/kg: n=8. (*) P<0.01, (**) P<0.001 vs control.

A combination of lysine and maleate (both 400 mg/kg) reduced the kidney dose of [^{111}In-DTPA^{0}]octreotide more than both compounds alone (P<0.005 vs sodium maleate and P<0.001 vs lysine alone). Rats receiving this combination ate 90.9% of control. The combination of lysine and sodium maleate together with administration of electrolytes (NaHCO3 or NH4Cl) in the food did not change kidney uptake of [^{111}In-DTPA^{0}]octreotide significantly compared to the combination of lysine and maleate alone (not shown).

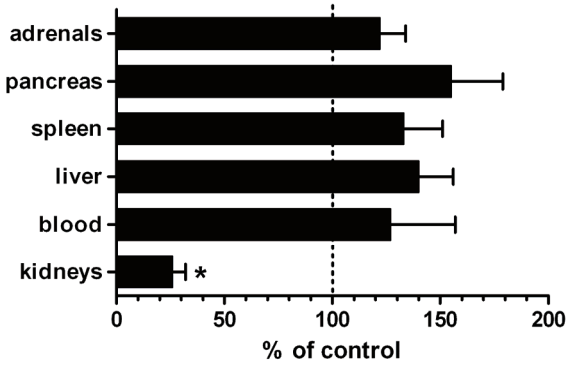


Figure 16. Influence of 400 mg/kg sodium maleate on the kidney uptake of [^{111}In-DTPA^{0}]octreotide in several organs (n=8); (*) P<0.01.

Figure 15 shows the effects of several other compounds as well, including acetazolamide (100 mg/kg), succinylacetone (100 mg/kg) and cystine dimethylester (340 mg/kg). These compound had no significant effect on the amount of radioactivity in the kidneys 20 h after [^{111}In-DTPA^{0}]octreotide administration.

Figure 16 shows the effects of 400 mg/kg sodium maleate on the uptake of [^{111}In-DTPA^{0}]octreotide in several other organs 20 h after administration, expressed as % of control values (Table 3). In contrast to the kidneys the dose in all other organs, including the blood, was increased after sodium maleate administration, though not significantly (range 121-155% of control values). In order to study if this increased uptake of [^{111}In-DTPA^{0}]octreotide represented specific binding, some rats were pretreated with 0.5 mg unlabelled octreotide before [^{111}In-DTPA^{0}]octreotide administration, combined or not with administration of sodium maleate (400 mg/kg). Unlabelled octreotide competitively inhibited the binding of [^{111}In-DTPA^{0}]octreotide to the somatostatin receptors, as is shown in Figure 17 for pancreas and adrenals. These organs contain somatostatin receptors and the amount of radioactivity after unlabelled octreotide treatment was less than 2% of the control values. It is further shown that the increase in radioactivity in pancreas and adrenals is inhibited by unlabelled octreotide, showing that the increase in organ radioactivity consisted of specific binding of [^{111}In-DTPA^{0}]octreotide to somatostatin receptors.

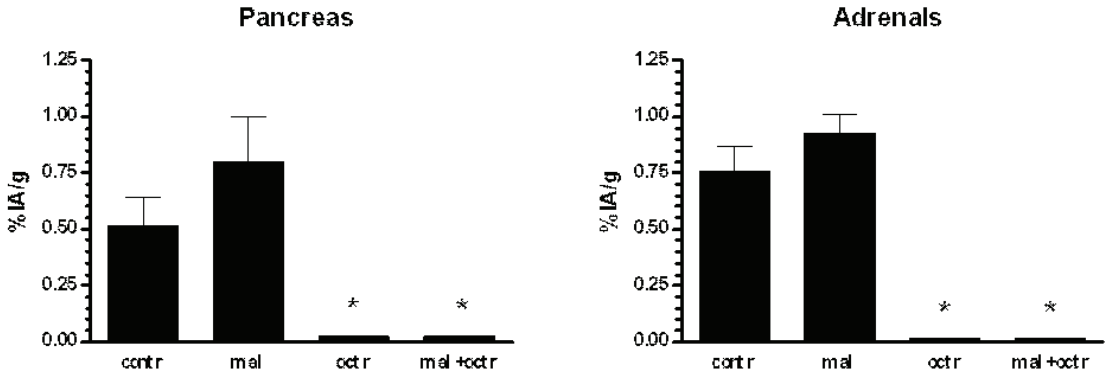


Figure 17. Uptake of [^{111}In-DTPA^{0}]octreotide in pancreas and adrenals after pretreatment with 0.5 mg unlabelled octreotide 40 min before [^{111}In-DTPA^{0}]octreotide administration, whether or not in combination with sodium maleate (400 mg/kg). (*) P<0.001 vs control. All groups n=3.

DISCUSSION

Peptides in the plasma are filtered through the capillaries in the kidneys and subsequently reabsorbed almost completely (≥ 90%) by the proximal tubular cells via receptor-mediated endocytosis. First, the ligand binds to a carrier on the membrane. The carrier-ligand complex is then internalized in an intracellular vesicle; the vesicle content becomes acidified, releasing the ligand from its receptor. Then, the ligand is routed to the lysosomes where degradation

takes place. These steps require energy [135]. Lysozomal degradation has also been described for [^{111}In-DTPA^{0}]octreotide. Its labelled degradation products are trapped in the lysosomes, because of the charged DTPA-complex [92]. This study demonstrates that the uptake of [^{111}In-DTPA^{0}]octreotide by the renal tubular cells after glomerular filtration can be reduced, in favor of the scintigraphic sensitivity of detection for small tumours in the perirenal region and of radiotherapy.

Increases in urine production, as shown in Figure 13, were induced by giving food suspension in water. However, re-uptake of [^{111}In-DTPA^{0}]octreotide in the renal tubules is apparently a very efficient process that is not influenced by increased urine production.

Addition of electrolytes to the diet, resulting in a change in pH of the urine, reduced kidney uptake of [^{111}In-DTPA^{0}]octreotide (Figure 14). Membranes of renal tubular cells contain negatively charged sited, to which positively charged amine or guanine residues of peptides can bind [95]. Changing the pH of the (primary) urine may influence these binding processes, decreasing the efficiency of the re-uptake process of [^{111}In-DTPA^{0}]octreotide.

Decreased binding to negatively charged membranes of renal tubular cells of [^{111}In-DTPA^{0}]octreotide after administration of the positively charged amino acids lysine and arginine can be explained by the same phenomenon [96]. In our study the effect of lysine was more pronounced than that of arginine (Figure 15), in accordance with the findings of Mogensen and Solling [95]. They reported that compounds with a positively charged group preferentially located terminally in the molecule inhibited instantaneously the tubular protein reabsorption. This finding of instantaneous inhibition is in concert with our finding that administration of lysine 30 min before [^{111}In-DTPA^{0}]octreotide injection was less effective in reduction of kidney uptake than administration just prior to injection of the radiolabelled compound.

Hammond et al. [96] reported that an infusion of synthetic amino acids containing lysine and arginine, among other amino acids, significantly reduced renal uptake of [^{111}In-DTPA^{0}]octreotide 4 h after injection, without effect on glomerular filtration rate. Also in mice, inhibition of the renal tubular reabsorption process, in the case of ^{111}In-labelled Fab fragment, could be effected by systemic administration of lysine [127]. This is in accordance with our findings in rats.

The most pronounced effect on renal uptake of [^{111}In-DTPA^{0}]octreotide was exerted by sodium maleate (Figure 15). Sodium maleate has been used to study renal tubular dysfunction comparable to Fanconi's syndrome in humans [128]. Maleate forms maleyl-CoA by reacting with succinyl-CoA, thereby reducing the cellular CoA supply and inhibiting the citric acid cycle in tubular cells. The resulting reduced ATP supply or the reaction of the maleyl-CoA with membrane proteins inhibits a variety of transport systems, including peptide reabsorption

[136, 137]. The effect of sodium maleate was dose dependent, and the combination of lysine and sodium maleate resulted in even greater inhibition of the re-uptake process.

The compounds cystine dimethylesther, succinylacetone and acetazolamide had no effect on reabsorption of [^{111}In-DTPA^{0}]octreotide. Increased excretion of peptides into the urine of rats in vivo, without causing any renal abnormalities, has been described after administration of cystine dimethylester [133], but in our study a single dose did not influence [^{111}In-DTPA^{0}]octreotide reabsorption. The same holds for succinylacetone, a compound that depressed oxygen consumption in the rat renal tubule [131] and inhibits peptide re-uptake [132] without any damage to mitochondria. Acetazolamide is known to increase intracellular pH, but did not influence [^{111}In-DTPA^{0}]octreotide reabsorption in our study.

The increasing effect of sodium maleate on the dose of [^{111}In-DTPA^{0}]octreotide in other organs may be explained by described inhibiting effects on the renal glomerular filtration rate [138]. However, although the increasing effect was consistent, it was not significantly different from the control situation because of rather large standard deviations. One may conclude from our findings, however, that during inhibition of glomerular filtration of [^{111}In-DTPA^{0}]octreotide a longer residence time in the plasma will occur leading to higher uptake in the organs. The effect of sodium maleate on organ uptake was also found in the case of [^{161}Tb-DTPA^{0}]octreotide [139]. Uptake in all organs, except for the kidneys was significantly increased after sodium maleate administration.

The effect of sodium maleate on glomerular filtration rate may not be explain the inhibition of kidney uptake of [^{111}In-DTPA^{0}]octreotide. Hysing et al. [138] studied the effects of sodium maleate on protein reabsorption at a glomerular filtration rate in dogs by altering the renal arterial perfusion pressure. Under these conditions, they still found the reduction of reabsorption by sodium maleate, showing that the effect of sodium maleate on tubular reabsorption is not caused by a decrease in glomerular filtration rate alone.

Figure 17 shows higher uptake of [^{111}In-DTPA^{0}]octreotide into the pancreas and adrenals after maleate administration, with both organs containing somatostatin receptors. This increase in binding can be completely prevented with unlabelled octreotide, showing that the higher uptake after maleate represented the higher specific binding of [^{111}In-DTPA^{0}]octreotide ot the somatostatin receptors.

Although maleate had inhibitory effects on renal re-uptake of [^{111}In-DTPA^{0}]octreotide, it may not be suitable for administration to humans because the toxic effects of this compound on the kidneys. Conflicting data have been published. Worthen [130] described abnormalities in the proximal tubules within 2 hours after injection to rats, the degree related to the dose of sodium maleate (480-1440 mg/kg) given. Normal renal histology returned after intervals of 24 to 72 hours, depending on the dose administered. Harrison and Harrison [128] also did not find

evidence of permanent injury in rat kidneys after a dose of 160 mg/kg body weight daily for a period of 2 to 3 weeks. Hysing et al. [138] found that maleate stopped protein reabsorption in dogs without a significant increase in brush border and lysosome marker enzymes in the urine. However, Verani et al. [140] observed in kidneys of rats treated with 200 or 400 mg/kg maleic acid infusion (1 hour) an immediate injury that progressed to necrosis by 24 hours after administration. No dose relationship was seen. We could not find histological abnormalities in kidneys of rats after a single dose of 400 mg/kg maleate (not shown). However, the cited findings caused us to abandon the idea of human administration.

It appeared possible to reduce re-uptake of [^{111}In-DTPA^{0}]octreotide in the rat kidney in vivo. The most pronounced effects were seen after administration of sodium maleate or lysine but, because of possible toxic effects of maleate, we will further study the effects of lysine in clinical studies only.

CHAPTER 3.2

SAFE AND EFFECTIVE INHIBITION OF RENAL UPTAKE OF RADIOLABELLED OCTREOTIDE BY A COMBINATION OF LYSINE AND ARGININE

Edgar J. Rolleman, Roelf Valkema, Marion de Jong, Peter P.M. Kooij and Eric P. Krenning.

Eur J Nucl Med Mol Imaging 2003; 30:9-15

ABSTRACT

Background

As scintigraphy with [^{111}In-DTPA^{0}]octreotide has become a standard technique in analysing somatostatin receptor-positive lesions such as neuro-endocrine tumours, a logical next step is peptide receptor radionuclide therapy (PRRT). Initial studies on PRRT were performed with high doses of [^{111}In-DTPA^{0}]octreotide, and recently other radionuclides coupled to other somatostatin analogues have been used for this purpose. However, the dose delivered to the kidney is a major dose-limiting factor. Amino acid solutions have previously been used to reduce renal uptake of radioactivity, but these solutions have some disadvantages, i.e. their hyperosmolarity and their propensity to cause vomiting and metabolic changes. In this study we tested various amino acid solutions in patients receiving [^{111}In-DTPA^{0}]octreotide PRRT patients in order to assess their safety and their capacity to inhibit the renal uptake of radioactivity.

Methods and results

Patients served as their own non-infused control. Renal radioactivity at 24 h after the injection of [^{111}In-DTPA^{0}]octreotide was inhibited by (1) a commercially available amino acid solution (AA) (21% ± 14%, P<0.02), (2) by 25 g (17% ± 9%, P<0.04), 50 g (15% ± 13%, P<0.04) or 75 g of lysine (44% ± 11%, P<0.001) and (3) by a combination of 25 g of lysine plus 25 g of arginine (LysArg) (33% ± 23%, P<0.01). Fluid infusion alone (500, 1000 or 2000 mL of saline/glucose) did not change renal uptake of radioactivity. In patients studied with 75 g of lysine (Lys75) and LysArg, serum potassium levels rose significantly. Maximal potassium levels were within the toxic range (6.3, 6.7 and 6.8 mmol/L) in three out of six patients infused with Lys75, whereas with LysArg the highest concentration measured was 6.0 mmol/L. Electrocardiographic analysis did not reveal significant changes in any of the patients. Vomiting occurred in 50% of patients infused with AA, but in only 6% of patients receiving no amino acid infusion (controls) and 9% of patients receiving LysArg.

Conclusion

Co-infusion of Lys75 or LysArg results in a significant inhibition of renal radioactivity in PRRT, allowing higher treatment doses and thus resulting in higher tumour radiation doses. Because Lys75 produced serious hyperkalaemia, it is not suitable for clinical use. LysArg however, is effective in offering renal protection in PRRT and is safe.

INTRODUCTION

Scintigraphy using the radiolabelled somatostatin analogue [^{111}In-DTPA^{0}]octreotide has great potential for the visualization of somatostatin receptor-positive lesions such as neuro-endocrine tumours, breast cancer and small cell lung cancer [16, 141, 142]. Its high sensitivity for neuro-endocrine tumours makes scintigraphy with [^{111}In-DTPA^{0}]octreotide the method of choice for the detection and staging of these tumours [141].

Another new and promising application of radiolabelled octreotide and other somatostatin analogues is peptide receptor radionuclide therapy (PRRT). Initial PRRT studies were performed using high doses of [^{111}In-DTPA^{0}]octreotide [28, 29], which emits Auger and internal conversion electrons. Recently, studies have been published on PRRT with the β-particle emitting radiopeptides [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34, 46] and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate [143-145].

However, the high renal uptake of the radiolabelled compounds may lead to renal radiation toxicity - a dose-limiting factor in PRRT [146]. In the aforementioned PRRT study with [^{111}In-DTPA^{0}]octreotide [29], no acute nephrotoxicity was noticed, confirming findings in rat experiments [147]. In contrast, recent reports have shown both acute and late renal side effects in studies on PRRT with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34, 44, 45, 48]. A cumulative dose of [^{90}Y-DOTA^{0}, Tyr^{3}] octreotide of more than 7,400 MBq/m^{2}, without renal protection, may be a risk factor for renal toxicity [34], whereas lower doses may cause late-onset renal insufficiency [44]. Unfortunately, these reports did not provide data on individual dosimetry, especially in those who had renal complications.

Since the radiation dose to the kidney is dose-limiting in PRRT, optimization of the tumour radiation dose while avoiding too much radiation damage to the kidneys is necessary, and will enlarge the therapeutic window. This goal may be reached when three conditions are met. Firstly, the tumour dose should be optimized by use of improved chelators, peptides and radiometals. Secondly, the individual maximal safe radiation burden to the kidney should be known and should not be exceeded. Thirdly, specific interactions with the radiopharmaceutical at the cellular level may lower the renal radiation burden. This last aspect was the subject of this study.

Radiolabelled peptides are filtered by the glomerulus and effectively taken up in proximal tubule cells by endocytosis [91, 115]. After uptake, the radioligand is translocated to the lysozomal apparatus for further handling [27]. Uptake of low-molecular-weight proteins can be inhibited by cationic compounds, for instance amino acids [95]. In a semi-quantitative manner, Hammond et al. [96] reported reduced renal parenchymal uptake of [^{111}In-DTPA^{0}]octreotide in humans after co-infusion of a mixture of amino acids. Behr et al. [97] showed that renal uptake of ^{111}In-labelled monoclonal antibodies could be inhibited by

cationic amino acids in mice. Furthermore, our rat studies showed that co-injection of 400 mg/kg L-lysine inhibited renal uptake of [^{111}In-DTPA^{0}]octreotide by about 40%, whereas arginine caused an inhibition of 20% [115].

Current PRRT protocols use commercially available mixtures of amino acids to reduce renal uptake, causing a reduction in the radiation dose to the kidney of about 40% [148, 149].

However, these amino acid solutions have some disadvantages. First, a large amount of non-relevant amino acids is infused, making the solution hyperosmolar. Therefore these solutions need to be diluted to a larger volume, which may give rise to haemodynamic problems in patients with cardiac disease. Secondly, infusion of large amounts of amino acids may cause metabolic changes, of which hyperkalaemia is the most important. Hyperkalaemia has been associated with infusion of hyperosmolar amino acids [150], cationic amino acids [151-154] or the lysine-like drug ε-aminocaproic acid [155]. In some cases, cardiac arrhythmias caused by hyperkalaemia after cationic amino acid administration are fatal. Thirdly, infusion of amino acid mixtures may produce profound vomiting [153].

In this report we studied various amino acid solutions in order to assess their effectiveness in reducing renal uptake of radioactivity in PRRT and their side effects.

PATIENTS AND METHODS

Study protocol

As frequent high-dose administration of [^{111}In-DTPA^{0}]octreotide has been found not to result in renal toxicity, even at calculated renal absorbed doses of 30 Gy or more [29, 143], we performed this study in patients receiving [^{111}In-DTPA^{0}]octreotide PRRT.

The study group comprised 26 patients with end-stage neuro-endocrine tumours who were enrolled in study protocols for treatment with multiple therapeutic doses of [^{111}In-DTPA^{0}]octreotide or were eligible for this treatment. They received repeated doses of either 220 MBq (diagnostic dose) or 7-10 GBq (therapeutic dose) of [^{111}In-DTPA^{0}]octreotide. In order to allow in-patient comparison, each patient received at least one dose of [^{111}In-DTPA^{0}]octreotide without any amino acid co-infusion.

To study the effect of volume administration alone, patients received 500, 1,000 or 2,000 mL of saline 0.45%/glucose 2.5% intravenously. To study the effects of different amino acid solutions (see below) on the renal uptake of radioactivity, patients were infused with these solutions. All infusions (whether 500, 1,000 or 2,000 mL, fluid alone or amino acids) lasted 4 hours; they were started 30 min prior to the [^{111}In-DTPA^{0}]octreotide injection, and a constant infusion rate was used throughout the infusion period.

Blood samples were taken and electrocardiographic analysis was performed during infusions with LysArg or 75 g of lysine only. ECG parameters assessed were the length of the P-R

interval, the height of the T-wave and the QRS–duration. For reasons of radiation safety and because infusions of 25 and 50 g of lysine were shown to be tolerated quite well [148, 153], biochemical and ECG data were not collected during infusions of 25 and 50 g of lysine.
Renal, tumour and spleen uptake was measured using planar scintigraphy at 24 and 48 h post injection (in diagnostic procedures) or at 24 and 72 h post injection (in therapeutic procedures) using a dual-head gamma camera (2000XP, Marconi Systems, Cleveland, Ohio) fitted with a medium-energy collimator. All patients gave their informed consent to participation in the study, which was approved by the medical ethical committee of the hospital.

Composition of amino acid solutions

Cocktail of amino acids (AA). The cocktail comprised 1,500 mL of Aminosteril N-Hepa 8% (Fresenius, Bad Homburg, Germany) plus 500 mL of Ringer lactate solution (Baxter, Uden, The Netherlands) plus 30 mL of 10% magnesium sulphate, yielding a total volume of 2,030 mL, with osmolarity of 700 mosmol/L and pH 7.4. This solution contained 10.32 g of lysine and 16.08 g of arginine.
Lysine preparations. Solutions of 500 mL of 5% L-lysine HCl were made, pH 7.4, containing 25 g of lysine. Patients received either 500 mL (25 g of lysine), 1,000 mL (50 g of lysine) or 1,500 mL (75 g of lysine) of this solution.
Combination of lysine and arginine (LysArg). Five hundred millilitres of L-lysine HCl 5% plus 250 mL L-arginine HCl 10% plus 250 mL saline were mixed and brought to pH 7.4. The osmolarity of this solution was 400 mosmol/L, and it contained 25 g of lysine and 25 g of arginine.

Labelling

Commercially available kits [$DTPA^0$]octreotide and $^{111}InCl_3$ were obtained from Tyco Health Care (Petten, The Netherlands). The radiolabelling procedure was performed in accordance with standard procedures [15, 16].

Statistics

Paired *t*-test was used for intra-patient comparisons. Unpaired *t*-test was used for comparisons between groups. *P*-values <0.05 were considered statistically significant.

RESULTS

Effects of saline infusion on renal radioactivity

As shown in Figure 18, renal uptake of [^{111}In-DTPA^{0}]octreotide was not altered by infusion of 1,000 mL saline/glucose solution over 4 h, nor did the infusion affect uptake in the spleen, liver or tumours. Similarly, infusion of 500 or 2,000 mL did not alter uptake in the organs in which it was measured (data not shown).

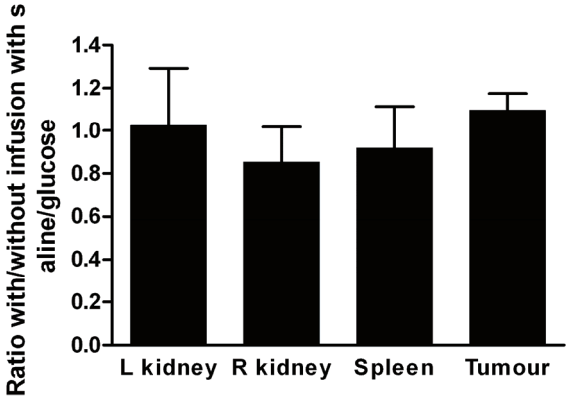


Figure 18. Radioactivity in the left and right kidney, spleen and tumour after [^{111}In-DTPA^{0}]octreotide injection with or without infusion of 1,000 mL of saline/glucose (*n*=5). Data from 24 h p.i. are expressed as the ratio of radioactivity with a saline/glucose infusion to radioactivity without such an infusion (mean ± SD).

Effects of infusion of different amino acid solutions on renal radioactivity

Figure 19 shows abdominal scintigraphy in one patient after 220 MBq of [^{111}In-DTPA^{0}]octreotide with and without co-infusion of 75 g of lysine. Figure 20 shows scintigraphy in another patient after 220 MBq of [^{111}In-DTPA^{0}]octreotide with and without co-infusion of LysArg. It is clearly shown that co-infusion with 75 g of lysine (Figure 19b) or LysArg (Figure 20b) lowered renal radioactivity.

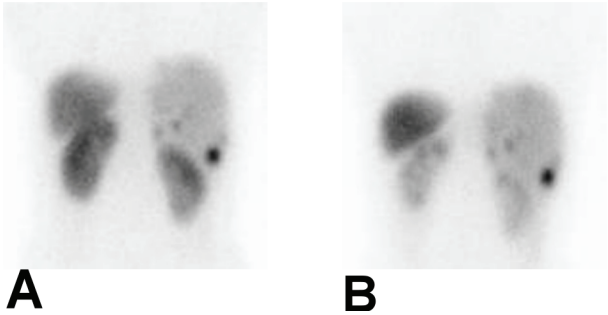


Figure 19. Abdominal scintigraphy in one patient after 220 MBq [^{111}In-DTPA^{0}]octreotide. The images were obtained without (A) and with (B) co-infusion of 75 g of lysine. Renal radioactivity was 59% of control when 75 g of lysine was infused.

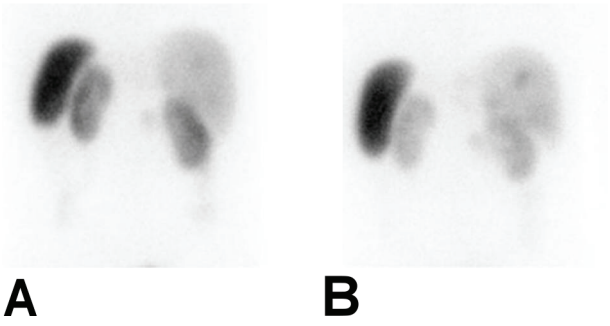


Figure 20. Abdominal scintigraphy in one patient after 220 MBq [^{111}In-DTPA^{0}]octreotide. The images were obtained without (A) and with (B) co-infusion of LysArg. Renal radioactivity was 52% of control when LysArg was infused.

Figure 21 shows the effects of infusion of the different amino acid preparations on left kidney radioactivity at 24 h after the injection of diagnostic or therapeutic doses of [^{111}In-DTPA^{0}]octreotide. Infusion of the mixture of amino acids (AA, see Materials and methods) or 25 g of lysine yielded an inhibition of about 20% (P<0.02, vs control). Doubling the dose of lysine to 50 g did not provide additional inhibition (25 vs 50 g of lysine: NS). The best inhibition of renal uptake of radioactivity was achieved with co-infusion of 75 g of lysine (44% ± 11, P<0.001). The combination of 25 g of lysine plus 25 g of arginine (LysArg) caused an inhibition of about 33% (P<0.01 vs. control, NS vs. 75 g of lysine).

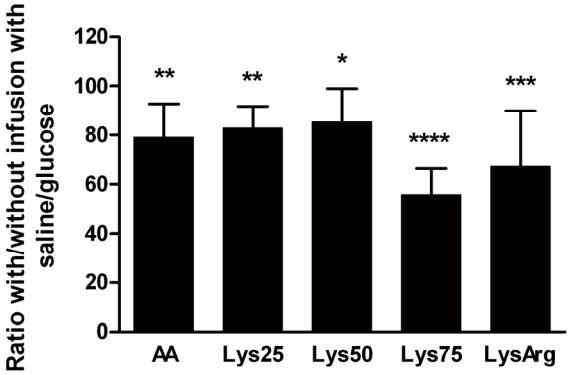


Figure 21. Effects of the different amino acid solutions on left renal uptake at 24 h p.i. of [^{111}In-DTPA^{0}]octreotide. Data are expressed as % of non-infused control value (mean ± SD).
AA: n=6; Lys25: n=5; Lys50: n=*7*; Lys75: n=*7*; LysArg: n=*9*.
(*) P<0.04; (**) P<0.02; (***) P<0.01; (****) P<0.001.

Figure 22 shows left kidney radioactivity at 48 hours p.i.. This was about 50% of control in patients infused with either LysArg or 75 g of lysine. All amino acid solutions tested resulted in a significant inhibition of left kidney radioactivity measured at 72 h p.i. (Figure 23).

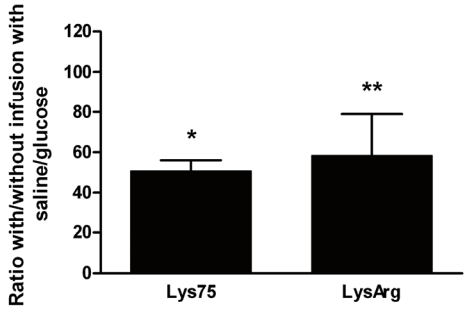


Figure 22. Effects of 75 g of lysine (n=3) or LysArg (n=9) on left kidney uptake at 48 h p.i. of [^{111}In-DTPA^{0}]octreotide. Data are expressed as % of non-infused control value (mean ± SD). Lys75: n=3; LysArg: n=9. (*) P<0.01; (**) P<0.001.

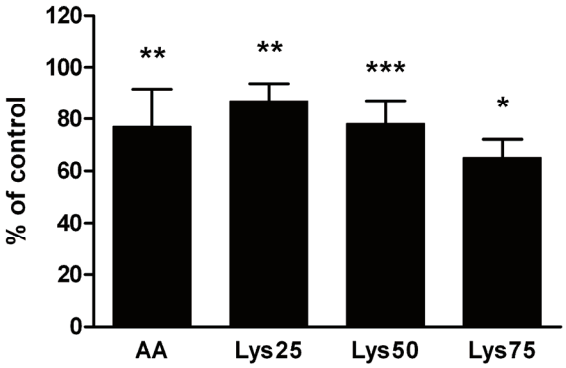


Figure 23. Effects of different amino acid solutions on left kidney uptake at 72 h p.i. of [^{111}In-DTPA^{0}]octreotide. Groups consist of 4 (Lys75), 5 (Lys25), 6 (AA) or 7 (LysArg) subjects. Data are expressed as % of non-infused control value (mean ± SD).
AA: *n*=6; Lys25: *n*=5; Lys50: n=7; Lys75: n=4. (*) *P*<0.01; (**) *P*<0.02; (***) *P*<0.001.

ECG and laboratory measurements during infusions of 75 g of lysine and LysArg

ECG analysis did not show any abnormalities during infusion of either 75 g of lysine or LysArg. During infusion of 75 g of lysine, mean serum potassium concentrations rose from 4.4 ± 0.5 mmol/L to maximal concentrations of 5.9 ± 0.8 mmol/L (*P*<0.02, basal vs maximal levels). During infusion of LysArg, serum potassium concentration rose from 4.2 ± 0.5 mmol/L to maximal concentrations of 5.1 ± 0.6 mmol/L (*P*<0.001, basal vs maximal). Maximal potassium concentrations were significantly higher in patients infused with 75 g of lysine than with LysArg (*P*<0.05). The highest potassium concentration in the LysArg group was 6.0 mmol/L, in one patient. In contrast, three out of six patients infused with 75 g of lysine had maximal potassium levels within the toxic range: 6.3, 6.7 and 6.8 mmol/L. Serum sodium, phosphate and chloride concentrations did not show significant changes in any of the groups studied.

Other side effects of amino acid infusions

In general, the amino acid infusions were well tolerated, except with regard to vomiting. During hyperkalemia, one patient infused with 75 g of lysine had some muscle weakness. Table 4 shows the rate of vomiting during PRRT or scintigraphy with concomitant infusion of the various amino acid solutions tested. Out of 11 patients infused with LysArg, one patient vomited (Table 4), but this was probably due to tumour necrosis and not caused by the LysArg infusion. She had four subsequent infusions with LysArg during PRRT without any problem. Vomiting occurred in 6% of patients receiving no amino acid infusion. Infusion of AA resulted in profound vomiting, whereas in the LysArg group vomiting only occurred in the aforementioned patient (9%).

Table 4. Rate of vomiting during scintigraphy or PRRT with radiolabelled octreotide: influence of concomitant infusion of different amino acid solutions.

	[^{111}In-DTPA^{0}]octreotide	*N*	Vomiting in	
			Patients	**Treatments**
Control	7-10 GBq	8		3/52 (6%)
AA	7-10 GBq	8	4 /8 (50%)	
Lys25 or	7-10 GBq	8		3/22 (9%)
LysArg	220 MBq	11	1 (9%) *	

(*) This patient had a subfebrile temperature, malaise and nausea before the first treatment. During this treatment she vomited twice, probably due to tumour necrosis. No problems occurred during four later infusions.

DISCUSSION

Radiolabelled somatostatin analogues are used in scintigraphic evaluation and in PRRT of neuro-endocrine tumours. After glomerular filtration, these peptides are efficiently taken up by proximal tubule cells and are retained to some extent. This may cause high renal radiation doses, possibly resulting in radiation nephropathy when high doses of radiolabelled somatostatin analogues are administered.

In external beam radiation, renal absorbed doses of 23 Gy may cause nephropathy in about 5% of patients within 5 years [70]. Acute radiation nephropathy occurs with a latent period of 6-12 months, while at lower doses chronic radiation nephropathy may occur between 1 and 5 years. Fractionation of external beam radiation lowers renal toxicity as compared with the same dose given as a single administration. However, since radionuclide therapy is applied as continuous low-dose radiation, data from external beam radiation may not be applicable for radionuclide therapy. At present it is unclear which radiation dose can be guided safely to the kidneys in PRRT. Dosimetry of the renal radiation dose remains important in enabling maximal doses of radiolabelled octreotide to be administered without damage to the kidney in the context of high-dose radioactivity.

There is evidence that differences in radionuclides may result in different levels of nephrotoxicity. In a dose-escalation animal study, high cumulative doses of [^{111}In-DOTA^{0},Tyr^{3}]octreotide did not cause microscopic damage [147]. Furthermore, several reports have been published on nephropathy after PRRT with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34, 44, 45, 48], but these reports did not provide data on renal absorbed doses. No renal toxicity of PRRT with [^{111}In-DTPA^{0}]octreotide was found [29]. The differences between ^{111}In (an Auger electron emitter) and ^{90}Y (a β-particle emitter) with regard to causation of radiation nephropathy may be explained by differences in the particle range (10 μm vs 10 mm). ^{111}In Auger electrons do not reach, and thus will not damage, the more radiosensitive glomeruli.

Recent reports show that cationic amino acids, like lysine and arginine, interfere with the proximal tubule cell protein reabsorption system in general [95], and with the uptake of radiolabelled somatostatin analogues and monoclonal antibodies in particular [91, 115]. The mechanism of the inhibition by these positively charged amino acids may be competition for negative charges on the tubule cell membrane between the amino acid and radiolabelled octreotide [91, 95]. Hammond et al. [96] reported inhibition of renal uptake of [^{111}In-DTPA^{0}]octreotide in patients with a commercially available mixture of amino acids containing, among others 2.46 g of lysine and 8.9 g of arginine. However, this inhibition was measured semi-quantitatively.

In a phase I study on PRRT with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, a marked difference was seen with regard to renal complications between the group that was concomitantly infused with a commercially available amino acid solution (which was similar to our AA solution) and the group that was not [34]. All patients who had renal complications, e.g. stable renal insufficiency or requiring haemodialysis, had not been infused with the amino acid solution. Recently, one patient from the same series was described with late radiation nephropathy leading to progressive end-stage renal disease. In only one out of four cycles of high-dose [^{90}Y-DOTA^{0},Tyr^{3}]octreotide administration was she concomitantly co-infused with the amino acid mixture [44]. A study by Paganelli et al. [46] reported WHO grade 1-2 renal toxicity in four patients treated with high-dose [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, but without a kidney protecting amino acid infusion. One patient, who suffered hypertension and nephropathy for 6 years, developed WHO grade 2 renal toxicity. The other three had WHO grade 1 nephrotoxicity. These data imply that reduction of the renal absorbed dose in PRRT may prevent radiation nephropathy, and may allow larger doses to be administered in PRRT.

Currently used mixtures of amino acids do have several disadvantages, namely the induction of severe vomiting, the risk of metabolic changes like serious hyperkaelemia and the large volume needed because of the hyperosmolarity of the fluid [150, 153]. Furthermore, administration of cationic amino acids like arginine and lysine has been associated with serious hyperkalaemia [151, 153, 155], as well as having the potential to cause fatal arrhythmias.

This study was performed in order to find a safe and effective amino acid infusion regimen to inhibit renal uptake of radioactivity in PRRT. From the data presented it is clear that all amino acid preparations tested had some inhibitory effect on renal uptake of radioactivity after injection of diagnostic and therapeutic doses of [^{111}In-DTPA^{0}]octreotide. As fluid administration alone did not significantly change renal uptake, the inhibition measured can be attributed to the amino acids alone. Lysine did not exhibit a linear dose-response relation. Doubling the amount of lysine from 25 to 50 g infused did not result in a significant

additional inhibitory effect on renal uptake of radioactivity. However, the best inhibition of kidney uptake was achieved using 75 g of lysine. The combination of 25 g of lysine plus 25 g of arginine (LysArg) resulted in an inhibition of about 33%. This inhibition is far better than that achieved with the approximately isomolar solution of 50 g of lysine, indicating a synergistic effect of combined administration. All amino acid solutions had long-lasting effects on renal radioactivity.

Barone et al. [153] also studied different amino acid solutions. Their data showed with infusion of their most effective amino acid solution, 40% higher doses of [^{86}Y-DOTA^{0},Tyr^{3}]octreotide could be administered without increasing the renal radiation dose. This amino acid solution consisted of 240 g of amino acids in a volume of 3,700 mL infused over 10 h. The solution contained about 50 g of lysine plus arginine. Our results regarding inhibition of renal uptake of radioactivity are similar to those reported by Barone et al. [153], but when infusing LysArg we administered a lower dose of amino acids in a smaller volume. Furthermore, a shorter and thus clinically more suitable infusion period was used in our study. Our data show no effects of the amino acid solutions on tumour uptake, which is in concordance with the results of other studies [144, 148].

Our study shows that profound hyperkalaemia occurred in 50% of the patients infused with 75 g of lysine, yet without serious symptoms. In contrast, in patients infused with LysArg no dangerous hyperkalaemia occurred. The induction of hyperkalaemia can be explained by the ketogenic characteristics of lysine, lowering intracellular pH and causing an outwardly directed K^{+}-flux [153]. Despite the severe hyperkalaemia seen in patients infused with 75 g of lysine, the ECG remained normal in all patients infused with LysArg or 75 g of lysine.

Our data on vomiting indicate that it was seen predominantly during infusion of the amino acid mixture AA. This was probably due to the large amount of amino acids infused (124 g) and the hyperosmolarity of the solution administered. Our experience with patients treated with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide co-infused with AA is comparable: about 42% of 26 patients, representing 20 out of 84 treatment cycles, had profound vomiting [146]. In one of these patients vomiting was so severe during the first two treatments that we had to change the renal protection regimen. In the later two treatments the patient was co-infused with 50 g of lysine. During these two treatments he vomited only once. Infusions with 25 and 50 g of lysine or with LysArg did not result in severe and unacceptable vomiting. Also, patients without any infusion vomited in about 6% of the treatments, probably due to tumour necrosis.

Recently, animal studies revealed that angiotensin converting enzyme inhibitors and AT II receptor antagonists are effective in prophylaxis against radiation-induced renal damage, perhaps via limitation of the consequences of endothelial cell injury [110]. The renal

protective effect of these medications remains to be confirmed in randomised human trials [44].

In conclusion, we tested various amino acid solutions in patients receiving PRRT with radiolabelled octreotide in order to assess their capacity to inhibit the renal uptake of radioactivity and their safety. Seventy-five grams of lysine resulted in the best inhibition of renal radioactivity, but it produced unacceptably severe hyperkalaemia. It is therefore not a suitable regimen for clinical use. The combination LysArg yielded the second best results with regard to inhibition of renal radioactivity but was far more safe: no serious hyperkalaemia was found and no vomiting occurred. Thus, inhibition of renal radioactivity, which is dose limiting for PRRT, with a combination of 25 g of lysine and 25 g of arginine (LysArg) is effective and safe. At present, infusion of this combination is used in our department as a standard procedure during PRRT.

CHAPTER 3.3

UPTAKE OF [^{111}In-DTPA^{0}]OCTREOTIDE IN THE RAT KIDNEY IS INHIBITED BY COLCHICINE AND NOT BY FRUCTOSE

Edgar J. Rolleman, Eric P. Krenning, Arthur van Gameren, Bert F. Bernard and Marion de Jong.

J Nucl Med 2004; 45:709-713

ABSTRACT

Background

The high renal uptake of radiolabelled somatostatin analogues is dose-limiting. Lowering this uptake permits higher radioactivity doses and, thus, tumour doses to be administered. We tested the effects of the microtubule drug colchicine on renal uptake of [^{111}In-DTPA^{0}]octreotide. Also, the effects of fructose were tested.

Methods

Organ radioactivity 24 h after injection of [^{111}In-DTPA^{0}]octreotide was determined in rats.

Results

Co-injection of 1 mg/kg colchicine did not influence renal uptake of [^{111}In-DTPA^{0}]octreotide, whereas this dose administered 5 hours before [^{111}In-DTPA^{0}]octreotide resulted in significant renal uptake reduction (63%). Lysine plus colchicine reduced the uptake by 76% (P<0.01 vs lysine alone). Liver and blood radioactivity levels were significantly elevated by colchicine. Fructose did not affect the biodistribution of [^{111}In-DTPA^{0}]octreotide.

Conclusion

Renal uptake of [^{111}In-DTPA^{0}]octreotide is dependent on microtubule function in rats. Addition of colchicine to amino acid protocols may permit administration of higher doses, improving the therapeutic window of peptide receptor radionuclide therapy.

INTRODUCTION

Radiolabelled somatostatin analogues have proven to be very helpful in the diagnostic work-up for neuro-endocrine tumours [141]. In addition, coupling Auger electron or beta-particle emitting radionuclides to these somatostatin analogues made internal radiation of these neuro-endocrine tumours possible, and several peptide receptor radionuclide therapy (PRRT) studies have been conducted using [^{111}In-DTPA^{0}]octreotide [29], [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34] and [^{177}Lu-DOTA^{0},Tyr3]octreotate [145].

The radioactivity dose to be administered in PRRT is limited by normal-organ toxicity, of which the kidneys are the principal dose-limiting organs. In the kidney, radiolabelled octreotide is efficiently reabsorbed by proximal tubular cells, causing high kidney radiation doses. Lowering kidney uptake of radioactivity may permit larger doses to be administered to tumours.

Hammond et al. [96] showed in a semi-quantitative method that a mixture of amino acids inhibited kidney uptake of radiolabelled octreotide. Further studies on animals and patients have shown that this inhibition of renal uptake of [^{111}In-DTPA^{0}]octreotide is mainly caused by the positively charged amino acids lysine and arginine [115, 116]. The effects of these positively charged amino acids are explained by competition for negatively charged binding sites at the proximal tubule cell surface.

We previously found indications that reabsorption of [^{111}In-DTPA^{0}]octreotide requires energy. Injection of a 400 mg/kg maleate dose, which inhibits the citric acid cycle, inhibited kidney uptake of [^{111}In-DTPA^{0}]octreotide by about 74% in rats [115].

However, the exact mechanism by which radiolabelled somatostatin analogues are taken up in the kidney is not fully understood. In general, peptide and protein reabsorption in the proximal tubule occur mostly via endocytosis of cell membrane after binding of the ligand. The complex is then transported to the lysozomal apparatus for proteolysis. Then, the scavenger receptor proteins are recycled to the cell surface and are again made available for endocytosis [93, 94].

We investigated, using fructose administration, whether kidney uptake of radiolabelled octreotide is adenosine triphosphate (ATP) dependent. Fructose can be administered to humans and is known to significantly lower cellular ATP in proximal tubule cells [156]. Also, we tested if microtubule-dependent endocytosis plays a role in kidney uptake of radiolabelled octreotide in rats, using colchicine administration. Colchicine prevents the return of the cell membrane parts to the cell surface by disrupting of cellular microtubules [98, 99, 157]. The subsequent result is lowered peptide and protein reabsorption in the proximal tubule. The results presented suggest microtubule-dependent uptake of [^{111}In-DTPA^{0}]octreotide in the proximal tubule of the rat kidney.

MATERIALS AND METHODS

Radiolabelling

Commercially available kits of [$DTPA^0$]octreotide and $^{111}InCl_3$ were obtained from Tyco Health Care (Petten, The Netherlands). The radiolabelling procedure was performed in accordance with standard procedures [134] .

Tissue Distribution of [^{111}In-$DTPA^0$]Octreotide

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

Male Wistar rats weighing 250-300 grams were used in these experiments. Rats received an intravenous injection of [^{111}In-$DTPA^0$]octreotide (3 MBq; 0.5 µg octreotide) into the dorsal vein of the penis. Twenty-four hours later, organs were isolated. Radioactivity was measured in isolated organs and in a blood sample taken at 24 h after injection. The experimental groups are described in Table 5.

Table 5. Description of experimental groups.

Abbreviation	Description	*n*
CON	Controls	12
FRUC 2	2 mmol/kg fructose i.p. t = 0 h	3
FRUC 20	20 mmol/kg fructose i.p. t = 0 h	3
FRUC 20 + LYS	20 mmol/kg fructose i.p. t = 0 h + 400 mg/kg i.v. t = 0 h	3
LYS	400 mg/kg D-lysine i.v. t = 0 h	12
Col 0.5	0.5 mg/kg colchicine i.p. t = -5 h	6
Col 1	1 mg/kg colchicine i.p. t = -5 h	9
Col 2	2 mg/kg colchicine i.p. t = -5 h	9
Col 1 + LYS	1 mg/kg colchicine i.p. t = -5 h + 400 mg/kg D-lysine i.v. t = 0 h	9
Col 1 AC	1 mg/kg colchicine i.v. t = 0 h	3
Col 3*0.3	0.3 mg/kg colchicine i.p. t = -5 h, -29 h and -53 h	6
Col 3*0.3 + LYS	Idem plus 400 mg/kg D-lysine i.v. t = 0 h	3

i.p.= intraperitoneally; t = time; i.v. = intravenously

Results represent four experiments with a total of 3-9 rats per experimental group. Data are expressed as mean ± SEM. Statistical evaluation was performed using one-way ANOVA. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Tissue distribution of [^{111}In-$DTPA^0$]octreotide 24 h after injection in control rats is shown in Table 6.

Figure 24 shows the kidney radioactivity 24 h after injection of [^{111}In-$DTPA^0$]octreotide and the effects of lysine (400 mg/kg), fructose (2 mmol/kg and 20 mmol/kg) and the

combination of lysine with fructose on the renal uptake of the radioligand. Administration of lysine clearly inhibited kidney radioactivity by about 40%. However, administration of both 2 and 20 mmol of fructose per kilogram had no effect. The inhibition of renal radioactivity by the combination of lysine and fructose was comparable to that by lysine alone. Fructose administration did not influence radioactivity in other organs tested as well.

Organ	%IA/g
Blood	0.001 ± 0.000
Spleen	0.027 ± 0.001
Pancreas	0.62 ± 0.08
Adrenals	1.32 ± 0.13
Kidney	2.18 ± 0.18
Liver	0.035 ± 0.003
Stomach	0.097 ± 0.007
Muscle	0.001 ± 0.000

Table 6. Tissue distribution of [^{111}In-DTPA^{0}]octreotide in control rats, 24 hours after injection. %IA = percentage injected activity. Data are expressed as mean ± SEM and represent 9 rats.

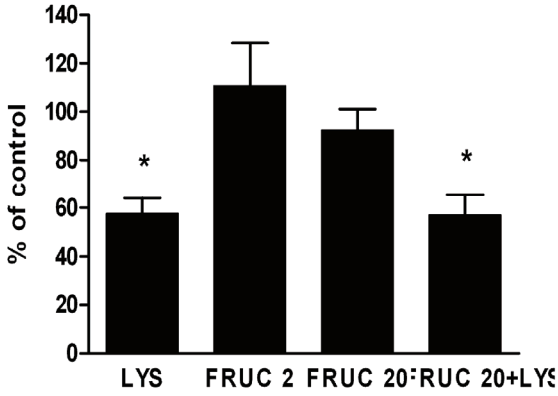


Figure 24. Effects of lysine (400 mg/kg), fructose (2 and 20 mmol/kg) and the combination of 400 mg lysine per kilogram plus 20 mmol/kg fructose on kidney radioactivity 24 h after injection of [^{111}In-DTPA^{0}]octreotide. Groups consist of 3 rats.
(*) P<0.01 vs control.

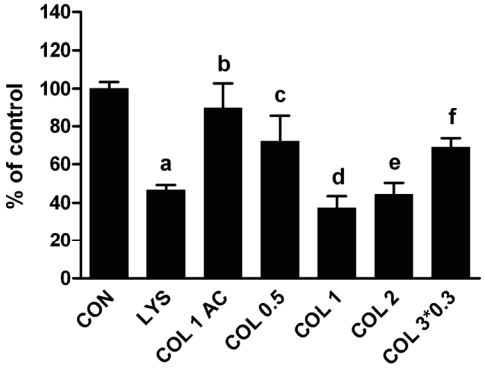


Figure 25. Effects of different treatment modalities with colchicine on kidney radioactivity 24 h after injection of [^{111}In-DTPA^{0}]octreotide. Groups consist of 6-9 rats. Table 1 describes the experimental groups and gives their abbreviations.
(a) P<0.001 vs control; (b) NS vs control; (c) P<0.01 vs control and P< 0.001 vs 1 mg/kg colchicine;
(d) P<0.001 vs control and NS vs 400 mg/kg lysine;
(e) P<0.001 vs control and NS vs 1 mg/kg colchicine;
(f) P<0.001 vs control and P<0.001 vs 1 mg/kg colchicine.

Figure 25 shows the effects of colchicine treatment on kidney uptake of [^{111}In-DTPA^{0}]octreotide. Co-injection of 1 mg of colchicine per kilogram with the radioligand did

not inhibit kidney radioactivity. However, injecting 0.5, 1 or 2 mg of colchicine per kilogram 5 h before injection of [^{111}In-DTPA^{0}]octreotide significantly reduced kidney uptake (respective inhibitions of 28% ± 13%, P<0.01 vs. control; 63% ± 6%, P<0.001 vs. control; 56% ± 6%, P<0.001 vs. control). A dose-response relationship was found: the inhibition caused by 1 mg/kg was significantly better than that caused by 0.5 mg of colchicine per kilogram. However, treatment with 2 mg of colchicine per kilogram did not augment the inhibition caused by 1 mg/kg. Repeated administration of colchicine (0.3 mg/kg at 5, 29 and 53 h before radioligand injection) inhibited of kidney radioactivity by about 31%, but this degree of inhibition was significantly lower than caused by the comparable dose of 1 mg/kg 5 h before the radioligand injection (63%, P<0.001).

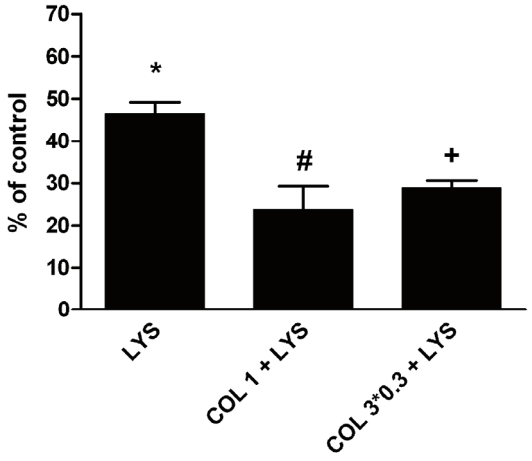


Figure 26. Effects of lysine (400 mg/kg) and two combinations of colchicine with lysine on kidney radioactivity 24 h after injection of [^{111}In-DTPA^{0}]octreotide. See Table 1 for description of experimental groups. Groups consist of 6-9 rats.
(*) P<0.001 vs control; (#) P<0.001 vs control and P<0.01 vs 400 mg/kg lysine; (**) P<0.001 vs control and NS vs 400 mg/kg lysine.

In Figure 26 the treatment combinations of colchicine and lysine versus lysine alone are compared with regard to their potential to inhibit kidney uptake of [^{111}In-DTPA^{0}]octreotide. Kidney-uptake reduction was more pronounced with 1 mg colchicine per kilogram plus 400 mg of lysine per kilogram than with 400 mg lysine per kilogram alone (76% ± 5% vs 54% ± 3%, P<0.01). There was a clear trend, although not statistically significant, for better inhibition of kidney radioactivity by the combination of 1 mg colchicine per kilogram and 400 mg/kg of lysine per kilogram than by colchicine alone (inhibitions of 76% ± 5% vs 63% ± 6%, NS). Administration of three doses of 0.3 mg of colchicine per kilogram at 24-h intervals plus lysine treatment was not better than lysine alone.

Table 7 shows the effects of the different colchicine doses on radioactivity in different organs. Except for the repeated dose group, all colchicine treatment modalities elevated the radioactivity level in blood and liver. Radioactivity in the spleen was higher only in the rats treated with 2 mg of colchicine per kilogram. The tissue content of [^{111}In-DTPA^{0}]octreotide in stomach, muscle, pancreas, and adrenals, of which the latter two are somatostatin receptor-positive organs, was not changed due to colchicine treatment.

Table 7. Effects of colchicine administration on tissue radioactivity after injection on [^{111}In-DTPA^{0}]octreotide. Data are expressed as mean ± SEM after normalization to percentage of control.
(a) $P<0.001$ vs control; (b) $P<0.01$ vs control.

	Blood	Spleen	Pancreas	Adrenals	Liver	Stomach	Muscle
CON	100 ± 5	100 ± 4	100 ± 6	100 ± 8	100 ± 3	100 ± 5	100 ± 2
LYS	110 ± 9	101 ± 4	88 ± 7	97 ± 11	105 ± 5	91 ± 6	103 ± 5
COL 0.5	154 ± 7[a]	108 ± 6	109 ± 14	128 ± 15	131 ± 6[a]	134 ± 11	94 ± 7
COL 1	167 ± 13[a]	109 ± 8	92 ± 8	101 ± 9	138 ± 6[a]	106 ± 11	108 ± 7
COL 2	268 ± 8[a]	150 ± 20[a]	109 ± 4	123 ± 8	127 ± 5[b]	137 ± 14	87 ± 3
COL 1 + LYS	172 ± 10[a]	119 ± 7	97 ± 18	83 ± 8	146 ± 9[a]	105 ± 14	107 ± 7
COL 3*0.3	116 ± 11	107 ± 7	107 ± 15	92 ± 10	145 ± 9[a]	104 ± 11	112 ± 7

DISCUSSION

Radiolabelled somatostatin analogues are efficiently excreted by the kidneys, but a significant amount is reabsorbed by proximal tubule cells [126]. This kidney uptake is the major dose-limiting factor in PRRT using these peptides [158] and lowering it will allow larger doses to be administered, thereby enlarging the therapeutic window of PRRT. However, the maximal safe kidney radiation dose is not known. At present, a maximal radiation dose of 23-27 Gy to the kidney is accepted in humans [158].

The positively charged amino acids lysine and arginine have been shown effective and safe in reducing kidney radiation dose during PRRT with radiolabelled octreotide [116]. The presumed mechanism of action is competition between the radiopharmaceutical and the amino acids for negatively charged membrane molecules.

This study was performed to gain a better insight in the uptake process in order to find new kidney protection methods to add to or substitute for currently used amino acid solutions.

In general, low-molecular-weight proteins and peptides are easily filtered and subsequently reabsorbed in the proximal tubule. This reabsorption process is mediated mainly by endocytosis [93]: after binding to a carrier molecule, an endosome is formed and transported through the cytoplasm to the lysozomal apparatus for hydrolysis. Then, the carrier molecule is transported back to the cell surface [94].

Several reports suggest that proper functioning of endocytosis is dependent on ATP via the endosomal acidification machinery [159, 160]. We have previously shown that re-uptake of [^{111}In-DTPA^{0}]octreotide in the rat kidney was inhibited by maleate [115], which is used to mimic the Fanconi's syndrome in rats. It causes a generalised tubule transport dysfunction, possibly by inhibiting the citric cell cycle [128] pointing to a possible ATP dependency of kidney uptake of radiolabelled somatostatin analogues. Maleate can not be used in humans, whereas fructose can. In the present study we tested if the ATP-lowering substance fructose would inhibit kidney uptake of [^{111}In-DTPA^{0}]octreotide. Burch et al. [156] showed that

administration of 20 mmol/kg fructose resulted in a 41% decline of ATP in rat proximal tubule cells within 40 minutes. However, our results show that fructose did not affect kidney uptake of [^{111}In-DTPA^{0}]octreotide. It can not be excluded that higher doses, repeated doses or an earlier dose of fructose may reduce kidney radioactivity.

Several reports have shown that the endocytosis process in the proximal tubule is also dependent on microtubule action [98, 157]. Colchicine is a drug that prevents polymerisation of microtubules in the cytoplasm and the nucleus. Its efficacy has been proved in patients with gout, Behcet's disease, familial Mediterranean fever, and cirrhosis [161]. Treatment with colchicine results in scattering of multiple vesicles throughout the proximal tubule cell containing specific uptake molecules [98, 157]. Normally, these vesicles would fuse to form dense apical tubules that later would insert into the apical membrane again [94]. However, transportation of these vesicles into dense apical tubule is inhibited because of microtubule disruption. In turn, this causes a defect in proximal tubule reabsorption of low-molecular-weight proteins and peptides [98, 157].

Our data on colchicine treatment show that colchicine efficiently blocked tubule uptake of [^{111}In-DTPA^{0}]octreotide. A dose-response relationship was found, with a maximal effect at a dose of 1 mg/kg. Effects of 1 mg of colchicine per kilogram on kidney [^{111}In-DTPA^{0}]octreotide uptake depend on the interval between colchicine administration and injection of [^{111}In-DTPA^{0}]octreotide. For instance, injection of colchicine 5 h before the radioligand inhibited kidney uptake by about 60%, whereas no effects were seen when colchicine was co-injected with the radioligand. This finding is in accordance with a previous study of Gutmann et al. [99], which found that the effects of colchicine on transport proteins did not appear before 4-8 hours after colchicine treatment, depending on the dose used. The investigators suggested that this interval may, rather than representing transcriptional factors, represent the effects of colchicine on recycling membrane parts, as is supported by the fact that effects of colchicine were reversed after 24 h [99]. We found indications for reversibility of the effects of colchicine as well. Rats treated with three doses of 0.3 mg/kg at 24-h intervals had higher kidney [^{111}In-DTPA^{0}]octreotide content than did rats treated with 1 mg/kg injected 5 h before the radioligand was injected. Thus, tubule cells may have recovered (partially) before the next dose was administered.

Colchicine treatment produced pronounced elevated radioactivity uptake in both blood and liver in almost all treatment groups. The elevated blood levels may indicate a higher bone marrow uptake of radioactivity and this may constitute a dose-limiting factor in PRRT. However, whether colchicine will similarly affect blood radioactivity levels in humans is unclear, especially when the lower colchicine doses that are used in humans are considered. The higher radioactivity distributed to the liver may reflect cellular damage by metabolism of

colchicine in the liver. Despite higher circulating radioactivity levels, radioactivity accumulation in other organs was not elevated.

We did not show any effect of colchicine on uptake in somatostatin receptor-positive organs pancreas and adrenals, but it might be interesting to test the effects of colchicine on tumour radioactivity uptake in rats bearing tumours positive for somatostatin receptor type 2.

From our study, we conclude that 1 mg of colchicine per kilogram adds significantly to the renal-protecting effects of 400 mg of lysine per kilogram, a finding that is promising for clinical use. However, colchicine doses used in our experimental study on rats are much higher than those applied clinically. Within the time frame of the study, we saw no toxic side effects on the rats treated with colchicine. It remains difficult, however, to extrapolate drug dosage schedules and toxicity data from one species to another, because of large differences in metabolic turnover of drugs and different susceptibility for toxic effects of drugs.

In humans, colchicine is usually administered orally at an initial dose of 1 mg, followed by 0.5 mg every 2 hours until gastrointestinal side effects occur or until a total dose of 6 mg has been given within several days. This procedure is usually safe, unless the patient has renal or liver disease or is elderly [162]. It might be of interest to find out if this clinical applicable dosage of colchicine can further enhance kidney protection during PRRT with radiolabelled octreotide.

CONCLUSION

Injection of fructose, known to lower proximal-tubule ATP content, did not reduce kidney radioactivity after injection of [^{111}In-DTPA^{0}]octreotide in rats. The microtubule-disrupting drug colchicine significantly inhibited kidney uptake of [^{111}In-DTPA^{0}]octreotide. The combination of lysine and colchicine significantly enhanced the reduction of renal uptake.

CHAPTER 3.4

MOLECULAR IMAGING OF REDUCED RENAL UPTAKE OF RADIOLABELLED [DOTA^0,Tyr^3]OCTREOTATE BY THE COMBINATION OF LYSINE AND GELOFUSINE IN RATS

Edgar J. Rolleman, Bert F. Bernard, Wout A.P. Breeman, Flavio Forrer, Erik de Blois, Jack Hoppin, Martin Gotthardt, Otto C. Boerman, Eric P. Krenning and Marion de Jong.

Nuklearmedizin 2007; *revised version re-submitted*

ABSTRACT

Aim

In peptide receptor radionuclide therapy (PRRT) using radiolabelled somatostatin analogues, kidney uptake of radiolabelled compound is the major dose-limiting factor. We studied the effects of Gelofusine (20 mg) and lysine (100 mg) and the combination of both after injection of therapeutic doses of radiolabelled [DOTA^0,Tyr^3]octreotate (60 MBq ^{111}In or 555 MBq ^{177}Lu labelled to 15 µg peptide) in male Lewis rats.

Methods

Kidney uptake was measured by single photon emission computed tomography (SPECT) scans with a four-headed multi-pinhole camera (NanoSPECT) at 24 h, 5 and 7 days p.i. and was quantified by volume of interest analysis. For validation the activity concentration in the dissected kidneys was also determined ex vivo using a gamma counter and a dose calibrator.

Results

Gelofusine and lysine both reduced kidney uptake of [^{177}Lu-DOTA^0,Tyr^3]octreotate significantly by about 40% at all time points. The combination of Gelofusine and lysine resulted in a 62% inhibition of kidney uptake (P<0.01 vs lysine alone). A weak but significant dose-response relationship for Gelofusine, but not for lysine, was found. In a study with [^{111}In-DOTA^0,Tyr^3]octreotate, conclusions drawn from NanoSPECT data were confirmed by biodistribution data.

Conclusions

Rat kidney uptake of radiolabelled somatostatin analogues can be monitored for a longer period in the same animal using animal SPECT. Gelofusine and lysine had equal potential to reduce kidney uptake of therapeutic doses of [^{177}Lu-DOTA^0,Tyr^3]octreotate. The combination of these compounds caused a significantly larger reduction than lysine or Gelofusine alone and may therefore offer new possibilities in PRRT. The NanoSPECT data were validated by standard biodistribution experiments.

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) using somatostatin analogues has become a most convincing new modality in the treatment of somatostatin receptor-positive tumours [32, 33, 37]. But, as most therapeutic interventions have their drawbacks, so does the approach of PRRT with somatostatin analogues. The radiolabelled peptides are filtered by the glomerulus and mostly excreted into the urine. However, a small percentage is reabsorbed and retained in the proximal tubules [92], creating prolonged irradiation of the kidneys. In PRRT, the maximum tolerable dose to the kidneys is not exactly known, but from external beam radiation therapy a limit of 23 Gy [70] has been adopted by several groups. The kidney is the major dose-limiting organ with regard to the total dose that can be administered [34, 71, 77, 163].

In recent years, we showed that positively charged amino acids, such as lysine and arginine, can lower kidney uptake of radiolabelled somatostatin analogues [115, 116, 164] and these compounds are used currently in PRRT protocols [35, 116]. Consequently, higher radioactivity doses can be administered to the patient within safe renal dose limits.

In our protocol, we use 25 grams of lysine and 25 grams of arginine infused over a 4 h period [116]. We previously showed that larger doses of lysine, 50 or 75 grams, induced a significantly higher kidney uptake reduction in humans, though these higher doses of lysine were accompanied by toxic effects, of which hyperkalemia is the most important [116]. Therefore, additional and different methods to reduce kidney uptake of radiolabelled somatostatin analogues are warranted.

Recently, it was shown that co-infusion of the gelatin-based plasma expander Gelofusine reduced kidney uptake of diagnostic doses of [^{111}In-DTPA^{0}]octreotide by approximately 40% in both rats [100] and humans [103], which is comparable to the reduction achieved currently using standard amino acid infusion. These studies were inspired by the finding that the administration of gelatin-based plasma expanders led to low-molecular-weight proteinuria [101, 102, 165].

The aim of the present study was to investigate whether renal uptake of therapeutic doses of radiolabelled [DOTA^{0},Tyr^{3}]octreotate is also reduced by Gelofusine, and to test the potential of the combination of Gelofusine and lysine.

MATERIALS AND METHODS

Radionuclides, peptides and chemicals

$^{177}LuCl_3$ was obtained from IDB (Baarle Nassau, The Netherlands). [DOTA^{0},Tyr^{3}]octreotate was supplied by BioSynthema (St Louis, MO, USA). [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and [^{111}In-DOTA^{0},Tyr^{3}]octreotate were labelled as previously described [144, 166].

Gelofusine was purchased from B.Braun Medical (Oss, The Netherlands). D-lysine was purchased from Sigma (St. Louis, MO, USA). $^{111}InCl_3$ and [$DTPA^0$]octreotide were obtained from Tyco Health Care (Petten, The Netherlands) and radiolabelling was performed in accordance with standard procedures [15, 166].

Peptide radionuclide therapy experiments using [^{177}Lu-$DOTA^0$,Tyr^3]octreotate

All animal experiments were approved by the governing Animal Welfare Committee and conducted according to institutional regulations. Male Lewis rats (Harlan, Horst, The Netherlands; weight 240-400 g) were used.

Twenty-one rats (5-6 rats per group) were injected with 555 MBq (15 µg) [^{177}Lu-$DOTA^0$,Tyr^3]octreotate with or without co-injection of 20 mg Gelofusine, 100 mg lysine or the combination of these compounds. Total injection volumes were 250 µL (control group), 500 µL (lysine group), 750 ul (Gelofusine group) and 1 mL (Gelofusine + lysine group). Doses corrected for body weight were 50-83 mg/kg and 246-415 mg/kg for Gelofusine and lysine, respectively. At 24 h, 5 and 7 days after injection of [^{177}Lu-$DOTA^0$,Tyr^3]octreotate a single photon emission computed tomography (SPECT) was made to determine retention of radioactivity in the kidneys.

In each individual experiment and at each time point, the measured activity per rat (mean of both kidneys) was expressed as a percentage of the mean of the 555 MBq alone group.

In an additional experiment, 12 rats (3 rats per group) were injected with [^{111}In-$DOTA^0$,Tyr^3]octreotate (60 MBq, 15 µg) with or without Gelofusine, lysine or both for kidney protection, similar to the experiments with [^{177}Lu-$DOTA^0$,Tyr^3]octreotate. A SPECT scan was made 24 hours post injection and directly thereafter rats were euthanized. Both kidneys were excised, weighed and the radioactivity was determined in a dose-calibrator (VDC-405, Veenstra Instruments, Joure, The Netherlands) and a gamma counter (Perkin Elmer, Wallac, 1480 Wizard 3, Turku, Finland). This gamma counter had the following specifications: the spectrum width for ^{111}In was set at 124-207 and the energy window at 128-711keV. Calibration is performed by the manufacturer twice a year using ^{125}I and ^{137}Cs. In the experiments, counting times were 60 seconds, and each complete kidney was counted separately in a tube. As total counts were far above 10000 counts, statistics were good. Volumes between 3 microliter and 2 milliliter do not require volume correction in the Wizard 3 gamma counter, according to the manufacturer's handbook.

Molecular imaging with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and [^{111}In-DOTA^{0},Tyr^{3}]octreotate

For SPECT imaging the four-headed multiplexing multi-pinhole NanoSPECT (Bioscan Inc., Washington D.C., USA) was used, see [167] for description of this system. The quantification factor was determined by imaging a cylindrical phantom filled with a known activity. This measurement was in turn performed for each combination of isotope and aperture. The diameter of the cylinder phantom was selected according to the size of the animals. Thus, the calibration measurement to determine the quantification factor corrects for attenuation within the animal. Voxel values in the reconstruction provide a proper estimate of the activity level without further calculation. In our model, the partial volume effect is negligible as the measured volume of the kidney (~1mL) is significantly larger than the resolution setting of the camera used in the experiment (~8 microL).

For ^{177}Lu the energy-peaks were set at 113 and 209 keV and for ^{111}In at 173 and 245 keV. The window width was ± 10%. An acquisition time of 40 seconds per view was chosen. Total acquisition times ranged from 10-15 minutes per animal (16-24 views). Data were reconstructed iteratively with HiSPECT© software (BioScan Inc., Washington D.C., USA), using a dedicated OSEM algorithm employing a multiplexing multi-pinhole acquisition technique. Regions of interest (ROI) were drawn manually around each kidney and the 3D activity distribution within the volume of interest (VOI) was then summed to determine the uptake in the kidneys. The quantification of the activity in the VOI was performed with the INTERVIEW XP© software. The counting efficiency for ^{111}In and ^{177}Lu is comparable: approximately 1100 cps/MBq.

Chromatography experiments

PD10 chromatography was performed to investigate the possible binding of radiolabelled somatostatin analogues to Gelofusine. 3.4 MBq [^{111}In-DTPA^{0}]octreotide (specific activity 11 MBq/μg) was incubated for an hour at 37 ºC with 1 mL blood, plasma, saline or 4% human serum albumin in saline, in the presence or absence of 2.45 or 4.9 g/L Gelofusine. After incubation, a 200 μL aliquot was eluted on a PD10 column (Pharmacia Fine Chemicals, Uppsala, Sweden) with saline. Twenty-four fractions of 0.5 mL were collected and counted in a Cobra Gammacounter (Perkin Elmer). PD10 colums separate large molecules (i.e. Gelofusine) from smaller ones (i.e. [^{111}In-DTPA^{0}]octreotide).

Statistical analysis

Data are expressed as mean ± SD. One-way ANOVA followed by Tukey's test was used to test significance of differences. *P*-values less than 0.05 were considered to indicate statistically significant differences.

RESULTS

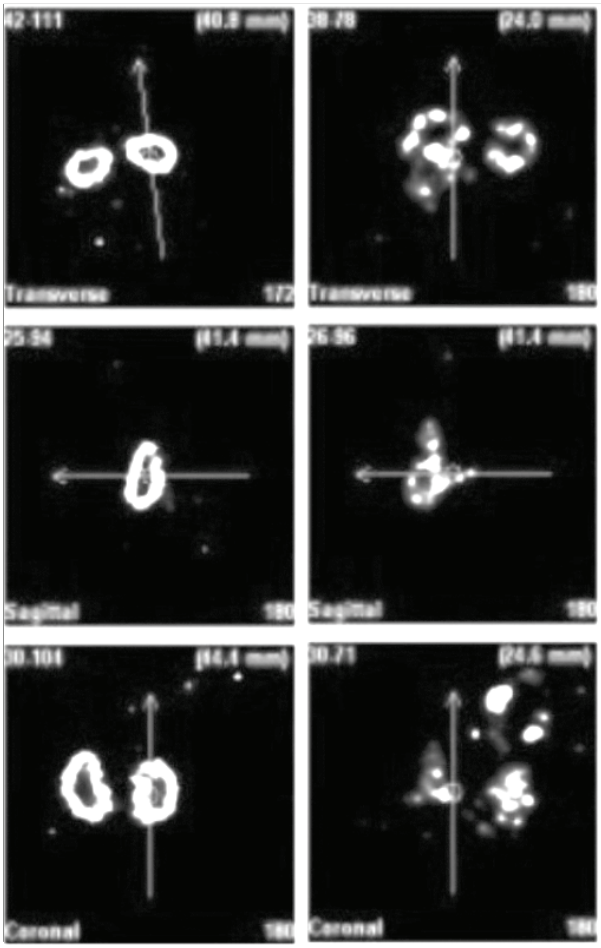


Figure 27. Transversal, axial and sagital slices obtained by SPECT imaging 5 days after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Slices of a control rat (left) and a rat treated with the combination of Gelofusine and lysine (right) are shown.

Figure 27 shows transaxial, coronal and sagittal slices of rat kidneys obtained via SPECT 5 days after the injection of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. A clear discrimination can be made between kidney cortex and medulla, implying that kidney uptake of radiolabelled somatostatin analogues is mainly located in the cortical regions of the kidneys. The left panel shows the SPECT of a rat injected with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate without co-injections whereas the rat displayed on the right was co-injected with lysine and Gelofusine. The figure shows a pronounced reduction in the renal uptake of the radioactivity. Both images are displayed with the same threshold settings.

Figure 28 shows the relative SPECT-measured kidney retained activity over time after injection of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, expressed as percentage of that in rats injected with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone. Lysine or Gelofusine co-injection reduced renal uptake by approximately 42% at all time points. The combination of Gelofusine and lysine reduced renal uptake by approximately 65%, which was significantly different from the effects of Gelofusine or lysine alone (P<0.05 vs Gelofusine co-injection and P<0.05 vs lysine co-injection) at all time points.

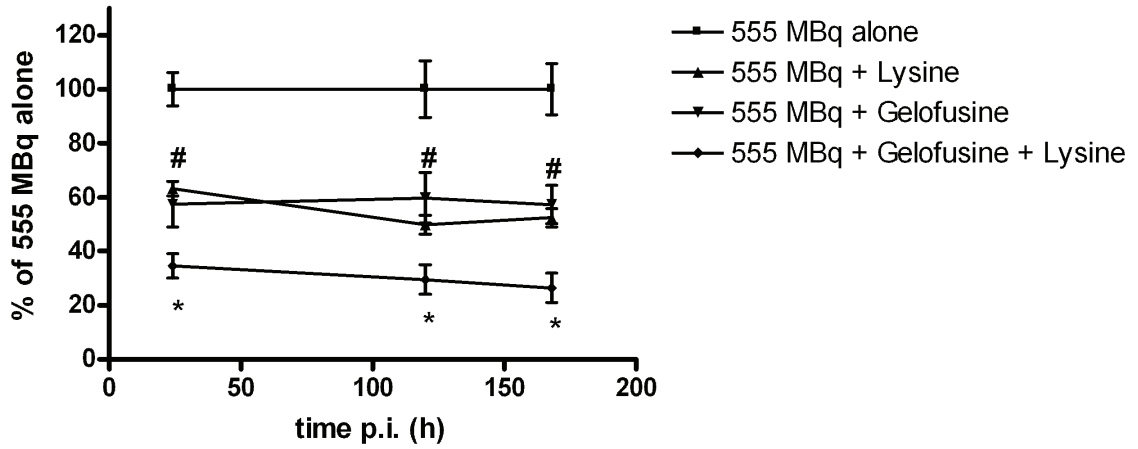


Figure 28. Time course of kidney retained radioactivity after injection of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with or without lysine, Gelofusine or the combination of both. Data are expressed as % of the corresponding 555 MBq alone group in the individual experiments. (*) P<0.001 vs 555 MBq alone, P<0.05 vs Gelofusine alone, or vs lysine alone; (#) P<0.001 vs 555 MBq alone.

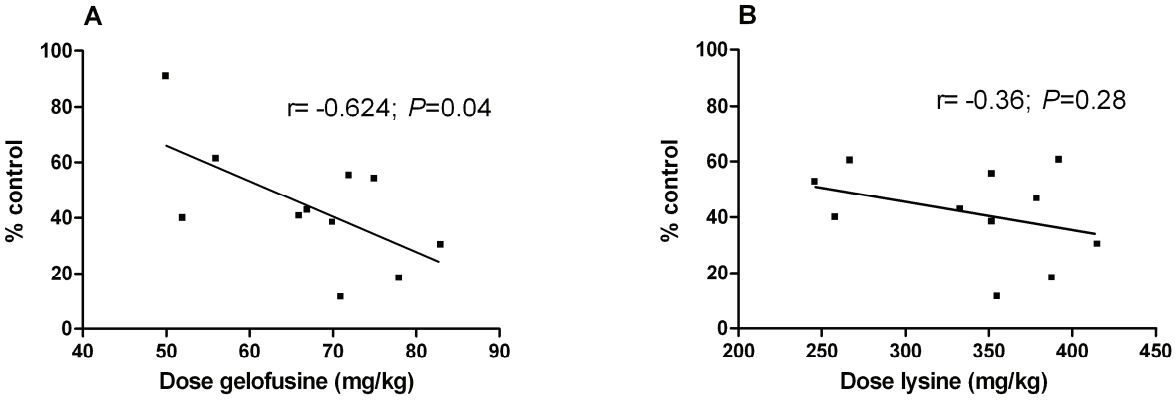


Figure 29. Dose-effect relationships of Gelofusine (panel A) and lysine (panel B) on the inhibition of renal uptake of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate after injection of 555 MBq. Percentage of control was calculated as the mean of the three scans in each rat. Data represent those of rats in the lysine alone (n=5), Gelofusine alone (n=5) and lysine+Gelofusine (n=6) groups.

Rats received a fixed dose of either Gelofusine and/or lysine. When the dose of Gelofusine, expressed as mg/kg, was plotted against the % inhibition of kidney uptake, a weak, but significant, dose-effect relation was found (r= -0.62, P=0.04; Figure 29). No dose-effect response was found for lysine (r= -0.36; P=0.28).

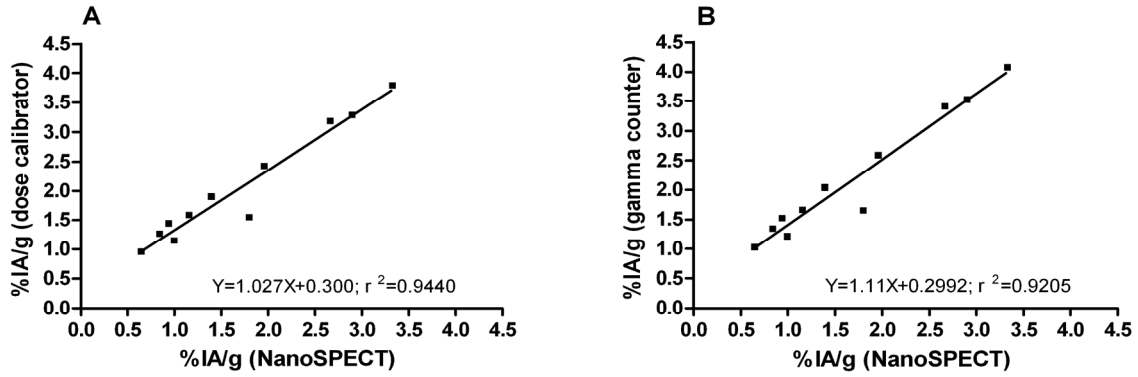


Figure 30. Correlation between NanoSPECT and dose calibrator (A) and between NanoSPECT and gamma counter (B). Kidneys of rats, injected with 60 MBq [^{111}In-DOTA^0,Tyr^3]octreotate with of without kidney protection, were measured for radioactivity by both SPECT (in NanoSPECT), dose calibrator and gamma counter and expressed as %IA/g.

The NanoSPECT measurements were validated by measuring the uptake in the dissected kidneys *ex vivo* (dose calibrator or gamma counter). Figure 30 shows strong correlation between *in vivo* (NanoSPECT) and *ex vivo* measurements of kidney-retained radioactivity 24 h after injection of 60 MBq [^{111}In-DOTA^0,Tyr^3]octreotate.

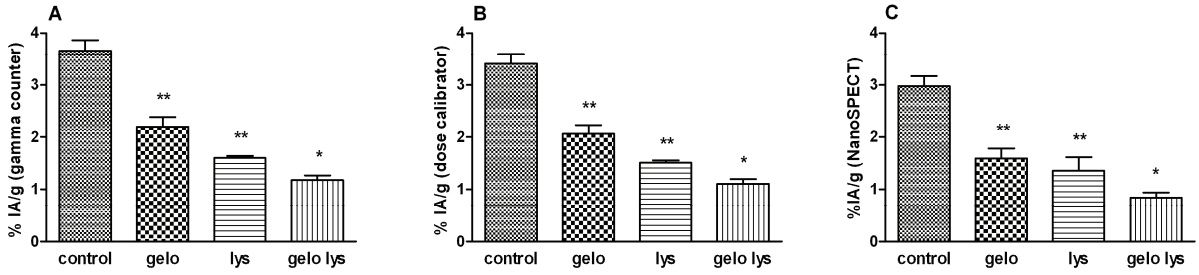


Figure 31. Effects of Gelofusine, lysine and the combination of both on the kidney uptake of 60 MBq [^{111}In-DOTA^0,Tyr^3]octreotate measured by gamma counter (panel A), dose calibrator (panel B) and NanoSPECT (panel C). Note the apparent similarity.
(**) P<0.01 vs control; (*) P<0.05 vs Gelofusine alone or lysine alone and P<0.001 vs control.

In Figure 31 the results of the NanoSPECT measurements and the biodistribution study 24 h after injection of 60 MBq [^{111}In-DOTA^0,Tyr^3]octreotate are compared. Data of both biodistribution settings resemble those of the NanoSPECT measurements: reduction of renal uptake by both Gelofusine and lysine alone, and a significantly larger reduction by the combination of lysine and Gelofusine. These data are consistent with the studies with [^{177}Lu-DOTA^0,Tyr^3]octreotate.

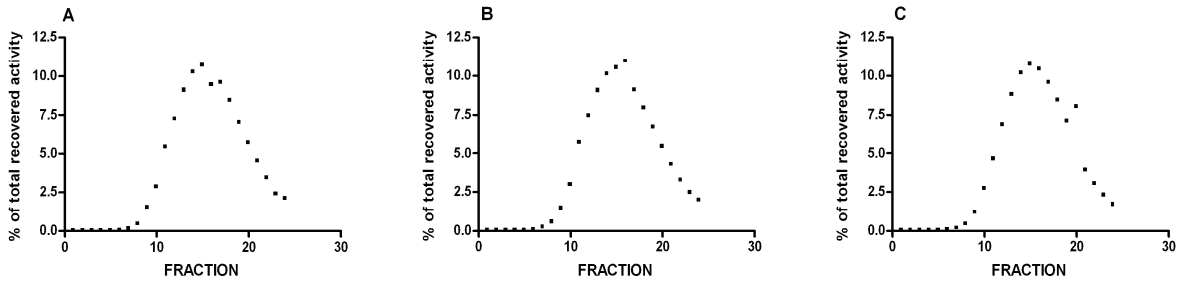


Figure 32. PD10 column analysis of incubations of blood (panels A-C) with [^{111}In-DTPA^{0}]octreotide in absence (panel A) or presence of 2.45 g/L Gelofusine (Panel B) or 4.9 g/L Gelofusine (panel C).

In order to further investigate the mechanism by which Gelofusine could reduce kidney uptake of radiolabelled somatostatin analogues, a PD 10 column analysis was performed. Large molecules (such as Gelofusine) will appear in fraction 5 to 8, whereas smaller molecules (radiolabelled somatostatin analogues) appear in later fractions. Figure 32 shows the radioactivity in the eluted fractions after incubation of [^{111}In-DTPA^{0}]octreotide in blood, in the presence or absence of two concentrations of Gelofusine. No early peak measurable, indicating that no radioactivity was bound to Gelofusine. Data for incubations in saline, serum and 4% human serum albumin were comparable (not shown).

DISCUSSION

Positively charged amino acids such as lysine and arginine are now commonly used for renal protection in peptide receptor radionuclide therapy (PRRT) in clinical studies. It has been shown that lysine and arginine inhibit the renal uptake of radiolabelled somatostatin analogues [115]. We recently showed that this consequently results in lower kidney damage after high dose therapy with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in rats [168], indicating that the use of these amino acids may result in the potential to administer higher radioactivity doses to the patient, and thus higher tumour radiation doses, without increased kidney toxicity.

Although positively charged amino acid infusions have been proven to result in significant dose-dependent reduction of renal uptake in PRRT, their use is limited by onset of side effects such as nausea, vomiting and hyperkalemia. We have shown that an increase in the lysine dose from 25 grams to 50 or 75 grams results in a reduction of the renal uptake of somatostatin analogues [116]. The resulting hyperkalemia (serum potassium concentration between 6.3-6.8 mmol/L) however, was unacceptable. Therefore, new methods are mandatory to further enhance the efficacy of PRRT.

It has previously been shown that the gelatin-based plasma expander Gelofusine can lower renal uptake of diagnostic amounts of [^{111}In-DTPA^{0}]octreotide to a level comparable to the currently used amino acid solutions [100, 103]. The idea for the experimental use of

Gelofusine was based on the finding that gelatin-based plasma expanders produce low-molecular- weight proteinuria as reported in various studies [101, 102, 165]. More specifically, Gelofusine infusion results in enhanced urinary excretion of specific megalin ligands, such as β_2-microglobuline. As it has been shown previously that megalin plays an essential role in the kidney uptake of somatostatin analogues [42], testing Gelofusine and other gelatin-based solutions for kidney protection in PRRT was logical.

In the present study we showed both with SPECT imaging and in biodistribution studies that the kidney uptake of therapeutic doses of [^{177}Lu-DOTA^0,Tyr^3]octreotate was successfully reduced by Gelofusine. Most importantly, the combination of lysine and Gelofusine resulted in a significantly increased inhibition of renal uptake.

We found a weak but significant dose-effect relationship for Gelofusine, but not for lysine. Although infusion of larger amounts of lysine results in higher reductions of renal uptake of radiolabelled somatostatin analogues in humans [116], this effect was not found in rats [164].

Gelofusine is a plasma expander used in critical emergencies and in the post-surgical setting. It consists of bovine bone derived heat-treated succinylated gelatin. As a result of the succinylation the protein fragments are negatively charged. The mechanism by which Gelofusine reduces the renal uptake of somatostatin analogues is not quite clear, but interference with the megalin/cubulin system is to be expected. It is not likely that the low lysine (4%) and arginine (8%) content of Gelofusine significantly attributes to the inhibitory effect. Binding of the radiolabelled peptide to Gelofusine, thereby hampering proximal tubular reabsorption, would be another possibility, though this is not supported by the PD10 column experiments in the present study. Therefore, the exact mechanism by which Gelofusine reduces kidney uptake of radiolabelled somatostatin analogues remains unclear.

As mentioned above, Gelofusine is of bovine origin and this might cause the allergic reactions reported in some patients, including anaphylactoid reactions [169, 170]. Although the incidences of these reactions is very low, in PRRT any allergic reaction is undesirable and doctors administering this compound must be aware of the possible occurrence of allergic reactions. Nevertheless, application of the combination of Gelofusine and lysine seems promising and we will study this phenomenon in a clinical study in the near future.

In the recent past, different small-animal SPECT systems have become available, amongst them the NanoSPECT system. Previously we showed that the NanoSPECT is a reliable tool for quantifying ^{99m}Tc-DMSA uptake *in vivo* [167]. In this study, we could confirm the accuracy of in vivo activity determination with an ^{111}In-labelled compound. The conclusions drawn from the SPECT studies were confirmed in the biodistribution studies. Therefore, the NanoSPECT appears to be a reliable tool for *in vivo* determination of organ-retained activity with different radionuclides.

CONCLUSIONS

This rat study showed that:

1. Co-injection of Gelofusine reduced the kidney uptake of radiolabelled [DOTA^0,Tyr^3]octreotate. The mechanism by which this occurs is still unclear, a megalin-mediated pathway might be involved. A weak dose-effect relationship was noticed.
2. The combination of Gelofusine and lysine reduced the kidney uptake of therapeutic doses of radiolabelled [DOTA^0,Tyr^3]octreotate significantly better than either compound alone. Therefore this combination might be helpful to improve PRRT.
3. Using the NanoSPECT, rat kidney uptake of radiolabelled somatostatin analogues could be monitored at different time points after injection in the same animal. Quantification of organ retained radioactivity using the NanoSPECT is a reliable method.

CHAPTER 3.5

SOMATOSTATIN RECEPTOR TYPE 2 MEDIATED UPTAKE OF RADIOLABELLED SOMATOSTATIN ANALOGUES IN THE HUMAN KIDNEY

Edgar J. Rolleman, Peter P.M. Kooij, Wouter W. de Herder, Roelf Valkema, Eric P. Krenning and Marion de Jong.

Eur J Nuclear Medicine Molecular Imaging 2007; *in press*

ABSTRACT

Purpose

Renal irradiation is a dose-limiting factor in peptide receptor radionuclide therapy using radiolabelled somatostatin analogues. This irradiation is mainly caused by reabsorption of radiolabelled peptides in the proximal tubule. In the human kidney somatostatin receptors are expressed in the vasa recta, tubuli and glomeruli. It is not clear to what extent these receptors contribute to the total kidney radioactivity uptake.

Methods

Retrospectively, [^{111}In-DTPA^{0}]octreotide scans of 10 selected patients with carcinoids (well-differentiated gastrointestinal endocrine tumour) with liver metastases were evaluated. For each patient, 2 scans were obtained; one scan was performed without (control) and one during treatment with unlabelled octreotide. Kidney, tumour, spleen and liver uptake was measured in both scans.

Results

The interval between the 2 scans per patient varied from 50-397 days. Octreotide treatment substantially lowered kidney [^{111}In-DTPA^{0}]octreotide uptake in 8 out of 10 patients. Kidney uptake in all patients was reduced to 82% ± 15% of control (P<0.01). A correlation between kidney uptake and spleen uptake was found (r=0.67, P<0.05). Serum creatinine was not changed. Surprisingly, tumour and liver [^{111}In-DTPA^{0}]octreotide uptake were not significantly influenced by unlabelled octreotide therapy, but spleen uptake was significantly lowered by treatment (30.6% of control, P<0.002).

Conclusion

We conclude that the somatostatin receptor plays a role in the total renal uptake of radiolabelled somatostatin analogues. The long interval between scans might explain the finding that tumour and liver metastasis uptake of [^{111}In-DTPA^{0}]octreotide was not changed. Further studies are needed to confirm and eludicate the results of this study.

INTRODUCTION

The finding that neuro-endocrine tumours have a high expression of somatostatin receptors has led to new diagnostic and therapeutic strategies for these tumours. In recent years many peptide receptor radionuclide therapy (PRRT) clinical trials have been conducted using radiolabelled somatostatin analogues with promising results [29, 34, 37, 171-173].

In PRRT, the kidney radiation dose is the major dose-limiting factor, being caused by uptake of a few percent of the injected dose after glomerular filtration and the subsequent long residence of radioactivity in the kidney cells. Based on external beam radiation data kidney radiation doses of 23-27 Gy are generally accepted as safe [70]. Follow-up studies after PRRT have suggested that the maximum safe kidney dose, calculated as the biological equivalent dose using the linear quadratic model, might be about 37 Gy [49, 84]. When renal uptake of radiolabelled somatostatin analogues can be significantly lowered, larger total radioactivity doses can be injected within the boundaries of these maximum renal radiation doses, thereby enlarging the therapeutic window for PRRT.

It was recently shown that renal uptake of somatostatin analogues can be inhibited by maleic acid which interferes with cellular energy supplies [115], by colchicine [174] due to dysfunction of proximal tubule reabsorption processes and by positively charged amino acids such as lysine and arginine [96, 97, 115]. Based on animal studies and clinical data [77, 116, 117, 164, 173] PRRT is nowadays performed by co-infusion of lysine and arginine for kidney protection. All these data point to the proximal tubule as the main site of kidney uptake, but the specific mechanism of renal uptake is not yet fully elucidated. From in vitro studies with opossum kidney cells it was suggested that megalin, a multiligand endocytic protein, is involved in the uptake process [41], which is strongly supported by our recent findings of low renal uptake of [^{111}In-DTPA^{0}]octreotide in kidney-specific megalin-deficient mice [42]. For more specific renal prevention strategies it is important to know the mechanism and site of renal uptake of somatostatin analogues.

Using binding and immunohistochemistry studies Reubi et al. [105] found that the vasa recta in human kidney express somatostatin type 2 (sst_2) receptors in a high density. Sst_2 were also demonstrated in tubular cells of the renal cortex, but to a lesser density. Balster et al. [104] showed mRNA of the somatostatin receptor subtypes 1 and 2 in 9 out of 9 human kidneys. With antisera they found sst_2, but not sst_1, being expressed on the glomeruli, whereas both subtypes could be demonstrated on distal tubule cells and on the thick ascending limb of the Henle's loop.

The aim of the present study was to establish the contribution of renal sst_2-receptors to the total kidney uptake of radiolabelled somatostatin analogues. Because a rat model is not suitable –the rat kidney does not express somatostatin receptors - we retrospectively examined

[^{111}In-DTPA^{0}]octreotide scans of carcinoid patients with and without high dose octreotide therapy.

MATERIALS AND METHODS

Patients

From our database, ten patients with a carcinoid tumour (well-differentiated gastrointestinal endocrine tumour) were selected who had been investigated with two diagnostic [^{111}In-DTPA^{0}]octreotide scans. One scan was performed without and one while treated with high dose octreotide for relieve of carcinoid syndrome symptoms. The interval between scans was 50-397 days (mean 275 days, SD 102 days). No other medical anti-tumour therapy or surgery was given in between scans. Octreotide was either administered as the short acting Sandostatine (Novartis, Brussels, Belgium) or the long acting Sandostatine LAR. Doses varied between 200-300 µg per day and 20-30 mg per 28 days, respectively. Patients initially were treated with octreotide as the gold standard in our hospital, but after the long-acting Sandostatine LAR became available this became the treatment modality of choice. Doses were adjusted individually on basis of symptoms. For the patients treated with the LAR-octreotide, the interval between the last octreotide-LAR injection and the scan was 4-21 days. Table 8 summarizes the patients' characteristics.

Table 8. Patient's characteristics.

Patient	Localisation	Octreotide	Octreotide dose
PB/75/F	Small intestine + appendix	LAR-octreotide	30 mg every 4 weeks
JD/75/F	Unknown origin	LAR-octreotide	20 mg every 4 weeks
AJ/52/M	Small intestine	LAR-octreotide	30 mg every 4 weeks
HM/64/F	Small intestine	LAR-octreotide	20 mg every 4 weeks
MS/75/M	Small intestine	LAR-octreotide	20 mg every 4 weeks
JJ/65/F	Small intestine	Octreotide	3 injections of 100 µg each day
AS/62/F	Unknown origin	Octreotide	3 injections of 100 µg each day
RW/60/M	Small intestine	Octreotide	2 injections of 100 µg each day
JB/47/M	Appendix	Octreotide	3 injections of 100 µg each day
PP/38/F	Unknown origin	Octreotide	3 injections of 100 µg each day

First column: patient's initials/age/gender;

LAR-octreotide: the long acting Sandostatine LAR.

Radiolabelling

Commercially available kits of [DTPA^{0}]octreotide and $^{111}InCl_3$ were obtained from Mallinckrodt (Petten, The Netherlands). The radiolabelling procedure was performed in accordance with standard procedures [15, 16].

Measurement of kidney uptake of [^{111}In-DTPA^{0}]octreotide

Uptake of [^{111}In-DTPA^{0}]octreotide in left kidney, spleen, liver and tumour was calculated as percentage uptake of the injected dose using conjugate-view abdominal planar scintigraphy at 24 h post injection of 220 MBq [^{111}In-DTPA^{0}]octreotide. Planar scintigraphy was performed with a dual-head gamma camera (2000XP, Philips Medical Systems, Eindhoven, The Netherlands) fitted with a medium-energy collimator. The energy windows were set at ± 15% and acquisition times were 15 minutes.

Statistics

Wilcoxon paired non-parametric test was used for intra-patient comparisons. *P*-values <0.05 were considered statistically significant.

RESULTS

Left kidneys were clearly visible on all scintigrams, but overprojection of the liver made measurement of the right kidney unreliable.

Table 8 shows the patients' characteristics of the ten included patients, who all had liver metastases.

Table 9. Left kidney uptake of [^{111}In-DTPA^{0}]octreotide during octreotide treatment expressed as % of the scan performed without octreotide treatment (% of control). (*) P<0.01vs control.

Patient	Kidney uptake (% of control)
B/75/F	83
D/75/F	91
AJ/52/M	74
HM/64/F	68
MS/75/M	109
JJ/65/F	74
AS/62/F	101
RW/60/M	80
JB/47/M	87
PP/38/F	58
MEAN	**82 ± 15 ***

Left kidney radioactivity at 24 h p.i. ranged from 0,2%-1.05% of the injected dose; during octreotide treatment left kidney radioactivity ranged from 0,2%-1.07% of the injected dose. Table 9 shows the left kidney uptake of [^{111}In-DTPA^{0}]octreotide during octreotide treatment, expressed as percentage of the scan performed without octreotide treatment. In 8 out of 10 patients substantial reductions in renal radioactivity were found, ranging from 58-91% of the scan without octreotide treatment, but in 2 out of 10 patients no reduction in kidney

radioactivity uptake was found. The kidney uptake in all patients was reduced by 18% to 82% ± 15% of the scan without octreotide treatment (P<0.01).

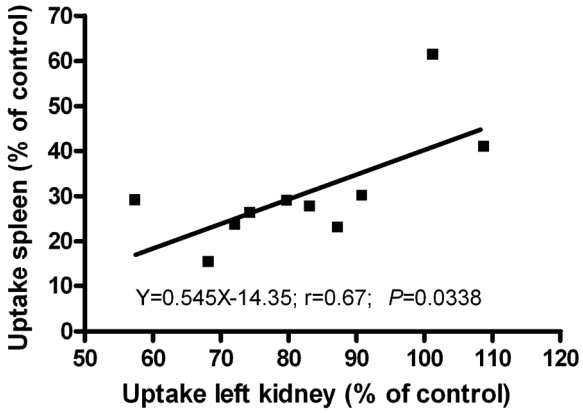


Figure 33. Correlation of effects of octreotide treatment on kidney uptake as well as on spleen uptake 24h after injection of 220 MBq [^{111}In-DTPA^{0}]octreotide. Data are expressed as percentage of the scan without octreotide treatment.

Figure 33 shows the weak, but significant correlation that was found between kidney and spleen uptake (r=0.67; P<0.05). There was no correlation between reduction of renal uptake of [^{111}In-DTPA^{0}]octreotide and the length of the interval between scans (not shown). In addition, no correlation was found between reduction of renal uptake of [^{111}In-DTPA^{0}]octreotide and the dose of octreotide administered (not shown).

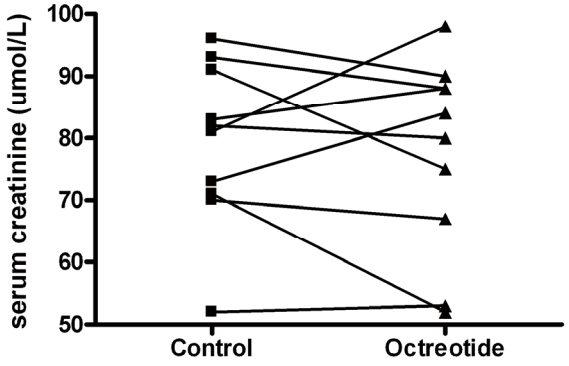


Figure 34. Serum creatinine values at times of [^{111}In-DTPA^{0}]octreotide scintigrams before and during octreotide treatment.

Figure 34 shows the serum creatinine values at the time of the standard scan and at the time of the scan during octreotide treatment. During octreotide treatment, the serum creatinine was not significantly different from that during the control situation.

Table 10 shows the uptake of [^{111}In-DTPA^{0}]octreotide in the left kidney, liver, spleen and tumour in patients receiving octreotide therapy, expressed as percentage of the uptake measured without this treatment. Spleen uptake was significantly reduced by octreotide treatment, but tumour and liver uptake were not.

Table 10. Spleen, liver and tumour uptake of [^{111}In-DTPA^{0}]octreotide during treatment with unlabelled octreotide expressed as percentage of uptake without treatment.
($) P<0.002 vs control; (#) NS vs control.

	Uptake (% of control)
Spleen	31% ± 13% $
Liver	83% ± 32% #
Tumour	85% ± 37% #

Table 11 summarizes the individual values for tumour uptake (expressed as percentage of control) in each patient. In Table 11 also the changes of disease in the interval between scans are given as measured by computer tomography (CT) or magnetic resonance imaging (MRI). No clear correlation could be found between increases in tumour uptake and changes in disease activity, e.g. progression.

Table 11. Tumour uptake of [^{111}In-DTPA^{0}]octreotide during treatment with unlabelled octreotide expressed as percentage of a scan without treatment in each individual patient. Also the course of the disease in the interval between scans is given.

Patient	Tumour uptake (% of control)	Disease
PB	119	Stable (MRI)
JD	145	Progression (MRI)
AJ	70	Stable (MRI)
HM	46	Stable (CT)[a]
MS	104	Stable (CT)[a]
JJ	70	Stable (CT)[b]
AS	93	Stable (CT)
RW	117	Regression (CT)[c]
JB	67	Stable (CT)
PP	22	Stable (MRI)

a) stable disease on CT, but new lesions on SMS scan
b) on CT the metastasis volumes were not changed, but less necrotic tissue in metastases
c) minimal reduction of metastasis size

The patients that were treated with LAR-octreotide had tumour uptake values of 97 ± 35 % of control, not significantly different from control. The patients that were treated with octreotide had tumour uptake values of 74 ± 32 % of control, respectively, both not significantly different from control.

DISCUSSION

During PRRT, the kidney radiation dose is the major factor that limits the total administered dose and the radiation dose to the tumour. Renal uptake of radiolabelled somatostatin analogues and subsequent long retention of radioactivity are responsible for this kidney radiation.

In order to interfere with this uptake successfully, it is prerequisite to understand the exact uptake mechanism. The major part of kidney uptake of radiolabelled somatostatin analogues is via proximal tubular reabsorption. This is supported by the finding of several groups that positively charged amino acids, like lysine and arginine, can reduce the kidney uptake of radiolabelled somatostatin analogues by about 40-50% [77, 115, 116]. These amino acids are known to interfere with the proximal tubular reabsorption of small proteins and peptides [95]. Furthermore we showed the kidney uptake of [^{111}In-DTPA^{0}]octreotide in megalin-deficient mice was significantly reduced as compared to normal mice [42]. Megalin is a multi-scavenger molecule that is essential for proximal tubular reabsorption of many low-molecular- weight proteins. Despite this, the exact localization and mechanism are not fully eludicated. As the renal uptake of somatostatin analogues can not be completely blocked, other mechanisms may be involved.

In the past, sst_2 have been demonstrated in cells of glomeruli [104], tubuli and vasa recta [105]. To elucidate the role of these receptors in renal uptake during scintigraphy and PRRT using somatostatin analogues the present study was performed. We showed that renal uptake of somatostatin analogues is significantly reduced by concomitant treatment with unlabelled octreotide for relief of carcinoid syndrome symptoms. This indicates that binding to the renal sst_2, plays a role in the total uptake of radiolabelled somatostatin analogues in the kidneys and thus on the resulting renal radiation dose. We could not find a clear dose-relationship in these ten patients, but the found correlation between spleen uptake and kidney uptake suggests a dose-dependency.

One could argue that the effects of octreotide treatment on kidney uptake of [^{111}In-DTPA^{0}]octreotide might not be caused by ousting of the radioligand from the sst_2 by the cold octreotide, but by other factors, such as hemodynamic changes due to high octreotide levels. There is some controversy in the literature on the effects of somatostatin and somatostatin analogues on kidney function parameters. Table 12 summarizes all human data on the effects of somatostatin or octreotide on kidney function. Somatostatin and octreotide have been described in healthy subjects and patients with diabetes mellitus and acromegaly to decrease renal plasma flow (RPF) and concomitantly decrease glomerular filtration rate (GFR) with unchanged filtration fraction [175-182]. However, some of these studies administered supraphysiological doses of somatostatin (100-420 µg/h IV, or 600 µg/d SC).

Table 12. Summary of human studies on renal effects of somatostatin or octreotide.

Authors	Subjects	Dosage	Major finding
Serri [175]	Diabetics (*n*=11)	Cont.SC infusion of 300 µg octreotide/day for 12 weeks	GFR 136 mL/min vs. 157 mL/min in placebo group
Luksch [176]	Healthy (*n*=3)	6 µg/kg/h somatostatine IV, 3 hours	Inulin clearance 131 → 124 mL/min
Schmidt [177]	Healthy (*n*=9)	6 µg/kg/h somatostatine IV, 3 hours	Inulin clearance 138 → 119 mL/min
Tulassay [178]	Healthy (*n*=7)	250 µg somatostatine/h IV, for 2 hours	Inulin clearance 131 → 62 mL/min
Vora [179]	Healthy (*n*=6) and diabetics (*n*=9)	100 µg somatostatine/h IV for 2 hours	^{51}Cr-EDTA clearance 112→95 mL/min
Dullaart [180]	Acromegaly (*n*=7)	T.i.d. 100 µg octreotide, 3 months	^{125}I-iothalamate clearance 132→117 mL/min
Kalambokis [181]	Cirrhotics (*n*=25)	B.i.d. 300 µg octreotide SC, 11 days	Higher RPF flow but ^{99m}Tc-DTPA clearance 95→79 mL/min
Kalambokis [182]	Cirrhotics (*n*=20)	B.i.d. 300 µg octreotide SC, 14 days	^{99m}Tc-DTPA clearance 79→72 mL/min
Castellino [183]	Healthy (*n*=18)	480 µg/h somatostatin IV, 3 hours	No effect of somatostatine on inulin clearance
Tulassay [184]	Healthy (*n*=8)	100 µg octreotide SC, once	Creatinine clearance 124 → 66 mL/min
Krempf [185]	Diabetics (*n*=5)	480 µg octreotide IV/hour, 10 hours	^{99m}Tc-DTPA clearance unchanged
Malesci [186]	Cirrhotics (*n*=11)	T.i.d. 100 µg octreotide SC, 2 weeks	Inulin clearance 99 → 99 mL/min
Ruggenenti [187]	Cystic kidneys (*n*=14)	40 mg LAR-octreotide SC every 4 weeks, for 6 months	Unchanged iohexol plasma clearance
Sabat [188]	Cirrotics (*n*=20)	B.i.d. 250 µg octreotide SC, 5 days	Creatinine clearance 61 → 65 mL/min
Dullaart [189]	GH-deficient patients (*n*=7)	10 µg/h IV plus 4 injections 100 µg octreotide SC	^{125}I-iothalamate clearance unchanged
Mountokalakis [190]	Cirrhotic (*n*=9)	40 µg/h octreotide IV, 2 hours	Creatinine clearance higher
Ottesen et al.[191]	Cirrhotics (*n*=25)	20 mg LAR-octreotide SC every 4 weeks	^{51}Cr-EDTA clearance 120→114 mL/min (NS)
Pomier et al. [192]	Hepatorenal syndrome (*n*=14)	50 µg/h IV, 2 days	Creatinine clearance unchanged

In contrast, the study by Castellino [183] applied 480 μg/h somatostatin in 18 healthy subjects and did not find any effect on the GFR. Studies that applied octreotide doses comparable with the doses in our patient group showed conflicting results as well. Tulassay et al. [184] administered 100 μg octreotide subcutaneously and found a significant decrease in GFR of 47%, that lasted up to 8 hours. Studies performed in cirrhotic patients, growth hormone deficient patients and patients with mild to moderate renal insufficiency did not find significant changes of either RPF or GFR [183, 185-192]. It must be stressed that the studies listed in Table 12 describe the relatively short term (hours to months) effects of somatoatatin or its analogues on kidney function. Patients suffering from neuro-endocrine tumours are usually treated for years with unlabelled somatostatin analogues. In our study, serum creatinine values did not change during treatment with octreotide, suggesting that there were no major changes in glomerular filtration rate in our patients that could have biased our findings.

In the present study, the variable effects of octreotide treatment on tumour and liver uptake were unexpected. As these tumours and metastases are somatostatin receptor-positive one would expect an unequivocal reduction in [^{111}In-DTPA^{0}]octreotide uptake in these tumours and metastases. A possible explanation for our findings could be progression of the tumour at the time of the scan during octreotide treatment. As shown in Table 11, in only one patient with an increase in tumour [^{111}In-DTPA^{0}]octreotide uptake during octreotide treatment, progression of the disease was present. Two patients with progressive disease, however, had a strong reduction of tumour and liver uptake. Patients with stable disease could have both a strong reduction and an increase in [^{111}In-DTPA^{0}]octreotide uptake during octreotide treatment.

Interestingly, patients treated with the long-acting LAR-octreotide seemed to have a smaller reduction (3% ± 35%) in tumour uptake of [^{111}In-DTPA^{0}]octreotide than those treated with octreotide (26% ± 32%), although this difference was not statistically significant and large variations were present in both groups. This, taken together with the reduced kidney uptake caused by both octreotide and LAR-octreotide, might provide a basis in the future for continuation of LAR-octreotide treatment during PRRT as it causes little reduction in tumour uptake but sustained kidney uptake reduction, enlarging the therapeutic window of PRRT. Larger studies that apply smaller intervals between the scans are needed before such a strategy can be implemented in PRRT protocols, however. In our study, the interval between the scan without and with octreotide treatment was long and it is hard to tell exactly what happens in the tumours at the level of receptor numbers and receptor density.

Dorr et al. [193] previously showed diminished kidney, liver and spleen uptake during octreotide treatment with 600 μg per day, yet improved tumour visualisation by somatostatin

analogue treatment. Our results are not in line with their findings: indeed we found significant reduction of kidney uptake, but in 4 of 5 patients treated with 3 daily injections of octreotide we also found a considerable reduction of tumour uptake, applying even a lower dose (200 or 300 μg/day) than Dorr et al. Due to the small number of patients and the large inter-individual variation, the mean tumour uptake was not significantly reduced by unlabelled octreotide treatment.

CONCLUSION

We showed that during treatment with octreotide for symptom relief, kidney uptake of [^{111}In-DTPA^{0}]octreotide was decreased by about 18 percent in 10 patients with carcinoid tumours. This indicates a substantial contribution of renal sst_2 to the total kidney uptake of radiolabelled somatostatin analogues. The effects of octreotide and LAR-octreotide on tumour and liver metastases were not expected: we did not find a significant reduction of tumour uptake. The large interval between scans might be a confounding factor as well as the small number of patients evaluated.

CHAPTER 4

MITIGATION OF RADIATION EFFECTS IN THE KIDNEY DURING PRRT

CHAPTER 4.1

AMIFOSTINE PROTECTS RAT KIDNEYS DURING PEPTIDE RECEPTOR RADIONUCLIDE THERAPY WITH [^{177}Lu-DOTA^{0},Tyr^{3}]OCTREOTATE

Edgar J. Rolleman, Flavio Forrer, Bert Bernard, Magda Bijster, Marcel Vermeij, Roelf Valkema, Eric P. Krenning, and Marion de Jong.

Eur J Nucl Med Mol Imaging 2007; *in press*

ABSTRACT

Purpose

In peptide receptor radionuclide therapy (PRRT) using radiolabelled somatostatin analogues, the kidneys are the major dose-limiting organs, because of tubular reabsorption and retention of radioactivity. Preventing renal uptake or toxicity will allow for higher tumour radiation doses. We tested the cytoprotective drug amifostine, that selectively protects healthy tissue during chemo- and radiotherapy, for its renoprotective capacities after PRRT with high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

Methods

Male Lewis rats were injected with 278 or 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate to create renal damage and were followed up for 130 days. For renoprotection, rats received either amifostine or co-injection with lysine. Kidneys, blood and urine were collected for toxicity measurements. At 130 days after PRRT a single photon emission computed tomography (SPECT) scan was performed to quantify tubular uptake of ^{99m}Tc-dimercaptosuccinic acid (DMSA), a measure of tubular function.

Results

Treatment with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate resulted in body weight loss, elevated creatinine and proteinuria. Amifostine and lysine treatment significantly prevented this rise in creatinine and the level of proteinuria, but did not improve the histological damage. In contrast, after 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, creatinine values were slightly, but not significantly, elevated compared to the control rats. Proteinuria and histological damage were different from controls and were significantly improved by amifostine treatment. Quantification of ^{99m}Tc-DMSA SPECT scintigrams at 130 days after [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate therapy correlated well with 1/creatinine (r^2=0.772, P<0.001).

Conclusion

Amifostine and lysine effectively decreased functional renal damage caused by high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Besides lysine, amifostine might be used in clinical PRRT as well as to maximize anti-tumour efficacy.

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues has shown convincing results in patients with somatostatin receptor-positive tumours, e.g. neuro-endocrine tumours [37]. Partial remission rates of up to 30% with sporadic complete remissions [32, 114], as well as significant improvements in quality of life [38] and survival [33], have been reported when using PRRT with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and [^{90}Y-DOTA^{0},Tyr^{3}]octreotide.

The major drawback of the use of these small peptides in PRRT is the radiation absorbed dose to the kidneys. The radiolabelled small peptides are eliminated by glomerular filtration and a fraction is subsequently taken up in the proximal tubules, where the chelator-radionuclide complex is retained in the lysozomes, causing kidney tissue irradiation [92]. Accordingly, Barone et al. [49] showed that in patients, renal uptake of [^{86}Y-DOTA^{0},Tyr^{3}]octreotide was about 2 % of the injected dose, which would result in a considerable radiation dose to the kidneys in high-dose PRRT. This proximal tubular uptake therefore represents a major dose-limiting factor in PRRT [163]. It is not exactly known which dose can be safely administered to the kidneys in PRRT. In the past a threshold of 23 Gy has been adopted, based on external beam radiation therapy experiences (this dose results in 5% renal failure after 5 years) [70].

In PRRT, the aim is to deliver maximum possible radiation doses to the tumour, within safe margins for the kidneys. Therefore, methods to lower renal radioactivity uptake after injection are essential. In both animals [115] and humans [116, 117] it has been shown that positively charged amino acids successfully reduce the renal uptake of radiolabelled somatostatin analogues, and these amino acids are now used on a large scale in PRRT protocols [35]. We recently showed that lysine co-injection indeed prevents functional kidney damage caused by PRRT with three weekly doses of 185 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in rats [168].

In addition, interference with other factors that play a role in the cascade of renal damage after irradiation might be helpful. As ionising radiation induces damage via generation of oxidative stress [55], anti-oxidants may serve as additional protectors acting by a different mechanism. In radio- and chemotherapy, amifostine has successfully been used to prevent damage in healthy tissues since higher treatment doses were possible with reduced side effects and fewer treatment discontinuations [106].

Amifostine is administered as a pro-drug and is rapidly dephosphorylised in the endothelium of capillaries by alkaline phosphatase to its active form WR-1065 [106], which is instantly taken up into the cells. This activation step takes place in healthy tissues and to a lesser extent in tumour tissue because of lower action of alkaline phosphatases in tumours [194]. Thirty minutes after administration, tumours have an up to 100-fold lower WR-1065 concentration compared with normal tissues, e.g. the kidney [107]. The differential uptake of WR-1065 will

result in protection of healthy tissue, and not of tumours. The mechanism of cytoprotection offered by amifostine is complex and not completely understood, but most probably due to scavenging of free radicals in competition with oxygen. Furthermore, the amifostine metabolite WR-33278 exhibits structural similarities to naturally occurring polyamines and may affect processes related to DNA-synthesis, DNA-repair, gene expression, and cell cycle progression [106].

The aim of the present study was to test the renoprotective effects of amifostine and those of lysine in rats during PRRT with high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

MATERIALS AND METHODS

Radionuclides, peptides and chemicals

^{177}Lu was obtained from IDB (Baarle Nassau, The Netherlands). [DOTA^{0},Tyr^{3}]octreotate was supplied by BioSynthema (St Louis, MO, USA). [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate was synthesised and labelled as previously described [144]. The ^{99m}Tc-dimercaptosuccinic acid (DMSA) kit was purchased from Tyco Healthcare (Petten, the Netherlands) and labelled according to the indicated procedure. Amifostine was purchased from MedImmune Oncology Inc. (Nijmegen, The Netherlands). D-lysine was purchased from Sigma (St. Louis, MO, USA).

Peptide radionuclide therapy experiments using [^{177}Lu-DOTA,Tyr^{3}]octreotate

All animal experiments were approved by the governing Animal Welfare Committee and conducted according to institutional regulations. Male Lewis rats (Harland, Horst, The Netherlands; weight 300-350 g) were divided into seven separate experimental groups. Rats were injected with indicated doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (specific activity 38 MBq/μg) into the dorsal vein of the penis (300 μL). For renal protection, rats were treated with either D-lysine (400 mg/kg, co-injection with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate) or amifostine (200 mg/kg, 30 minutes prior to PRRT, followed by 25 mg/kg subcutaneously once daily for 7 days after PRRT). The groups were as follows:

1. 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (*n*=9)
2. 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate + lysine (*n*=15)
3. 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate + amifostine (*n*=6)
4. 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (*n*=10)
5. 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate + amifostine (*n*=8)
6. Only amifostine, no radioactivity (*n*=4)
7. control group, no treatment (*n*=9)

Recent dosimetry studies showed that the radioactivity doses applied resulted in kidney total organ absorbed doses of about 70 and 35 Gy, after 555 and 278 MBq [^{177}Lu-

DOTA^0,Tyr^3]octreotate respectively [83], and caused profound renal damage [168]. These doses had excellent anti-tumour efficacy in tumour-bearing rats [118].
Weekly, rats were weighed and 24-h urine samples were obtained using metabolic cages. At day 90 blood samples were taken by orbital puncture for determination of kidney function.
Rats were euthanised according to the protocol on day 130 after therapy, or earlier when they had significant loss of body weight (>20%). In most of the rats (group 1: *n*=8, group 2: *n*=13 , group 3: *n*=4, group 4 : *n*=6, group 5, *n*=7, groups 6+7: *n*=6), single photon emission computed tomography (SPECT) scans were performed using ^{99m}Tc-DMSA for kidney function evaluation (see below), just prior to sacrifice. At sacrifice, another blood sample was taken by cardiac puncture for determination of haematological parameters and renal function. Kidneys were analysed histologically.

Analytical procedures

Kidneys were fixed in 10% buffered formalin, trimmed and processed by standard techniques for embedding in paraffin. Four-micron sections were cut and stained with haematoxylin-eosin (HE) and periodic acid-Schiff reagent (PAS). Microscopically, a renal damage score (RDS) was graded from 0 (no damage) to 4 (severe renal damage) according to criteria listed in the M&M section of Chapter 2.
Urinary protein was measured in 24-h urine samples with a commercially available colorimetric method (BioRad, Hercules, CA, USA). Blood chemistry parameters were determined by standard hospital analysis procedures.

Kidney function with ^{99m}Tc-DMSA

For SPECT imaging the four-headed multiplexing multi-pinhole NanoSPECT (Bioscan Inc., Washington D.C., USA) was used; see [167] for description and validation of this system. At 130 days after treatment with [^{177}Lu-DOTA,Tyr^3]octreotate, rats were injected with 50 MBq ^{99m}Tc-DMSA into the dorsal vein of the penis. Four hours post injection a helical SPECT of the kidneys was acquired. The energy-peak was set at 140 keV, the window width was ± 10%. An acquisition time of 30 s per view was chosen. Total acquisition times ranged from 6 to 9 min per animal. Data were reconstructed iteratively with the HiSPECT software, using a dedicated OSEM algorithm for multiplexing multi-pinhole reconstruction. Regions of interest were drawn manually around each kidney and the 3D activity distribution within the volume of interest (VOI) was then summed to determine the uptake in the kidneys. Quantification of the activity in the VOI was performed with the INTERVIEW XP (Mediso, Budapest, Hungary) software.

Statistical analysis

Data are expressed as mean ± SD. One- or two- way ANOVA followed by Tukey's test or Student's *t*-test was used to test significance of differences. *P*-values less than 0.05 were considered to indicate statistically significant differences.

RESULTS

The renal damage score, levels of serum creatinine and blood urea nitrogen (BUN), and urinary protein excretion of the rats treated with amifostine alone were equal to those of control rats, indicating no toxic effects of amifostine at the applied dose regimen. Therefore both groups were further considered as one control group.

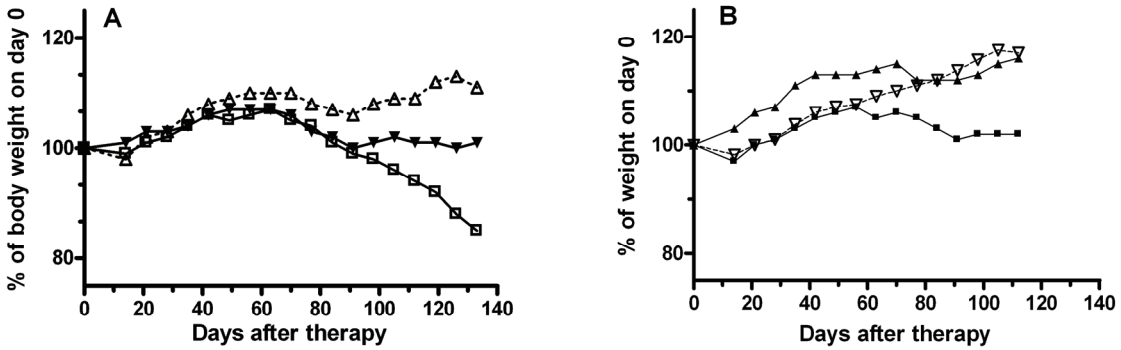


Figure 35. Body weight expressed as % of initial body weight in rats treated high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. In panel **a** data of rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (■), rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus amifostine (▲) and rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus lysine (▼) are shown. In panel **b,** data of rats treated with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (■), 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus amifostine (▲), and control rats (○) are shown.

Figure 35 shows the mean body weight of all treatment groups as a percentage of body weight at start of therapy. The rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate showed a significant decline in body weight, indicating severe impairment of general condition, most likely attributed to renal failure. This body weight loss after therapy was largely prevented by amifostine and lysine co-administration. Rats treated with 278 MBq showed a slower increase in body weight than control rats, which were without significant weight loss. Amifostine treatment significantly counteracted this slower body weight increase.

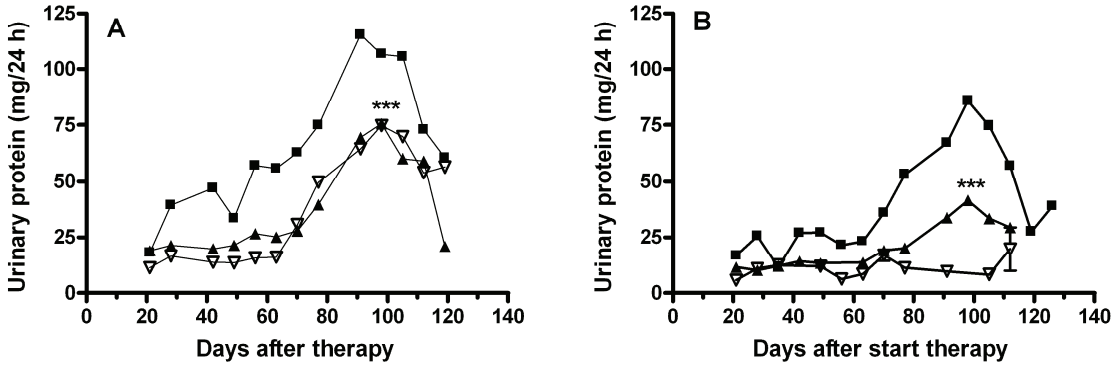


Figure 36. Proteinuria expressed in mg protein/24 h in rats treated high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. In panel **a**, data of rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (■), rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus amifostine (▲) and rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus lysine (○) are shown. In panel **b**, data of rats treated with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (■), 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus amifostine (▲), and control rats (○) are shown. (***) P<0.05 vs groups without renoprotection.

Figure 36 shows urinary protein excretion per 24 h in all experimental groups. Proteinuria was significantly lower in the 278-MBq group than in the 555-MBq group (P<0.001). In addition, amifostine significantly lowered proteinuria when given to rats treated with 278 and 555 MBq. Also, lysine co-injection significantly lowered proteinuria.

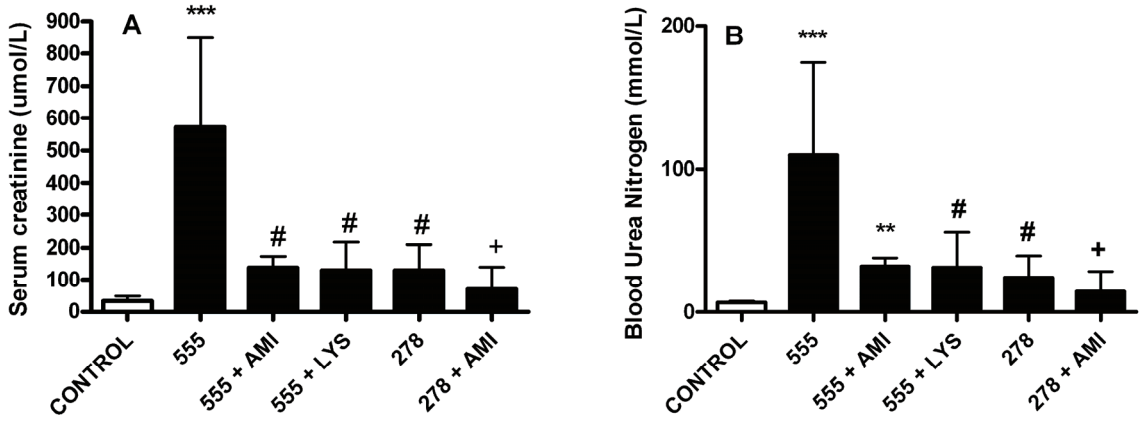


Figure 37. Serum creatinine (**a**) and BUN (**b**) at sacrifice of rats treated with 278 or 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with or without amifostine (*AMI*) or lysine (*LYS*) as renoprotective drugs. (***) P<0.001 vs control; (#) P<0.001 vs 555 MBq alone; (+) NS vs 278 MBq alone; (**) P<0.01 vs 555 MBq alone.

Renal function, monitored by serum creatinine and BUN values, was not significantly altered at 90 days after any treatment (data not shown). In Figure 37, both serum creatinine and BUN

at 130 days after treatment are depicted. Administration of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate caused severe renal insufficiency, as reflected by high creatinine and BUN serum values. This was largely prevented by both amifostine and lysine treatment. The creatinine and BUN serum concentrations of rats in the 278-MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate group were significantly lower than those of rats in the 555-MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate group and not statistically different from control rats. Therefore, no significant favourable effect of amifostine treatment on creatinine and BUN serum concentrations could be shown in rats treated with 278 MBq of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

Table 13. Effect of amifostine and lysine treatment on renal damage score (RDS) 130 days after treatment with high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. (*) P<0.01 vs 278 MBq alone.

Treatment	Renal Damage Score	Number of rats
Control	0.5 ± 0.9	13
555 MBq alone	4.0 ± 0.0	9
555 MBq + amifostine	4.0 ± 0.0	6
555 MBq + lysine	3.5 ± 1.0	15
278 MBq alone	3.7 ± 0.7	10
278 MBq + amifostine	2.1 ± 1.5 *	8

Histological damage was evaluated using a renal damage score (RDS) system. Data are shown in Table 13. Control rats had a mean RDS of 0.5 at sacrifice. All rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate had the maximal RDS of 4, indicating severe kidney damage. This score was not significantly decreased by amifostine or lysine treatment. In contrast, the rats treated with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone had comparable RDS to the ones with the 555 MBq treatment but at this lower radioactivity dose amifostine reduced the renal damage significantly.

For further kidney function evaluation, several rats per group underwent SPECT with ^{99m}Tc-DMSA at day 130 after PRRT. Figure 38a shows the percentage injected activity (%IA) in the left kidneys measured 4 hours after injection of 50 MBq ^{99m}Tc-DMSA. Uptake of ^{99m}Tc-DMSA in the kidneys was significantly decreased at 130 days after both 278 and 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate compared with that in control rats, indicating severe tubular dysfunction. Both amifostine + PRRT-treated and lysine + PRRT-treated rats had significantly higher uptake of ^{99m}Tc-DMSA than the corresponding groups that received only PRRT. Figure 38b shows the correlation between 1/creatinine values (a measure of glomerular filtration rate) and %IA in the left kidney 4 hours after injection of ^{99m}Tc-DMSA, as measured by SPECT. The shown line has a correlation coefficient of r^2= 0.772, P<0.001.

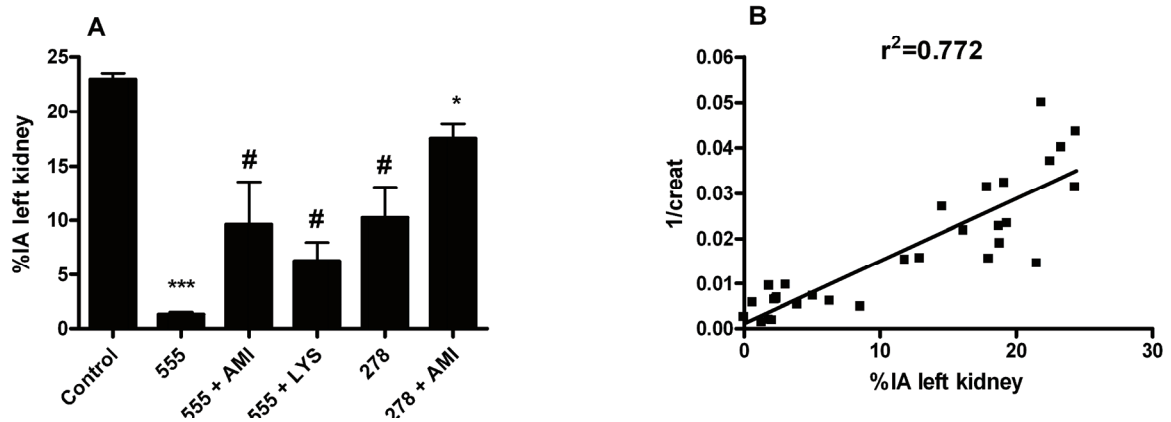


Figure 38. a Percentage injected activity (%IA) in the left kidney, 4 h after injection of 50 MBq ^{99m}Tc-DMSA in rats. Scans were performed 130 days after treatment with 278 or 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with or without amifostine (*AMI*) or lysine (*LYS*) as renoprotective drugs. Data represent measurements with the four headed multiple pinhole NanoSPECT. (***) P<0.001 vs control; (#) P<0.05 vs 555 MBq alone; (*) P<0.05 vs 278 MBq alone. **b** Linear correlation of %IA in the left kidney 4 h after injection of 50 MBq ^{99m}Tc-DMSA measured with NanoSPECT (*x-axis*) and 1/creatinine (*creat*) values (*y-axis*).

Haematological analysis revealed a significantly lower haemoglobin concentration in the 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate-treated rats (5.6 ± 1.5 mmol/L) as compared to control rats (9.2 ± 0.9 mmol/L; P<0.001) (Figure 39a). Rats treated with 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate had haemoglobin values of 7.8 ± 0.5 mmol/L, which was not significantly different from control values. Amifostine and lysine treatment significantly improved the haemoglobin concentrations in 555 MBq-treated rats; the same trend, but not significantly different, was observed in the 278 MBq-treated rats. Figure 39b shows the correlation between haemoglobin values and creatinine values 130 days after PRRT with 555 or 278 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate: a significant correlation was found, with r^2 of 0.723. Platelet counts and leucocytes counts were not significantly lowered at 130 days post therapy. Amifostine treatment therefore could not exert significant improving effects on these haematological parameters.

DISCUSSION

Renal irradiation is an undesired effect in PRRT, caused by reabsorption and retention of radiolabelled peptides by proximal tubule cells. Although significant renal damage is found in only a small fraction of treated patients, the renal radiation dose remains a major concern as this determines the maximum tolerated injected radioactivity and therefore the maximum achievable radiation dose to the tumour. At present, the maximum tolerated kidney radiation dose is not exactly known, but a 23-Gy limit has been adopted based on external beam radiation therapy experiences.

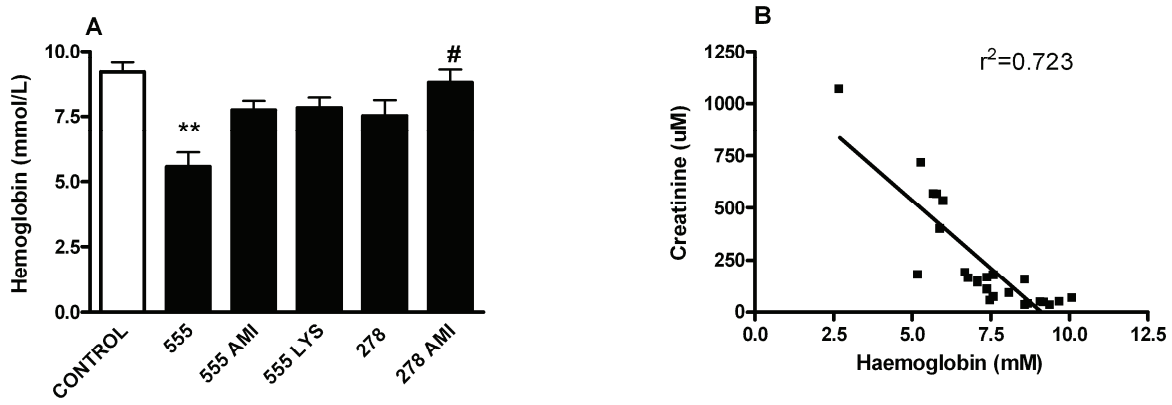


Figure 39 a Haemoglobin concentrations in rats 130 days after treatment with 278 or 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. (**) P<0.001 vs control and P<0.05 vs rats treated with 555 MBq plus amifostine (*AMI*) or lysine (*LYS*); (#) not significantly different from 278 MBq without amifostine. **b** Correlation between serum creatinine values and haemoglobin concentrations in treated rats.

However, these two types of radiation (PRRT and external beam radiation) differ in various respects. In external beam radiation therapy, the radiation to the kidneys is homogenously delivered at high dose rate to that part of the kidney that is in the field of radiation. In contrast, kidney irradiation in PRRT occurs at a lower dose rate and is inhomogeneous, dependent on specific retention (mainly in the renal cortex) and energetic characteristics of the radiolabelled compound [168].

For PRRT, few data are available that correlate the onset of renal toxicity with dosimetric calculations. Barone et al. showed a correlation between the calculated biological equivalent dose (BED) and renal function loss per year, using the linear quadratic (LQ) model and taking into account fractionation of the dose and inhomogeneous distribution of the radiolabelled peptide in the kidney [49]. From that study it can be concluded that a calculated BED between 27 and 42 Gy correlates with kidney function loss rates of up to 10% per year, whereas a BED higher than 45 Gy resulted in yearly kidney function loss of 26-56%. Also, it was shown that co-existing factors, like hypertension, diabetes mellitus or previous chemotherapy, influenced the dose-response rate of kidney function to the applied doses [49]. One should realise that these calculated BED values can not easily be compared with radiation absorbed doses using classical calculation methods. Also, this dose-effect relationship and possible critical dose limits were determined in patients after [^{90}Y-DOTA^{0},Tyr^{3}]octreotide treatments and might be different in patients treated with other somatostatin analogues or radionuclides. For instance, beta-emitters with shorter energy ranges, like ^{177}Lu, might produce less profound damage than those with longer energy ranges.

The doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (555 and 278 MBq) used in this study were chosen on the basis of our previous studies, in which they were proved to cure rats that had

CA20948 tumour [118] or AR42J tumour [168] in their flank. As a result of these administered high doses, renal toxicity occurred, both histologically and functionally [168]. The whole kidney absorbed dose with the activities injected were estimated to be 35 and 70 Gy [83].

This rat model reflects the clinical situation: at present most patients can not be cured unless higher treatment doses are used. However, these higher doses may be accompanied by severe renal toxicity, as in our rats.

It has been shown that lysine (and arginine) co-injection/infusion reduces kidney uptake of radiolabelled peptide in patients and animal models [35, 115-117]. In rats it significantly reduced proteinuria and serum creatinine after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, fractionated in three doses [168]. Lysine results in the present study are comparable to those previously reported [168].

So far the research on kidney protection for PRRT has mainly focussed on methods to reduce renal uptake of the radiopharmaceutical. A different approach would be to reduce the effects of the permanent state of oxidative stress that is caused by the irradiation of tissue, as indicated by recent studies [55]. This oxidative stress leads to changes in the cell and to fibrosis and apoptosis that may occur late after initial irradiation. Therefore, we investigated whether free radical scavengers or anti-oxidants, e.g. amifostine, might ameliorate or prevent PRRT-induced radiation damage to the kidneys. In this study, we showed that amifostine effectively decreased both functional damage (proteinuria and serum creatinine) after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and histological damage in rats treated with 278 Mq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

The mechanisms of action by which lysine and amifostine protect the kidneys are different. Lysine inhibits the reabsorption of radiolabelled peptides in the proximal tubule, thus lowering the radiation burden, whereas amifostine mitigates radiation effects by interference with free radicals and DNA damage caused by radiation. Based on the present findings, a dual-modality approach to protect the kidneys in PRRT seems conceivable.

Besides offering kidney protection, amifostine has been reported to have beneficial effects of amifostine bone marrow toxicity [195, 196]. In the present study, significantly lowered haemoglobin concentrations were observed only in the highest [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate treatment group, with significant protection by amifostine and lysine. We previously showed that significant haematological toxicity was apparent only beyond 200 days after PRRT [168]. Based on this, as well as on the strong correlation between creatinine values and haemoglobin concentrations and on the beneficial effects of lysine on haemoglobin concentrations, it is more likely that the lowered haemoglobin concentrations observed were caused by the renal insufficiency in these rats resulting in lowered erythropoietin, leading to anemia.

The used dose and the administration schedule of amifostine was chosen based on dose-response effects in other preclinical animal studies [197]. In rat studies, it was shown that exact timing is essential [197, 198] because of de drug's short half-time. In fact, the drug must be administered just before irradiation in order to have high tissue concentrations of the compound. As PRRT is characterized by a prolonged radiation owing to the specific tissue localisation of the peptide and the decay characteristics of the radionuclide, we chose to administer amifostine for the following 7 days to promote further protection while radiation is still present.

There has been a lot of debate on the safety and toxicity of amifostine. In general, amifostine is well tolerated at the recommended dose and schedule [199]. The side effects that are most frequently experienced include nausea and vomiting on the day of therapy and transient reductions in systolic blood pressure during infusion; these are usually reversible and manageable [199]. Also, three reports have been published that amifostine treatment might induce anaphylactoid reactions [200-202], but the incidence has yet to be established. Severe cutaneous (allergic) reactions like Stevens-Johnson syndrome or toxic epidermal necrolysis can occur, but their occurrence is comparable to that of similar reactions when using other drugs, as reported by a committee on the cutaneous effects of amifostine [203]. In the present study, no signs of toxicity of the amifostine treatment were noted. Koukourakis et al. reported that subcutaneous injection has a better profile than commonly applied intravenous administration with regard to adverse effects [204] and that subcutaneous administration is feasible in patient care. It is associated with less side effects like vomiting and hypotension, but more cutaneous reactions can occur [205]. Clinical and preclinical studies have shown equal efficacy of subcutaneous and intravenous administration of amifostine [197, 204, 205].

Another concern in the use of amifostine could be undesired tumour protective effects. Some preclinical studies showed tumour protection, whereas others did not [206]. A recently published meta-analysis of clinical studies did not confirm tumour protection by amifostine [207]. In the present study, non-tumour bearing rats were used; therefore it is unknown whether amifostine protects tumours in PRRT. In the near future, experiments studying this aspect will be performed in our laboratory.

CONCLUSION

Lysine and amifostine were successfully used as kidney protectors in high-dose PRRT using [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in rats. Amifostine may be an additional tool to optimise the therapeutic window of PRRT in patients.

CHAPTER 4.2

KIDNEY PROTECTION BY AN ANGIOTENSINE II BLOCKER PLUS LYSINE IN A RAT MODEL OF RADIATION NEPHROPATHY

Preliminary results

Edgar J. Rolleman, Roelf Valkema, E. P. Krenning, Bert F. Bernard, Magda Bijster, Marleen Melis, Flavio Forrer and Marion de Jong.

ABSTRACT

Background

Treatment with high doses of somatostatin analogues radiolabelled with beta-particle emitting radionuclides can result in radiation nephropathy due to reabsorption of the radiolabelled peptides in the proximal tubule. Prevention of renal damage will allow higher doses of radiolabelled somatostatin analogues to be administered to cancer patients to achieve a higher tumour absorbed dose. The renine-angiotensine-aldosterone system (RAAS) is believed to play a key role in the development of renal radiation damage. We investigated the renoprotective effects of the angiotensine converting enzyme (ACE) inhibitor captopril and the angiotensine II receptor blocker L158,809 in a rat model of radiation nephropathy after treatment with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

Methods

Male Lewis rats were injected with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. For renoprotection L158,809 (20 mg/L) or captopril (500 mg/L) was added to the drinking water. One group received the combination of L158,809 (20 mg/L) plus co-injection of lysine (400 mg/kg). Urinary protein was measured weekly. At 130 days (end of study) after [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate injection, rats were euthanized and a blood sample was obtained and kidney histology was performed.

Results

Fifty percent of the rats treated with captopril + [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate or L158,809 + [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate had to be euthanized before 130 days of treatment due to poor physical condition. In these groups proteinuria was not lower than in the rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone. Rises in serum creatinine at 130 days after [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate were not prevented by captopril (206 ± 47 μmol/L vs 162 ± 14 μmol/L). In contrast, L158,809 alone, or the combination of L158,809 plus lysine co-injection partially prevented the rise in serum creatinine (83 ± 32 μmol/L and 54 ± 19 μmol/L, respectively, both P<0.001 vs 555 MBq alone). All rats treated with L158,809, captopril or 555 MBq alone showed maximal renal damage scores of 4 based on histological examination. In contrast, the rats treated with the combination of L158,809 plus lysine had significantly lower histological damage scores (2.5 ± 1.5, P<0.001 vs 555 MBq alone).

Conclusion

Co-treatment with L158,809 or captopril worsened renal damage after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Adding lysine to L158,809 treatment, however, resulted in significant reductions in proteinuria, serum creatinine and histological damage scores. This is interesting since in previous studies, lysine co-injection could not reduce the histological damage after 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Therefore, combined efforts applying both amino acids and RAAS-inhibition might be a new tool to prevent renal damage in high dose peptide receptor radionuclide therapy.

INTRODUCTION

In peptide receptor radionuclide therapy (PRRT) using somatostatin analogues that are radiolabelled with beta-particle emitting radionuclides, the kidney delivered radiation dose due to reabsorption and renal retention of the radiolabelled compound is a major limiting factor for the maximum amount of radioactivity that can be administered to patients.

Kidney protection in PRRT is therefore warranted to prevent renal damage and to enable higher amounts of radioactivity to be administered to patients within safe limits for the kidneys. In the last decade positively charged amino acids have been used extensively to lower the renal radiation dose [77, 115, 116]. These amino acids reduce the reabsorption of the radiolabelled peptides in the proximal tubule. We previously showed in rats that this approach indeed results in less functional kidney damage [168, 208].

Another approach might be to allow high radiation doses to the kidneys and to prevent the development of subsequent cellular damage using drugs that interfere with the cascade of damage. In addition to an acute cytotoxic effect, radiation results in an ongoing state of oxidative stress that ultimately leads to interstitial fibrosis [64]. Interference with this oxidative stress is an attractive option. We previously showed that the radioprotective drug amifostine mitigated the effects of radiation after high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and decreased functional kidney damage [208].

Angiotensine II appears to play a key role in the promotion of renal damage. This is not only the case in diabetic and proteinuric nephropathies [68], but also in radiation nephropathy [55]. In external beam radiation therapy experiments in rats, renal failure could be prevented in about 70% of rats due to treatment with an ACE-inhibitor or an angiotensine 2 receptor blocker (ARB) [109]. In addition, Jaggi et al. showed that the aldosteron antagonistic drug spironolacton prevented the onset of renal damage after injection of an antibody radiolabelled with an alpha-particle emitting radionuclide in mice [113].

The aim of this study was to investigate if the use of drugs that interfere with the renine angiotensine aldosteron system (RAAS) can be used to prevent kidney dysfunction and histological damage in PRRT after high dose [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate.

MATERIALS AND METHODS

Radionuclides, peptides and chemicals

^{177}Lu was obtained from IDB (Baarle Nassau, The Netherlands). [DOTA^{0},Tyr^{3}]octreotate was supplied by BioSynthema (St Louis, MO, USA). [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate was synthesized and labelled as previously described [145]. The ^{99m}Tc-DMSA (Dimercaptosuccinic acid) kit was purchased from GE Healthcare (Roosendaal, the Netherlands) and labelled according to the indicated procedure.

Peptide radionuclide therapy experiments using [^{177}Lu-DOTA,Tyr^3]octreotate

All animal experiments were approved by the governing Animal Welfare Committee and conducted according to institutional regulations. Male Lewis rats (Harlan, Horst, The Netherlands; weight 300-350 g) were divided over 6 experimental groups.

Groups were as follows:

1. 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate (*n*=6)
2. 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate + L158,809 in drinking water (*n*=6)
3. 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate + captopril in drinking water (*n*=5)
4. 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate + lysine co-injection + L158,809 in drinking water (*n*=6)
5. no radioactivity, L158,809 in drinking water (*n*=3)
6. no radioactivity, captopril in drinking water (*n*=3)

Rats from group 1-4 were injected with 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate into the dorsal vein of the penis. For kidney protection, rats were treated with either an ARB (L158,809; 20 mg/L in drinking water) or an ACE-inhibitor (captopril; 500 mg/L in drinking water), both started 10 days before the radioactivity was administered, and continued during the experiment. Group 4, treated with 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate plus L158,809, was co-injected with lysine 400 mg/kg as well.

Recent dosimetry studies showed that administration of 555 MBq [^{177}Lu-DOTA^0,Tyr^3]octreotate caused a kidney cortex absorbed radiation dose of about 95 Gy [83], and produced profound renal damage [168]. Also, these doses resulted in excellent anti-tumour efficacy in rats [118].

Weekly, rats were weighed and a 24-h urine sample was obtained in metabolic cages from day 82 until day 130 days after treatment. Rats were euthanised on day 130 after therapy, or earlier if they had lost >10% of initial body weight or if they were in poor physical condition. A single photon emission computed tomography (SPECT) scan was performed using ^{99m}Tc-DMSA for evaluation of tubular function (see later) at 90 days p.i. and just prior to sacrifice. At sacrifice, a blood sample was taken by cardiac punction for determination of renal function. Kidneys were analysed histologically (see below).

L158,809 was kindly provided by Merck (J. Obenchain, New Jersey, USA). Captopril was purchased from BUFA Pharmaceutical Products, Uitgeest, The Netherlands. D-lysine was purchased from Sigma (St. Louis, MO, USA).

Measurement of tubular function with ^{99m}Tc-DMSA

For SPECT imaging the four-headed multiplexing multi-pinhole NanoSPECT (Bioscan Inc., Washington D.C., USA) was used, see [167] for description and validation of this system.

At 90 and 130 days after treatment with [^{177}Lu-DOTA,Tyr^{3}]octreotate, rats were injected with 50 MBq ^{99m}Tc-DMSA into the dorsal vein of the penis. Four hours post injection, a helical SPECT of the kidneys was acquired. The energy peak was set at 140 keV, the window width was ± 10%. An acquisition time of 30 seconds per view was chosen. Acquisition times ranged from 6-9 minutes per animal. Data were reconstructed iteratively with the HiSPECT® software, using a dedicated OSEM algorithm for multiplexing multi-pinhole reconstruction. Regions of interest were drawn manually around each kidney and the 3D activity distribution within the volume of interest (VOI) was then summed to determine the uptake in the kidneys. Quantification of the activity in the VOI was performed with the INTERVIEW XP® software.

Analytical procedures

Kidneys were fixed in 10% buffered formalin, trimmed and processed by standard techniques for embedding in paraffin. Four-micron sections were cut and stained with haematoxylin eosin (HE) and periodic acid-Schiff reagent (PAS). Microscopically, renal damage score (RDS) was graded from 0 (no damage) to 4 (severe damage) according to previously described criteria [168, 208].

Urinary protein was measured in 24-h urine samples with a commercially available colorimetric method (BioRad, Veenendaal, The Netherlands). Serum creatinine and blood urea nitrogen (BUN) were determined by standard hospital analysis procedures.

Statistical analysis

Data are expressed as mean ± SD. One- or two-way ANOVA followed by Tukey's test or Student's *t*-test was used to test significance of differences. *P*-values less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Rats from the groups that had not been treated with radioactivity, but only received L158,809 or captopril in the drinking water, had comparable levels of proteinuria, serum creatinine, serum BUN and renal damage score. The data of these two groups were thus merged and further assigned as a single control group.

From the group treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone, one rat had to be sacrificed because of poor physical condition at 123 days after injection. Its creatinine and BUN values were 608 umol/L and 133 mmol/L, respectively, indicating severe renal insufficiency.

From the group treated with L158,809 plus 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, one rat lost more than 10% of body weight and had to be sacrificed before the first ^{99m}Tc-DMSA scan.

Three others died shortly after the ^{99m}Tc-DMSA scan at 90 days. All were in poor physical condition. Serum creatinine and BUN in one of them were 111 µmol/L and 16 mmol/L, respectively. Only two rats of this group could be evaluated at 130 days after injection.

From the 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus captopril group, two rats were euthanized because of poor physical condition, just after the first ^{99m}Tc-DMSA scan. Serum creatinine and BUN values in these two rats were 109 and 112 µmol/L, and 21.7 and 26.1 mmol/L, respectively. Three rats could be evaluated at 130 days after injection.

None of the rats treated with 555 [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and lysine plus L158,809 as kidney protection had to be euthanized because of weight loss or poor physical condition.

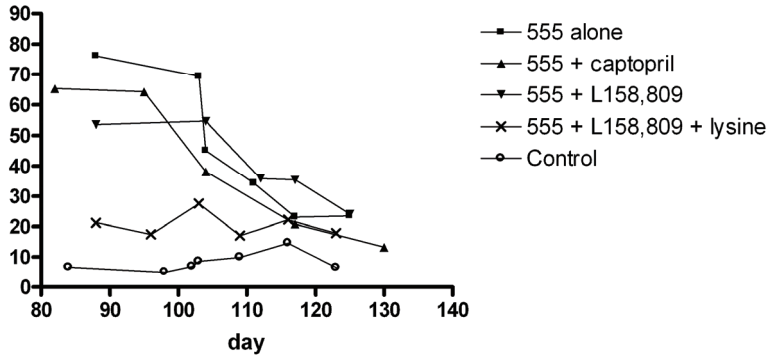


Figure 40. Proteinuria in indicated groups. For clarity SD are not shown. The data of 555 + L158,809 + LYS are significantly different from 555 MBq alone and not significantly different from control.

Figure 40 shows the mean 24-h urinary protein excretion values of the five experimental groups. Co-treatment with L158,809 or captopril did not significantly influence the proteinuria, whereas the combination of lysine plus L158,809 exerted a clearly lower level of proteinuria.

Figure 41 shows the serum creatinine values of rats that were euthanized at 130 days after injection of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Administration of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (*n*=5) resulted in a significant rise of the serum creatinine and BUN levels. This rise could not be prevented by co-treatment of captopril (*n*=3), but L158,809 (*n*=2) treatment significantly lowered serum creatinine and BUN values. The combination of lysine and L158,809 (*n*=6) further decreased the serum creatinine and BUN levels. Values in this group are not significantly different from values in the control group.

Figure 42 shows the ^{99m}Tc-DSMA uptake at 90 and 130 days. After 90 days, the ^{99m}Tc-DMSA in the group that received 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone was already substantially lower than in control rats. This decline was not significantly prevented by L158,809 or captopril (Figure 42a). However, the combination of L158,809 plus lysine significantly prevented the decrease of ^{99m}Tc-DMSA uptake.

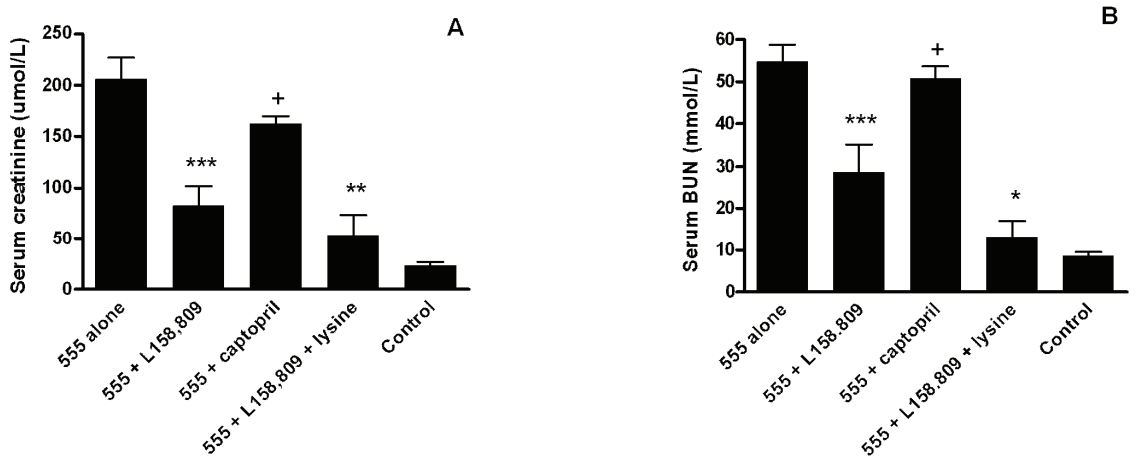


Figure 41. Serum creatinine (A) and BUN (B) values of rats 130 days after start of treatment with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with or without kidney protection.
(***) P<0.001 vs control; (**) P<0.001 vs 555 control, NS vs 555 L158,809 and NS vs control; (+) NS vs 555 control; (*) P<0.001 vs 555 control, P<0.05 vs 555 L158,809 and NS vs control.

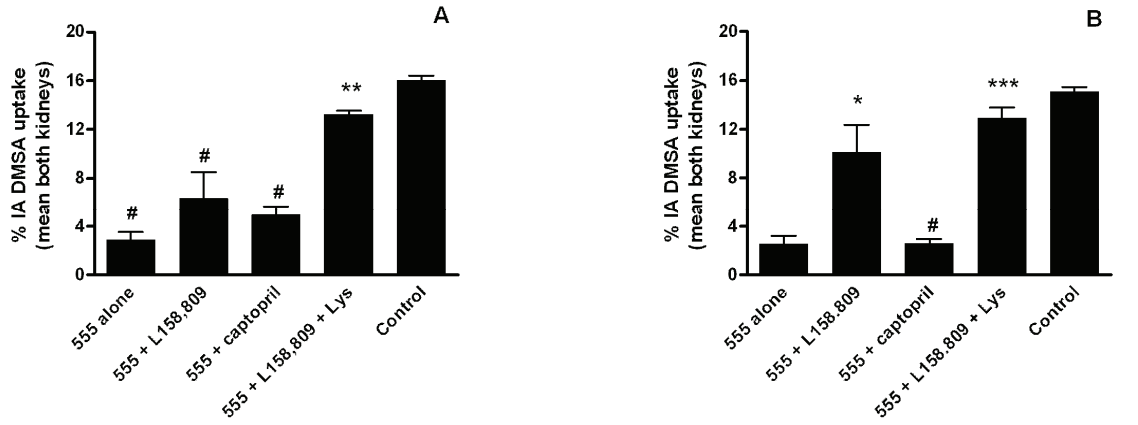


Figure 42. ^{99m}Tc-DMSA uptake expressed as percentage of the injected activity (%IA) at 90 days (A) and 130 days (B) after the start of therapy. (#) NS vs 555 alone; (*) P<0.001 vs 555 alone; (**) P<0.001 vs 555 alone, P<0.001 vs 555 + L158,809 and NS vs control; (***) P<0.001 vs 555 alone, NS vs 555 + L158,809 and vs control.

At 130 days (Figure 42b), when some rats already had died due to poor physical condition, the mean ^{99m}Tc-DMSA uptake was significantly higher in the L158,809 group than in the group that was treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone. Again, captopril did not prevent the decreased ^{99m}Tc-DMSA uptake. The rats in the L158,809 plus lysine group had a significantly higher ^{99m}Tc-DMSA uptake than rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone.

In Table 14 the histological findings are shown. Kidneys from rats treated with 555 MBq of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and those that received L158,809 and captopril as kidney protection all showed the maximum renal damage score, grade 4.

In contrast, the rats protected with the combination of L158,809 plus lysine had a significantly lower renal damage score.

Table 14. Renal damage scores of the four experimental groups.

Group	Renal damage score	N	
555 MBq alone	4.0 ± 0.0	6	
555 MBq + L158,809	4.0 ± 0.0	5	
555 MBq + Captopril	4.0 ± 0.0	5	
555 MBq + L158,809 + Lysine	2.5 ± 1.5	6	$P<0.001$

DISCUSSION

Radiation nephropathy is a risk in the treatment with high activities of radiolabelled somatostatin analogues and therefore the kidney absorbed radiation dose, caused by renal uptake of these radiolabelled peptides, is a dose-limiting factor. As it is the aim to administer the highest possible dose to the tumour without renal toxicity, kidney protection is warranted.

The first step in the development of renal damage (Figure 43) is the renal uptake and retention of the radiolabelled somatostatin analogues via a megalin-dependent mechanism [41, 42]. Consequently, kidney cells are irradiated. This irradiation causes chemical reactions and the generation of free radical species resulting in vascular and tissue damage. Fibroblasts transform to myofibroblasts under influence of growth factors and cytokines. Collagen and other extra-cellular matrix proteins are formed, ultimately leading to fibrosis (Figure 43).

Nowadays, positively charged amino acids are routinely used to partially block the renal uptake of radiolabelled peptides [76, 77, 116]. Recently, we showed that the radioprotective drug amifostine reduced functional kidney damage in rats after high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate [208]. Amifostine is a free radical scavenger and one of its metabolites has some effects on DNA repair [106].

From animal models of radiation nephropathy induced by external beam radiation therapy evidence has been reported that angiotensine II plays a key role in promoting the histopathologic changes in the kidney, leading to fibrosis [55]. Infusion of angiotensine II accelerated the onset of radiation induced damage and changes were more severe [212]. Although tissue and blood levels of angiotensine II are reported not to be elevated in radiation nephropathy [69, 213], treatment with an angiotensine II receptor blocker, and to a lesser extent an ACE-inhibitor, resulted in prevention [109] and successful treatment of radiation nephropathy [214]. Angiotensine II is known to promote vasoconstriction by inactivating the vasodilator nitric oxide. It also stimulates free radical generation [68].

Convincing and accumulating evidence increasingly indicates that angiotensine II and other members of the RAAS activate the TGF-β (transforming growth factor β, a profibrogenic factor) axis in the kidney by both direct and indirect mechanisms [211] and treatment with angiotensine II receptor blockers eliminates the rise in TGF-β [67].

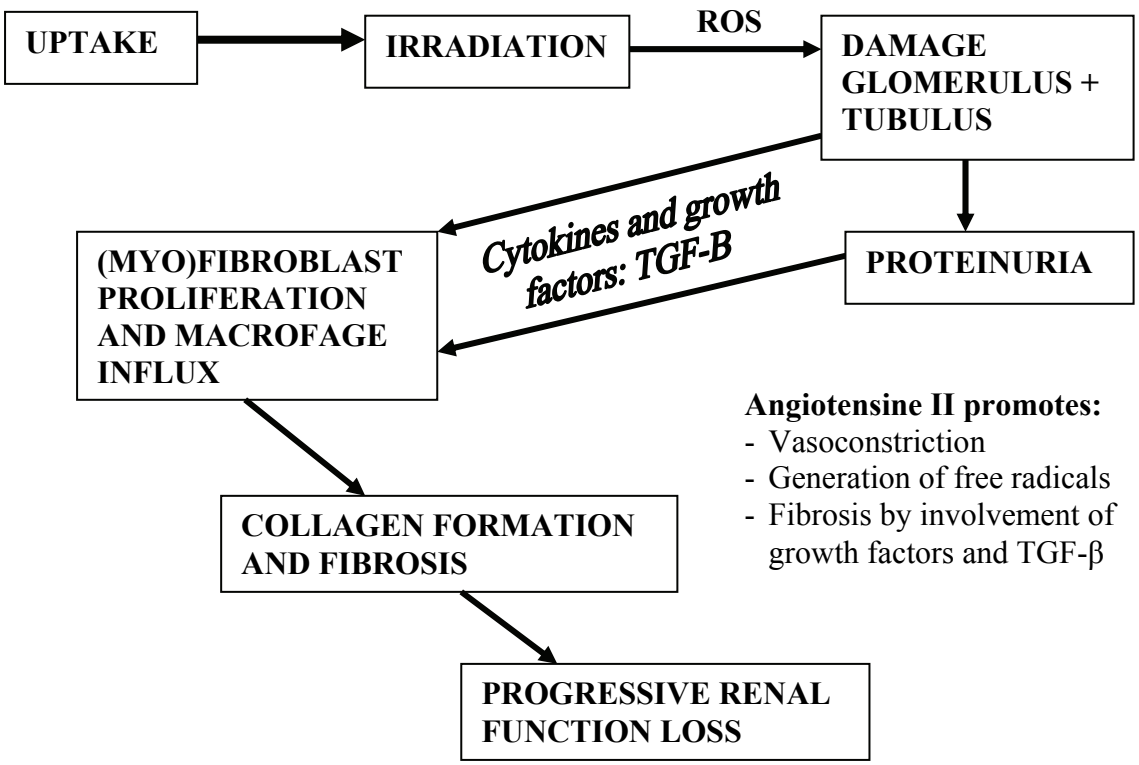


Figure 43. Simplified schedule of mechanisms that lead to radiation induced kidney damage in PRRT. Adapted from [55, 68, 209-211].

In this study we tested the protective effects of ACE-inhibition and angiotensine II receptor blockade on the kidney in a rat model of PRRT. ACE-inhibitors inhibit the angiotensine converting enzyme (ACE) that transforms angiotensine I to angiotensine II, and will thus lead to lowered blood and tissue angiotensine II concentrations. Blocking of the angiotensine II receptor with an angiotensine II receptor blocker (ARB) will result in less harmful effects of angiotensine II. Although aiming at kidney protection, about 50% of the rats in both the captopril and L158,809 group had to be euthanized prematurely due to poor physical condition, which is greater than expected from our previous experience and also higher than the current group treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone. Clinically, no indications of infections were present. Dosage schedules were similar to previously published work [109]. The rats that were treated with the drugs but did not receive the radioactivity remained healthy throughout the study, indicating that a problem with the drinking water or the administration

of the drugs was not likely. However, all rats that had to be killed prematurely had significant renal damage, as evidenced by a grade 4 histological damage score.

Our findings are similar to the findings of Jaggi et al. [113] who recently showed that the renal damage caused by an alpha-emitter labelled antibody was exacerbated by treatment with the ACE-inhibitor captopril. In their study, treatment with L158,809 had a moderate renoprotective effect [113], and the aldosterone antagonist spironolacton had significant renoprotective effects, as shown by both lower creatinine and BUN values and by lower histological damage scores.

Three mechanisms might explain the findings of Jaggi et al. [113] and our present findings.

1. During long-term ACE-inhibitor therapy, a compensatory increase in intrarenal production of angiotensine II by non-ACE-dependent pathways can occur in a diseased kidney [215].
2. Treatment with an ACE-inhibitor and angiotensine II receptor blockers causes aldosteron levels initially to decline, but after prolonged treatment aldosterone levels return to or might even exceed baseline values, the so called 'aldosterone escape' phenomenon [215-217]. Aldosterone promotes renal tissue remodelling by mechanisms that are independent of effects on blood pressure or salt homeostasis. It induces expression of growth factors and endothelin-1 [218-220]. Endothelin-1 is believed to play a key role in extracellular matrix deposition and mitotic cell death of renal tubule cells [221, 222].
3. A lowered renal and intra-glomerular perfusion pressure caused by both ACE-inhibitors and angiotensine II receptor blockers might further enhance and sustain renal damage.

Another possible cause of the poor outcome in the captopril and L158,809 groups could be higher initial kidney uptake of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate caused by these drugs. This was not tested, however.

In contrast to the rats treated with radioactivity plus captopril or L158,809, the group of rats treated with radioactivity plus lysine and L158,809 lived until the end of the study and remained in good health. In these rats, the kidney absorbed dose was lowered by 40% by lysine and they had clearly better renal function, lower amounts of proteinuria and lower histological damage scores as compared to rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. This is interesting, since we recently reported that lysine co-injection could reduce the functional but **not** the histological damage exerted by 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate [168, 208]. The present findings suggest that two methods that work

at different points in the cascade can be additive to one another: neither lysine alone, nor L158,809 alone could reduce the renal histological damage after injection of 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, but the combination is capable to reduce the histological damage. This might provide a basis for clinical use of a combined strategy: lowering radioactivity uptake by lysine, and prevention of the damage cascade by RAAS inhibition. In addition, it would be very interesting to test the effects of aldosterone antagonists in PRRT setting, referring to the data of Jaggi et al. [113] and to the growing insight that aldosterone blockade is an effective strategy for abrogating progressive renal disease [220].

CONCLUSION

In this rat study, co-treatment with the ACE-inhibitor captopril worsened renal damage caused by 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, as shown by early loss of health and early histological damage. Rats treated with the angiotensine II receptor blocker L158,809 lost clinical condition as well, although creatinine values were lower than the group treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone. Histologically, L158,809 alone did not result in renoprotection. Combining lysine and L158,809 resulted in lowered amounts of proteinuria, lowered serum creatinine and BUN values and significantly less histological damage

CHAPTER 5

DIFFERENT ASPECTS OF RENAL PROTECTION IN PRRT

CHAPTER 5.1

CUBULIN AND MEGALIN IN RADIATION-INDUCED RENAL INJURY WITH LABELLED SOMATOSTATIN ANALOGUES: ARE WE JUST DEALING WITH THE KIDNEY?

Edgar J. Rolleman, Roelf Valkema, Marleen Melis, Eric P. Krenning, Theo J. Visser and Marion de Jong

Eur J Nucl Med Mol Imaging 2006; 33:749-750

Dear Sir,

We read with great interest the editorial commentary by Dr. Moncayo [223] accompanying our article on the localisation and mechanism of renal retention of radiolabelled somatostatin analogues [39]. This editorial commentary embeds the field of peptide receptor radionuclide therapy (PRRT) in a somewhat greater biological perspective, for which we are thankful. However, we would like to comment on three important issues in this editorial.

First, Dr. Moncayo suggests that intervention with the cubilin/megalin system -in order to protect kidney tissue in PRRT- may have serious implications for other essential biological processes. In PRRT with radiolabelled somatostatin analogues, the kidneys are the major dose-limiting organ with respect to the amount of radioactivity that can be administered to the patient. Radiolabelled somatostatin analogues are largely excreted in the urine. A small amount is taken up and retained in the proximal tubule, causing a high radiation burden to the kidney. In PRRT, the kidneys are critical organs and the renal damage caused by excessively high radiation doses is irreversible and has serious consequences for patients. Therefore, it is important to find new methods for kidney protection and to improve the old ones, with the ultimate aim of increasing the radiation dose to the tumours at safe levels for the kidneys and other organs.

Recent data indicate that renal radioactivity uptake is mainly due to a megalin-dependent pathway as shown in studies with opossum kidney cells [41], in immunohistological studies [39] and in experiments with kidney-specific megalin-deficient mice [42]. Megalin is therefore an attractive target to reduce renal accumulation of radiolabelled somatostatin analogues. We do not think that interventions on the cubilin/megalin system, aimed to protect the kidneys in PRRT, will cause serious problems in other organs, in particular the thyroid axis. We base this opinion on the following three arguments:

1. After injection of radioactivity, there is only a short period of time with a high rate of renal uptake of the radioactive peptides. This implies that only a short lasting (4 to 10 hours), reversible, blockade of the reabsorption process is necessary. Such a short duration of megalin intervention unlikely causes major problems elsewhere in the body.
2. In hundreds of patients, we have routinely administered a combination of lysine and arginine over a limited period, starting 30 minutes before and continuing 3.5 hours after infusion of the radioactivity [116]. In these patients no alterations in thyroid hormone levels could be detected.
3. The kidney is the organ with the highest megalin expression [224].

The second issue raised by Dr. Moncayo is that of the patient's anti-oxidant status in view of secondary (undesired) radiation injury. Dr. Moncayo recommends including this factor when planning PRRT or other therapy. We do agree that it is important to consider this factor, but presently we would not recommend prescribing anti-oxidants or free radical scavengers in PRRT, before taking into account the pharmacologic actions of such a compound. If an anti-oxidant or radical scavenger is equally taken up in all tissues, including the tumour, one might expect undesired tumour protection by this compound. So, candidate free radical scavengers must be selected that have a differential uptake/metabolism pattern: no uptake in the tumour, and high uptake in healthy tissues, e.g. the kidneys. Amifostine [225] is such a compound, which is already applied in clinical practice [207]. We recently initiated studies to evaluate the renoprotective effects of amifostine in high-dose PRRT in rats and the first results of these studies are currently analysed.

Finally, we would like to comment on the selenium (Se) issue mentioned by Dr. Moncayo. He suggests that the high expression of somatostatin receptors on tumours might be a reflection of Se deficiency. However, currently there is very little published evidence to support this theory. Expression of receptors for all kinds of growth factors and inhibitory signals on tumours represent a complex process. It is unlikely that Se deficiency alone would be responsible for the somatostatin receptor status on the tumour cell surface. Dr. Moncayo cites his own publication in which six patients with latent hypothyroidism are cured after Se supplementation [226]. In the original publication, the autoimmune serologic values from only one patient are mentioned. Therefore, we question Dr. Moncayo's view that cure of these hypothyroid patients was caused by a better immune system function due to selenium supplementation.

CHAPTER 5.2

INHIBITION OF KIDNEY UPTAKE OF RADIOLABELLED SOMATOSTATIN ANALOGUES: AMINO ACIDS OR GELOFUSINE?

Edgar J. Rolleman, Marion de Jong, Roelf Valkema, Dik Kwekkeboom, Boen Kam and Eric P. Krenning.

J Nucl Med 2006; 47:1730-1731

TO THE EDITOR: With great interest we read the papers by Van Eerd et al. [100] and Vegt et al. [103] on the effects of the succinylated gelatin plasma expander Gelofusine (B. Braun Medical) on renal uptake of [^{111}In-DTPA^{0}]octreotide. We congratulate the authors on their innovative work, as we believe this is fundamental and interesting research.

The authors reported that Gelofusine significantly inhibited kidney uptake of [^{111}In-DTPA^{0}]octreotide to a level comparable to the level of inhibition by currently applied amino acid solutions. This further expands on the previous clinical observation that Gelofusine infusion results in tubular proteinuria [101, 102, 165] of both albumin and β_2-microglobulin. Although the mechanism for this proteinuria is not completely understood, involvement of the megalin receptor system is likely, since both β_2-microglobuline and albumin are ligands for this receptor. The megalin system was recently shown to be essential for kidney uptake of radiolabelled somatostatin analogues [42], making interventions at the megalin level interesting potential targets for renal protection during peptide receptor radionuclide therapy (PRRT). The new findings of the group in Nijmegen [100, 103] offer an additional way for further research this subject.

We would like to comment on some conclusions and statements brought forward in the two papers:

On the basis of several reports, the authors state that amino acid infusion for kidney protection may have several side effects like vomiting and potentially fatal hyperkalemia. We previously reported on the safety and side effects of different amino acid solutions [116]. On the basis of that study, we now use a combination of 25 g of L-lysine and 25 g of L-arginine, dissolved in 1 L (LysArg), as a standard 4-h infusion protocol for kidney protection during PRRT. During infusion with LysArg, the highest serum potassium level measured was 6.0 mmol/L in one out of eleven patients. No electrocardiography changes were seen. Vomiting occurred in 1 patient, but this was not drug related [116]. We then concluded that this LysArg solution was safe enough to be used as the standard procedure in our PRRT protocols. In the years following this publication, we have infused the LysArg solution more than 2,000 times in the PRRT setting. In our clinical setting, we have not encountered severe side effects, underlining the good toxicity profile for LysArg. In particular, no symptoms of volume overload have occurred in patients and no drug-related emergencies and fatalities have been registered. Vomiting occurs in about 15%, nausea in about 30% of patients [114], but it should be noted that vomiting may be caused in part by other factors, as we reported vomiting in at least 6% of patients who did not receive an amino acid infusion [116].

The authors stated that lysine itself may produce renal failure, and they cited wo studies that indeed showing that administration of lysine produced significant renal impairment [227, 228]. However, the doses used in these animal studies were approximately 4-6 times higher than the dose of lysine used in our [115] and their [100] animal experiments (400 mg/kg). To our knowledge, no studies have been published that describe toxicity from lysine in our dose range or in human subjects.

Most publications on plasma expanders deal with administration to critically ill patients - for instance in septic shock, in hemorrhagic shock, or after surgery. Little is known, however, about infusion of plasma expanders in healthy subjects and patients with a normal circulation. The authors report that infusion of Gelofusine volumes did not cause side effects in five healthy volunteers; it was not stated, however, which parameters in addition to blood pressure and heart rate were investigated [103].

An important point is that the incidence of allergic reactions 12-fold higher for Gelofusine than for human albumin infusion [229], possibly because of the bovine origin of the gelatin molecules in the fluid. More than 40 reports have been published on anaphylactic reactions that were due to the use of gelatin-derived plasma expanders. Also, a cross reactivity exists between the different gelatin solutions [230]. A 0.038% frequency of severe reactions (shock, cardiac, and/or respiratory arrest) has been reported for gelatin solutions [231]. The incidence of all grades of allergic reactions is between 0.06 and 0.78% [231-233]. Although this incidence is low, any anaphylactic reaction in the PRRT setting is unwanted.

In conclusion, lowering the renal uptake of radiolabelled peptides, such as somatostatin analogues, using the plasma expander Gelofusine may be a promising method to protect the kidneys in PRRT. Although no side effects were noted in five healthy subjects, we must be aware that infusion of gelatin-based plasma expanders may cause side effects, such as anaphylactic reactions, in the target group of patients. Further validation studies on larger groups of healthy subjects and patients must be performed and compared with the current method using amino acid solutions to find out whether this new strategy is also safe and effective in the PRRT setting.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The discovery of somatostatin and the cloning and characterisation of its five receptor subtypes have led to many intriguing developments in clinical nuclear medicine. It was found that somatostatin administration resulted in inhibition of hormonal overproduction syndromes [5], which are found in several neuro-endocrine tumours. In addition, these tumours were shown to exhibit a high expression of somatostatin receptors [8-10].

Somatostatin itself can not be used for treatment purposes because it is metabolised very rapidly [5]. Analogues were made, of which octreotide was the most important. This eight-amino acid peptide has a longer plasma half-life and is now used for treatment of neuro-endocrine tumour-related hormonal overproduction syndromes [5]. The next step was the development of specific targeting and visualisation of the somatostatin receptor on the tumour cell surface. Octreotide was radiolabelled with the gamma-emitter ^{111}In, using the attached chelator DTPA ([^{111}In-DTPA^{0}]octreotide, Octreoscan®). In 1994, Octreoscan® was approved for diagnostic use by the U.S. Federal Drug Administration in patients and it has become one of the most important imaging investigations in the initial identification and staging of gastroenteropancreatic neuro endocrine tumours [16-18, 234].

Patients with neuro-endocrine tumours have a number of therapeutic options (surgery, unlabelled somatostatin analogues, hepatic artery embolisation, ablation, chemotherapy and interferon-alpha), but these seldom result in cure [5, 235]. So, it was aimed to deliver therapeutic radioactivity to the tumour, adding a new therapeutic modality to the current available treatment options.

The first peptide receptor radionuclide therapy (PRRT) studies were performed at our department administering high doses of [^{111}In-DTPA^{0}]octreotide [28]. These studies showed encouraging results, but few objective responses [29-31]. Different groups, including our group, performed PRRT studies with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, that takes advantage of the beta-emitter ^{90}Y [34, 46, 84]. This radionuclide has a longer tissue penetration which results in considerable absorbed doses to cells that do not bear somatostatin receptors. Higher objective response rates were observed in studies with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide as compared to studies with [^{111}In-DTPA^{0}]octreotide. Recently, studies with another somatostatin analogue labelled with the beta-particle emitter ^{177}Lu ([^{177}Lu-DOTA^{0},Tyr^{3}]octreotate) were performed showing similar objective results as obtained in studies using [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [114, 171], including significant improvements in quality of life [38].

In addition to the highly specific uptake of radiolabelled somatostatin analogues in the somatostatin receptor-positive tumour cells, radioactivity is also taken up by the kidney. Although this is a small percentage [49], significant kidney absorbed radiation doses are the consequence, limiting the maximum administrable activity to patients and thus to the tumour

[49, 163]. Several reports have shown kidney toxicity, mostly after treatment with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34, 44, 45, 47, 49, 84]. The aim of the studies described in this thesis was to investigate methods that protect the kidneys in order to enlarge the total dose that can be administered to the patient within safe kidney radiation dose limits.

Chapter 2 describes rat studies in which high renal radiation doses (35 and 70 Gy) were applied by administration of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotide. These high doses resulted in proteinuria and elevation of serum creatinine and BUN levels later than 100 days after injection. Histological damage consisted merely of tubular damage: tubule dilatation, thickening of the basement membrane and protein cylinders in the tubule lumen. The extent of damage was dose-dependent. Fractionation of the total dose into two or three doses had significant beneficial effects on functional renal damage. Furthermore, three rats co-injected with the amino acid lysine had lower serum creatinine levels and a lower degree of proteinuria. The histological damage score was not significantly influenced by either fractionation or lysine co-injection.

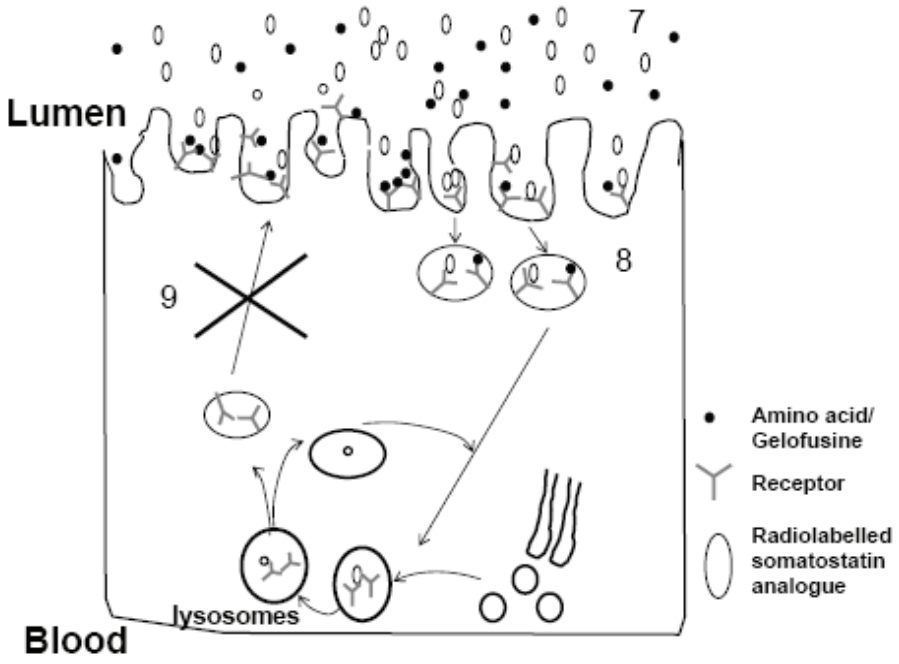


Figure 44. Schematic reproduction of two methods to reduce the uptake of radiolabelled somatostatin analogues in a proximal tubule cell. Adapted from Behr et al. [91]. Infused molecules of Gelofusine or positively charged amino acids are freely filtered and enter the tubular fluid (step 7). This results in megalin-mediated reabsorption of these two compounds, preventing reabsorption of freely filtered radiolabelled somatostatin analogues (step 8). Consequently, a lower amount of radiolabelled metabolites is to be retained in the lysozomes. Colchicine inhibits the return of the megalin-receptor to the cells surface (step 9).

One way to achieve kidney protection is to reduce the kidney uptake of radiolabelled somatostatin analogues. **Chapter 3** describes the different strategies that have been tested for this purpose. Uptake reduction by amino acids, colchicine and Gelofusine are schematically in Figure 44.

Chapter 3.1 reports studies on the effects of positively charged amino acids (lysine and arginine). Injection of lysine and arginine significantly reduced the kidney uptake of [^{111}In-DTPA^{0}]octreotide in rats. These amino acids are known to inhibit the proximal tubular reabsorption of peptides and low-molecular-weight proteins [95], indicating that this mechanism is important in the kidney uptake of somatostatin analogues. Also, the renal uptake of [^{111}In-DTPA^{0}]octreotide could significantly be inhibited by maleic acid in rats. This strategy is less applicable in humans as various reports suggest severe toxicity of this compound [130, 140].

In **Chapter 3.2** studies are described that were performed to test five different amino acid solutions in patients with neuro-endocrine tumours after injection with a diagnostic dose of [^{111}In-DTPA^{0}]octreotide. Infusion of a commercially available amino acid solution (1500 mL, containing 10.3 grams lysine and 16 grams of arginine) reduced the kidney uptake by 21%. Infusion of 25, 50 and 75 grams of lysine resulted in reductions of 17%, 15% and 44%, respectively. When 25 grams of lysine were combined with 25 grams of arginine, a reduction of 33% was achieved. Two signs of toxicity were noted in this study. Patients often suffered from nausea and vomiting, probably caused by the high osmolarity of the infusion fluids. In this study the frequency of nausea and vomiting was the highest (50% of patients) during infusion of the commercially available amino acid solution. Vomiting and nausea was less frequent when the other solutions were infused. The second sign of toxicity was the onset of hyperkalemia. This might be caused by an extracellularly directed shift of potassium secondary to an increased production of ketonic bodies in an acidic environment, as lysine is a ketogenic amino acid [153, 236, 237]. In our study, infusion of 75 grams of lysine resulted in elevated potassium concentrations (6.3, 6.7 and 6.8 mmol/L) in 3 out of 8 patients. The highest potassium concentration in the group infused with 25 grams of lysine + 25 grams of arginine was 6.0 mmol/L.

Reabsorption of different peptides and low-molecular-weight proteins in the proximal tubule is mediated by different carrier-molecules or receptors [238], of which the megalin/cubulin complex is the most important one [238, 239]. After binding of the ligand to the receptor, this complex is internalised and transported into the lysozomal apparatus [93].

There, the ligand-receptor bond is hydrolysed and the ligand is metabolised [93]. The receptor-molecule is transported back to the cell surface to be usable for a new reabsorption event [93]. The return of the receptor to the cell membrane is dependent on microtubule function as shown by several authors [98, 99, 157]. In **Chapter 3.3** we show that this process is also important in the kidney uptake of somatostatin analogues. Colchicine reduced the kidney [^{111}In-DTPA^{0}]octreotide uptake by about 62% in rats, and the combination of lysine plus colchicine was significantly more effective than lysine alone. This indicates that the kidney uptake of radiolabelled somatostatin analogues is dependent on proper microtubule function. The doses used in these studies were considerably high, resulting in liver toxicity. Before this drug may be introduced to kidney protection strategies in PRRT, two questions need to be addressed. First, is administration of safe human colchicine doses capable of reducing the renal radiolabelled somatostatin analogue uptake? Second, does colchicine influence tumour somatostatin receptor trafficking? Preliminary *in vitro* internalisation experiments with CA 20948 cells did not show effects of colchicine on the internalisation of [^{111}In-DTPA^{0}]octreotide, but this finding needs to be confirmed in additional studies, as well as in biodistribution-experiments in tumour-bearing rats.

Recently it was found that the plasma expander Gelofusine, that consists of succinylated bovine gelatin molecules, could inhibit kidney uptake of [^{111}In-DTPA^{0}]octreotide in rats and humans to a level comparable of that of lysine [100, 103]. In **Chapter 3.4** we describe rat studies on the effects of the combination of Gelofusine and lysine on the renal uptake of [^{111}In-DOTA^{0},Tyr^{3}]octreotate and [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Kidney uptake of these two radiolabelled somatostatin analogues was reduced by Gelofusine or lysine by about 40%. Combined co-injection of Gelofusine and lysine resulted in a significantly greater reduction of renal radioactivity uptake of about 65%. Clinical studies are warranted, however, before this combination can be implemented in routine kidney protection regimens during PRRT.

Several authors have reported expression of the somatostatin subtype 2 receptor in parts of the human kidney: on tubuli, glomeruli and blood vessels [104, 105]. This could imply that the kidney uptake of radiolabelled somatostatin analogues is in part mediated by human kidney sst_2 receptors. In **Chapter 3.5**, [^{111}In-DTPA^{0}]octreotide scans made during treatment with high doses of unlabelled octreotide were compared with scans made without this treatment, on an intra-patient basis. In 8 out of 10 patients, the kidney uptake of [^{111}In-DTPA^{0}]octreotide was significantly reduced, resulting in an overall reduction of 18% (P<0.01). Surprisingly, the tumour uptake in these patients was not significantly reduced while treated with unlabelled octreotide, but this was a retrospective study with long intervals between the two scans.

Therefore, the conclusion that unlabelled octreotide treatment might enlarge the therapeutic window of PRRT -by reducing kidney but not tumour uptake of radioactivity- is too preliminary and this should be evaluated in a controlled study. However, the results of this study indicate that the kidney somatostatin receptor subtype 2 plays a role in the total uptake of radiolabelled somatostatin analogues.

In addition to the methods to reduce the renal radioactivity uptake, reduction of the damaging effects of radiation can provide kidney protection in PRRT. In **Chapter 4** two such methods are described: the use of the radioprotective drug amifostine and the use of drugs that interfere with the action of angiotensine II.
Amifostine is a radioprotector used in external beam radiation therapy to protect the salivary glands when these organs are in the irradiation field [106]. Also, amifostine is used in cisplatin-based chemotherapy to reduce renal damage [240]. The drug is administered as a pro-drug to be dephosphorylized to WR-1065 [106]. This active metabolite is rapidly taken up into cells, exerting its protective action by radical scavenging and effects on DNA-repair [106]. The concentration in healthy tissues is about 100-fold higher than that in tumours, possibly by a lower alkaline phosphatase action in tumour tissue [240, 241]. In **Chapter 4.1** we studied the effects of amifostine and lysine on the kidney damage caused by injection of high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Amifostine and lysine treated rats had significantly lower amounts of proteinuria, body weight loss and serum levels of creatinine. However, the histological damage score was only lower in rats treated with 278 MBq plus amifostine and not in rats treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate plus amifostine. Recent findings in our laboratory suggest that amifostine does not protect the tumours.

From the literature it is well known that angiotensine II is a pro-inflammatory molecule [68] that drives the cascade of damage after irradiation [55]. We studied the effects of the angiotensine II receptor blocker L158,809 and the angiotensine converting-enzyme (ACE) inhibitor captopril in the model of radiation nephropathy evoked by high doses of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, described in **Chapter 4.2**. Both drugs will prevent angiotensine II action: via receptor antagonism or lower production, respectively. In this rat study, co-treatment with the ACE-inhibitor captopril worsened renal damage caused by 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate, as shown by early loss of health and early maximum renal damage scores. Rats treated with the angiotensine II receptor blocker L158,809 lost clinical condition as well, although creatinine values were lower than the group treated with 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate alone. L158,809 alone did not reduce the histological renal

damage. In contrast, the combination of lysine and L158,809 resulted in lowered amounts of proteinuria, lowered serum creatinine and BUN values and significantly less histological damage.

CONCLUSIONS

High doses of radiolabelled somatostatin analogues can produce late profound kidney damage: proteinuria, high serum creatinine and BUN serum concentrations and histologically damaged tubules, and to a lower extent damaged glomeruli.

The uptake of radiolabelled somatostatin analogues can successfully be reduced by positively charged amino acids like lysine and arginine. The combination of 25 grams of lysine and 25 grams of arginine is a safe and powerful method to be used in routine PRRT in patients. The side effects are moderate and mostly easy to handle. In our institution, we have not encountered any serious problem in thousands of routinely LysArg administrations in hundreds of patients. Some reports show renal damage after lysine administration in rats [227, 228, 242], whereas another shows beneficial effects of amino acid solutions in acute tubular necrosis [243]. The long term toxicity of amino acid solutions remains to be determined in humans.

Other strategies to reduce the renal uptake of radiolabelled somatostatin analogues comprise of Gelofusine and colchicine. The safety and effective dosage of colchicine in patients needs to be elucidated, as well. Furthermore, it has to be excluded that tumour radioactivity is reduced by colchicine treatment. The combination of lysine and Gelofusine seems very attractive and effective, clinical studies with this combination are advancing. Functional renal damage exerted by high doses of radiolabelled somatostatin analogues can be prevented by lysine and the cytoprotective drug amifostine. Interference with the renine-angiotensine-aldosterone system might be effective in the lower radiation dose range or in combination with lysine.

HOOFDSTUK 7

SAMENVATTING EN CONCLUSIES

De ontdekking van somatostatine en het klonen en karakteriseren van de vijf somatostatine receptoren heeft geleid tot fascinerende ontwikkelingen binnen de klinische (nucleaire) geneeskunde. Zo werd ontdekt dat toedienen van somatostatine resulteerde in remming van hormonale overproductie [5], die gezien wordt in het kader van diverse neuro-endocriene tumoren. Deze tumoren worden gekenmerkt door een verhoogde expressie van somatostatine receptoren [8-10].

Somatostatine zelf is ongeschikt voor klinisch gebruik, omdat het erg snel gemetaboliseerd wordt [5]. Er werden analoga gemaakt, waarvan octreotide de meest belangrijke is. Dit acht aminozuren bevattende peptide heeft een langere plasma halfwaardetijd en wordt nu gebruikt bij behandeling van hormonale overproductie-syndromen die gerelateerd zijn aan neuro-endocriene tumoren [5]. De volgende stap was het targetten en visualiseren van de somatostatine receptoren aan de oppervlakte van de tumorcellen. Octreotide werd gelabeld met de gamma-emitter ^{111}In via de chelator DTPA. [^{111}In-DTPA^{0}]octreotide (Octreoscan®) is in 1994 door de U.S. Federal Drug Administration goedgekeurd voor diagnostische doeleinden bij patiënten en is één van de meest belangrijke beeldvormende onderzoeken in de eerste identificatie en stagering van gastroenteropancreatische neuro-endocriene tumoren [16-18, 143, 234].

Patiënten met een neuro-endocriene tumor hebben een aantal behandelingsoptie: chirurgische resectie, ongelabelde somatostatine analoga, arteria hepatica embolisatie, Radio Frequency Ablation, chemotherapie en interferon-alfa, maar deze therapeutische opties resulteren zelden in genezing van de ziekte [5, 235]. Recent is een nieuwe therapievorm ontwikkeld die ernaar streeft om selectief therapeutische doses radioactiviteit op de tumor te richten.

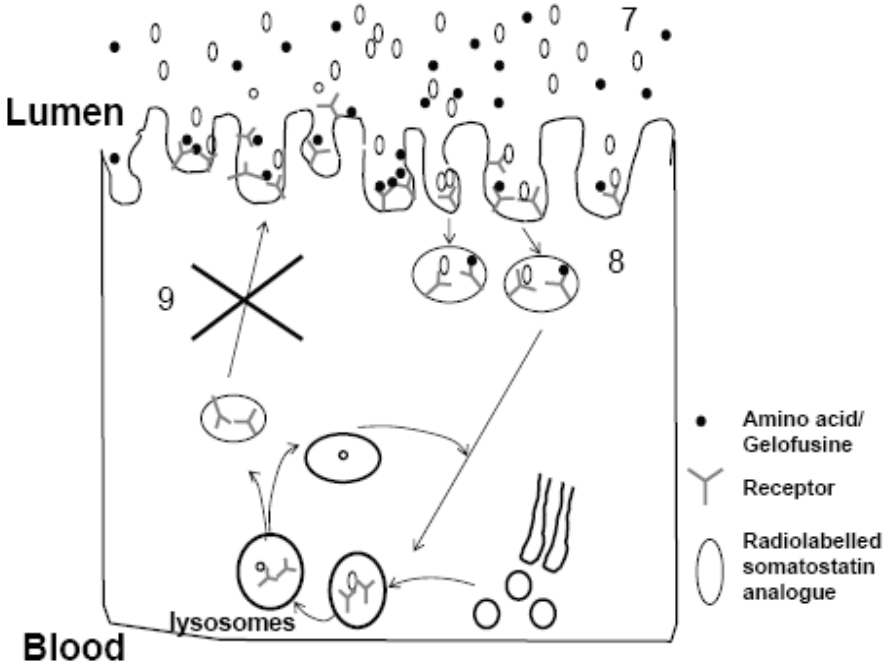
De eerste peptide receptor radionuclide therapie (PRRT) studies werden op onze afdeling uitgevoerd met hoge doses [^{111}In-DTPA^{0}]octreotide [28]. Deze studies lieten bemoedigende resultaten zien, hoewel slechts in een klein aantal patiënten een objectieve respons werd gemeten [29-31]. Diverse groepen, waaronder de onze, voerden daarna PRRT studies uit met [^{90}Y-DOTA^{0},Tyr^{3}]octreotide, daarbij profiterend van de beta-emitter ^{90}Y [34, 46, 84]. Dit radionuclide heeft een langere dracht waardoor omgevende cellen die geen somatostatine receptoren bezitten toch ook aanzienlijke stralingsdoses ontvangen. Betere objectieve respons is gemeld in studies met [^{90}Y-DOTA^{0},Tyr^{3}]octreotide in vergelijking tot [^{111}In-DTPA^{0}]octreotide. Recent zijn studies met een ander somatostatine analogon, dat gelabeld is met de beta-emitter ^{177}Lu ([^{177}Lu-DOTA^{0},Tyr^{3}]octreotate), uitgevoerd. Met dit gelabelde analogon werd een vergelijkbare objectieve respons verkregen als in studies met [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [114, 171], en ook werd een aanzienlijke verbetering in kwaliteit van het leven gemeld [38].

Naast de zeer specifieke opname van radioactief gelabelde somatostatine analoga in somatostatine receptor-positieve tumorcellen, wordt ook in de nieren radioactiviteit opgenomen. Hoewel dit een klein percentage is [49], resulteert dit in een aanzienlijke stralingsbelasting voor de nieren. De consequentie hiervan is dat de maximale activiteit die aan patiënten, en dus aan tumoren kan worden toegediend, gelimiteerd is [49, 163].
Diverse auteurs hebben nierschade na PRRT gemeld, meestal na behandeling met [^{90}Y-DOTA^{0},Tyr^{3}]octreotide [34, 44, 45, 47, 49, 84]. Het onderzoek dat in dit proefschrift is beschreven, had tot doel te zoeken naar methoden die de nieren beschermen zodat de totale hoeveelheid radioactiviteit die toegediend kan worden aan de patiënt op een veilige manier verhoogd kan worden.

Hoofdstuk 2 beschrijft studies met ratten die een hoge dosis nierbestraling (35 and 70 Gy) kregen door toediening van [^{177}Lu-DOTA^{0},Tyr^{3}]octreotide. Toediening van deze hoge doses resulteerde in proteïnurie en verhoogde creatinine en ureum waarden, later dan honderd dagen na injectie. De histologische schade was voornamelijk tubulair: tubulus dilatatie, verdikking van de membraan en eiwitcylinders in het tubulaire lumen. De omvang van de schade was sterk afhankelijk van de dosis die werd toegediend. Het verdelen van de totale dosis over twee of drie giften had een reducerend effect op functionele nierschade. Bovendien hadden drie ratten die met het aminozuur lysine waren geïnjecteerd, lagere creatinine waarden en minder proteïnurie. De histologische schade werd niet significant beïnvloed door het fractioneren van de dosis of door het toedienen van lysine.

Nierbescherming kan onder andere bereikt worden door de nieropname van de radioactief gelabelde somatostatine analoga te verminderen. **Hoofdstuk 3** beschrijft de verschillende strategieën die voor dit doeleinde getest zijn. Reductie van opname door aminozuren, colchicine en Gelofusine zijn schematisch weergegeven in Figuur 45.

Hoofdstuk 3.1 laat studies naar de effecten van positief geladen aminozuren (lysine en arginine) zien. Toediening van lysine en arginine verminderde de opname van [^{111}In-DTPA^{0}]octreotide in rattennieren aanzienlijk. Deze twee aminozuren staan bekend om het verhinderen van de proximale tubulaire reabsorptie van peptiden en laag-moleculaire eiwitten [95]. Dit suggereert dat dit mechanisme belangrijk is voor de opname van somatostatine analoga in de nieren. Ook kon de nieropname van [^{111}In-DTPA^{0}]octreotide aanzienlijk verminderd worden door maleaat. Eerder gepubliceerde data over ernstige toxiciteit van maleaat maken deze stof minder geschikt voor klinische toepassing [130, 140].



Figuur 45. Schematische weergave van twee methoden om de opname van radioactief gelabelde somatostatine analoga in een proximale tubulus cel te reduceren. Overgenomen van Behr et al. [91].
Gelofusine moleculen of positief geladen aminozuren die per infuus worden toegediend, worden glomerulair gefiltreerd en gaan de tubulaire vloeistof binnen (stap 7). Dit resulteert in megalin-gemedieerde reabsorptie van deze twee stoffen, hetgeen de reabsorptie van gefiltreerde radioactief gelabelde somatostatine analoga reduceert (stap 8). Hierdoor zal een kleinere hoeveelheid metabolieten achterblijven in de lysosomen. Colchicine verhindert de terugkeer van de megalin-receptor naar het celoppervlak (stap 9).

Hoofdstuk 3.2 beschrijft studies waarin het effect op de nieropname van een diagnostische hoeveelheid [^{111}In-DTPA^{0}]octreotide door vijf verschillende aminozuuroplossingen werd getest in patiënten met neuro-endocriene tumoren. Infusie van een in de handel verkrijgbare aminozuur oplossing (1500 mL bevat 10,3 gram lysine en 16 gram arginine) verminderde de nieropname met 21%. Infusie met 25, 50 en 75 gram lysine resulteerde in opnamereducties van respectievelijk 17%, 15% en 44%. Wanneer 25 gram lysine gecombineerd werd met 25 gram arginine, werd een reductie van 33% behaald. Binnen deze studie werden twee tekenen van toxiciteit gezien. Patiënten hadden soms last van misselijkheid en braken, waarschijnlijk veroorzaakt door de hoge osmolariteit van de infuusvloeistoffen. De frequentie van misselijkheid en braken was het hoogst (50% van de patiënten) gedurende de infusie van de commercieel verkrijgbare aminozuuroplossing. De frequentie was lager bij infusie van de andere oplossingen. Het tweede teken van toxiciteit was het ontstaan van hyperkaliëmie. Het ontstaan van hyperkaliëmie kan verklaard worden door verplaatsing van kalium naar buiten de cel, als respons op een toename in productie van ketonlichamen in een zure omgeving,

omdat lysine een ketogeen aminozuur is [153, 236, 237]. In onze studie resulteerde een infusie met 75 gram lysine in een verhoogde kaliumconcentratie (6.3, 6.7 en 6.8 mmol/L) in 3 van de 8 patiënten. In de groep die 25 gram lysine plus 25 gram arginine kreeg toegediend was de hoogste kaliumconcentratie 6.0 mmol/L.

Reabsorptie van verschillende peptiden en laag-moleculaire eiwitten in de proximale tubulus wordt mogelijk gemaakt door verschillende carrier-moleculen of receptoren [238] waarvan het megalin/cubulin complex de meest belangrijke is [238, 239]. Nadat het ligand aan de receptor gebonden is, wordt dit complex geïnternaliseerd en getransporteerd naar het lysozomale apparaat [93]. Na hydrolyse wordt de receptor teruggetransporteerd naar het celoppervlak voor een nieuwe reabsorptie-cyclus [93]. De terugkeer van de receptor naar de celmembraan is afhankelijk van de microtubulus functie zoals beschreven door diverse auteurs [98, 99, 157]. In **hoofdstuk 3.3** tonen we aan dat dit proces ook belangrijk is voor de nieropname van somatostatine analoga. Colchicine remde de nieropname van [^{111}In-DTPA^{0}]octreotide met ongeveer 62% in ratten. De combinatie van lysine en colchicine was aanzienlijk effectiever dan lysine alleen. Dit toont aan dat de nieropname van radioactief gelabeld somatostatine analoga afhankelijk is van goed functionerende microtubuli. De in deze studie gebruikte doses waren behoorlijk hoog met levertoxiciteit tot gevolg. Voordat colchicine geïntroduceerd kan worden als nierbeschermend bij PRRT moeten twee vragen worden beantwoord. Ten eerste: is een veilige dosis colchicine bij mensen in staat om de nieropname van somatostatine analoga te verminderen? Ten tweede: beïnvloedt colchicine het intracellulaire transport van de somatostatine receptor in de tumorcellen? Bij recente *in vitro* internalisatie experimenten met CA 20948 werd geen effect van colchicine op de internalisatie van [^{111}In-DTPA^{0}]octreotide gezien, maar deze bevindingen moeten nader worden bevestigd in aanvullende experimenten en in biodistributie-experimenten in tumordragende ratten.

Recent is ontdekt dat de colloïdale vloeistof Gelofusine, dat bestaat uit gesuccinyleerde runder gelatine moleculen, de nieropname van [^{111}In-DTPA^{0}]octreotide in ratten en mensen kan remmen tot een niveau dat vergelijkbaar is met dat door lysine [100, 103].
In **hoofdstuk 3.4** beschrijven we de effecten die de combinatie van Gelofusine en lysine heeft op de nieropname van [^{111}In-DOTA^{0},Tyr^{3}]octreotaat en [^{177}Lu-DOTA^{0},Tyr^{3}]octreotaat in rattenstudies. De nieropname van deze twee radioactief gelabelde somatostatine analoga werd gereduceerd door Gelofusine of lysine met ongeveer 40%. Gecombineerde toediening van Gelofusine en lysine remde de nieropname van radioactiviteit significant beter (ongeveer

65%). Klinische studies zijn nodig voordat deze combinatie geïmplementeerd kan worden in de routine nierbescherming gedurende PRRT.

Diverse auteurs hebben beschreven dat de somatostatine subtype-2 receptor op sommige onderdelen van de humane nier tot expressie wordt gebracht: tubuli, glomeruli en bloedvaten [104, 105]. Dit zou kunnen betekenen dat de opname van radioactief gelabelde somatostatine analoga in nieren van mensen gedeeltelijk gemedieerd wordt door sst_2 receptoren. In **hoofdstuk 3.5** zijn [^{111}In-DTPA^0]octreotide scans van 10 patiënten, die zijn gemaakt tijdens behandeling met hoge doses ongelabeld (koud) octreotide, vergeleken met de scans die bij dezelfde patiënten werden gemaakt zonder deze behandeling. Bij 8 van de 10 patiënten was de opname van [^{111}In-DTPA^0]octreotide in de nieren duidelijk gereduceerd, resulterend in een over-all reductie van 18% (P<0.01). Verrassend genoeg was de opname in de tumor bij deze patiënten niet significant gereduceerd tijdens behandeling met koud octreotide. Dit betrof echter een retrospectieve studie met grote intervallen tussen de twee scans. Het is te voorbarig om de conclusie te trekken dat behandeling met koud octreotide het therapeutisch window van PRRT vergroot - door reductie van nieropname van radioactiviteit maar niet in de tumor – en dus zal dit verder geëvalueerd moeten worden in een gecontroleerde studie. De resultaten van deze studie geven wel aan dat de subtype 2 somatostatine receptoren een rol spelen bij de totale nieropname van radioactief gelabelde somatostatine analoga.

Naast methoden om de nieropname van radioactiviteit te verminderen, kan de reductie van de schadelijke effecten van bestraling ook nierbescherming tijdens PRRT bewerkstelligen. In **hoofdstuk 4** zijn twee van deze methoden beschreven: het gebruik van het stralingsbeschermende middel amifostine en het gebruik van het geneesmiddelen die de werking van angiotensine II belemmeren.
Amifostine is een stralingsbeschermer dat gebruikt wordt bij conventionele bestraling, o.a. om de speekselklieren te beschermen als deze organen binnen het bestralingsgebied vallen [106]. Amifostine wordt ook gebruikt bij cisplatin-bevattende chemotherapie om de beschadiging van de nieren te reduceren [240]. Het medicijn wordt toegepast als een pro-drug om gedefosforyleerd te worden tot WR-1065 [106]. Deze actieve metaboliet wordt snel opgenomen in de gezonde cellen en oefent ter plaatse zijn beschermende werking uit door het vangen van vrije zuurstof radicalen en door herstel van DNA [106]. De concentratie in gezond weefsel is ongeveer 100 keer groter dan in tumorweefsel en dit wordt mogelijk veroorzaakt door een lager alkalisch fosfatase gehalte in tumorweefsel [240, 241].
In **hoofdstuk 4.1** hebben we de effecten van amifostine en lysine op de nierschade door injectie van hoge doses [^{177}Lu-DOTA^0,Tyr^3]octreotaat bestudeerd.

Ratten die behandeld waren met amifostine of lysine hadden veel minder proteïnurie, minder gewichtsverlies en lagere serum creatinine concentraties. De histologische schade was echter alleen lager in ratten die behandeld waren met 278 MBq plus amifostine en niet in ratten die behandeld waren met 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotaat plus amifostine. Recente data suggereren dat amifostine de tumoren niet beschermt.

Uit de literatuur is bekend dat angiotensine II een pro-inflammatoir molecuul is [68] dat de cascade van schadelijke effecten na bestraling aanjaagt [55]. We bestudeerden het effect van de angiotensine II receptor blokker L158,809 en de angiotensine converting enzyme (ACE)-remmer captopril op het ontstaan van radiatie nefropathie door een hoge dosis van [^{177}Lu-DOTA^{0},Tyr^{3}]octreotaat in **hoofdstuk 4.2**. Beide middelen verhinderen de werking van angiotensine II via respectievelijk een lagere angiotensine II productie en receptor antagonisme. In deze rattenstudie verslechterde de door [^{177}Lu-DOTA^{0},Tyr^{3}]octreotaat veroorzaakte nierschade wanneer de ACE-remmer captopril werd gegeven. Dit werd zichtbaar in verslechtering van de gezondheidstoestand en het vroeg ontstaan van maximale nierschade-scores. De klinische toestand van ratten die behandeld werden met de angiotensine II receptor blokker L158, 809, verslechterde ook, maar de creatinine waarden waren lager dan bij de groep die alleen met 555 MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotaat behandeld waren. L158,809 alleen reduceerde de histologische nierschade niet. De combinatie van lysine en L158,809 verlaagde de proteïnurie, verbeterde de serum creatinine en ureum concentraties en verminderde de histologische schade.

Conclusies

Hoge doses radioactief gelabelde somatostatine analoga kunnen late en ernstige nierschade veroorzaken: proteïnurie, hoge serum creatinine en ureum concentraties en histologisch beschadiging van tubuli en in mindere mate van glomeruli.

De opname van radioactief gelabelde somatostatine analoga kan succesvol worden gereduceerd door positief geladen aminozuren zoals lysine en arginine. De combinatie van 25 gram lysine en 25 gram arginine is een veilige en krachtige methode, en wordt tegenwoordig routinematig gebruikt tijdens PRRT bij patiënten. De bijwerkingen zijn minimaal en in de meeste gevallen makkelijk te hanteren. In ons instituut hebben zich nog geen serieuze problemen voorgedaan bij ondertussen duizenden routine LysArg toedieningen bij honderden patiënten. Sommige publicaties laten nierschade na lysine toediening in ratten zien [227, 228, 242], terwijl een andere studie gunstige effecten van aminozuur oplossingen bij acute tubulusnecrose liet zien [243]. De toxiciteit van aminozuur oplossingen op de lange termijn zal verder moeten worden bepaald in mensen.

Andere strategieën om de opname van radioactief gelabelde somatostatine analoga te reduceren omvatten het gebruik van Gelofusine en colchicine. De veilige en effectieve dosis van colchicine bij patiënten moet nog nader worden bepaald. Tevens moet uitgesloten worden dat tumoropname van radioactiviteit wordt gereduceerd door colchicine. De behandeling met de combinatie van lysine en Gelofusine lijkt erg aantrekkelijk en effectief. Klinische studies hieromtrent worden momenteel uitgevoerd.

Functionele nierschade die veroorzaakt wordt door hoge doses radioactief gelabelde somatostatine analoga, kan worden voorkomen door lysine en het celbeschermende middel amifostine. Remming van het renine – angiotensine – aldosteron systeem kan effectief zijn bij lagere bestralingsdoses of in combinatie met lysine.

CHAPTER 8

REFERENCES

1. Teunissen JJ, Kwekkeboom DJ, de Jong M, Esser JP, Valkema R, Krenning EP. Endocrine tumours of the gastrointestinal tract. Peptide receptor radionuclide therapy. Best Pract Res Clin Gastroenterol 2005; 19:595-616.
2. Krenning EP, Bakker WH, Breeman WA, Koper JW, Kooij PP, Ausema L, Lameris JS, Reubi JC, Lamberts SW. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet 1989; 1:242-4.
3. Reubi JC, Krenning E, Lamberts SW, Kvols L. In vitro detection of somatostatin receptors in human tumors. Digestion 1993; 54:76-83.
4. Reubi JC, Schar JC, Waser B, Wenger S, Heppeler A, Schmitt JS, Macke HR. Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. Eur J Nucl Med 2000; 27:273-82.
5. de Herder WW, Lamberts SW. Somatostatin and somatostatin analogues: diagnostic and therapeutic uses. Curr Opin Oncol 2002; 14:53-7.
6. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, Petcher TJ, Pless. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci 1982; 31:1133-40.
7. Lamberts SW, Oosterom R, Neufeld M, del Pozo E. The somatostatin analog SMS 201-995 induces long-acting inhibition of growth hormone secretion without rebound hypersecretion in acromegalic patients. J Clin Endocrinol Metab 1985; 60:1161-5.
8. Reubi JC, Lang W, Maurer R, Koper JW, Lamberts SW. Distribution and biochemical characterization of somatostatin receptors in tumors of the human central nervous system. Cancer Res 1987; 47:5758-64.
9. Reubi JC, Schaer JC, Markwalder R, Waser B, Horisberger U, Laissue J. Distribution of somatostatin receptors in normal and neoplastic human tissues: recent advances and potential relevance. Yale J Biol Med 1997; 70:471-9.
10. Reubi JC, Lamberts SW, Maurer R. Somatostatin receptors in normal and tumoral tissue. Horm Res 1988; 29:65-9.
11. Bruns C, Weckbecker G, Raulf F, Kaupmann K, Schoeffter P, Hoyer D, Lubbert H. Molecular pharmacology of somatostatin-receptor subtypes. Ann N Y Acad Sci 1994; 733:138-46.
12. Hoyer D, Bell GI, Berelowitz M, Epelbaum J, Feniuk W, Humphrey PP, O'Carroll AM, Patel YC, Schonbrunn A, Taylor JE, Reisine T. Classification and nomenclature of somatostatin receptors. Trends Pharmacol Sci 1995; 16:86-8.
13. Reisine T, Bell GI. Molecular biology of somatostatin receptors. Endocr Rev 1995; 16:427-42.
14. Bakker WH, Krenning EP, Breeman WA, Kooij PP, Reubi JC, Koper JW, de Jong M, Lameris JS, Visser TJ, Lamberts SW. In vivo use of a radioiodinated somatostatin analogue: dynamics, metabolism, and binding to somatostatin receptor-positive tumors in man. J Nucl Med 1991; 32:1184-9.
15. Bakker WH, Krenning EP, Reubi JC, Breeman WA, Setyono-Han B, de Jong M, Kooij PP, Bruns C, van Hagen PM, Marbach P, Visser TJ, Lamberts SWJ. In vivo application of [111In-DTPA-D-Phe1]-octreotide for detection of somatostatin receptor-positive tumors in rats. Life Sci 1991; 49:1593-601.
16. Krenning EP, Kwekkeboom DJ, Bakker WH, Breeman WA, Kooij PP, Oei HY, van Hagen M, Postema PT, de Jong M, Reubi JC, et al. Somatostatin receptor scintigraphy with [111In-DTPA-D-Phe1]- and [123I- Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients. Eur J Nucl Med 1993; 20:716-31.
17. Kwekkeboom DJ, Krenning EP. Somatostatin receptor imaging. Semin Nucl Med 2002; 32:84-91.
18. Gibril F, Jensen RT. Diagnostic uses of radiolabelled somatostatin receptor analogues in gastroenteropancreatic endocrine tumours. Dig Liver Dis 2004; 36 Suppl 1:S106-20.
19. Oberg K, Eriksson B. Nuclear medicine in the detection, staging and treatment of gastrointestinal carcinoid tumours. Best Pract Res Clin Endocrinol Metab 2005; 19:265-76.

20. Gabriel M, Decristoforo C, Maina T, Nock B, vonGuggenberg E, Cordopatis P, Moncayo R. 99mTc-N4-[Tyr3]Octreotate Versus 99mTc-EDDA/HYNIC-[Tyr3]Octreotide: an intrapatient comparison of two novel Technetium-99m labeled tracers for somatostatin receptor scintigraphy. Cancer Biother Radiopharm 2004; 19:73-9.
21. Decristoforo C, Maina T, Nock B, Gabriel M, Cordopatis P, Moncayo R. 99mTc-Demotate 1: first data in tumour patients-results of a pilot/phase I study. Eur J Nucl Med Mol Imaging 2003; 30:1211-9.
22. Hofmann M, Maecke H, Borner R, Weckesser E, Schoffski P, Oei L, Schumacher J, Henze M, Heppeler A, Meyer J, Knapp H. Biokinetics and imaging with the somatostatin receptor PET radioligand (68)Ga-DOTATOC: preliminary data. Eur J Nucl Med 2001; 28:1751-7.
23. Kowalski J, Henze M, Schuhmacher J, Macke HR, Hofmann M, Haberkorn U. Evaluation of positron emission tomography imaging using [68Ga]-DOTA-D Phe(1)-Tyr(3)-Octreotide in comparison to [111In]-DTPAOC SPECT. First results in patients with neuroendocrine tumors. Mol Imaging Biol 2003; 5:42-8.
24. Hofland LJ, van Koetsveld PM, Waaijers M, Lamberts SW. Internalisation of isotope-coupled somatostatin analogues. Digestion 1996; 57 Suppl 1:2-6.
25. De Jong M, Bernard BF, De Bruin E, Van Gameren A, Bakker WH, Visser TJ, Macke HR, Krenning EP. Internalization of radiolabelled [DTPA0]octreotide and [DOTA0,Tyr3]octreotide: peptides for somatostatin receptor-targeted scintigraphy and radionuclide therapy. Nucl Med Commun 1998; 19:283-8.
26. Andersson P, Forssell-Aronsson E, Johanson V, Wangberg B, Nilsson O, Fjalling M, Ahlman H. Internalization of indium-111 into human neuroendocrine tumor cells after incubation with indium-111-DTPA-D-Phe1-octreotide. J Nucl Med 1996; 37:2002-6.
27. Duncan JR, Stephenson MT, Wu HP, Anderson CJ. Indium-111-diethylenetriaminepentaacetic acid-octreotide is delivered in vivo to pancreatic, tumor cell, renal, and hepatocyte lysosomes. Cancer Res 1997; 57:659-71.
28. Krenning EP, Kooij PP, Bakker WH, Breeman WA, Postema PT, Kwekkeboom DJ, Oei HY, de Jong M, Visser TJ, Reijs AE, Lamberts SWJ. Radiotherapy with a radiolabeled somatostatin analogue, [111In-DTPA-D- Phe1]-octreotide. A case history. Ann N Y Acad Sci 1994; 733:496-506.
29. Valkema R, De Jong M, Bakker WH, Breeman WA, Kooij PP, Lugtenburg PJ, De Jong FH, Christiansen A, Kam BL, De Herder WW, Stridsberg M, Lindemans J, Ensing G, Krenning EP. Phase I study of peptide receptor radionuclide therapy with [In- DTPA]octreotide: the Rotterdam experience. Semin Nucl Med 2002; 32:110-22.
30. Anthony LB, Woltering EA, Espenan GD, Cronin MD, Maloney TJ, McCarthy KE. Indium-111-pentetreotide prolongs survival in gastroenteropancreatic malignancies. Semin Nucl Med 2002; 32:123-32.
31. Buscombe JR, Caplin ME, Hilson AJ. Long-term efficacy of high-activity 111in-pentetreotide therapy in patients with disseminated neuroendocrine tumors. J Nucl Med 2003; 44:1-6.
32. Waldherr C, Pless M, Maecke HR, Schumacher T, Crazzolara A, Nitzsche EU, Haldemann A, Mueller-Brand J. Tumor response and clinical benefit in neuroendocrine tumors after 7.4 GBq (90)Y-DOTATOC. J Nucl Med 2002; 43:610-6.
33. Valkema R, Pauwels S, Kvols LK, Barone R, Jamar F, Bakker WH, Kwekkeboom DJ, Bouterfa H, Krenning EP. Survival and response after peptide receptor radionuclide therapy with [90Y-DOTA0,Tyr3]octreotide in patients with advanced gastroenteropancreatic neuroendocrine tumors. Semin Nucl Med 2006; 36:147-56.
34. Otte A, Herrmann R, Heppeler A, Behe M, Jermann E, Powell P, Maecke HR, Muller J. Yttrium-90 DOTATOC: first clinical results. Eur J Nucl Med 1999; 26:1439-47.

35. Bodei L, Cremonesi M, Zoboli S, Grana C, Bartolomei M, Rocca P, Caracciolo M, Macke HR, Chinol M, Paganelli G. Receptor-mediated radionuclide therapy with 90Y-DOTATOC in association with amino acid infusion: a phase I study. Eur J Nucl Med Mol Imaging 2003; 30:207-16.
36. Bushnell D, O'Dorisio T, Menda Y, Carlisle T, Zehr P, Connolly M, Karwal M, Miller S, Parker S, Bouterfa H. Evaluating the clinical effectiveness of 90Y-SMT 487 in patients with neuroendocrine tumors. J Nucl Med 2003; 44:1556-60.
37. Kwekkeboom DJ, Mueller-Brand J, Paganelli G, Anthony LB, Pauwels S, Kvols LK, O'Dorisio T M, Valkema R, Bodei L, Chinol M, Maecke HR, Krenning EP. Overview of results of Peptide receptor radionuclide therapy with 3 radiolabeled somatostatin analogs. J Nucl Med 2005; 46:62S-6S.
38. Teunissen JJ, Kwekkeboom DJ, Krenning EP. Quality of life in patients with gastroenteropancreatic tumors treated with [177Lu-DOTA0,Tyr3]octreotate. J Clin Oncol 2004; 22:2724-9.
39. Melis M, Krenning EP, Bernard BF, Barone R, Visser TJ, de Jong M. Localisation and mechanism of renal retention of radiolabelled somatostatin analogues. Eur J Nucl Med Mol Imaging 2005; 32:1136-43.
40. De Jong M, Valkema R, Van Gameren A, Van Boven H, Bex A, Van De Weyer EP, Burggraaf JD, Korner M, Reubi JC, Krenning EP. Inhomogeneous localization of radioactivity in the human kidney after injection of [(111)In-DTPA]octreotide. J Nucl Med 2004; 45:1168-71.
41. Barone R, Van Der Smissen P, Devuyst O, Beaujean V, Pauwels S, Courtoy PJ, Jamar F. Endocytosis of the somatostatin analogue, octreotide, by the proximal tubule-derived opossum kidney (OK) cell line. Kidney Int 2005; 67:969-76.
42. De Jong M, Barone R, Krenning E, Bernard B, Melis M, Visser T, Gekle M, Willnow TE, Walrand S, Jamar F, Pauwels S. Megalin is essential for renal proximal tubule reabsorption of 111In-DTPA-octreotide. J Nucl Med 2005; 46:1696-700.
43. Stahl A, Lutz J, Wagner B, Essler M, Blechert B, Poethko T, Schwaiger M. Probenecid reduces renal uptake of 111In-DOTATOC in rats [abstract]. J Nucl Med 2006; 47:528P.
44. Cybulla M, Weiner SM, Otte A. End-stage renal disease after treatment with 90Y-DOTATOC. Eur J Nucl Med 2001; 28:1552-4.
45. Moll S, Nickeleit V, Mueller-Brand J, Brunner FP, Maecke HR, Mihatsch MJ. A new cause of renal thrombotic microangiopathy: yttrium 90-DOTATOC internal radiotherapy. Am J Kidney Dis 2001; 37:847-51.
46. Paganelli G, Zoboli S, Cremonesi M, Bodei L, Ferrari M, Grana C, Bartolomei M, Orsi F, De Cicco C, Macke HR, Chinol M, de Braud F. Receptor-mediated radiotherapy with 90Y-DOTA-D-Phe1-Tyr3-octreotide. Eur J Nucl Med 2001; 28:426-34.
47. Paganelli G, Zoboli S, Cremonesi M, Macke HR, Chinol M. Receptor-mediated radionuclide therapy with 90Y-DOTA-D-Phe1-Tyr3- Octreotide: preliminary report in cancer patients. Cancer Biother Radiopharm 1999; 14:477-83.
48. Stoffel MP, Pollok M, Fries J, Baldamus CA. Radiation nephropathy after radiotherapy in metastatic medullary thyroid carcinoma. Nephrol Dial Transplant 2001; 16:1082-3.
49. Barone R, Borson-Chazot F, Valkema R, Walrand S, Chauvin F, Gogou L, Kvols LK, Krenning EP, Jamar F, Pauwels S. Patient-specific dosimetry in predicting renal toxicity with 90Y-DOTATOC: Relevance of kidney volume and dose rate in finding a dose-effect relationship. J Nucl Med 2005; 46:99S-106S.
50. Lewis JS, Wang M, Laforest R, Wang F, Erion JL, Bugaj JE, Srinivasan A, Anderson CJ. Toxicity and dosimetry of (177)Lu-DOTA-Y3-octreotate in a rat model. Int J Cancer 2001; 94:873-7.
51. Luxton RW. Radiation nephritis. Q J Med 1953; 22:215-42.
52. Luxton RW. Radiation nephritis. A long-term study of 54 patients. Lancet 1961; 2:1221-4.
53. Luxton RW, Kunkler PB. Radiation Nephritis. Acta Radiol Ther Phys Biol 1964; 2:169-78.

54. Kunkler PB, Farr RF, Luxton RW. The limit of renal tolerance to x-rays; an investigation into renal damage occurring following the treatment of tumours of the testis by abdominal baths. Br J Radiol 1952; 25:192-201.
55. Cohen EP, Robbins ME. Radiation nephropathy. Semin Nephrol 2003; 23:486-99.
56. Cassady JR. Clinical radiation nephropathy. Int J Radiat Oncol Biol Phys 1995; 31:1249-56.
57. Avioli LV, Lazor MZ, Cotlove E, Brace KC, Andrews JR. Early effects of radiation on renal function in man. Am J Med 1963; 34:329-37.
58. Robbins ME, Bonsib SM. Radiation nephropathy: a review. Scanning Microsc 1995; 9:535-60.
59. Krochak RJ, Baker DG. Radiation nephritis. Clinical manifestations and pathophysiologic mechanisms. Urology 1986; 27:389-93.
60. Emery EW, Denekamp J, Ball MM, Field SB. Survival of mouse skin epithelial cells following single and divided doses of x-rays. Radiat Res 1970; 41:450-66.
61. Schmidt-Ullrich RK, Dent P, Grant S, Mikkelsen RB, Valerie K. Signal transduction and cellular radiation responses. Radiat Res 2000; 153:245-57.
62. Cohen EP, Bonsib SA, Whitehouse E, Hopewell JW, Robbins ME. Mediators and mechanisms of radiation nephropathy. Proc Soc Exp Biol Med 2000; 223:218-25.
63. Robbins ME, O'Malley Y, Zhao W, Davis CS, Bonsib SM. The role of the tubulointerstitium in radiation-induced renal fibrosis. Radiat Res 2001; 155:481-9.
64. Robbins ME, Zhao W, Davis CS, Toyokuni S, Bonsib SM. Radiation-induced kidney injury: a role for chronic oxidative stress? Micron 2002; 33:133-41.
65. Turi S, Nemeth I, Torkos A, Saghy L, Varga I, Matkovics B, Nagy J. Oxidative stress and antioxidant defense mechanism in glomerular diseases. Free Radic Biol Med 1997; 22:161-8.
66. Oikawa T, Freeman M, Lo W, Vaughan DE, Fogo A. Modulation of plasminogen activator inhibitor-1 in vivo: a new mechanism for the anti-fibrotic effect of renin-angiotensin inhibition. Kidney Int 1997; 51:164-72.
67. Datta PK, Moulder JE, Fish BL, Cohen EP, Lianos EA. TGF-beta 1 production in radiation nephropathy: role of angiotensin II. Int J Radiat Biol 1999; 75:473-9.
68. Das UN. Is angiotensin-II an endogenous pro-inflammatory molecule? Med Sci Monit 2005; 11:RA155-62.
69. Cohen EP, Fish BL, Moulder JE. The renin-angiotensin system in experimental radiation nephropathy. J Lab Clin Med 2002; 139:251-7.
70. Emami B, Lyman J, Brown A, Coia L, Goitein M, Munzenrider JE, Shank B, Solin LJ, Wesson M. Tolerance of normal tissue to therapeutic irradiation. Int J Radiat Oncol Biol Phys 1991; 21:109-22.
71. Boerman OC, Oyen WJ, Corstens FH. Between the Scylla and Charybdis of peptide radionuclide therapy: hitting the tumor and saving the kidney. Eur J Nucl Med 2001; 28:1447-9.
72. O'Donoghue J. Relevance of external beam dose-response relationships to kidney toxicity associated with radionuclide therapy. Cancer Biother Radiopharm 2004; 19:378-87.
73. Konijnenberg MW. Is the renal dosimetry for [90Y-DOTA0,Tyr3]octreotide accurate enough to predict thresholds for individual patients? Cancer Biother Radiopharm 2003; 18:619-25.
74. Pauwels S, Barone R, Walrand S, Borson-Chazot F, Valkema R, Kvols LK, Krenning EP, Jamar F. Practical Dosimetry of Peptide Receptor Radionuclide Therapy with 90Y-Labeled Somatostatin Analogs. J Nucl Med 2005; 46:92S-8S.
75. Forster GJ, Engelbach MJ, Brockmann JJ, Reber HJ, Buchholz HG, Macke HR, Rosch FR, Herzog HR, Bartenstein PR. Preliminary data on biodistribution and dosimetry for therapy planning of somatostatin receptor positive tumours: comparison of (86)Y-DOTATOC and (111)In-DTPA-octreotide. Eur J Nucl Med 2001; 28:1743-50.

76. Cremonesi M, Ferrari M, Bodei L, Tosi G, Paganelli G. Dosimetry in Peptide radionuclide receptor therapy: a review. J Nucl Med 2006; 47:1467-75.
77. Jamar F, Barone R, Mathieu I, Walrand S, Labar D, Carlier P, De Camps J, Schran H, Chen T, Smith MC, Bouterfa H, Valkema R, Krenning EP, Kvols LK, Pauwels S. 86Y-DOTA(0)- d-Phe(1)-Tyr(3)-octreotide (SMT487)-a phase 1 clinical study: pharmacokinetics, biodistribution and renal protective effect of different regimens of amino acid co-infusion. Eur J Nucl Med Mol Imaging 2003; 30:510-8.
78. Cremonesi M, Ferrari M, Zoboli S, Chinol M, Stabin MG, Orsi F, Maecke HR, Jermann E, Robertson C, Fiorenza M, Tosi G, Paganelli G. Biokinetics and dosimetry in patients administered with (111)In-DOTA- Tyr(3)-octreotide: implications for internal radiotherapy with (90)Y- DOTATOC. Eur J Nucl Med 1999; 26:877-86.
79. Stahl A, Schachoff S, Beer A, Winter A, Wester HJ, Scheidhauer K, Schwaiger M, Wolf I. [(111)In]DOTATOC as a dosimetric substitute for kidney dosimetry during [(90)Y]DOTATOC therapy: results and evaluation of a combined gamma camera/probe approach. Eur J Nucl Med Mol Imaging 2006; 33:1328-36.
80. Forrer F, Uusijarvi H, Waldherr C, Cremonesi M, Bernhardt P, Mueller-Brand J, Maecke HR. A comparison of (111)In-DOTATOC and (111)In-DOTATATE: biodistribution and dosimetry in the same patients with metastatic neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2004; 31:1257-62.
81. Helisch A, Forster GJ, Reber H, Buchholz HG, Arnold R, Goke B, Weber MM, Wiedenmann B, Pauwels S, Haus U, Bouterfa H, Bartenstein P. Pre-therapeutic dosimetry and biodistribution of 86Y-DOTA-Phe1-Tyr3-octreotide versus 111In-pentetreotide in patients with advanced neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2004; 31:1386-92.
82. Barone R, Jamar F, Walrand S, Labar D, Carlier P, Smith C, Kvols L, Krenning EP, Pauwels S. Can 111In-DTPA-octreotide (In-OC) predict kidney and tumor exposure during treatment with 90Y SMT487 (OctreoTher TM)? J Nucl Med 2000; 41:110P.
83. Konijnenberg MW, Bijster M, Krenning EP, De Jong M. A stylized computational model of the rat for organ dosimetry in support of preclinical evaluations of peptide receptor radionuclide therapy with (90)Y, (111)In, or (177)Lu. J Nucl Med 2004; 45:1260-9.
84. Valkema R, Pauwels SA, Kvols LK, Kwekkeboom DJ, Jamar F, de Jong M, Barone R, Walrand S, Kooij PP, Bakker WH, Lasher J, Krenning EP. Long-Term Follow-Up of Renal Function After Peptide Receptor Radiation Therapy with 90Y-DOTA0,Tyr3-Octreotide and 177Lu-DOTA0, Tyr3-Octreotate. J Nucl Med 2005; 46:83S-91S.
85. Stewart FA, Lebesque JV, Hart AA. Progressive development of radiation damage in mouse kidneys and the consequences for reirradiation tolerance. Int J Radiat Biol Relat Stud Phys Chem Med 1988; 53:405-15.
86. Stewart FA, Oussoren Y, Luts A, Begg AC, Dewit L, Lebesque J, Bartelink H. Repair of sublethal radiation injury after multiple small doses in mouse kidney: an estimate of flexure dose. Int J Radiat Oncol Biol Phys 1987; 13:765-72.
87. van Rongen E, Kuijpers WC, Madhuizen HT, van der Kogel AJ. Effects of multifraction irradiation on the rat kidney. Int J Radiat Oncol Biol Phys 1988; 15:1161-70.
88. Williams MV, Denekamp J. Radiation induced renal damage in mice: influence of fraction size. Int J Radiat Oncol Biol Phys 1984; 10:885-93.
89. Williams MV, Stewart FA, Soranson JA, Denekamp J. Time-dependent recovery in the kidney. Radiother Oncol 1986; 5:165-7.
90. Safwat A, Nielsen OS, el-Badawy S, Overgaard J. Late renal damage after total body irradiation and bone marrow transplantation in a mouse model: effect of radiation fractionation. Eur J Cancer 1995; 31A:987-92.

91. Behr TM, Goldenberg DM, Becker W. Reducing the renal uptake of radiolabeled antibody fragments and peptides for diagnosis and therapy: present status, future prospects and limitations. Eur J Nucl Med 1998; 25:201-12.
92. Duncan JR, Welch MJ. Intracellular metabolism of indium-111-DTPA-labeled receptor targeted proteins. J Nucl Med 1993; 34:1728-38.
93. Christensen EI, Nielsen S. Structural and functional features of protein handling in the kidney proximal tubule. Semin Nephrol 1991; 11:414-39.
94. Nielsen S. Endocytosis in proximal tubule cells involves a two-phase membrane- recycling pathway. Am J Physiol 1993; 264:C823-35.
95. Mogensen CE, Solling. Studies on renal tubular protein reabsorption: partial and near complete inhibition by certain amino acids. Scand J Clin Lab Invest 1977; 37:477-86.
96. Hammond PJ, Wade AF, Gwilliam ME, Peters AM, Myers MJ, Gilbey SG, Bloom SR, Calam J. Amino acid infusion blocks renal tubular uptake of an indium-labelled somatostatin analogue. Br J Cancer 1993; 67:1437-9.
97. Behr TM, Sharkey RM, Juweid ME, Blumenthal RD, Dunn RM, Griffiths GL, Bair HJ, Wolf FG, Becker WS, Goldenberg DM. Reduction of the renal uptake of radiolabeled monoclonal antibody fragments by cationic amino acids and their derivatives. Cancer Res 1995; 55:3825-34.
98. Elkjaer ML, Birn H, Agre P, Christensen EI, Nielsen S. Effects of microtubule disruption on endocytosis, membrane recycling and polarized distribution of Aquaporin-1 and gp330 in proximal tubule cells. Eur J Cell Biol 1995; 67:57-72.
99. Gutmann EJ, Niles JL, McCluskey RT, Brown D. Colchicine-induced redistribution of an apical membrane glycoprotein (gp330) in proximal tubules. Am J Physiol 1989; 257:C397-407.
100. van Eerd JE, Vegt E, Wetzels JF, Russel FG, Masereeuw R, Corstens FH, Oyen WJ, Boerman OC. Gelatin-based plasma expander effectively reduces renal uptake of 111In-octreotide in mice and rats. J Nucl Med 2006; 47:528-33.
101. ten Dam MA, Branten AJ, Klasen IS, Wetzels JF. The gelatin-derived plasma substitute Gelofusine causes low-molecular-weight proteinuria by decreasing tubular protein reabsorption. J Crit Care 2001; 16:115-20.
102. Veldman BA, Schepkens HL, Vervoort G, Klasen I, Wetzels JF. Low concentrations of intravenous polygelines promote low-molecular weight proteinuria. Eur J Clin Invest 2003; 33:962-8.
103. Vegt E, Wetzels JF, Russel FG, Masereeuw R, Boerman OC, van Eerd JE, Corstens FH, Oyen WJ. Renal uptake of radiolabeled octreotide in human subjects is efficiently inhibited by succinylated gelatin. J Nucl Med 2006; 47:432-36.
104. Balster DA, O'Dorisio MS, Summers MA, Turman MA. Segmental expression of somatostatin receptor subtypes sst(1) and sst(2) in tubules and glomeruli of human kidney. Am J Physiol Renal Physiol 2001; 280:F457-65.
105. Reubi JC, Horisberger U, Studer UE, Waser B, Laissue JA. Human kidney as target for somatostatin: high affinity receptors in tubules and vasa recta. J Clin Endocrinol Metab 1993; 77:1323-8.
106. Andreassen CN, Grau C, Lindegaard JC. Chemical radioprotection: a critical review of amifostine as a cytoprotector in radiotherapy. Semin Radiat Oncol 2003; 13:62-72.
107. Capizzi R. Amifostine: the preclinical basis for broad-spectrum selective cytoprotection of normal tissues from cytotoxic therapies. Semin Oncol 1996; 23:2-17.
108. Moulder JE, Fish BL, Cohen EP. Brief pharmacological intervention in experimental radiation nephropathy. Radiat Res 1998; 150:535-41.
109. Molteni A, Moulder JE, Cohen EP, Fish BL, Taylor JM, Veno PA, Wolfe LF, Ward WF. Prevention of radiation-induced nephropathy and fibrosis in a model of bone marrow transplant by an angiotensin II receptor blocker. Exp Biol Med (Maywood) 2001; 226:1016-23.

110. Moulder JE, Fish BL, Cohen EP. Angiotensin II receptor antagonists in the treatment and prevention of radiation nephropathy. Int J Radiat Biol 1998; 73:415-21.
111. Cohen EP, Fish BL, Moulder JE. Successful brief captopril treatment in experimental radiation nephropathy. J Lab Clin Med 1997; 129:536-47.
112. Cohen EP. Radiation nephropathy after bone marrow transplantation. Kidney Int 2000; 58:903-18.
113. Jaggi JS, Seshan SV, McDevitt MR, Sgouros G, Hyjek E, Scheinberg DA. Mitigation of radiation nephropathy after internal alpha-particle irradiation of kidneys. Int J Radiat Oncol Biol Phys 2006; 64:1503-12.
114. Kwekkeboom DJ, Teunissen JJ, Bakker WH, Kooij PP, de Herder WW, Feelders RA, van Eijck CH, Esser JP, Kam BL, Krenning EP. Radiolabeled somatostatin analog [177Lu-DOTA0,Tyr3]octreotate in patients with endocrine gastroenteropancreatic tumors. J Clin Oncol 2005; 23:2754-62.
115. De Jong M, Rolleman EJ, Bernard BF, Visser TJ, Bakker WH, Breeman WA, Krenning EP. Inhibition of renal uptake of indium-111-DTPA-octreotide in vivo. J Nucl Med 1996; 37:1388-92.
116. Rolleman EJ, Valkema R, De Jong M, Kooij PP, Krenning EP. Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. Eur J Nucl Med Mol Imaging 2003; 30:9-15.
117. Barone R, Pauwels S, De Camps J, Krenning EP, Kvols LK, Smith MC, Bouterfa H, Devuyst O, Jamar F. Metabolic effects of amino acid solutions infused for renal protection during therapy with radiolabelled somatostatin analogues. Nephrol Dial Transplant 2004; 19:2275-81.
118. De Jong M, Breeman WA, Bernard BF, Bakker WH, Schaar M, van Gameren A, Bugaj JE, Erion J, Schmidt M, Srinivasan A, Krenning EP. [177Lu-DOTA(0),Tyr3] octreotate for somatostatin receptor-targeted radionuclide therapy. Int J Cancer 2001; 92:628-33.
119. Breeman WA, Mearadji A, Capello A, Bernard BF, van Eijck CH, Krenning EP, de Jong M. Anti-tumor effect and increased survival after treatment with [177Lu-DOTA0,Tyr3]octreotate in a rat liver micrometastases model. Int J Cancer 2003; 104:376-9.
120. Bernard BF, Krenning E, Breeman WA, Visser TJ, Bakker WH, Srinivasan A, de Jong M. Use of the rat pancreatic CA20948 cell line for the comparison of radiolabelled peptides for receptor-targeted scintigraphy and radionuclide therapy. Nucl Med Commun 2000; 21:1079-85.
121. Behr TM, Sharkey RM, Sgouros G, Blumenthal RD, Dunn RM, Kolbert K, Griffiths GL, Siegel JA, Becker WS, Goldenberg DM. Overcoming the nephrotoxicity of radiometal-labeled immunoconjugates: improved cancer therapy administered to a nude mouse model in relation to the internal radiation dosimetry. Cancer 1997; 80:2591-610.
122. Mayer SA, Aledort LM. Thrombotic microangiopathy: differential diagnosis, pathophysiology and therapeutic strategies. Mt Sinai J Med 2005; 72:166-75.
123. Capello A, Krenning E, Bernard B, Reubi JC, Breeman W, de Jong M. 111In-labelled somatostatin analogues in a rat tumour model: somatostatin receptor status and effects of peptide receptor radionuclide therapy. Eur J Nucl Med Mol Imaging 2005; 32:1288-95.
124. Behe M, Koller S, Pusken M, Gross M, Alfke H, Keil B, Henzel M, Neidel HO, Schramm N, Gotthardt M, Behr TM, Engelhart-Cabillic E. Irradiation-induced upregulation of somatostatin and gastrin receptors in vitro and in vivo. Eur J Nucl Med Mol Imaging 2004; 31:S237-S38.
125. Krenning EP, Kwekkeboom D, Pauwels S, *Somatostatin receptor scintigraphy*, in *Nuclear Medicine annual*, L.M. Freeman, Editor. 1995, Raven Press: New York. p. 1-50.
126. Krenning EP, Bakker WH, Kooij PP, Breeman WA, Oei HY, de Jong M, Reubi JC, Visser TJ, Bruns C, Kwekkeboom DJ, et al. Somatostatin receptor scintigraphy with indium-111-DTPA-D-Phe-1-octreotide in man: metabolism, dosimetry and comparison with iodine-123- Tyr-3-octreotide. J Nucl Med 1992; 33:652-8.

127. Pimm MV, Gribben SJ. Prevention of renal tubule re-absorption of radiometal (indium-111) labelled Fab fragment of a monoclonal antibody in mice by systemic administration of lysine. Eur J Nucl Med 1994; 21:663-5.
128. Harrison HE, Harrison HC. Experimental production of renal glycosuria, phosphaturia and aminoaciduria by injection of maleic acid. Science 1954; 120:606-08.
129. Kramer HJ, Gonick HC. Experimental Fanconi syndrome. I. Effect of maleic acid on renal cortical Na-K-ATPase activity and ATP levels. J Lab Clin Med 1970; 76:799-808.
130. Worthen HG. Renal Toxicity of Maleic Acid in the Rat: Enzymatic and Morphologic Observations. Lab Invest 1963; 12:791-801.
131. Roth KS, Spencer PD, Higgins ES, Spencer RF. Effects of succinylacetone on methyl alpha-D-glucoside uptake by the rat renal tubule. Biochim Biophys Acta 1985; 820:140-6.
132. Spencer PD, Roth KS. Effects of succinylacetone on amino acid uptake in the rat kidney. Biochem Med Metab Biol 1987; 37:101-9.
133. Foreman JW, Bowring MA, Lee J, States B, Segal S. Effect of cystine dimethylester on renal solute handling and isolated renal tubule transport in the rat: a new model of the Fanconi syndrome. Metabolism 1987; 36:1185-91.
134. Bakker WH, Albert R, Bruns C, Breeman WA, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B, Koper JW, et al. [111In-DTPA-D-Phe1]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991; 49:1583-91.
135. Wall DA, Maack T. Endocytic uptake, transport, and catabolism of proteins by epithelial cells. Am J Physiol 1985; 248:C12-20.
136. Neuhaus OW. Acetoacetate does not prevent maleate-induced proteinuria in rats. Nephron 1989; 53:83-4.
137. Rogulski J, Pacanis A. Effects of maleate on CoA metabolism in rat kidney. Curr Probl Clin Biochem 1977; 8:406-15.
138. Hysing J, Ostensen J, Tolleshaug H, Kiil F. Effect of maleate on tubular protein reabsorption in dog kidneys. Ren Physiol 1988; 10:338-51.
139. De Jong M, Breeman WA, Bernard BF, Rolleman EJ, Hofland LJ, Visser TJ, Setyono-Han B, Bakker WH, van der Pluijm ME, Krenning EP. Evaluation in vitro and in rats of 161Tb-DTPA-octreotide, a somatostatin analogue with potential for intraoperative scanning and radiotherapy. Eur J Nucl Med 1995; 22:608-16.
140. Verani RR, Brewer ED, Ince A, Gibson J, Bulger RE. Proximal tubular necrosis associated with maleic acid administration to the rat. Lab Invest 1982; 46:79-88.
141. Gibril F, Reynolds JC, Doppman JL, Chen CC, Venzon DJ, Termanini B, Weber HC, Stewart CA, Jensen RT. Somatostatin receptor scintigraphy: its sensitivity compared with that of other imaging methods in detecting primary and metastatic gastrinomas. A prospective study. Ann Intern Med 1996; 125:26-34.
142. Kwekkeboom D, Krenning EP, de Jong M. Peptide receptor imaging and therapy. J Nucl Med 2000; 41:1704-13.
143. Kaandorp YAM, Jacobs JJA, Valkema R, Kwekkeboom DJ, Krenning EP. Renal uptake and renal function, before and after In-111-DTPA-octreotide therapy. Eur J Nucl Med 2000; 27:1224, PS_855.
144. Kwekkeboom DJ, Bakker WH, Kooij PP, Konijnenberg MW, Srinivasan A, Erion JL, Schmidt MA, Bugaj JL, de Jong M, Krenning EP. [177Lu-DOTAOTyr3]octreotate: comparison with [111In-DTPA0]octreotide in patients. Eur J Nucl Med 2001; 28:1319-25.

145. Kwekkeboom DJ, Kam BLR, Bakker WH, Kooij PPM, Srinivasan A, Erion JL, Bugaj JE, Schmidt MA, deJong M, Krenning EP. Treatment with 177Lu-DOTA0,Tyr3-octreotate in patients with somatostatin receptor postive tumors:preliminary results [abstract]. J Nucl Med 2001; 42:37P.
146. Valkema R, De Jong M, Kooij PPM, Kwekkeboom DJ, Krenning EP. Effective and safe inhibition of renal uptake of radiolabeled peptides by combined lysine and arginine infusion. J Nucl Med 2001; 42:37P.
147. Krenning BJ, Konings IR, Norenberg JP, De Jong M, Kvols L. Long-term histological organ damage in animals following peptide receptor radionuclide therapy (PRRT) with high doses of 90Y- and 111In-labeled [DOTA0,TYR3}octreotide (DOTATOC). J Nucl Med 2001; 42:37P.
148. Barone R, Jamar F, Walrand S, Labar D, Carlier P, Smith C, Kvols L, Krenning EP, Pauwels S. Amino acids infusion for renal protection during radiolabeled peptide therapy: comparison between different infusion regimens. Eur J Nucl Med 2000; 27:976, OS__325.
149. Chinol M, Cremonesi M, Ferrari M, Zoboli S, Maecke HR, Paganelli G. Evaluation of amino acid infusion to reduce renal toxicity during 90Y-DOTATOC therapy. J Nucl Med 2000; 41:267P.
150. Sartori S, Nielsen I, Pennacchio G, Pazzi P, Trevisani L. Hyperkalaemia during infusion of hyperosmolar amino acid solutions enriched with branched chain amino acids. Report of two cases. Recenti Prog Med 1991; 82:275-7.
151. Massara F, Cagliero E, Bisbocci D, Passarino G, Carta Q, Molinatti GM. The risk of pronounced hyperkalaemia after arginine infusion in the diabetic subject. Diabete Metab 1981; 7:149-53.
152. Massara F, Martelli S, Ghigo E, Camanni F, Molinatti GM. Arginine-induced hypophosphatemia and hyperkaliemia in man. Diabete Metab 1979; 5:297-300.
153. Barone R, De Camps J, Smith C, Kvols L, Krenning EP, Pauwels S, Jamar F. Metabolic effect of amino acids (AA) solutions infused for renal radioprotection. J Nucl Med 2000; 41:94P.
154. Bushinsky DA, Gennari FJ. Life-threatening hyperkalemia induced by arginine. Ann Intern Med 1978; 89:632-4.
155. Perazella MA, Biswas P. Acute hyperkalemia associated with intravenous epsilon-aminocaproic acid therapy. Am J Kidney Dis 1999; 33:782-5.
156. Burch HB, Choi S, Dence CN, Alvey TR, Cole BR, Lowry OH. Metabolic effects of large fructose loads in different parts of the rat nephron. J Biol Chem 1980; 255:8239-44.
157. Baus M, Medjugorac-Popovski M, Brown D, Sabolic I, Mirela B, Mila MP, Ivan S. In colchicine-treated rats, cellular distribution of AQP-1 in convoluted and straight proximal tubule segments is differently affected. Pflugers Arch 2000; 439:321-30.
158. De Jong M, Krenning E. New advances in peptide receptor radionuclide therapy. J Nucl Med 2002; 43:617-20.
159. Takano M, Nakanishi N, Kitahara Y, Sasaki Y, Murakami T, Nagai J. Cisplatin-induced inhibition of receptor-mediated endocytosis of protein in the kidney. Kidney Int 2002; 62:1707-17.
160. Marshansky V, Bourgoin S, Londono I, Bendayan M, Maranda B, Vinay P. Receptor-mediated endocytosis in kidney proximal tubules: recent advances and hypothesis. Electrophoresis 1997; 18:2661-76.
161. Ben-Chetrit E, Levy M. Colchicine: 1998 update. Semin Arthritis Rheum 1998; 28:48-59.
162. Molad Y. Update on colchicine and its mechanism of action. Curr Rheumatol Rep 2002; 4:252-6.
163. De Jong M, Kwekkeboom D, Valkema R, Krenning EP. Radiolabelled peptides for tumour therapy: current status and future directions. Plenary lecture at the EANM 2002. Eur J Nucl Med Mol Imaging 2003; 30:463-9.
164. Bernard BF, Krenning EP, Breeman WA, Rolleman EJ, Bakker WH, Visser TJ, Macke H, de Jong M. D-lysine reduction of indium-111 octreotide and yttrium-90 octreotide renal uptake. J Nucl Med 1997; 38:1929-33.

165. O'Reilly DS, Parry ES, Whicher JT. The effects of arginine, dextran and Haemaccel infusions on urinary albumin, beta 2-microglobulin and N-acetyl-beta-D-glucosaminidase. Clin Chim Acta 1986; 155:319-27.
166. Breeman WA, De Jong M, Visser TJ, Erion JL, Krenning EP. Optimising conditions for radiolabelling of DOTA-peptides with 90Y, 111In and 177Lu at high specific activities. Eur J Nucl Med Mol Imaging 2003; 30:917-20.
167. Forrer F, Valkema R, Bernard B, Schramm NU, Hoppin JW, Rolleman E, Krenning EP, de Jong M. In vivo radionuclide uptake quantification using a multi-pinhole SPECT system to predict renal function in small animals. Eur J Nucl Med Mol Imaging 2006; 33:1214-7.
168. Rolleman EJ, Bernard B, De Visser M, Bijster M, Visser TJ, Vermeij M, Lindemans J, Krenning EP, De Jong M. Long term toxicity of [177Lu-DOTA0,Tyr3]octreotate in rats. Eur J Nucl Med Mol Imaging 2007; In press:
169. Rolleman EJ, de Jong M, Valkema R, Kwekkeboom D, Kam B, Krenning EP. Inhibition of kidney uptake of radiolabeled somatostatin analogs: amino acids or gelofusine? J Nucl Med 2006; 47:1730-1.
170. van Eerd JE, Vegt E, Wetzels JF, Russel FG, Masereeuw R, Corstens FH, Boerman OC, Oyen WJ. Reply: inhibition of kidney uptake of radiolabeled somatostatin analogs: amino acids or gelofusine? J Nucl Med 2006; 47:1731.
171. Kwekkeboom DJ, Bakker WH, Kam BL, Teunissen JJ, Kooij PP, de Herder WW, Feelders RA, van Eijck CH, de Jong M, Srinivasan A, Erion JL, Krenning EP. Treatment of patients with gastro-entero-pancreatic (GEP) tumours with the novel radiolabelled somatostatin analogue [177Lu-DOTA(0),Tyr3]octreotate. Eur J Nucl Med Mol Imaging 2003; 30:417-22.
172. Virgolini I, Britton K, Buscombe J, Moncayo R, Paganelli G, Riva P. In- and Y-DOTA-lanreotide: results and implications of the MAURITIUS trial. Semin Nucl Med 2002; 32:148-55.
173. Bodei L, Cremonesi M, Grana C, Rocca P, Bartolomei M, Chinol M, Paganelli G. Receptor radionuclide therapy with 90Y-[DOTA]0-Tyr3-octreotide (90Y-DOTATOC) in neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2004; 31:1038-46.
174. Rolleman EJ, Krenning EP, Van Gameren A, Bernard BF, De Jong M. Uptake of [111In-DTPA0]octreotide in the rat kidney is inhibited by colchicine and not by fructose. J Nucl Med 2004; 45:709-13.
175. Serri O, Beauregard H, Brazeau P, Abribat T, Lambert J, Harris A, Vachon L. Somatostatin analogue, octreotide, reduces increased glomerular filtration rate and kidney size in insulin-dependent diabetes. Jama 1991; 265:888-92.
176. Luksch A, Polak K, Matulla B, Dallinger S, Kapiotis S, Rainer G, Wolzt M, Schmetterer L. Glucose and insulin exert additive ocular and renal vasodilator effects on healthy humans. Diabetologia 2001; 44:95-103.
177. Schmidt A, Pleiner J, Schaller G, Roden M, Dallinger S, Mayer G, Schmetterer L, Wolzt M. Renal hemodynamic effects of somatostatin are not related to inhibition of endogenous insulin release. Kidney Int 2002; 61:1788-93.
178. Tulassay T, Tulassay Z, Rascher W, Szucs L, Seyberth HW, Nagy I. Effect of somatostatin on kidney function and vasoactive hormone systems in health subjects. Klin Wochenschr 1991; 69:486-90.
179. Vora J, Owens DR, Luzio S, Atiea J, Ryder R, Hayes TM. Renal response to intravenous somatostatin in insulin-dependent diabetic patients and normal subjects. J Clin Endocrinol Metab 1987; 64:975-9.
180. Dullaart RP, Meijer S, Marbach P, Sluiter WJ. Effect of a somatostatin analogue, octreotide, on renal haemodynamics and albuminuria in acromegalic patients. Eur J Clin Invest 1992; 22:494-502.

181. Kalambokis G, Economou M, Fotopoulos A, Al Bokharhii J, Pappas C, Katsaraki A, Tsianos EV. The effects of chronic treatment with octreotide versus octreotide plus midodrine on systemic hemodynamics and renal hemodynamics and function in nonazotemic cirrhotic patients with ascites. Am J Gastroenterol 2005; 100:879-85.
182. Kalambokis G, Economou M, Fotopoulos A, Bokharhii JA, Katsaraki A, Tsianos EV. Renal effects of treatment with diuretics, octreotide or both, in non-azotemic cirrhotic patients with ascites. Nephrol Dial Transplant 2005; 20:1623-9.
183. Castellino P, Giordano C, Perna A, DeFronzo RA. Effects of plasma amino acid and hormone levels on renal hemodynamics in humans. Am J Physiol 1988; 255:F444-9.
184. Tulassay Z, Tulassay T, Szucs L, Nagy I. Effects of long acting somatostatin analogue on renal functions. Horm Metab Res 1990; 22:555-6.
185. Krempf M, Ranganathan S, Remy JP, Charbonnel B, Guillon J. Effect of long-acting somatostatin analog (SMS 201-995) on high glomerular filtration rate in insulin dependent diabetic patients. Int J Clin Pharmacol Ther Toxicol 1990; 28:309-11.
186. Malesci A, Tacconi M, Valentini A, Basilico M, Lorenzano E, Salerno F. Octreotide long-term treatment in patients with portal hypertension: persistent inhibition of postprandial glucagon response without major changes in renal function. J Hepatol 1997; 26:816-25.
187. Ruggenenti P, Remuzzi A, Ondei P, Fasolini G, Antiga L, Ene-Iordache B, Remuzzi G, Epstein FH. Safety and efficacy of long-acting somatostatin treatment in autosomal-dominant polycystic kidney disease. Kidney Int 2005; 68:206-16.
188. Sabat M, Guarner C, Soriano G, Bulbena O, Novella MT, Ortiz J, Ricart E, Villanueva C, Rosello J, Rodriguez J, Balanzo J. Effect of subcutaneous administration of octreotide on endogenous vasoactive systems and renal function in cirrhotic patients with ascites. Dig Dis Sci 1998; 43:2184-9.
189. Dullaart RP, Meijer S, Marbach P, Sluiter WJ. Renal reserve filtration capacity in growth hormone deficient subjects. Eur J Clin Invest 1992; 22:562-8.
190. Mountokalakis T, Kallivretakis N, Mayopoulou-Symvoulidou D, Karvountzis G, Tolis G. Enhancement of renal function by a long-acting somatostatin analogue in patients with decompensated cirrhosis. Nephrol Dial Transplant 1988; 3:604-7.
191. Ottesen LH, Aagaard NK, Kiszka-Kanowitz M, Rehling M, Henriksen JH, Pedersen EB, Flyvbjerg A, Bendtsen F. Effects of a long-acting formulation of octreotide on renal function and renal sodium handling in cirrhotic patients with portal hypertension: a randomized, double-blind, controlled trial. Hepatology 2001; 34:471-7.
192. Pomier-Layrargues G, Paquin SC, Hassoun Z, Lafortune M, Tran A. Octreotide in hepatorenal syndrome: a randomized, double-blind, placebo-controlled, crossover study. Hepatology 2003; 38:238-43.
193. Dorr U, Rath U, Sautter-Bihl ML, Guzman G, Bach D, Adrian HJ, Bihl H. Improved visualization of carcinoid liver metastases by indium-111 pentetreotide scintigraphy following treatment with cold somatostatin analogue. Eur J Nucl Med 1993; 20:431-3.
194. Calabro-Jones PM, Fahey RC, Smoluk GD, Ward JF. Alkaline phosphatase promotes radioprotection and accumulation of WR-1065 in V79-171 cells incubated in medium containing WR-2721. Int J Radiat Biol Relat Stud Phys Chem Med 1985; 47:23-7.
195. Asna N, Lewy H, Ashkenazi IE, Deutsch V, Peretz H, Inbar M, Ron IG. Time dependent protection of amifostine from renal and hematopoietic cisplatin induced toxicity. Life Sci 2005; 76:1825-34.
196. Capizzi RL, Scheffler BJ, Schein PS. Amifostine-mediated protection of normal bone marrow from cytotoxic chemotherapy. Cancer 1993; 72:3495-501.

197. Cassatt DR, Fazenbaker CA, Kifle G, Bachy CM. Subcutaneous administration of amifostine (ethyol) is equivalent to intravenous administration in a rat mucositis model. Int J Radiat Oncol Biol Phys 2003; 57:794-802.
198. Capizzi RL. The preclinical basis for broad-spectrum selective cytoprotection of normal tissues from cytotoxic therapies by amifostine. Semin Oncol 1999; 26:3-21.
199. Culy CR, Spencer CM. Amifostine: an update on its clinical status as a cytoprotectant in patients with cancer receiving chemotherapy or radiotherapy and its potential therapeutic application in myelodysplastic syndrome. Drugs 2001; 61:641-84.
200. Vardy J, Wong E, Izard M, Clifford A, Clarke SJ. Life-threatening anaphylactoid reaction to amifostine used with concurrent chemoradiotherapy for nasopharyngeal cancer in a patient with dermatomyositis: a case report with literature review. Anticancer Drugs 2002; 13:327-30.
201. Lin A, Lawrence TS. An anaphylactoid reaction from amifostine. Radiother Oncol 2006; 79:352.
202. Demiral AN, Yerebakan O, Simsir V, Alpsoy E. Amifostine-induced toxic epidermal necrolysis during radiotherapy: a case report. Jpn J Clin Oncol 2002; 32:477-9.
203. Boccia R. Improved tolerability of amifostine with rapid infusion and optimal patient preparation. Semin Oncol 2002; 29:9-13.
204. Koukourakis MI, Kyrias G, Kakolyris S, Kouroussis C, Frangiadaki C, Giatromanolaki A, Retalis G, Georgoulias V. Subcutaneous administration of amifostine during fractionated radiotherapy: a randomized phase II study. J Clin Oncol 2000; 18:2226-33.
205. Anne PR. Phase II trial of subcutaneous amifostine in patients undergoing radiation therapy for head and neck cancer. Semin Oncol 2002; 29:80-3.
206. Block KI, Gyllenhaal C. Commentary: the pharmacological antioxidant amifostine -- implications of recent research for integrative cancer care. Integr Cancer Ther 2005; 4:329-51.
207. Sasse AD, Clark LG, Sasse EC, Clark OA. Amifostine reduces side effects and improves complete response rate during radiotherapy: results of a meta-analysis. Int J Radiat Oncol Biol Phys 2006; 64:784-91.
208. Rolleman E, Forrer F, Bernard HF, al. e. Amifostine protects rat kidneys from damage after high dose [177Lu-DOTA0,Tyr3]octreotate. Eur J Nucl Med Mol Imaging 2007; In press:
209. Brenner BM. AMGEN International Prize: the history and future of renoprotection. Kidney Int 2003; 64:1163-8.
210. Perico N, Codreanu I, Schieppati A, Remuzzi G. Pathophysiology of disease progression in proteinuric nephropathies. Kidney Int Suppl 2005; S79-82.
211. Wolf G. Renal injury due to renin-angiotensin-aldosterone system activation of the transforming growth factor-beta pathway. Kidney Int 2006; 70:1914-9.
212. Cohen EP, Fish BL, Moulder JE. Angiotensin II infusion exacerbates radiation nephropathy. J Lab Clin Med 1999; 134:283-91.
213. Moulder JE, Fish BL, Cohen EP. ACE inhibitors and AII receptor antagonists in the treatment and prevention of bone marrow transplant nephropathy. Curr Pharm Des 2003; 9:737-49.
214. Moulder JE, Fish BL, Cohen EP. Treatment of radiation nephropathy with ACE inhibitors. Int J Radiat Oncol Biol Phys 1993; 27:93-9.
215. Wolf G, Ritz E. Combination therapy with ACE inhibitors and angiotensin II receptor blockers to halt progression of chronic renal disease: pathophysiology and indications. Kidney Int 2005; 67:799-812.
216. Sato A, Saruta T. Aldosterone breakthrough during angiotensin-converting enzyme inhibitor therapy. Am J Hypertens 2003; 16:781-8.
217. Sato A, Saruta T, Funder JW. Combination therapy with aldosterone blockade and renin-angiotensin inhibitors confers organ protection. Hypertens Res 2006; 29:211-6.

218. Krug AW, Grossmann C, Schuster C, Freudinger R, Mildenberger S, Govindan MV, Gekle M. Aldosterone stimulates epidermal growth factor receptor expression. J Biol Chem 2003; 278:43060-6.
219. Gumz ML, Popp MP, Wingo CS, Cain BD. Early transcriptional effects of aldosterone in a mouse inner medullary collecting duct cell line. Am J Physiol Renal Physiol 2003; 285:F664-73.
220. Epstein M. Aldosterone blockade: an emerging strategy for abrogating progressive renal disease. Am J Med 2006; 119:912-9.
221. Clozel M, Salloukh H. Role of endothelin in fibrosis and anti-fibrotic potential of bosentan. Ann Med 2005; 37:2-12.
222. Hocher B, Rohmeiss P, Thone-Reineke C, Schwarz A, Burst V, van der Woude F, Bauer C, Theuring F. Apoptosis in kidneys of endothelin-1 transgenic mice. J Cardiovasc Pharmacol 1998; 31 Suppl 1:S554-6.
223. Moncayo R. Cubilin and megalin in radiation-induced renal injury with labelled somatostatin analogues: are we just dealing with the kidney? Eur J Nucl Med Mol Imaging 2005; 32:1131-5.
224. Nagai J, Takano M. Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokinet 2004; 19:159-70.
225. Seed TM. Radiation protectants: current status and future prospects. Health Phys 2005; 89:531-45.
226. Moncayo R, Moncayo H, Kapelari K. Nutritional treatment of incipient thyroid autoimmune disease. Influence of selenium supplementation on thyroid function and morphology in children and young adults. Clin Nutr 2005; 24:530-1.
227. Racusen LC, Whelton A, Solez K. Effects of lysine and other amino acids on kidney structure and function in the rat. Am J Pathol 1985; 120:436-42.
228. Racusen LC, Finn WF, Whelton A, Solez K. Mechanisms of lysine-induced acute renal failure in rats. Kidney Int 1985; 27:517-22.
229. Barron ME, Wilkes MM, Navickis RJ. A systematic review of the comparative safety of colloids. Arch Surg 2004; 139:552-63.
230. Russell WJ, Fenwick DG. Anaphylaxis to Haemaccel and cross reactivity to Gelofusin. Anaesth Intensive Care 2002; 30:481-3.
231. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977; 1:466-9.
232. Beez M, Dietl H. [Retrospective consideration of the incidence of anaphylactoid reactions following administration of Plasmasteril and Longasteril]. Infusionsther Klin Ernahr 1979; 6:23-6.
233. Davies MJ. Polygeline. Dev Biol Stand 1987; 67:129-31.
234. Oberg K. Neuroendocrine tumors of the gastrointestinal tract: recent advances in molecular genetics, diagnosis, and treatment. Curr Opin Oncol 2005; 17:386-91.
235. van der Lely AJ, de Herder WW. Carcinoid syndrome: diagnosis and medical management. Arq Bras Endocrinol Metabol 2005; 49:850-60.
236. D'Andrea G. Classifying amino acids as gluco(glyco)genic, ketogenic, or both. Biochem. Educ. 2000; 28:27-28.
237. Noda C, Ichihara A. Control of ketogenesis from amino acids. IV. Tissue specificity in oxidation of leucine, tyrosine, and lysine. J Biochem (Tokyo) 1976; 80:1159-64.
238. Christensen EI, Birn H, Verroust P, Moestrup SK. Membrane receptors for endocytosis in the renal proximal tubule. Int Rev Cytol 1998; 180:237-84.
239. Christensen EI, Birn H, Verroust P, Moestrup SK. Megalin-mediated endocytosis in renal proximal tubule. Ren Fail 1998; 20:191-9.
240. Capizzi RL. Amifostine reduces the incidence of cumulative nephrotoxicity from cisplatin: laboratory and clinical aspects. Semin Oncol 1999; 26:72-81.

241. Yuhas JM. Active versus passive absorption kinetics as the basis for selective protection of normal tissues by S-2-(3-aminopropylamino)-ethylphosphorothioic acid. Cancer Res 1980; 40:1519-24.
242. Malis CD, Racusen LC, Solez K, Whelton A. Nephrotoxicity of lysine and of a single dose of aminoglycoside in rats given lysine. J Lab Clin Med 1984; 103:660-76.
243. Abel RM, Beck CH, Jr., Abbott WM, Ryan JA, Jr., Barnett GO, Fischer JE. Improved survival from acute renal failure after treatment with intravenous essential L-amino acids and glucose. Results of a prospective, double-blind study. N Engl J Med 1973; 288:695-9.

HOOFDSTUK 9

DANKWOORD, CURRICULUM VITAE EN PUBLICATIELIJST

DANKWOORD

Zonder dankwoord is een proefschrift geen proefschrift! Omdat onderzoek teamwerk is en de schrijver van een proefschrift altijd wordt ondersteund door anderen, ook hier een woord van dank aan alle mensen die direct of indirect hebben bijgedragen aan de totstandkoming van dit proefschrift. Met het risico dat ik iemand vergeet, wil ik een aantal mensen meer persoonlijk bedanken:

Allereerst mijn promotoren: prof.dr.ir. M.de Jong en prof.dr. E.P. Krenning.
Beste Marion, dit proefschrift kreeg vorm onder jouw bezielende begeleiding. Je hebt me destijds aangemoedigd om op de rijdende trein van de Nucleaire te stappen, en dat is goed bevallen! Meerdere malen zag jij kaas in door mij verloren gewaande resultaten, en moedigde je me aan om eigen ideeën uit te werken. Manuscripten kwamen super snel gecorrigeerd terug. Ik heb veel van je geleerd over netwerken, presenteren en hoe reviewers te kietelen! Je bent altijd in voor een anekdote, over vallen…. en opstaan, skiën (ik doe geen beloften…) en vakanties. Bedankt voor je begeleiding, de samenwerking en de gezelligheid!

Beste Eric, dank je wel voor de gelegenheid om te mogen werken aan jouw kindje: therapie met gelabelde somatostatine analoga. Het was om meerdere redenen fantastisch dat je me vorig jaar in staat stelde het onderzoek af te ronden door een tijdelijke aanstelling op de afdeling. Bedankt!

De leden van de kleine commissie, de professoren Weimar, Visser en Pauwels, wil ik hartelijk danken voor de snelle beoordeling van het manuscript. Ook (kersvers) professor Otto Boerman wil ik bedanken voor het zitting nemen in de promotiecommissie.

Het hele onderzoeksavontuur was nooit begonnen als professor Hennemann tijdens keuze-onderwijs niet zo enthousiast sprak over zijn onderzoek. Het aanstekelijke enthousiasme vloog over, en ook ik werd bevangen door het ‘transport-virus’. Beste Jorg, van huis uit heb ik grote bewondering voor je klinisch en wetenschappelijk vakmanschap, en dat is in mijn schildklierlab-periode niet minder geworden. Ik ben zeer vereerd dat je wilt deelnemen aan de oppositie op 14 juni!

Heel veel dank ben ik verschuldigd aan de collega’s in de ‘prekliniek-groep’.
Bert Bernard: beste Bert, we kennen elkaar al vanaf mijn eerste momenten op het schildklierlab. Samen hebben we veel experimenten uitgevoerd met T_4 en T_3, later stapten we over naar de Nucleaire Geneeskunde. Een kei op lab- en proefdiergebied, altijd vriendelijk en

hulpvaardig; een groot gedeelte van ‘mijn’ studies is door jou ondersteund…….je bent een kanjer, ik kan je niet genoeg bedanken!
Marleen Melis: dank je wel voor de gesprekken over van alles en nog wat, over nieren en nieropname. Dank je wel voor je support. Ik bewonder je gedrevenheid. Succes in alles!
Magda Bijster: bedankt voor alle uurtjes turen in de microscoop! Ria van den Berg: dank voor alle gekraakte praktische noten, de hand- en spandiensten en de gezelligheid.
Suzanne Verwijnen en Monique de Visser: bedankt voor jullie vrolijke noot in de groep, succes met de laatste loodjes, ik ben er bij!
Flavio Forrer: thanks for the cooperation during the past 16 months; I wish you a lot of success in finishing your thesis!
Wout Breeman en Eric de Bloois: ontelbare malen hebben jullie het labelen verzorgd dat nodig was om onze experimenten tot een goed einde te brengen. Ook bij gierende spoed was van alles mogelijk, super!
Ex-CIL-ers Michael Schaar en Arthur van Gameren:bedankt voor jullie hulp bij experimenten!
De andere CIL-ers (Rogier, Ingrid, Cristina, Stefan): bedankt voor de gezelligheid! Stagiaires Esther Slottje en Satish Boedhoe: bedankt voor jullie hulp bij (stinkende) urine-eiwit bepalingen. De dierverzorgers van het EDC (Ron Briegoos, Esther Fijneman, Dennis de Meulder en Agnes Boer): bedankt voor de hulp bij een aantal van onze experimenten.

Roelf Valkema wil ik bedanken voor het in extenso lezen van manuscripten, bedankt voor alle aanwijzingen, discussies en support. Peter Kooij: ik maakte het je vaak niet makkelijk: elke klus een spoedklus… altijd weer succesvol opgelost, TOP!
Ambroos, Ronald, Ron, Tuki en Paul: helping hands in barre soft- en hardwaretijden!
Martijn van Essen, gewaardeerd paranimf, en (ondertussen ex-)kamergenoot: je werd opeens geconfronteerd met een rare snuiter vanuit de prekliniek: je aanpassingsvermogen is subliem!
De collega’s van de ‘groep van Willem’ (tegenwoordig ‘groep van Erica’?) wil ik bedanken voor alle keren lutetium-octreotaat en technetium-DMSA labellen: jullie hebben heel wat bequerelletjes doorgegeven.
Alle NMW’ers en baliemedewerkers: bedankt voor de acquisities en de gezelligheid!

Adri Edenburg: een pijltje niet goed, de kleur niet naar mijn zin, iets te groot of juist te klein…. de grafische vormgeving van de omslag is nu precies wat ik wilde. Merci!

Johannes de Graaff: bedankt voor je supersnelle hulp bij het bouwrijp maken van het manuscript.

Opleiders, internisten en collega-assistenten in het MCRZ: bedankt voor de mentale ondersteuning en het aanleren van een kritische houding.

Mijn broer(tje) Imre: dank je wel voor je gezelligheid en steun bij de voorbereiding van deze promotie. Ik ben blij dat jij op deze dag een van mijn paranimfen wilt zijn!

Helaas kan mijn moeder dit hoogtepunt niet meer meemaken. Ik ben mijn ouders heel dankbaar voor hun liefdevolle aanmoedigingen en de gelegenheid om te gaan studeren. Zij maakten het mogelijk dat ik me heb ontwikkeld tot de persoon die ik nu ben: zonder hen was het niet gelukt zo ver te komen.

Last but most prominent: mijn lieve vrouw Thera en zonen Timon en Koen.
Het combineren van een drukke baan met het schrijven van een proefschrift betekende dat jij er vaak alleen voor stond, en dat viel best zwaar, zeker met de verdrietige gebeurtenissen van de afgelopen maanden. Dank je wel voor je opoffering, je hulp, je mentale steun, je liefde! Uiteindelijk werd het ook een project van ons beiden: dank je voor de hulp bij de lay-out en de vertaling. Zonder jou was het zinloos en onmogelijk. Ik hou van je!
Timon (bedankt voor stelling 11!) en Koen, het is klaar! Stoeien?

SPONSORING

Financiële ondersteuning voor de totstandkoming van dit proefschrift werd verleend door onderstaande sponsoren, waarvoor hartelijk dank!

Amgen Nederland B.V.
Astellas Pharma B.V. (producent van o.a. Prograf®)
Astra Zeneca B.V.
B. Braun Medical B.V.
Baxter B.V.
Bristol-Myers Squibb B.V.
Fresenius Medical Care Nederland B.V.
Gambro-Hospal B.V.
GE Healthcare Medical Diagnostics
Genzyme Nederland
Harlan Netherlands B.V.
Menarini Pharma Nederland
Nierstichting Nederland
Novartis Pharma B.V.
Novo Nordisk Farma B.V.
Roche Pharmaceuticals Nederland B.V.
Servier Nederland Farma B.V.
Shire Pharmaceuticals - Benelux
Tyco Healthcare/Mallinckrodt Medical B.V.
UNO Roestvrijstaal B.V.
Veenstra Instruments B.V.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 9 juni 1972 geboren te Rotterdam. In 1990 behaalde hij het VWO-Gymnasium diploma aan Scholengemeenschap 'De Krimpenerwaard' te Krimpen aan den IJssel. In dat zelfde jaar begon hij met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam.

Tijdens de doctoraalfase deed hij onderzoek op de laboratoria van de afdelingen Interne Geneeskunde (het schildklierlab, begeleiders: dr.ir. M. de Jong en prof.dr. G. Hennemann) en Nucleaire Geneeskunde (begeleiders: prof.dr. E.P. Krenning en dr.ir. M. de Jong). Het doctoraal examen werd behaald in 1996, en het arts-examen volgde in 1998.

Daarna was hij werkzaam als arts-assistent op de afdeling Interne Geneeskunde van het Albert Schweitzer ziekenhuis te Dordrecht en op de afdeling Chirurgie van het IJssellandziekenhuis te Capelle aan den IJssel. Vanaf november 2001 werd begonnen met het promotieonderzoek dat beschreven is in dit proefschrift en in mei 2002 startte de opleiding tot internist in het Medisch Centrum Rijnmond-Zuid (opleider: dr. A. Berghout).

Van februari t/m december 2006 werd de opleiding tot internist tijdelijk onderbroken om het in dit proefschrift beschreven onderzoek af te ronden. In januari 2007 werd begonnen met de aandachtsgebied-opleiding tot internist-nefroloog in het Medisch Centrum Rijnmond-Zuid (opleiders: dr. M.A. van den Dorpel en dr. A. Berghout), vanaf juli 2007 te vervolgen in het Erasmus MC (opleiders: prof.dr. W. Weimar, dr. R. Zietse en dr. J. van Saase).

PUBLICATIELIJST

De Jong M, Breeman WAP, Bernard HF, **Rolleman EJ**, Hofland LJ, Visser TJ, Setyono-Han B, Bakker WH, Vd Pluijm ME, Krenning EP. **1995** Evaluation in vitro and in rats of Tb-161-DTPA-octreotide, a somatostatin analogue with potential for intraoperative scanning and radiotherapy. Eur J Nucl Med 22:609-616.

De Jong M, **Rolleman EJ**, Bernard HF, Visser TJ, Bakker WH, Breeman WAP, Krenning EP. **1996** Inhibition of renal uptake of indium-111-DTPA-octreotide in vivo. J Nucl Med 37:1388-1392.

Bernard BF, Krenning EP, Breeman WAP, **Rolleman EJ**, Bakker WH, Visser TJ, Macke H, De Jong M. **1997** D-Lysine reduction of indium-111 octreotide and yttrium-90 octreotide renal uptake. J Nucl Med 38:1929-1933.

Breeman WAP, De Jong M, Bernard BF, Bakker WH, **Rolleman EJ**, Kwekkeboom DJ, Visser TJ, Krenning EP. **1997** Effects of ligand priming and multiple-dose injection on tissue uptake of ^{111}In-pentetreotide in rats. Nuclear Medicine & Biology 24: 749-753.

Rolleman EJ, De Jong M, Van Toor H, Schoenmakers C, Krenning EP, Hennemann G. **2000** Changes in renal triiodothyronine and thyroxine handling during fasting. Eur J Endocrinology 142:125-130.

Rolleman EJ, Valkema R, De Jong M, Kooij PPM, Krenning EP. **2003** Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. Eur J Nucl Med Mol Imag 30:9-15.

Rolleman EJ, Krenning EP, Van Gameren A, Bernard BF, De Jong M. **2004** Uptake of [^{111}In-DTPA^{0}]octreotide in the rat kidney is inhibited by colchicine and not by fructose. J Nucl Med 45:709-713.

Rolleman EJ, Valkema R, Melis M, Krenning EP, Visser TJ, De Jong M. **2006** Cubilin and megalin in radiation-induced renal injury with labelled somatostatin analogues: are we just dealing with the kidney? Eur J Nucl Med Mol Imaging 33:749-750.

Rolleman EJ, De Jong M, Valkema R, Kwekkeboom DJ, Kam BL, Krenning EP. **2006** Inhibition of kidney uptake of radiolabeled somatostatin analogs: amino acids or gelofusine? J Nucl Med 47:1730-1731.

Forrer F, Valkema R, Bernard B, Schramm NU, Hoppin JW, **Rolleman E**, Krenning EP, de Jong M. **2006** *In vivo r*adionuclide uptake quantification using a multi-pinhole SPECT system to predict renal function in small animals. Eur J Nucl Med Mol Imaging 33:1214-1217.

Rolleman EJ, Krenning EP, Bernard HF, De Visser M, Bijster M, Visser TJ, Vermeij M, Lindemans J, De Jong M. **2007** Long term toxicity of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in rats. Eur J Nucl Med Mol Imaging 34:219-227.

Rolleman EJ, Forrer F, Bernard B, Bijster M, Vermeij M, Valkema R, Krenning EP, De Jong M. **2007** Amifostine protects rat kidneys in peptide receptor radionuclide therapy with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Eur J Nucl Med Mol Imaging. *In press*.

Rolleman EJ, Bernard HF, Breeman WAP, Forrer F, de Blois E, Gotthardt M, Boerman O, Krenning EP, De Jong M. **2007** Molecular imaging of reduced renal uptake of radiolabelled [$DOTA^0$,Tyr^3]octreotate by the combination of lysine and Gelofusine in rats. Nuklearmedizin. *Submitted*.

Rolleman EJ, Forrer F, Bernard B, Valkema R, Krenning E, De Jong M. **2006** Molecular Imaging of renal damage using ^{99m}Tc-DMSA SPECT. Erasmus MC Oncology Magazine Issue 1:24-25.

Gotthardt M, Van Eerd-Vismale J, Oyen WJG, De Jong M, Zhang H, **Rolleman E**, Maecke HR, Behe M, Boerman O. **2007** Kidney uptake of different radiopeptides occurs via different mechanisms. J Nucl Med. 48:596-601.

Forrer F, **Rolleman E**, Bijster M, Melis M, Bernard B, Krenning EP, De Jong M. **2007** 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 Radiother. *In press*.

Rolleman EJ, Forrer F, Deckers J, De Groot H, Valkema R, De Jong M, Krenning EP. **2007** Anaphylactoid reaction to amifostine – Letter to the Editor. Radiother Oncol 82:110-111.

Forrer F, **Rolleman EJ**, Schramm N, Krenning EP and De Jong M. **2007** Is it possible to predict renal function in small animals using a multi-pinhole SPECT system? Reply. Eur J Nucl Med Mol Imaging. *In press*

Rolleman EJ, Kooij PPM, De Herder WW, Krenning EP, De Jong M. **2007** Somatostatin receptor subtype 2 mediated uptake of radiolabelled somatostatin analogues in the human kidney. Eur J. Nuclear Med Mol Imaging. *In press*.

Melis M, Krenning EP, Bernard HF, De Visser M, **Rolleman E**, De Jong M. **2007** Renal uptake and retention of radiolabelled somatostatin, bombesin, neurotensin, minigastrin and CCK analogues: species and gender differences. Nuclear Medicine Biology. *Submitted*.