Uptake kinetics of the somatostatin receptor ligand [86Y]DOTA-DPhe1-Tyr3-octreotide ([86Y]SMT487) using positron emission tomography in non-human primates and calculation of radiation doses of the 90Y-labelled analogue

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Abstract. [90Y]DOTA-DPhe1-Tyr3-octreotide ([90Y]-SMT487) has been suggested as a promising radiotherapeutic agent for somatostatin receptor-expressing tumours. In order to quantify the in vivo parameters of this compound and the radiation doses delivered to healthy organs, the analogue [86Y]DOTA-DPhe1-Tyr3-octreotide was synthesised and its uptake measured in baboons using positron emission tomography (PET). [86Y]DOTA-DPhe1-Tyr3-octreotide was administered at two different peptide concentrations, namely 2 and 100 µg peptide per m2 body surface. The latter concentration corresponded to a radiotherapeutic dose. In a third protocol [86Y]DOTA-DPhe1-Tyr3-octreotide was injected in conjunction with a simultaneous infusion of an amino acid solution that was high in L-lysine in order to lower the renal uptake of radioyttrium. Quantitative whole-body PET scans were recorded to measure the uptake kinetics for kidneys, liver, lung and bone. The individual absolute uptake kinetics were used to calculate the radiation doses for [90Y]DOTA-DPhe1-Tyr3-octreotide according to the MIRD recommendations extrapolated to a 70-kg human. The highest radiation dose was received by the kidneys, with 2.1–3.3 mGy per MBq [90Y]DOTA-DPhe1-Tyr3-octreotide injected. For the 100 µg/m2 SMT487 protocol with amino acid co-infusion this dose was about 20%–40% lower than for the other two treatment protocols. The liver and the red bone marrow received doses ranging from 0.32 to 0.53 mGy and 0.03 to 0.07 mGy per MBq [90Y]DOTA-DPhe1-Tyr3-octreotide, respectively. The average effective dose equivalent amounted to 0.23–0.32 mSv/MBq. The comparatively low estimated radiation doses to normal organs support the initiation of clinical phase I trials with [90Y]DOTA-DPhe1-Tyr3-octreotide in patients with somatostatin receptor-expressing tumours.

Key words: Yttrium-86 – Yttrium-90 – Octreotide – Positron emission tomography – Dosimetry

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Introduction

[90Y]DOTA-DPhe1-Tyr3-octreotide ([90Y]SMT487) is a radiotherapeutic somatostatin analogue that has the capability to induce shrinkage and even complete remission of somatostatin receptor-expressing tumours, as demonstrated in animal experiments [1]. The pharmacokinetics and the biodistribution of this compound were previously evaluated in rodents by counting the activity accumulated in each organ after the animals had been sacrificed. Organ distributions of [111In]DOTA-DPhe1-Tyr3-octreotide in rats bearing a pancreatic tumour were reported [2]. A first clinical case report on the therapeutic efficacy of [90Y]DOTA-DPhe1-Tyr3-octreotide was recently published [3, 4].

The aim of this study was to evaluate the radiation dosimetry of [90Y]DOTA-DPhe1-Tyr3-octreotide in primates prior to clinical trials with [90Y]DOTA-DPhe1-Tyr3-octreotide. The determination of the radiation doses requires the measurement of the uptake kinetics of [90Y]DOTA-DPhe1-Tyr3-octreotide. As 90Y is a pure β^--emitting isotope without accompanying γ-radiation it hardly allows quantitative evaluation from outside the body. In the past only estimates of the quantitative up-

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take kinetics of ^{90}Y radiopharmaceuticals and of the resulting individual human radiation doses have been reported, based on approaches such as (a) extrapolating pharmacokinetic data measured ex vivo in animals to humans; (b) using measurements of urinary excretion and blood clearance in humans to draw conclusions as to whole-body radiation doses [5, 6]; (c) measuring human uptake kinetics by means of bremsstrahlung registration [7–10]; (d) substituting ^{90}Y by γ-emitting isotopes such as ^{87}Y or ^{88}Y and performing measurements by means of γ-scintigraphy [11]; or (e) extrapolating from pharmacokinetic data of chemically similar indium-111 radiopharmaceuticals for the dosimetric calculation of the ^{90}Y radiopharmaceuticals [12–17]. As regards approach (e), differences in the biodistribution between ^{111}In and ^{90}Y-labelled antibodies were recently reported [18], these differences possibly being due to the different in vivo stabilities of the chemically different co-ordination compounds.

While all these imaging approaches provide qualitatively relevant data they are less appropriate to provide quantitative uptake data. However, it is the inherent advantage of positron emission tomography (PET) to assess the uptake kinetics for individual organs quantitatively in vivo. This approach was already successfully applied to analyse the pharmacokinetics of yttrium-90 radiopharmaceuticals in humans, using the positron-emitting isotope yttrium-86 ($T_{1/2}$= 14.74 h, 33% β^+) as isotopic surrogate [19,20]. Consequently, we regarded the use of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide and PET to be the most authentic quantitative approach to measure the pharmacokinetics of [^{90}Y]DOTA-DPhe^1-Tyr^3-octreotide in baboons as a prerequisite for the calculation of radiation doses caused by the [^{90}Y]DOTA-DPhe^1-Tyr^3-octreotide analogue. At the time the study was designed, [^{90}Y]DOTA-DPhe^1-Tyr^3-octreotide was not yet approved for human use. This confined us to the presented comparably limited study protocol in primates.

Materials and methods

Synthesis of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide. The ^{86}Y was produced as described previously [21, 22] at the Jülich compact cyclotron CV28. DOTA-DPhe^1-Tyr^3-octreotide (SMT487) was synthesised at Novartis Pharma, Switzerland [23]. The chemical structure is illustrated in Fig. 1.

A stock solution of 1.47 m*M* SMT 487 in H_2O/HAc_{conc}/MeOH (100/1/1) was prepared. Depending on the experiment, small amounts of the stock solution were added to defined portions of 0.15 *M* NH_4OAc solution, pH 4.5, containing 0.3% bovine serum albumin (BSA). After transferring about 1–3 mCi of the no-carrier-added (n.c.a.) ^{86}Y in 0.15 *M* α-hydroxyisobutyric acid, the vial was closed and the reaction mixture was heated for 15 min at 100°C in a water bath. After complexation had been completed, 1 µl of the reaction mixture was added to 100 µl of the labelling buffer.

Quality control. Five microlitres of this solution was mixed with 100 µl of a 4 m*M* DTPA solution, pH 4.5, and quality control was performed using high-performance liquid chromatography (HPLC) [Nucleosil RP C-18, 5 µ; buffer A: 0.025 *M* NH_4OAc, pH 4.5; buffer B: MeCN/H_2O/1 *M* NH_4OAc, pH 4.5 (60/40/2); 1.2 ml/min] with a gradient of 0–15 min; 0–60% B, 15–20 min 60% B, 20–25 min 60–80% B (Table 1). Elution profiles were recorded by UV as well as by radioactivity detectors. Under these conditions two peaks were found with retention times of 3.6±0.2 min (^{86}Y-DTPA) and 13.1±0.2 min ([^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide).

The labelling was highly effective, with typical labelling yields of >98.5%. Specific activities of 28 GBq/µ*M* and 0.5 GBq/µ*M* were reached for the two labelling conditions of 2 and 100 µg SMT487 per m^2 body surface, respectively. Prior to the administration to the baboons the reaction mixture was diluted with a buffer solution (10 m*M* HEPES, pH 7.6; 1% BSA) to a final injection volume of 500 µl per monkey. For each protocol, the baboons received 10–40 MBq [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide.

Animals. Two male and one female baboons (*Papio hamadryas*) took part in this study. Their ages were between 3 and 4 years and the weights 12.4 kg, 9.1 kg and 7.9 kg. The baboons came from the German primate centre in Göttingen and stayed in the Forschungszentrum Jülich for 4 weeks. The principles of laboratory animal care were followed.

Treatment groups. All animal experiments were carried out according to the *Deutsches Tierschutzgesetz*. In particular, only one

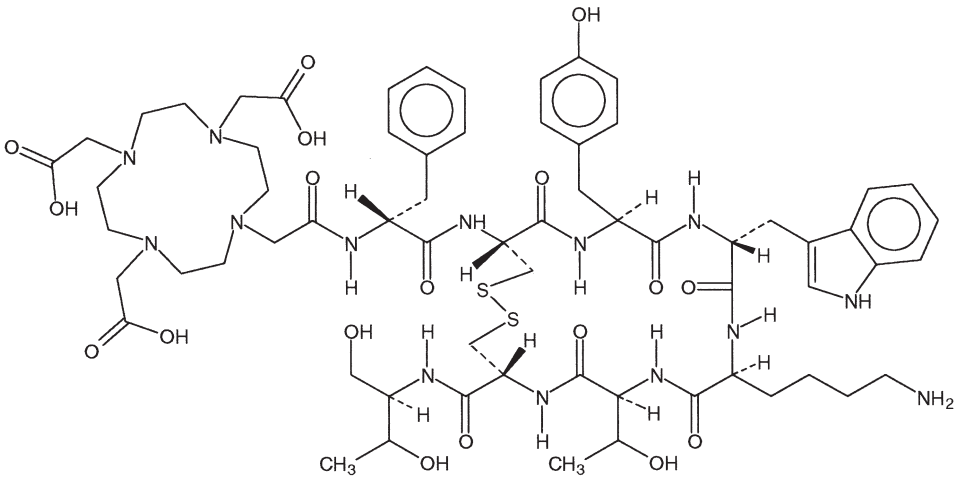

Fig. 1. Structure of DOTA-DPhe^1-Tyr^3-octreotide (SMT487)

Table 1. Treatment groups and protocols

Baboon	Body weight (kg)	PET measurement (h p.i.)	Activity injected (MBq[a])		
			100 µg/m² SMT487	100 µg/m² SMT487 + amino acid infusion	2 µg/m² SMT487
A (male)	12.4	0–1.5	38.3	17.5	14.6
B (female)	9.1	5	27.1	22.6	9.9
C (male)	7.9	24 or 17[b]	27.1	23.4	16.3[b]

[a] 1 MBq = 27 µCi
[b] PET measurement for baboon C in the 2 µg/m² SMT487 protocol at 17 h instead of 24 h

anaesthetic procedure per week was permissible, lasting for only some hours per experiment and animal. Therefore the same animal could not be imaged several times over a period of 24 h. It was, therefore, the strategy of the study to analyse one and the same animal at identical periods post injection (p.i.) of the tracer and to compare the biokinetic data for three different experimental protocols. Each experimental protocol was performed at 1 week, the three different experimental protocols thus being carried out within three consecutive weeks. For each protocol the three baboons (A, B, C) were injected nearly simultaneously, but measured at different time points p.i.. Table 1 gives an overview of the different treatment protocols.

In the first and second weeks, [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide was administered at two different compositions with regard to the concentration of the peptide, namely 2 and 100 µg SMT487 per m² body surface. The 100 µg SMT487 per m² dose reflects the peptide dose in a radiotherapeutic dose of [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide, whereas the 2 µg SMT487 per m² dose corresponds to the diagnostic dose range of the peptide of <10 µg/m² as used for ^{111}In-OctreoScan. An average body surface of a baboon of about 0.4 m² was assumed. Therefore, in the experiments using 100 µg/m² SMT487 the injected solution contained 40 µg of SMT487 while in the experiment using 2 µg/m² SMT487 the injected solution contained 0.8 µg SMT487, in both cases in 500 µl buffer.

In the third-week protocol the 100 µg/m² SMT487 dose was administered together with an amino acid solution (Nephroplasmal N7%) that was high in L-lysine (8 g/l). We assumed that the pharmacokinetics of L-lysine are similar to those of small peptides (or even faster). From rodent experiments it was known that high doses of L-lysine (2 g/kg in mice) were required [24] and that the blood clearance of radiolabelled SMT 487 was fast. Since the duration of the anaesthesia in primates was restricted, the amino acid infusion was maintained for 30 min, starting 15 min before and ending 15 min after the administration of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide with an infusion rate of 0.5 ml/min.

Anaesthesia. General anaesthesia was induced using ketamine (10 mg/kg, i.m.). Salivary secretion was reduced by intramuscular glycopyrrolate (Robinul 0.02 mg/kg). Prior to intubation 2–3 mg/kg methohexital was injected intravenously. During the transportation to the PET scanner the anaesthesia was maintained by further bolus injections of methohexital (2 mg/kg i.v.). During the PET measurement anaesthesia was maintained at a constant level by inhalation anaesthesia (isoflurane, nitrous oxide and oxygen). The animals were monitored by EKG, pulse oxymetry, capnography and invasive and non-invasive measurements of blood pressure. After the PET measurements the animals were transported back to their cages and continuously supervised until they woke up.

Blood and urine measurements. Blood samples were taken from the baboons before the injection as well as at 24 h and at 4 weeks post injection of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide and subjected to conventional clinical chemistry analysis. Differential blood counts, measures of kidney function (creatinine, urea, uric acid, cholesterol, triglycerides, bilirubin, total protein, Fe, Na, K, Ca, Cl, P), liver enzymes (aspartate aminotransferase, alanine, transaminase, γ-glutamyl transpeptidase and alkaline and acidic phosphatases) and blood sugar were determined. The blood cell counts in the baboons were normal for all time points, showing no major differences from the values as measured in normal males/females.

Simultaneously with the PET measurements, blood was sampled from the femoral artery at 0.5, 1, 1.5, 2, 4, 6, 10, 15, 30, 60 and 90 min p.i. for baboon A, at 5 and 6.5 h for baboon B and at 24 or 17 h p.i. for baboon C. Aliquots of each of these samples were subjected to radiochemical analysis (see below). The radioactivity in the blood pool at each time was calculated from the measured activity per unit volume in the blood samples and the estimated blood volume as 7% of total body weight. The blood-activity curves were fitted by two-exponential functions from 0 to 90 min p.i. Later blood data of baboons B and C were not included in these fits because of possible errors due to the inter-individual variabilty. The time constants resulting from the fits were converted into elimination half-lifes $T_{1/2\alpha}$ and $T_{1/2\beta}$, describing a fast and a slow elimination phase for the kinetics measured in baboon A, i.e. covering the blood clearance kinetics up to 1.5 h post injection.

Individual blood radioactivity data are expressed as %ID/ml. Any individual errors and significance tests are included because of the one-animal-per-experiment character of the study. However, individual experimental errors can be derived for every individual measurement. The uptake values given in %ID for kidney, liver and skeleton are derived from PET measurements with individual errors of less than 15%. The blood clearance and urinary excretion kinetics were calculated by γ-spectroscopy with average errors of less than 10% for time points up to 5 h p.i. and less than 15% at 24 (or 17) h p.i. Because these values became the basis for further calculations, elimination half-life errors are of the same order of magnitude.

Urine was collected via catheter (baboons A and C) or by periodically pressing the bladder (female baboon B) only during the period of the corresponding PET measurements. Radioactivity in 1-ml aliquots of urine was measured by γ-spectrometry. The urine could not be collected continuously over the whole period of investigation, i.e. for one entire day after injection, because this would have required catheterisation of the animal under repeated anaesthesia, which is not allowed by the *Deutsches Tierschutzgesetz*.

Stability of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide in vivo. Blood samples were centrifuged at 4300 rpm for 20 min without clotting

protection. The supernatant serum was treated with a mixture of MeOH/MeCN (1/1) and again centrifuged to separate the precipitated serum proteins. Finally, 100 µl of the supernatant protein-free solution was mixed with 50 µl of a 4 m*M* DTPA solution, pH 4.5, and analysed by HPLC for quality control. The [^{86}Y]DTPA and the [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide fractions were collected and the amount of the two fractions was determined in percent of injected activity. Blood platelets, serum proteins and the collected fraction were assayed by an automatic NaI(Tl) counter. Urine samples were taken 60 min, 90 min, 5 h and 17 h p.i. Fifty microlitres of a 4 m*M* DTPA solution was added to 100–200 µl of the urine. This mixture was analysed by HPLC.

PET measurements. The PET measurements were performed with a GE scanner 4096 plus, which records 15 image planes simultaneously within an axial field of view of 10.5 cm [25]. Prior to the emission measurements, transmission scans were acquired over 10 min using a rotating line source filled with germanium-68, so that measured attenuation correction could be applied. Each series of emission scans was started with an injection of 10–40 MBq [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide. In baboon A dynamic PET scans were acquired after tracer injection for 90 min. During a repetitive sequence of three scans of 2–5 min each the scanner was positioned over the head, the abdomen and the pelvis. The head position was included to look for possible accumulation of the tracer in the pituitary. The abdominal position included the liver and kidneys, while the pelvic position included the caudal parts of the liver, the kidneys and the bladder. At 1 h a continuous whole-body scan ranging from the head to the pelvis was done so that the upper and lower thorax could also be imaged. This sequence of PET measurements was performed in animal A in each week.

Baboon B was measured at about 5 h after injection. In this case the whole-body transmission measurement was followed by a whole-body emission scan, again ranging from the head to the pelvis, so that the bladder was included as well. The same procedure was repeated in baboon C at 17 h or 24 h p.i.

Image reconstruction. The resolution of the PET images reconstructed with filtered backprojection after attenuation correction was about 7 mm. Using all 75 image planes of the whole-body scans, reprojected body views [19] were calculated in order to delineate areas with enhanced tracer uptake. Regions of interest (ROIs) were placed over areas where distinct activity concentrations became visible, i.e. over the kidneys, liver, spine, humerus and bladder. All regions were defined in at least three adjacent images. By applying all ROIs to the dynamic image data, decay-corrected time-radioactivity curves were derived in the unit of activity concentration, i.e. kBq/ml.

The ROI over the liver was marked within the organ. For kidneys and bladder the ROI borders coincided with the organs' borders estimated from the activity distribution. ROIs over the bone were defined using an isocontour level of 60% of the image maximum. In addition all transverse slices of the whole-body scans at 5 h and 24 or 17 h p.i. were evaluated as described above to obtain the cumulative whole-body activity. In the scans of baboon A the pituitary could just be identified, but was overlaid with so much noise that no attempt was made at analysis.

To determine the whole-body activity, the whole-body scans recorded at 4 h and 24 h or 17 h were directly evaluated. In all transverse slices, ROIs were defined comprising the body shape as visible in the transmission scan. The product of ROI mean (unit kBq/cm^{3}) and the ROI area (unit cm^{2}) was multiplied by the slice thickness of 6.4 mm to obtain the overall radioactivity of ^{86}Y in each slice. The summation of the activity of all slices resulted in the whole-body activity.

Radiation dose calculations. In order to calculate estimates for the radiation doses of the therapeutic [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide, the decay-corrected time-activity curves of the ^{86}Y-labelled analogue for the organs with visible radioactivity uptake and for the remainder of the body were first determined. The resulting time-activity curves were extrapolated until seven half-times of ^{90}Y (i.e. 448 h) and then multiplied with the decay function of ^{90}Y, so that the residence times could be calculated by integration and by relating the integral to the injected activity. In order to obtain the activity uptake of the single organs the radioactivity concentration data measured by PET had to be multiplied by the organ volumes. In the case of kidneys and the liver the volumes were derived from the areas of ROIs placed along the borders of the organs in all image planes in which the organ was visible. This approach was not possible for the skeleton; its volume was estimated from the volume of the human skeleton by scaling it with the ratio of the baboon's weight and the 70 kg of the reference man [26].

As already explained, it was not possible to scan one animal continuously over 24 h or three times within 24 h. Therefore, the kinetic data of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide had to be combined from all three baboons; this procedure, however, neglects possible variations among the animals. For this reason a conservative exponential function estimation was applied. Instead of fitting the slowly decreasing (decay-corrected) kinetics for liver and kidneys we assumed a constant tracer uptake, calculated as the average of the uptake data at 1 h, 5 h and 24 h p.i. (or 17 h for the 100 µg/m^{2} [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide injection).

In contrast to the kidneys and the liver a considerable release of radioactivity from the bone was observed. Consequently, two-exponential fits were applied to describe its uptake kinetics. Using the time-activity data of the whole body as shown in Table 3, the residence time for the urinary bladder contents was determined according to the model suggested by Cloutier et al. [27]. The residence time for the remainder of the body was calculated as the difference between the whole-body activity and the sum of the radioactivity found in liver, kidneys and bone.

After having determined the residence times for kidneys, liver, bone, bladder and remainder of the body, the radiation doses for a 70-kg adult were calculated by means of the MIRDOSE3 program [28]. The residence time calculated for total bone was equally attributed to cortical and trabecular bone. The bladder dose was determined assuming voiding intervals of 1 h or 0.1 h. However, 1 h is regarded as the shortest feasible interval for discrete voiding. The dose to the bladder might be further reduced by catheterisation. For the purpose of the MIRDOSE3 calculation the voiding interval of 0.1 h is considered as equivalent to catheterisation.

Results and discussion

Blood pharmacokinetics

Individual blood radioactivity data for [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide are summarised in Table 2. The simultaneous infusion of amino acids resulted in an about 1.5-fold decrease in blood pool activity during the first hour p.i. when compared with the administration of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide without co-infusion of amino acids. Within this period more than 90% of the

Table 2. Blood radioactivity of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide ([^{86}Y]SMT487) in individual baboons

Baboon	Time p.i	Blood radioactivity (% ID/ml)		
		100 µg/m^2 SMT487	100 µg/m^2 SMT487 + amino acid infusion	2 µg/m^2 SMT487
A	0.5 min	0.101	0.046	0.034
A	1 min	0.049	0.041	0.037
A	1.5 min	0.054	0.033	0.035
A	2 min	0.043	0.026	0.027
A	4 min	0.028	0.019	0.017
A	6 min	0.026	0.020	0.016
A	10 min	0.019	0.017	0.015
A	15 min	0.017	0.010	0.009
A	30 min	0.013	0.006	0.007
A	60 min	0.008	0.005	0.006
A	90 min	0.004		0.004
B	5 h	0.003	0.003	
B	6 h	0.002		0.005
C	24 (17) h	0.00004	0.0001	0.003

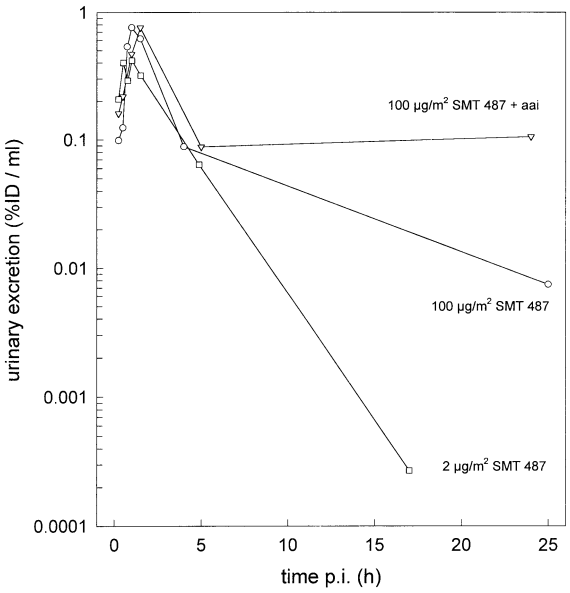

Fig. 2. Rate of urinary excretion of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide ([^{86}Y]SMT487) in percent of the injected dose (%ID) per ml urine in individual baboons. All animals received 10–40 MBq [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide per baboon containing different peptide concentrations. *aai*, Tracer injection with simultaneous amino acid co-infusion

injected [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide was cleared from the circulation after all three treatment protocols. At later time points a greater percentage remained in the blood after the 2 µg/m^2 dose than after either of the 100 µg/m^2 doses.

For all three treatment protocols the clearance of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide from blood was biphasic during the first 90 min p.i. In the initial rapid phase the elimination half-life $T_{1/2\alpha}$ was 0.42 min and 0.95 min for 100 µg/m^2 without and with amino acid infusion, respectively, and 2.4 min for 2 µg/m^2. The second elimination phase was similar in the case of 100 µg/m^2 both with and without amino acid infusion, at 20 min and 23 min, respectively. This slow elimination half-life $T_{1/2\beta}$ was again slower, at 58 min, in the case of the 2 µg/m^2 treatment protocol. Thus, the elimination of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide from the blood was about 2.5 times slower when low peptide amounts (2 µg/m^2) were used. The percentages of the first exponential component were 81%, 66% and 73% for the protocols with 100 µg/m^2 with and without amino acid and with 2 µg/m^2, respectively. The corresponding percentages of the second exponential component were 19%, 34% and 27%, respectively.

Urinary excretion

The individual urinary excretion kinetics of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide in the baboons are shown in Fig. 2 as %ID/ml urine. The rate of excretion peaked at about 1 h p.i. at 0.76 %ID/ml, when [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide was administered without co-infusion of amino acids. After administration of 100 µg/m^2 with simultaneous amino acid infusion, however, the highest excretion rate was observed at 1.5 h p.i. This may point to a lower retention of the peptide within the kidneys owing to the interaction of the amino acids (preferentially L-lysine) with the peptide uptake mechanism located in the kidney cortex [29]. The same observation was made at later time points (e.g. 5 h p.i.) and in particular at about 1 day p.i.. At 24 h (or 17 h) p.i. the urinary excretion rate decreased to very low values of 0.007% ID/ml, 0.1% ID/ml and 0.0003% ID/ml for the protocols with 100 µg/m^2, with 100 µg/m^2 including simultaneous amino acid infusion and with 2 µg/m^2 [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide, respectively. This indicates the importance of the infusion of the amino acid simultaneously with the injection of the radiolabelled octreotide analogue.

Cumulatively, about 17% ID and 33% ID of [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide was excreted within 1.5 h p.i. by the baboons treated with 100 µg/m^2 without and with simultaneous amino acid infusion, respectively. After administration of 2 µg/m^2 [^{86}Y]DOTA-DPhe^1-Tyr^3-octreotide 16% ID was excreted within 1.5 h p.i.

From the whole-body evaluation it is concluded that at 5 h p.i. 80%–84% and at 24 (or 17) h p.i. 93%–95% of the injected activity is excreted probably mainly via the urine and perhaps to a smaller amount via the faeces (Table 3). The different routes of excretion could not be controlled with the animals awake in their cages.

Table 3. [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide ([^{86}Y]SMT487) accumulation in total organs (kidneys, liver and skeleton) and whole-body activity of individual baboons measured in percent of injected activity

Time p.i.	Activity (%ID)											
	100 µg/m^2 SMT487				100 µg/m^2 SMT487 + amino acid infusion				2 µg/m^2 SMT487			
	Kidneys	Liver	Skeleton	Whole body	Kidneys	Liver	Skeleton	Whole body	Kidneys	Liver	Skeleton	Whole body
0 min	0	0	0	100	0	0	0	100	0	0	0	100
3 min	2.4	3.2			2.9	3.0			2.1	3.7		
10 min	1.8	2.0			2.7	2.0			1.6	2.4		
15 min	1.5	1.7			3.5	2.0			1.5	2.0		
25 min	1.5	1.5			2.4	1.6			1.6	2.0		
40 min	1.4	1.3			3.1	1.5			1.6	1.8		
50 min	1.2	1.3	2.2		1.7	1.8	2.0		1.7	1.6	1.5	
80 min	1.2	1.1			1.7	1.5			1.6	1.5		
95 min	1.1	1.0		83	1.4	1.3		70	1.5	1.4		84
ca. 5 h	2.0	1.6	0.9	16	1.2	1.6	1.2	16	2.4	2.6	1.4	20
24 (17) h	1.6	0.8	0.6	7	1.2	0.8	0.4	5	1.7	1.5	1.0	7.4

Stability analysis

The [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide was stable in vitro up to 12 h after labelling. Radiochemical analysis of the serum water fractions showed that at 1.5 h >95% and at 5 h >90% of the remaining activity corresponded to the intact [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide. Negligible amounts of the tracer were bound to blood cells. The in vivo stability of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide in the urine was >99% under all treatment protocols up to 90 min p.i. and >97.5% up to 5 h p.i.

Pharmacokinetics of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide as measured with PET

In the whole-body images predominantly the bladder and kidneys were visible, with faint representation of the liver and skeleton, especially the joints (Fig. 3). The quantitative [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide accumulation in percent of the injected activity in kidneys, liver and skeleton is summarised in Table 3. In the liver a primary uptake of about 4% of the injected activity was found, which rapidly decreased to 1.5%–2%ID for the 100 µg/m^2 protocol both with and without amino acid infusion, whereas the comparable values for the 2 µg/m^2 dose were slightly above 2%ID.

The kidneys are the organ with the highest activity concentration of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide. The uptake ranged from 3.5%–2.1%/ID after 3–30 min p.i. and decreased with time. Up to 1 h p.i. this uptake was higher after administration of 100 µg/m^2 with amino acid co-infusion compared with the other protocols. This effect may reflect the higher clearance rate of the radioligand during the amino acid infusion. However, this uptake kinetics is more correlated to an enhanced renal blood flow as a consequence of the amino acid infusion [30, 31]. In the present study, kidney function was stimulated 15 min before injection, resulting in a much higher tubular filtration rate in the experimental protocol with amino acid co-infusion. Consequently, the kidney uptake between 10 and 40 min p.i. was about twice the uptake measured for the two protocols without amino acid infusion. At about 30 min after the amino acid infusion was stopped (15 min p.i.) and the kidney uptake fell to a more "normal" level, comparable to that with the two other protocols. This seems to have occurred in parallel with to the normalisation of the renal blood flow. After 5 h the activity accumulation had dropped further to levels about half those measured for the two other protocols without amino acid infusion (Table 3). This result is interpreted as an effect of the blockade of binding places in the kidney cortex by cationic amino acid infusion. This, consequently, becomes responsible for the lower radiation dose (see below).

The activity distribution to the kidneys did not markedly decrease at about 1 day p.i. in any of the three experimental protocols. Whereas in the early images radioactivity was seen in the renal pelvis, in the late images it was mainly found in the renal cortex. This is illustrated in the transverse PET images (Fig. 4). Upon administration of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide containing 100 µg/m^2 of the peptide with amino acid co-infusion the highest rate of activity accumulation in the bladder as derived from the PET measurements was found within the first hour p.i.. A lower influx rate was found in the 2 µg/m^2 treatment protocol and in the 100 µg/m^2 protocol without amino acid co-infusion.

Data for activity distribution to the bone were evaluated at the time of the whole-body scans. The total skeletal

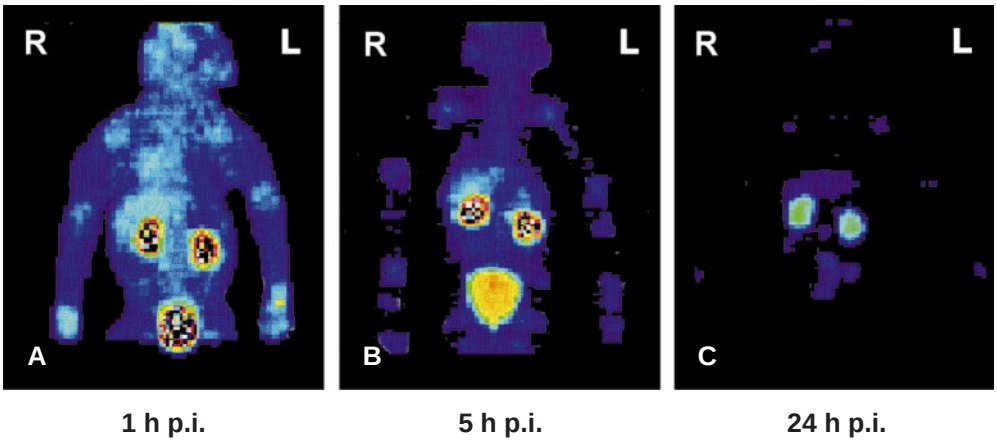

Fig. 3. Whole-body PET scans of baboons A, B and C injected with 10–40 MBq [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide per baboon containing 100 µg/m^2 peptide without simultaneous amino acid co-infusion, recorded at 1 h p.i. (baboon A), 5 h p.i. (baboon B) and 24 h p.i. (baboon C).

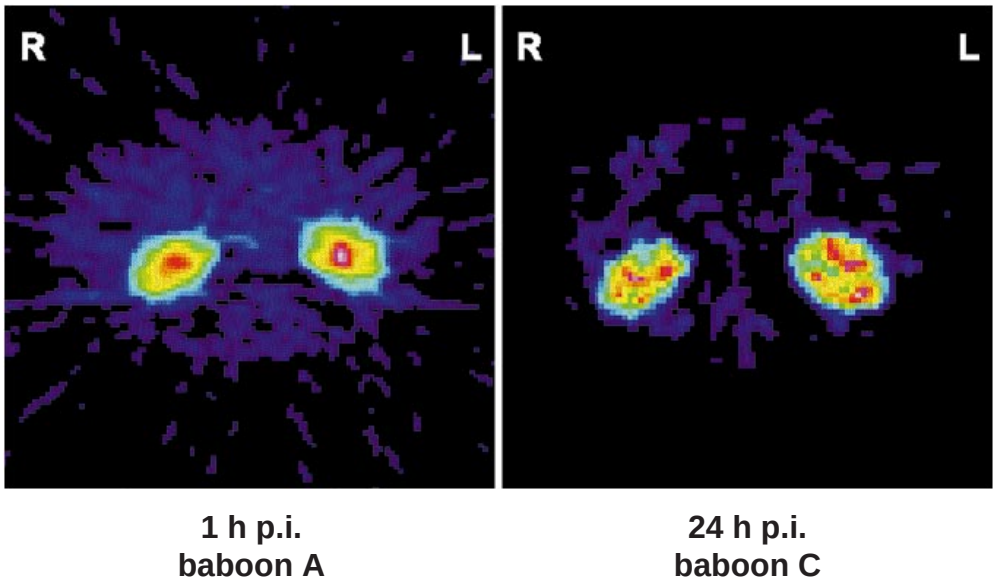

Fig. 4. Transverse PET scans of the kidneys of baboons A and C injected with 10–40 MBq [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide per baboon containing 100 µg/m^2 peptide without simultaneous amino acid co-infusion, recorded at 1 h p.i. (baboon A) and 24 h p.i. (baboon C)

Table 4. Residence times of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide ([^{86}Y]SMT487) in kidneys, liver, lung, bone, urinary bladder and remainder of the body in baboons

Organ	Residence times (h)		
	100 µg/m^2 SMT487	100 µg/m^2 SMT487 + amino acid infusion	2 µg/m^2 SMT487
Kidneys	1.56	1.17	1.83
Liver	1.14	1.20	1.88
Lung	0.16	0.08	0.14
Bone	0.33	0.25	0.43
Urinary bladder[a]	0.27	0.45	0.42
Remainder of the body	5.9	3.1	6.4

[a] 1 h voiding interval

uptake was 1.5%–2.2%ID for the three treatment protocols at 50 min p.i. (Table 3). The activity in the skeleton decreased to about 0.9%–1.4%ID and 0.4%–1.0%ID at 5 h p.i. and 24 (or 17) h p.i., respectively. The residence times for these organs are summarised in Table 4.

The distribution of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide activity to the spleen of the primates was not higher than the background from the abdomen. This is in contrast to the biodistribution and dosimetry found after administration of ^{111}In-OctreoScan to patients [32]. However, it is in line with the distribution of [^{111}In]DOTA-DPhe^{1}-Tyr^{3}-octreotide to the primate spleen [unpublished results].

Radiation doses

Due to study design, the main emphasis was on the comparison of biokinetic data for one and the same animal, but for three different experimental protocols. Because not only one and the same animal but also identical measurement periods were considered, errors in comparison of the biokinetic data (blood clearance, urinary excretion, individual organ uptake kinetics) are negligible. For the first baboon A, i.e. for the early period up to 1.5 h p.i., the increasing effect of amino acid co-infusion on the tracer accumulation in the kidneys could be clearly shown. On the other hand, animal B was used to com-

Table 5. Radiation dose estimates for [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide ([^{90}Y]SMT487), in an adult of 70 kg body weight

Organ	Calculated individual radiation doses (mGy per MBq [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide injected)		
	100 µg/m^2 SMT487	100 µg/m^2 SMT487 + amino acid infusion	2 µg/m^2 SMT487
Kidneys	2.81	2.11	3.30
Liver	0.32	0.34	0.53
Bone marrow	0.07	0.03	0.04
Bladder			
Voiding interval 1 h	0.64	0.65	0.64
Voiding interval 0.1 h	0.09	0.09	0.09
EDE (mSv/MBq)			
Voiding interval 1 h	0.28	0.23	0.32
Voiding interval 0.1 h	0.25	0.20	0.29

[a] EDE, effective dose equivalent

pare organ uptakes for medium time points, i.e. about 5 h p.i; in this case the reduced tracer uptake in the kidneys could obviously be compared for the three different experimental protocols.

Quantification was important for the estimated radiation dose. In this case the organ activity accumulation was averaged for the time points of 95 min, 5 h and 24 (17) h for the three baboons. Thus, inter-individual changes could not significantly affect the calculations. As stated, the resulting values thus reflect maximum estimated radiation doses.

The radiation doses estimated for the therapeutic [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide are listed in Table 5.

The critical organs are the kidneys, with values between 2.1 and 3.3 mGy per MBq injected [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide. The bladder receives average doses of 0.6 mGy/MBq (voiding interval 1 h). The average effective dose equivalent is 230–320 µSv/MBq. The 100 µg/m^2 protocol with amino acid infusion resulted in a kidney dose about 20% less (230 µSv/MBq) than that of the same protocol without amino acid infusion (280 µSv/MBq). The protocol with the lower peptide amount present in the radioligand preparation (2 µg/m^2) resulted in increased radiation doses. This might have been due to the higher specific activity in this preparation.

Conclusion

Using the β^+-emitting ^{86}Y as a substitute for β^--emitting ^{90}Y and in vivo PET measurements the pharmacokinetics and radiation doses of [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide were determined in non-human primates. Although no individual errors and significance tests were considered because of the one-animal-per-experiment character of the study, some conclusions may be derived. The octreotide analogue [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide is highly stable in vivo both in serum and in urine. Binding of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide to blood cells and serum proteins is negligible. Rapid clearance of intact [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide from the blood was observed.

As calculated for a 70-kg human, the highest radiation dose will be delivered to the kidneys and was extrapolated to amount to less than 3 mGy per MBq [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide injected with the 100 mg peptide per m^2 body surface dose. Simultaneous co-infusion of an amino acid solution that is high in cationic amino acids, e.g. L-lysine, will accelerate the whole-body clearance of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide and thereby lower the radiation dose to the kidneys by about 20%. This effect is in accordance with the finding of a reduction in kidney distribution of the radiodiagnostic octreotide analogue ^{111}In-OctreoScan in patients after an amino acid infusion that is high in L-lysine [33]. The reason for this may lie in the competition of the peptide moiety of [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide for uptake into the renal cortex with the cationic amino acids infused during radioligand administration.

Taken together, the high in vivo stability and the comparatively low estimated radiation doses to normal organs are encouraging for the further evaluation of somatostatin receptor-targeted radiotherapy in patients using [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide. A phase I trial is now being conducted to utilise [^{90}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide ([^{90}Y]SMT487) for endoradiotherapy, which is preceded by PET studies using [^{86}Y]DOTA-DPhe^{1}-Tyr^{3}-octreotide ([^{86}Y]SMT487).

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