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PRE-CLINICAL COMPARISON OF [DTPA^0] OCTREOTIDE, [DTPA^0,Tyr^3] OCTREOTIDE AND [DOTA^0,Tyr^3] OCTREOTIDE AS CARRIERS FOR SOMATOSTATIN RECEPTOR-TARGETED SCINTIGRAPHY AND RADIONUCLIDE THERAPY

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We have evaluated the potential usefulness of radiolabelled [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide as radiopharmaceuticals for somatostatin receptor–targeted scintigraphy and radiotherapy. *In vitro* somatostatin receptor binding and *in vivo* metabolism in rats of the compounds were investigated in comparison with [^{111}In-DTPA^0] octreotide. Comparing different peptide–chelator constructs, [DTPA^0,Tyr^3]octreotide and [DOTA^0, Tyr^3]octreotide were found to have a higher affinity than [DTPA^0]octreotide for subtype 2 somatostatin receptors (sst$_2$) in mouse AtT20 pituitary tumour cell membranes (all IC$_{50}$ values obtained were in the low nanomolar range). *In vivo* studies in CA20948 tumor-bearing Lewis rats revealed a significantly higher uptake of both ^{111}In-labelled [DOTA^0,Tyr^3]octreotide and [DTPA^0,Tyr^3]octreotide in sst$_2$-expressing tissues than after injection of [^{111}In-DTPA^0]octreotide, showing that substitution of Tyr for Phe at position 3 in octreotide results in an increased affinity for its receptor and in a higher target tissue uptake. Uptake of ^{111}In-labelled [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide in pituitary, pancreas, adrenals and tumour was decreased to less than 7% of control by pre-treatment with 0.5 mg unlabelled octreotide/rat, indicating specific binding to sst$_2$. Comparing different radionuclides, [^{90}Y-DOTA^0,Tyr^3]octreotide had the highest uptake in sst$_2$-positive organs, followed by the [^{111}In-DOTA^0,Tyr^3]octreotide, whereas [DOTA^0,^{125}I-Try^3]octreotide uptake was low compared to that of the other radiopharmaceuticals, when measured 24 hr after injection. Renal uptake of ^{111}In-labelled [DTPA^0]octreotide, [DTPA^0, Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide was reduced over 50% by an i.v. injection of 400 mg/kg D-lysine, whereas radioactivity in blood, pancreas and adrenals was not affected.
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[^{111}In-DTPA^0]octreotide is a radiopharmaceutical, consisting of the octapeptide octreotide, the chelator DTPA (diethylenetriaminepentaacetic acid) that enables radiolabelling with a radiometal and the radionuclide ^{111}In. It is being used for scintigraphic imaging of somatostatin receptor–positive lesions (Krenning *et al.,* 1993). A new application is the use of radiolabelled octreotide for radionuclide therapy; promising results have been reported in human and rat studies, in which octreotide radiolabelled with ^{111}In, ^{90}Y or ^{64}Cu was used, respectively (Krenning *et al.,* 1996; Stolz *et al.,* 1996; Anderson *et al.,* 1996). Favourable effects on tumour growth and on survival were shown. However, the Auger electron emitter ^{111}In, used in the human studies, is probably not the optimal radionuclide for radiotherapy; the β^- particle emitter ^{90}Y with a maximum β^- energy of 2.3 MeV and a half-life of 64 hr may be more suitable. As ^{90}Y-DTPA is not stable, resulting in haematopoietic toxicity *in vivo* (Rowlinson *et al.,* 1989; Jowsey *et al.,* 1958), octreotide has been derivatized recently with the DOTA (tetraazacyclododecanetetraacetic acid) chelator for stable radiolabelling with ^{90}Y. In addition, Phe^3 in octreotide was replaced by Tyr^3 to increase hydrophilicity and to enable radioiodination. Initial experiments with this compound showed favourable characteristics with regard to receptor binding and biodistribution in rats (De Jong *et al.,* 1997).

We have now studied the binding of [DOTA^0,Tyr^3]octreotide and [DTPA^0,Tyr^3]octreotide to subtype 2 somatostatin receptors (sst$_2$) on murine AtT20 pituitary tumour cell membranes in comparison with that of [DTPA^0]octreotide. This enabled us to discriminate between the effects caused by different chelators (DTPA *vs.* DOTA) and those caused by Phe^3→Tyr replacement. Biodistribution in rats of ^{111}In-labelled [DOTA^0,Tyr^3]octreotide and [DTPA^0, Tyr^3] octreotide was compared with that of [^{111}In-DTPA^0]octreotide. Furthermore, the effect of radiolabelling of [DOTA^0,Tyr^3]octreotide with ^{90}Y and ^{125}I on biodistribution was investigated and compared to ^{111}In-labelling.

[^{111}In-DTPA^0]octreotide is cleared rapidly from the body, mostly by the kidneys. However, a significant amount of the dose accumulates in the kidneys, reducing both the scintigraphic sensitivity of [^{111}In-DTPA^0]octreotide for detection of small tumours in the peri-renal region in the abdomen and its application for radionuclide therapy. It has been reported repeatedly that renal accumulation of peptides or proteins labelled with radiometals can be reduced by L-lysine (Hammond *et al.,* 1993; Pimm and Gribben, 1994; Behr *et al.,* 1995; De Jong *et al.,* 1995, 1996; Bernard *et al.,* 1997). We compared the effects of D-lysine on kidney uptake of ^{111}In-labelled [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide as well as on uptake of the radiopharmaceuticals in sst$_2$-positive organs.

MATERIAL AND METHODS

Labelling of octreotide derivatives

[DTPA^0]octreotide and $^{111}InCl_3$ were provided by Mallinckrodt (Petten, The Netherlands) and octreotide by Sandoz (Basel, Switzerland). [DOTA^0,Tyr^3]octreotide was synthesised by one of us (H.M., the synthesis details will be published elsewhere), and [DTPA^0,Tyr^3]octreotide was from Mallinckrodt Medical (St. Louis, MO). $^{90}YCl_3$ was obtained from Nordion (Kanata, Canada). ^{111}In-labelling of [DTPA^0]octreotide and [DTPA^0,Tyr^3]octreotide (Bakker *et al.,* 1991), ^{90}Y- and ^{111}In-labelling of [DOTA^0, Tyr^3]octreotide (De Jong *et al.,* 1997) and ^{125}I-labelling of [DOTA^0,Tyr^3]octreotide (Bakker *et al.,* 1990) were performed as described.

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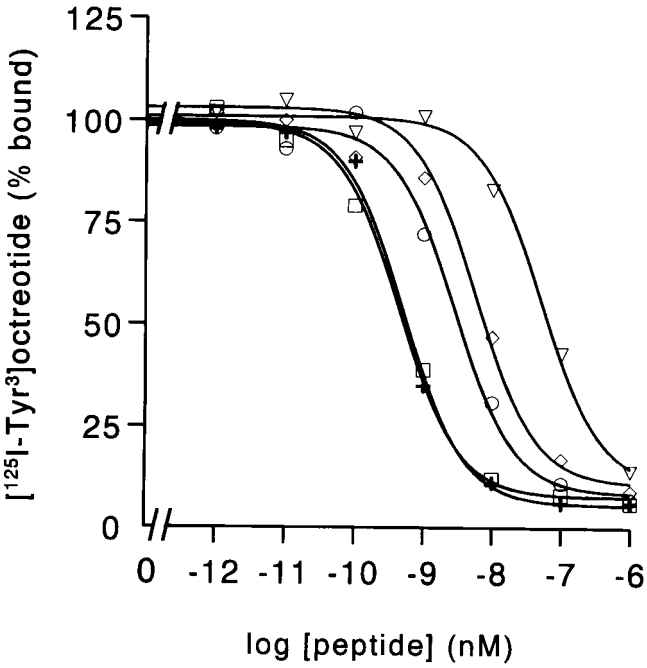

FIGURE 1 – Displacement curve of binding of [^{125}I-Tyr^3]octreotide to murine AtT20 pituitary cell membranes. Results are means of triplicate measurements. □, octreotide; +, [Tyr^3]octreotide; ○, [DOTA^0, Tyr^3]octreotide; ◇, [DTPA^0,Tyr^3]octreotide; ▽, [DTPA^0] octreotide.

In vitro *receptor-binding studies*

Receptor-binding assays were carried out using [^{125}I-Tyr^3]octreotide (2,200 Ci/mmol) as a radioligand and murine AtT20 pituitary tumour cell membrane preparations as a source of sst_2 (Hofland *et al.*, 1992).

In vivo *tissue distribution*

Animal experiments were performed in compliance with the regulations of our institution and with generally accepted guidelines governing such work. Male Lewis rats, bearing the CA20948 pancreatic tumour, or male Wistar rats (200 to 250 g) were used in the experiments. Rats were injected under ether anaesthesia with about 3 MBq (range 1 to 8 MBq, peptide mass 0.5 µg) of ^{90}Y-, ^{125}I- or ^{111}In-labelled [DOTA^0,Tyr^3]octreotide, [^{111}In-DTPA^0, Tyr^3] octreotide or [^{111}In-DTPA^0]octreotide in 200 µl saline into the dorsal vein of the penis. To determine non-specific binding of the radiopharmaceutical, a separate group of rats was injected s.c. with 0.5 mg octreotide/rat in 1 ml 0.05 M acetic acid in saline, 30 min before injection of the labelled peptide. For reduction of kidney uptake, 400 mg/kg D-lysine chloride (Sigma, St. Louis, MO) were co-injected with the radiopharmaceutical. At the indicated time points, rats were killed under ether anaesthesia. Organs and blood were collected, and the radioactivity in these samples was determined (in the case of ^{90}Y as bremsstrahlung after compensation for differences in tissue volumes) using an LKB (Bromma, Sweden)-1282-Compu-gammasystem.

Statistical analysis

Results are expressed as mean ± SEM of at least 4 rats per group. Statistical evaluation was performed using 1-way ANOVA followed by comparison among class means and Student's *t*-test, corrected for multiple pairwise comparisons between means.

TABLE I – RADIOACTIVITY IN ORGANS, BLOOD AND TUMOUR 4, 24 OR 48 HR AFTER INJECTION OF [^{111}In-DOTA^0,Tyr^3]OCTREOTIDE (DOTATOC), [^{111}In-DTPA^0,Tyr^3]OCTREOTIDE (DTPATOC) OR [^{111}In-DTPA^0]OCTREOTIDE (DTPAOC) IN CA20948 TUMOUR-BEARING RATS (FOR EACH GROUP n ≥ 4)

	Time (hr)	Radioactivity (% ID/g)		
		DTPAOC	DTPATOC	DOTATOC
Blood	4	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
	24	0.003 ± 0.000	0.003 ± 0.001	0.002 ± 0.001
	48	0.002 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Kidneys	4	2.02 ± 0.05	1.54 ± 0.07[1]	2.58 ± 0.07[1,2]
	24	1.91 ± 0.11	1.52 ± 0.05[1]	2.32 ± 0.13[1,2]
	48	1.32 ± 0.05	1.09 ± 0.04	2.01 ± 0.07[1,2]
Liver	4	0.09 ± 0.006	0.06 ± 0.003[1]	0.08 ± 0.001
	24	0.05 ± 0.003	0.03 ± 0.001	0.05 ± 0.000
	48	0.02 ± 0.002	0.03 ± 0.002	0.03 ± 0.005
Pancreas	4	0.99 ± 0.02	3.51 ± 0.17[1]	2.57 ± 0.08[1,2]
	24	0.69 ± 0.06	2.40 ± 0.08[1]	1.70 ± 0.13[1,2]
	48	0.47 ± 0.01	1.95 ± 0.04[1]	1.15 ± 0.08[1,2]
Spleen	4	0.03 ± 0.002	0.03 ± 0.001	0.03 ± 0.001
	24	0.03 ± 0.002	0.03 ± 0.002	0.04 ± 0.001
	48	0.02 ± 0.001	0.02 ± 0.001	0.03 ± 0.002
Adrenals	4	1.69 ± 0.04	6.53 ± 0.52[1]	3.62 ± 0.14[1,2]
	24	1.33 ± 0.10	5.83 ± 0.12[1]	3.19 ± 0.27[1,2]
	48	0.59 ± 0.01	3.35 ± 0.12[1]	1.80 ± 0.13[1,2]
Pituitary	4	0.53 ± 0.02	2.10 ± 0.02[1]	1.48 ± 0.07[1,2]
	24	0.51 ± 0.01	1.65 ± 0.06[1]	1.22 ± 0.03[1,2]
	48	0.25 ± 0.02	1.68 ± 0.08[1]	1.19 ± 0.03[1,2]
Stomach	4	0.14 ± 0.006	0.92 ± 0.02[1]	0.52 ± 0.01[1,2]
	24	0.15 ± 0.01	0.48 ± 0.03[1]	0.25 ± 0.04[1]
	48	0.05 ± 0.004	0.24 ± 0.03[1]	0.15 ± 0.02[1]
Femur	4	0.03 ± 0.005	0.13 ± 0.01[1]	0.07 ± 0.01[1,2]
	24	0.03 ± 0.003	0.09 ± 0.01[1]	0.05 ± 0.01[1,2]
	48	0.02 ± 0.002	0.09 ± 0.01[1]	0.05 ± 0.01[1,2]
Thymus	4	0.02 ± 0.002	0.08 ± 0.004[1]	0.04 ± 0.005[1,2]
	24	0.01 ± 0.002	0.03 ± 0.002[1]	0.03 ± 0.004[1]
	48	0.007 ± 0.005	0.02 ± 0.001[1]	0.02 ± 0.005[1]
Tumour	4	0.79 ± 0.01	0.94 ± 0.1	1.15 ± 0.16[1]
	24	0.58 ± 0.08	0.96 ± 0.1[1]	1.12 ± 0.11[1]
	48	0.27 ± 0.04	0.46 ± 0.03[1]	0.51 ± 0.04[1]

[1]$p < 0.01$ *vs.* [^{111}In-DTPA^0]octreotide.–[2]$p < 0.01$ *vs.* [^{111}In-DTPA^0,Tyr^3]octreotide.

RESULTS

Labelling of [DOTA^0,Tyr^3]octreotide

The ^{90}Y and ^{111}In-labelling efficiency of [DOTA^0,Tyr^3]octreotide, [DTPA^0,Tyr^3]octreotide and [DTPA^0]octreotide ranged from 97% to 100%. After labelling with ^{125}I, 100% ^{125}I-labelled compound was obtained by SEP-PAK purification as described previously (Bakker *et al.*, 1990). The radiopharmaceuticals were found to be pure by high-performance liquid chromatography (HPLC).

In vitro *receptor-binding studies*

Figure 1 shows the progressive displacement of [^{125}I-Tyr^3]-octreotide in murine AtT20 pituitary cell membranes along with increasing concentrations of unlabelled peptides. The IC_{50} values obtained for [DTPA^0,Tyr^3]octreotide, [DOTA^0,Tyr^3]octreotide, octreotide and [Tyr^3]octreotide were in the nanomolar range and significantly lower than that of [DTPA^0]octreotide.

Tissue distribution of ^{111}In-labelled octreotide derivatives in rats

Table I and Figure 2*a* present the radioactivity in organs, tumours and blood expressed as percent injected dose (ID) per gram tissue 4, 24 and 48 hr after injection of [^{111}In-DOTA^0,Tyr^3]octreotide, [^{111}In-DTPA^0,Tyr^3]octreotide or [^{111}In-DTPA^0]octreotide. Uptake in the sst_2-expressing pituitary, pancreas, adrenals and tumours at the time points tested was highest with [^{111}In-DTPA^0,Tyr^3]octretotide and lowest with [^{111}In-DTPA^0]octreotide. Figure 2*b* shows that radioactivity in the target organs 24 and 48 hr after injection, expressed as a percent of the 4 hr value, is comparable for the 3 compounds tested.

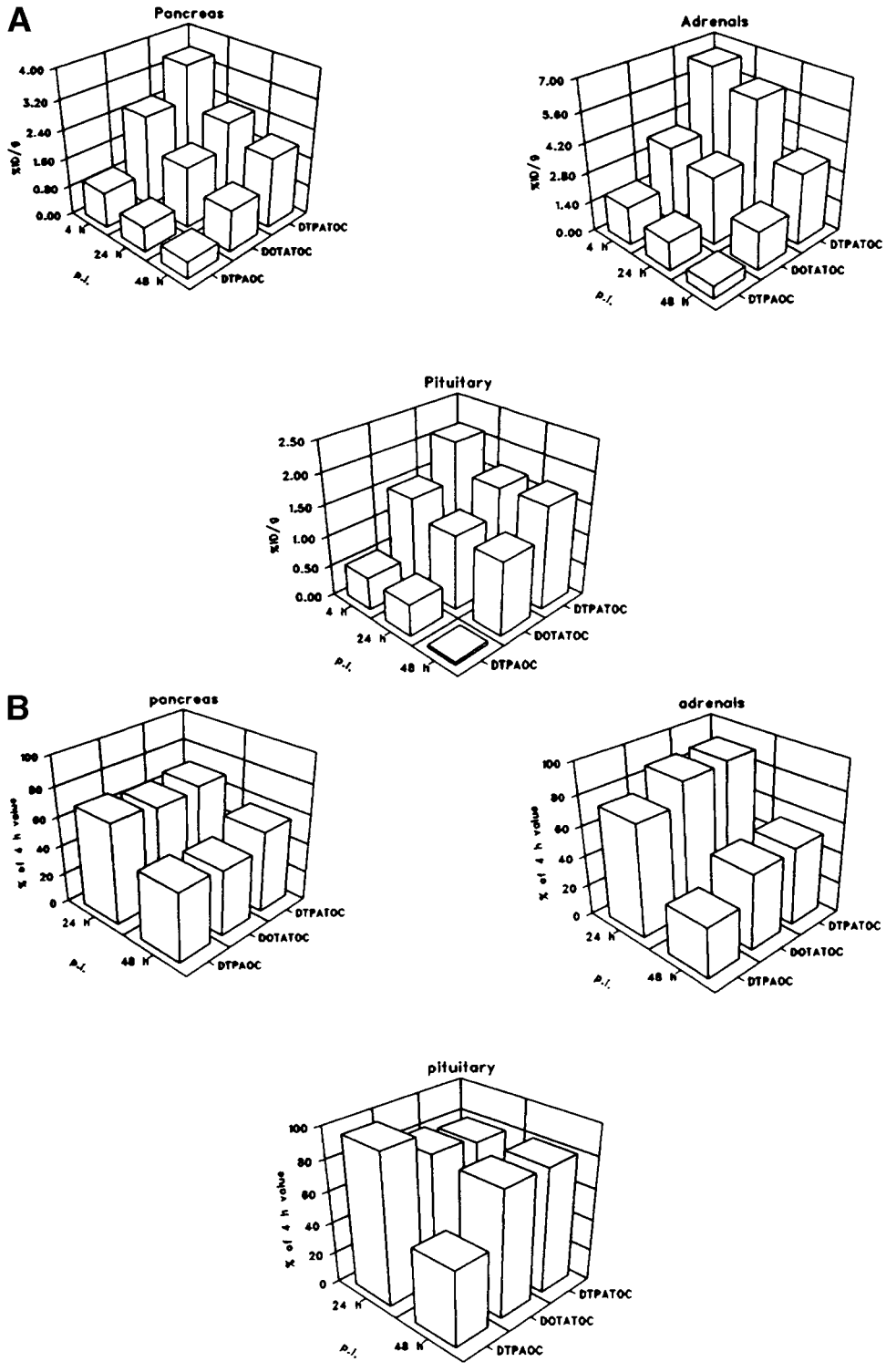

FIGURE 2 – *(a)* Radioactivity in sst_2-expressing organs 4, 24 or 48 hr after injection of [^{111}In-DOTA^0,Tyr^3]octreotide (DOTATOC), [^{111}In-DTPA^0,Tyr^3]octreotide (DTPATOC) or [^{111}In-DTPA^0]octreotide (DTPAOC) in rats (data from Table I). *(b)* Radioactivity in sst_2-expressing organs 24 or 48 hr after injection of [^{111}In-DTPA^0]octreotide (DTPAOC) in rats [^{111}(data from Table I, expressed as % of 4 hr value).

Specific binding in vivo

Table II shows that uptake of ^{111}In-labeled [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide in pancreas, pituitary, adrenals and tumours represents mostly specific binding to the sst_2 as uptake was decreased to less than 7% of control by pre-treatment of rats with 0.5 mg unlabelled octreotide. This pre-treatment had no significant effects on radioactivity in sst_2-negative organs.

TABLE II – RADIOACTIVITY IN SOMATOSTATIN RECEPTOR-POSITIVE ORGANS OF CA20948 TUMOUR-BEARING RATS 24 HR AFTER ADMINISTRATION OF [^{111}In-DOTA^0,Tyr^3]OCTREOTIDE (DOTATOC), [^{111}In-DTPA^0,Tyr^3]OCTREOTIDE (DTPATOC) OR [^{111}In-DTPA^0]OCTREOTIDE (DTOAOC)

	Radioactivity (% of control)		
	DTPAOC	DTPATOC	DOTATOC
Pituitary	6.9 ± 0.7[1]	4.7 ± 0.4[1]	1.6 ± 0.04[1]
Pancreas	3.5 ± 0.03[1]	0.9 ± 0.03[1]	1.0 ± 0.02[1]
Adrenals	1.5 ± 0.02[1]	4.0 ± 0.3[1]	1.0 ± 0.04[1]
Tumour	4.3 ± 0.3[1]	4.8 ± 0.4[1]	3.6 ± 0.3[1]

Labelled compound was injected 30 min after s.c. injection of 0.5 mg unlabelled octreotide. Tissue radioactivity in octreotide-pre-treated rats is expressed as % of that in controls (for each group n ≥ 4).–[1] $p < 0.001$ *vs.* control.

TABLE III – RADIOACTIVITY IN SEVERAL ORGANS AND BLOOD OF RATS 24 HR AFTER ADMINISTRATION OF [^{111}In-DOTA^0,Tyr^3]OCTREOTIDE (DOTATOC), [^{111}In-DTPA^0,Tyr^3]OCTREOTIDE (DTPATOC) OR [^{111}In-DTPA^0]OCTREOTIDE (DTPAOC) WITHOUT (CONTROL) OR WITH CO-INJECTION OF 400 mg/kg D-LYSINE

	Radioactivity (% of control)		
	DTPAOC	DTPATOC	DOTATOC
Blood	109.3 ± 6.6	89.0 ± 6.2	100.3 ± 11.3
Kidneys	45.5 ± 2.4[1]	47.8 ± 0.9[1]	37.3 ± 1.6[1]
Pancreas	110.9 ± 9.9	99.8 ± 2.3	102.5 ± 11.4
Adrenals	107.3 ± 8.2	102.9 ± 2.6	115.6 ± 9.4

Data from D-lysine-treated rats are expressed as % of those in controls.–[1] $p < 0.01$ *vs.* control.

Reduction of kidney uptake

Table III shows the distribution of radioactivity in control rats 24 hr after administration of ^{111}In-labelled [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide or [DOTA^0,Tyr^3]octreotide, without or with co-injection of 400 mg/kg D-lysine. Renal uptake of the different labelled peptides was reduced by 54% to 62% by D-lysine, whereas radioactivity in blood, pancreas and adrenals (both sst_2-positive) and sst_2-negative organs (not shown) was not affected.

Tissue distribution of [DOTA^0,Tyr^3]octreotide labelled with different radionuclides

Table IV presents the radioactivity in organs, tumours and blood 24 hr after injection of ^{125}I-, ^{90}Y-, or ^{111}In-labelled [DOTA^0,Tyr^3]octreotide. Uptake in the sst_2-positive organs was highest for ^{90}Y-labelled [DOTA^0,Tyr^3]octreotide, followed by that of [^{111}In-DOTA^0,Tyr^3]octreotide, whereas radioactivity 24 hr after injection of [DOTA^0,^{125}I-Tyr^3]octreotide was the lowest of the 3 compounds tested ($p < 0.001$ compared to the other 2 radiopharmaceuticals).

TABLE IV – RADIOACTIVITY IN ORGANS, BLOOD AND TUMOUR 24 HR AFTER INJECTION OF [^{111}In-DOTA^0,Tyr^3]OCTREOTIDE (^{111}In), [^{90}Y-DOTA^0,Tyr^3]OCTREOTIDE (^{90}Y) OR [DOTA^0,^{125}I-Tyr^3]OCTREOTIDE (^{125}I) IN CA20948 TUMOUR-BEARING RATS (FOR EACH GROUP n ≥ 4)

	Radioactivity (% ID/g)		
	^{125}I	^{111}In	^{90}Y
Blood	0.01 ± 0.001	0.002 ± 0.00[1]	0.0008 ± 0.000[1,2]
Kidneys	0.08 ± 0.003	2.32 ± 0.13[1]	1.40 ± 0.05[1,2]
Liver	0.02 ± 0.001	0.05 ± 0.00[1]	0.06 ± 0.01[1]
Pancreas	0.24 ± 0.011	1.70 ± 0.13[1]	1.97 ± 0.17[1]
Spleen	0.02 ± 0.001	0.04 ± 0.00[1]	0.07 ± 0.02[1,2]
Adrenals	0.16 ± 0.013	3.19 ± 0.27[1]	6.76 ± 0.27[1,2]
Pituitary	0.81 ± 0.033	1.22 ± 0.03[1]	2.79 ± 0.21[1,2]
Stomach	0.05 ± 0.000	0.25 ± 0.04[1]	0.35 ± 0.04[1]
Femur	0.007 ± 0.000	0.05 ± 0.01[1]	0.08 ± 0.03[1]
Thymus	0.007 ± 0.000	0.03 ± 0.00[1]	0.03 ± 0.00[1]
Tumour	n.a.[3]	1.12 ± 0.11[1]	1.63 ± 0.25[1,2]

[1] $p < 0.01$ or better *vs.* [DOTA^0-^{125}I-Tyr^3]octreotide.–[2] $p < 0.01$ or better *vs.* [^{111}In-DOTA^0,Tyr^3]octreotide.–[3] n.a. = not available.

DISCUSSION

Somatostatin receptors (sst) are integral membrane glycoproteins. Five human sst types that bind native somatostatin (SS_{14}) and its 28-amino-acid precursor (SS_{28}) with high affinity have been cloned, but their affinity for somatostatin analogues differs considerably (Yamada *et al.,* 1993; Bruno and Berelowitz, 1993). Octreotide binds with high affinity to sst_2 and with low affinity to sst_3 and sst_5, while it does not bind to sst_1 and sst_4 (Yamada *et al.,* 1993; Bruno and Berelowitz, 1993). Octreotide scintigraphy, therefore, is based on the visualisation of octreotide-binding somatostatin receptors, predominantly sst_2. Currently, radionuclide therapy of sst_2-positive lesions is explored by repeated administration of high doses of radiolabelled octreotide. Promising results have been reported in both humans and rats (Krenning *et al.,* 1996; Stolz *et al.,* 1996; Anderson *et al.,* 1996). Anderson *et al.* (1996) found up to 100% longer survival of tumour-bearing rats after radionuclide therapy with [^{64}Cu-TETA^0]octreotide. Stolz *et al.* (1996) obtained similar findings after treatment of rats with [^{90}Y-DTPA^0-benzyl-acetamido,Tyr^3]octreotide. We have investigated the effect of radionuclide therapy in rats, using [^{111}In-DTPA^0]octreotide, on the development and growth of sst_2-positive tumours (CA20948) inoculated in the liver. Significantly fewer tumours were found in animals treated on day 1 and/or 8 after inoculation with 370 MBq [^{111}In-DTPA^0]octreotide (data not shown). As for radionuclide therapy in humans, we treated 20 end-stage patients with mainly neuroendocrine tumours with a cumulative dose of up to 53 GBq [^{111}In-DTPA^0]octreotide per patient in a phase I trial (Krenning *et al.,* 1996). There were no major side effects after up to 2 years of treatment, and positive effects were found on clinical symptoms, hormone production and tumour proliferation in 12 patients (Krenning *et al.,* 1996). However, ^{111}In is not the most appropriate radionuclide for radionuclide therapy; it lacks the preferable, higher energies of β^- particles. ^{90}Y, with its maximum β^- energy of 2.3 MeV and high affinity for the DOTA chelator, is probably a better candidate. Radiotherapeutic use of [^{90}Y-DOTA^0,Tyr^3]octreotide should lead to a higher and more evenly distributed radiation dose to the tumour because of its larger particle range and tissue penetration. Even tumours with a non-homogeneous distribution of receptors may respond favourably to treatment with such a radiopharmaceutical.

We investigated the characteristics of radiolabelled [DOTA^0,Tyr^3]octreotide, with Tyr replacing Phe^3 to increase hydrophilicity and enabling radioiodination, with respect to *in vitro* binding to sst_2 on murine AtT20 pituitary cell membranes and *in vivo* tissue distribution. For reasons of comparison, we included in these experiments [DTPA^0,Tyr^3]octreotide and [DTPA^0]octreotide.

The results of the *in vitro* studies demonstrated that [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide are high-affinity ligands for sst_2, with an affinity higher than that of [DTPA^0]octreotide. Specific uptake of [^{111}In-DOTA^0,Tyr^3]octreotide and [^{111}In-DTPA^0,Tyr^3]octreotide in sst_2-expressing tissues also was demonstrated *in vivo.* Our findings further show that uptake of ^{111}In-labelled [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide in sst_2-expressing tissues is significantly higher than that of [^{111}In-DTPA^0]octreotide at the time points tested, in agreement with the finding that replacement of Phe^3 in DTPA-octreotide by Tyr leads to increased affinity for the sst_2, as found in the *in vitro* experiments. In this tumour-bearing animal model, [^{111}In-DTPA^0,Tyr^3]octreotide showed the highest uptake of the ^{111}In-labelled compounds in the sst_2-positive organs at all time points tested, making it very promising for visualisation of sst_2-positive lesions in patients. Radioactivity in the target organs 24 and 48 hr after injection, expressed as a percent of the 4 hr value, was in the

same range for the 3 compounds tested, showing that retention times of the compounds in the target organs were comparable.

Uptake of [^{90}Y-DOTA^0,Tyr^3]octreotide in the sst$_2$-positive organs is mostly higher than that of [^{111}In-DOTA^0,Tyr^3]octreotide, suggesting that the DOTA-chelator is more appropriate for complexation of ^{90}Y than of ^{111}In. From these findings, we hypothesise that ^{90}Y-[DOTA^0,Tyr^3]octreotide is a very promising radiopharmaceutical for radionuclide therapy of patients with sst$_2$-positive lesions such as most neuroendocrine tumours. At radiotherapeutic levels, the high uptake of radioactivity in the somatostatin receptor–positive normal organs should be considered. Currently, we are performing radionuclide therapy studies in normal rats with [DOTA^0,Tyr^3]octreotide radiolabelled with different radionuclides to investigate possible radiotoxic effects on normal organs.

For the success of radionuclide therapy, it is important that the radiopharmaceutical is internalised by the tumour cells. We have reported *in vitro* results using AtT20 murine pituitary tumour cells for detection of internalisation of [^{125}I-Tyr^3]octreotide (Hofland *et al.,* 1995). Experiments using the same method showed that [^{111}In-DTPA^0]octreotide, [^{111}In-DOTA^0,Tyr^3]octreotide and ^{90}Y-[DOTA^0,Tyr^3]octreotide were internalised in these cells as well (data not shown).

Peptides and proteins below 60 kDa in plasma are filtered through the glomerular capillaries in the kidneys and subsequently reabsorbed almost completely (≥99%) by the proximal tubular cells *via* receptor-mediated endocytosis. This involves the binding of ligand to a carrier on the plasma membrane of the cells. The carrier–ligand complex subsequently is internalised in an intracellular vesicle, the content of which becomes acidified, releasing the ligand from its receptor. The ligand is then routed to the lysosomes, where degradation may take place. Lysosomal degradation also has been described for ^{111}In-DTPA peptides. Their radiolabelled degradation products are retained in the lysosomes and likely transferred to intracellular metalloproteins (Behr *et al.,* 1995). However, in contrast to the intracellular fate of the radiometals, radioiodine is liberated and released quickly from the cells, explaining our findings of low radioactivity in the kidneys 24 hr after injection of [DOTA^0,^{125}I-Tyr^3]octreotide. It has been shown that octreotide derivatives bound to its receptor in target tissues are internalised as well (Hofland *et al.,* 1995) and that the lysosomal degradation pathway also takes place in the receptor-positive target cells (Duncan *et al.,* 1997). This explains the findings of longer retention time of radioactivity in the sst$_2$-positive organs after injection of ^{111}In- or ^{90}Y-labelled [DOTA^0,Tyr^3]octreotide as compared to that after injection of [DOTA^0,^{125}I-Tyr^3]octreotide.

It has been shown repeatedly that renal accumulation of peptides or proteins can be reduced by administration of amino acids, among others lysine and arginine, which block renal tubular peptide or protein re-absorption (Hammond *et al.,* 1993; Pimm and Gribben, 1994; Behr *et al.,* 1995; De Jong *et al.,* 1995, 1996; Bernard *et al.,* 1997). It was concluded that inhibited tubular reabsorption is the mechanism of the reduction of the renal uptake of radiolabelled peptides (Behr *et al.,* 1995). We have shown that L- and D-lysine were equally potent at inhibiting kidney uptake of [^{111}In-DTPA^0]octreotide (Bernard *et al.,* 1997). D-Lysine, however, seems to be the preferred agent for inhibition of kidney uptake of [^{111}In-DTPA^0]octreotide as toxicity of lysine at high doses seems to be restricted to the L-isomer (Friedman, 1991; Gullino *et al.,* 1995). In the experiments described here, D-lysine administration resulted in a significant reduction of labelled [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide and [DOTA^0,Tyr^3]octreotide uptake in the kidneys without affecting uptake in receptor-positive tissues, which is favourable for both visualisation of lesions in the kidney region and radionuclide therapy, thus bringing these applications further within reach.

These biodistribution studies in rats show that small changes in the chemical structure of octreotide (DOTA *vs.* DTPA as chelator, Tyr instead of Phe^3, radiolabelling with different radionuclides) have significant effects on uptake and retention of the radiolabelled compounds in sst$_2$-expressing organs *in vivo*. The effects may be caused by the changes in receptor affinity observed *in vitro*, but changes in internalisation, metabolism and cellular retention of metabolites also may play a role.

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