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IN VIVO APPLICATION OF [^{111}IN-DTPA-D-PHE^{1}]-OCTREOTIDE FOR DETECTION OF SOMATOSTATIN RECEPTOR-POSITIVE TUMORS IN RATS

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Summary

Radioiodinated somatostatin analogues are useful ligands for the in vitro and in vivo detection of somatostatin receptors. [^{111}In-DTPA-D-Phe^{1}]-octreotide, a somatostatin analogue labeled with a different radionuclide, also binds specifically to somatostatin receptors in vitro. In this study we investigated its in vivo application in the visualization of somatostatin receptor-positive tumors in rats. The distribution of the radiopharmaceutical was investigated after intravenous injection in normal rats and in rats bearing the somatostatin receptor-positive rat pancreatic carcinoma CA 20948. After injection the radiopharmaceutical was rapidly cleared (50 % decrease in maximal blood radioactivity in 4 min), predominantly by the kidneys. Excreted radioactivity was mainly in the form of the intact radiopharmaceutical. Ex vivo autoradiographic studies showed that specific accumulation of radioactivity occurred in somatostatin receptor-containing tissue (anterior pituitary gland). However, in contrast to the adrenals and pituitary, the tracer accumulation in the kidneys was not mediated by somatostatin receptors. Increasing radioactivity over the somatostatin receptor-positive tumors was measured rapidly after injection and the tumors were clearly visualized by gamma camera scintigraphy. In rats pretreated with 1 mg octreotide accumulation of [^{111}In-DTPA-D-Phe^{1}]-octreotide in the tumors was prevented. Because of its relatively long effective half-life, [^{111}In-DTPA-D-Phe^{1}]-octreotide is a radionuclide-coupled somatostatin analogue which can be used to visualize somatostatin receptor-bearing tumors efficiently after 24 hr, when interfering background radioactivity is minimized by renal clearance. This is an advantage over the previously used [^{123}I-Tyr^{3}]-octreotide which has a shorter effective half-life and shows high abdominal interference due to its hepato-biliary clearance. Therefore, [^{111}In-DTPA-D-Phe^{1}]-octreotide seems a better alternative for scintigraphic imaging of somatostatin receptor-bearing tumors.

Radioiodinated analogues have been used extensively in the detection of somatostatin

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receptors in vitro (1). Recently one of these analogues, [^{123}I-Tyr^3]-octreotide, intravenously administered, was shown to visualize somatostatin receptor-positive tumors in vivo by means of gamma camera scintigraphy (2, 3, 4). However, because of a number of drawbacks of this radioiodinated compound, such as its short effective half-life in the blood circulation and high background radiation in the abdominal region, a search was made for an alternative somatostatin analogue, which could be labeled with a different radionuclide, ^{111}In. Predominant advantages of ^{111}In over ^{123}I (half-life 13.2 hr) are its ready availability as well as its attractive physical properties, such as 173 keV and 246 keV gamma radiation, appropriate for scintigraphy, and half-life of 2.8 d, enabling scintigraphy at longer intervals after injection. Therefore, the somatostatin analogue [DTPA-D-Phe^1]-octreotide has been synthesized and the specific binding properties of this peptide, labeled with ^{111}In, to somatostatin receptors have been demonstrated (5).

Materials and methods

Somatostatin analogues

Somatostatin analogues [DTPA-D-Phe^1]-octreotide (SDZ 215-811), [Tyr^3]-octreotide (SDZ 204-090) and octreotide (SMS 201-995) were obtained from Sandoz (Basle, Switzerland). Radiolabeling of [Tyr^3]-octreotide and [DTPA-D-Phe^1]-octreotide with respectively ^{125}I and ^{111}In and consecutive quality control were performed as described before (3, 5). The radiochemical purity of the radiolabeled somatostatin analogues [^{125}I-Tyr^3]-octreotide and [^{111}In-DTPA-D-Phe^1]-octreotide was greater than 95 %.

Animals and tumors

Nine male Lewis rats were inoculated in both upper left- and right hindlegs with the transplantable rat pancreatic tumor CA 20948, which was previously shown to possess somatostatin receptors (6). The growth of this tumor is inhibited by octreotide treatment (7). All conditions were as described before (3). Twelve control animals were studied in parallel. The tumor-bearing animals were divided into two groups: (a) 4 animals without pretreatment and (b) 5 animals which were pretreated subcutaneously with 1 mg octreotide, 30 min before injection of the radiopharmaceutical. All tumor-bearing animals and 10 control animals, used for gamma camera scintigraphy and tissue radioactivity measurements, received 18.5 MBq (0.5 - 1 μg) [^{111}In-DTPA-D-Phe^1]-octreotide in 0.5 - 0.8 ml 154 mM NaCl intravenously via the dorsal vein of the penis. Two control animals used for ex vivo autoradiography received 74 MBq (1 μg) [^{111}In-DTPA-D-Phe^1]-octreotide. For injection and scintigraphy the animals were anaesthetized with ether.

Data acquisition and analysis

The gamma camera and computer system were as described before (3). A medium-energy parallel-hole collimator was used. The analyzer was set to both ^{111}In peaks: 173 keV and 246 keV, window 20 %. Dynamic acquisition took place in 1 min intervals during the first 30 min after injection of [^{111}In-DTPA-D-Phe^1]-octreotide. For the disappearance of radioactivity from the blood the percentage of the injected dose measured over the heart area was calculated. The renal excretion during the first 30 min after injection, measured over the kidneys together with the bladder, was also calculated as percentage of the injected dose. Over less well-defined regions such as tumor, head and lower right hindleg fixed areas were chosen in which the time course of radioactivity was expressed relative to that measured during the first min immediately after injection. Static images were obtained 30 min, 4 hr and 24 hr after injection. On the basis of the static digital images of the animals and proper standards, estimates were made of whole body, kidney and tumor retentions. The 24-hr results of the gamma camera measurements were compared with determinations of radioactivity in isolated tissues using a semi-conductor (GeLi)

detector connected to a multichannel analyzer (Series 40, Canberra). The distance to the detector was 20 cm. The uptake of the radionuclide was calculated as a percentage of the dose and as a percentage of the dose/gram tissue. Urine samples of four different control rats were obtained 30 min, 2 hr and 24 hr after injection of [^{111}In-DTPA-D-Phe^1]-octreotide for analysis by high performance liquid chromatography (HPLC) as previously described (5). For this purpose urine samples were diluted 1:10 in HPLC solvent, i.e. 40 % methanol in 0.05 M acetate buffer, pH 5.5, and applied to the C_{18} column.

Two control animals were perfused with 100 ml 2.5 % glutaraldehyde solution 2 hr, respectively 24 hr after injection of the radiopharmaceutical. Kidneys and pituitaries were isolated and cut with a cryostate (Leitz, Wetzlar, Germany) or a freezing microtome (Jung, Heidelberg, Germany) into 10 - 15 μm thick sections. The sections were then apposed to [^{3}H]-LKB ultrofilm as described previously (8). Kidneys of control rats were tested for the presence of somatostatin receptors by means of in vitro receptor autoradiography using [^{125}I-Tyr^3]-octreotide as ligand (1).

The statistical significance of differences was determined with Student's t-test or by analysis of variance. Differences were considered significant if $p < 0.05$. All data are reported as mean $\pm$ SD.

Results

[^{111}In-DTPA-D-Phe^1]-octreotide is rapidly cleared from the blood as is indicated by the decreasing radioactivity above the heart area (Fig. 1). During the fifth min after injection radioactivity over the heart had decreased to 50 % of that during the first min. Dynamic gamma camera observations show that the radionuclide is cleared almost exclusively via the kidneys. Already 4 min after injection radioactivity appears in the bladder. Also in Fig. 1 the

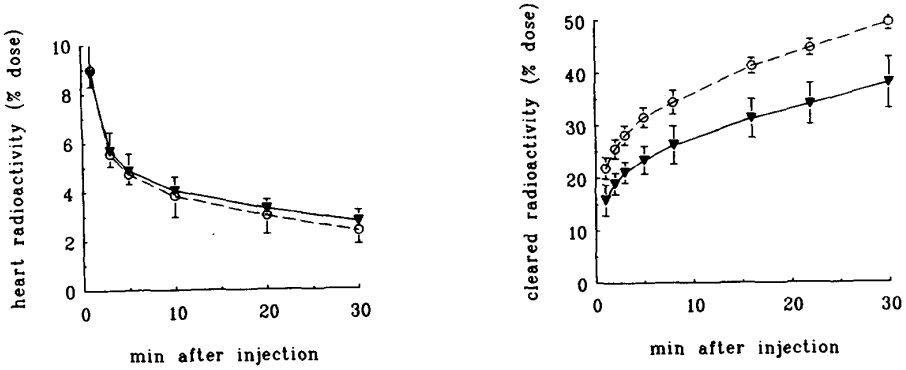

FIG. 1

Disappearance of [^{111}In-DTPA-D-Phe^1]-octreotide from the blood (left) expressed as mean $\pm$ SD percentage of the administered dose measured over the heart area and renal clearance (right) expressed as mean $\pm$ SD percentage of the administered dose measured over the kidneys and the bladder together in 4 control rats (○) and 9 tumor-bearing rats (▼).

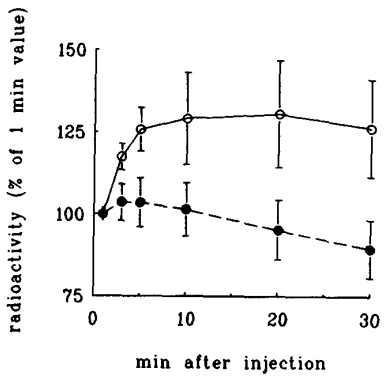

FIG. 2
Radioactivity as function of time, expressed as mean ± SD percentage of the 1-min value, measured above 8 tumors in 4 untreated rats (○) and 10 tumors in 5 rats, which were pretreated with 1 mg octreotide (●).

renal activity together with the excretion of the radionuclide in the bladder, is presented as a function of time. In control rats about 50 % of the injected dose had already been cleared within 30 min via the kidneys, whereas in tumor-bearing rats this clearance was significantly slower ($p < 0.05$). Pretreatment with 1 mg octreotide did not influence blood clearance or renal clearance (data not shown). During the first 30 min after injection radioactivity measured above the head and lower right hindleg (without tumor) of all investigated animals showed decreasing blood pool radioactivity (data not shown). Increasing radioactivity was observed over the tumors of the animals immediately after injection, whereas this was not the case in the octreotide-pretreated group ($p < 0.001$, Fig. 2). Figure 3 presents static analogue images, 30 min and 24 hr after injection, of one untreated and one octreotide-pretreated animal. It is evident that the clear visualization of this transplantable pancreatic carcinoma (notably after 24 hr) was prevented by pretreatment with the high dose of unlabeled somatostatin analogue.

From digital static images obtained 24 hr after injection, whole body retention of radioactivity appeared to be about 10 % (Table I), which was mainly localized in the kidneys (7 %). This quantity closely parallels measurements in isolated kidneys using the semi-conductor detector (Table I). Radioactivity measured over the kidneys remained constant between 4 and 24 hr after injection. The presence of tumors did not significantly influence 24-hr kidney accumulation of radioactivity. Table I also gives the results of the measurements of radioactivity in the tumors with the gamma camera and the semi-conductor detector. In the tumors of the untreated animals significantly higher percentages of the injected dose were found in comparison with the tumors of the octreotide-pretreated group.

Results of radioactivity measurements in a number of tissues (of animals which were not pretreated with octreotide), isolated 24 hr after injection, are reported in Table II. The highest tissue radioactivity concentrations were found in the kidneys and the adrenals. There was no significant difference between radioactivity concentrations in most tissues of control and octreotide-pretreated animals. However, after pretreatment with octreotide the radioactivity concentrations were much lower in the adrenals and in the tumors.

Ex vivo autoradiography of the pituitary glands of control rats, obtained 2 and 24 hr after in vivo administration of [^{111}In-DTPA-D-Phe^1]-octreotide demonstrated accumulation of radioactivity in the anterior lobe but not in the posterior lobe (Fig. 4), confirming previous studies using in vitro or ex vivo autoradiography (9, 10). Interestingly, ex vivo autoradiography of kidney tissue of the same animals clearly showed the presence of radioactivity in the proximal tubules but not in the glomeruli (Fig. 5). However, with in vitro autoradiography using [^{125}I-Tyr^3]-octreotide as ligand, no specific somatostatin receptors were detected in the kidney (data not shown).

HPLC of a 30-min and a 2-hr urine sample of two different control rats showed that the excreted radioactivity was in the form of the intact [^{111}In-DTPA-D-Phe^{1}]-octreotide. Of two late urine samples obtained from control rats 24 hr after injection of the radiopharmaceutical (when most radioactivity already had been excreted, see Table I) more than 90 % of the radioactivity was not peptide-bound and eluted in the void volume, probably representing ^{111}In-DTPA.

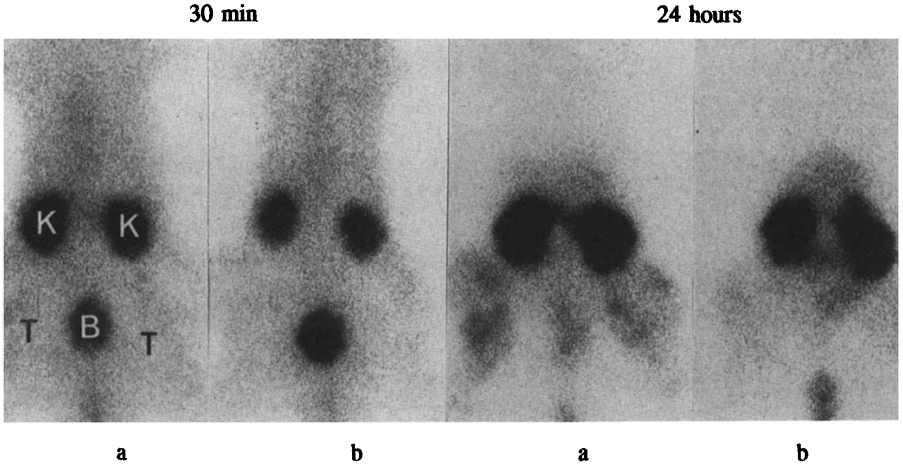

FIG. 3
Static images, 30 min and 24 hr after injection, of one untreated (a) and one octreotide-pretreated animal (b), showing accumulation in the kidneys (K) and/or urinary bladder (B). Accumulation of radioactivity in tumors (T) in both hindlegs is noted in a, not in b.

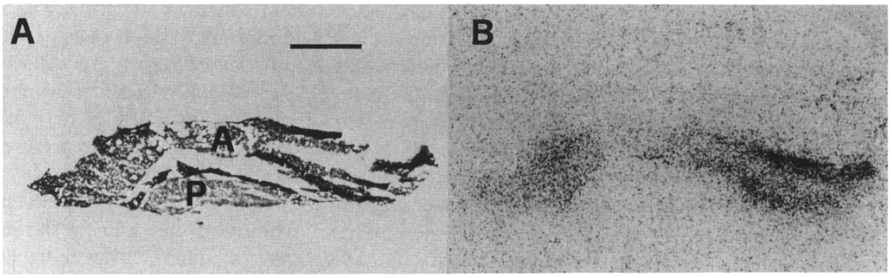

FIG. 4
Ex vivo autoradiography 24 hr after injection, showing the somatostatin receptors in the rat pituitary gland: A) hematoxylin-eosin stained section showing anterior (A) and posterior (P) pituitary gland. B) autoradiogram showing binding in the anterior pituitary but not in the posterior lobe. Bar = 1 mm.

Discussion

After injection of [^{111}In-DTPA-D-Phe^{1}]-octreotide the radionuclide disappears rapidly from the circulation. Blood clearance (measured over the heart) during the first 30 min was not noticeably influenced by octreotide-pretreatment nor by the presence of tumors. Gamma

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