Preclinical and Initial Clinical Evaluation of ^{111}In-Labeled Nonsulfated CCK_8 Analog: A Peptide for CCK-B Receptor-Targeted Scintigraphy and Radionuclide Therapy

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The presence of cholecystokinin (CCK)-B (gastrin) receptors has been shown in more than 90% of medullary thyroid cancers (MTCs) and in a high percentage of small cell lung cancers, stromal ovarium cancers and several other tumor types. **Methods:** The aim of this study was to evaluate in vitro and in vivo whether ^{111}In-labeled CCK-B receptor-specific CCK_8 analog [D-Asp^{26},Nle^{28,31}]CCK_{26-33} (D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2) is suitable for CCK-B receptor scintigraphy based on the finding that unlabeled nonsulfated diethylenetriamine pentaacidic acid [DTPA^0]CCK_8 and tetraazacyclododecanetetraacetic acid [DOTA^0]CCK_8 analogs show high and specific binding for CCK-B receptors in human tumors. Fifty percent inhibitory concentrations were in the low nanomolar range. **Results:** In vitro, [^{111}In-DOTA^0]CCK_8 showed specific internalization in CCK-B receptor-positive rat pancreatic tumor cells AR42J. Internalization of the analog appeared to be time and temperature dependent and receptor specific. From the data obtained with [^{111}In-DOTA^0]CCK_8 and ^{125}I-gastrin, the latter being a specific ligand for the CCK-B receptor, the rat pancreatic cell line CA20948 also appeared to be CCK-B receptor positive. This provides an in vitro and in vivo rat tumor model because this cell line can be grown to solid tumors in Lewis rats. In vivo biodistribution experiments in CA20948 tumor-bearing Lewis rats showed rapid clearance of [^{111}In-DOTA^0]CCK_8, and specific uptake was found in the CCK-B receptor-expressing stomach and tumor. Furthermore, comparing [^{111}In-DOTA^0]CCK_8 with the radioiodinated nonsulfated CCK_{10} analog (D-Tyr-Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2), both ligands having high affinity for the CCK-B receptor, tumor-to-blood ratios were significantly higher for [^{111}In-DOTA^0]CCK_8 than for ^{125}I-CCK_{10}, analogous to the findings with radioiodinated and ^{111}In-labeled octreotide. The study in humans with [^{111}In-DTPA^0]CCK_8 showed receptor-specific uptake in the CCK-B receptor-positive stomach and in metastases in the neck region up to 48 h after injection. **Conclusion:** [^{111}In-DOTA^0]CCK_8 is most promising for scintigraphy and, after coupling to therapeutic radionuclides, for radionuclide therapy of human CCK-B receptor-positive tumors such as MTC and small cell lung cancer.

Key Words: cholecystokenin receptors; [DOTA^0]CCK_8 [DTPA^0]CCK_8; ^{111}In; tumor scintigraphy; radionuclide therapy; medullary thyroid cancers

J Nucl Med 1999; 40:2081–2087

Radiolabeled tumor receptor-binding peptides can be used for in vivo scintigraphic imaging. The peptides most widely used now are stable somatostatin analogs that bind to their receptors on tumors of neuroendocrine origin (*1*). An example is the octapeptide [^{111}In-DTPA^0]octreotide, consisting of octreotide and the chelator diethylenetriamine pentaacetic acid (DTPA), enabling instant radiolabeling with a radiometal such as ^{111}In. We have described its use for scintigraphic imaging of somatostatin receptor-positive lesions, such as gastroenteropancreatic neuroendocrine tumors, neuroblastoma, pheochromocytoma, breast cancer, Hodgkin's lymphoma and small cell lung cancer (*2,3*). However, unlike in other neuroendocrine tumors, somatostatin receptor expression is rather low in medullary thyroid cancer (MTC) and is completely absent in clinically aggressive forms of the disease (*4,5*). Recently, the presence of cholecystokinin (CCK)-B (gastrin) receptors was shown in more than 90% of MTCs, and the presence of these receptors was shown in a high percentage of small cell lung cancers, stromal ovarium cancers, astrocytomas and several other tumor types (*6*). On the basis of these findings, Behr et al. (*7*) evaluated the suitability of radioiodinated gastrin, a specific ligand for the CCK-B receptor, for targeting CCK-B receptor-expressing tumors in vivo. Their data suggest that gastrin and its analogs may represent a useful new class of receptor-binding peptides for diagnosis and therapy of a variety of tumor types, including MTC. Furthermore, Reubi et al. (*8*) developed DTPA-conjugated CCK-B receptor-binding CCK analogs, evaluated their receptor-binding characteristics and obtained initial preclinical biodistribution data in nontumor-bearing rats. For the tetraazacyclododecanetetraacetic acid (DOTA) counterpart of the most

Received Dec. 14, 1998; revision accepted Apr. 9, 1999.

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promising analog, a high CCK-B receptor affinity was found. They concluded that CCK analogs are promising for human CCK-B receptor scintigraphy as well (*8*).

A new application is the use of radiolabeled peptides for radionuclide therapy. Promising results in tumor growth inhibition using [^{111}In-DTPA^0]octreotide have been reported in humans (*9*). Besides Auger electron emitters, such as ^{111}In, β^- particle emitters, such as ^{90}Y, may appear suitable for this purpose. The ^{90}Y-DTPA complex is unstable, resulting in hematopoietic toxicity in vivo. Therefore, the DOTA chelator, which forms stable complexes with ^{90}Y and ^{111}In, was coupled to CCK_8, enabling future radionuclide therapy of MTC.

The success of the therapeutic strategy relies on the amount of radioligand that can be concentrated within tumor cells and thus the rates of internalization, degradation and recycling of both ligand and receptor. Binding of several peptide hormones to specific surface receptors is generally followed by internalization of the ligand-receptor complex. Williams et al. (*10*) and Svoboda et al. (*11*) have reported internalization of unchelated CCK. The internalization process of this peptide appears to be CCK receptor specific and temperature dependent. One aim of this study was to evaluate internalization of [^{111}In-DOTA^0]CCK_8 in rat pancreatic tumor cells in vitro.

Biodistribution and tumor visualization also were investigated in vivo in tumor-bearing animals. Biodistribution of [^{111}In-DOTA^0]CCK_8 was compared with that of ^{125}I-CCK_{10} analog (*8*), analogous to our studies using radioiodinated and ^{111}In-labeled octreotide, to compare the differences in biodistribution and cellular retention of the peptides radiolabeled with either a residualizing radiolabel like ^{111}In or with a nonresidualizing radiolabel like ^{125}I. We performed toxicity studies with unlabeled [DOTA^0]CCK_8 and [DTPA^0]CCK_8 in rats and mice, and, thereafter, an initial evaluation of [^{111}In-DTPA^0]CCK_8 in humans was performed.

MATERIALS AND METHODS

Compounds

^{125}I-gastrin (74×10^{12} Bq/mmol) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). Synthetic gastrin was from Bachem (Bubendorf, Switzerland). This is a heptadecapeptide, specific for the CCK-B/gastrin receptor with an affinity constant in the subnanomolar range, whereas its affinity for the CCK-A receptor is lower by more than four orders of magnitude. Mallinckrodt, Inc. (Petten, The Netherlands) provided $^{111}InCl_3$. DOTA and DTPA analogs of CCK_8 (D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2) were synthesized according to previously described methods (*8*). ^{125}I-CCK_{10} (D-Tyr-Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2) was synthesized by Chiron (*8*). ^{111}In-labeling of the DTPA and DOTA analogs was as described for [DTPA^0]octreotide (*2*) and [DOTA^0,Tyr^3]octreotide (*12*), respectively. ^{125}I-labeling of ^{125}I-CCK_{10} was performed as described for [Tyr^3]octreotide (*13*).

Internalization

AR42J cells were grown in RPMI-1640 medium (Gibco, Grand Island, NY), CA20948 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco) and ARO cells were grown in DMEM/F12 medium (Gibco). For all cell lines, medium was supplemented with 2 mmol/L glutamine and 10% fetal calf serum (Gibco). Before the experiment, subconfluent cell cultures were transferred to six-well plates.

The binding of the radiolabeled peptides to tumor cells and subsequent internalization were studied as described (*14*). Cells were washed and incubation was started by addition of 1 mL internalization medium per well (culture medium without fetal calf serum but with 1% bovine serum albumin [Sigma, St. Louis, MO]) with about 80 kBq of radiotracer. Peptide concentration range in the different experiments was 0.1–1 μmol/L. Cells were incubated at 37°C for indicated periods of time. To determine nonspecific internalization, cells were incubated with an excess unlabeled peptide (0.1 μmol/L). Cellular uptake was stopped by removing medium from the cells and washing with 2 mL ice-cold phosphate-buffered saline. To discriminate between internalized and noninternalized (surface-bound) radiopharmaceutical, intact cells were incubated with 1 mL 20 mmol/L sodium acetate. The internalized and noninternalized fractions were determined by measuring radioactivity. The internalized fraction was expressed as percentage of the applied dose per milligram cellular protein. The latter was determined using a commercially available kit (Bio-Rad, Veenendaal, The Netherlands).

Data are expressed as mean ± SD for incubations assayed in triplicate, with each experiment performed at least three times.

In Vivo Tissue Distribution

Animal experiments were performed in compliance with the regulations of this institution and with generally accepted guidelines governing such work. Male Lewis rats (200–250 g), bearing the CA20948 pancreatic tumor, were used in the experiments. Rats were injected under ether anesthesia with 3 MBq (0.5 μg) ^{111}In-labeled peptide in 200 μL saline into the dorsal vein of the penis. To determine nonspecific binding of the radiopharmaceutical, a separate group of rats was coinjected intravenously with 0.1 mg [DOTA^0]CCK_8. At the indicated time points, rats were killed under ether anesthesia. Organs and blood were collected, and the radioactivity in these samples was determined. Results are expressed as mean ± SD of at least six rats per group.

Toxicity Study of Unlabeled [DOTA^0]CCK_8 and [DTPA^0]CCK_8

On experimental day 0, eight treatment groups of five Wistar rats and five BALB/c mice were injected intravenously with saline (0 times), 10 times, 100 times or 1000 times the human concentration of 15 μg/75 kg of [DOTA^0]CCK_8 or [DTPA^0]CCK_8, respectively. All test solutions were injected through the penis vein at a rate not exceeding 1 mL/min. Until 24 h after injection the animals were monitored for changes in behavior (eating, sleeping, motion, posture) and signs of toxicosis. Any reaction to the treatment was recorded. All rats were killed by ether narcosis 24 h after injection and subjected to detailed macroscopic postmortem examination. After examination of the external surfaces, the chest and abdomen were opened by midline incision. Thoracic and abdominal viscera were examined for abnormalities. Aberrations were recorded. Organs were investigated macroscopically (e.g., for bleedings). Liver, kidneys, stomach, spleen, lungs and intestines were isolated and fixed in 4% buffered formalin. The organs were sectioned, stained and evaluated by light microscopy.

Initial Human Evaluation of [^{111}In-DTPA^0]CCK$_8$

A 45-y-old woman with local recurrence of MTC was injected with 222 MBq ^{111}In-labeled [DTPA^0]CCK$_8$ (10 μg peptide). Scans were acquired 4, 24 and 48 h after injection as described (*2,3*). Urine was collected during the first 24 h after injection.

RESULTS

Radiolabeling

^{111}In-labeling efficiency of the different peptides and radioiodination efficiency of ^{125}I-CCK$_{10}$ ranged from 95% to 100%. Radiochemical purity always exceeded 90%.

In Vitro Internalization Studies

Figure 1A shows the time- and temperature-dependent internalization of [^{111}In-DOTA^0]CCK$_8$ in the CCK-B receptor-positive AR42J rat pancreatic tumor cells and in the CCK-B receptor-negative ARO human anaplastic thyroid tumor cell line. Internalization was dose dependent and was reduced in the presence of increasing concentrations of unlabeled peptide, indicating that this process is receptor specific. Furthermore, it was reduced at 6°C versus 37°C, indicating temperature dependence, and increased over time. The acid-removable ("surface-bound") uptake was about 5%–10% of the internalized fraction (not shown). The ARO cells were used as negative controls. In these cells, internalization of [^{111}In-DOTA^0]CCK$_8$ was indeed low and showed no specific temperature-dependent accumulation. Figure 1B shows the inhibitory effect in AR42J cells of excess medium gastrin concentrations on internalization of ^{125}I-gastrin, a specific ligand with high affinity for CCK-B receptors, indicating specific internalization in these AR42J cells.

Figure 2A shows the time- and temperature-dependent internalization of [^{111}In-DOTA^0]CCK$_8$ in the CA20948 rat pancreatic tumor cells. Internalization was reduced in the presence of increasing concentrations of unlabeled peptide. Furthermore, it was reduced at 6°C, indicating temperature dependence, and increased over time. The acid-removable (surface-bound) uptake was about 5%–10% of the internalized fraction (not shown). Figure 2B shows internalization of ^{125}I-gastrin in the presence or absence of excess medium gastrin concentrations. In the presence of unlabeled gastrin, internalization was significantly reduced, indicating the presence of CCK-B receptors on the CA20948 rat pancreatic tumor cells.

Tissue Distribution in Rats

Table 1 compares the radioactivity in several organs after injection of [^{111}In-DOTA^0]CCK$_8$ and [^{111}In-DTPA^0]CCK$_8$. A rapid clearance from the blood through renal excretion was found for both radiolabeled compounds, with a pronounced uptake in the kidneys. Receptor-negative organ radioactivity paralleled blood clearance, resulting in a very low background radioactivity 24 h after injection. Comparison of biodistribution data and tumor uptake of [^{111}In-DOTA^0]CCK$_8$ and [^{111}In-DTPA^0]CCK$_8$ showed no major differences.

Figure 3 shows that uptake of [^{111}In-DOTA^0]CCK$_8$ in stomach and tumor, 4 h after injection, was at least partially receptor specific because uptake was significantly reduced after coinjection with 0.1 mg unlabeled peptide. Uptake in the other organs and radioactivity in blood did not change significantly after coinjection of unlabeled peptide (not shown).

Figure 4 shows a comparison of tissue radioactivity after injection of [^{111}In-DOTA^0]CCK$_8$ and ^{125}I-CCK$_{10}$, 1 and 4 h after injection, in several rat organs. Clearance from the blood was slower for ^{125}I-CCK$_{10}$ than for [^{111}In-DOTA^0]CCK$_8$, and uptake in receptor-negative organs, of which the liver is shown as an example, was higher for ^{125}I-CCK$_{10}$ than for [^{111}In-DOTA^0]CCK$_8$. A higher uptake of ^{125}I-CCK$_{10}$ than of [^{111}In-DOTA^0]CCK$_8$ was also found in the receptor-positive stomach, which seemed to be favorable for ^{125}I-CCK$_{10}$. However, the stomach-to-blood ratio, which represents the target-to-background ratio, was significantly lower for ^{125}I-CCK$_{10}$ than for [^{111}In-DOTA^0]CCK$_8$ at 4 h after injection.

Figure 5 shows a gamma camera scan, made 20 min after injection, of two tumor-bearing rats and a control rat,

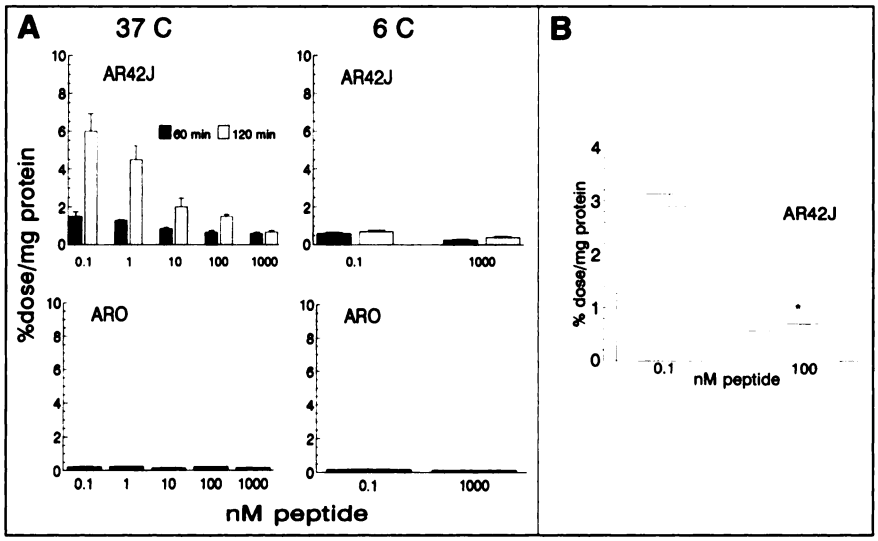

FIGURE 1. (A) Internalization of [^{111}In-DOTA^0]CCK$_8$ in CCK-B receptor-positive AR42J rat pancreatic tumor cells and in CCK-B receptor-negative ARO human anaplastic thyroid tumor cell line. Data are expressed as percentage added dose/mg protein (mean ± SD). (B) Internalization, after 60-min incubation, of ^{125}I-gastrin, specific ligand with high affinity for CCK-B receptor, in AR42J cells. Data are expressed as percentage added dose/mg protein (mean ± SD).

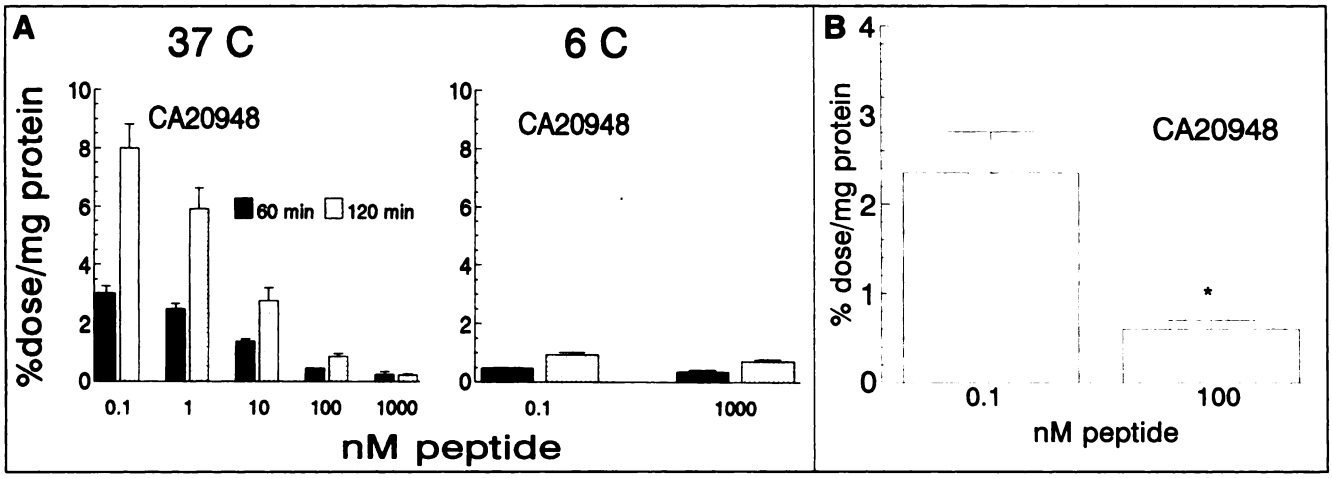

FIGURE 2. (A) Internalization of [^{111}In-DOTA^0]CCK$_8$ in CCK-B receptor-positive CA20948 rat pancreatic tumor cells. Data are expressed as percentage added dose/mg protein (mean ± SD). (B) Internalization, after 60-min incubation, of ^{125}I-gastrin, specific ligand with high affinity for CCK-B receptor, in CA20948 cells. Data are expressed as percentage added dose/mg protein (mean ± SD).

injected with [^{111}In-DOTA^0]CCK$_8$ without or with excess unlabeled peptide or with ^{125}I-CCK$_{10}$. The radioiodinated analog had a different distribution pattern with much higher liver uptake than did [^{111}In-DOTA^0]CCK$_8$, which is cleared through the kidneys. Furthermore, the CCK-B receptor-positive tumor was clearly visualized in the left rat but was not detected after coinjection with unlabeled peptide, as shown in the middle rat.

Toxicity Study

No abnormalities were found in animal behavior during the 24 h after injection. No macroscopic pathology was observed. Microscopic examination of representative tissue sections from rats treated with [DOTA^0]CCK$_8$ revealed no abnormalities that could be attributed to the treatment.

TABLE 1
Radioactivity in Organs and Tumor of CA20948 Tumor-Bearing Rats 1, 4 and 24 Hours After Administration of [^{111}In-DOTA^0]CCK$_8$ or 24 Hours After Administration of [^{111}In-DTPA^0]CCK$_8$

	DOTA* time administration			DTPA* time administration
Tissue	1 h	4 h	24 h	24 h
Blood	0.099 (0.010)	0.018 (0.002)	0.005 (0.001)	0.005 (0.001)
Spleen	0.041 (0.004)	0.034 (0.004)	0.033 (0.003)	0.023 (0.003)
Pancreas	0.037 (0.002)	0.013 (0.001)	0.011 (0.000)	0.012 (0.000)
Adrenals	0.086 (0.016)	0.017 (0.002)	0.019 (0.000)	0.018 (0.000)
Kidneys	0.706 (0.11)	0.439 (0.061)	0.379 (0.050)	0.322 (0.03)
Liver	0.040 (0.011)	0.027 (0.004)	0.026 (0.005)	0.045 (0.007)
Stomach	0.08 (0.02)	0.044 (0.004)	0.028 (0.006)	0.035 (0.007)
Colon	0.041 (0.013)	0.013 (0.003)	0.037 (0.008)	0.01 (0.001)
Muscle	0.012 (0.001)	0.003 (0.000)	0.003 (0.000)	0.003 (0.000)
Femur	0.035 (0.002)	0.014 (0.001)	0.012 (0.001)	0.011 (0.001)
Pituitary	0.012 (0.002)	0.004 (0.001)	0.003 (0.000)	
Tumor	0.160 (0.021)	0.130 (0.020)	0.082 (0.011)	0.094 (0.02)

*Tissue radioactivity is expressed as percentage injected dose/g tissue (mean ± SD). For each group, n ≥ 4.

DOTA = tetraazacyclododecanetetraacetic acid CCK$_8$ analog; DTPA = [^{111}In]diethylenetriaminepentaacetic acid CCK$_8$ analog.

Initial Human Evaluation of [^{111}In-DTPA^0]CCK$_8$

Intravenous injection of 10 μg peptide was well tolerated by the patient, with no adverse reactions. Figure 6 shows a

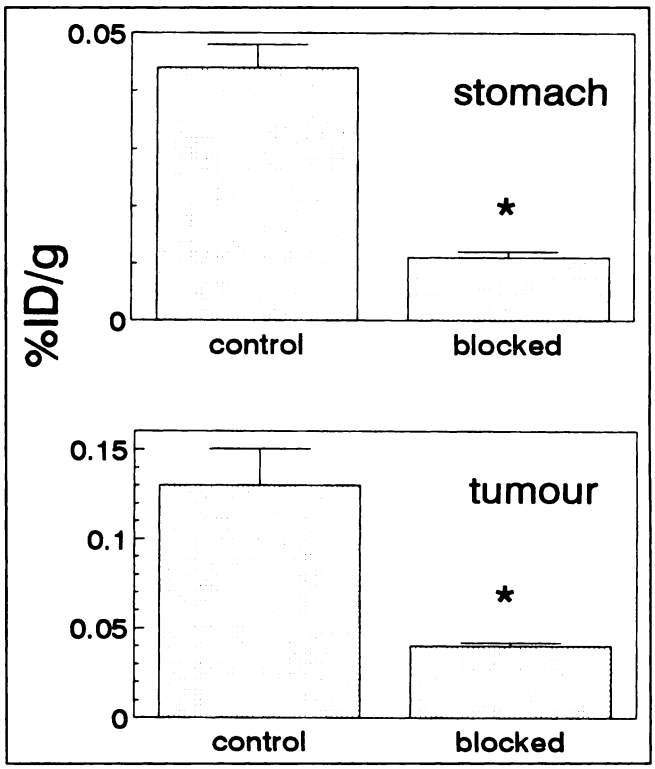

FIGURE 3. Uptake in rat stomach and CA20948 tumor, 4 h after injection of [^{111}In-DOTA^0]CCK$_8$, with or without coinjection with 100 μg unlabeled peptide. %ID/g = percentage injected dose/g tissue (mean ± SD).

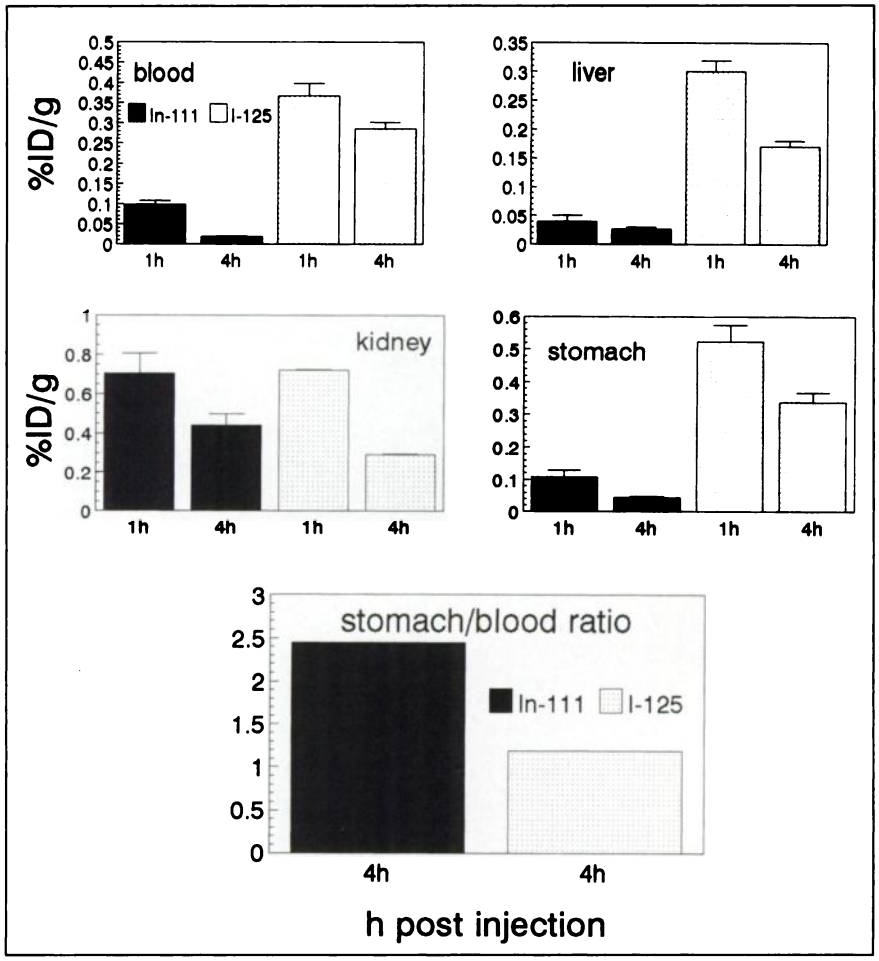

FIGURE 4. Radioactivity in several rat organs, 1 and 4 h after injection of ^{125}I-CCK_{10} and [^{111}In-DOTA^0]CCK_8. %ID/g = percentage injected dose/g tissue (mean ± SD).

scan of the upper abdomen and neck, taken 48 h after injection. As in the preclinical studies in rats, receptor-specific uptake was seen in the CCK-B receptor-expressing fundus of the stomach and in metastases in the neck region. Cumulative excretion of radioactivity in the urine was more than 90% of the dose at 24 h after injection.

DISCUSSION

Autoradiographic studies by Reubi et al. (*6*) revealed the presence of CCK-B receptors in more than 90% of MTCs and in a high percentage of other tumors. Therefore, CCK-B receptors appeared to represent a new and promising target for radiolabeled peptides for tumor scintigraphy and radionuclide therapy. Behr et al. (*7*) evaluated the suitability of radioiodinated gastrin, a specific ligand for the CCK-B receptor, for targeting CCK-B receptor-expressing tumors in vivo. Reubi et al. (*8*) also developed DOTA- and DTPA-conjugated CCK-B receptor-binding CCK analogs, evaluated their receptor binding characteristics and obtained initial preclinical biodistribution data using [DTPA^0]CCK_8 in nontumor-bearing control rats. They concluded that the peptide analogs used were promising for human CCK-B receptor scintigraphy and radionuclide therapy.

For these studies, we chose nonsulfated octapeptide CCK_8, derivatized with either DOTA or DTPA, because this peptide has shown high affinity for CCK-B receptors but low affinity for CCK-A receptors (*8*), the latter in contrast to sulfated analogs that bear a sulfate ester attached to the Tyr moiety. CCK-A receptors are expressed at a much higher density in normal tissues, especially in the abdomen. The selective CCK-B receptor affinity of the chosen nonsulfated analog will result therefore in a favorable low background radioactivity during scanning.

For the success of radionuclide therapy, it is important that the tumor cells internalize the radiopharmaceutical after binding to the receptor. We demonstrated receptor-specific, time- and temperature-dependent internalization of [^{111}In-DOTA^0]CCK_8 in AR42J cells, indicating that the DOTA group does not prevent the CCK analog from internalization. In this study we also demonstrated receptor-specific internalization of ^{125}I-gastrin and [^{111}In-DOTA^0]CCK_8 in CA20948 cells in culture, consistent with the presence of CCK-B receptors in these tumor cells. This provides a tumor model for research on CCK analogs in vivo because these cells can also be grown to solid tumors in vivo in Lewis rats.

For [^{111}In-DTPA^0]octreotide, the internalization pathway proceeds through endosomes fusing with lysosomes where degradation of the radiolabeled peptide occurs. After dissociation of the ligand, the receptor protein may return to the plasma membrane, whereas ^{111}In-DTPA-containing degrada-

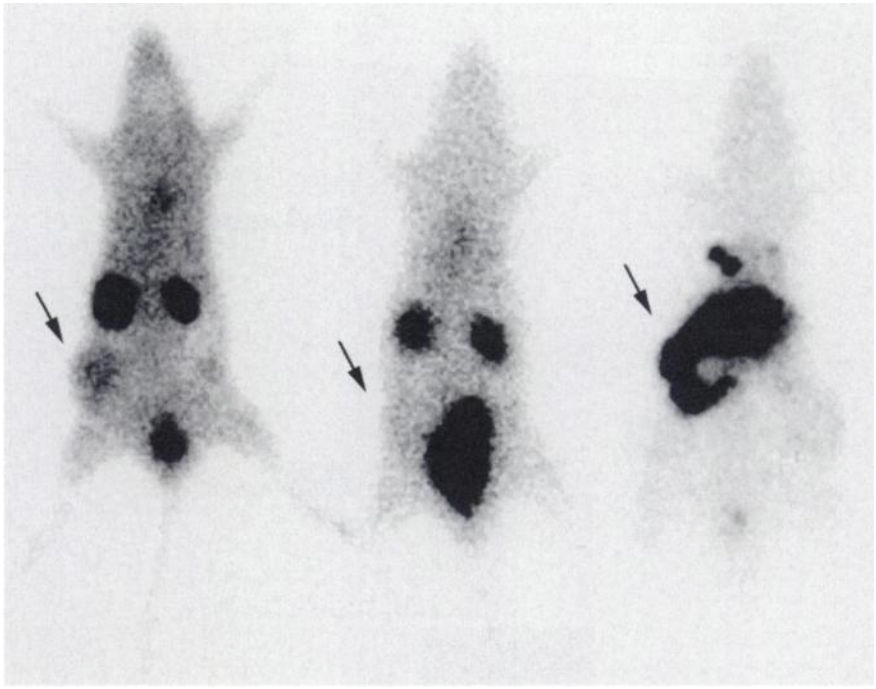

FIGURE 5. Scan, made 20 min after injection, of two tumor-bearing rats (left and middle) and control rat (right), injected with [^{111}In-DOTA^0]CCK$_8$ (left), [^{111}In-DOTA^0]CCK$_8$ in the presence of excess unlabeled peptide (middle) or ^{125}I-CCK$_{10}$ (right). Left and middle arrows indicate tumor uptake; right arrow indicates liver uptake.

tion products are retained in the lysosomes, causing the long retention time of radioactivity in receptor-positive cells (*15*). The assumption that this intracellular route holds also for CCK-B receptor-specific peptides is in accordance with the long retention time of the radiolabel shown in the human study, where CCK-B receptors in stomach and metastases were visualized up to 48 h after injection.

Uptake of the ^{111}In-labeled peptide in CCK-B receptor-expressing tissues (stomach and CA20948 tumor) in vivo in rats was also found to be specific because uptake was reduced significantly in the presence of excess unlabeled hormone. Furthermore, in agreement with the assumption that unsulfated CCK analogs will result in low background radioactivity, uptake in receptor-negative organs was indeed low, which is favorable during scintigraphy and radionuclide therapy. These findings also showed that the tumor-to-background ratio for [^{111}In-DOTA^0]CCK$_8$ already was significantly higher than that for ^{125}I-CCK$_{10}$ at 4 h after injection, analogous to the findings with octreotide (*16*). Because we know that the intracellular retention of radioactivity is much longer for the residualizing ^{111}In-chelator peptides than for radioiodinated peptides (*16*), this tumor-to-background ratio will be increasingly favorable for the ^{111}In-labeled peptide. Furthermore, the high liver uptake found with the radioiodinated compound is very unfavorable during scintigraphy of tumors in the upper abdomen. Behr et al. (*7*), using ^{131}I-gastrin, found that a tumor-to-blood ratio of about 5 could be reached in nude mice bearing the human MTC tumor. In this study, a tumor-to-blood ratio of 16 was reached 24 h after injection, showing that residualizing radiolabels, such as the radiometal ^{111}In, have advantages over ^{131}I. This was also shown in human studies. After injection of ^{131}I-gastrin, good tumor-to-nontumor ratios were maintained for about 3 h (*7*), whereas in this study, using the ^{111}In-labeled peptide, the receptor-positive stomach (*17*) and tumor metastases were clearly visualized even 48 h after injection.

Despite the fact that the excretion of [^{111}In-DOTA^0]CCK$_8$ was through renal excretion, the uptake of radioactivity in the kidneys was about one order of magnitude lower than that of [^{111}In-DTPA]octreotide, a DTPA-coupled peptide also excreted in the urine. [^{111}In-DOTA^0]CCK$_8$ contains two

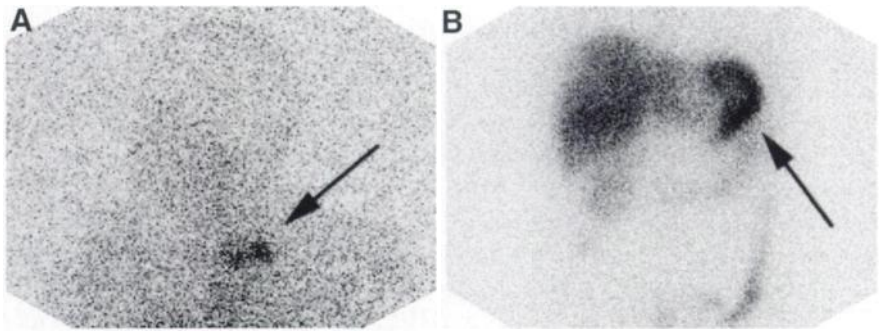

FIGURE 6. Visualization of CCK-B receptors in 45-y-old woman with MTC after intravenous administration of 222 MBq [^{111}In-DTPA^0]CCK$_8$ (10 μg peptide). Scans at 48 h after injection show uptake in lymph node metastases in neck region (A, arrow) and in receptor-positive stomach (B, arrow).

Asp moieties, giving an anionic charge to the molecule, whereas octreotide contains the positively charged Lys moiety. Negatively charged peptides apparently have a lower renal uptake in the environment of negatively charged membranes of tubular cells than neutral or cationic ones, consistent with the finding that positively charged amino acids can block peptide reabsorption by binding to the negatively charged membranes of the tubular cells (*18*). This low kidney uptake is very favorable during scintigraphy in the perirenal region; during radionuclide therapy studies, it will prevent renal radiotoxicity.

CONCLUSION

[^{111}In-DOTA^0]CCK$_8$ is most promising for scintigraphy and, after coupling to therapeutic radionuclides, for radionuclide therapy of CCK-B receptor-positive tumors, such as MTC and small cell lung cancer.

ACKNOWLEDGMENTS

The authors thank Prof. W.J. Mooi for sharing his pathology expertise in the evaluation of tissue slices from the toxicology studies and Marcel van der Pluijm, Arthur van Gameren, Michael Schaar and Elisa de Bruin for their excellent help during the experiments.

REFERENCES

1. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin receptor imaging in the localization of endocrine tumors. *New Engl J Med.* 1990;323:1246–1249.
2. Krenning EP, Kwekkeboom DJ, Bakker WH, et al. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe^1]- and [^{123}I-Tyr^3]-octreotide: the Rotterdam experience with more than 1000 patients. *Eur J Nucl Med.* 1993;20:716–773.
3. Krenning EP, Kwekkeboom DJ, Pauwels S, Kvols LK, Reubi JC. Somatostatin receptor scintigraphy. In: Freeman, LH, ed. *Nuclear Medicine Annual.* New York, NY: Raven Press; 1995:1–50.
4. Reubi JC, Chayvialle JA, Franc B, Cohen R, Calmettes C, Modigliani E. Somatostatin receptors and somatostatin content in medullary thyroid carcinomas. *Lab Invest.* 1991;64:567–573.
5. Kwekkeboom DJ, Reubi JC, Lamberts SWJ, et al. In vivo somatostatin receptor imaging in medullary thyroid carcinoma. *J Clin Endocrinol Metab.* 1993;76:1413–1417.
6. Reubi JC, Schaer JC, Waser B. Cholecystokinin (CCK)-A and CCK-B/gastrin receptors in human tumors. *Cancer Res.* 1997;57:1377–1386.
7. Behr TM, Jenner N, Radetzky S, et al. Targeting of cholecystokinin-B/gastrin receptors in vivo: preclinical and initial clinical evaluation of the diagnostic and therapeutic potential of radiolabelled gastrin. *Eur J Nucl Med.* 1998;25:424–430.
8. Reubi JC, Waser B, Schaer JC, et al. Unsulfated DTPA- and DOTA-CCK analogs as specific high affinity ligands for CCK-B receptor-expressing human and rat tissues in vitro and in vivo. *Eur J Nucl Med.* 1998;25:481–490.
9. Krenning EP, Kooij PPM, Pauwels S, et al. Somatostatin receptor scintigraphy and radionuclide therapy. *Digestion.* 1996;57:57–61.
10. Williams JA, Bailey AC, Roach E. Temperature dependence of high-affinity CCK receptor binding and CCK internalization in rat pancreatic acini. *Am J Physiol.* 1988;254(suppl):G513-G521.
11. Svoboda M, Dupuche MH, Lambert M, Bui D, Christophe J. Internalization-sequestration and degradation of cholecystokinin (CCK) in tumoral rat pancreatic AR4–2J cells. *Biochim Biophys Acta.* 1990;1055:207–216.
12. de Jong M, Krenning EP, Bakker WH, et al. ^{90}Y and ^{111}In labelling, receptor binding and biodistribution of [DOTA^0,D-Phe^1,Tyr^3]octreotide, a promising somatostatin analogue for radionuclide therapy. *Eur J Nucl Med.* 1997;24:368–371.
13. Bakker WH, Krenning EP, Breeman WAP, et al. Receptor scintigraphy with a radioiodinated somatostatin analogue: radiolabeling, purification, biologic activity and in vivo application in animals. *J Nucl Med.* 1990;31:1501–1509.
14. de Jong M, Bernard HF, De Bruin E, et al. Internalization of radiolabeled [DTPA^0]octreotide and [DOTA^0,Tyr^3]octreotide: peptides for somatostatin receptor-targeted scintigraphy and radionuclide therapy. *Nucl Med Commun.* 1998;19:283–288.
15. Duncan JR, Stephenson MT, Wu HP, Anderson CJ. Indium-^{111}-diethylenetriamine-pentaacetic acid-octreotide is delivered in vivo to pancreatic, tumor cell, renal and hepatocyte lysosomes. *Cancer Res.* 1997;57:659–671.
16. Krenning EP, Bakker WH, Kooij PPM, et al. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe^1]-octreotide in man: metabolism, dosimetry and comparison with [^{123}I-Tyr^3]-octreotide. *J Nucl Med.* 1992;33:652–658.
17. Reubi JC, Waser B, Laderach U, et al. Localization of cholecystokinin A and cholecystokinin G-gastrin receptors in the human stomach. *Gastroenterology.* 1997;112:1197–1205.
18. Mogensen CE, Solling K. Studies on renal tubular protein reabsorption: partial and near complete inhibition by certain amino acids. *Scan J Clin Lab Invest.* 1977;37:477–486.