Hepatobiliary Handling of Iodine-125-Tyr^3-Octreotide and Indium-111-DTPA-D-Phe^1-Octreotide by Isolated Perfused Rat Liver

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Radiolabeled bioactive peptides may show receptor-mediated binding to tumors, making them suitable for scintigraphic imaging. The liver is an important organ for peptide clearance. To gain insight into the uptake and intracellular processing of somatostatin analogs, we compared the hepatobiliary handling of ^{125}I-Tyr^3-octreotide and ^{111}In-DTPA-D-Phe^1-octreotide, which are successfully used to image somatostatin receptor-positive tumors in vivo in isolated recirculating perfused rat livers. Sixty minutes following administration of the radiolabeled peptides, perfusion medium and biliary radioactivity were analyzed. Radioiodinated Tyr^3-octreotide was rapidly cleared by the liver and 60% of the dose was excreted intact into the bile after 60 min. In contrast, ^{111}In-DTPA-D-Phe^1-octreotide was not cleared by the liver; medium radioactivity levels remained about constant and only 2% of the dose was found in the bile. These results are in agreement with in vivo findings in rats and humans. We concluded that isolated rat liver perfusion is a good system to rapidly gain insight into the hepatic handling of radiopharmaceuticals.

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Tumor receptor-binding radiolabeled peptides are interesting recent developments in nuclear medicine, as they can be used for in vivo scintigraphic imaging of tumors. An example is somatostatin (Fig. 1A), which binds to its receptors on tumors of neuro-endocrine origin (*1*).

This native peptide is susceptible to very rapid enzymatic degradation (*2*), and therefore is not very useful for in vivo application. For that reason, more stable synthetic somatostatin analogs have been developed. The octapeptide octreotide (SMS 201-995 or Sandostatin®, Fig. 1B) fulfils this criterion (*3*). Large numbers of high-affinity binding sites for native somatostatin and synthetic octreotide have been detected on most endocrine-active tumors (*4*). Since octreotide cannot be radiolabeled easily with a gamma-emitting radionuclide, a synthetic analog (Tyr^3-octreotide) has been developed in which a phenylalanine has been replaced by tyrosine, allowing radioiodination of the molecule (Fig. 1C). This compound, radiolabeled with ^{125}I or ^{123}I, has been used successfully for in vitro somatostatin receptor studies (*4–7*) and tumor scintigraphy in animals (*6,8*) and humans (*1,9,10*). Another radioactive analog of somatostatin is ^{111}In-DTPA-D-Phe^1-octreotide (Fig. 1D), which is also used for in vivo scintigraphy. The last compound lacks some drawbacks of ^{123}I-Tyr^3-octreotide (*7,8*).

The liver is an important organ in the degradation of many circulating peptides. We compared liver handling and excretion into bile of ^{125}I-Tyr^3-octreotide and ^{111}In-DTPA-D-Phe^1-octreotide in isolated recirculating perfused rat liver. By studying the uptake and intracellular handling of octreotide analogs in an isolated rat liver perfusion system, we hope to further extend our understanding of the pharmacokinetic behavior of these compounds.

MATERIALS AND METHODS

Materials

The radiopharmaceuticals used were NA^{125}I (Amersham International, UK), $^{111}InCl_3$ (Mallinckrodt Medical BV, Petten, The Netherlands), Tyr^3-octreotide and DTPA-D-Phe^1-octreotide (Sandoz Pharma AG, Basel, Switzerland), ^{131}I-human serum albumin (Sorin Biomedica, Tronzano, Italy) and bovine serum albumin (Organon Teknika, Oss, The Netherlands). Bovine serum albumin (BSA) and human serum albumin (HSA) were highly purified and free of fatty acids. All other reagents were of the highest purity commercially available.

Radiolabeling

Radioiodination of Tyr^3-octreotide with ^{125}I was performed with the chloramine-T method as described by Bakker et al. (*6*). Labeling of ^{111}In-DTPA-D-Phe^1-octreotide with ^{111}In was carried out as previously described (*7*).

Isolated Perfused Rat Liver System

Livers of male Wistar rats (200–250 g) were isolated and perfused in a recirculating system at 37°C as described by Docter et al. (*11*). The magnetically stirred perfusion medium used in all experiments was 150 ml of Krebs-Ringer buffer with with NaCl (118 mmol/liter), KCl (5 mmol/liter), $MgSO_4$ (1.1 mmol/liter), $CaCl_2$ (2.5 mmol/liter), KH_2PO_4 (1.2 mmol/liter) and $NaHCO_3$

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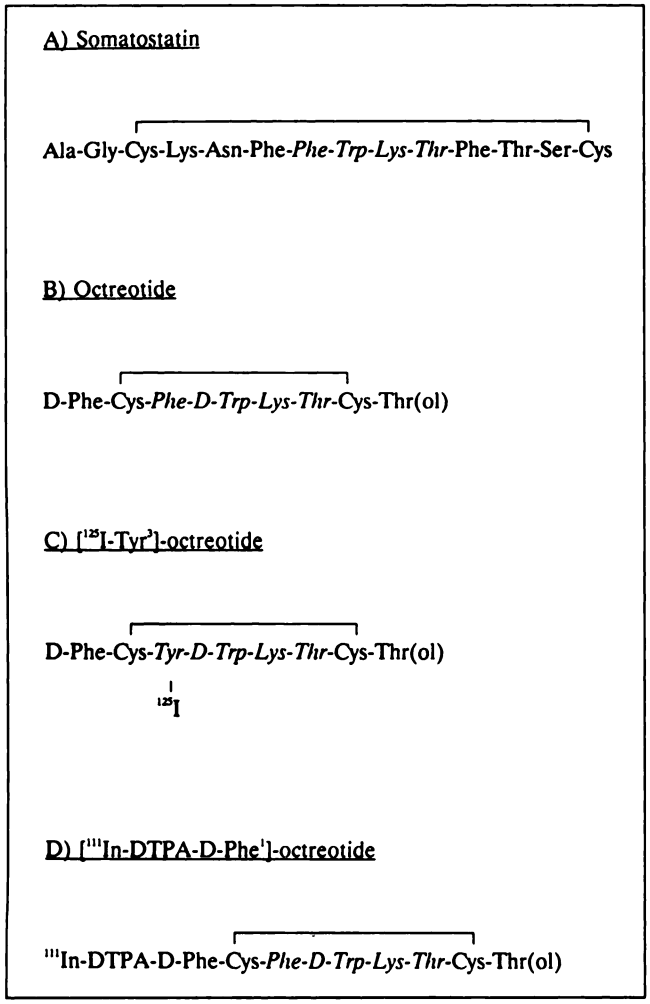

FIGURE 1. Somatostatin and analogs with the supposed bioactive site printed in italics.

mmol/liter) supplemented with 10 m*M* of glucose and 1% BSA. The pH of the medium was maintained at 7.43 by gassing with carbogen (95% CO_2 and 5% O_2, 400 ml/min). Liver function was monitored by its outer appearance, hydrostatic pressure necessary to maintain a perfusion medium flow of 40 ml/min, bile flow and pH of the perfusion medium. Livers were preperfused for 30 min. The experiment was started by adding 370 kBq of tracer (10^{-9} mole) to the stirred medium in the central reservoir. Subsequently, 0.5-ml medium samples were taken at 1–10 min and 15, 20, 25, 30, 40, 50 and 60 min from a smaller medium reservoir, with hydrostatic pressure determined by height. For the determination of correct curve-fitting, more samples were taken in the first minutes of some experiments. Bile samples were collected during 10-min intervals. The samples were stored at −20°C until analysis.

Perfusion Medium and Bile Sample Analysis

The chemical status of the radionuclide in the perfusion medium and bile samples was analyzed as a function of time using SEP-PAK C18 chromatography. The perfusion medium and bile samples were applied to SEP-PAK C18 columns, which had been activated with 2-propanol (5 ml). Elution of the different fractions was performed with 5 ml of distilled water and 5 ml of 0.5 *M* acetic acid to remove free ^{125}I, and 5 ml of 96% ethanol to elute peptide-bound radioactivity. Fractions were collected and counted for radioactivity. Radiochemical composition of the different samples was confirmed by HPLC analysis with a Waters 600 E multisolvent delivery system connected to a μ-Bondapak-C18 reversed-phase column (300 × 3.9 mm, particle size 10 μm). Before HPLC, bile samples were diluted 1:10 with 40% methanol in 154 m*M* of NaCl. Elution was carried out at a flow of 1 ml/min with a linear gradient of 40% to 80% methanol in 154 m*M* of NaCl in 20 min. The latter composition was maintained for another 5 min. Collected fractions were measured by routine scintillation counting.

Calculations

Curve-fitting of the two-exponential medium tracer disappearance curve was done as described previously (*11*). A peel-off system of curve-fitting was used (Fig. 3). All data are reported as mean ± s.d. of each parameter obtained in replicate studies (n = 4–8). Statistical evaluation was performed using Student's t-test.

RESULTS

Iodine-125-Tyr^3-Octreotide

Figure 2 shows typical time courses of total radioactivity, peptide-bound radioactivity and liberated ^{125}I in the medium and bile after administering ^{125}I-Tyr^3-octreotide to the perfusion medium. Results are expressed as a percentage of the administered dose. After tracer administration, radioactivity rapidly disappears from the perfusion medium followed by rapid excretion into the bile. Of the administered radioactivity, 27% of peptide-bound radioactivity is left in the perfusion medium after 60 min, while about 1% consists of free iodide (Table 1). Free iodide in the bile accounts for only 1% of the administered radioactivity; most of the radioactivity (60% of the total dose) is excreted in peptide-bound form in the bile. HPLC analysis revealed that the peptide-bound radioactivity in the bile is completely intact ^{125}I-Tyr^3-octreotide. In all experiments, the disappearance of ^{125}I-Tyr^3-octreotide could be fitted to the sum of two exponentials. In Figure 3, this two-exponential medium disappearance curve is shown and the half-lives of the fast and slow component were calculated.

The distribution time through the system was estimated by perfusing livers with ^{131}I-HSA, a substance that is not taken up into the hepatocytes. The half-life of the fast component of the medium ^{131}I-HSA curve was 0.29 (0.02) min, whereas the half-life of the slow component was very long as almost no radioactivity disappeared from the perfusion medium. In the case of ^{125}I-Tyr^3-octreotide, the half-life of the fast component was 0.28 (0.04) min (representing distribution through the perfusion system) and 36.2 (3.9) min of the slow component (representing uptake and metabolism, Table 2).

Indium-111-DTPA-D-Phe^1-Octreotide

Figure 4 shows typical time courses of total radioactivity, peptide-bound radioactivity and nonpeptide-bound breakdown products after administration of ^{111}In-DTPA-D-Phe^1-octreotide to the perfusion medium. After 60 min, 95% of the administered radioactivity still consists of peptide-bound tracer in the perfusion medium, which is identical to intact ^{111}In-DTPA-D-Phe^1-octreotide as demonstrated by HPLC (not shown) (Table 1). Only a small

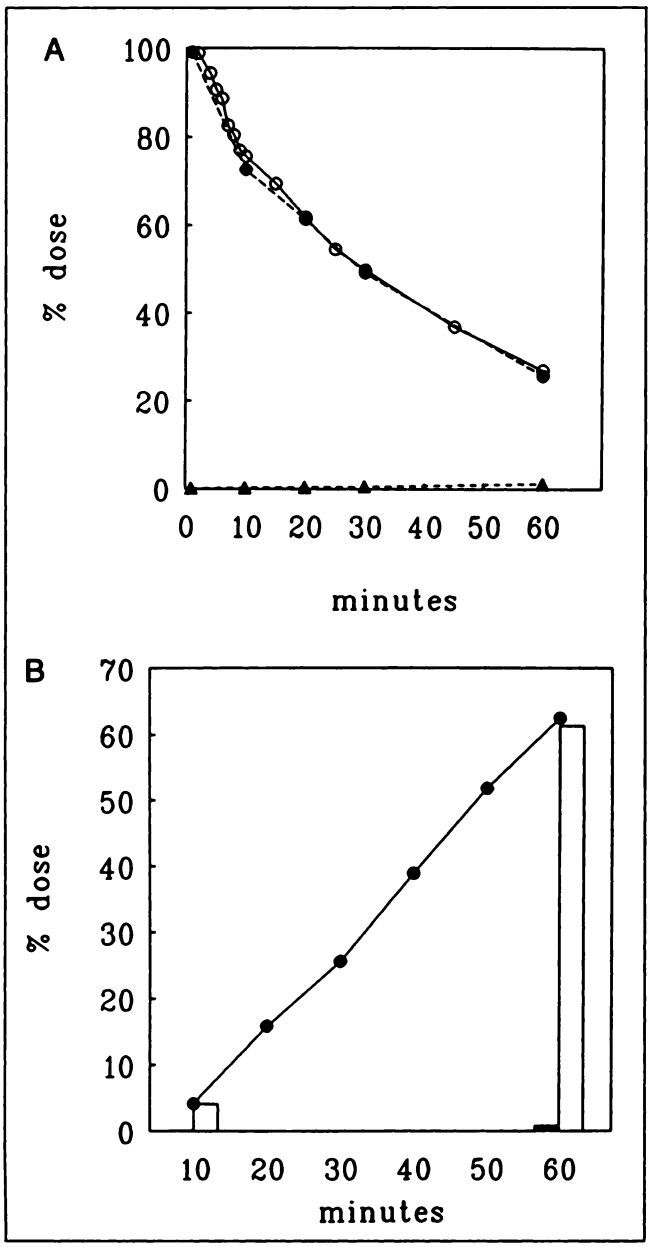

FIGURE 2. (A) Rat liver perfusion: a typical example of disappearance of total (○) and peptide-bound (●) radioactivity from the medium and appearance of ^{125}I (▲) in medium after administration of ^{125}I-Tyr^3-octreotide. (B) Typical example of cumulative excretion by the perfused rat liver of total radioactivity (●), divided in peptide-bound radioactivity (open bar) and ^{125}I (solid bar), into the bile after administration of ^{125}I-Tyr^3-octreotide.

portion (2%) of the administered radioactivity is excreted in the bile. Furthermore, the half-lives of the fast and slow components of the biphasic disappearance from the medium (which is described as the sum of two exponentials) are 0.25 (0.08) min (distribution) and unmeasurably long, respectively (Table 2).

DISCUSSION

Radiolabeled octreotide analogs bind to the somatostatin receptors on neuro-endocrine tumor cells and are suitable for scintigraphic imaging of these tumors. Although these

TABLE 1
Original Peptides and Break-Down Products

	PBR* (medium)	NPBR† (medium)	PBR (bile)	NPBR (bile)
^{125}I-Tyr^3-octreotide	26.9 (1.9)	1.2 (0.0)	60.4 (3.7)	1.0 (0.1)
^{111}In-DTPA-D-Phe^1-octreotide	94.9 (0.8)‡	0.9 (0.1)‡	2.2 (0.2)‡	0.2 (0.0)‡

*PBR = peptide-bound radioactivity.
†NPBR = nonpeptide-bound radioactivity, e.g., ^{125}I and ^{111}In-DTPA, in perfusion medium and bile after 60 min of perfusion with ^{125}I-Tyr^3-octreotide or ^{111}In-DTPA-D-Phe^1-octreotide. Results are given as mean (s.d.) % dose (n = 4–8).
‡$p < 0.001$ versus ^{125}I-Tyr^3-octreotide.

radiopharmaceuticals have been investigated in vitro (*4–7*) and in vivo (*1,6,8–10*), little is known about the metabolism of these radioligands, especially in the liver. However, there is much information regarding the hepatic metabolism of the native peptides somatostatin-14 and somatostatin-28. They are degraded through the action of hepatic aminopeptidases and endopeptidases as studied in the perfused rat liver (*12,13*). The perfused rat liver is very suitable for investigating several parameters of liver metabolism, such as the disappearance from the perfusion medium and the appearance of degradation products and biliary excretion. Therefore, in this study we compared the liver handling of ^{125}I-Tyr^3-octreotide and ^{111}In-DTPA-D-Phe^1-octreotide in this system.

Iodine-125-Tyr^3-octreotide is rapidly taken up into the

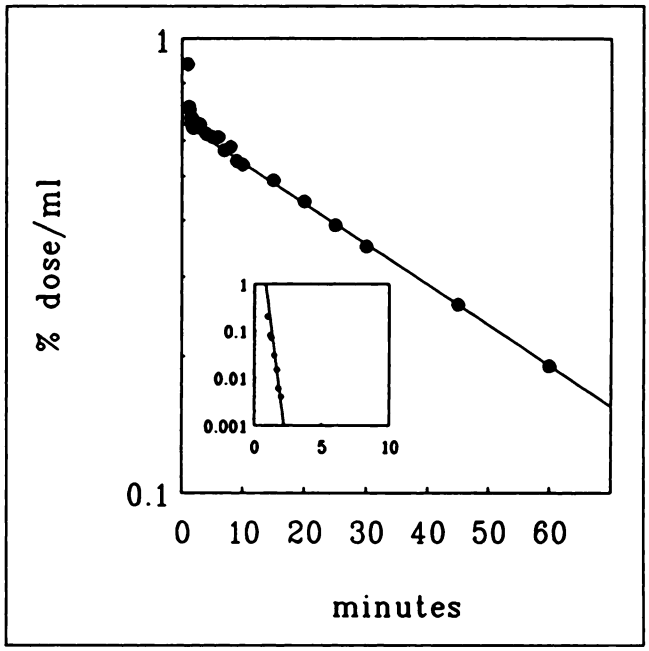

FIGURE 3. Typical example of the curve-fitting of a ^{125}I-Tyr^3-octreotide disappearance curve, which represents the sum of two exponentials, by a two-exponential model. Plot of log %dose/ml versus time, with the least-squares regression line on the final straight part of the curve (slow component). Inset: log plot of the fast component; data values were obtained after subtracting the slow component with the least-squares regression line.

TABLE 2
Half-Lives of the Fast and Slow Disappearance Components from the Medium of ^{131}I-HSA, ^{125}I-Tyr^3-Octreotide and ^{111}In-DTPA-D-Phe^1-Octreotide*

	Fast component	Slow component
^{131}I-HSA	0.29 (0.02)	∞†
^{125}I-Tyr^3-octreotide	0.28 (0.04)	36.2 (3.9)
^{111}In-DTPA-D-Phe^1-octreotide	0.25 (0.08)	∞†

*Results are given in mean (s.d.) minutes (n = 4–8).
†$p < 0.001$ versus ^{125}I-Tyr^3-octreotide.

liver, immediately followed by intact excretion into the bile (Fig. 3). Because the half-life of the fast component is the same as that of ^{131}I-HSA—which is not transported into the hepatocytes but is passively distributed into the liver interstitium—it is predominantly determined by distribution of the tracer through the system and the extracellular liver compartment. The half-life of the second component is mainly determined by transport and metabolism in the liver. We recently reported transport and metabolism of thyroid hormones in the perfused rat liver where an uptake and metabolism component could be distinguished clearly in the biphasic disappearance curve from the medium after the distribution phase (*11*). In the case of ^{125}I-Tyr^3-octreotide, uptake is immediately followed by excretion into the bile and therefore not seen as a distinct component. Medium and bile chromatography showed that hardly any free radioactive iodide is found, while the majority of the administered dose is already excreted intact into the bile within 60 min. Very little of the administered radioactivity accumulates in the liver.

An internalized peptide can reach the bile by two pathways: (1) a lysosomal, or indirect pathway or (2) a nonlysosomal, or direct pathway (*14*). In general, molecules processed by the first pathway are not excreted intact into the bile. Compounds that utilize the second pathway are generally excreted into the bile as intact molecules and appear in the bile sooner than those excreted by the lysosomal pathway. Our results show that ^{125}I-Tyr^3-octreotide is translocated across the hepatocytes into the bile by the direct pathway, thereby bypassing the lysosomes, because the radiopharmaceutical is excreted intact into the bile and because there is no lag time before excretion into the bile. These findings are in accordance with ^{125}I-Tyr^3-octreotide's stability against (hepatic) enzymatic degradation, contrary to the native peptides, somatostatin-14 and somatostatin-28 (*12,13*). This is probably due to the introduction of a D-amino acid at the N-terminal and an amino-alcohol substituent at the C-terminal end of the peptide chain (*3*).

Recently, it has been reported that uptake into isolated rat hepatocytes is a carrier-mediated process which is related to the multispecific bile acid transporter for several types of cyclosomatostatins (*15*). Further transcellular bile-acid transport to the bile after uptake into the cells has been elucidated by electron microscopic autoradiography, showing that these substances are excreted rapidly into the bile in a chemically unchanged form (*16*). We did not investigate possible carrier-mediated transport properties of our somatostatin analogs, but intact excretion into the bile after a rapid transcellular transport agrees with our findings in the perfused rat liver.

The results of our study are in agreement with rat and

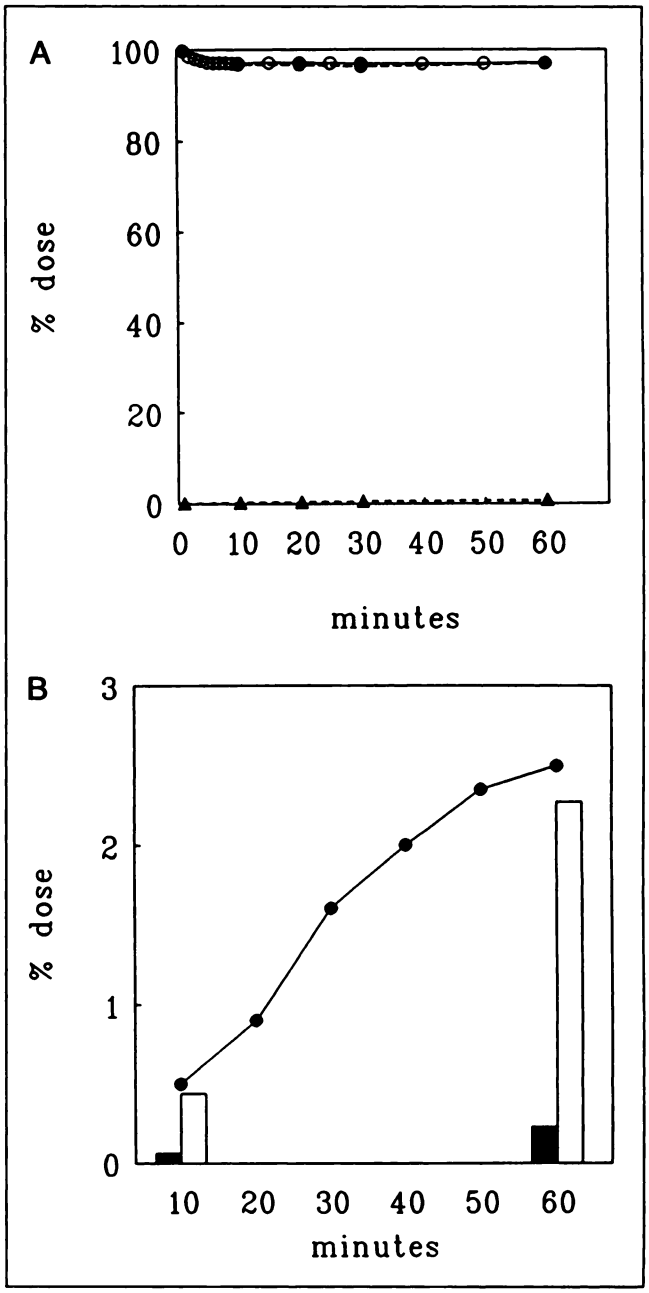

FIGURE 4. (A) Rat liver perfusion: a typical example of disappearance of total (○) and peptide-bound (●) radioactivity from the medium and appearance of nonpeptide-bound breakdown products (▲) in medium after administration of ^{111}In-DTPA-D-Phe^1-octreotide. (B) A typical example of cumulative excretion by the perfused rat liver of total radioactivity (●), divided in peptide-bound radioactivity (open bar) and nonpeptide-bound breakdown products (solid bar) into the bile after ^{111}In-DTPA-D-Phe^1-octreotide administration.

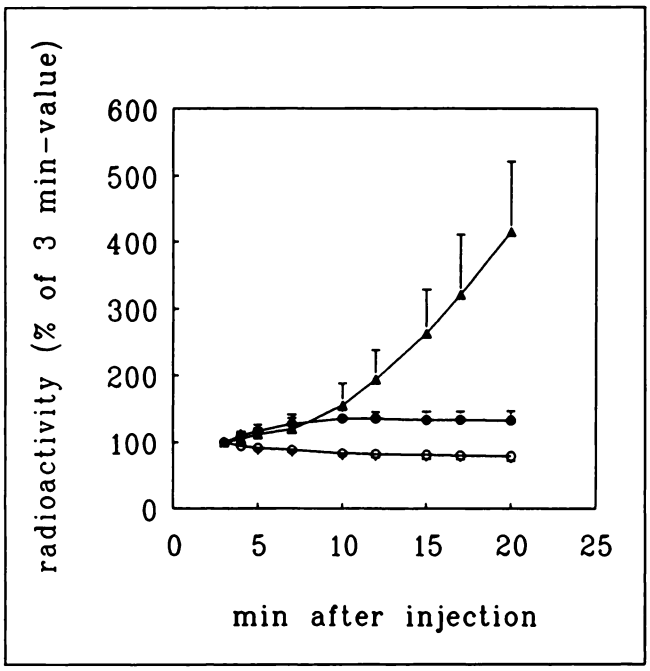

FIGURE 5. Radioactivity, expressed as mean ± s.d. percentage of the 3-min value, measured above ROIs in humans, i.e., liver tissue without major bile ducts (after ^{123}I-Tyr^3-octreotide (●, n = 5) and ^{111}In-DTPA-D-Phe^1-octreotide (○, n = 3)) and gallbladder (after ^{123}I-Tyr^3-octreotide (▲, n = 5)). After injection of ^{111}In-DTPA-D-Phe^1-octreotide, no bile-related radioactivity was measured in major bile-ducts or in the gallbladder.

human in vivo investigations with ^{123}I-Tyr^3-octreotide, which show that disposal occurs predominantly by rapid uptake into the liver and excretion via the bile into the intestines (*6,9*). In Figure 5, metabolism in the human liver is shown. Data are derived from studies by Bakker et al. (*9*). Iodine-123-Tyr^3-octreotide is rapidly cleared from the circulation by the liver, immediately followed by hepatobiliary excretion; radioactivity in a nonmajor bile duct-containing part of the liver does not increase above 150% of the 3-min value, but a sharp increase of radioactivity is seen above the gallbladder during the first 20 min after administration.

Indium-111-DTPA-D-Phe^1-octreotide is not taken up by the isolated perfused rat liver because almost no tracer disappears from the perfusion medium. The half-life of the fast component is again determined by distribution through the system, whereas the half-life of the slow component is very long, indicating very slow handling in the liver. Furthermore, hardly any radioactivity is found in the liver and excretion into the bile is negligible. This may be due to the addition of the relatively large and very hydrophylic DTPA group to the octapeptide molecule, favoring renal excretion of the latter, like the radiolabeled chelate itself (i.e., ^{99m}Tc-DTPA), which is excreted exclusively by glomerular filtration (*17*). These findings are in excellent accordance with in vivo studies (*8,10*). In Figure 5, metabolism in the human liver is shown using data derived from studies by Krenning et al. (*10*). After ^{111}In-DTPA-D-Phe^1-octreotide administration, the radioactivity measured above the liver does not increase, but decreases, due to clearance by the kidneys rather than the liver (*8,10*). Likewise, no radioactivity could be measured scintigraphically above the gallbladder.

Indium-111-DTPA-D-Phe^1-octreotide is the preferred analog for in vivo scintigraphy because it has several advantages compared to ^{123}I-Tyr^3-octreotide: general availability, simple one-step radiolabeling, longer physiological half-life in plasma and a more suitable metabolism. In order to visualize a tumor by receptor binding in vivo, the specific activity expressed in counts per unit of area must exceed the local background radiation. Unlike radioiodinated Tyr^3-octreotide, ^{111}In-DTPA-D-Phe^1-octreotide is not cleared via the liver and causes no accumulation of radioactivity in the biliary and digestive tracts. The latter radiopharmaceutical is more suitable for visualization of tumor-receptor accumulation in the upper abdominal region where the small endocrine gastro-entero-pancreatic target tumors are located.

This study showed that the results obtained in the perfusion system agreed with in vivo results and could predict in vivo clearance for both rats and humans. Currently we are exploring the use of other radiolabeled peptides which will be used for imaging endocrine tumors and immunological disorders. As we prefer to use peptides that are not degraded in or cleared by the liver and therefore cause no accumulation of radioactivity in the biliary and digestive tracts, the liver perfusion system can be used to test liver metabolism of these peptides rapidly. During in vivo studies in rats and humans with ^{125}I-Tyr^3-octreotide and other peptides, it is difficult to determine which fraction of plasma radioactivity is still composed of intact material and where peptide degradation takes place—in the liver, the intestines or the kidneys. Rat liver perfusion studies may provide insight into this matter. Furthermore, when it is known where and how a compound is degraded, possible inhibition of early and undesirable degradation can be investigated to save intact compounds for binding to their receptors on tumors. The rat liver perfusion system can also be used for these studies.

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EDITORIAL

Hepatic Handling of Radiopharmaceuticals: Is the In Vitro Model Useful?

Somatostatin is a hyothalamic polypeptide that inhibits the secretion of the pituitary growth hormone. It also inhibits the secretion of prolactin and thyroid-stimulating hormone and has a variety of other inhibitory effects. These divergent functions of hormones are generally related to the biodistribution of their corresponding receptors. The receptors for somatostatin are expressed in the brain, the anterior lobe of pituitary gland, acinar and islet cells of the pancreas, stomach mucosa, intestinal mucosa and the adrenal gland. In addition, these receptors are also expressed on tumor cells of neuroendocrine origin, including meningioma, gastrinoma, carcinoid and insulinoma. Naturally, somatostatin is considered as a reagent useful for in vivo scintigraphic imaging as well as for therapy of such tumors.

However, native forms of somatostatin that are 14 or 28 amino acid peptides (somatostatin-14 and somatostatin-28, respectively) have very short biological half-lives; therefore, their clinical usefulness is limited. They are metabolized very rapidly through the action of aminopeptidases and endopeptidases principally in the liver. Synthetic somatostatin analogs were synthesized to increase their stability in vivo (*1*). An octapeptide octreotide, SMS 201-995, is a somatostatin analog that possesses D-isomer of phenylalanine (D-Phe) and amino alcohol of threonine (Thr(ol)) at N-terminal and C-terminal end, respectively. This analog is resistant to proteolysis and has a long half-life. It has been used for the treatment of growth hormone-producing pituitary adenoma and gastrinoma (*2,3*). Furthermore, derivatives of this analog have been labeled with radionuclides to visualize somatostatin receptors expressed in tumors of neuroendocrine origin. Initially, a phenylalanine of octreotide has been replaced by tyrosine to allow iodination (^{125}I or ^{123}I-Tyr[3]-octreotide), and nuclear imaging of endocrine-related tumors was tested (*4*). However, high abdominal background was a major drawback. Radioiodinated Tyr[3]-octreotides were rapidly cleared from the circulation principally through the liver and secreted into the biliary system. This hepatobiliary clearance resulted in high hepatic and intestinal accumulation (*5*). Subsequently, a diethylenetriaminepentaacetic acid (DTPA) has been conjugated to phenylalanine of octreotide for ^{111}In labeling ([^{111}In-DTPA-D-Phe[1]]-octreotide). In contrast to radioiodinated Tyr[3]-octreotide, ^{111}In-DTPA-D-Phe[1]-octreotide was cleared predominantly by the kidneys and hepatic accumulation was not observed (*6*). The modification of the N-terminal D-Phe residue with the ^{111}In-DTPA group appears to have inhibited hepatic clearance. The difference in metabolism and biodistribution between [^{123}I-Tyr[3]]-octreotide and [^{111}In-DTPA-D-Phe[1]]-octreotide was confirmed by an in vivo system in man (*7*). Thus, the modifications of peptides with different radionuclides may change their metabolism and biodistribution in vivo.

The liver and the kidney are the two major organs for metabolism and clearance but more so the liver because it is located in the center of the abdominal cavity and by itself causes a high background. In addition, metabolized radionuclides may be secreted into the intestine through the biliary tract, thereby increasing abdominal background and interfering with nuclear imaging. As the modifications of the peptides with different radionuclides seem to change their metabolism in the liver, the availability of an in vitro system to examine hepatic handling of modified reagents will help us to understand the pharmacokinetics of bioactive reagents and may be useful in predicting their behaviors in vivo. Such a system also will provide information on the uptake and intracellular processing of reagents that are difficult to study in vivo.

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