Gelatin-Based Plasma Expander Effectively Reduces Renal Uptake of ^{111}In-Octreotide in Mice and Rats

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^{111}In-Diethylenetriaminepentaacetic acid-octreotide generally is used for the scintigraphic imaging of neuroendocrine and other somatostatin receptor–positive tumors. On the basis of the successful targeting of octreotide, radiolabeled somatostatin analogs, such as ^{90}Y-(1,4,7,10-tetraazacyclododecane-*N*,*N′*,*N″*,*N‴*-tetraacetic acid [DOTA])^{0}-Tyr^{3}-octreotide and ^{177}Lu-DOTA^{0}-Tyr^{3}-octreotate, were developed for peptide receptor radionuclide therapy. However, the maximum tolerated doses of these analogs are limited because of the high and persistent renal uptake that leads to relatively high radiation doses in the kidneys. Renal uptake can be reduced by coinfusion of basic amino acids or polypeptides. However, high doses of basic amino acids can induce severe side effects. It was reported that the infusion of gelatin-based plasma expanders resulted in increased low-molecular-weight proteinuria, suggesting that these plasma expanders interfere with the tubular reabsorption of peptides and proteins. In the present study, we analyzed the effects of several plasma expanders on the renal uptake of ^{111}In-octreotide in rats and mice. **Methods:** Wistar rats and BALB/c mice were injected with 0.5 or 0.1 mL of plasma expander, respectively. Thereafter, the animals received ^{111}In-octreotide intravenously. Animals were killed at 20 h after the injection of the radiopharmaceutical. Organs were dissected, and the amount of radioactivity in the organs and tissues was measured. **Results:** The administration of 20 mg of Gelofusine in rats or 4 mg in mice was as effective in reducing the renal uptake of ^{111}In-octreotide as the administration of 80 or 20 mg of lysine in rats or mice, respectively, without reducing ^{111}In-octreotide uptake in receptor-positive organs. Plasma expanders based on starch or dextran had no effect on the renal uptake of ^{111}In-octreotide. **Conclusion:** The gelatin-based plasma expander Gelofusine significantly reduced the kidney uptake of ^{111}In-octreotide as effectively as did lysine. Because Gelofusine is a well-known and generally used blood volume substitute that can be applied safely without the induction of toxicity, evaluation of this compound for its potential to reduce the kidney uptake of radiolabeled peptides in patients is warranted.

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The elimination of hydrophilic peptide–based radiopharmaceuticals from the body usually occurs through the kidneys. For scintigraphic imaging and also for therapeutic applications, rapid renal excretion of these radiopharmaceuticals leads to images with low background activity and relatively low radiation doses compared with what is seen with gastrointestinal elimination (*1*). On the other hand, retention of a radiopharmaceutical in the kidneys may hamper the clinical use of the compound. For imaging applications, the renal uptake of radiolabeled peptides reduces the sensitivity for detection in the vicinity of the kidneys (*2*). This situation often applies to radiolabeled peptides used for specific receptor targeting of tumors, such as ^{111}In-octreotide (*3*). For therapeutic applications, the renal accumulation of radiolabeled peptides limits the maximum tolerated activity doses that can be administered without the induction of radiation nephrotoxicity. In most cases of peptide receptor radionuclide therapy (PRRT), the kidneys are the dose-limiting organs (*2*).

Renal uptake and retention of (radiolabeled) low-molecular-weight proteins and peptides is a complex mechanism that occurs through glomerular filtration and tubular reabsorption (*4,5*). It is thought that after filtration, the peptides are reabsorbed by the proximal tubular cells and transferred to lysosomes for proteolytic digestion (*2,4*). Subsequently, metabolized amino acids reappear in the bloodstream. In contrast, radiometal-chelated amino acids are trapped in lysosomes, leading to high absorbed doses of radiation in the tubular cells of the kidneys (*2*). Several studies have aimed to develop a strategy to reduce the renal retention of radiolabeled peptides and antibody fragments (*6,7*). These studies have indicated that the

coadministration of basic compounds, especially amino acids such as lysine and arginine, significantly reduces the radioactivity concentrations in the kidneys, in some cases up to 60% (*2,8*). However, the maximum tolerated doses of these amino acids are limited, because at high doses, these compounds can cause side effects (arrhythmias, nausea, and hyperkalemia) and induce nephrotoxicity themselves (*3,6*).

Recently, it was found that infusions of low doses of polygelines (e.g., Gelofusine [Braun]) caused low-molecular-weight proteinuria. It was hypothesized that specific components in these plasma expanders might attenuate the tubular reabsorption process (*9,10*). In the present study, we evaluated whether blood volume substitutes reduced the uptake of ^{111}In-octreotide in the kidneys of rats and mice.

MATERIALS AND METHODS

Radiolabeling of Octreotide

Diethylenetriaminepentaacetic acid-D-Phe-1-octreotide was obtained from Tyco Health Care (Octreoscan). Labeling was performed as described by the manufacturer. In brief, 37 MBq of ^{111}In-Cl_3 was transferred to the octreotide reaction vial (10 μg). After incubation for 30 min at room temperature, quality control was performed. Radiochemical purity was checked by reversed-phase high-pressure liquid chromatography with a C_{18} column (Zorbax Rx-C_{18}; 4.6 mm × 25 cm; Agilent Technologies). A gradient was applied to the column from 40%–80% ammonium acetate (pH 5.5; 0.05 mol/L) to methanol over 20 min (1 mL/min). The unbound ^{111}In eluted with a retention time of 2.7 min, whereas ^{111}In-octreotide showed a retention time of 14 min.

Plasma Expanders

Plasma expanders were obtained commercially. Gelofusine is a succinylated gelatin solution (40 g/L). Voluven (Fresenius Kabi AG) is a plasma expander based on poly(*O*-hydroxyethyl)starch molecules (60 g/L). Rheomacrodex solution (NPBI International BV) contains dextran 40 (100 g/L). Haemaccel (Aventis Pharma BV) is a plasma expander based on degraded gelatin cross-linked with urea (35 g/L).

Lysine Solutions

A lysine solution of 160 mg/mL (rats) or 200 mg/mL (mice) was prepared in phosphate-buffered saline (PBS).

Animal Studies

For all experiments, groups of 5 female BALB/c mice (weight, 18–24 g) or 4 male Wistar rats (weight, 200–220 g) were used. Animals were injected intravenously with a plasma expander (0.1 mL in mice or 0.5 mL in rats) via the tail vein. At 2–5 min after injection of the plasma expander or PBS, ^{111}In-octreotide was injected intravenously (0.4 MBq per mouse or 2 MBq per rat). Animals were killed 20 h later, a blood sample was drawn, and organs were dissected. Tissues were weighed, and the amount of radioactivity was measured to determine the concentration of radioactivity. The activity in tissues was measured by use of a shielded well-type γ-counter (Wizard; Pharmacia-LKB) together with the injection standards. The radioactivity concentration was expressed as the percentage injected dose per gram of tissue (%ID/g).

In a first experiment, the effects of Gelofusine and lysine on the renal uptake of ^{111}In-octreotide in rats were analyzed. In a second experiment, the levels of kidney uptake of ^{111}In-octreotide in rats and mice after injection of PBS, lysine, or Gelofusine were compared. A third experiment was performed to analyze the effects of several plasma expanders on the ^{111}In-octreotide biodistribution in rats. In this experiment, animals were injected with 0.5 mL of PBS, lysine (80 mg), Gelofusine (20 mg), Voluven (20 mg), or Rheomacrodex (30 mg) 2–5 min before the injection of ^{111}In-octreotide. In a last experiment with rats, ^{111}In-octreotide was injected 3, 15, or 60 min after the injection of Gelofusine to determine the optimal time interval between the administration of the plasma expander and the administration of the tracer.

Statistical Analysis

Values are presented as mean ± SD. Analysis was performed with a 1-way ANOVA. Results were corrected for multiple datasets with the Bonferroni multiple-comparison test. The significance level was set at 0.05.

RESULTS

The biodistributions of ^{111}In-octreotide in rats injected 2–5 min after injection with PBS, lysine, or Gelofusine are shown in Figure 1. Although the uptake of ^{111}In-octreotide

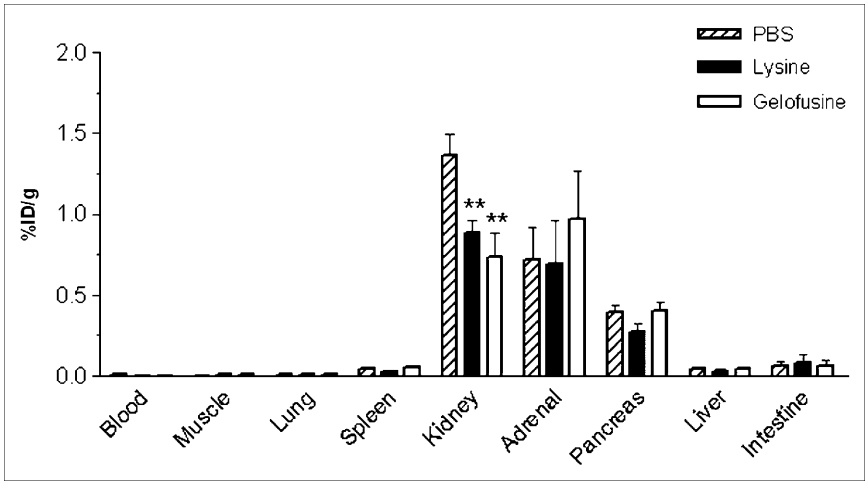

FIGURE 1. Biodistribution data obtained 20 h after intravenous injection of ^{111}In-octreotide in rats. Groups of 4 rats received 0.5 mL of PBS, 0.5 mL of lysine (80 mg), or 0.5 mL of Gelofusine (20 mg) intravenously 2–5 min before injection of ^{111}In-octreotide. Results are presented as mean %ID/g; error bars indicate SDs. $^{**}P < 0.01$.

in most organs and tissues (liver, spleen, lungs, intestine, muscle, and blood) was very low, increased uptake was observed in the adrenal glands, pancreas, and kidneys of the rats in all groups. Injection of PBS, lysine, or Gelofusine resulted in similar ^{111}In-octreotide concentrations in all tissues and organs except for the kidneys. The amount of radioactivity in the kidneys was significantly higher (1.36 ± 0.14 %ID/g) in the control animals (preinjected with PBS) than in the rats that received lysine (0.88 ± 0.08 %ID/g) or Gelofusine (0.73 ± 0.15 %ID/g) ($P < 0.001$). No significant difference in kidney uptake was found between animals injected with lysine and those injected with Gelofusine.

Because Gelofusine reduced the kidney uptake of ^{111}In-octreotide in rats as effectively as did lysine, we evaluated whether this agent had similar effects in mice. Table 1 shows ^{111}In-octreotide concentrations measured in the organs and tissues of rats and mice after injection of PBS, lysine (80 mg), or Gelofusine (20 mg). The effects of lysine and Gelofusine on the renal uptake of ^{111}In-octreotide in mice were similar to those observed in rats. Compared with that of PBS, the intravenous administration of lysine and Gelofusine reduced kidney uptake in rats by 35% and 46%, respectively. Like that in rats, the administration of Gelofusine in mice demonstrated the most prominent reduction in kidney uptake (16% for 20 mg of lysine and 61% for 4 mg of Gelofusine). In mice, relatively higher radioactivity concentrations were present in the lungs whereas the concentrations in the adrenal glands and the pancreas were considerably lower than those in rats. Although the administration of lysine and Gelofusine in mice had similar effects on the renal uptake of ^{111}In-octreotide, further experiments were performed with rats because the high uptake in receptor-positive tissues (adrenal glands and pancreas) allowed us to monitor possible effects of plasma expanders on receptor-mediated ^{111}In-octreotide uptake.

Next, we determined the effects of various plasma expanders on the kidney uptake of ^{111}In-octreotide. Figure 2 shows the biodistributions of ^{111}In-octreotide in rats preinjected with various blood volume substitutes. Although lysine, Gelofusine, and Haemaccel reduced kidney uptake significantly ($P < 0.001$), no significant reduction in kidney uptake was seen after the injection of Voluven or Rheomacrodex. None of the plasma expanders affected the uptake of ^{111}In-octreotide in receptor-positive tissues significantly. The levels of uptake in the other organs, except for the kidneys, were comparable.

Finally, we determined the optimal time interval between Gelofusine administration and the injection of radiolabeled octreotide. The biodistribution of ^{111}In-octreotide is shown in Figure 3. Only the uptake in the kidneys is shown because the levels of uptake in the other organs were similar to those observed in the previous experiments. No significant differences in the radioactivity concentrations in the adrenal glands and the pancreas were measured. When Gelofusine was administered 3 and 15 min before the injection of ^{111}In-octreotide, the radioactivity concentration in the kidneys was significantly lower than when PBS was administered ($P < 0.01$ and $P < 0.05$, respectively). The radioactivity concentration in the kidneys of rats injected with ^{111}In-octreotide 1 h after Gelofusine administration was similar to that in the control rats. Gelofusine administration apparently did not reduce the kidney uptake of ^{111}In-octreotide when the time between the injections exceeded 15 min.

DISCUSSION

PRRT is a relatively new and promising therapeutic modality for the treatment of cancer. In general, the applicability of PRRT is limited by the renal uptake of the radiolabeled peptides, which can cause renal toxicity. In the

TABLE 1
^{111}In-Octreotide Biodistribution at 20 Hours After Injection*

	Mean ± SD %ID/g in:					
	Rats			Mice		
Organ	PBS	Lysine	Gelofusine	PBS	Lysine	Gelofusine
Blood	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.00
Muscle	0.005 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Lungs	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.59 ± 0.12	0.79 ± 0.25	0.69 ± 0.10
Spleen	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.10 ± 0.01	0.08 ± 0.06	0.11 ± 0.01
Kidneys	1.36 ± 0.14	0.88 ± 0.08†	0.73 ± 0.15†	1.47 ± 0.38	1.23 ± 0.19‡	0.58 ± 0.23†
Adrenal glands	0.72 ± 0.20	0.69 ± 0.27	0.97 ± 0.30	0.13 ± 0.01	0.12 ± 0.03	0.18 ± 0.04
Pancreas	0.39 ± 0.05	0.27 ± 0.05‡	0.40 ± 0.05	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02
Liver	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.00	0.10 ± 0.01	0.10 ± 0.02	0.11 ± 0.02
Intestine	0.06 ± 0.03	0.08 ± 0.05	0.06 ± 0.04	0.09 ± 0.02	0.10 ± 0.02	0.11 ± 0.02

*PBS, lysine, or Gelofusine was injected 2–5 min before injection of radiolabel. Biodistribution data were collected at 20 h after intravenous injection of ^{111}In-octreotide in rats or mice. Groups of 4 rats received 0.5 mL of PBS or Gelofusine (20 mg). Groups of 5 mice received 0.1 mL of PBS or Gelofusine (4 mg).

†$P < 0.01$.

‡$P < 0.05$.

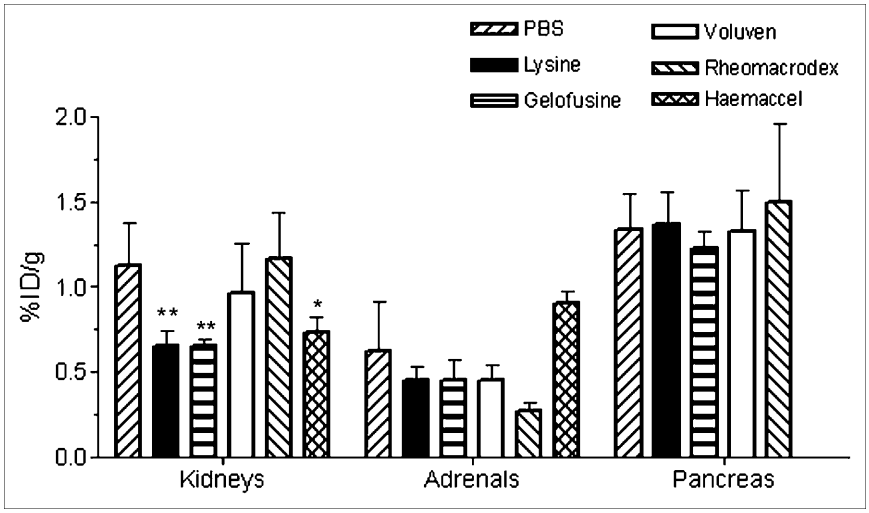

FIGURE 2. Biodistribution data obtained with various plasma expanders 20 h after intravenous injection of ^{111}In-octreotide in rats. Rats received PBS, lysine (80 mg), Gelofusine (20 mg), Voluven (20 mg), Rheomacrodex (30 mg), or Haemaccel (17.5 mg) (0.5 mL intravenously) 2–5 min before injection of ^{111}In-octreotide. Results are presented as mean %ID/g; error bars indicate SDs. $^{*}P < 0.05$. $^{**}P < 0.01$.

last decade, several studies have been performed to develop a strategy to reduce the renal retention of radiolabeled peptides to enhance the therapeutic window of PRRT (*1,2*). On the basis of the observation that individuals infused with polygeline-based plasma expanders developed low-molecular-weight proteinuria, we studied the effects of blood volume substitutes on the renal uptake of ^{111}In-octreotide. We demonstrated that the administration of Gelofusine efficiently reduced the kidney uptake of ^{111}In-octreotide in rats. A bolus injection of 0.5 mL of Gelofusine (20 mg) before the injection of the tracer reduced the radioactivity concentration by 46%. The reduction in kidney uptake was comparable to that obtained with 80 mg of lysine. Because various animal models have been used in preclinical PRRT experiments, we compared the effects of Gelofusine administration in rats and mice (*1,11,12*). In both species, the initial kidney uptake of the radiolabeled peptide was high, and the administration of Gelofusine effectively reduced the renal uptake of ^{111}In-octreotide. In both rats and mice, Gelofusine was at least as effective as lysine in reducing the renal uptake of the radiopharmaceutical.

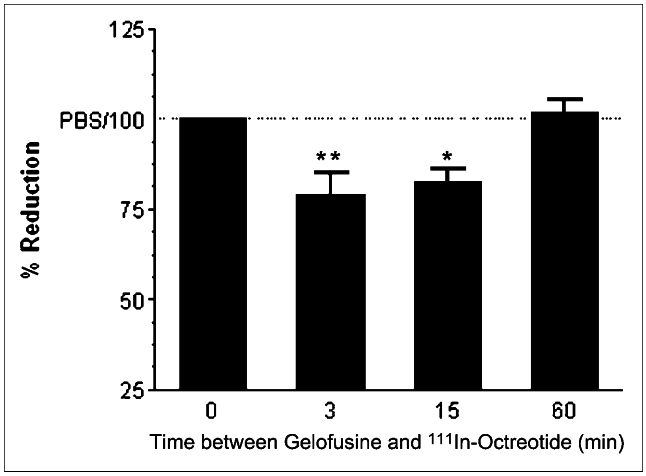

FIGURE 3. ^{111}In-Octreotide uptake in kidneys of rats (4 per group) 20 h after injection. Rats received PBS or Gelofusine (20 mg) (0.5 mL intravenously) 3, 15, or 60 min before injection of ^{111}In-octreotide. Results are presented as mean %ID/g; error bars indicate SDs. $^{*}P < 0.05$. $^{**}P < 0.01$.

Because the radioactivity concentration of ^{111}In-octreotide in the kidneys and receptor-positive organs of rats was high whereas in other organs the concentration was very low, we performed additional experiments with rats. A comparison of several different blood volume substitutes indicated that only blood volume substitutes based on modified gelatin (Gelofusine and Haemaccel) reduced the renal uptake of ^{111}In-octreotide; those based on poly(*O*-hydroxyethyl)starch (Voluven) or polydextrose (Rheomacrodex) did not. Thus, the effects of Gelofusine and Haemaccel on the renal concentration of ^{111}In-octreotide were not attributable primarily to the colloid osmotic pressure of the solution, as all blood volume substitutes induced similar hemodynamic effects. Because neither Rheomacrodex nor Voluven induced a reduction in the radioactivity concentration in the kidneys, apparently a specific component(s) in Gelofusine and Haemaccel is responsible for the reduction in ^{111}In-octreotide uptake.

The kidney uptake of ^{111}In-octreotide and the mechanism of renal processing of this radiolabeled peptide have been studied intensively (*1,13*). It is assumed that ^{111}In-octreotide is taken up by the proximal tubular cells, partly through fluid-phase endocytosis and partly through receptor-mediated endocytosis, in which the megalin and cubilin receptor is supposed to play a major role (*13*). Melis et al. demonstrated that ^{111}In-diethylenetriaminepentaacetic acid-octreotide was retained in the proximal tubules and colocalized with megalin staining, a finding that confirmed the possible role of megalin in this process (*14*). Megalin (gp330) is a very large (660-kDa) multiligand transmembrane receptor that facilitates the endocytosis of a broad series of compounds (*5*). The receptor consists of several binding domains, binds a broad spectrum of proteins and peptides,

and plays a significant role in the reabsorption of many filtered peptides and proteins (*15*). Several studies demonstrated that megalin binds to proteins rich in positively charged amino acids as well as cationic drugs (*16,17*). These data support the concept that lysine and arginine interfere with ^{111}In-octreotide uptake through binding to megalin. The reduction in kidney uptake by lysine and arginine is believed to interfere with the process of renal reabsorption (*7,14,18*). These cationic amino acids are expected to interact with negatively charged (receptor) sites, thereby blocking the receptor-mediated endocytosis of radiolabeled peptides.

The results that we obtained with Gelofusine, however, agree only partially with this theory. Gelofusine consists of succinylated gelatin and contains mainly negatively charged molecules. In addition, degraded gelatin, the main component of Gelofusine, consists of peptide helices composed mainly of glycine, proline, and hydroxyproline, which are neutral rather than positively charged amino acids. Of course, other (cationic) amino acids or groups may be present in gelatin (*19*). On the other hand, polygelines may interact with megalin through domains other than cationic amino acids. To understand the mechanism by which polygeline-based blood volume substitutes reduce the kidney uptake of ^{111}In-octreotide, identification of the responsible component(s) in these plasma expanders is required. The results of the study by Veldman et al. suggesting that Gelofusine induces the competitive inhibition of tubular reabsorption are in agreement with our findings (*9*). Furthermore, their results also implied that megalin plays a role in this process because Gelofusine infusion induced the urinary excretion of β_2-microglobulin, a well-known ligand for megalin. Under physiologic conditions, β_2-microglobulin is reabsorbed efficiently by the proximal tubular cells (*9,18,20*). Besides the composition of the blood volume substitute, the time interval between the Gelofusine and ^{111}In-octreotide injections appeared to be relevant. The short half-life of the reducing effect of Gelofusine further suggests that a specific compound in Gelofusine interferes with the renal reabsorption process. We expect that when Gelofusine is applied to reduce the kidney uptake of radiolabeled peptides in patients, the radiolabel should be injected almost immediately after the injection of Gelofusine. In patients, the effect of Gelofusine probably will last for more than 15 min, because its elimination is slower in humans than in small animals.

The administration of Gelofusine is safe. Even in large amounts, Gelofusine administration does not provoke side effects (*21,22*). Although very rare, mild hypersensitivity reactions may be elicited by Gelofusine (*23,24*). These properties are in sharp contrast to the severe side effects reported after the administration of lysine or arginine (*3,25,26*). Gelofusine appears to be a potent and very safe agent for reducing the uptake of radiolabeled peptides in the kidneys. This effect was seen in both mice and rats and continued in rats until 15 min after the injection of Gelofusine.

CONCLUSION

The present study demonstrates that gelatin-based blood volume substitutes significantly reduced the kidney uptake of ^{111}In-octreotide in mice and rats at least as effectively as did lysine. Gelofusine is a well-known, widely used plasma expander that can be administered safely. Evaluation of this compound for reducing the renal uptake of radiolabeled peptides in patients is warranted.

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