Development of a Novel ^{99m}Tc-Chelate–Conjugated Bisphosphonate with High Affinity for Bone as a Bone Scintigraphic Agent

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In bone scintigraphy using ^{99m}Tc with methylenediphosphonate (^{99m}Tc-MDP) and hydroxymethylenediphosphonate (^{99m}Tc-HMDP), it takes 2–6 h after an injection before imaging can start. This interval could be shortened with a new radiopharmaceutical with higher affinity for bone. Here, based on the concept of bifunctional radiopharmaceuticals, we designed a ^{99m}Tc-mercaptoacetylglycylglycylglycine (MAG3)-conjugated hydroxy-bisphosphonate (HBP) (^{99m}Tc-MAG3-HBP) and a ^{99m}Tc-6-hydrazinopyridine-3-carboxylic acid (HYNIC)-conjugated hydroxy-bisphosphonate (^{99m}Tc-HYNIC-HBP). **Methods:** ^{99m}Tc-MAG3-HBP was prepared by complexation of MAG3-HBP with ^{99m}Tc using $SnCl_2$ as a reductant. The precursor of ^{99m}Tc-HYNIC-HBP, HYNIC-HBP, was obtained by deprotection of the Boc group after the coupling of Boc-HYNIC to a bisphosphonate derivative. ^{99m}Tc-HYNIC-HBP was prepared by a 1-pot reaction of HYNIC-HBP with $^{99m}TcO_4^-$, tricine, and 3-acetylpyridine in the presence of $SnCl_2$. Affinity for bone was evaluated in vitro by hydroxyapatite-binding assays for ^{99m}Tc-HMDP, ^{99m}Tc-MAG3-HBP, and ^{99m}Tc-HYNIC-HBP. Biodistribution experiments for the 3 ^{99m}Tc-labeled compounds were performed on normal rats. **Results:** ^{99m}Tc-MAG3-HBP and ^{99m}Tc-HYNIC-HBP were each prepared with a radiochemical purity of >95%. In the in vitro binding assay, ^{99m}Tc-MAG3-HBP and ^{99m}Tc-HYNIC-HBP had greater affinity for hydroxyapatite than ^{99m}Tc-HMDP. In the biodistribution experiments, ^{99m}Tc-MAG3-HBP and ^{99m}Tc-HYNIC-HBP had higher levels of radioactivity in bone than ^{99m}Tc-HMDP. ^{99m}Tc-MAG3-HBP was cleared from the blood slower than ^{99m}Tc-HMDP, whereas there was no significant difference in clearance between ^{99m}Tc-HYNIC-HBP and ^{99m}Tc-HMDP. Consequently, ^{99m}Tc-HYNIC-HBP showed a higher bone-to-blood ratio than ^{99m}Tc-HMDP. **Conclusion:** We developed a novel ^{99m}Tc-chelate–conjugated bisphosphonate with high affinity for bone and rapid clearance from blood, based on the concept of bifunctional radiopharmaceuticals. The present findings indicate that ^{99m}Tc-HYNIC-HBP holds great potential for bone scintigraphy.

Key Words: bone scintigraphy; bisphosphonate; ^{99m}Tc; MAG3; HYNIC

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Over the last quarter of a century, complexes of ^{99m}Tc with methylenediphosphonate (^{99m}Tc-MDP) and hydroxymethylenediphosphonate (^{99m}Tc-HMDP) have been widely used as radiopharmaceuticals for bone scintigraphy in cases of metastatic bone disease, Paget's disease, fractures in osteoporosis, and so forth (*1–4*). With these ^{99m}Tc-labeled bisphosphonates, however, an interval of 2–6 h is needed between injection and bone imaging (*3*). Shorting this interval would lessen the burden on patients in terms of the total length of the examination and the dose of radiation absorbed. To enable imaging at an earlier time after injection, a radiopharmaceutical with higher affinity for bone is required.

Bisphosphonate analogs accumulate in bone because their phosphonate groups bind to the Ca^{2+} of hydroxyapatite crystals (*5*). In the case of ^{99m}Tc-MDP and ^{99m}Tc-HMDP, the phosphonate groups coordinate with technetium (*6*), which might decrease the inherent accumulation of MDP and HMDP in bone. Thus, we hypothesized that bone affinity of ^{99m}Tc-labeled bisphosphonate would be increased by the design of a bisphosphonate in which the phosphonate groups do not coordinate with technetium, and we attempted to design a ^{99m}Tc-chelate–conjugated bisphosphonate based on the concept of bifunctional radiopharmaceuticals (Fig. 1). In this study, mercaptoacetylglycylglycylglycine (MAG3) and 6-hydrazinopyridine-3-carboxylic acid (HYNIC) were chosen as chelating sites because they have been widely used for ^{99m}Tc labeling of proteins and peptides (*7–11*) and conjugated to 4-amino-1-hydroxybutylidene-1,1-bisphosphonate. Then, ^{99m}Tc-[[[(4-hydroxy-4,4-diphosphonobutyl)carbamoylmethyl]carbamoylmethyl]carbamoylmethyl]carbamoylmethanethiolate (^{99m}Tc-MAG3-HBP; Fig. 1A) and [^{99m}Tc][[4-[(6-hydrazinopyridine-3-carbonyl)amino]-1-hydroxy-1-phosphonobutyl]phosphonic acid](tricine)(3-acetylpyridine) (^{99m}Tc-HYNIC-HBP; Fig. 1B) were

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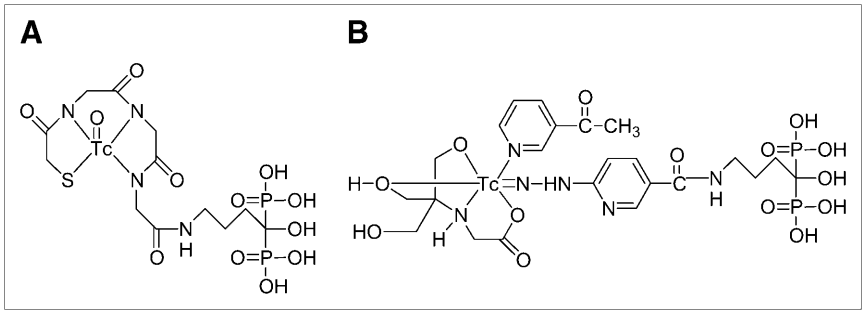

FIGURE 1. Chemical structures of ^{99m}Tc-MAG3-HBP (A) and ^{99m}Tc-HYNIC-HBP (B).

prepared by coordination with ^{99m}Tc, and their properties in vitro and in vivo were compared with these of ^{99m}Tc-HMDP.

MATERIALS AND METHODS

Materials

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AC-200 spectrometer (JEOL Ltd.), and the chemical shifts were reported in parts per million downfield from an internal 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt standard. Electrospray ionization mass spectra (ESI-MS) were obtained with a LCMS-QP8000α (Shimadzu). Thin-layer chromatographic analyses were performed with silica plates (Silica gel 60; Merck KGaA) with acetone as a developing solvent. ^{99m}Tc-Pertechnetate ($^{99m}TcO_4^-$) was eluted in a saline solution on a daily basis from generators (Daiichi Radioisotope Laboratories, Ltd.). ^{99m}Tc-HMDP was prepared by reconstitution with a conventional HMDP labeling kit (Nihon Medi-Physics Co., Ltd.) with a $^{99m}TcO_4^-$ solution. Other reagents were of reagent grade and were used as received.

Preparation of ^{99m}Tc-MAG3-HBP

The precursor of ^{99m}Tc-MAG3-HBP, [1-hydroxy-1-phosphono-4-[2-[2-[2-(2-tritylmercaptoacetylamino)-acetylamino]acetylamino] acetylamino]butyl]phosphonic acid (Tr-MAG3-HBP) was synthesized according to procedures described previously (*12*). The trityl group of Tr-MAG3-HBP (0.1 mg) was dissolved in 190 μL of trifluoroacetic acid (TFA) and 10 μL of triethylsilane. After removal of the solvent under a stream of N_2, 80 μL of 0.1 mol/L borate buffer (pH 9.5) were added to the residue. Next, 3 μL of $SnCl_2{\cdot}2H_2O$ solution in 0.1 mol/L citrate buffer (pH 5.0) (1 mg/mL) and 200 μL of $^{99m}TcO_4^-$ solution were added, and the reaction mixture was vigorously stirred and allowed to react at 95°C for 1 h. ^{99m}Tc-MAG3-HBP was purified by reversed-phase high-performance liquid chromatography (RP-HPLC) performed with a Cosmosil 5C_{18}-AR-300 column (4.6 × 150 mm; Nacalai Tesque) at a flow rate of 1 mL/min with a mixture of 0.2 mol/L phosphate buffer (pH 6.0) and ethanol (90:10) containing 10 mmol/L tetrabutylammonium hydroxide.

Preparation of [[[[(4-Hydroxy-4,4-Diphosphonobutyl) Carbamoylmethyl]Carbamoylmethyl]Carbamoylmethyl] Carbamoylmethanethiolate] Oxorhenium(V) (Re-MAG3-HBP)

Re-MAG3-HBP was synthesized according to procedures described previously (*12*). ESI-MS calculated for $C_{12}H_{20}N_4O_{12}P_2{}^{187}ReS$ $(M-H)^-$: m/z 692. Found: 692 $C_{12}H_{20}N_4O_{12}P_2{}^{185}ReS$ $(M-H)^-$: m/z 690. Found: 690.

Preparation of ^{99m}Tc-HYNIC-HBP

2,3,5,6-Tetrafluorophenyl 6-(tert-butoxycarbonyl)-hydrazinopyridine-3-carboxylate (Boc-HYNIC-TFP) and 4-amino-1-hydroxybutylidene-1,1-bisphosphonate were synthesized according to procedures described preciously (*12,13*). 4-Amino-1-hydroxybutylidene-1,1-bisphosphonate (10.3 mg, 41.3 μmol) was suspended in 1.33 mL of distilled water, and triethylamine (25.1 mg, 248 μmol) was added to the suspension. After a few seconds of stirring at room temperature, Boc-HYNIC-TFP (17.8 mg, 44.4 μmol) dissolved in 1.33 mL of acetonitrile was added. The reaction mixture was stirred for 3 h at room temperature. RP-HPLC was performed with a Hydrosphere 5C18 column (20 × 150 mm; YMC Co., Ltd) at a flow rate of 16 mL/min with a mixture of water, acetonitrile, and formic acid (90:10:1). Chromatograms were obtained by monitoring the ultraviolet (UV) adsorption at a wavelength of 254 nm. The fraction containing [4-[[6-(tert-butoxycarbonyl)-hydrazinopyridine-3-carbonyl]amino]-1-hydroxy-1-phosphonobutyl]phosphonic acid (Boc-HYNIC-HBP) was identified by mass spectrometry and collected. The solvent was removed by lyophilization to provide Boc-HYNIC-HBP (178 mg, 36.7%) as white crystals. ^{1}H NMR (D_2O): δ 8.42 (1H, s), 8.33 (1H, d), 7.21 (1H, d), 3.43 (2H, t), 1.92−2.04 (4H, m), 1.47 (9H, s). ESI-MS calculated for $C_{15}H_{26}N_4O_{10}P_2$ $(M-H)^-$: m/z 483. Found: 483.

Boc-HYNIC-HBP (10.4 mg, 21.5 μmol) was stirred in a mixed solution of TFA (720 μL) and anisole (80 μL) for 10 min at room temperature. After removal of the solvent under a stream of N_2, the residue was washed with dry ether to produce [4-[(6-hydrazinopyridine-3-carbonyl)amino]-1-hydroxy-1-phosphonobutyl]phosphonic acid (HYNIC-HBP) quantitatively as white crystals. ^{1}H NMR (D_2O): δ 8.33 (1H, s), 8.13 (1H, d), 7.01 (1H, d), 3.42 (2H, t), 1.94−2.04 (4H, m). ESI-MS calculated for $C_{10}H_{18}N_4O_8P_2$ $(M-H)^-$: m/z 383. Found: 383.

Forty microliters of HYNIC-HBP solution (3.75 mg/mL in 0.1 mol/L borate buffer, pH 9.5) were mixed successively with 200 μL of tricine solution (30 mg/mL in 10 mmol/L citrate buffer, pH 5.2), 200 μL of 3-acetylpyridine solution (10 μL/mL in 10 mmol/L citrate buffer, pH 5.2), 200 μL of $^{99m}TcO_4^-$ solution, and 25 μL of $SnCl_2$ solution (1.0 mg/mL in 0.1N HCl). The reaction mixture was vigorously stirred and allowed to react at 95°C for 35 min. ^{99m}Tc-HYNIC-HBP was purified by RP-HPLC under the same conditions as for the preparation of ^{99m}Tc-MAG3-HBP.

Hydroxyapatite-Binding Assay

The hydroxyapatite-binding assay was performed according to procedures described preciously with a slight modification (*14–16*). In brief, hydroxyapatite beads (Bio-Gel; Bio-Rad) were suspended in Tris/HCl-buffered saline (50 mmol/L, pH 7.4) at 1,

2.5, and 10 mg/mL. For the solutions of ^{99m}Tc-labeled compounds (^{99m}Tc-MAG3-HBP, ^{99m}Tc-HYNIC-HBP, and ^{99m}Tc-HMDP), the bisphosphonate concentrations were adjusted to 0.60 μmol/L. One hundred microliters of each solution of ^{99m}Tc-labeled compound were added to 100 μL of the hydroxyapatite suspension, and samples were gently shaken for 1 h at room temperature because it has been reported that 1 h was enough to attain binding equilibrium (*14*). After centrifugation at 10,000*g* for 5 min, the radioactivity of the supernatant was measured with an auto well γ-counter (ARC-2000; Aloka). Control experiments were performed with a similar procedure in the absence of hydroxyapatite beads. The rate of binding was determined as follows:

$$\text{Hydroxyapatite binding (\%)} = (1 - [\text{radioactivity of supernatant of each sample}]/[\text{radioactivity of supernatant in the respective control}]) \times 100.$$

Biodistribution Experiments

Animal experiments were conducted in accordance with our institutional guidelines, and the experimental procedures were approved by the Kyoto University Animal Care Committee. Biodistribution experiments were performed with an intravenous administration of 250 μL of each diluted tracer solution (370–740 kBq) to male Wistar rats (200–230 g). Groups of at least 4 rats each were sacrificed by decapitation at 5, 10, 30, and 60 min after injection. Organs of interest were removed and weighed, and radioactivity counts were determined with an auto well γ-counter and corrected for background radiation and physical decay during counting.

Serum Protein-Binding Assay

The binding of ^{99m}Tc-labeled compounds to serum protein was evaluated by ultrafiltration. Male Wistar rats (200–230 g) received a bolus of ^{99m}Tc-labeled compound by intravenous injection. At 3 min after the injection, the rats were anesthetized with ether and blood was collected by heart puncture. Each serum sample was prepared and applied to Centrifree units (Millipore). The units were centrifuged at 1,000*g* at room temperature. The radioactivity counts of the initial samples and filtrates were determined with an auto well γ-counter.

Statistical Analysis

Data are expressed as means ± SD where appropriate. Results of biodistribution experiments were statistically analyzed using a 1-way ANOVA followed by the Dunnett post hoc test. Differences were considered statistically significant when P values were less than 0.05.

RESULTS

Preparation of ^{99m}Tc-Labeled Compounds

^{99m}Tc-MAG3-HBP was prepared by complexation with ^{99m}Tc using $SnCl_2$ as a reductant. The radiochemical yield of ^{99m}Tc-MAG3-HBP was 73%. After purification by RP-HPLC, ^{99m}Tc-MAG3-HBP had a radiochemical purity of >95%.

HYNIC-HBP, the precursor of ^{99m}Tc-HYNIC-HBP, was synthesized by the coupling of the carboxyl group of Boc-HYNIC with the amino group of the bisphosphonate derivative and the subsequent deprotection of the Boc group. ^{99m}Tc-HYNIC-HBP was prepared by a 1-pot reaction of HYNIC-HBP with $^{99m}TcO_4^-$, tricine, and 3-acetylpyridine in the presence of $SnCl_2$. The radiochemical yield of ^{99m}Tc-HYNIC-HBP was 39%. After purification by RP-HPLC, ^{99m}Tc-HYNIC-HBP had a radiochemical purity of >95%.

Hydroxyapatite-Binding Assay

Figure 2 shows the percentage of each ^{99m}Tc-labeled compound bound to hydroxyapatite beads. With an increase in the amount of hydroxyapatite, the rate of binding rose. Both ^{99m}Tc-chelate−comjugated bisphosphonates (^{99m}Tc-MAG3-HBP and ^{99m}Tc-HYNIC-HBP) showed a significantly higher rate of binding than ^{99m}Tc-HMDP.

Biodistribution Experiments

The biodistributions of ^{99m}Tc-MAG3-HBP, ^{99m}Tc-HYNIC-HBP, and ^{99m}Tc-HMDP in normal rats are presented in Tables 1, 2, and 3. All ^{99m}Tc-labeled compounds accumulated rapidly and resided a long time in the femur. ^{99m}Tc-Chelate−conjugated bisphosphonates, ^{99m}Tc-MAG3-HBP and ^{99m}Tc-HYNIC-HBP, accumulated in bone in significantly larger amounts than ^{99m}Tc-HMDP. However, the bone-to-blood ratio of ^{99m}Tc-MAG3-HBP was decreased because its clearance from blood was delayed compared with that of ^{99m}Tc-HMDP. ^{99m}Tc-HYNIC-HBP had a significantly higher bone-to-blood ratio than ^{99m}Tc-HMDP because its clearance was equivalent to that of ^{99m}Tc-HMDP.

Serum Protein-Binding Assay

The proportions of ^{99m}Tc-MAG3-HBP and ^{99m}Tc-HYNIC-HBP bound to serum protein were 97.7% ± 0.2% and 88.7% ± 2.7%, respectively.

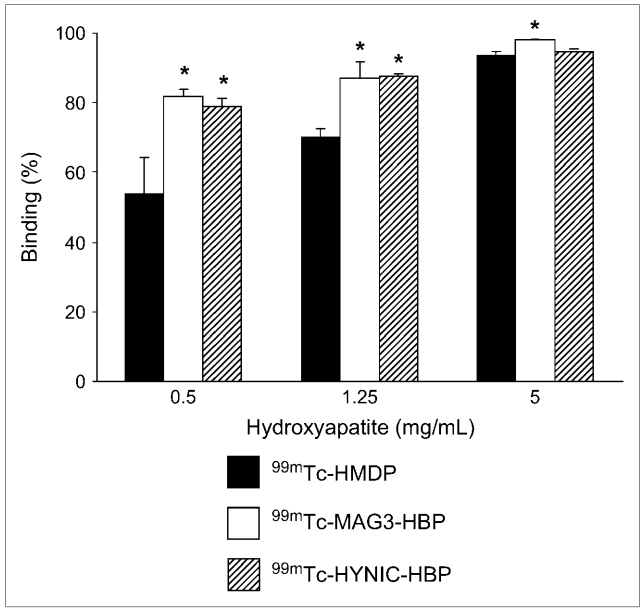

FIGURE 2. Binding of hydroxyapatite to ^{99m}Tc-MAG3-HBP, ^{99m}Tc-HYNIC-HBP, and ^{99m}Tc-HMDP. Data are expressed as mean ± SD for 3 or 4 experiments. Asterisks indicate statistically significant differences compared with ^{99m}Tc-HMDP with the Dunnett test ($P < 0.05$).

TABLE 1
Biodistribution of Radioactivity After Intravenous Administration of ^{99m}Tc-MAG3-HBP in Rats

Tissue	Time after administration (min)			
	5	10	30	60
Blood	0.98 ± 0.09*	0.77 ± 0.06	0.29 ± 0.01	0.07 ± 0.01*
Liver	0.32 ± 0.00	0.29 ± 0.03	0.18 ± 0.01	0.11 ± 0.02
Kidney	2.15 ± 0.33	1.87 ± 0.40	0.85 ± 0.05*	0.50 ± 0.08*
Intestine	0.16 ± 0.03*	0.15 ± 0.03*	0.13 ± 0.01*	0.17 ± 0.03*
Femur	1.80 ± 0.24	2.31 ± 0.22	3.73 ± 0.18*	4.23 ± 0.25*
F/B ratio†	1.86 ± 0.38*	3.02 ± 0.21*	13.1 ± 0.85*	59.7 ± 10.41

*Significant differences ($P < 0.05$) from ^{99m}Tc-HMDP were identified with the Dunnett test.
†Femur-to-blood ratio.
Each value is mean ± SD for 4 animals.

DISCUSSION

Radiopharmaceuticals with greater affinity for bone are expected to be effective in shortening the time interval between injection and bone imaging. Thus, based on the concept of bifunctional radiopharmaceuticals, ^{99m}Tc-chelate–conjugated bisphosphonates were designed in this study. First, MAG3 was chosen as a ^{99m}Tc-chelating group because this N_3S ligand forms a relatively compact, hydrophilic, and stable complex with ^{99m}Tc in high yields (*17*). As expected, ^{99m}Tc-MAG3-HBP bound to the hydroxyapatite beads in vitro and accumulated in the rat femur in vivo to a greater extent than ^{99m}Tc-HMDP. However, the level of ^{99m}Tc-MAG3-HBP in blood was higher than that of ^{99m}Tc-HMDP, which resulted in a decreased bone-to-blood ratio. We attribute this high radioactivity of ^{99m}Tc-MAG3-HBP in blood to the binding of serum proteins because the ^{99m}Tc-MAG3 complex shows strong binding to serum proteins (*18,19*). In fact, the level of binding of ^{99m}Tc-MAG3-HBP to protein was very high.

HYNIC is a representative bifunctional chelating agent used to prepare ^{99m}Tc-labeled proteins and peptides with tricine as a coligand (*10,20–22*). However, it has been reported that the complex [^{99m}Tc](HYNIC)(tricine)$_2$ is not stable and exists in multiple forms, and the pharmacokinetics could be affected by the exchange reaction between tricine and protein in the plasma and tissues (*22–24*). To solve these problems, Liu et al. used several pyridine derivatives as coligands to form ternary ligand complexes, [^{99m}Tc](HYNIC)(tricine)(pyridine derivative), with greater stability and fewer isomers (*25*). Furthermore, in a previous study, we showed that little binding to plasma protein was displayed by ^{99m}Tc-HYNIC-labeled polypeptides derivatized with this ternary ligand complex (*13*). Then, ^{99m}Tc-HYNIC-HBP in which [^{99m}Tc](HYNIC)(tricine)(3-acetylpyridine) was conjugated with a bisphosphonate derivative, was designed for high bone affinity and rapid washout from the blood.

The rate of binding of ^{99m}Tc-HYNIC-HBP to serum protein was significantly lower than that of ^{99m}Tc-MAG3-HBP. When these values were expressed as a nonprotein−binding rate, the value of ^{99m}Tc-HYNIC-HBP (11.3%) is about 5 times the value of ^{99m}Tc-MAG3-HBP (2.3%). The difference in the binding of the compound to the serum protein affects its blood clearance (*26–28*). In fact, the blood clearance of ^{99m}Tc-HYNIC-HBP was significantly faster than that of ^{99m}Tc-MAG3-HBP and was equivalent to that

TABLE 2
Biodistribution of Radioactivity After Intravenous Administration of ^{99m}Tc-HYNIC-HBP in Rats

Tissue	Time after administration (min)			
	5	10	30	60
Blood	0.56 ± 0.05	0.39 ± 0.04*	0.10 ± 0.01	0.03 ± 0.01
Liver	0.18 ± 0.02	0.12 ± 0.01*	0.07 ± 0.01*	0.05 ± 0.01
Kidney	3.19 ± 1.02	1.43 ± 0.09	0.45 ± 0.05	0.26 ± 0.10
Intestine	0.15 ± 0.01*	0.10 ± 0.01	0.10 ± 0.07	0.05 ± 0.02
Femur	1.90 ± 0.21*	2.59 ± 0.22*	3.71 ± 0.30*	3.96 ± 0.36*
F/B ratio†	3.40 ± 0.50*	6.69 ± 0.43	37.1 ± 4.01*	123.20 ± 18.30*

*Significant differences ($P < 0.05$) from ^{99m}Tc-HMDP were identified with the Dunnett test.
†Femur-to-blood ratio.
Each value is mean ± SD for 6 animals.

TABLE 3
Biodistribution of Radioactivity After Intravenous Administration of ^{99m}Tc-HMDP in Rats

Tissue	Time after administration (min)			
	5	10	30	60
Blood	0.61 ± 0.03	0.32 ± 0.04	0.10 ± 0.01	0.04 ± 0.01
Liver	0.15 ± 0.02	0.08 ± 0.01	0.03 ± 0.00	0.04 ± 0.05
Kidney	3.11 ± 0.23	1.59 ± 0.42	0.46 ± 0.08	0.24 ± 0.03
Intestine	0.12 ± 0.00	0.09± 0.02	0.04 ± 0.01	0.03 ± 0.01
Femur	1.52 ± 0.14	1.97 ± 0.18	2.73 ± 0.15	3.16 ± 0.51
F/B ratio*	2.49 ± 0.15	6.27 ± 0.94	29.16 ± 4.86	93.40 ± 19.81

*Femur-to-blood ratio.
Each value is mean ± SD for 4 or 5 animals.

of ^{99m}Tc-HMDP, although factors other than protein binding may also be responsible for rapid blood clearance. Furthermore, ^{99m}Tc-HYNIC-HBP accumulated in larger amounts in femur than did ^{99m}Tc-HMDP, which could be attributable to its design based on the bifunctional radiopharmaceutical concept. Consequently, the bone-to-blood ratio of ^{99m}Tc-HYNIC-HBP was significantly higher than that of ^{99m}Tc-HMDP.

We assume that ^{99m}Tc-chelate−conjugated bisphosphonates in bone remain intact because their ^{99m}Tc complexes are stable. On the other hand, previous studies suggested that there was a separation of the ^{99m}Tc-bisphosphonate complex into its ^{99m}Tc and bisphosphonate components before incorporation into the mineral phase (*29,30*). However, the separation of ^{99m}Tc-bisphosphonate must occur at the bone site because reduced ^{99m}Tc alone was not taken up by bone (*30*). Namely, there is little difference from the point of view that bisphosphonate is used as a carrier to bone in both cases. Thus, we hypothesized that the affinity of bisphosphonate for hydroxyapatite is important for bone accumulation and it would be increased by the design of a bisphosphonate in which the phosphonate groups do not coordinate with technetium. In fact, ^{99m}Tc-chelate–conjugated bisphosphonates had better affinity for hydroxyapatite and accumulated in the rat femur in vivo to greater extent than ^{99m}Tc-HMDP. These results support our hypothesis. However, other factors such as the blood circulation and delivery of the agents may also be important. Further study may be required to elucidate the mechanism for bone localization.

Because MAG3-HBP and HYNIC-HBP contain a bisphosphonate site, there is the possibility that ^{99m}Tc coordinates not with the MAG3 moiety or the HYNIC moiety but rather with the bisphosphonate moiety. To ascertain that ^{99m}Tc is chelated with only the MAG3 moiety, Re-MAG3-HBP was prepared by the coupling of nonradioactive Re-MAG3 complexed previously with the bisphosphonate analog. By RP-HPLC analysis, identical retention times between ^{99m}Tc-MAG3-HBP and Re-MAG3-HBP were exhibited (Fig. 3), revealing their structural analogy. In the case of the HYNIC complex, because it is difficult to synthesize the corresponding stable Re-HYNIC complex, ^{99m}Tc-HYNIC-HBP was prepared by the coupling of [^{99m}Tc](HYNIC-TFP)(tricine)(3-acetylpyridine) with the bisphosphonate derivative. RP-HPLC analysis revealed this ^{99m}Tc-labeled product to be identical to that obtained from the reaction of HYNIC-HBP with $^{99m}TcO_4^-$, tricine, and 3-acetylpyridine in the presence of $SnCl_2$ (Fig. 4). These findings exclude the possibility of complexation between technetium and the bisphosphonate structure and indicate the chelation of ^{99m}Tc with only the MAG3 moiety in MAG3-HBP and the HYNIC moiety in HYNIC-HBP.

CONCLUSION

As a radiopharmaceutical that accumulates at high level in bone and is rapidly cleared from blood, we designed a novel ^{99m}Tc-chelate−conjugated bisphosphonate, ^{99m}Tc-HYNIC-HBP. ^{99m}Tc-HYNIC-HBP had good affinity for hydroxyapatite crystals and showed lower binding to serum proteins. In rat biodistribution experiments, ^{99m}Tc-HYNIC-HBP had a higher bone-to-blood accumulation ratio of radioactivity at early times after injection than ^{99m}Tc-HMDP.

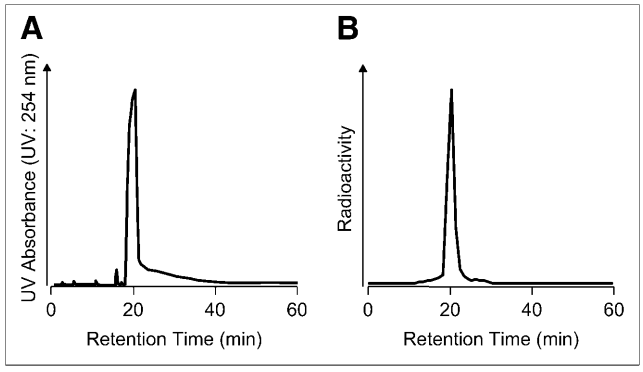

FIGURE 3. RP-HPLC chromatograms of nonradioactive Re-MAG3-HBP (A) and ^{99m}Tc-MAG3-HBP (B). Conditions: flow rate of 1 mL/min with 10% ethanol in 200 mmol/L phosphate buffer (pH 6.0) containing 10 mmol/L tetrabutylammoniumhydroxide.

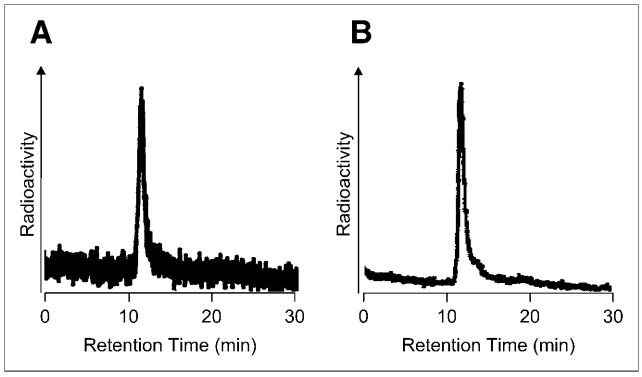

FIGURE 4. RP-HPLC chromatograms of ^{99m}Tc-HYNIC-HBP prepared by coupling of [^{99m}Tc](HYNIC-TFP)(tricine)(3-acetylpyridine) with bisphosphonate derivative (A) and labeling of HYNIC-HBP with $^{99m}TcO_4^-$, tricine, and 3-acetylpyridine (B). Conditions: flow rate of 1 mL/min with 10% ethanol in 200 mmol/L phosphate buffer (pH 6.0) containing 10 mmol/L tetrabutylammoniumhydroxide.

These results indicate that ^{99m}Tc-HYNIC-HBP holds great potential for bone scintigraphy.

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