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Research Article

Synthesis, characterization and biodistribution of $^{99m}Tc(CO)_3$–ABP and comparison with ^{99m}Tc–ABP

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Abstract: The (l-hydroxy-4-amino-butylidene-l,l-bisphosphate) (ABP) is a compound that inhibits bone resorption, and a highly effective drug in the treatment of metastatic bone disease. The fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was reacted with ABP in saline (pH = 3–4) at 45°C for 15 min to produce the $^{99m}Tc(CO)_3$–ABP complex. The radiochemical purity (RCP) of the product was over 90% as measured by thin layer chromatography and high-performance liquid chromatography. No decomposition of the complex at room temperature (25°C) was observed over a period of 6 h. Its partition coefficient indicated that it was a weak hydrophilic complex. The biodistribution in normal mice of $^{99m}Tc(CO)_3$–ABP complex differed greatly from that of ^{99m}Tc–ABP, and the former had a lower bone uptake as compared with that of the latter. The experiment results showed that the incorporation of the $[^{99m}Tc(CO)_3]^+$ core into the ABP ligand may drastically change the characterization and biological features as compared with ^{99m}Tc–ABP. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: bisphosphonates; ^{99m}Tc-carbonyl; labeling; biodistribution; $^{99m}Tc(CO)_3$–ABP

Introduction

Bisphosphonates (BPs) are analogues of pyrophosphate in which the hydrolytically labile P–O–P linkage has been replaced by the hydrolysis-resistant P–C–P bond. The diphosphonate groups impart sufficient affinity to the mineral phase of bone and result in significant deposition to the osseous tissue. BPs are widely used in the management of skeletal disorder, such as osteoporosis, malignant hypercalcemia and Paget's disease.[1,2] ^{99m}Tc-labeled BP is also considered useful as a radiotracer for judgment of the therapeutic effect of BP on bone metastases by determining the degree of accumulation in metastases bone lesions.[3]

The l-hydroxy-4-amino-butylidene-l,l-BP, Alendronate (ABP) known as amino BP is an important representative BP. ABP is an inhibitor of bone resorption which shows a significant reduction in fractures of the spine and hip in clinical trials.[4,5] Because of its amino group, ABP seems to be a good chelating agent for ^{99m}Tc and forms a stable complex with technetium-99m. ABP was labeled with ^{99m}Tc, shown as a promising bone imaging agent, and the resulting images were similar to those obtained with ^{99m}Tc-MDP.[6,7] Compared with ^{99m}Tc-MDP, ^{99m}Tc-ABP has similar bone uptake and faster blood clearance, a high ratio of bone to soft tissue.[8]

Since Alberto and his colleague developed a convenient high-yield synthesis for ^{99m}Tc-tricarbonyl complexes, a large number of such complexes have been reported.[9,10] Major characteristics of ^{99m}Tc-tricarbonyl complexes are the presence of three stable CO groups and three labile water molecules which can be replaced by a variety of donating ligands.[11] Some studies suggested that the ideal ligands for the precursor $[^{99m}Tc(OH_2)_3(CO)_3]^+$ should contain amino groups, phosphine group or carboxylic acid group, because the water molecules of precursor are readily substituted by those functional groups, such as amine, imines, thiols and phosphines.[12] Compared with conventional ^{99m}Tc-labeled complexes, ^{99m}Tc-tricarbonyl complexes show particular characteristics. First, the clinical assessment[13] of a ^{99m}Tc-tricarbonyl complex in humans revealed the potential clinical usefulness of $^{99m}Tc(CO)_3$(LAN) complexes as an excellent renal imaging agent. Furthermore, developed as a myocardial imaging agent, the biodistribution study in mice and preliminary SPECT imaging studies in dogs both

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indicated that the heart/liver ratio of $[^{99m}Tc(CO)_3(MIBI)_3]^+$ is better than that of the known complex ^{99m}Tc-MIBI.[14]

It is of interest to have a comparative evaluation of ^{99m}Tc-tricarbonyl BPs complexes with the classic ^{99m}Tc-labeled BPs complexes. In this study, we have investigated the preparation of $^{99m}Tc(CO)_3$–ABP by using ^{99m}Tc-tricarbonyl precursor and comparison of the biodistribution of $^{99m}Tc(CO)_3$–ABP in normal mice with that of ^{99m}Tc–ABP. Till now, to our knowledge, no report about preparation and biological properties of this new class of $^{99m}Tc(CO)_3$ BPs complexes has been described.

Results and discussion

Preparation of $^{99m}Tc(CO)_3$–ABP complex and ^{99m}Tc–ABP complex

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor could be prepared over 93% yield, and this precursor was used without further purification. The radiochemical purities (RCPs) of $^{99m}Tc(CO)_3$–ABP complex and ^{99m}Tc–ABP complex were over 90% after the preparation.

The RCP of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, $^{99m}Tc(CO)_3$–ABP complex and ^{99m}Tc–ABP complex was routinely checked by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). R_f values (polyamide strip) and HPLC retention times (R_t) for some selected complexes are shown in Table 1. When TLC was performed on a polyamide strip with acetonitrile as the mobile phase, $^{99m}Tc(CO)_3$–ABP moved to the solvent front ($R_f = 0.7-1.0$), ^{99m}Tc–ABP remained at the origin ($R_f = 0-0.1$), $^{99m}TcO_2 \cdot nH_2O$ is retained at the origin ($R_f = 0$) and R_f value for $^{99m}TcO_4^-$ was about 0.3–0.5.

According to the HPLC analyses performed on the C18 reversed-phase column, the retention times were 4.1 min for $^{99m}Tc(CO)_3$–ABP and 3.5 min for ^{99m}Tc–ABP, respectively. Rattat *et al.*'s study[15] showed, in the same HPLC-system, the shorter retention time means higher hydrophilic complex. The reverse-phase-high performance liquid chromatography (RP-HPLC) measurement suggested that ^{99m}Tc–ABP is more hydrophilic than that of $^{99m}Tc(CO)_3$–ABP.

Characterization of $^{99m}Tc(CO)_3$–ABP complex and ^{99m}Tc–ABP complex

To determine the charge of ^{99m}Tc–ABP and $^{99m}Tc(CO)_3$–ABP, electrophoresis experiments were performed. The results of paper electrophoresis are shown in Table 2.

$^{99m}Tc(CO)_3$–ABP remains at the point of spotting, while ^{99m}Tc–ABP complex migrated towards the anode. The results indicated that the $^{99m}Tc(CO)_3$–ABP complex is neutral and the^{99m}Tc–ABP complex is negative.

The partition coefficient of the $^{99m}Tc(CO)_3$–ABP complex ($\log P = -0.76$) was much more than that of the ^{99m}Tc–ABP complex ($\log P = -3.39$); suggesting that the incorporation of the $[^{99m}Tc(CO)_3]^+$ core into the ABP ligand may increase the lipophilicity of the complex greatly.

The stability experiment demonstrated that $^{99m}Tc(CO)_3$–ABP complex and ^{99m}Tc–ABP complex were stable within 6 h at room temperature.

Biodistribution study

Figures 1 and 2 present the biological distribution data for three mice at each of three time intervals after the injection of $^{99m}Tc(CO)_3$–ABP and ^{99m}Tc–ABP, respectively.

The $^{99m}Tc(CO)_3$–ABP complex showed high liver, kidney , lungs and blood uptake, a certain bone uptake, low brain and flesh uptake at 30 min post injection. The hepatic and renal uptakes reached their peak activities of 20.13(%ID/g) and 11.08(%ID/g) at 30 min post injection, respectively, and their uptakes remained high at 180 min post injection, suggesting that the main excretion routes were through the kidneys and liver. Although the lungs have a high initial uptake, the clearance is rapid. The complex showed steady retention of activity in bone, and the radioactivity in bone increased as time progressed. The

Table 2 Paper electrophoresis pattern of $^{99m}Tc(CO)_3$–ABP and ^{99m}Tc–ABP

Component	Cathode (%)	Origin (%)	Anode (%)
^{99m}Tc–ABP	0.6	4.9	94.5
$^{99m}Tc(CO)_3$–ABP	1.9	88.5	9.5

Table 1 Result of TLC and HPLC for $^{99m}Tc(CO)_3$–ABP and other components

Component	$[^{99m}TcO_4]^-$	$^{99m}TcO_2 \cdot nH_2O$	$[^{99m}Tc(CO)_3(H_2O)_3]^+$	^{99m}Tc–ABP	$^{99m}Tc(CO)_3$–ABP
R_f	0.3–0.5	0.1	0.1	0–0.1	0.7–1.0
R_t (min)	2.8	—	3.6	3.5	4.1

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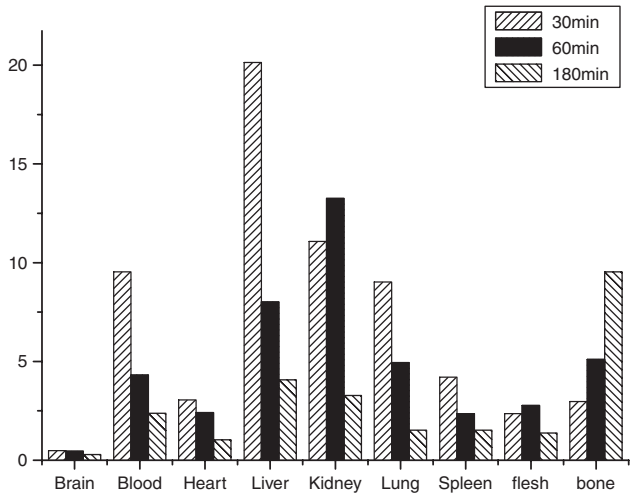

Figure 1 Biodistribution results for $^{99m}Tc(CO)_3$–ABP in normal mice ($n = 3$, values represent the means of the percent injected dose per gram).

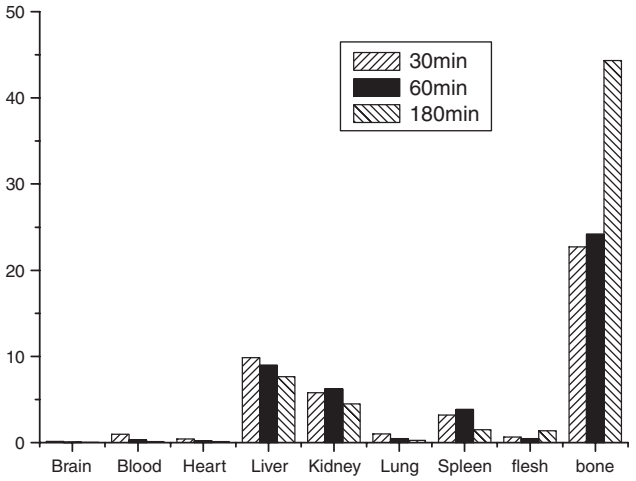

Figure 2 Biodistribution results for ^{99m}Tc–ABP in normal mice ($n = 3$, values represent the means of the percent injected dose per gram).

radioactivity in the bone reaches a maximum of 9.53(%ID/g) at 180 min.

The differences in biodistribution of complexes ^{99m}Tc–ABP and $^{99m}Tc(CO)_3$–ABP are significant. As can be seen in Table 3, compared with the ^{99m}Tc–ABP complex, the $^{99m}Tc(CO)_3$–ABP complex exhibited lower bone/liver, bone/kidney, bone/blood, bone/brain and bone/flesh ratios at each of the three time intervals. Labeling ABP with a $^{99m}Tc(CO)_3$-core also leads to a longer retention in blood, and the higher ratios of bone/brain and bone/flesh may also reflect the high blood activity instead of a real organ uptake.

The above fact suggested that the introduction of the $[^{99m}Tc(CO)_3]^+$ core into the ABP molecular increases its lipophilicity significantly and greatly alters its biological behavior.

Experimental

$^{99}Mo/^{99m}Tc$ generator was obtained from the China Institute of Atomic Energy (CIAE). Sodium borohydride, Na/K tartrate and sodium carbonate were purchased from Beijing Chemical Reagents Company. All other chemicals were of reagent grade and were used without further purification. Normal Kunming mice, 18–22 g, were obtained from Animal Center of Peking University. ^1H, ^{31}P and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer operating in D_2O or $+Na_2CO_3$ at 500.1, 202.5 and 125.8 MHz, respectively. TMS was used as an internal standard for ^1H NMR, ^{13}C NMR and 85% H_3PO_4 as an external standard for ^{31}P NMR spectroscopy. Infrared spectrum was performed on a Nicolet-170SX.

Synthesis of ABP

ABP was synthesized as the reported procedure.[16,17]

IR (KBr, ν, cm^{-1}): 3483 (–NH), 3300–2900 (P–OH), 1177 (P = O), 1065 (C–N), 1055. ^1H-NMR(D_2O+Na_2CO_3, δ, ppm):1.60 (2H, t, $J = 5.69$, CH_2), 1.75

Table 3 Biodistribution comparison of ^{99m}Tc–ABP complex and $^{99m}Tc(CO)_3$–ABP complex ($n = 3$)

Organ ratio	^{99m}Tc–ABP			$^{99m}Tc(CO)_3$–ABP		
	Post injection time (min)					
	30	60	180	30	60	180
Bone/liver	2.31	2.70	5.79	0.15	0.64	2.34
Bone/kidney	3.88	3.84	9.72	0.27	0.39	2.91
Bone/blood	25.01	80.25	376.47	0.31	1.18	4.01
Bone/brain	143.92	633.88	965.14	6.08	11.12	32.84
Bone/ flesh	34.39	52.66	213.78	1.26	1.84	6.92

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(2H, m, CH_2), 2.46 (2H, m, CH_2). ^{13}C-NMR (D_2O+Na_2CO_3, δ, ppm): 27.5, 33.4, 41.9, 76.6(t, ^1Jc-p = 134 z). ^{31}P-NMR (D_2O+Na_2CO_3, δ, ppm): 15.6.

Preparation of the precursor fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$

The precursor was prepared according to the procedure published by Alberto and his coworkers[18] with minor modification: $NaBH_4$ (10 mg), $NaCO_3$ (5 mg) and potassium sodium tartrate (15 mg) were added to a 10 ml glass vial. The vial was sealed and a needle was introduced through the rubber stopper to equilibrate with the atmospheric pressure. Carbon monoxide gas was purged through the vial for 15 min. After the addition of 1 ml of generator eluate containing 37–74 MBq of $^{99m}TcO_4^-$, the vial was heated at 80°C for 30 min. The solution was cooled down to room temperature. The RCP of the precursor was evaluated by TLC. The TLC was performed on a polyamide strip with acetonitrile as the mobile phase.

Preparation of ^{99m}Tc(CO)$_3$–ABP complex

A 0.5 ml of freshly prepared [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor was added to 0.5 ml of saline containing 4 mg of ABP. HCl (0.1 N) was added and the pH of the mixture was maintained between 3 and 4. The mixture was heated at 45°C for 15 min. After cooling to room temperature, the mixture was incubated at room temperature for 10 min. The RCP of the mixture was evaluated by TLC and HPLC. TLC was performed on a polyamide strip eluted with acetonitrile.

Preparation of ^{99m}Tc–ABP complex

Saline (1 ml) containing [$^{99m}TcO_4$]$^-$ (37 MBq) was added to a vial containing 0.1 mg of $SnCl_2$•$2H_2O$ dissolved in 1 ml HCl (1 M). Then 0.1 ml of NaOH solution containing 1 mg of ABP was added and the pH was maintained at 2. The resulting reaction was heated at 50°C for 15 min. After cooling to room temperature, the mixture was left standing for 10 min at room temperature.

HPLC analysis

RP-HPLC was performed on a (Shimadu Lc-10Avp, Japan) chromatograph equipped with a flow scintillation analyzer (Packard Bioscience Company). Chromatographic analyses were carried out using a C18 reversed-phase column(4.6 mm × 250 mm, Alltima). The column was eluted at a flow rate of 1 mL/min using linear gradient mixtures of 0.2 mol/L phosphate buffer (pH 6.0) and CH_3OH (t= 0 min, 10% CH_3OH; t= 10 min, 25%CH_3OH; t= 20 min, 70% CH_3OH; t= 30 min, 90%CH_3OH v/v).

TLC analysis

The RCP of the ^{99m}Tc–ABP complex and ^{99m}Tc(CO)$_3$–ABP complex was evaluated by TLC. The chromatography analyses were performed on a polyamide film with acetonitrile as the mobile phase.

Paper electrophoresis

Samples of 1 μl were spotted on a Whatman 1 chromatography paper, saturated with 0.05 M pH 7.4 phosphate buffer in an electrophoresis bath. One hundred and fifty volt was applied across a 15 cm strip for 120 min. The strips were dried and the distribution of radioactivity on the strip was determined.

Determination of the partition coefficient

The partition coefficient was determined by mixing the complex with an equal volume of 1-octanol and phosphate buffer (0.025 M, pH 7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then centrifuged at 5000 rpm for 5 min. From each phase, 0.1 ml of the liquid was pipetted and assayed in a well γ-counter. The measurement was repeated three times. Care was taken to avoid cross contamination between the phases. The partition coefficient, P, was calculated by

$$P = (\text{cpm in octanol} - \text{cpm in background})/(\text{cpm in buffer} - \text{cpm in background})$$

Usually the final partition coefficient value was expressed as log P.

Stability studies

The stability of the complex was determined by measuring the RCP at room temperature (25°C) at different times after preparation.

Biodistribution studies

Biodistribution studies were carried out with ^{99m}Tc(CO)$_3$–ABP as well as ^{99m}Tc–ABP. A solution of (0.1 ml, 740 kBq) ^{99m}Tc(CO)$_3$–ABP or ^{99m}Tc–ABP was administered via a tail vein to Kunming mice (18–22 g) and the injected radioactivity was measured with a well-type NaI (Tl) detector. The mice were sacrificed at 30, 60 and 180 min post injection. Selected organs and the blood were collected, weighed and measured for radioactivity. The accumulated radioactivity in the

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tissue of organs was calculated in terms of percentage of injected dose per gram organ (%ID/g). All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

Conclusion

A novel $^{99m}Tc(CO)_3$–ABP complex was prepared via $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor with a low ligand concentration of ABP in high yields (>90%). The $^{99m}Tc(CO)_3$–ABP complex was neutral and stable in saline for 6 h at room temperature. The biodistribution study in normal mice showed that $^{99m}Tc(CO)_3$–ABP complex had a lower bone uptake and high liver, kidney and lungs uptake as compared with that of the ^{99m}Tc–ABP complex. From these above biodistribution data, it may be concluded that the newly developed ^{99m}Tc-tricarbonyl BPs derivative is not suitable as potential bone tracer agents.

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