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Research Article

Synthesis, separation and biodistribution of ^{99m}Tc-CO-MIBI complex

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Summary

^{99m}Tc-CO-MIBI was prepared by a two-step procedure involving the convenient preparation of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor and followed by the substitution of the water molecules by the MIBI (2-methoxyisobutylisonitrile) ligands. In a second step, the reaction solution was adjusted to different pH values, and then the product, ^{99m}Tc-CO-MIBI, was confirmed to be a mixture of two complexes: complex A and complex B, whose labeling yields could be over 90%. The ratio of complex B to the sum of A and B could increase gradually from 0 to 1 when pH was shifted from 3.0 to 9.0. These changes were monitored by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The two complexes were stable within 8 h at room temperature *in vitro*. The partition coefficient of the two complexes indicated that there was distinct difference between them. Biodistribution in mice demonstrated that complex B showed better myocardial imaging properties than that of complex A. The heart/liver ratios of complex A, the mixture, and complex B were 1.57, 1.93, and 2.33, respectively, for 30 min post-injection. The discovery of chemical and biological properties of ^{99m}Tc-CO-MIBI would certainly promote the research on a new promising myocardial perfusion-imaging agent.

Key Words: biodistribution; tricarbonyl core; MIBI; myocardial perfusion-imaging agent

Introduction

Technetium-99 m, an excellent imaging isotope, has played an essential role in the diagnostic nuclear medicine. There are some successful technetium cores such as 'naked' Tc atom, $[Tc=O]^{3+}$ core, $[Tc{\equiv}N]^{2+}$ core, $[O=Tc=O]^+$ core and [Tc]-HYNIC core. However, technetium cores in its low oxidation did not

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receive adequate attention until the $[Tc(CO)_3]^+$ core was developed. Several years ago Alberto *et al.* reported a one-step synthesis of the complex $[^{99m}Tc(CO)_3(OH_2)_3]^+$ by direct reduction of $^{99m}TcO_4^-$ with $NaBH_4$ in aqueous solution in the presence of CO at 1 atm.[1,2] $[^{99m}Tc(CO)_3(OH_2)_3]^+$ is proved to be a versatile intermediate for technetium labeling.

So far, there are several radiopharmaceuticals used widely in myocardial imaging. ^{99m}Tc-MIBI is one of them though it has comparatively high liver uptake and slow liver washout. Thus we utilized the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor and MIBI to prepare ^{99m}Tc-CO-MIBI, which was reported by Marmion[3] and Liu[4,5] independently almost at the same time. Dyszlewski also reported it as a transport substrate of the multidrug resistance *P*-glycoprotein.[6] Control experiments of ^{99m}Tc-CO-MIBI vs. $[^{99m}Tc(MIBI)_6]^+$ were carried out in mice[4] and dogs[7] earlier, in which ^{99m}Tc-CO-MIBI showed to be a promising myocardial perfusion-imaging agent with good myocardial uptake and fast liver washout. Subsequently, we found that ^{99m}Tc-CO-MIBI was a mixture with two main components in different reaction conditions whose biodistribution results were distinctively different.[8] The aim of this study was to further the study of the chemical and biological properties of ^{99m}Tc-CO-MIBI.

Experimental

Materials

The MIBI ligand was synthesized following the previously published procedure.[9–11] Pure CO gas was purchased from NRCCRM. ^{99}Mo/^{99m}Tc generator was obtained from the Beijing Syncor Medical Corporation. All other chemicals were obtained from Beijing Chemical Reagents Company and Aldrich Co.

Preparation of $[^{99m}Tc(CO)_3(OH_2)_3]^+$

The preparation of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor was carried out as follows:[2] 4 mg Na_2CO_3 and 5.5 mg $NaBH_4$ were added to a 10 ml vial, which was tightly closed and flushed with pure CO for 15 min. A 3 ml quantity of generator elute containing up to 7.4 GBq $Na[^{99m}TcO_4]$ in saline was added. And the solution was heated to 75°C for 30 min. The reaction mixture was finally cooled to room temperature.

Preparation of ^{99m}Tc-CO-MIBI

^{99m}Tc-CO-MIBI (complex A and complex B) were prepared in the following general procedure: 1.0 mg MIBI, dissolved in 0.5 ml water, was added to 1 ml $[^{99m}Tc(CO)_3(OH_2)_3]^+$ solution, which was adjusted to a certain pH value with

0.5 N HCl solution. The mixture was heated to 100°C for 15 min, then cooled to room temperature.

The labeling yields of ^{99m}Tc-CO-MIBI COMPLEX were calculated by TLC. The chromatographic analyses were performed on polyamide film with acetonitrile (Sys 1) and saline : acetone : strong aqua ammonia = 9 : 1 : 0.1 (v/v/v) (Sys 2) as mobile phases.

The stability of complex A and complex B was determined at room temperature by measuring their yields at different times (1, 2, 3, 4, 5, 6, 7 and 8 h) after preparation.

HPLC analysis

Radio high-pressure liquid chromatography (HPLC) experiments were performed by using a SHIMADZU System with SCL-10Avp HPLC pump system and Park radioflow detector. The column (Waters RP C18, 250 × 4.6 mm, 5 μm) was eluted at a flow rate of 1.0 ml/min. Characterization of $[^{99m}Tc(CO)_3(OH_2)_3]^+$:

Mobile phase:[12]

a. TEAP 0.05 M, pH = 2.25,

b. MeOH 100%.

Gradient: 0–3 min 100% a, 3–6 min from 100 to 75% a, 6–9 min from 75 to 66% a, 9–20 min from 34 to 100% b, 20–27 min 100% b, 27–30 min from 100% b to 100% a.

Characterization of complex A and complex B:

Mobile phase: a : b = 35 : 65.

Determination of the partition coefficient for the complexes

The lipophilicity of the complex A (or B) with yields over 95% was determined as follows: 0.1 ml complex A (or B) solution was mixed with 2 ml 1-octanol and 1.9 ml PBS (0.01 M, pH = 7.4) in a centrifuge tube. The tube was vortexed at room temperature for 3 min and then was centrifuged at high speed for 10 min. 0.1 ml samples of both phases were pipetted into other test tubes with adequate care to avoid cross contamination between the phases and were counted in a well γ-counter. The measurement was repeated for three times. The partition coefficient, P, was calculated using the following equation:

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

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

Biodistribution studies

Samples (about 740 kBq in 0.1 ml solution) were injected through the tail vain into mice (18–22 g, female, obtained from Animal Center of Peking

University). The mice were sacrificed at 5, 15, 30, and 60 min post-injection. Selected organs were collected for weighing and counting. The accumulated radioactivity in the tissue of organs was calculated in terms of percentage of injected dose per gram organ (%ID/g). The biodistribution experiments were performed in three groups (I: complex A; II: the ^{99m}Tc-CO-MIBI mixture; III: complex B).

Results and discussion

Preparation of ^{99m}Tc complexes

Over 95% labeling yields of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor evaluated by reverse phase HPLC were found (Retention time: 5.5 min).

^{99m}Tc-CO-MIBI was prepared by substituting the MIBI ligands for the water molecules of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor. Labeling yields of the final products were evaluated by TLC and HPLC. The R_f values in TLC analysis are shown in Table 1.

The precursor solution was adjusted to various pH values ranging from 3.0 to 9.0, and varying final products were obtained with labeling yields over 90% evaluated by the TLC-Sys1. When the precursor solution was nearly neutral, the product was a mixture of complex A and complex B. The preparation of complex A or B was determined by the pH conditions of the solution during the substitution reaction. When pH is between 3.0 and 5.0, complex A was the main product. When pH was close to 9.0, complex B was almost the exclusive product. In both conditions mentioned above the yields of complex A and complex B calculated by the TLC-Sys2 could be over 90%. The variation of the ratios of B/(A + B) with the pH values of the reaction solution is shown in Figure 1. The experiments demonstrated that it was feasible to obtain pure complex A, pure complex B, or a mixture of A and B with a certain ratio (Figure 2) by adjusting the pH of the reaction mixture. The products obtained under these conditions were used in the biodistribution studies below.

Stability experiment demonstrated that the two products A and B were stable within 8 h at room temperature.

Determination of the partition coefficient for the complexes

The partition coefficient values ($\log P$) of complex A and complex B were — 0.172 and 0.125, respectively.

Table 1. R_f values for some complexes in TLC analysis

Mobile phase[a]	$^{99m}TcO_4^-$	$^{99m}TcO_2.nH_2O$	Complex A	Complex B
Sys1	0.4–0.6	0.0	0.9–1.0	0.9–1.0
Sys2	0.1–0.2	0.0	0.6–0.8	0.3–0.4

[a] Sys1: acetonitrile; Sys2: saline : acetone : strong aqua ammonia = 9 : 1 : 0.1(v/v/v).

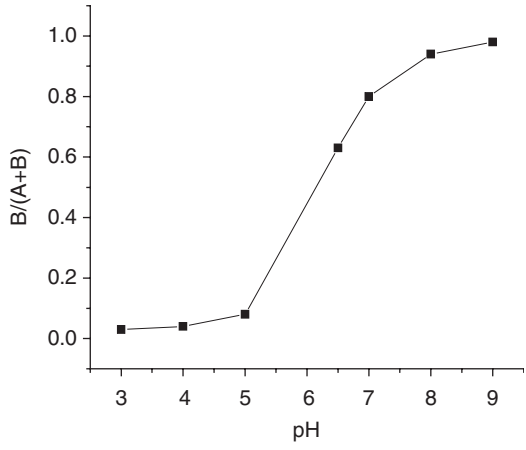

Figure 1. The ratios of complex B to the sum of complex A and complex B

Biodistribution studies

The results of biodistribution experiments of I (complex A, yields 95%), II (complex A, yields 67%; complex B, yields 30%) and III (complex B, yields 90%) are shown in Table 2 and Figure 3. Unlike the previous experiments in which the samples were obtained by separating the ^{99m}Tc-CO-MIBI mixtures by HPLC,[8] the samples of Group I, II and III were prepared directly by adjusting the pH values of the precursor solution and without further purification or separation by HPLC. The present experimental method was more convenient and more feasible for studying chemical and biological characters of complex A and complex B. Complex B showed higher myocardial uptake and faster liver washout than complex A, and therefore complex B had considerably better heart/liver ratios in mice. For 30 min post-injection the heart/liver ratios of Group I, II, III and ^{99m}Tc-MIBI were 1.57, 1.93, 2.33 and 0.90,[13] respectively. Since complex B had distinct improvement over ^{99m}Tc-MIBI in heart/liver ratios, it was possible to make the heart imaging at early post injection. Earlier, Liu *et al*. carried out the comparative pharmacology study of the ^{99m}Tc-CO-MIBI mixtures and ^{99m}Tc-MIBI in dogs,[7] and they got the same results. All these showed that ^{99m}Tc-CO-MIBI could be a promising myocardial perfusion-imaging agent.

Though there was no direct evidence to prove the structure of complex A and complex B, we presumed that complex A was $[^{99m}Tc(CO)_3(MIBI)_2(H_2O)]^+$ and complex B was $[^{99m}Tc(CO)_3(MIBI)_3]^+$ from the existing experimental results. There were differences between complex A and complex B in the retention time, the partition coefficient value and biodistribution in mice. The partition coefficient values showed that complex B had stronger lipophilicity than complex A. Furthermore, the three CO ligands of

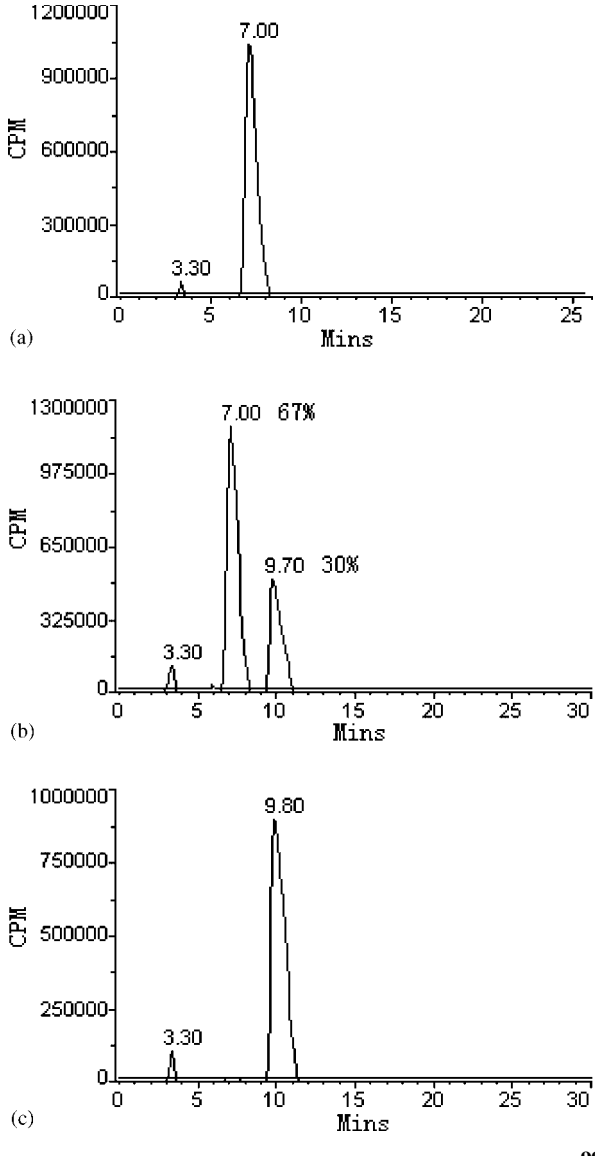

Figure 2. HPLC chromatograms of (a) complex A (b) the ^{99m}Tc-CO-MIBI mixture (complex A, complex B) (c) complex B

$[^{99m}Tc(CO)_3(OH_2)_3]^+$ were too stable to be substituted by any other incoming ligand generally.

Conclusion

^{99m}Tc-CO-MIBI was prepared by using a two-step procedure and was confirmed to be a mixture of two complexes (A and B). Furthermore, either complex A or B in the final products could be obtained with yields greater than 90% if the solution pH in the second step was adjusted to a certain value. This

Table 2. Biodistribution of Group I, II and III in mice (%ID/g, mean ± SE, $n = 3$)

Tissue	Groups[a]	Post-injection time (min)			
		5	15	30	60
Blood	I	1.39 ± 0.09	0.67 ± 0.03	0.50 ± 0.03	0.41 ± 0.02
	II	1.69 ± 0.07	0.93 ± 0.06	0.74 ± 0.02	0.59 ± 0.03
	III	1.22 ± 0.58	1.00 ± 0.07	0.61 ± 0.06	0.43 ± 0.00
Heart	I	17.16 ± 1.13	15.72 ± 0.53	16.22 ± 0.58	15.61 ± 1.57
	II	18.96 ± 1.49	22.08 ± 0.72	23.37 ± 1.12	18.57 ± 0.13
	III	18.45 ± 4.45	24.05 ± 1.40	22.29 ± 2.86	21.28 ± 3.95
Liver	I	8.96 ± 0.35	8.50 ± 0.45	10.42 ± 1.42	10.30 ± 0.63
	II	13.96 ± 1.25	13.84 ± 0.89	12.26 ± 1.82	11.95 ± 1.75
	III	11.21 ± 2.42	12.07 ± 1.64	10.02 ± 2.85	8.27 ± 1.31
Lungs	I	4.02 ± 0.60	3.72 ± 0.24	2.79 ± 0.25	2.44 ± 0.38
	II	5.34 ± 0.47	4.56 ± 0.87	3.77 ± 1.01	2.84 ± 0.09
	III	5.91 ± 0.77	5.02 ± 0.74	4.86 ± 1.00	2.88 ± 0.66
Kidneys	I	30.59 ± 2.12	24.08 ± 5.54	17.98 ± 2.57	14.92 ± 2.62
	II	49.28 ± 4.60	39.67 ± 5.44	30.30 ± 4.12	26.14 ± 7.24
	III	71.82 ± 16.38	59.61 ± 11.13	34.78 ± 0.26	29.26 ± 4.26
Brain	I	0.20 ± 0.00	0.15 ± 0.02	0.16 ± 0.01	0.15 ± 0.01
	II	0.29 ± 0.05	0.32 ± 0.16	0.25 ± 0.03	0.25 ± 0.03
	III	0.19 ± 0.05	0.27 ± 0.09	0.18 ± 0.02	0.20 ± 0.03
Spleen	I	2.38 ± 0.54	2.48 ± 0.19	2.94 ± 0.63	1.40 ± 0.03
	II	4.78 ± 1.10	3.87 ± 0.58	2.50 ± 0.25	2.57 ± 0.05
	III	5.96 ± 0.33	5.60 ± 0.79	3.21 ± 0.70	2.07 ± 0.32
Muscle	I	7.04 ± 0.53	6.20 ± 0.62	4.91 ± 0.62	6.49 ± 0.83
	II	7.21 ± 2.75	7.25 ± 1.55	9.78 ± 1.76	6.72 ± 0.41
	III	5.72 ± 1.44	9.62 ± 5.03	8.19 ± 1.78	6.39 ± 1.15
Bone	I	2.13 ± 0.64	1.92 ± 0.58	1.73 ± 0.37	1.37 ± 0.32
	II	3.64 ± 0.46	2.26 ± 1.02	3.07 ± 0.20	2.28 ± 0.30
	III	4.76 ± 0.21	2.63 ± 1.48	2.85 ± 0.58	1.79 ± 0.47

[a] I: complex A, yields 95%; II: complex A, yields 67%, complex B, yields 30%; III: complex B, yields 90%.

allows a much more convenient way to study the chemical and biologic properties of ^{99m}Tc-CO-MIBI. The biodistribution in mice demonstrates that complex B is better than complex A for myocardial imaging. However, there are still two main indefinite questions to be studied further. One is how the pH of the solution influences the synthesis of ^{99m}Tc-CO-MIBI, and the other is the structural information of complex A and complex B. Both of them are in process.

Acknowledgements

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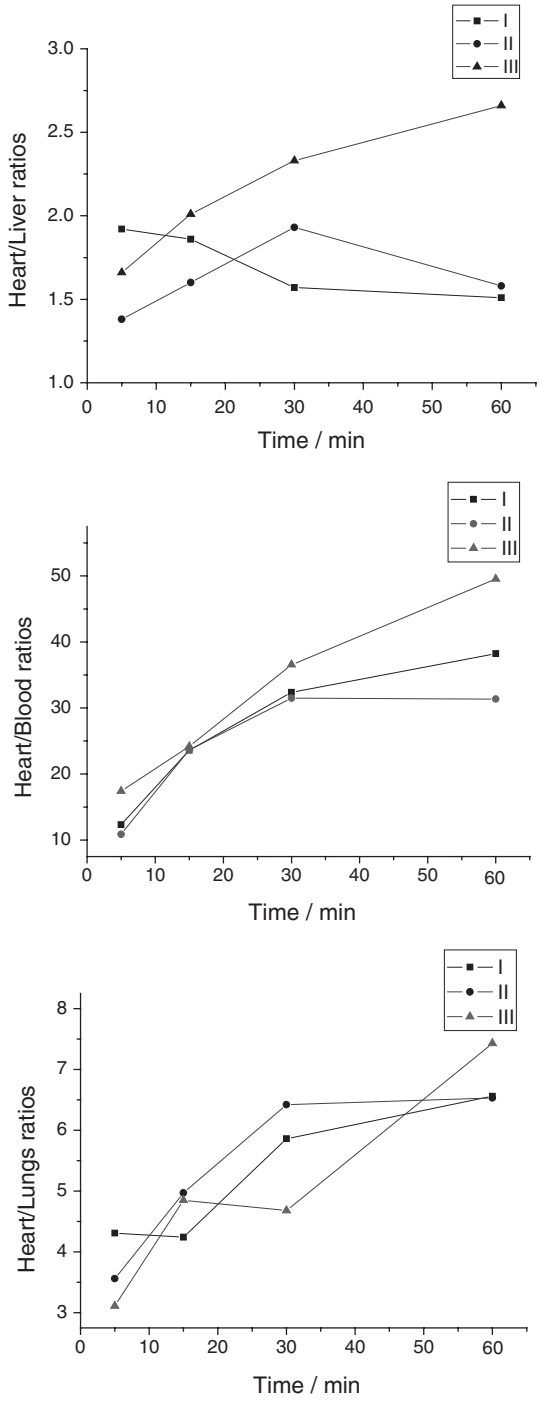

Figure 3. Target/non-target ratios for the Group I, II and III*. * I: complex A, yields 95%; II: complex A, yields 67%; complex B, yields 30%; III: complex B. yields 90%

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