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Research Article

Preparation and biodistribution of novel $^{99m}Tc(CO)_3$-CNR complexes for myocardial imaging

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Abstract: We evaluated lipophilicity and biodistribution of a series of $^{99m}Tc(CO)_3$-ether isonitrile complexes to determine whether different lipophilicity and structure of isonitrile ligands would improve the imaging properties of the radiopharmaceutical for the heart. Novel $^{99m}Tc(CO)_3$-MIBI analogs were prepared and analyzed by radio-HPLC, and their lipophilicity was determined. These new complexes could be bi- or tri-substituted in specified pH conditions like $^{99m}Tc(CO)_3$-MIBI. These new complexes exhibited low liver, lungs and blood uptake compared with $[^{99m}Tc(CO)_3(MIBI)_3]^+$ though their heart uptake was not so high. Among these complexes, $[^{99m}Tc(CO)_3(EPI)_2(OH_2)]^+$ showed higher target to non-target ratios at 5 and 30 min post-injection than that of $[^{99m}Tc(CO)_3(MIBI)_3]^+$.

Keywords: Tc-99m; tricarbonyl; isonitrile; myocardial imaging

Introduction

Recently, Alberto[1] reported the synthesis and applications of the astonishing complex fac-$[^{99m}Tc(CO)_3(OH_2)_3]^+$. This simple complex combines a 'lower' organometallic half with three carbonyl ligands with an 'upper' Werner-type half with three water molecules as ligands.[2] It has favorable properties mainly attributed to its unusual ligand set. The σ-bound water ligands are readily displaced by other ligands, but in combination with other donor–acceptor ligands, the carbonyl groups impart a high kinetic stability on $[Tc(CO)_3]^+$ derivatives. In addition, a high kinetic stability results from the d^6 low-spin electron configuration of the Tc^I complex. Although it was used in hexakis(2-methoxyisobutyl isonitrile) $^{99m}Tc(I)$ (^{99m}Tc-MIBI), the oxidation state +1 is rather unusual in ^{99m}Tc imaging agents and difficult to stabilize with Werner-type ligands (typically with an N, O, or S donor set) alone. In this respect, organometallic complexes with CO ligands expand the available range of compounds significantly. There has been a lot of published research work utilizing this new precursor to develop new radiopharmaceuticals.[3,4]

^{99m}Tc-MIBI is widely used in myocardial perfusion imaging and human tumor imaging. Therefore, preparation and preliminary evaluations of $^{99m}Tc(CO)_3$-MIBI as a myocardial imaging agent[5–8] and a functional probe of Pgp transport activity[9] followed the introduction of the attractive precursor of fac-$[^{99m}Tc(CO)_3(OH_2)_3]^+$. Our group independently designed $^{99m}Tc(CO)_3$-MIBI as a myocardial imaging agent which showed high potential both in mice and dogs.

Examination of the uptake mechanism in myocardial and carcinoma cells indicates that the lipophilicity, cationic charge and the ligand of MIBI itself of ^{99m}Tc-MIBI play a significant role in its accumulation and retention. Moreover, it can be seen from our previous research that $^{99m}Tc(CO)_3$-MIBI might be bi-, tri-substituted product or both in specified pH conditions.[7] It is still unknown whether this radiolabeling reaction phenomenon is only specific for MIBI or not. Therefore, we prepared novel $^{99m}Tc(CO)_3$-ether isonitrile complexes to study this experimental phenomenon and

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the effects of different lipophilicity and ligand structures on biodistribution.

Results and discussion

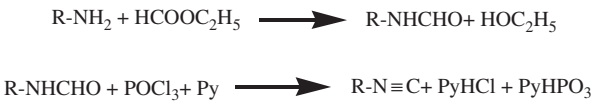
Synthesis

The general synthetic approach for the isonitrile derivatives (CNR) is shown in Scheme 1. The isonitrile derivatives could be prepared conveniently from commercial available alkoxyl amines in a two-step synthesis (Table 1).[10–12] The use of $POCl_3$/pyridine for dehydration of formamide was more convenient and safer than other dehydration systems. The two steps were both exothermic reactions, so the good cooling conditions should be guaranteed. The Vigreux column was necessary to collect the products with high purity, though there would be a little loss in yields. All these isonitriles could be stored at −20°C under N_2 for radiolabeling.

Radiolabeling

The $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor was prepared in > 98% yield according to the method of Alberto *et al.*[1] $^{99m}Tc(CO)_3$-CNR complexes were prepared as described in the experimental section (Table 1). They were analyzed by radio-TLC and radio-HPLC, and the results were shown in Table 2. Besides, there was one radio-HPLC peak corresponding to the mono-complex that appeared at the beginning of radiolabeling course and would disappear quickly with the substitution going on. When pH=9.0–10.0, all three H_2O ligands of was shown to be readily substituted by isonitrile ligands. However, the sequential reaction would stop at bi-substitution when pH=3.0–4.0. When pH lied between the two scopes of 3.0–4.0 and 9.0-10.0, tri-substitution only partially occurred and the ratios of bi- to tri-complex were determined by the pH values. These results above mentioned were same as that of $^{99m}Tc(CO)_3$-MIBI,[7] so it should be a common characteristic for ether isonitriles reacting with $[^{99m}Tc(CO)_3(OH_2)_3]^+$.

All complexes were stable within 6 h at room temperature. Their lipophilicity was determined by partition between 1-octanol and PBS (Table 3). Most of them showed low lipophilicity because of the hydrophilicity of $^{99m}Tc(CO)_3^+$ core. The lipophilicity turned higher when the substituent number changed from two to three for the same isonitrile ligand. The



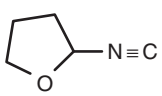
Scheme 1 Synthesis of isonitrile ligands.

Table 2 Results of radiolabeling reaction in pH=3.0–4.0 and 9.0–10.0

pH condition	Complex	HPLC elution condition TETA[a]: MeOH (v:v)	R_t (min)	Yield (%)
3.0–4.0	1a	50:50	7.9	>98%
	2a	40:60	9.0	
	3a	30:70	6.9	
	4a	20:80	5.6	
	5a	30:70	7.1	
9.0–10.0	1b	50:50	9.5	>98%
	2b	40:60	10.7	
	3b	30:70	10.0	
	4b	20:80	7.9	
	5b	30:70	9.2	

[a]TETA: triethylamine phosphate buffer, 0.05 M, pH 2.25.

Table 1 The isonitrile ligands and $^{99m}Tc(CO)_3$-CNR complexes

	Ligand	Complex	No.
MEI	$CH_3OCH_2CH_2NC$	$[^{99m}Tc(CO)_3(MEI)_2(OH_2)]^+$	1a
		$[^{99m}Tc(CO)_3(MEI)_3]^+$	1b
MPI	$CH_3OCH_2CH_2CH_2NC$	$[^{99m}Tc(CO)_3(MPI)_2(OH_2)]^+$	2a
		$[^{99m}Tc(CO)_3(MPI)_3]^+$	2b
EPI	$CH_3CH_2OCH_2CH_2CH_2NC$	$[^{99m}Tc(CO)_3(EPI)_2(OH_2)]^+$	3a
		$[^{99m}Tc(CO)_3(EPI)_3]^+$	3b
IPPI	$(CH_3)_2CHOCH_2CH_2CH_2NC$	$[^{99m}Tc(CO)_3(IPPI)_2(OH_2)]^+$	4a
		$[^{99m}Tc(CO)_3(IPPI)_3]^+$	4b
THFMI		$[^{99m}Tc(CO)_3(THFMI)_2(OH_2)]^+$	5a
		$[^{99m}Tc(CO)_3(THFMI)_3]^+$	5b
MIBI	$CH_3OC(CH_3)_2CH_2NC$	$[^{99m}Tc(CO)_3(MIBI)_2(OH_2)]^+$	6a
		$[^{99m}Tc(CO)_3(MIBI)_3]^+$	6b

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tri-substituted $[^{99m}Tc(CO)_3(IPPI)_3]^+$ had the highest lipophilicity, and the lipophilicity of $[^{99m}Tc(CO)_3(MEI)_2(OH_2)]^+$ was the lowest. The lipophilicity would increase with the number of carbon in ligands increasing for MEI, MPI, EPI and IPPI. At the same time, the sequences of lipophilicity of the corresponding complexes were as below: 1a < 2a < 3a < 4a, 1b < 2b < 3b < 4b. Therefore, the structure of the isonitriles strongly affects the lipophilicity of the complexes.

Biodistribution

Table 4 showed the tissue distributions of the novel $^{99m}Tc(CO)_3$-CNR complexes. Tissues, including heart, blood, liver, lungs, kidneys and spleen, were collected at 5, 30, and 60 min post-injection. Ten complexes showed distinct accumulation in heart, liver and lungs etc. The heart uptake of the new complexes except $[^{99m}Tc(CO)_3(EPI)_2(OH_2)]^+$ and $[^{99m}Tc(CO)_3(IPPI)_2(OH_2)]^+$ lay in a low level, and the activity in heart did not show a good retention from 5 to 30 min post-injection. For ^{99m}Tc-MIBI and $[^{99m}Tc(CO)_3(MIBI)_3]^+$ the heart uptake at 30 min post-injection in mice could be 25 and 22%ID/g,[13] respectively. However, clearance from liver, lungs and blood was very fast for the new complexes during the early stage post-injection, which resulted in apparently low uptake of liver, lungs and blood at 30 and 60 min post-injection. Therefore, the new complexes exhibited lower liver, lungs and blood

Table 3 Partition coefficients of $^{99m}Tc(CO)_3$-CNR complexes

Complex	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b
P	0.09	0.10	0.31	0.62	0.58	1.58	2.79	10.86	0.14	0.36
log *P*	−1.04	−1.00	−0.51	−0.21	−0.23	0.20	0.44	1.04	−0.87	−0.44

Table 4 Tissue distributions of all the complexes at 5, 30, and 60 min post-injection

Complex	Time(min)	%ID/g in tissues					
		Blood	Heart	Liver	Lungs	Kidneys	Spleen
	5	1.29 ± 0.16	3.04 ± 0.34	10.24 ± 1.64	2.16 ± 0.22	28.11 ± 7.05	0.85 ± 0.03
1a	30	0.39 ± 0.02	2.10 ± 0.02	6.80 ± 0.85	1.27 ± 0.12	9.85 ± 2.04	0.44 ± 0.12
	60	0.31 ± 0.01	1.63 ± 0.25	7.06 ± 0.63	1.34 ± 0.19	4.58 ± 0.37	0.43 ± 0.04
	5	2.32 ± 0.20	7.74 ± 1.31	14.00 ± 1.92	3.60 ± 0.37	34.22 ± 1.58	1.65 ± 0.23
1b	30	0.87 ± 0.11	4.32 ± 0.48	8.39 ± 0.38	2.10 ± 0.12	11.19 ± 1.15	0.97 ± 0.18
	60	0.39 ± 0.02	2.02 ± 0.82	5.82 ± 1.02	1.25 ± 0.20	6.03 ± 0.62	0.62 ± 0.23
	5	1.09 ± 0.24	11.44 ± 2.14	11.62 ± 1.24	2.64 ± 0.40	31.71 ± 4.19	1.72 ± 0.42
2a	30	0.36 ± 0.01	10.35 ± 1.14	5.26 ± 0.71	1.70 ± 0.36	9.19 ± 1.77	1.18 ± 0.13
	60	0.20 ± 0.02	7.54 ± 2.18	4.99 ± 2.64	1.19 ± 0.37	5.36 ± 0.25	0.81 ± 0.19
	5	2.08 ± 0.27	15.39 ± 1.04	14.67 ± 1.02	4.72 ± 0.82	41.42 ± 1.74	2.93 ± 0.18
2b	30	0.44 ± 0.07	9.71 ± 2.24	4.34 ± 1.17	1.70 ± 0.14	10.12 ± 1.35	1.29 ± 0.09
	60	0.21 ± 0.02	5.26 ± 0.15	2.48 ± 0.05	0.97 ± 0.04	6.53 ± 0.21	1.02 ± 0.23
	5	1.44 ± 0.10	28.87 ± 3.19	8.60 ± 1.30	6.56 ± 0.40	62.91 ± 16.95	3.99 ± 1.34
3a	30	0.42 ± 0.03	19.48 ± 1.96	5.35 ± 0.45	2.98 ± 0.30	16.91 ± 1.28	2.40 ± 0.31
	60	0.26 ± 0.02	12.39 ± 0.59	5.08 ± 0.49	1.96 ± 0.26	9.44 ± 0.69	1.22 ± 0.17
	5	3.66 ± 0.67	15.98 ± 6.61	57.06 ± 15.74	7.12 ± 1.65	112.35 ± 26.3	7.37 ± 1.22
3b	30	0.75 ± 0.01	7.14 ± 1.12	13.00 ± 4.17	2.15 ± 0.37	42.14 ± 6.71	2.60 ± 0.76
	60	0.42 ± 0.02	4.71 ± 1.58	5.77 ± 1.27	0.99 ± 0.14	17.78 ± 3.98	1.40 ± 0.38
	5	1.63 ± 0.19	17.03 ± 4.45	14.41 ± 0.74	6.01 ± 1.86	70.61 ± 19.65	5.70 ± 1.03
4a	30	0.47 ± 0.07	17.08 ± 3.90	8.53 ± 0.68	2.46 ± 0.30	50.95 ± 9.11	3.99 ± 0.54
	60	0.94 ± 0.89	14.99 ± 3.09	9.77 ± 1.45	1.96 ± 0.73	46.60 ± 12.76	1.61 ± 0.04
	5	6.65 ± 0.74	5.77 ± 1.34	104.10 ± 8.39	9.83 ± 0.96	71.78 ± 9.51	7.74 ± 1.63
4b	30	1.18 ± 0.04	1.99 ± 0.27	19.07 ± 2.98	1.69 ± 0.22	26.36 ± 5.89	2.72 ± 1.39
	60	0.63 ± 0.08	1.18 ± 0.28	11.23 ± 4.66	1.01 ± 0.34	14.53 ± 3.80	0.92 ± 0.10
	5	0.60 ± 0.06	4.68 ± 0.53	7.67 ± 1.98	1.50 ± 0.25	18.00 ± 3.90	0.70 ± 0.15
5a	30	0.14 ± 0.02	2.55 ± 0.20	1.95 ± 0.12	0.59 ± 0.05	3.18 ± 0.46	0.29 ± 0.06
	60	0.08 ± 0.01	2.16 ± 0.18	1.57 ± 0.08	0.61 ± 0.25	2.53 ± 0.03	0.29 ± 0.11
	5	1.00 ± 0.13	5.00 ± 1.02	6.03 ± 1.30	1.78 ± 0.32	22.49 ± 6.27	0.97 ± 0.36
5b	30	0.23 ± 0.01	4.67 ± 0.78	2.48 ± 0.14	1.06 ± 0.16	7.75 ± 1.75	0.61 ± 0.23
	60	0.11 ± 0.06	2.52 ± 0.73	1.09 ± 0.09	0.73 ± 0.15	4.36 ± 0.60	0.45 ± 0.06

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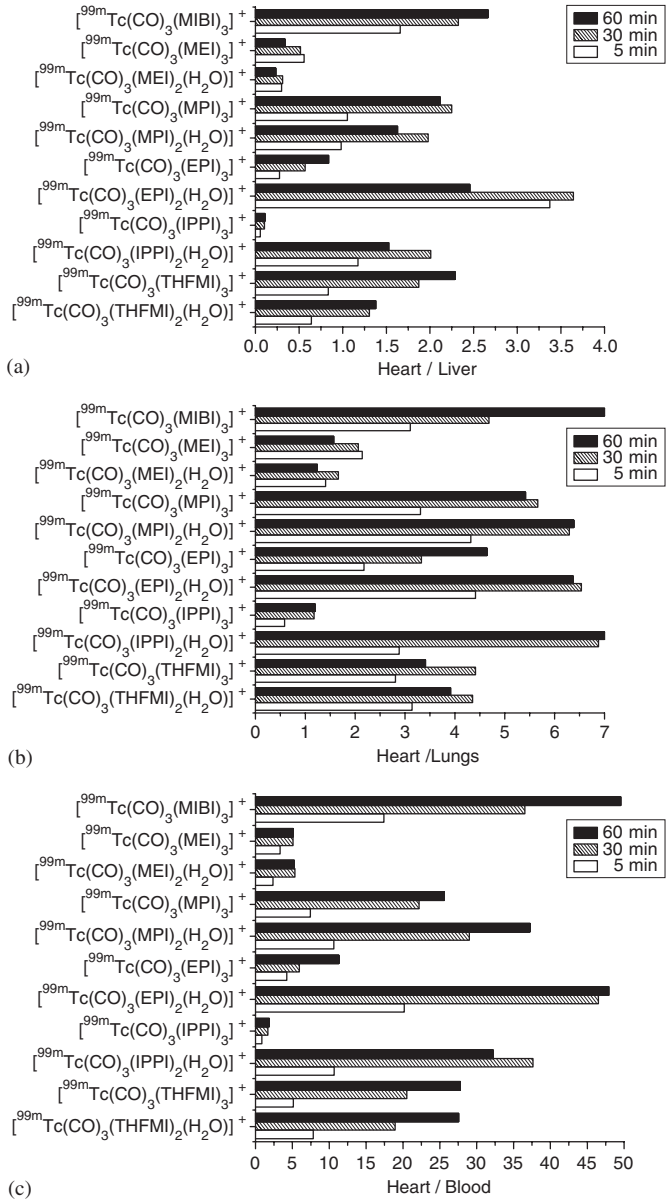

Figure 1 The heart to non-target ratios of the $^{99m}Tc(CO)_3$-CNR complexes: (a) heart/liver; (b) heart/lungs; (c) heart/blood.

uptake compared with $[^{99m}Tc(CO)_3(MIBI)_3]^+$, which made the target to non-target (T/NT) ratios of several new complexes were higher than that of $[^{99m}Tc(CO)_3(MIBI)_3]^+$ (Figure 1). The heart to liver ratio of $[^{99m}Tc(CO)_3(EPI)_2(OH_2)]^+$ was 3.37 that was about double ratio of $[^{99m}Tc(CO)_3(MIBI)_3]^+$ at 5 min post injection, and for ^{99m}Tc-MIBI this ratio was usually less than 1.0 within 1 h. $[^{99m}Tc(CO)_3(EPI)_2(OH_2)]^+$ exhibited almost the best performance in several important aspects for myocardial imaging among the new designed complexes. To be successful myocardial imaging agents the new complexes chiefly need to improve their heart uptake and retention to a little higher level as ^{99m}Tc-MIBI and keep their current high target to non-target ratios at the same time.

$[^{99m}Tc(CO)_3(IPPI)_3]^+$ with highest lipophilicity also had the highest liver, lungs and blood uptake at 5 min post-injection, but it had low heart uptake. The complex with the lowest lipophilicity, $[^{99m}Tc(CO)_3(MEI)_2(OH_2)]^+$, had low heart uptake though its liver, lungs and blood uptake were also low. $^{99m}Tc(CO)_3$THFMI

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with the greatest difference in ligand structure did not show good biodistribution. For $^{99m}Tc(CO)_3$-MIBI, the tri-substituted product showed much better performance in myocardial imaging than bi-complex. On the contrary, for EPI and IPPI the bi-complex had much more better imaging properties. Therefore, the substituent number was not crucial factor in biodistribution for $^{99m}Tc(CO)_3$-CNR.

Usually, the biodistribution of homologous complexes of complex is affected by their lipophilicity, size and the ligand itself, etc. During the course of developing ^{99m}Tc-isonitrile complexes as myocardial imaging agents, $[^{99m}Tc(MIBI)_6]^+$ showed much better properties than others such as $[^{99m}Tc(TBI)_6]^+$ and $[^{99m}Tc(CPI)_6]^+$. It proves that the ligand plays an important role to the biological behavior of the complex, and MIBI is the best among the isonitrile analogs. It might explain from one aspect why the new complexes in this investigation did not exhibited the same high heart uptake and retention as $[^{99m}Tc(CO)_3(MIBI)_3]^+$. In addition, the lipophilicity also showed marked influence on the biodistribution. $[^{99m}Tc(CO)_3(IPPI)_3]^+$ and $[^{99m}Tc(CO)_3(MEI)_2(OH_2)]^+$ with highest qand lowest lipophilicity, respectively, did not show good biodistribution in mice. Therefore, there is a most appropriate scope of lipophilicity to achieve the good balance of high uptake in heart and fast clearance from non-target tissues for $^{99m}Tc(CO)_3$-CNR complexes. To get this scope, more $^{99m}Tc(CO)_3$-CNR complexes should be prepared and studied in the future.

Conclusion

Like $^{99m}Tc(CO)_3$-MIBI, there are bi- and tri-substituted products when the five isonitriles react with fac-$[^{99m}Tc(CO)_3(OH_2)_3]^+$ in specified pH conditions. The changes from lipophilicity and ligand itself did bring great effects on the properties of the $^{99m}Tc(CO)_3$-CNR complexes for myocardial imaging. Among these new complexes, $[^{99m}Tc(CO)_3(EPI)_2(OH_2)]^+$ showed the most favorable imaging characteristics in mice.

Experimental

2-methoxyethaneamine, 3-ethoxypropaneamine, 3-iso propoxypropan-1-amine, tetrahydro-furfurylamine were purchased from Acros Organics Co. 3-methoxypropaneamine was purchased from Aldrich Chemical Co. Other chemicals were purchased from Beijing Chemical Reagents Company. Pure CO gas was purchased from NRCCRM, China. $^{99}Mo/^{99m}Tc$ generator was obtained from the Beijing Syncor Medical Corporation. ICR mice, 18–20 g, female, were obtained from Animal Center of Peking University. Proton nuclear magnetic resonance spectroscopy was performed on Bruker Avance 500 MHz.Infrared spectrum was performed on Nicolet-170SX. The automatic gamma counts were carried out by WALLAC/WIZARD 1470, Perkin Elmer Wallac. HPLC was performed on a SHIMADZU system (SCL-10Avp pumps and SPD-10Avp UV detector) and Park Radioow detector. TLC was run on polyamide film using acetonitrile as mobile phase.

Synthesis of isonitriles (CNR)

A solution of ethyl formate (8.0 ml, 100 mmol), precooled to approximately 0°C, was added slowly to a stirred solution of amine analogs (95 mmol) in an ice/NaCl bath. After the slightly exothermic reaction ceased, the solution was allowed to warm slowly to room temperature and refluxed overnight. The solution was distilled through a Vigreux column to give formamide analogs determined by infrared spectroscopy. 50 mmol formamide was dissolved in methylene chloride (45 ml). Triethylamine (20.1 ml, 0.25 mol) was added and the clear solution was cooled in an ice/water bath. Phosphorus oxychloride (2.75 ml, 30 mmol) was added dropwise to the cooled formamide solution. The resulting suspension was stirred and allowed to slowly warm to room temperature for 1 h, and at reflux temperature for 15 min. 20 ml cold water was added and the organic layer separated. The organic layer was washed with a saturated solution of sodium bicarbonate, water and dried with anhydrous sodium sulfate. Evaporation of the methylene chloride left a dark brown liquid. The dark brown liquid was distilled through a Vigreux column under vacuum to give the isonitrile analogs. They were determined by IR and [^{1}H]NMR.

Preparation of $^{99m}Tc(CO)_3$-CNR

The $[^{99m}Tc(CO)_3(OH_2)_3]^+$ precursor was prepared according to the method of Alberto *et al.* 1.0 mg isonitrile, dissolved in 0.5 ml water, was added to 1 ml $[^{99m}Tc(CO)_3(OH_2)_3]^+$ solution which was adjusted to pH=3.0–4.0 or 9.0–10.0 with 0.5 N HCl solution. The solution was allowed in a boiling water bath for 15 min and examined by TLC (R_f = 0.9–1.0 for $[^{99m}Tc(CO)_3(CNR)_2(OH_2)]^+$ and $[^{99m}Tc(CO)_3(CNR)_3]^+$, R_f = 0–0.1 for $[^{99m}Tc(CO)_3(OH_2)_3]^+$ and R_f = 0.4–0.5 for $^{99m}TcO_4^-$) and radio HPLC (Alltima C18 RP column, 250 × 4.6 mm, 5 μm, flow rate 1 ml/min).

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Stability

The labeled complexes were incubated at room temperature for up to 6 h. Aliquots were taken and analyzed by radio-HPLC to assess the stability.

Determination of the partition coefficient

The lipophilicity of the complex with RCP over 95% was determined as follows: 0.1 ml complex 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 taken out and counted in a well-counter. The measurement was repeated for three times. The partition coefficient, *P*, was calculated using the following equation:

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

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

Biodistribution

Samples (about 740 kBq in 0.1 ml solution) were injected through the tail vain into ICR mice (18–20 g, female). The mice were sacrificed at 5, 30, and 60 min post-injection. Selected organs were collected for weighing and counting. The accumulated radioactivity in the tissues was calculated in terms of percentage of injected dose per gram organ (%ID/g).

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