SYNTHESIS, CHARACTERIZATION AND BIODISTRIBUTION OF NEW ^{99m}Tc NITRIDO COMPLEXES WITH DERIVATIVES OF ALKYL XANTHATES

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SUMMARY

We have synthesized a series of neutral ^{99m}Tc alkyl xanthates complexes with the $[Tc \equiv N]^{2+}$ core, in which the xanthate ligand substituents are varied from pure alkyls to aminoalkyls ($R^1 R^2 NR-$). Analyses by TLC, ion exchange, partition coefficient (PC) determination and paper chromatography show that the complexes are neutral and lipid soluble. The ^{99m}Tc complexes of dialkylaminoxanthates have a higher myocardial uptake than their counterparts of unsubstituted xanthate, but still lower than that of the $[^{99m}Tc \equiv N]^{2+}$ complexes of dithiocarbamate.

Key words: Technetium-99m / Technetium radiopharmaceuticals / Technetium nitrido complex.

INTRODUCTION

In recent years much research have been devoted to the synthesis of ^{99m}Tc radiopharmaceuticals with the $[Tc \equiv N]^{2+}$ core. Baldas et al.[1] successfully synthesized and characterized the first technetium nitrido complex $[TcN(Et_2NCS_2)_2]$ (TcN-DEDC) at the macroscopic level. The first method for the preparation of ^{99m}Tc radiopharmaceuticals with the $[Tc \equiv N]^{2+}$ core was proposed and successively applied to the synthesis of various technetium-nitrido complexes at no carrier added level[2,3]. This procedure is based on the reaction of ^{99m}Tc pertechnetate with excess of NaN_3 in the presence of concentrated HCl under reflux, and a consequent reduction-substitution reaction with suitable ligand.

A more recent procedure for the preparation of $[^{99m}Tc \equiv N]^{2+}$ radiopharmaceuticals proposed by Pasqualini et al.[4] is by the initial reaction of $^{99m}TcO_4^-$ with S-methyl N-methyl dithiocarbazate $[H_2NNMeC(=S)SMe]$ in the presence of PR_3 / HCl, followed by addition of the ligand. It was shown that the formation of the $[Tc \equiv N]^{2+}$ core in this reaction, is independent upon both the choice of reducing agent and pH condition. This suggests that the basic reaction of pertechnetate with N-methyl S-methyl dithiocarbazate is of general applicability and can be easily used to prepare ^{99m}Tc radiopharmaceuticals with the $[Tc \equiv N]^{2+}$ core in a wide range of labeling conditions, especially under sterile and apyrogenic conditions.

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Abram et al.[5] had prepared a series of technetium complexes with different alkyl xanthates. Solution studies showed the complexes are neutral and lipid soluble. Recently, Pasqualini et al.[6] prepared a series of neutral bis(dithiocarbamato) nitrido technetium(V) complexes of general formula $^{99m}TcN(L)_2$ [L=$R^1(R^2)NCS_2^-$], where the R^1 and R^2 groups on the >NC(=S)S^- moiety were varied by different organic functional groups such as ether and alkoxy etc.. We report here the synthesis and biodistributions of ^{99m}TcN complexes of dialkylamino xanthates [$R^1R^2NR^3OCS_2^-$].

EXPERIMENTAL

Materials

$H_2NNHCSSCH_3$ and TPPS were synthesized according to a previously reported procedure[7,8].The potassium salt of the xanthate ligands { KL, L=$ROCS_2^-$ } used in the preparations are shown in Table 2. Potassium isopropyl xanthate is commercially available. The other ligands were prepared by reacting the corresponding alcohol with an equivalent amount of carbon disulfide in aqueous KOH solutions. The following procedure, utilized for the preparation of the potassium salt $(CH_3)_2N(CH_2)_2OCS_2K$ is a representative one. 2.5 g of KOH was added into 25ml of $(CH_3)_2N(CH_2)_2OH$, and gently warmed until the solid was no longer dissolved, then allowed to cool to room temperature. The residue was removed and 10 ml of CS_2 was added. The solution turned orange and a precipitate was formed immediately. The mixture evolved gas and heat, and was stirred for 30 min in a ice-salt bath, then allowed to cool down to room temperature. Dried diethylether was then added, the resulted yellow precipitate was filtered off, washed with diethylether and dried. The crude product was recrystallized from ethanol to yield crystals of potassium salt $(CH_3)_2N(CH_2)_2OCS_2K$.

$^{99}Mo/^{99m}Tc$ generator was purchased from the China Institute of Atomic energy (CIAE) .All other chemicals were of laboratory grade and used without further purificaton.

Preparation of $^{99m}TcN(L)_2$ Complexes.

1 ml of saline containing $[^{99m}TcO_4]^-$ (activity ranging from 1.0 MBq to 1.0 GBq) was added to a vial containing 3.0 mg of TPPS and 1.0 mg of $H_2NNH(C=S)SCH_3$ dissolved in 1.0 ml 0.1 M HCl. The resulting solution was heated at 100 °C for 15 min and then cooled to room temperature. The pH of the solution was adjusted to 8.0 by adding 1.0 ml 0.2M phosphate buffer and then 1.0 ml of water solution containing 10 mg of potassium salt of the appropriate ligand was added. After heating at 60 °C for 15 min and then cooling, the preparation was ready for use.

Analytical methods

Elemental analysis for ligands was performed by the Institute of Chemistry, Chinese Academy of Sciences. Analysis by paper chromatography was conducted using 2 × 20 cm filter paper eluted with saline and toluene respectively. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminium-backed plates and with toluene as an eluent.

Ion exchange analysis was performed with both cation and anion exchange resins. Cation exchange resin: HHY-10K, large hole, exchange capacity: 4.5 mmol/g, water content 56%, humidity density 0.8 g, applicable acidity pH=1-14, applicable temperature <120°C. Anion exchange resin: HHY-10A, exchange capacity: 4.1 mmol/g, water content 58%, humidity density 0.69 g, applicable acidity pH=1-14, applicable temperature <60°C(OH^-), <75°C(Cl^-).

The partition coefficients in octanol or cyclohexane and water phases of the five $^{99m}TcN^{2+}$ complexes were measured according to the procedure of Mutalib [9] et al. The measurement was repeated three times. Care was taken to prevent cross-contamination between the phases.

Biodistribution studies

An amount of 0.1 ml of the preparation (ca. 60-90 kBq) was administered i.v. via tail to Yun Lan mice (20-30g) and the injected activity measured with a well-type NaI(Tl) detector. Mice were sacrificed at 5 and 30 min postinjection respectively. Organs of interest and blood were collected, weighed and measured for radioactivity. The original injected activity was corrected for the activity found in the tail (<3% in all case). Blood activity was calculated on the assumption that the overall blood volume represents 7% of total body weight.

RESULTS AND DISCUSSION

Characterization

Table1. Elemental analysis and ^1H NMR* of ligands

Ligands	Assayed values(%)			Caculated values(%)		
	C	H	N	C	H	N
$(CH_3)_2N(CH_2)_2OCS_2K$	28.74	5.10	6.72	28.15	4.92	6.73
$(C_2H_5)_2N(CH_2)_2OCS_2K$	33.48	5.60	5.32	33.72	5.62	5.62
	^1H NMR (ppm,peak multiplicity)					
$(CH_3)_2N(CH_2)_2OCS_2K$	CH_3: 2.2, 1 (6H)		CH_2: 2.68,3(2H);	4.47,3(2H)		
$(C_2H_5)_2N(CH_2)_2OCS_2K$	CH_3: 0.97, 3 (6H)		CH_2: 2.53,4(4H);	2.82,3(2H);	4.48,3(2H)	

* D_2O, ppm relative to TMS

All prepared compounds are characterized by elemental analysis, ^1H NMR (only two new ligands are listed in Table 1) and infrared spectrascopy, $H_2NNHCSSCH_3$ and TPPS are further assayed by mass spectroscopy [FAB^+: $(M+H)^+$/123; M^+/569 respectively]. The chemical identity of all $^{99m}TcN(L)_2$ products prepared at tracer level is determined by their chromatographic behavior, partition coefficients and ionic charge. The results are shown in Table 2. All the preparations are neutral and lipophilic complexes and of > 95% purity.

Table 2 . Characterization of $^{99m}TcN(L)_2$ complexes

Ligands	Rf (Paper chromatography)		Rf (Silica gel)	Ion exchanger (Ionic charge)		Partition coefficients	
	Saline	Toluene	Toluene	Cation	Anion	lgP_{oct}	lgP_{cyhex}
$CH_3OCS_2^-$	0	0.9-1.0	0.2-0.4	~ 0	~ 0	1.12	0.78
$C_2H_5OCS_2^-$	0	0.9-1.0	0.3-0.4	~ 0	~ 0	0.76	0.72
$(CH_3)_2CHOCS_2^-$	0	0.9-1.0	0.3-0.4	~ 0	~ 0	1.43	0.95
$(CH_3)_2N(CH_2)_2OCS_2^-$	0	0.9-1.0	0.4-0.5	~ 0	~ 0	1.07	0.53
$(C_2H_5)_2N(CH_2)_2OCS_2^-$	0	0.9-1.0	0.4-0.5	~ 0	~ 0	1.36	0.61

Biodistribution

Table 3 . Biodistribution of nitrido ^{99m}Tc complexes at 5 min in mice (ID% / tissue)

Complexes	1	2	3	4	5	TcN-DEDC[6]
Heart*	0.46±0.11	0.26±0.06	0.24±0.04	1.16±0.14	0.62±0.09	3.78±0.15
Lung*	0.70±0.13	0.38±0.05	0.51±0.07	3.46±0.41	1.22±0.22	5.19±0.85
Liver*	5.82±0.47	6.24±0.58	5.38±0.64	12.5±1.80	10.8±1.23	17.7±1.3
Blood*	7.23±0.88	8.36±0.97	8.23±0.92	11.4±1.92	13.2±2.41	2.10±0.30
Kidneys	2.43±0.31	2.12±0.43	1.75±0.21	4.82±0.52	4.24±0.50	5.24±0.59
Stomach	0.57±0.06	0.48±0.06	0.50±0.06	0.32±0.06	0.41±0.05	
Spleen	1.62±0.20	1.35±0.11	1.46±0.12	2.11±0.18	2.65±0.32	
Brain*	0.08±0.02	0.07±0.02	0.05±0.02	0.10±0.03	0.12±0.03	
Heart-to-blood	1.13	0.43	0.36	1.37	0.74	
Heart-to-liver	1.22	0.56	0.53	1.14	0.73	2.0
Heart-to-lung	0.66	0.62	0.85	0.67	0.81	0.7

Mean±s.d. ; n=4; * n=8

1.$(CH_3OCS_2)_2TcN$, 2.$(C_2H_5OCS_2)_2TcN$ 3.$[(CH_3)_2CHOCS_2]_2TcN$,
4.$[(CH_3)_2N(CH_2)_2OCS_2]_2TcN$ 5.$[(C_2H_5)_2N(CH_2)_2OCS_2]_2TcN$

Biological distribution results in mice for the five nitrido ^{99m}Tc complexes are shown in Table 3 and Table 4. All $^{99m}TcN(L)_2$ complexes accumulate more or less in the myocardium, but their biodistribution are dependent on the functional groups bonded to the uncoordinated oxygen atom of the xanthate ligand. Obviously, complexes 4 and 5, which have tertiary amine radical groups, exhibit higher uptake in the heart than complexes 1, 2 and 3. However, they are all quickly cleaned out of the tissues, especially from blood resulting in a higher heart-to-blood ratio, whereas complexes 4 and 5 show slow clearance from liver and lungs.

As Pasqualini[6] reported, for the TcN-dithiocarbamate complexes, the highest values of myocardial uptake were found with the derivatives having $R^1 = R^2 = C_2H_5-$ and $R^1 = C_2H_5-$, $R^2 = C_2H_5O-$ as lateral groups. By increasing the length and size of the alkyl substituents, a concomitant decrease of myocardial localization was observed. Biodistribution results in this work, for either TcN complexes

Table 4 . Biodistribution of nitrido ^{99m}Tc complexes at 30 min in mice (ID% / tissue)

Complexes	1	2	3	4	5	TcN-DEDC[6]
Heart*	0.09±0.02	0.04±0.02	0.08±0.03	0.25±0.04	0.09±0.03	3.05±0.25
Lung*	0.21±0.03	0.21±0.04	0.22±0.04	2.73±0.33	0.66±0.08	2.30±0.42
Liver*	1.55±0.17	0.68±0.12	1.42±0.21	9.80±1.02	4.76±0.56	24.8±1.90
Blood*	1.82±0.16	2.32±0.34	1.66±0.23	2.03±0.28	1.78±0.23	2.58±0.61
Kidneys	1.52±0.24	1.96±0.22	1.62±0.17	4.50±0.46	3.66±0.43	5.02±0.20
Stomach	0.42±0.05	0.28±0.03	0.32±0.05	0.21±0.03	0.24±0.06	
Spleen	1.20±0.14	1.10±0.09	1.20±0.11	1.43±0.13	1.87±0.14	
Brain*	0.06±0.03	0.04±0.02	0.04±0.02	0.07±0.03	0.05±0.02	
Heart-to-blood	0.86	0.28	0.68	1.72	0.63	
Heart-to-liver	0.79	0.41	0.67	0.38	0.25	1.4
Heart-to-lung	0.45	0.37	0.62	0.21	0.22	1.1

Mean±s.d. ; n=4; * n=8

1.$(CH_3OCS_2)_2TcN$, 2.$(C_2H_5OCS_2)_2TcN$ 3.$[(CH_3)_2CHOCS_2]_2TcN$,
4.$[(CH_3)_2N(CH_2)_2OCS_2]_2TcN$ 5.$[(C_2H_5)_2N(CH_2)_2OCS_2]_2TcN$

of unsubstituted xanthate (complexes 1, 2 and 3) or the other two complexes of dialkylaminoxanthate(complexes 4 and 5), are in agreement with the structure – uptake correlation reported by Pasqualini. The highest values of myocardial uptake were found with the derivatives having shortest carbon chain in each analog, such as R=CH_3 – of complex 1 compared with 2 and 3 and R=$(CH_3)_2N$– of complex 4 compared with 5. However, complexes 4 and 5 carrying longer chains of $(CH_3)_2N$– and $(C_2H_5)_2N$– side groups show the higher heart uptake. Presumably, the structural N atom plays a predominant role in modifying the biological behaviour of the complexes for increasing of the myocardium uptake.

Transient retention of neutral, lipophilic complexes would be expected on the basis of a nonspecific partitioning of these agents into the hydrophobic environment of the cell[6]. In the Pasqualini's paper, $[(Et)_2NCS_2]_2TcN$, with $lgP_{oct}=1.3$[5], was uptaken in heart by a peak value of 3.78 ID%[6] in an optimum condition. In this work, both less lipophilic complex 2 and more lipophilic complex 3 show lower heart uptake, while complex 1 with moderate lipophilicity presents higher myocardium localization. Seemingly, it is better that the lipophilicity should be moderate. However, complex 4 and complex 1 with similar lipophilicity show very different heart uptake (see Table 2). This may mainly be attributed to their different steric structure of the ligands, R-O-CS_2^- and $(R^1)_2N$-(R^2)-O-CS_2^-.

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