BFCA for labeling with the $M(CO)_3$ precursor (M = Re, ^{99m}Tc) : Cysteine toward a tridentate complex

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1. Introduction

Organometallic complexes of techneutium in its low oxidation states received little attention till the development of very stable water-soluble organometallic Tc(I) complexes using monodentate isonitrile ligands. The Tc(I) oxidation state is pratically advantegeous because of the kinetic inertness inherent in its low-spin d_6 configuration and good stability in aqueous media. [1] That is, Tc(I) or Re(I) tricarbonyl complexes are ideal candidates for the radiolabeling of biomolecules which $M(CO)_3$ core allows the high efficient labelling with the retention of the biological affinity. [2, 3]

To be used as a BFCA, it should possess not only both a strong metal-binding moiety and a group that binds to a biomolecules, but also the metal complex should maintain the high stability *in vitro* and *in vivo*.
In this investigation, to estimate the usefulness of thiol modified L-cysteine as a new BFCA with M(CO)3 precursor for the conjugation to biomolecules while maintaining high stability, their non-carrier added and macroscopic level complexes of L-cycteine (L_1), S-methyl L-cysteine (L_2), L-methionine (L_3), and S-carboxymethyl-L-cysteine (L_4) were prepared for the evaluation of radiolabelling efficiency with $^{99m}Tc(CO)_3$ and macroscopic characteristics. Furthermore, in order to study *in vivo* pharmacokinetics, scintigraphic imaging scans using gamma camera were acquired after intravenous administration of each $^{99m}Tc(CO)_3$ complex and biodistribution study on $^{99m}Tc(CO)_3$-L_4 was implemented using ICR mice.

2. Methods and Results

2-1. Preparation ofmacroscopic $[Re(CO)_3]$ Complex

The $Re(CO)_3$-complexes were synthesized. Complex formation was monitored by means of HPLC. The portion containing the product was collected and evaporated under reduced pressure to give the white solid.

2-2. Radiolabeling with $[^{99m}Tc(CO)_3(H_2O)_3]^+$

For the radiolabeling with $^{99m}Tc(CO)_3$ moiety, 50 μl of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was injected into the prepared ligand vial followed by incubation at 75 °C for 30 min and cooled in ice bath. The ligand concentrations were ranged from 10^{-3} to 10^{-8} M in the reaction mixture.

The radiolabeling efficiency and radiochemical purity were analyzed by a reverse-phase high performance liquid chromatography (RP-HPLC).
The HPLC profile of all reactions on the non-carrier-added level showed the formation of the same product as characterization on the macroscopic level. *(Table 1)*
The IR spectra were confirmed that all ligands formed well-defined complexes.

Table 1. HPLC retention time of complexes labeled with $^{99m}Tc(CO)_3$ or $Re(CO)_3$: $Re(CO)_3$-complexes were monitored at 254 nm

Ligand	Retention time (min) of metal tricarbonyl complex	
	Macroscopic level (254 nm)	Non-carrier added level
L-cycteine (L_1)	12.2	11.5
S-methyl L-cysteine (L_2)	14.0	13.9
L-methionine (L_3)	14.4	14.8
S-carboxymethyl L-cysteine (L_4)	16.0	15.8

2.3. In Vivo Pharmacokinetics

2-3-1. Scintigraphic Imaging

Six week-old New Zealand white male rabbits (average 2.73 kg) were used for the imaging studies. After being anesthetized with ketamine and xylazine, 0.5 ml of $^{99m}Tc(CO)_3$ complex solution (37 MBq) was injected into each rabbit via the left ear vein.
The major excretion pathway of each complex was urinary excretion. The serial static image scans showed that most of the injected dose was excreted via urinary system and low uptake in liver.

2-3-2. Biodistribution Studies

$[^{99m}Tc(CO)_3 \cdot L_4]$ 4 complex solution with 7.4 ± 0.7 MBq (0.2 ± 0.02 mCi) adjusted to physiological conditions with PBS buffer (pH 7.4) was injected into 6-week-old ICR male mice (33.47±1.07 g, n = 4, SPF grade) through a lateral tail vein.
To determine the radioactive concentration in the tissues and organs, the animals were sacrificed after being anesthetized at 24 hrs after administration. The tissues and organs were excised and weighed. The radioactivity

in the samples was counted for 1 min using a gamma well counter (Canberra, USA).
The results of the biodistribution of $^{99m}Tc(CO)_3L_4$are summarized in Table 2 as the percent of the injected dose to each selected organ of the ICR male mice (%ID/g).

Table 2. Biodistribution of $^{99m}Tc(CO)_3$-complex 24 hr post intravenous administration

	S-carboxymethyl L-cysteine [a]	Histidine [b]
Blood	0.03 ± 0.01	0.02 ± 0.01
Heart	0.02 ± 0.01	0.01 ± 0.01
Lung	0.05 ± 0.02	0.92 ± 0.20
Spleen	0.02 ± 0.01	0.36 ± 0.20
Kidney	0.14 ± 0.01	0.22 ± 0.07
Stomach	0.02 ± 0.01	0.19 ± 0.10
Intestine	0.20 ± 0.08	0.06 ± 0.02
Liver	0.15 ± 0.03	0.16 ± 0.10

Data are mean ± SD and are expressed in percentage injected dose per gram tissue (%ID/g)
[a] 6-week-old ICR male mice (33.47±1.07 g, n = 4, SPF grade)
[b] Reference [4]

3. Conclusion

The thiol-modified cysteine for $M(CO)_3$ precursor can be the appropriate candidate conjugated to biomolecules as a bifunctional chelating agent having $M(CO)_3$-core.
The $[M(CO)_3(H_2O)_3]^+$ (M= ^{99m}Tc, ^{188}Re) complex with S-R cysteine produces a tridentate complex [Re(Cys-R)$(CO)_3$] : whereas R is a chemical bearing a free carboxylic group or a amine which can be used for attachment of biomolecules.
Within a peptide chain, the thiol modified cysteine can be positioned either at the N-terminus of C-terminus dependant on the chemical used in the modification of cysteine. Also, it can be easily adapted to the other ligand for radiolabeling with $M(CO)_3$ (M= ^{99m}Tc, ^{188}Re) precursor.

Reference

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