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Preparation of ^{99m}Tc-$C_{60}(OH)_x$ and its biodistribution studies

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Abstract

The biological behavior of fullerene derivatives shows their considerable potential for medical applications. In order to provide a C_{60} derivative for biodistriburtion studies, the ^{99m}Tc-labeling of $C_{60}(OH)_x$ was optimized. Gamma counting and single photon emission computed tomography (SPECT) were used to assess the biodistribution of the ^{99m}Tc-labeled compound in mice and rabbits. Biodistribution studies in mice and imaging of rabbits indicated that ^{99m}Tc-$C_{60}(OH)_x$ was widely distributed in all tissues. A significant percentage of total activity was retained for 48 h, particularly in the kidneys, bone, spleen, and liver. All tissues displayed a slow clearance over 48 h, except for bone, which showed slightly increasing localization within 24 h. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: $C_{60}(OH)_x$; ^{99m}Tc-labeled; activity test; SPECT imaging; biodistribution

1. Introduction

Since the discovery of fullerenes in 1985 [1], C_{60} has elicited intense interest and recent intensive chemical studies have revealed the diverse reactivity of C_{60}. Biological applications have included the studies of the inhibition of human immunodeficiency virus (HIV) protease, site-selective DNA cleavage and photodynamic therapy [2–4]. Although significant information on the chemical properties of C_{60} is available, only limited studies have reported the biological activity, biodistribution and metabolism of C_{60}. This could result from insolubility of C_{60} in aqueous solution [5] and lack of analytical technique of high sensitive analysis. Two of the principle challenges in developing fullerene-base drugs are thus the development of water soluble for *vivo* use and finding appropriate analytical and test methods.

Research involving the biodistribution of fullerenes *in vivo* is limited to studies on C_{60} and La@C_{82} suspensions [6], ^{166}Ho@$C_{82}(OH)_x$ [7–8] and several water-soluble C_{60} derivatives (9–10). Animal studies with these derivatives demonstrated rapid distribution and long-term liver localization. Sine fullerols have polar hyodroxy groups and are water soluble, they have potential applications in aqueous solution chemistry, electrochemistry and biochemistry, as well as in synthesis of new fullerene derivatives [11]. Fullerenols show excellent efficiency in eliminating superoxide radicals (O^{2-}) generated by xanthine and xanthine oxidase, suggesting potential use of these compounds as novel potent free radical scavengers in biological systems [12]. In order to determine the biodistribution and metabolism behavior of $C_{60}(OH)_x$ quickly and conveniently, and to supply pharmacokinetic foundation of C_{60} derivatives as drug or drug carriers, $C_{60}(OH)_x$ was labeled in the present work with ^{99m}Tc ($T_{1/2}$=6.02h, E_γ=141keV(90%)). The biological behavior of this derivative was studied by assaying the radioactivity of tissues of mice and SPECT imaging of New Zealand rabbits.

2. Materials and methods

2.1. Agents and apparatus

C_{60} (99.9%, Wuhai University), $C_{60}(OH)_x$ used in these studies was synthesized by a reported procedure [13]. Stannous chloride, acetone, saline, Vc and sodium hydroxide were of guaranteed grade. A ^{99}Mo/^{99m}Tc generator was obtained from the China Institute of Atomic Energy (CIAE). A solid scintillation counter with NaI (Tl) crystal and Elscint Apex SP-6 SPECT were used for radioactivity analysis.

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2.2. Animals

The biodistribution study was performed on Kunming mice weighing 18–22 g (female, 5~6 weeks old). New Zealand rabbits (obtained from Shanghai Medical University) used in SPECT image study were 6 weeks old.

2.3. Synthesis of ^{99m}Tc-$C_{60}(OH)_x$

$^{99m}TcO_4^-$ was eluted from $^{99}Mo/^{99m}Tc$ generator and reduced to Tc (V) by stannous chloride. The radio labeling procedures were as follows: to a $C_{60}(OH)_x$ aqueous solution, ascorbic acid aqueous solution, a stannous chloride and $^{99m}TcO_4^-$ solution were added. After the pH was adjusted to 5, the mixture was allowed to react in boiling water for 40 min. Radiochemical yields of ^{99m}Tc-$C_{60}(OH)_x$, $^{99m}TcO_4^-$ and reduced $^{99m}TcO_2$ were determined by paper chromatography using Whatman 1 chromatography paper strips (1.5cm × 15cm). 10 μl portions of the test solution were applied at 1.5 cm from the lower end of the strips. The strips were developed in acetone and 0.9% saline respectively until the solvent reach the top of the strips. The strips dried, cut into 1-cm long equal segments, and the radioactivity was measured by using NaI(Tl) scintillor. $^{99m}TcO_4^-$, $^{99m}TcO_2$ and ^{99m}Tc-$C_{60}(OH)_x$ migrated with respective R_f values of 0.7, 0 and 1 in saline, and 0.9, 0 and 0 in acetone, respectively.

2.4. Biodistribution of ^{99m}Tc-$C_{60}(OH)_x$ in mice

Biodistribution studies were performed in female Kunming mice weighing 18–22 g, which were randomly divided into 5 groups. ^{99m}Tc-$C_{60}(OH)_x$ in ~ 0.2 ml volume was injected through a tail vein and the mice were sacrificed at different time intervals. The important tissues and organs such as blood, liver, spleen, kidney and cortical bone were excised, weighed and counted. Distribution of the radioactivity in different tissues and organs was calculated as the percent of the injected dose per gram of the tissue. All the biodistribution studies were carried out in compliance with the national regulations related to the conduct of experimentation.

2.5. SPECT imaging of ^{99m}Tc-$C_{60}(OH)_x$ in New Zealand rabbits

After 5 mCi ^{99m}Tc-$C_{60}(OH)_x$ was injected through the otic vein, whole-body dynamic imaging was immediately initiated, and 500 K counts were collected in anterior and posterior position. Biodistribution of ^{99m}Tc-$C_{60}(OH)_x$ was analyzed in rabbits at 1 h, 1.5 h, 14 h and 40 h, when whole-body static imaging was conducted.

3. Results

3.1. Optimization labeling

The influence of time on the formation of ^{99m}Tc-$C_{60}(OH)_x$ was studied in boiling water. The yield of ^{99m}Tc-$C_{60}(OH)_x$ ($[C_{60}(OH)_x]$ = 5 mg/ml) at mid pH (~ 5) increased rapidly and attained a relatively constant value of more than 92% after 40 min. The influence of pH on the radiolabeling yields was investigated at a concentration of 5 mg/ml $C_{60}(OH)_x$ as shown in Figure 1. The yields show a maximum in the pH 4–6 range and then decrease above pH 6. As seen from Figure 2, the radiolabeling yield is low when the amount of stannous chloride is less than 25 μg or more than 100 μg. Because at the amount of 50 μg, the radiolabeling yield obtain the highest value of 92%, the amount of stannous chloride was fixed at 50 μg in following procedures.

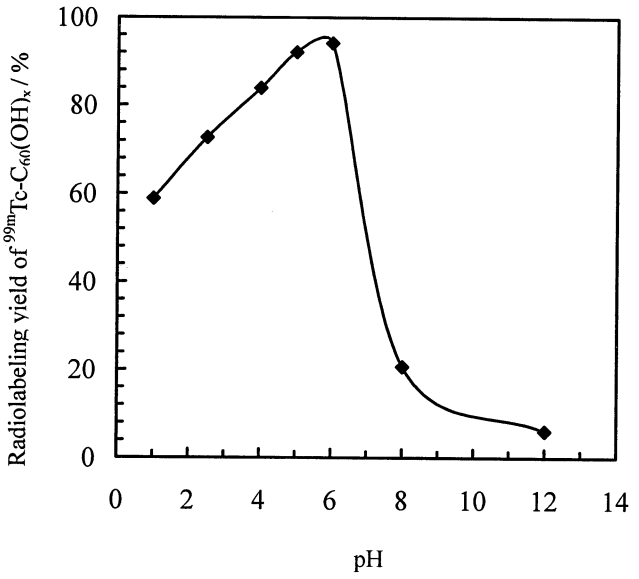

Fig. 1. Influence of pH on the yield of ^{99m}Tc-$C_{60}(OH)_x$ ($[SnCl_2 \bullet 2H_2O]$ = 50 μ g, $[C_{60}(OH)_x]$ = 5 mg/ml, [ascorbic acid] = 10 mg/ml).

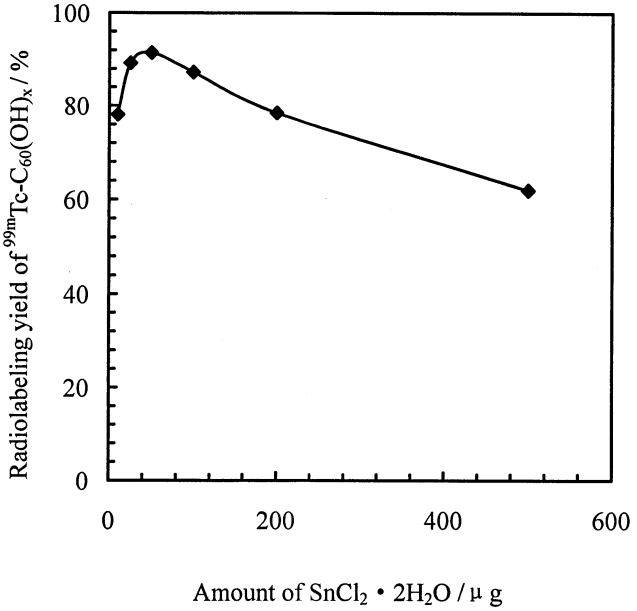

Fig. 2. Influence of amount of $SnCl_2 \bullet 2H_2O$ on the yield of ^{99m}Tc-$C_{60}(OH)_x$ (pH ~ 5, $[C_{60}(OH)_x]$ = 5 mg/ml, [ascorbic acid] = 10 mg/ml).

Table 1
Biodistribution of ^{99m}Tc-$C_{60}(OH)_x$ in mice (%ID/g, mean ± SD)

Tissue	T (h)				
	1	3	6	24	48
Blood	2.59 ± 0.59	1.22 ± 0.20	0.82 ± 0.20	0.47 ± 0.10	0.20 ± 0.03
Heart	1.18 ± 0.15	0.97 ± 0.22	0.82 ± 0.05	0.69 ± 0.20	0.69 ± 0.05
Lung	3.11 ± 0.70	1.84 ± 0.16	1.47 ± 0.17	1.67 ± 0.39	0.91 ± 0.37
Liver	9.92 ± 1.87	13.95 ± 1.23	13.02 ± 2.20	9.36 ± 2.28	6.12 ± 0.75
Spleen	6.44 ± 1.55	6.06 ± 1.28	6.17 ± 1.20	7.18 ± 1.70	5.53 ± 0.92
Kidney	7.62 ± 0.82	6.10 ± 1.35	5.72 ± 0.39	5.25 ± 0.81	2.96 ± 0.32
Muscle	0.71 ± 0.13	0.63 ± 0.07	0.56 ± 0.08	0.57 ± 0.10	0.58 ± 0.08
Bone	7.67 ± 1.48	7.31 ± 1.69	8.72 ± 1.27	8.35 ± 1.18	7.30 ± 1.06
Intestines	2.03 ± 0.36	2.04 ± 1.13	1.48 ± 0.53	2.08 ± 0.40	1.85 ± 0.70
Bone	0.12 ± 0.07	0.05 ± 0.07	0.04 ± 0.01	0.03 ± 0.01	0.07 ± 0.02

The optimized labeling conditions are summarized as follows: 0.2 ml 5 mg/ml $C_{60}(OH)_x$, 50 μg stannous chloride, 50 μl 10 mg/ml ascorbic acid aqueous solution, pH ~ 5. The reaction mixture was heated in a boiling water bath for 40 min. The radiolabeling yield of ^{99m}Tc-$C_{60}(OH)_x$ was more than 92%. By paper chromatography about 7% of radioactivity was assigned to $^{99m}TcO_4^-$ and $^{99m}TcO_2$. Since the radio labeling yield was significant high, and the amount of $C_{60}(OH)_x$ used in the experiment did not result in any observed biotoxicity, further separation was not required before the biological studies.

3.2. Biodistribution of ^{99m}Tc-$C_{60}(OH)_x$ in mice

The results of the distribution studies of ^{99m}Tc-$C_{60}(OH)_x$ in mice is given in Table 1. ^{99m}Tc-$C_{60}(OH)_x$ was delivered to all organs quickly and was present in measurable levels throughout the entire body except for those tissues with limited blood flow such as the brain and muscle. A significant percentage of total activity was retained throughout the 48 h of the study, particularly in the kidneys, bone, spleen, and liver. All tissues displayed a slow clearance over 48 h, except for bone which showed slightly increasing localization within 24 h. Relatively high radioacitivity in liver, kidney and intestines indicated that ^{99m}Tc-$C_{60}(OH)_x$ might excreted through urine and gut.

3.3. SPECT imaging of ^{99m}Tc-$C_{60}(OH)_x$ in New Zealand rabbits

^{99m}Tc-$C_{60}(OH)_x$ was present mainly in liver, kidney, bone especially in cortical bone, spine, and bone joints (Fig. 3). The radioactivity in heart reached background at 14 h, thus cortical bone, breastbone, spine were imaged clearly. The intestinal tract was not imaged throughout the test procedure, we could expect that most of ^{99m}Tc-$C_{60}(OH)_x$ was excreted through urine. The results of SPECT imaging supported the measurements obtained in the biodistribution performed in mice.

4. Discussion

Cagle et al have studied the biodistribution and metabolism of an endohedral $^{166}Ho_x@C_{82}(OH)_y$ metallofullerol using BALB/c mice and Fischer rats, and established that $^{166}Ho_x@C_{82}(OH)_y$ has a blood pool residence time of over an

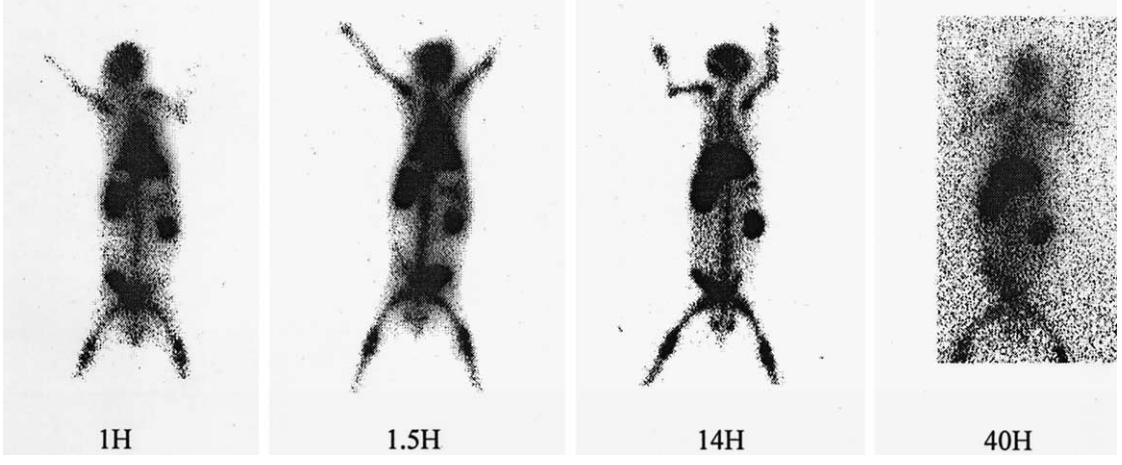

Fig. 3. SPECT imaging of ^{99m}Tc-$C_{60}(OH)_x(O)_y$ in New Zealand rabbits.

hour with nearly total clearance from blood shortly thereafter, localized high levels of radioactivity in liver and bone, with negligible accumulation in the brain [7–8]. These results are consistent with the date from the present work. The biologcal properties would not be expected to change by incorporation of a metal atom with C_{82} cage. Although the structure and component of $C_{82}(OH)_y$ have changed, the results of biodistribution of $^{166}Ho_x@C_{82}(OH)_y$ should accurately reflect the biological behavior of $C_{82}(OH)_y$. Since the biological behavior of ^{99m}Tc-$C_{60}(OH)_x$ was similar with that of $^{166}Ho_x@C_{82}(OH)_y$, we expect that $C_{60}(OH)_x$ labeled with ^{99m}Tc did not change its biological properties and that the biodistribution results for ^{99m}Tc-$C_{60}(OH)_x$ probably indicate the biological behavior of $C_{60}(OH)_x$.

One explanation for ^{99m}Tc-$C_{60}(OH)_x$ localized in the liver, spleen and bone is that small particles are recognized and trapped by reticuloendothelial cells, and thus retained in those organs. Empty fullerols and other polyhydroxylated compounds have also demonstrated a high affinity for cortical bone [7].

^{99m}Tc-$C_{60}(OH)_x$ is much more evenly distributed in body than a ^{14}C-labeled trimethylene methane derivative of C_{60}, which was retained mostly in the liver (90%), with little clearance after 48 h [3]. There are three major differences in the biological behaviors of these two molecules: Yamago et al confirmed that fullerene carboxylic acid derivative eventually retained in muscle and fur and was also able to penetrate the blood-brain barrier, but the present work indicated that $C_{60}(OH)_x$ distributed mainly in bone, liver and spleen, with little uptake in muscle and fur, and could not penetrate the blood-brain barrier. There are also difference in excretion since the fullerene carboxylic acid derivative was excreted through intestinal tract. The results of the present work showed that $C_{60}(OH)_x$ was excreted mainly through urine no matter the radioactivity test for rats or the imaging for rabbits. Finally, difference in retention was also observed since most of fullerene carboxylic acid derivative retained in the body after one week, and only 2.4% was excreted with the feces.

The possible reason responsible for the difference between two C_{60}-based derivatives is very complex. However, it is definitely true that fullerene derivatives with different water-solubilizing substituents have different biobehavior, and that there is the possibility that fullerene cages can be selectively tissue-targeted through choice of the water-solubilizing substituent, possible because of recent advances in fullerene synthetic chemistry.

5. Conclusion

Fullerol was labeled with ^{99m}Tc for the first time and the present findings suggest the feasibility of using ^{99m}Tc-labeled fullerene derivatives to monitor the fate of fullerene derivatives in animals. Unlike endohedral fullerol $^{166}Ho_x@C_{82}(OH)_y$, ^{99m}Tc-labeled derivatives can be conveniently prepared. The studies indicates that the clearance pathways, tissue retention, and tissue distribution for $C_{60}(OH)_x$ make it and other fullerol-like materials potential candidates as therapeutic agents for treating leukemia, bone cancer, or bone pain. Because of suitable chemical and biological properties of fullerene derivatives, it could increase the utility and decrease of toxicity of the drug by combining fullerene with therapeutic drugs and targeting-functional groups delivering the drugs to the desirable focus tissue directly.

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