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Synthesis of ^{99m}Tc-ciprofloxacin by different methods and its biodistribution

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Abstract

We describe here the synthesis of ^{99m}Tc-ciprofloxacin by four different methods and its biodistribution. All of the methods gave high radiochemical yields of $\geqslant 90\%$ and high stability of $\geqslant 90\%$ at 6 h after preparation. However HPLC analysis, bacterial binding assay, and in vivo distribution for the four ^{99m}Tc-ciprofloxacins showed different results. Among these methods, the use of formamidine sulfinic acid with microwave heating (Method A) was fast and easy, and gave more desirable biological properties than the other methods.

Keywords: Ciprofloxacin; ^{99m}Tc; Formamidine sulfinic acid; Microwave; Infection

1. Introduction

The preparation of ^{99m}Tc-labeled radiopharmaceuticals generally requires the initial reduction of ^{99m}Tc-pertechnetate, followed by reaction with added complexing agents. For this procedure, the reducing agent must be non-toxic and water-soluble and must suppress ^{99m}Tc-colloid formation (Baldas et al., 1982). Stannous(II) chloride is most widely used nowadays, while hydrazine and sodium borohydride have also been suggested together with an electrolyzed method. Fritzberg et al. proposed formamidine sulfinic acid (FSA) as an alternative reducing agent to stannous chloride (Fritzberg et al., 1977). FSA is less toxic than a metallic reducing agent such as stannous(II) chloride because it does not contain tin metal and which makes it possible to avoid problems associated with stannous salts and which sometimes lead to production of hydrolyzed and oxidized products (Ballinger et al., 1989; Jones et al., 1980). However, FSA is an organic compound, so it may compete with the added ligand during complex formation with reduced ^{99m}Tc. Another difference is that FSA may decompose during heating, and these decomposed compounds sometimes produce ^{99m}Tc-complex with reduced ^{99m}Tc. As a result, the ^{99m}Tc-labeled products obtained with FSA may have unexpected chemical structures with different biodistribution data than ^{99m}Tc-complexes made using stannous chloride (Fritzberg et al., 1977; Ballinger et al., 1989; Jones et al., 1980). Therefore, the selection of a reducing agent with a specific ligand for the formation of a specific radioisotope-labeled complex is an important consideration to achieve proper biodistribution.

^{99m}Tc-labeled ciprofloxacin, which has a 4-fluoroquinolone backbone, was developed as a biologically active radiopharmaceutical to diagnose infection, based on its broad spectrum of antibacterial activity toward not only Gram-positive but also Gram-negative bacteria (Goodman, 1991; Vinjamuri et al., 1996). The antibacterial action of ciprofloxacin is mediated via strong binding to and inhibition of bacterial DNA gynase. Recently, ^{99m}Tc-labeled ciprofloxacin has shown many advantages

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over ^{99m}Tc-HM-PAO WBC for diagnostic scans, in that it is more specific for bacterial infection, is easier to prepare, and gives better image quality (Vinjamuri et al., 1996; Britton et al., 1997; Hall et al., 1998). In previous papers, ^{99m}Tc-ciprofloxacin was prepared from ciprofloxacin with FSA as a reducing agent under water bath heating (Vinjamuri et al., 1996; Britton et al., 1997; Hall et al., 1998). However, this method gives a low radiochemical yield. As a result, additional purification and longer preparation time are needed.

To improve the properties of ^{99m}Tc-labeled ciprofloxacin as an agent for diagnosing infection, we prepared ^{99m}Tc-ciprofloxacin using four different methods. We used FSA or tin(II) chloride as a reducing agent and two different heating methods. We also evaluated the product of each synthetic method in terms of radiochemical properties, bacteria binding assay and in vivo biodistribution in animal studies.

2. Materials and methods

2.1. Synthesis of ^{99m}Tc-ciprofloxacin

In the synthesis of ^{99m}Tc-ciprofloxacin, the amount of ciprofloxacin (Il-Dong Pharmaceutical Company, Seoul, Korea) was fixed at 2 mg, as previously reported (Vinjamuri et al., 1996; Yu et al., 1994). To determine the optimal amount of reducing agent, 0.2–1.2 mg of FSA (Aldrich Chemical Co., WI, USA) or 10–500 μg of $SnCl_2 \cdot 2H_2O$ (Aldrich Chemical Co., WI, USA) was used. After addition of all reagents to the 10 ml vial with 370 MBq $^{99m}TcO_4^-$ in 1 ml saline, they were heated using a water bath for 10 min. After we determined the optimal amounts of reducing agent, ^{99m}Tc-ciprofloxacin was synthesized by the following four methods.

(A) *Microwave heating with FSA*: In this method, FSA was used as a reducing agent. A 10 ml serum glass vial (Wheaton Science Products, Millville, NJ, USA) containing 1 mg of FSA, 2 mg of ciprofloxacin and 1110 MBq $^{99m}TcO_4^-$ in 1 ml saline was sealed with an aluminum cap, and most of the air in it was withdrawn to prevent it from breaking (Hung, 1992). The vial was then placed in a plastic bottle with a screw cap, the solution was heated in a microwave oven (MM-M301; 600 W power out-put; LG electronics, Seoul, Korea) for 10–15 s. The vessel was cooled to room temperature and the reaction mixture was filtered through a sterile 0.22 μm filter.

(B) *Water bath or oil bath heating with FSA*: 2 mg of ciprofloxacin was mixed with 1 mg of FSA and 1110 MBq/ml of $^{99m}TcO_4^-$ solution in a serum glass vial (Wheaton Science Products, Millville, NJ, USA). The reaction mixture was kept in a 100°C water bath for 5–10 min. After cooling to room temperature, it was filtered through a sterile 0.22 μm filter.

For oil bath heating, we used silicon oil; the reaction vial was heated at 120°C for 10 min. Radiochemical yield and purity were checked by TLC and HPLC. For HPLC anaylsis, purification and biodistribution test, we used ^{99m}Tc-ciprofloxacin with water bath heating.

(C) *Microwave heating with $SnCl_2 \cdot 2H_2O$*: In this procedure, we used 100 μl (100 μg) of $SnCl_2 \cdot 2H_2O$ solution in 0.01 N HCl as a reducing agent. The method was otherwise the same as Method A. The reaction mixture was heated in a microwave for 15 s.

(D) *Room-temperature incubation with $SnCl_2 \cdot 2H_2O$*: 2 mg of ciprofloxacin was mixed with 100 μg of $SnCl_2 \cdot 2H_2O$ dissolved in 100 μl of 0.01 N HCl in a glass vial (Wheaton Science Products, Millville, NJ, USA). The reaction mixture was kept at room temperature for 10 min, and then collected in a sterile vial through a sterile 0.22 μm filter.

3. Quality control and stability

Radiochemical yield and stability were checked by ITLC-SG, Whatman paper 1 and reversed-phase silica gel TLC. The stability of each ^{99m}Tc-ciprofloxacin was checked for 6 h in 0.9% saline and 20% human plasma solution. The distribution of radioactivity on the TLC plates was measured using a gamma counter (Cobra II, Packard Instrument Co., Meriden, CT, USA).

4. HPLC analysis and purification

HPLC analysis was carried out using a reversed-phase method as follows. An HPLC system (ThermoQuest Corp., San Jose, CA, USA) equipped with UV (254 nm) and radioisotope detector (Bioscan, Inc., Washington, DC, USA) was used. Analysis was carried out using a Luna C_{18} reversed-phase column (4.6 × 150 mm, 5 μm) with a 10 mm guard column. The column was eluted at 1 ml/min with a linear gradient of 100% water to 100% acetonitrile for 30 min. Data were collected and analyzed using Chromquest software from ThermoQuest.

For animal experiments, we purified four ^{99m}Tc-ciprofloxacin by HPLC. Purification was carried out using an Alltech C_{18} column (10 × 250 mm, 10 μm) with 10 mm guard column and we used the same solvent and detector conditions, which had been used in the analysis procedure.

5. In vitro binding of ^{99m}Tc-ciprofloxacins to bacteria

Binding of ^{99m}Tc-ciprofloxacins to bacteria was assessed by the method described previously (Welling et al., 2000). Briefly, 0.1 ml of 0.1 M Na-PB containing 2.59 MBq of ^{99m}Tc-ciprofloxacin prepared by each of

the four different methods without any HPLC purification was transferred to a test tube. Next, 0.8 ml of 50% (v/v) of 0.01 M acetic acid in Na-PB containing approximately 1×10^8 viable *S. aureus* (ATCC 9213) were added. The mixture was incubated for 1 h at 4°C and then centrifuged for 5 min at 2000*g* at 4°C. The supernatant was removed and the bacterial pellet was gently resuspended in 1 ml of ice-cold Na-PB and re-centrifuged as above. The supernatant was removed, and the radioactivity in the bacterial pellet was determined by a gamma counter. The radioactivity related to bacteria was expressed in percent of the added ^{99m}Tc activity bound to viable bacteria in regard to total ^{99m}Tc.

6. Experimental thigh muscle infection and biodistribution

Male Sprague-Dawley rats weighing 120–150 g were used in all of the animal studies. A turbid suspension containing 2×10^8 organisms of *S. aureus* in 0.2 ml of Miller-Hilton broth was injected into the right thigh muscle of the rats. 24 h later, 0.1 ml of ^{99m}Tc-ciprofloxacin (37 MBq) was injected via the tail vein. Five rats were used for each of the four different preparations of ^{99m}Tc-cirofloxacin. 4 h after the injection of ^{99m}Tc-ciprofloxacin, the rats were sacrificed and biodistribution was determined. Samples of infected muscle, contralateral normal muscle, blood, liver, spleen, lung, kidney, stomach, intestine, brain, bone, and heart were weighed, and the radioactivity was measured using a gamma counter. The results were expressed as the percent uptake of injected dose per gram of tissue (%ID/g), the infected-to-normal muscle ratio and the infected muscle-to-blood ratio. All animal experiments were conducted following the principles of laboratory animal care (NIH publication no. 86–23, revised 1985). Our handling of animals was approved by the Unit of Animal Laboratory at Asan Medical Center.

To obtain in vivo distribution of purified ^{99m}Tc-ciproflxacin, we conducted another animal experiment. After HPLC purification of the four ^{99m}Tc-ciprofloxacin from Method A–D, we evaluated in vivo distribution of the four ^{99m}Tc-ciproflxacin with same methods.

7. Statistical analysis

Data are expressed as means ± SD. Statistical analysis was performed using the unpaired *t*-test or analysis of variance (ANOVA) with multiple comparison tests. A probability of less than 0.05 was considered to be significant.

8. Results

8.1. Synthesis of ^{99m}Tc-ciprofloxacin and stability

The radiochemical yield of ^{99m}Tc-ciprofloxacin was dependent on the amount of reducing agent, as shown in Figs. 1 and 2. With 1 mg of FSA, we obtained a radiochemical yield of 96.9 ± 0.78%. However, with 1.2 mg of FSA, the amount of radiochemical impurity on TLC increased leading to a lower radiochemical yield of 94.7 ± 0.90%. Similar results were seen with stannous chloride. With 100 μg of $SnCl_2 \cdot 2H_2O$, we obtained a radiochemical yield of 95.5 ± 0.60%. With more than 100 μg of reducing agent, the radiochemical yield was slightly increased, although this large amount sometimes produced a ^{99m}Tc-labeled colloid portion in the final product.

The labeling efficiency and stability of ^{99m}Tc-ciprofloxacins prepared by the four methods are shown in Fig. 3. All of the ^{99m}Tc-ciprofloxacins showed a radiochemical yield above 95% ($n = 10$), and a stability of above 90% up to 6 h after synthesis in the saline and 80% up to 6 h in the human plasma ($n = 3$).

In Method A, the radiochemical yield was highly dependent on the duration of microwave heating. With 10 and 13 s of heating, the radiochemical yields were

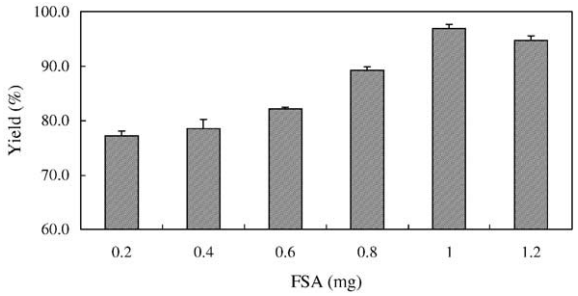

Fig. 1. Radiochemical yield by the amount of FSA in the synthesis of ^{99m}Tc-ciprofloxacin.

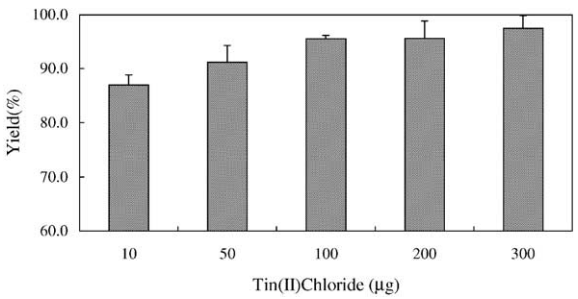

Fig. 2. Radiochemical yield by the amount of $SnCl_2 \cdot 2H_2O$ in the synthesis of ^{99m}Tc-ciprofloxacin.

80±2.5% and 87±0.5%, respectively. To increase the radiochemical yield, we tried to extend the duration of microwave heating to over 15 s. However, under these conditions the reaction vial bursts due to high internal pressure. In Method B, the radiochemical yield was again dependent on the duration of heating. With 5, 7 and 10 min of heating in a water bath, the radiochemical yields were 55.4±0.5, 79.5±1.2 and 95.6±1.5%, respectively. Based on these results, we applied heat for 15 s in Methods A and C, and for 10 min in Method B.

High-temperature heating with silicon oil bath in Method B achieved the same radiochemical yield of 96±2.4% as water bath heating. But this procedure suffered some disadvantage due to longer preparation time of oil bath heating.

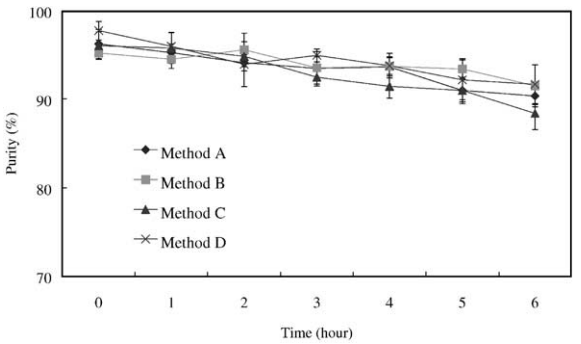

Fig. 3. Stability of ^{99m}Tc-ciprofloxacin prepared by the four different methods.

8.2. HPLC analysis

The retention time of ^{99m}Tc-ciprofloxacin was 2.63 min. The radiochemical purity based on HPLC analysis depended on the preparation method, as shown in Figs. 4 and 5. Method A gave a radiochemical purity of 99.3% and the least amount of organic impurities, while Method B from water bath and oil bath heating gave a similar radiochemical purity of only 80.5% and 82.6%, respectively. Method A shows only one radioactive peak, while Method B shows both radiochemical and organic impurities. While Methods C and D also gave only one radioactive peak and high radiochemical purities (99.2% and 99.5%, respectively), they also gave more organic impurities than Method A. HPLC purification of four ^{99m}Tc-ciprofloxacins also gave the same results.

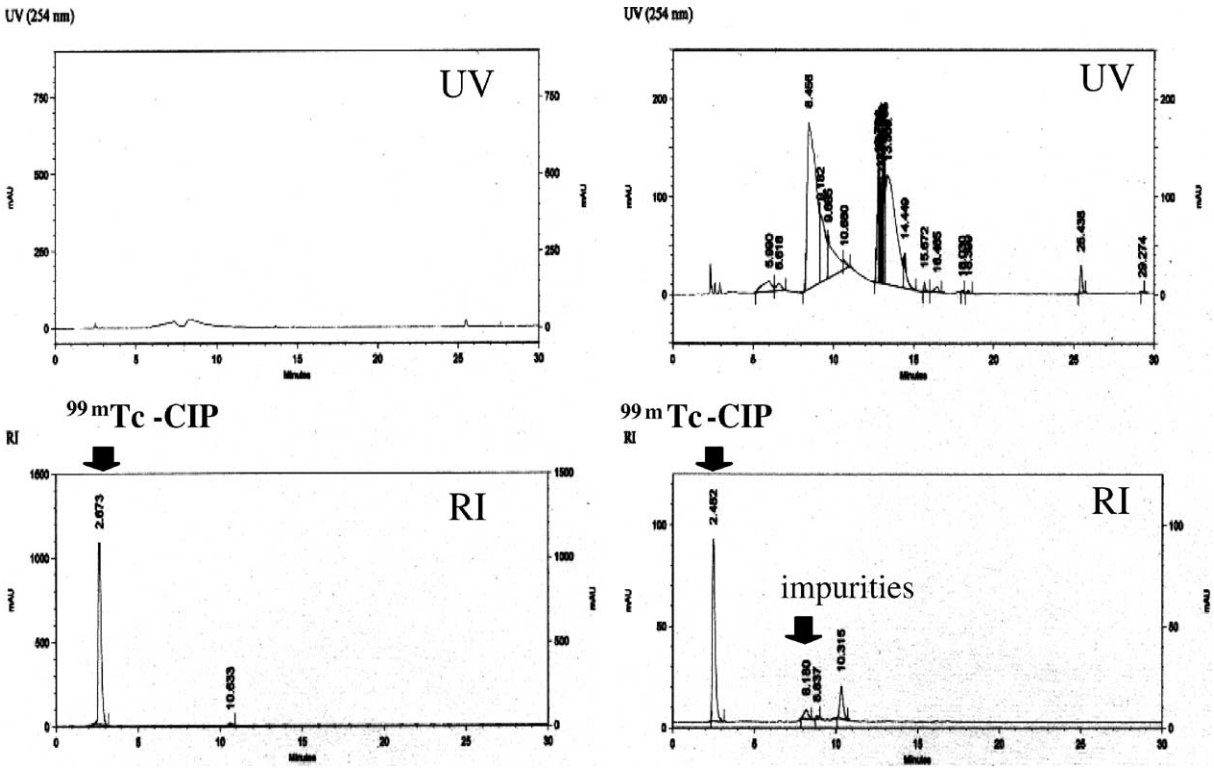

Fig. 4. HPLC chromatograms of the products of Methods A and B (^{99m}Tc-CIP: ^{99m}Tc-ciprofloxacin; UV: UV chromatogram at 254 nm; RI: Radioactive chromatogram).

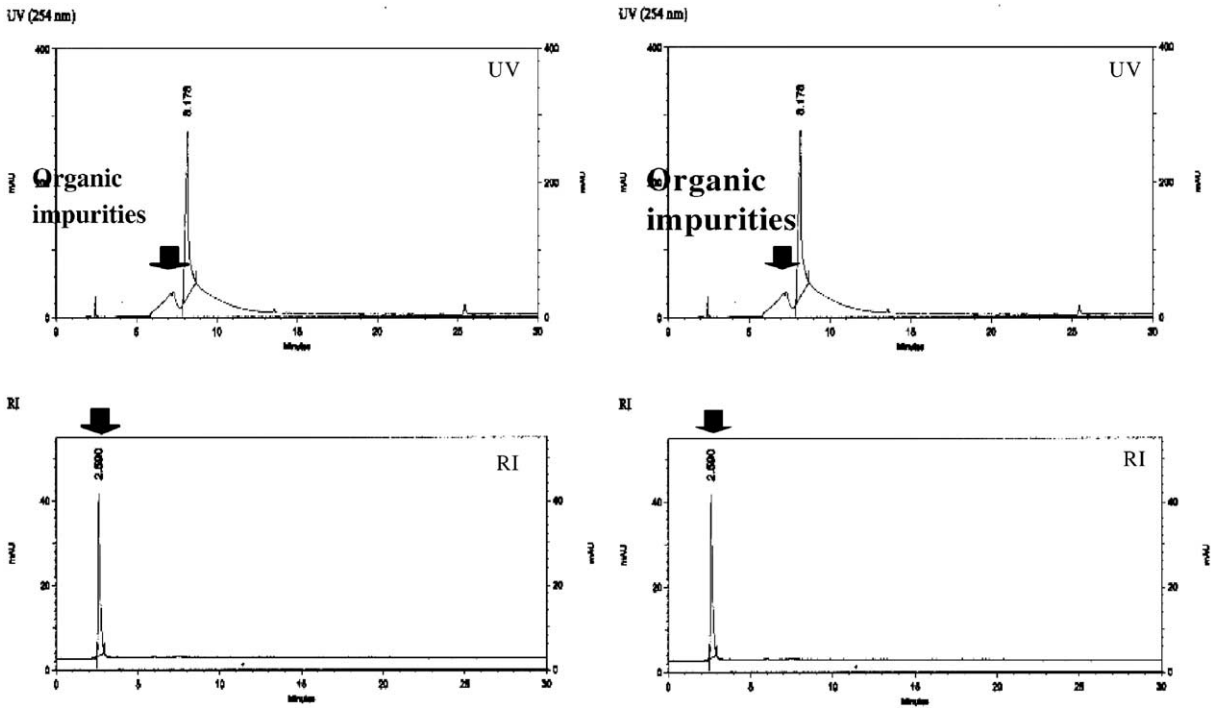

Fig. 5. HPLC chromatograms of the products of Methods C and D (^{99m}Tc-CIP: ^{99m}Tc-ciprofloxacin; UV: UV chromatogram at 254 nm; RI: Radioactive chromatogram).

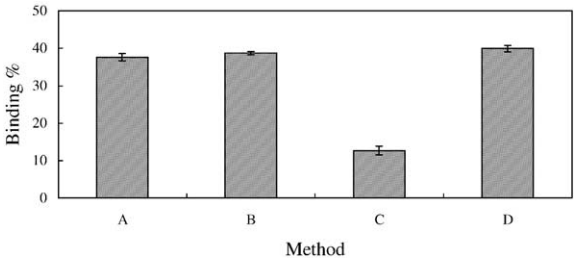

Fig. 6. In vitro binding of the ^{99m}Tc-labelled ciprofloxacins to 1×10^8 viable *S. aureus*. Mean percentage of ^{99m}Tc-ciprofloxacin bound with *S. aureus*. ($n = 10$ per experiment); A: microwave heating with FSA; B: water bath heating with FSA; C: microwave heating with $SnCl_2 \cdot 2H_2O$; D: room temperature incubation with $SnCl_2 \cdot 2H_2O$.

8.3. In vitro bacteria binding assay

Bacteria binding assay of four ^{99m}Tc-ciprofloxacin showed markedly different results depending on the labeling method. The ^{99m}Tc-ciprofloxacin produced by Methods A, B and D showed no significant differences in binding to bacteria (ANOVA, $P < 0.001$), whereas that produced by Method C showed significantly lower binding (Fig. 6).

8.4. Biodistribution study

Table 1 shows the organ distribution of ^{99m}Tc-ciprofloxacin at 4 h after injection. The ^{99m}Tc-ciprofloxacin prepared using Method A showed the highest accumulation in the infected region. The product of Method B showed the highest liver uptake and the lowest background muscle uptake. In general, ^{99m}Tc-ciprofloxacin synthesized with heating, as in Methods A, B and C, showed high liver and spleen uptake, while that prepared without heating (Method D) had the highest kidney uptake. The target-to-muscle (T/M) and target-to-blood (T/B) ratios of the products of the four different methods are shown in Table 2. Among the four methods, Method A gave ^{99m}Tc-ciprofloxacin with the highest target-to-blood and -muscle ratios.

In comparison with biodistribution results of ^{99m}Tc-ciprofloxacin which was not purified, purified ^{99m}Tc-ciprofloxacins produced very similar biodistribution results in all of the four ^{99m}Tc-ciprofloxacins. The results are shown in Table 3. ^{99m}Tc-ciprofloxacins without any purification exhibited high liver, spleen and kidney uptake and different biodistribution result according to preparation methods, but purified ^{99m}Tc-ciprofloxacins exhibited very similar biodistribution in all of the preparation methods and gave similar low liver and spleen uptake values ($P > 0.05$). In infection muscle

Table 1
Biodistribution of ^{99m}Tc-ciprofloxacins prepared by the four different methods

	%ID/g ($n = 4$)			
	Method A	Method B	Method C	Method D
Infected muscle	0.466±0.075	0.149±0.038	0.290±0.042	0.409±0.112
Normal muscle	0.124±0.002	0.052±0.006	0.125±0.018	0.170±0.079
Liver	5.314±0.396	9.213±0.432	4.595±1.775	2.677±0.823
Spleen	3.073±1.240	5.860±0.675	4.097±1.183	0.837±0.265
Lung	0.806±0.022	1.482±0.436	1.414±1.207	0.625±0.176
Kidney	4.250±1.505	0.832±0.125	3.151±0.326	5.108±0.952
Stomach	0.327±0.028	0.248±0.130	0.609±0.037	0.346±0.084
Intestine	0.286±0.028	0.130±0.011	0.339±0.013	0.407±0.065
Brain	0.012±0.004	0.009±0.004	0.014±0.003	0.018±0.004
Bone	0.270±0.075	0.107±0.076	0.249±0.036	0.116±0.017
Heart	0.182±0.020	0.137±0.038	0.203±0.111	0.230±0.049
Blood	0.227±0.072	0.295±0.012	0.704±0.109	0.415±0.100

Table 2
T/N and T/B ratios of ^{99m}Tc-ciprofloxacins prepared by the four different methods ($n = 4$)

	Method A	Method B	Method C	Method D
T/N ratio	3.759±0.613	2.877±0.822	2.456±0.490	1.020±0.067
T/B ratio	2.236±0.880	0.507±0.149	0.232±0.150	0.983±0.063

T/N = target (infected site)-to-non-target (muscle).
T/B = target-to-blood.

Table 3
Biodistribution of purified ^{99m}Tc-ciprofloxacins prepared by the four different methods

	%ID/g ($n = 4$)			
	Method A	Method B	Method C	Method D
Infected muscle	0.518±0.090	0.459±0.046	0.524±0.064	0.544±0.040
Normal muscle	0.055±0.018	0.050±0.010	0.045±0.006	0.055±0.014
Liver	0.426±0.089	0.586±0.070	0.590±0.125	0.575±0.124
Spleen	0.177±0.037	0.460±0.110	0.456±0.042	0.359±0.051
Lung	0.281±0.052	0.371±0.036	0.314±0.046	0.311±0.037
Kidney	2.029±0.322	1.355±0.193	2.606±0.098	2.304±0.176
Stomach	0.132±0.034	0.103±0.015	0.158±0.011	0.149±0.025
Intestine	0.218±0.030	0.113±0.019	0.211±0.039	0.211±0.044
Brain	0.018±0.005	0.012±0.005	0.023±0.003	0.010±0.001
Bone	0.149±0.023	0.107±0.012	0.169±0.042	0.200±0.034
Heart	0.119±0.016	0.090±0.011	0.220±0.016	0.145±0.006
Blood	0.250±0.032	0.251±0.014	0.184±0.085	0.198±0.021

uptake, purified ^{99m}Tc-ciprofloxacin was also shown to achieve three times higher uptake compared to ^{99m}Tc-ciprofloxacin which was not purified in Method B.

9. Discussion

In this study, ^{99m}Tc-ciprofloxacins were prepared by four different methods and evaluated with regard to their in vitro and in vivo characteristics. None of these preparation methods required any further purification steps to give high radiochemical purity products that retained more than 90% of their initial activity 6 h after synthesis. Among the four different methods, Method A, with FSA as a reducing agent and microwave heating, was preferable since it is fast and easy, and gives ^{99m}Tc-ciprofloxacin with favorable biological characteristics.

With FSA as a reducing agent (Methods A and B), heating turned the ^{99m}Tc-ciprofloxacin solutions yellow, which may have been due to the decomposition of FSA. FSA is an organic compound and may decompose upon heating, unlike a metal reducing agent such as tin(II) chloride (Fritzberg et al., 1977; Ballinger et al., 1989; Jones et al., 1980). This was observed in the HPLC chromatograms of the products of Methods A and B. Method B, which had a longer duration of water heating, gave more organic impurities than Method A. We applied oil bath heating for Method B to reduce ^{99m}Tc-labeled impurities but we obtained the same results as with the water bath heating. This ^{99m}Tc-labeled impurities difference may be due to the difference in the heating methods. As a result, a very short duration of heating with microwave may lead to less decomposition of organic compounds. In contrast, when a solution containing organic compounds is heated in a water bath or oil bath, the organic materials are also heated and it is more likely that the organic materials will decompose due to heating. To avoid this decomposition, we tried to synthesize ^{99m}Tc-ciprofloxacin with FSA at room temperature, but were not successful. Although FSA, unlike stannous chloride, is inert toward oxygen and is highly soluble in neutral conditions, the longer duration of heating increased the amount of ^{99m}Tc-labeled impurities. To find the chemical structure of ^{99m}Tc-labeled ciprofloxacin, we tried to synthesize Re-labeled ciprofloxacin, but this was not successful. Although Cu-labeled ciprofloxacin was assigned the chemical formula of $[Cu(ciporfloxacin)_2]Cl_2 \cdot 6H_2O$ in a previous report (Iztok et al., 1994), ^{99m}Tc-labeled ciprofloxacin may have a different chemical structure due to different oxidation state of metal and different coordinated atom.

In the bacterial binding assay, ^{99m}Tc-ciprofloxacins prepared by Methods A, B and D gave similar results, while that prepared by Method C had weaker binding properties. Methods C and D differed in that incubation was performed with heating or at room temperature, respectively. Furthermore, Methods A and B were conducted under neutral conditions, while Methods C and D involved acidic conditions. If we consider these reaction conditions, the assay results suggest that heating in acidic conditions might have changed the properties of ciprofloxacin through the formation of polar salts, which resulted in relatively poor bacteria cell binding. This was not observed in neutral media with heating.

In the biodistribution study, the four different preparation methods without any purification gave different results. But purified ^{99m}Tc-ciporlfoxacins produced very similar biodistribution results. The product of Method B without purification showed the highest liver and lowest kidney uptake, which is inconsistent with a previous report that it was excreted mainly by the kidney (Goodman, 1991). Method B involves heating for 10 min, which could lead to the decomposition of ciprofloxacin (Yu et al., 1994). But purified ^{99m}Tc-ciprofloxacin from Method B gave low liver, spleen and high kidney uptake values and these data were very similar to results for purified ^{99m}Tc-ciporlfoxacins from the other three preparation methods. As a result, ^{99m}Tc-labeled-ciprofloxacin decomposition products which were produced during preparation may cause increased liver and spleen uptake and decreased kidney uptake. A short duration of heating or room-temperature standing may produce a small quantity of ^{99m}Tc-labeled-ciprofloxacin decomposition products and they gave products that showed high uptake at the sites of infection. However, ^{99m}Tc-ciprofloxacin prepared with a short duration of heating with $SnCl_2 \cdot 2H_2O$ as a reducing agent (Method C) showed lower uptake at the site of infection than those prepared by Methods A and D, and also gave poor results in the bacteria cell binding assay. Although the four purified ^{99m}Tc-ciprofloxacins have some advantages such as high infected muscle uptake and low liver uptake compared to ^{99m}Tc-ciprofloxacin without purification, this purification procedure was very cumbersome in routine clinical procedures and might lead to low radiochemical yield.

10. Conclusion

^{99m}Tc-ciprofloxacins were prepared by various methods and showed different chemical and biological properties. ^{99m}Tc-ciprofloxacin prepared with FSA and microwave heating showed the most desirable characteristics.

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