99mTc-ciprofloxacin for diagnosis of bacterial infection

A Aunguraratt1, T Ngamprayad, M Dangprasert, S Phumkem and B Jowanaridhi
Radioisotope Center, Thailand Institute of Nuclear Technology, Bangkok, Thailand
E-mail: jangkanan@yahoo.com

Abstract. Preparation of 99mTc-ciprofloxacin for diagnosis of bacterial infection was investigated by varying factors which affected this compound. The optimum conditions for preparation of 99mTc-ciprofloxacin and a lyophilized kit for Tc-99m labelling were studied. The results from biodistribution study showed that the percentages of the injected dose per gram tissues of infected area at 1 and 3 hours after injection were around 0.25–0.56. 99mTc-ciprofloxacin was found sterile, pyrogen-free and non-toxic. Radiochemical purity was greater than 90% with greater than 6 hours of stability.

1. Introduction
At present, infectious diseases remain a major health problem and cause of death worldwide, particularly in developing countries. Diagnosis, therapy and control of the infection are challenges faced by medical personnel. Nuclear medicine techniques, because of their sensitivity, are often used in the diagnosis of fever of unknown origins or suspected bacterial infection especially deep-seated infection such as intra-abdominal abscesses, endocarditis and osteomyelitis.

The radiopharmaceuticals routinely used for infection imaging include 67Ga-citrate, 99mTc- or 111In-labelled leukocytes and 99mTc- or 111In-labelled human immunoglobulin (HIG). However, each of them has limitations such as a low blood clearance, the time-consuming procedure, difficulties in blood handling or a viral infection risk [1-2]. Therefore, a novel approach using 99mTc-labelled antibiotics has gained much attention owing to its antibacterial activity.

The first antibiotic that was commercialized for infection imaging was ciprofloxacin [3-4]. Ciprofloxacin, a fluoroquinolone broad spectrum antibiotic, binds to and inhibits DNA gyrase of bacteria. It is metabolized in the liver and excreted via the kidneys. The molecular structure of ciprofloxacin is shown in figure 1. The preparation of 99mTc-ciprofloxacin is much easier than that of 99mTc-labelled leukocytes. These features make 99mTc-ciprofloxacin potentially useful for infection imaging. As previously reported, 99mTc-ciprofloxacin has been extensively evaluated with good sensitivity for diagnosis and localization to a wide varieties of bacterial infection lesions [5-11]. Moreover, 99mTc-ciprofloxacin showed high sensitivity that could aid the physicians in detecting the lesions in their early stages and would be useful for monitoring clinical response.

1 To whom any correspondence should be addressed.
The objectives of this study were to optimize the condition for radiolabeling of $^{99m}$Tc-ciprofloxacin and to develop a lyophilized kit for $^{99m}$Tc labelling. On the other hand, the capability of $^{99m}$Tc-ciprofloxacin to distinguish between bacterial infection and sterile inflammation is still controversial [12-15]. Therefore, the biodistribution of $^{99m}$Tc-ciprofloxacin in septic and sterile inflammation-induced mice was also evaluated.

2. Materials and methods

2.1 Materials

The chemicals used in this study were obtained from commercial sources (Sigma, Aldrich, Fluka and Merck). The $^{99}$Mo/$^{99m}$Tc generator used in this study was the product of Sam Young Unitech Co., Ltd., Korea.

2.2 Optimization of the labelling condition

SnCl$_2$·2H$_2$O was added into ciprofloxacin solution. Then, sodium pertechnetate was added into the solution after the pH was adjusted. After incubation, the mixture was filtered through 0.22 µm cellulose acetate membrane filter and then the quality control was performed.

Various labelling conditions were studied including the amount of both SnCl$_2$·2H$_2$O (10 – 400 µg) and ciprofloxacin (1 – 10 mg), pH 2 – 10, activity of Na$^{99m}$TcO$_4$ (10 – 50 mCi), the incubation temperature (25, 60 and 100°C) and the total volume of the reaction mixture (1 – 5 mL) [5,8].

The radiochemical purity (RCP) was analysed by ITLC-SG or Whatman#31ET in acetone ($R_f$ of free TcO$_4^-$ = 0.9 – 1.0) and ITLC-SG in a mixture of ethanol : H$_2$O : NH$_4$OH (2:5:1) ($R_f$ of H.R. and tin colloid = 0 – 0.1). The radiochemical purity of $^{99m}$Tc-ciprofloxacin should be not less than 90.0%. Whereas labelling efficiency was obtained by the same procedure using the pre-filtered solution.

2.3 Stannous kit formulation

A mixture of SnCl$_2$·2H$_2$O and sodium tartrate was formulated as a lyophilized kit. The mole ratio of SnCl$_2$·2H$_2$O and sodium tartrate at 1:1, 1:2, 1:3, 1:4 and 1:5 when SnCl$_2$·2H$_2$O were 400 and 500 µg were studied.

2.4 Stability test

The stability of $^{99m}$Tc-ciprofloxacin was analyzed by ITLC at 0.5, 2, 4 and 6 h after labelling.

2.5 Biodistribution in mice

$^{99m}$Tc-ciprofloxacin biodistribution in male ICR mice, weighted 25 – 30 g, was performed in 5 groups (6 mice per group). Each group was pre-treated at the right thigh muscle 1 day prior to the test with Staphylococcus aureus (S. aureus), Pseudomonas aerugenosa (P. aerugenosa), heat-killed S. aureus, heat-killed P. aerugenosa and turpentine.

When the inflammation of the inoculated muscle was apparent, the mice were injected intravenously with 20 – 25 µCi / 0.1 mL $^{99m}$Tc-ciprofloxacin. The urine of each mouse was collected and counted in the gamma counter. Three mice of each group were killed at 1 and 3 h after injection. Tissue samples were dissected and measured in the gamma counter. The results were expressed as percentages of the injected dose per gram (%ID/g).
2.6 Sterility, pyrogen and toxicity test
The sterility test was performed according to the compendial method stated in the United State Pharmacopeia (USP). Briefly, the test samples were incubated with both soybean casein digest medium (SCDM) and fluid thioglycollate medium (FTM) for 14 days at 22.5±2.5°C and 32.5±2.5°C respectively.

The pyrogen test is a test for bacterial endotoxin using the gel-clot technique. The LAL test kits from Lonza USA were used in this study.

The toxicity was tested in 5 healthy male ICR mice, weighing 25 - 30 g. One mg of ciprofloxacin solution was intravenously injected into each mouse. The mice were observed over 24 hours following injection. All mice must survive and show no symptom of abnormal reaction.

2.7 High performance liquid chromatography (HPLC) analysis
The compounds were analysed by HPLC using 4 x 250 µ-Bondapack connected to an ultraviolet-visible (UV-VIS) spectrometer PDA at 254 nm and a flow cell YtSi detector for gamma energy less than 70 keV, eluted with 1.5 mL/min of a mixture of acetonitrile (eluent A) and 0.025 M H₃PO₄ pH 3 (eluent B). The gradient was as follows: 0 – 3 min of 100% eluent A, 4 – 5 min of 13% eluent A and 6 – 20 min of 100% eluent A [16].

3. Experimental results

3.1 Results
The optimum condition for wet labelling was 2 mg ciprofloxacin, 40 µg SnCl₂·2H₂O, pH 4 – 5 labelled with 10 – 30 mCi Na⁹⁹ᵐTcO₄⁻ in a total volume of 1 – 4 mL. The solution was incubated in boiling water for 10 min and let stand at room temperature for 20 min. Finally, the reaction mixture was filtered through 0.22 µm cellulose acetate membrane filter. The RCP of ⁹⁹ᵐTc-ciprofloxacin was not less than 90% up to 6 h at room temperature.

The effect of SnCl₂·2H₂O quantity was shown in figure 2. The labelling efficiency was greater than 90% when the quantity of SnCl₂·2H₂O was 30 - 60 µg.

The effect of ciprofloxacin quantity was shown in figure 3. The labelling efficiency was greater than 90% when the quantity of ciprofloxacin was 1 - 6 mg.

The effect of pH was shown in figure 4. The labelling efficiency was greater than 90% when pH of ⁹⁹ᵐTc-ciprofloxacin was 4 – 5.

The effect of total volume of ⁹⁹ᵐTc-ciprofloxacin was shown in figure 5. The RCP was greater than 90% when total volume of ⁹⁹ᵐTc-ciprofloxacin was 1 – 4 mL.
The effect of activity of Na$^{99m}$TcO$_4$ was shown in figure 6. The RCP was greater than 90% when the radioactivity of $^{99m}$Tc-ciprofloxacin was 10 – 30 mCi.

The effect of incubation temperature was shown in figure 7 - 8. The RCP was greater than 90% when incubation was performed at 60°C and 100°C for 10 min.

For lyophilized kit, the mixtures of 1:1, 1:2, 1:3, 1:4 and 1:5 mole ratio of 400 µg and 500 µg SnCl$_2$·2H$_2$O : sodium tartrate were lyophilized and labelled with NaTcO$_4$ by the same condition as wet labelling. $^{99m}$Tc-ciprofloxacin from lyophilized kit was sterile, pyrogen-free, non-toxic and having not less than 90% RCP when left at room temperature for 6 h (figure 9).

From HPLC analysis, the UV chromatogram of ciprofloxacin showed a peak with the retention time of 8.8 min (figure 10a) and the same peak position was found on $^{99m}$Tc-ciprofloxacin radiochromatogram (Figure 10b). The peak at 1.3 – 1.6 min on radiochromatogram represented free TcO$_4^-$ (figure 10c).

The biodistribution of $^{99m}$Tc-ciprofloxacin in mice was shown in figures 11 and 12. The infected:uninfected muscle ratio showed no differences between septic and turpentine-induced inflammation.
Figure 10. HPLC chromatogram (a) ciprofloxacin (b) $^{99m}$Tc-ciprofloxacin (c) Na$^{99m}$TcO$_4$. 
Figure 11. Biodistribution of $^{99m}$Tc-ciprofloxacin in mice 1 hr after injection.

Figure 12. Biodistribution of $^{99m}$Tc-ciprofloxacin in mice 3 hr after injection.

4. Discussion and conclusion

Chemical and biological quality control results of $^{99m}$Tc-ciprofloxacin preparation in this study were satisfactory. The optimization of wet labelling condition was preliminary to the preparation of lyophilized kit. The SnCl$_2$·2H$_2$O : sodium tartrate lyophilized kit was developed to ease the labelling procedure and reduce radiation exposure to workers.

In this study, $^{99m}$Tc-ciprofloxacin was a clear colorless solution with pH 4–5, having not less than 90% RCP up to 6 h at room temperature, sterile, pyrogen-free and non-toxic. It is promising for detection of septic and aseptic inflammation.

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