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Preparation of ^{99m}Tc-cefoperazone complex, a novel agent for detecting sites of infection

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Prompt localization of infection sites is essential for initiating appropriate therapeutic measures. There have been major advances in the management of patients suffering from infective and/or inflammatory disorders as a result of introduction of newer drugs with high sensitivity and specificity. Since the last decade, ^{99m}Tc-ciprofloxacin was used as a biologically active radiopharmaceutical to diagnose inflammation but it has some problems related to radiochemical purity and stability. The aim of this study is to develop simple and easy formulation of cefoperazone (other broad spectrum antimicrobial agent) with ^{99m}Tc a ready to use labeling kit for infection imaging. The optimum condition that gives high labeling yield of ^{99m}Tc-cefoperazone complex, 97.9%, was achieved by using 3 mg cefoperazone, 100 μg Sn(II), at pH 8 and 10-minute reaction time. For in vivo binding of ^{99m}Tc-cefoperazone pharmacokinetic studies were carried in experimentally induced infection, in the left thigh, using *Staphylococcus aureus* in rats. Both thighs of the rats were dissected and counted and the ratio of bacterial infected thigh/contralateral thigh was then evaluated. The time for maximum accumulation of ^{99m}Tc-cefoperazone at the site of infection ($T/NT = 4.5$) was 45-minute post intravenous injection, followed by gradual decline. So, ^{99m}Tc-cefoperazone complex is simple and stable preparation for infection imaging after 45-minute post injection.

Introduction

Bacterial infection is a major problem worldwide, and especially in the developing countries. Many a time these processes result in significant patient morbidity, permanent disability or even death.[1] One of the greatest challenges in dealing with this problem is exact localization of the infective focus. Several imaging methods like ultrasonography (US), computer tomography (CT) and magnetic resonance imaging (MRI) are available and have been used for the past several decades for the localization of infection. But it is well known that these are not the best of methods for the localization of infection at early stages. These procedures detect the morphologic alterations of the tissues after significant process has taken place in the infective process leading to abscess formation.[2] The radiopharmaceuticals routinely used for scintigraphic detection include ^{67}Ga-citrate,[3,4] ^{99m}Tc or ^{111}In-labeled leukocytes,[5] ^{99m}Tc-nano-colloid,[6] ^{99m}Tc or ^{111}In labeled HIG (human polyclonal immunoglobulin)[7,8] and ^{99m}Tc-ubiquicidin 29-41.[9–11] Other preparations, mainly small receptor-specific proteins and peptides, are currently tested. However, none of the preparations is capable of distinguishing in a clinically useful manner between infections and inflammatory lesions.[12] One of the most important radiopharmaceuticals which are now available currently for imaging infection, the antimicrobial agent ciprofloxacin labeled with ^{99m}Tc has probably shown the best results. But the fundamental problems of ^{99m}Tc-ciprofloxacin preparation discussed in the literature[13–17] are related to radiochemical purity as well as to the stability of the labeled complex. ^{99m}Tc-ciprofloxacin kit was formed by using formamidine sulfinic acid (FSA) or redox polymer as a reducing agent where the reaction kinetics and labeling efficiency depend primarily on the degree of dextran matrix cross linking and on the qualitative nature of the redox polymer end groups. Also redox polymer is suspended with the ciprofloxacin solution and is finally most removed by ultra filtration after incubation. FSA is an organic compound, so it may compete with the added ligand during complex formation with reduced ^{99m}Tc. FSA may decompose during heating, and these decomposed compounds sometimes produce ^{99m}Tc-complex with reduced ^{99m}Tc. Some trails to label ciprofloxacin using Sn(II) as a reducing agent but the obtained ^{99m}Tc-ciprofloxacin preparation must be purified to increase its labeling yield (81% before purification).[16] Cefoperazone is a third generation semi-synthetic cephalosporin with a broad spectrum of activity against the majority of aerobic and anaerobic gram positive and gram negative pathogenic bacteria. In this work, cefoperazone was labeled with ^{99m}Tc to be used as infection imaging agent.

Experimental

Cefoperazone was purchased from Pfizer Company, USA, and all other chemicals were purchased from Merck and they were of reactive grade.

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Kit preparation and labeling

The required amount of cefoperazone was transferred to an evacuated penicillin vial. The required amount of tin(II) solution were added and the pH of the mixture was adjusted to the proper value, then the volume of the mixture was adjusted to 1 ml by N_2-purged distilled water. One ml of freshly eluated $^{99m}TcO_4^-$ (400 MBq) was added to the above mixture. The reaction mixture was vigorously shaken and allowed to react at room temperature for sufficient time required to complete the reaction.

Analysis

Ascending chromatography was carried out using ITLC-SG paper strips of size 1.2×10 cm^2. Pencil lines were drawn on the paper at distance of 2 cm from the bottom, at which the labeled cefoperazone was applied and this point was called origin and a second line after an interval of 4 cm. About 10 µl of ^{99m}Tc-cefoperazone was applied at the origin, letting it migrate by capillary action with solvents acetone and saline. After the solvent migrate to the top of the strip, it was allowed to dry with a hair dryer. The strips were cut in the middle, the bottom part representing the origin and the upper part representing the front of the solvent. Each parts were count in a well-type γ-scintillation counter. Pertechnetate moved with the solvent front and hydrolyzed-reduced technetium remained at the point of application in both solvents. ^{99m}Tc-cefoperazone remained at the origin in acetone, but moved with the solvent front in saline. The labeling efficiency was determined by subtracting the sum of the amounts that migrated in acetone and that remained at the origin in saline from 100%. The radiochemical yield is the mean value of three experiments.

Bio-distribution studies

The biodistribution of ^{99m}Tc-cefoperazone was evaluated in male Sprague-Dawley rats (body mass 130–160 g). To induce the inflammation, approximately 10^5–10^6 colony forming units of *Staphylococcus aureus* suspended in 0.2 ml of saline was administered into the left thigh (consume about 24 hours to completely induce the inflammation). For quantitative determination of organ distribution, five rats were used for each experiment and 0.1 ml of about 18 MBq of ^{99m}Tc-cefoperazone solution was injected into the tail vein of rats then the rats were killed and blood was obtained by cardiac puncture. Samples of infected muscle, contralateral normal muscle, blood, liver, spleen, lung, kidney, stomach, intestine, bone and heart were dissected and weighed, and their activity was measured in a shielded well-type γ-scintillation counter. To correct for physical decay and to calculate the uptake of the radiolabel in each tissue sample as a fraction of the injected dose, aliquots of the injected dose were counted simultaneously. The results were expressed as percentage injected dose per gram of organ or tissue (%ID/g).[18]

Results and discussion

Factors affecting on the labeling yield

Effect of reaction time: Figure 1 describes the effect of time after labeling on the labeling yield of ^{99m}Tc-cefoperazone complex. At 1-minute post labeling, the yield was small and equal to 82.5% which increased with time till reaching its maximum value of 97.9% at 10 minutes. The yield remains stable at 97.9% for time up to 6 hours after that the yield decreased again.

Effect of cefoperazone amount: The labeling yield of ^{99m}Tc-cefoperazone complex was increased with increasing the amount of cefoperazone which increased from 95.3% at 1 mg cefoperazone till reaching the maximum value of 97.9% at 3 mg; after that, the formed complex remained stable with increasing the amount of cefoperazone up to 7 mg. So the optimum amount of cefoperazone was 3 mg.

Effect of $SnCl_2$ concentration: As shown in Fig. 2, Sn(II) content is the most important factor affecting the labeling yield of ^{99m}Tc-cefoperazone complex. At low Sn(II) content, 10 µg, the labeling yield was small and equal to 12.6% due to the amount of Sn(II) which was insufficient to reduce all $^{99m}TcO_4^-$, so the amount of $^{99m}TcO_4^-$ was 84.6%. By increasing the amount of Sn(II), the labeling yield increased till it attained the maximum value of 97.9% at 100 µg Sn(II). When Sn(II) increased above 100 µg, the yield decreased again till it became 55.8% at 150 µg Sn(II) due to colloid formation (41.5%).

Effect of pH of the reaction mixture: As shown in Fig. 3, at pH 6 the labeling yield of ^{99m}Tc-cefoperazone complex was small and equal to 68.3% and this yield was increased with increasing the pH of the reaction mixture where pH 8 gave the maximum labeling yield of 97.9%. By increasing the pH greater than 8, the labeling yield decreased again to 87.4% at pH 9.

Biodistribution

Table 1 shows the biodistribution of ^{99m}Tc-cefoperazone in important body organs and fluids. The amount of accumulated activity in left thigh inflamed tissue was nearly four and half fold higher than that in the right thigh control tissue.

The accumulation of ^{99m}Tc-cefoperazone complex at site of infection was expressed as a target-to-nontarget (T/NT) ratio. As shown in Fig. 4, the accumulation of

^{99m}Tc-cefoperazone complex at the site of infection was maximized at 45 minutes after intravenous injection where *T*/*NT* ratio was equal to 4.66±0.53 which was nearly similar to that of ^{99m}Tc-ciprofloxacin. The accumulation of activity at the site of infection was slightly decreased with time until *T*/*NT* equal to 2.9±0.75 at 5-hour post injection. The reason for this early maximum tracer accumulation followed by gradual decline may be subsequent bacterial killing by the antimicrobial activity of cefoperazone, followed by clearance from circulation.

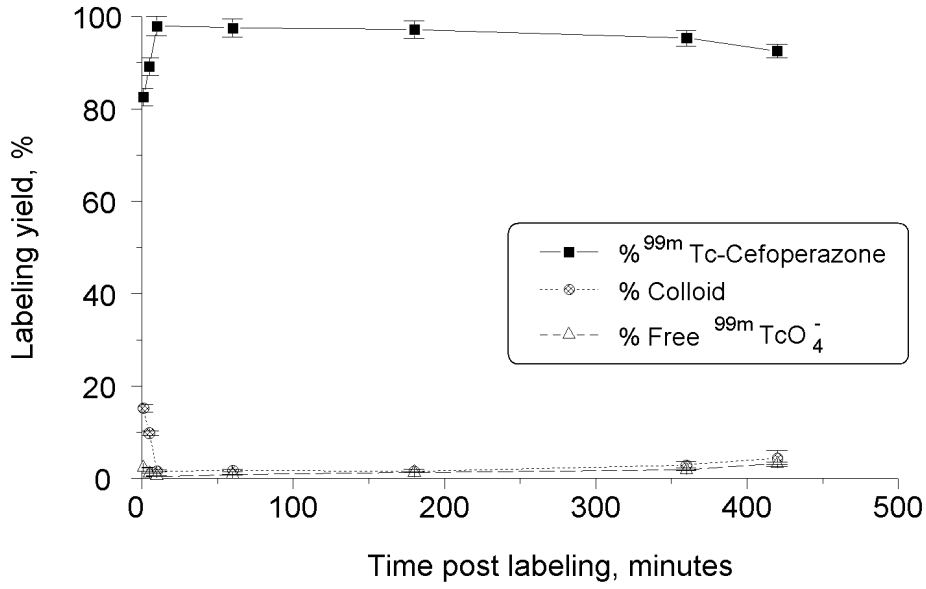

Fig. 1. Effect of reaction time on the labeling yield of ^{99m}Tc-cefoperazone complex. Conditions: 3 mg cefoperazone, 100 µg $SnCl_2$, pH 8 and 0–8 hours reaction time, $n = 3$

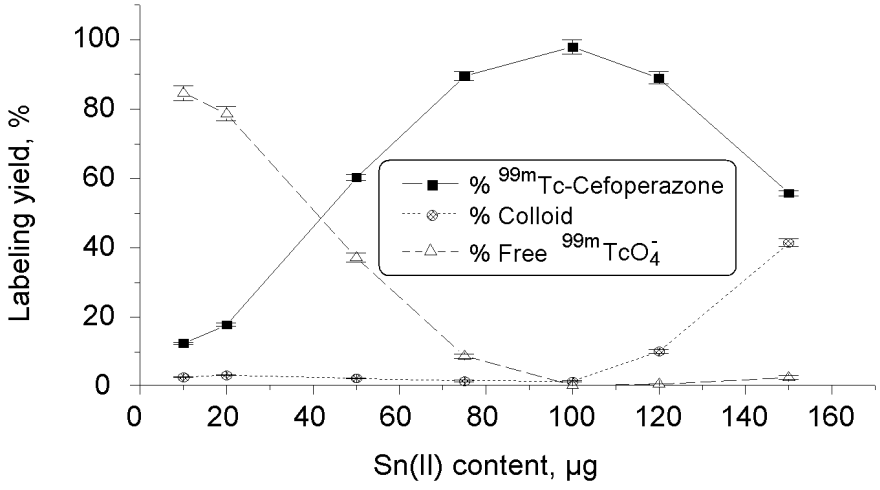

Fig. 2. Effect of Sn(II) content on the labeling yield of ^{99m}Tc-cefoperazone complex. Conditions: 3 mg cefoperazone, 10–150 µg $SnCl_2$, pH 8 and 10 minutes reaction time, $n = 3$

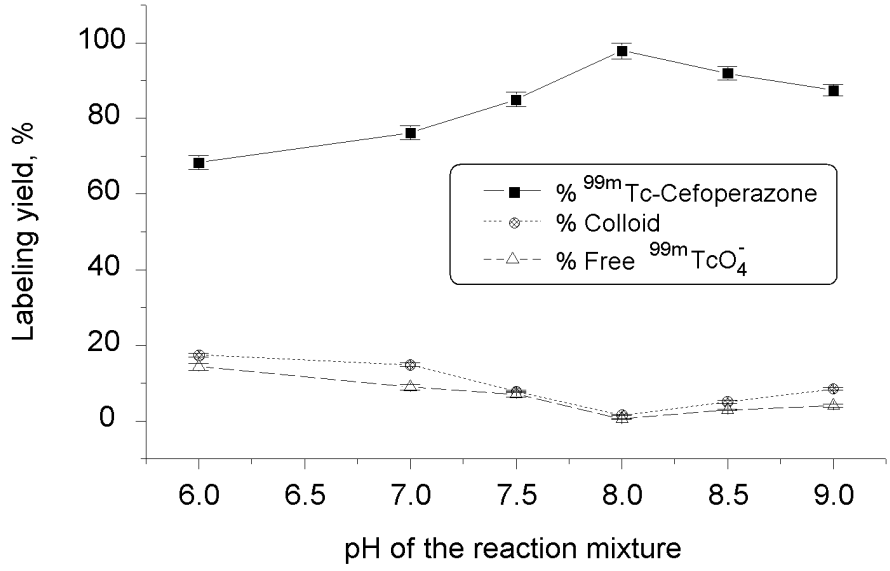

Fig. 3. Effect of pH on the labeling yield of ^{99m}Tc-cefoperazone complex. Conditions: 3 mg cefoperazone, 100 μg $SnCl_2$, pH 6–9 and 10 minutes reaction time, $n = 3$

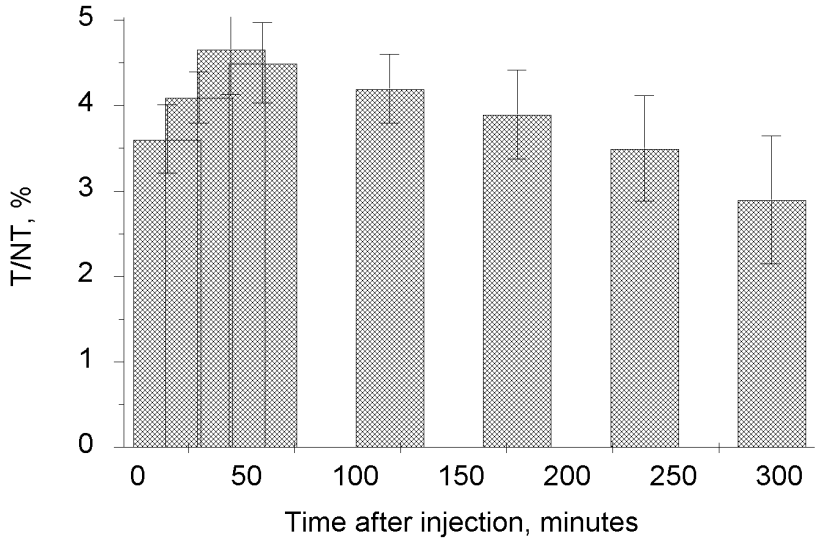

Fig. 4. Activity of infected site to non infected site at different time post injection. Same condition as for Fig. 3, $n = 3$

Table 1. Biodistribution of ^{99m}Tc-cefoperazone complex

Organs or body fluids	%ID/g ($n = 5$)
Inflamed muscle	0.513 ± 0.12
Control muscle	0.110 ± 0.02
Blood	0.306 ± 0.052
Liver	0.392 ± 0.031
Spleen	0.159 ± 0.02
Lung	0.228 ± 0.04
Kidney	3.101 ± 0.54
Stomach	0.124 ± 0.031
Intestine	0.226 ± 0.052
Bone	0.176 ± 0.044
Heart	0.098 ± 0.032

Same condition as for Fig. 4.

Conclusions

Nuclear medicine can make an important contribution to diagnose infection provided that radiopharmaceutical allowing imaging of infection at the early stages. In mid 1990s, ^{99m}Tc-ciprofloxacin was introduced in nuclear medicine as infection imaging agent but ^{99m}Tc-ciprofloxacin kit have some disadvantages related to stability and radiochemical purity. Cefoperazone is antimicrobial agent that easily labeled with ^{99m}Tc using $SnCl_2$ as a reducing agent instead of redox polymer or FSA where $SnCl_2$ present favorable characteristics over other reducing agents.

^{99m}Tc was added to cefoperazone solution containing 3 mg cefoperazone and 100 µg Sn(II) at pH 8 to give high labeling yield of 97.9% after 10-minute incubation time without any heating. The biological distribution of ^{99m}Tc-cefoperazone shows that, low activity in liver corresponds to the low content of the hydrolyzed form of ^{99m}Tc. After 45 minutes of administration, there is a four and half time higher radioactivity in the induced inflammation tissue compared to normal tissue indicating that ^{99m}Tc-cefoperazone showed promise in localizing foci of infection with optimal visualization at 45 minutes.

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