Optimized preparation and evaluation of ^{99m}Tc-Streptomycin

Original Article

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ABSTRACT

Introduction: The endemic occurrence of new strains of tuberculosis around the globe has initiated new research on developing diagnostic radiopharmaceutical based on antibiotics.

Methods: Tc-99m labeled streptomycin was prepared using freshly milked $^{99m}TcO_4^-$ and various concentrations of streptomycin, stannous chloride at various temperatures in high yield radiochemical purity shown by HPLC/RTLC.

Results: At various pHs tested (3-10) the best labeling yield at optimized conditions were at 6-8 range, however pH.7 was considered the best. The radiochemical purity at the best optimized conditions was >95% as shown by RTLC and >97% using HPLC (specific activity; 1-3 GBq/mmole). The tracer proved to be stable in the final product and in presence of human serum at 37°C up to 2h. However, biodistribution and high resolution SPECT studies in wild type mice showed instability of the complex due to high stomach and thyroid activity uptake.

Conclusion: Regardless the reported methods in the literature the direct ^{99m}Tc-labeling of aminoglycosides did not proved a suitable tracer.

Key words: Technetium-99m; Streptomycin; SPECT; Biodistribution

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INTRODUCTION

The emerging need for the detection of infection sites in various patients including tuberculosis infection has led into the introduction of various radiopharmaceuticals during the last 2 decades, including ^{99m}Tc-ciprofluxacin [1], radiolabeled human polyclonal antibodies [2] and radiolabeled chemotactic peptides [3]. Among these compounds, radiolabeled antibiotics demonstrate a significant discrimination between infection and inflammation sites.

Streptomycin (STP) is a water-soluble aminoglycoside with the chemical name of *D*-streptamine, O-2-deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-O-5-deoxy-3-C-formyl-α-*L*-lyxofuranosyl-(1→4)-N,N'-*bis*(aminoiminomethyl)-sulfate (2:3 salt) (empirical formula; $(C_{21}H_{39}N_7O_{12})_2$•$3H_2SO_4$; MW: 1457.38) [4].

Streptomycin (STP) binds to 30S subunit of the bacterial ribosome, specifically to the S12 protein which is involved in the initiation of protein synthesis. This evidently accounts for its antibacterial activity but does not explain its bactericidal effects, which distinguishes streptomycin and other aminoglycosides from most other protein synthesis inhibitors (Figure 1).

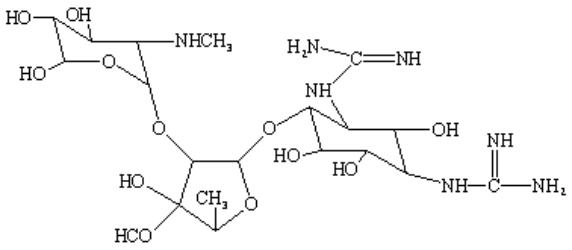

Fig 1. Chemical structure of Streptomycin.

The idea of developing radiolabeled antibiotics as infection imaging agents has led to development of various agents including Infecton. There are several reports on the development and application of radiolabeled STP antibiotic in the literature. Radiolabeled STP has been used in antibiotic drug interference studies [5]. In another study, ^{99m}Tc-streptomycin (^{99m}Tc–STP) has been prepared with greater than 98% labeling efficiency and reported stability up to 24 h and significant target:non target however no further applications of the labeled compounds has been reported [6].

In this work, we optimized ^{99m}Tc–STP formation conditions, followed by stability studies of the complex in presence of human serum up to 2h. Finally, the optimized tracer was administered to normal mice for biodistribution studies.

METHODS

Tc-99m pertechnetate was obtained from the commercially available 100 mCi generator, JaberTec™ from Pars Isotope Co. Tehran, Iran. All chemicals were of analytical grade and were purchased from Merck Chemical Company (Darmstadt, Germany). Streptomycin sulfate (Streptocin®) was purchased from Al-Havi pharmaceutical Co. Iran. Radio-thin-layer-chromatography (RTLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20×20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). Analytical high performance liquid chromatography (HPLC) used to determine the specific activity, was performed by a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250×4.6 mm, Whatman, NJ (USA).Normal saline and sodium acetate used for labeling were of high purity and were filtered through 0.22 µ Cativex filters. The approval of NSTRI Ethics Committee was obtained for conducting this research. The wild-type mice (NMRI) were purchased from Pasteur Institute of Iran, Karaj, and all weighing 20-25 g and were acclimatized at proper rodent diet and 12h/12h day/night ligh/darkness.

Labeling of streptomycin with Tc-99m pertechnetate

Streptomycin sulfate (Streptocin®; 2-5mg) was weighed into a clean sterilized borosilicate glass vial and dissolved in 10 ml of double distilled water. 600µl portions of the solution were added to separate vials. Portions of freshly prepared $SnCl_2.2H_2O$ solution (2µg/µl; 2.5µl, 25µl, 62.5µl and 100µl) were added to each antibiotic containing vials and pH of the solutions were exactly fixed to 3, 4, 5, 7, 10. Immediately 100µl of freshly eluted $^{99m}TcO4^-$ solution (10 mci) was added each vial. The whole process was done under a blanket of nitrogen. The mixtures were shaken gently and incubated for 30min at room temperature. Thereafter radiopharmaceutical purity was determined through TLC procedure.

Quality control

Paper chromatography: Radiochemical purity of ^{99m}Tc–STP was assessed by ascending paper chromatography. For determination of free $^{99m}TcO_4^-$ in ^{99m}Tc–STP preparation Whatman No.1 as stationary phase and methyl ethyl ketone (MEK) as a

mobile phase were used. The distribution of radioactivity was measured by RTLC scanner.

Determination of reduced and hydrolyzed activity in the preparation of ^{99m}Tc–STP was determined using Whatman No.1 as stationary phase and 0.9% NaCl as mobile phase. High performance liquid chromatography: HPLC was performed with a flow rate of 1 mL/min, pressure: 130 kgF/cm^2 for 20 min. HPLC was performed on the final preparation using a mixture of water:acetonitrile 3:2(v/v) as the eluent by means of reversed phase column Whatman Partisphere C_{18} 4.6 × 250 mm.

Stability of ^{99m}Tc–STP complex in the final product

A sample of ^{99m}Tc–STP (0.5 mCi) was kept at room temperature for 2 h, while checked by RTLC every 0.5 hours. A micro-pipette sample (5 µl) was taken from the shaking mixture and Whatman No.1 was used as stationary phase and methyl ethyl ketone (MEK) as mobile phase.

In vitro stability of ^{99m}Tc–STP in presence of human serum

Final solution (200 µCi, 50 µL) was incubated in the presence of freshly prepared human serum (300 µL) (Purchased from Iranian Blood Transfusion Organization, Tehran) and kept at 37ºC for 2 H. Every 30 min to a portion of the mixture (50 µL), trichloroacetic acid (10%, 100µl) was added and the mixture was centrifuged at 3000 rpm for 5 min followed by decanting the supernatant from the debris. The stability was determined by performing frequent ITLC analysis of supernatant using above mentioned ITLC system.

Sterility and apyrogenicity of the radiopharmaceutical

Sterility was controlled on a random sampling following decay of radioactivity. The *Limulus amoebocyte* lysate (LAL) test was used for validation of radiopharmaceutical production according to the European protocol [7].

Biodistribution in normal rats

Animal studies were performed in accordance with the United Kingdom Biological Council's *Guidelines on the Use of Living Animals in Scientific Investigations,* 2nd edn. The distribution of ^{99m}Tc–STP among tissues was determined for healthy NMRI male rats immediately after imaging. The total amount of activity injected into each rat was measured by counting the 1-ml syringe before and after injection in a dose calibrator with fixed geometry. The animals were sacrificed by CO_2 asphyxiation at selected times after injection (1, 2 h), the tissues (blood, heart, lung, brain, intestine, urine, skin, stomach, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline. The activity of each sample (and activity per gram tissue thereof) was determined by an HPGe detector equipped with a sample holder device using the area under curve (AUC) of the 140 keV peak.

Imaging of ^{99m}Tc–STP in normal rats

Mice injected with ^{99m}Tc–STP were used for imaging. Images were taken at various time intervals after administration of the tracer by a dual-head SPECT system. The mouse-to-high energy septa distance was 12 cm. The useful field of view (UFOV) was 540 mm×400 mm. The spatial resolution was 10 mm FWHM at the CFOV. Sixty four projections were acquired for 30 seconds.

RESULTS AND DISCUSSION

Radiolabeling of streptomycin

In order to get the best radiochemical purity, various fractions including $SnCl_2$, temperature and pH were tested. At a given amount of STP used 10, 30, 50, 100 and 200 µg of freshly dissolved $SnCl_2$ in HCl under a blanket of nitrogen was used and the best radiochemical purity (89%) was obtained at 200 µg at un-optimized conditions (Figure 2).

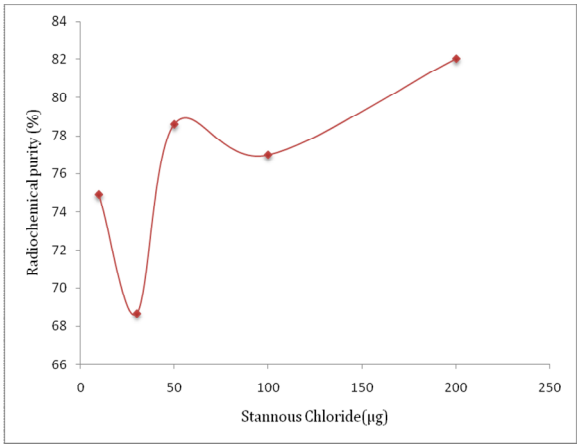

Fig 2. Radiochemical purity of ^{99m}Tc–Stereptomycin solution at various $SnCl_2$ amounts.

There is no explanation for the need of applying this amount of $SnCl_2$, however as reported previously, the stoichiometry of the Sn: ligand: ^{99m}TcO4 ratios is not well understood and is fluctuating based on laboratory settings.

At various pHs tested (3-10) the best labeling yield at optimized conditions were at 6-8 range, however pH

7 was considered the best. Considering guanidine moieties as functional groups labile to TcO_4^- labeling, pH<4 is believed to lead to the protonation of NH groups and lower labeling yield. While pH>9 was showed to lead to the colloid formation (Figure 3).

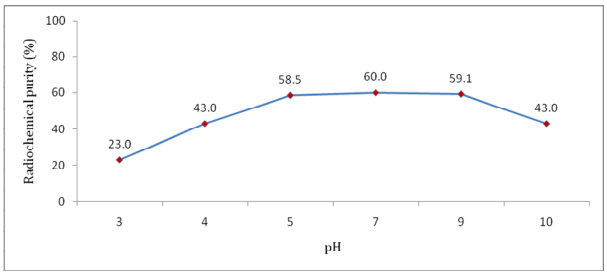

Fig 3. The radiochemical purity of radiolabeling reaction at various acidities.

The labeling reaction was performed at 25, 50 and 75° C and there were no significant difference among the temperatures used. The radiolabeling protocol was performed at room temperature. The radiochemical purity at the best optimized conditions was >95% as shown by RTLC in saline used as the solvent system ^{99m}Tc–STP retains at the base (Rf. 0.1) while ^{99m}TcO4 migrated to higher Rfs (0.7) (Figure 4).

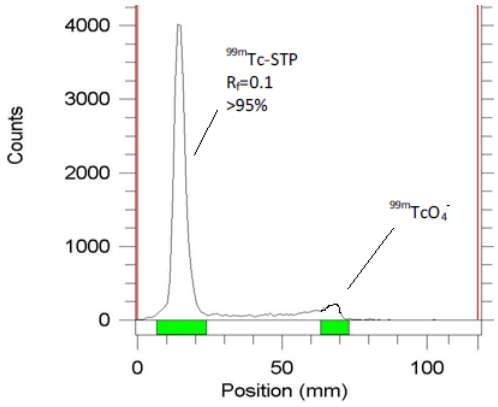

Fig 4. RTLC of ^{99m}Tc–Streptomycin solution in normal saline as the eluent on Whatman No. 2.

In another chromatography system colloids are shown as all remain at the base while ^{99m}TcO2 and radiolabeled compound migrates to the higher R_fs. In order to better demonstrate the radiochemical purity, HPLC on reverse phase column using water: acetonitrile was also used. A fast eluting species at 4.43 min was $^{99m}TcO_4^-$ or other unlabeled Tc-99m radiochemical impurities while the complex was eluted at 12.34 min. the radiochemical purity was >97% in this method immediately after labeling (Figure 5).

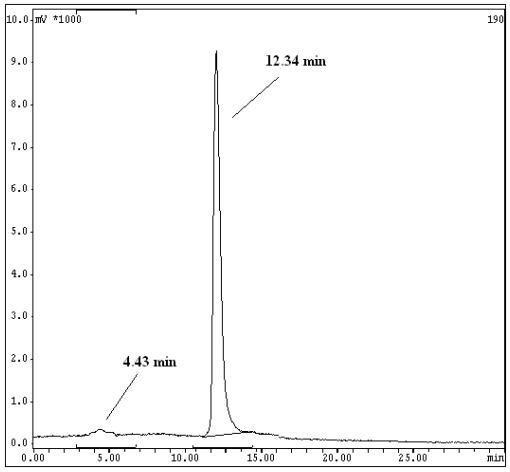

Fig 5. HPLC chromatogram of ^{99m}Tc–Streptomycin solution after labeling at optimized conditions.

However HPLC chromatogram after 4h showed different radiochemical purity due to instability of the complex (Figure 6). The only present species was shown to be unlabeled Tc-99m species.

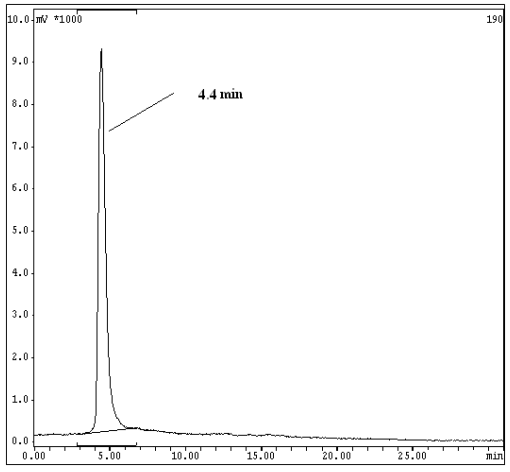

Fig 6. HPLC chromatogram of ^{99m}Tc–STP solution 4 h after labeling at optimized conditions in final solution.

Stability tests

The final product:human serum mixture was incubated at 37°C for up to 24 h and samples underwent RTLC tests to study the complex integrity after precipitation by trichloroacetic acid addition. No change in stability was observed in 1 h and the patterns for ^{99m}Tc–species and ^{99m}Tc–STP did not change. At optimized conditions according to the experiments using 200 µg of $SnCl_2$ at pH 7, while adding 2 mg of sterile streptomycin powder at room temperature, the best radiochemical purity (>95%,

n=10) was obtained, the formulation prepared using this conditions was then sent for biological studies.

Biodistribution studies in normal rats

The dissection studies demonstrated high stomach (>70% in 2h) as well as significant thyroid uptake. These findings were consistent with $^{99m}TcO_4$ biodistribution [8] and can be a sign of complete instability of the complex in the animal body due to enzymatic and/or trans-chelation effects (Figure 7).

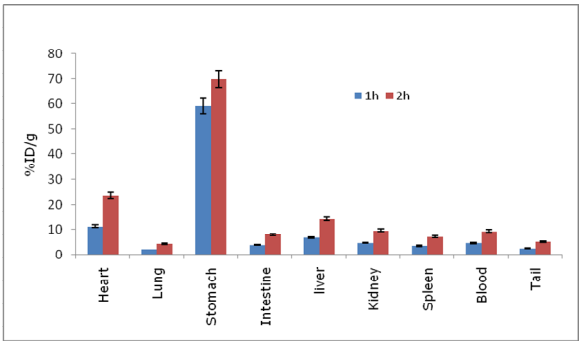

Fig 7. Biodistribution of ^{99m}Tc–Streptomycin in normal rats 1-2 h post-injection.

Using a high resolution animal SPECT, the biodistribution of the ^{99m}Tc–STP complex in animal body was studied. As Figure 8 shows the major activity accumulation was observed in stomach and thyroid. These findings are in agreement with the dissection studies results.

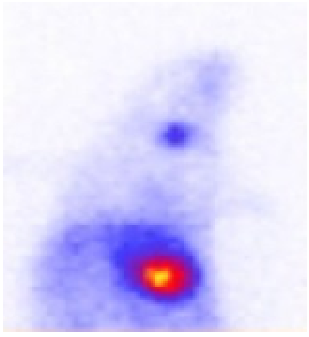

Fig 8. Biodistribution of ^{99m}Tc–Streptomycin in normal rat 2 h post-injection.

Interestingly, some researchers formerly reported the production of this labeled compound without any success in a real infectious model and instead used turpentine oil-induced models which normally should be in-responsive to radiolabeled antibiotics, however the maximum abscess-to-contralateral tissue ratios by ROIs over respective areas on scintigrams was 2.38 for ^{99m}Tc-STP [6] and concluded that the uptake mechanism might be infiltration into interstitial space due to increased capillary permeability. According to the present study, we believe that the radiolabeled complex was not stable and the major uptake was as a result of free $^{99m}TcO_4$ cation infiltration into the lesion rather than whole radiolabeled complex.

CONCLUSION

The radio-labeled ^{99m}Tc–STP complex was prepared after condition optimization for acidity, stannous amount, ligand amount, temperature and time. ^{99m}Tc–STP was stable in the final product and in presence of human serum at 37°C up to 2h. However, biodistribution and high resolution SPECT studies in wild type mice showed instability of the complex evidenced by high stomach and thyroid activity uptake. Regardless the reported methods in the literature the direct ^{99m}Tc-labeling of aminoglycosides did not proved a suitable tracer.

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