J Radioanal Nucl Chem (2013) 295:841–844
DOI 10.1007/s10967-012-2016-3

Synthesis of ^{99m}Tc-cationic steroid antimicrobial-107 and in vitro evaluation

Rizwana Zahoor · Samina Roohi · Mushtaq Ahmad · Zafar Iqbal · Nagina Amir · Saima Tariq · Paul. B. Savage

Received: 3 May 2012 / Published online: 17 August 2012

Abstract Ceragenins/cationic steroid antimicrobials (CSAs) are a group of cholic acid derivatives with many properties that make them favourable for application as anti-infective agents. CSA-107 is also a member of this group that was labelled with ^{99m}Tc by using $SnCl_2{\cdot}2H_2O$ as reducing agent and Na–K tartrate as transchelating agent. Labelling efficiency was optimized by varying the amount of reducing agent, pH, and time of incubation. Labelling efficiency and the stability of ^{99m}Tc-CSA-107 in human serum was determined by paper and thin layer chromatography, which were >95 and >90 % respectively. In vitro binding of ^{99m}Tc-CSA-107 was >95 % determined by using *Staphylococcus aureus* bacteria.

Keywords ^{99m}Tc labelled CSA · Ceragenins · Labelling efficiency · Infection · Cationic steroid antimicrobials · Chromatography · In vitro evaluation

Introduction

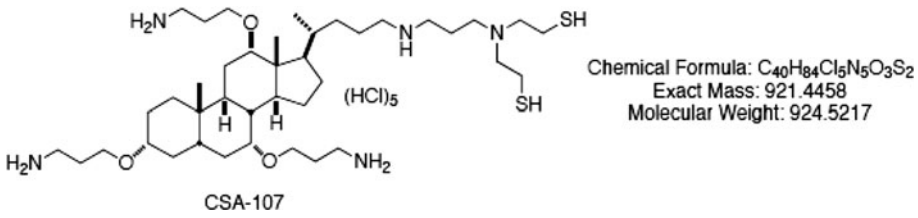
Infectious diseases remain a major health problem and cause of death worldwide. Over the years ^{67}Ga citrate that localizes in inflammation associated with infection sites have been used for infection imaging [1]. The most well established 'specific' agent that is regarded as the 'gold-standard' for infection imaging is ^{111}In labelled leukocytes. In view of the cost, limited availability, and not so favorable nuclear properties for imaging of ^{111}In, techniques for ^{99m}Tc labelling of leukocytes have also been developed and used. Other radiopharmaceuticals include ^{111}In/^{99m}Tc-HIG, radiolabeled monoclonal antibodies, bacterial chemotactic peptides, nanocolloids, liposomes, streptavidin–biotin, [18] FDG, and antimicrobial agents [2]. Several ^{99m}Tc labelled compounds have been developed for infection imaging purposes [3–11].The prevalence of drug resistant bacteria drives the quest for new antimicrobials, especially those that are not expected to readily engender resistance. One option is to mimic Nature's most ubiquitous means of controlling bacterial growth, anti microbial peptides that have evolved over eons. In general human antimicrobial peptides play a central role in innate immunity. Antibacterial/antimicrobial peptides have been isolated from a wide range of organisms including mammals [12], amphibians [13], insects [14], plants [15] and even bacteria [16]. However, clinical use of antimicrobial peptides is hampered by issues of cost and stability. The development of nonpeptide mimics (ceragenins) of antimicrobial peptides may provide the best option [17].

The property of selective toxicity is the basis of the use of antimicrobial compounds/antibiotics, to treat infections and have been live saving in this respect. The same property provides the potential for these agents to be exploited as radiopharmaceuticals for infection imaging. The principle is quite simple; the specificity for infection is provided by the antibiotic binding to the microbe, which in turn can be visualized by a gamma camera, if the antimicrobial agent is labelled with a gamma emitter, such as ^{99m}Tc.

R. Zahoor · S. Roohi · M. Ahmad (✉) · Z. Iqbal · N. Amir · S. Tariq
Isotope Production Division,
Pakistan Institute of Nuclear Science and Technology,
P.O. Nilore, Islamabad 45650, Pakistan
e-mail: amushtaq1@hotmail.com; mushtaqa@pinstech.org.pk

Paul. B. Savage
Department of Chemistry and Biochemistry,
Brigham Young University, C100BNSN, Provo,
UT 84602, USA

Fig. 1 Structure of CSA-107



Ceragenins Cationic steroid antibiotic (CSA)-8 and CSA-13 have previously been demonstrated to possess broad spectrum activities against multidrug resistant bacteria [18]. Now with some modifications in the structure of CSA-13 a new compound named CSA-107 (structure of CSA-107 is given in Fig. 1) has been labelled with ^{99m}Tc. Bacteria binding and human serum stability of ^{99m}Tc-CSA-107 were studied.

Experimental

Materials and methods

CSA-107 was obtained from Paul B. Savage Laboratory, Department of Chemistry and Biochemistry, Brigham Young, University. *Staphylococcus aureus* bacteria (American type culture collection, ATCC 25923) were obtained from the National Institute of Health (NIH), Islamabad, Pakistan. ^{99m}Tc was obtained from locally produced fission based PAKGEN ^{99}Mo/^{99m}Tc generator system. All the chemicals used were AR grade and purchased from Merck, Germany.

Radiolabelling of CSA-107 with ^{99m}Tc

Stock solution of CSA-107 was prepared by dissolving 1 mg of CSA-107 in 1 mL distilled water and an aliquot of 0.2 mL containing 200 μg of CSA-107 was mixed with 0.1 mL solution of Na–K tartrate (100 μg), 5–40 μg of $SnCl_2{\cdot}2H_2O$ was also added followed by addition of 1 mL of $^{99m}TcO_4^-$. pH of the solution was adjusted to 2–7 with NaOH solution. The mixture was prepared in inert environment (in nitrogen atmosphere) then incubated for different time periods at room temperature (23 ± 2 °C).

Determination of labelling efficiency of ^{99m}Tc-CSA-107

Radiochemical purity assessment of ^{99m}Tc-CSA-107 was made by ascending thin layer and paper chromatographic methods. Free $^{99m}TcO_4^-$ was determined by using Whatman paper no. 3 as stationary phase and acetone as mobile phase. Reduced/hydrolyzed activity was determined by using ITLC-SG strips as stationary phase and 0.05 M NaOH as mobile phase. The distribution of radioactivity on chromatographic strips was measured by a 2π Scanner (Berthold, Germany). Alternatively, the strips were cut into 1 cm segments and counted by a gamma-counter. The stability of ^{99m}Tc-CSA-107 was also checked using chromatography up to 24 h at room temperature.

Stability in human serum

Normal human serum 1 mL was mixed with 0.2 mL of ^{99m}Tc labelled CSA-107. The mixture was incubated at 37 °C. Sample was taken during incubation after different time intervals up to 24 h and subjected to instant thin layer and paper chromatography. Any increase in the impurity was considered to be due to degradation of labelled compound.

In vitro binding of ^{99m}Tc-CSA-107

Binding of ^{99m}Tc-CSA-107 to *S. aureus* bacteria was assessed by the following method. Briefly 0.1 mL of 0.2 M sodium phosphate buffer (Na–PB) of pH 7 was added in three different tubes to which 10, 50 and 100 μg of ^{99m}Tc labelled CSA-107 were added separately. 0.8 mL of 50 % (v/v) 0.2 M acetic acid in (Na–PB) containing approximately 1×10^8 viable *S. aureus* bacteria were also added in each tube. Tubes were incubated at 4 °C for 30 min, 1, 2 and 24 h. After completion of incubation time, the material was centrifuged for 5 min and supernatant was removed while bacterial pellet was resuspended in 1 mL of ice cooled (Na–PB) and centrifuged again. Supernatant was removed and then activity in the bacterial pellet was measured by gamma counter. Radioactivity related to bacteria was expressed in percent of the added ^{99m}Tc activity bound to viable bacteria in regard to total ^{99m}Tc activity.

Results and discussion

CSAs are synthetically produced small molecules that display broad spectrum antibacterial activity. These compounds are comprised of steroids backbone appended with amino group, amino acids and other chemical groups attached to them [19]. In order to form bonds with reduced

^{99m}Tc, the chelator must contain electron donors like nitrogen, oxygen or sulphur. CSA-107 has several functional groups such as –NH_2, –NH, –SH, and –O– to form bonds with ^{99m}Tc (Fig. 1), hence ^{99m}Tc-CSA-107 is assumed to be a chelate complex with one or more CSA-107 ligands attached to reduced ^{99m}Tc.

A compound of same class CSA-13 has already been labelled with ^{99m}Tc [20] but problem was its instability in serum for long duration. To overcome this problem CSA-107 was synthesized having mercapto group in addition to amino group as compared to CSA-13. CSA-107 is more stable in serum for up to 24 h. During synthesis of ^{99m}Tc-CSA-107, the labelling efficiency, radiochemical purity and the stability were assessed by ascending paper chromatography and instant thin layer chromatography. In paper chromatography the mobile phase was acetone. In this system free $^{99m}TcO_4^-$ moved towards the solvent front ($R_f = 1$) while ^{99m}Tc-CSA-107 and reduced/hydrolyzed ^{99m}Tc remained at the origin. In another system, ITLC/SG was used as stationary phase, while the mobile phase was 0.05 M NaOH. In this system free $^{99m}TcO_4^-$ and ^{99m}Tc-CSA-107 moved towards solvent front ($R_f = 1$) leaving behind reduced/hydrolyzed ^{99m}Tc at the origin.

The effect of pH on the labelling efficiency is shown in Fig. 2. CSA-107 (200 μg) was labelled at different pH (3–7) using 30 μg of $SnCl_2{\cdot}2H_2O$ as reducing agent in presence of 100 μg Na–K tartrate. At pH 3 the compound was poorly labelled and with increase in pH the labelling efficiency increased. At pH 4 maximum labelling >90 % was noted, hence further experiments were performed at this pH.

Correlation between the amount of reducing agent and labelling efficiency was investigated as shown in Fig. 3. Maximum labelling was achieved by using 15–30 μg of $SnCl_2{\cdot}2H_2O$ as reducing agent in presence of 100 μg Na–K tartrate. To avoid formation of colloid, 15 μg $SnCl_2{\cdot}2H_2O$ was selected.

Rate of complexation and the stability of ^{99m}Tc-CSA-107 were checked at room temperature as shown in Fig. 4.

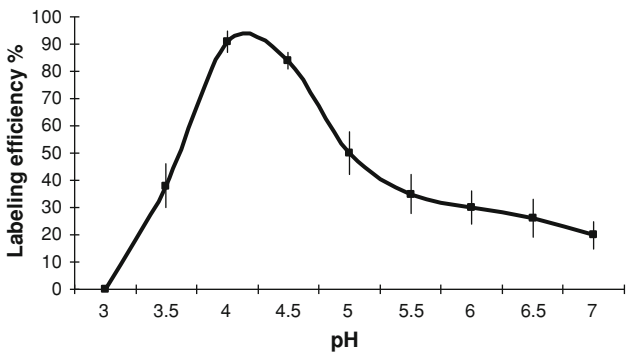


Fig. 2 Effect of pH on labelling efficiency of ^{99m}Tc-CSA-107 ($n = 4$)

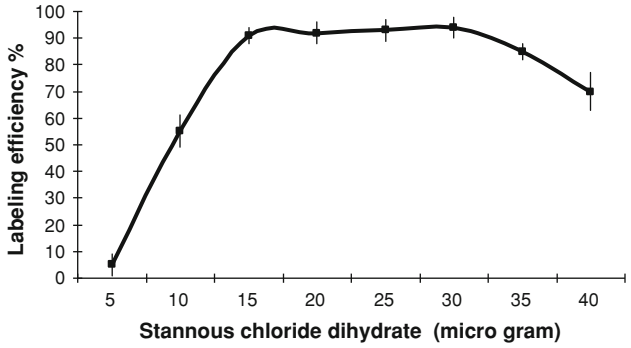


Fig. 3 Effect of amount of reducing agent $SnCl_2{\cdot}2H_2O$ on labelling efficiency of ^{99m}Tc-CSA-107 at pH 4 ($n = 4$)

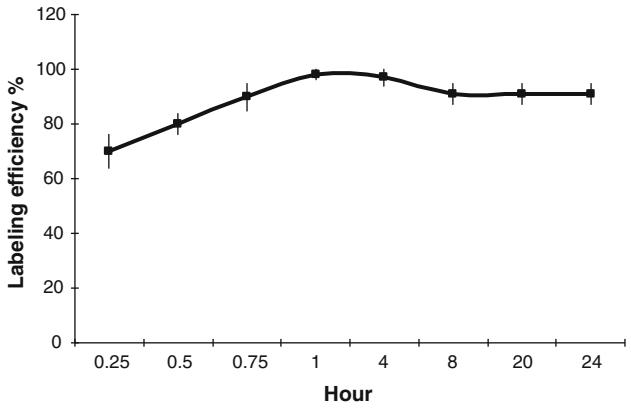


Fig. 4 Rate of complexation and stability of ^{99m}Tc-CSA-107 (The amount of reactants and other parameters; CSA-107 (200 μg), Na–K tartrate (100 μg), of $SnCl_2{\cdot}2H_2O$ (15 μg), 10 mCi sodium pertechnetate, pH 4 and incubation at 23 ± 2 °C), $n = 4$

It was 70 % in 15 min, while maximum labelling efficiency of ^{99m}Tc-CSA-107 (>95 %) was attained after 1 h, indicating the completion of transchelation from labelled tartrate to ^{99m}Tc-CSA-107, which remained >90 % up to 24 h.

The amount of reactants and other parameters, which gave >95 % labelling efficiency of ^{99m}Tc-CSA-107 were, 200 μg of CSA-107, 100 μg Na–K tartrate, 15 μg of $SnCl_2{\cdot}2H_2O$, 10 mCi sodium pertechnetate, pH 4 and incubation time at room temperature was 1 h.

The stability of ^{99m}Tc-CSA-107 was also checked in human serum. The labelled CSA was stable (>90 %) in serum throughout the period of observation that was from 10 min to 24 h. Paper and ITLC ascending chromatography was used to determine the labelling efficiency of ^{99m}Tc-CSA-107 in human serum. It was encouraging that the stability of ^{99m}Tc-CSA-107 in human serum was far better than ^{99m}Tc-CSA-13, which was more readily labelled with ^{99m}Tc [20].

In vitro binding of ^{99m}Tc-CSA-107 gave excellent results with *S. aureus* bacteria as shown in Fig. 5. Above

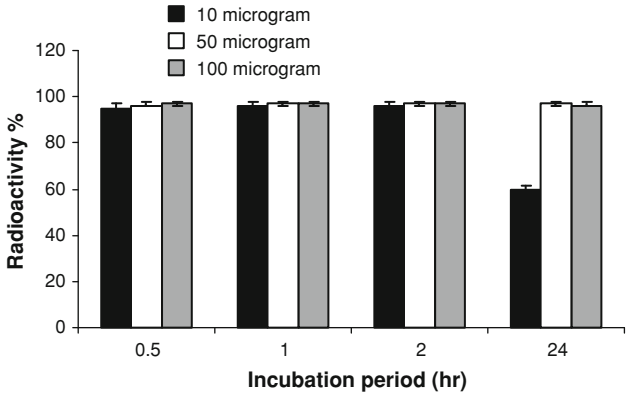


Fig. 5 In vitro binding of ^{99m}Tc-CSA-107 to viable *S. aureus* bacteria ($n = 4$)

95 % binding was achieved for all used amounts of CSA-107 (50, 100 μg) from 30 min of incubation time to 24 h, while 10 μg CSA-107 initially gave 95 % binding, which was reduced to 60 % after 24 h.

Conclusion

More than 95 % labelling of CSA-107 with ^{99m}Tc can be achieved by using transchelating agent Na–K tartrate. The human serum stability and binding capability with S. aureus bacteria for ^{99m}Tc-CSA-107 is quite high, >90 and >95 % respectively, hence warrants further studies in animal models.

References

1. Hoffer P (1980) J Nucl Med 21:282
2. Das SS, Hall AV, Wareham WW, Britton KE (2002) Braz Archiv Biol Technol 45:25
3. Roohi S, Mushtaq A, Salman AM (2005) Radiochim Acta 93:415
4. Roohi S, Mushtaq A, Jehangir M, Salman AM (2006) J Radioanal Nucl Chem 267(3):561
5. Roohi S, Mushtaq A, Jehangir M, Salman AM (2006) Radiochim Acta 94:147
6. Qaiser SS, Khan AU, Khan MR (2010) J Radioanal Nucl Chem 284:189
7. Lambrecht FY, Yilmaz O, Unak P, Seytigolu B, Durkan K, Baskan H (2008) J Radioanal Nucl Chem 277:491
8. Ibrahin IT, Motaleb MA, Attalah KM (2010) J Radioanal Nucl Chem 285:431
9. El-Ghany EA, Amine AM, El-Sayed AS, El-Kolaly MT, Abdel-Gelil F (2005) J Radioanal Nucl Chem 266:125
10. Motaleb MA, Alabdullah ES, Zaghary WA (2011) J Radioanal Nucl Chem 287:61
11. Motaleb MA (2007) J Radioanal Nucl Chem 272:95
12. Ganz T, Lehrer RIL (1997) Antimicrobial peptides of leukocytes. Curr Opin Hematol 4:53–58
13. Csordas A, Michl A (1970) Monatsh Chem 101:182
14. Habermann E (1972) Science 177:314
15. Fernandez de Caleya R, Gonzalez-Pascual B, Garcia-Olmedo F, Carborero P (1972) Appl Microb 23:998
16. Storm DR, Rosenthal KS, Swanson PE (1977) Ann Rev Biochem 46:723
17. Zhong LX, Yanshu F, Jacob P, Judy NC, Michael J, Robert RB, Raquel FE, Richard ME, Paul BS (2008) Acc Chem Res 41:1233
18. Chin JN, Rybak MJ, Cheung CM, Savage PB (2007) Antimicrob Agents Chemother 51(4):1268
19. Savage PB, Li C, Taotafa U, Ding B, Guan Q (2002) FEMS Microbiol Lett 217:1
20. Roohi S, Amir N, Mushtaq A, Savage PB, Salahuddin SM, Jehangir M (2009) Radiochim Acta 97(1):57