J Radioanal Nucl Chem (2012) 293:303–307
DOI 10.1007/s10967-012-1662-9

Direct labeling of doxorubicin with technetium-99m: its optimization, characterization and quality control

Faheem A. Rizvi · Tanveer H. Bokhari · S. Roohi · A. Mushtaq

Received: 17 January 2012 / Published online: 21 February 2012

Abstract Doxorubicin (DOX) is an anthracycline antineoplastic and one of the most potent and widely used drugs in clinical oncology. It is used in the treatment of a wide variety of cancers. The aim of this study was the direct labeling of DOX with ^{99m}Tc; its optimization, characterization and quality control of the radiolabeled DOX. Labeling efficiency was determined by paper chromatography. More than 92% labeling was obtained at pH 6–7, 10–12 μg stannous chloride and 200 μg of DOX. The stability of ^{99m}Tc–DOX was studied up to 5 h. All the experiments were performed at room temperature (25± 2 °C). The characterization of the labeling compound was performed by HPLC and electrophoresis. Electrophoresis indicates that labeled DOX has no charge and HPLC shows single specie of labeled compound.

Keywords ^{99m}Tc labeling · Doxorubicin · Characterization · Quality control · HPLC · Electrophoresis · Paper chromatography

Introduction

Elemental radionuclides are combined with other elements to form chemical compounds, or else combined with existing pharmaceutical compounds, to form radiopharmaceuticals. The radiopharmaceutical, once administered to the patient, can localize to specific organs or cellular receptors allowing imaging the extent of a disease-process in the body, based on the cellular function and physiology, rather than relying on physical changes in the tissue anatomy. In some diseases nuclear medicine studies can identify medical problems at an earlier stage than other diagnostic tests [1, 2]. Several ^{99m}Tc-labeled compounds have been developed for imaging purposes [3–11] and some of them are routinely employed in diagnostic nuclear medicine [12–15].

Doxorubicin (DOX) is an anthracycline antibiotic with potent antineoplastic properties effective against a broad spectrum of malignancies, such as non-Hodgkin's lymphoma, acute lymphoblastic leukemia, breast carcinoma, and several other types of cancers [16]. Doxorubicin interacts with DNA by intercalation and inhibition of macromolecular biosynthesis [17, 18]. The mechanism of cytotoxicity involves specific interaction of the planar anthracycline nucleus of DOX to the DNA double helix, resulting in prevention of further DNA replication [19, 20]. It has also been one of the most widely reported agents used in localized therapy approaches. In combination with ethiodized oil and gelatin sponge, DOX has shown response rates of up to 50% [21]. Children and adolescents are particularly susceptible to the cardiotoxic effects of anthracyclines chemotherapy and a significant portion of children treatment with DOX develop cardiomyopathy a year or more after cessation of chemotherapy [22–24].

In this paper, the direct labeling of DOX with ^{99m}Tc is described. Labeling efficiency, stability, quality control, and characterization were also studied.

F. A. Rizvi · T. H. Bokhari
Department of Chemistry, GC University, Faisalabad, Pakistan

S. Roohi · A. Mushtaq (✉)
Isotope Production Division, PINSTECH, Nilore, Islamabad, Pakistan
e-mail: mushtaqa@pinstech.org.pk

Experimental

Materials and methods

Doxorubicin hydrochloride for intravenous injection was obtained from Zhejiang Hisun Pharmaceutical Co. Ltd. Karachi, Pakistan. ^{99m}Tc was obtained from a locally produced fission based Pakgen ^{99}Mo/^{99m}Tc generator. All chemicals used were AR grade.

Synthesis of ^{99m}Tc–DOX

Optimization of labeling efficiency of DOX with ^{99m}Tc was performed by varying the amounts of DOX 100–500 μg, $SnCl_2{\cdot}2H_2O$ 5–20 μg and pH range 4–9. The pH was adjusted by using 0.5 M NaOH. Reaction mixture volume used in all experiments was 1.5 ± 0.2 mL. After addition of all reagents ~370 MBq $Na^{99m}TcO_4$ in saline was injected into the vial. All experiments were carried out at room temperature of 25 ± 2 °C and in darkness because the ligand was photosensitive.

Quality control

Radiochemical yield of ^{99m}Tc–DOX was checked by thin layer chromatographic method using Whatman No. 3 paper and ITLC-SG strips (Gelman Sciences). Free $^{99m}TcO_4^-$ in the preparation was determined by using Whatman No. 3 paper as the stationary phase and acetone as the mobile phase. Reduced and hydrolyzed activity was determined by using instant thin layer chromatography (ITLC-SG strips) as the stationary phase and THF as a mobile phase. Radiocolloids were also determined by passing the preparation through 0.22 μm bacteria filter (Millipore Filter Corp.). Activity remaining on the filter and in the solution was counted by a gamma-counter (Ludlum). The stability of ^{99m}Tc–DOX was checked for 5 h at room temperature. 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.

In vitro stability

Stability of the ^{99m}Tc–DOX was studied in vitro. 1.8 mL of normal human serum was mixed with 0.2 mL of ^{99m}Tc–DOX and incubated at 37 °C. 0.2 mL aliquots were withdrawn during the incubation at different time intervals up to 24 h and subjected to chromatography for determination of ^{99m}Tc–DOX, reduced/hydrolyzed ^{99m}Tc and free $^{99m}TcO_4^-$.

Electrophoresis of ^{99m}Tc–DOX

The charge on ^{99m}Tc–DOX was studied by using Deluxe electrophoresis chamber (Gelman) system. The Phosphate buffer of pH 6.8 was used in this experiment. Whatman No. 1 paper of 30 cm long strip was used. A drop of ^{99m}Tc–DOX at the middle of the strip was put and electrophoresis was run for 30–60 min at a voltage of 300 V. After completion of electrophoresis, the strip was scanned by using 2π scanner to know the charge on labeled DOX.

HPLC of ^{99m}Tc–DOX

HPLC of radiotracer ^{99m}Tc–DOX was studied by using D-200 Elite HPLC system. The column of C-18 was used as stationary phase and a mixture of acetonitrile and disodium hydrogen phosphate buffer (pH adjusted up to 4–5 by using 0.5 M NaOH) ratio 45:55 (v/v %) was used as mobile phase. The flow rate was adjusted up to 1 mL/min at a wavelength of 254 nm.

Results and discussion

The DOX molecule contains (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy] and 8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy bonded with 5,12-naphthalenedione hydrochloride. The structure of DOX HCl is shown in Fig. 1. The direct method of labeling of DOX with ^{99m}Tc was exploited which is simple, rapid, efficient and does not require bifunctional chelating agents. The various chelates of ^{99m}Tc, which serve as radiopharmaceuticals are formed by interaction between specific chelating agents and a reduced form of ^{99m}Tc. In order to form bonds with technetium, the chelator must contain electron donors like nitrogen, oxygen and sulfur. Space between multiple electron donor atoms is required to allow several bonds to form with the central metal. DOX has several functional groups, such as $–NH_2$, –OH, –O– to form bonds with ^{99m}Tc. Although the chemistry of formation and molecular structure are unknown, ^{99m}Tc–DOX is assumed to be a chelator complex with one or more DOX ligands attached to reduced ^{99m}Tc.

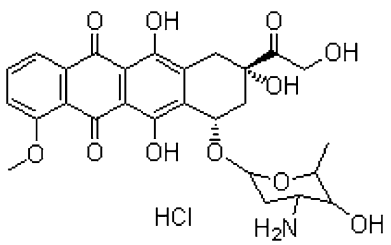

Fig. 1 Structure of DOX HCl

Labeling efficiency, radiochemical purity, and stability were determined by a combination of ascending paper chromatography and ITLC on silica gel. In paper chromatography using acetone as the solvent, free $^{99m}TcO_4^-$ moved towards the solvent while ^{99m}Tc–DOX and reduced/hydrolyzed ^{99m}Tc remained at the point of spotting. In ITLC-SG chromatography using THF as solvent, ^{99m}Tc–DOX and free $^{99m}TcO_4^-$ moved towards the solvent, while reduced/hydrolyzed ^{99m}Tc remains at the point of spotting. Radiocolloids were also determined by passing the preparation through sterile filters (0.22 μm). In this method radiocolloids were retained in the pores of filter while ^{99m}Tc–DOX and free $^{99m}TcO_4^-$ were passed through the filter. The results obtained from both methods were in excellent agreement. The amount of radiocolloid in the final preparations was ≤2.0%.

The effects of pH are shown in Fig. 2. At low pH (4–6) the minimum labeling efficiency is 70%, while at pH 6–7 the labeling efficiency of ^{99m}Tc–DOX is >92%. In basic media at pH 8–9 the labeling efficiency is decreased (68–53%). Hence further experiments were performed at pH 6–7.

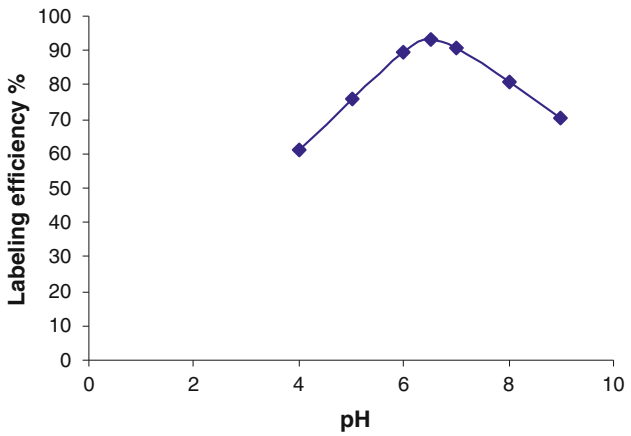

Fig. 2 Effect of pH on the labeling efficiency of ^{99m}Tc–DOX

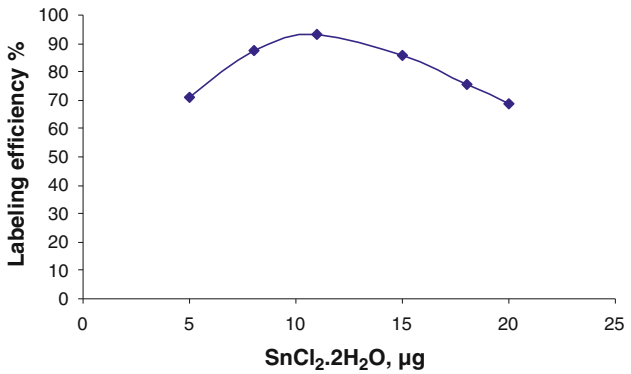

Fig. 3 Effect of $SnCl_2{\cdot}2H_2O$ amount on the labeling efficiency of ^{99m}Tc–DOX

The amount of the reducing agent, $SnCl_2{\cdot}2H_2O$, which gave the highest labeling efficiency was 12 μg (Fig. 3). Experiments were performed with varying amount of the ligand (DOX), and 200 μg gave the maximum labeling efficiency of >92% (Fig. 4). To avoid colloid formation,

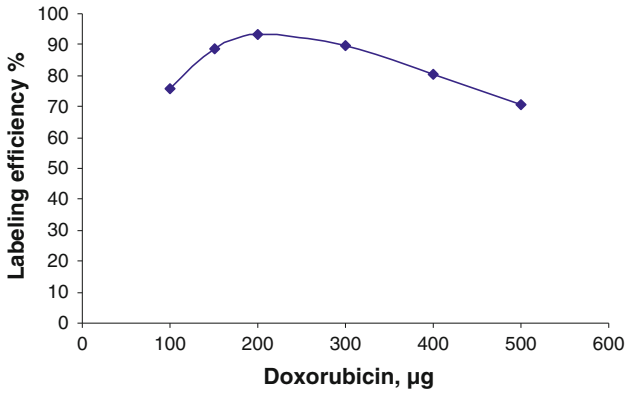

Fig. 4 Effect of ligand amount on the labeling efficiency of ^{99m}Tc–DOX

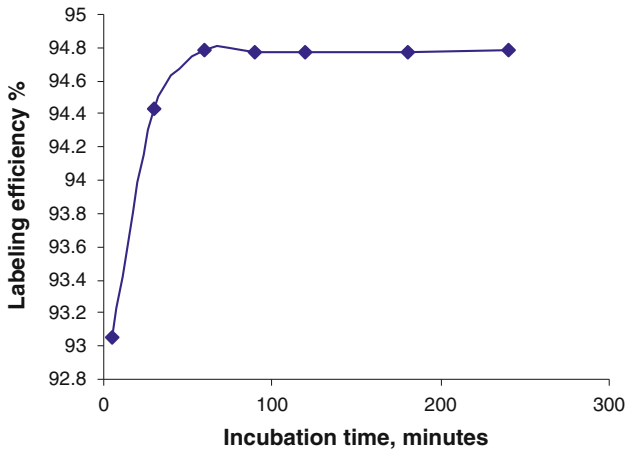

Fig. 5 Rate of complexation of ^{99m}Tc with DOX and stability of ^{99m}Tc–DOX

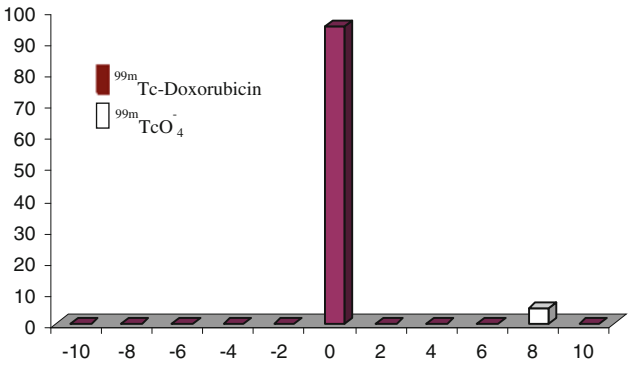

Fig. 6 Electrophoresis of DOX indicates its neutral nature

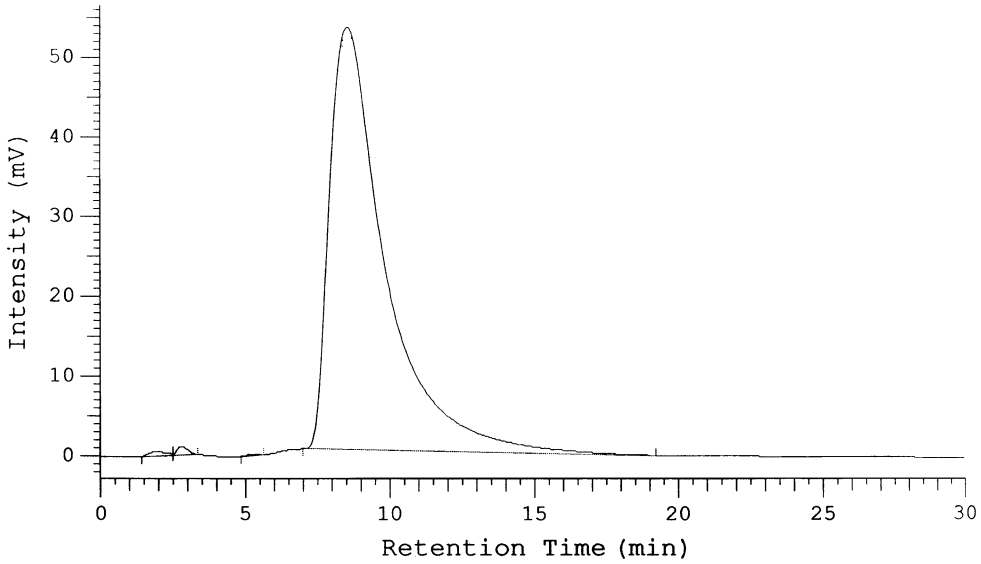

Fig. 7 HPLC analysis of Ligand shows >98% purity of ligand

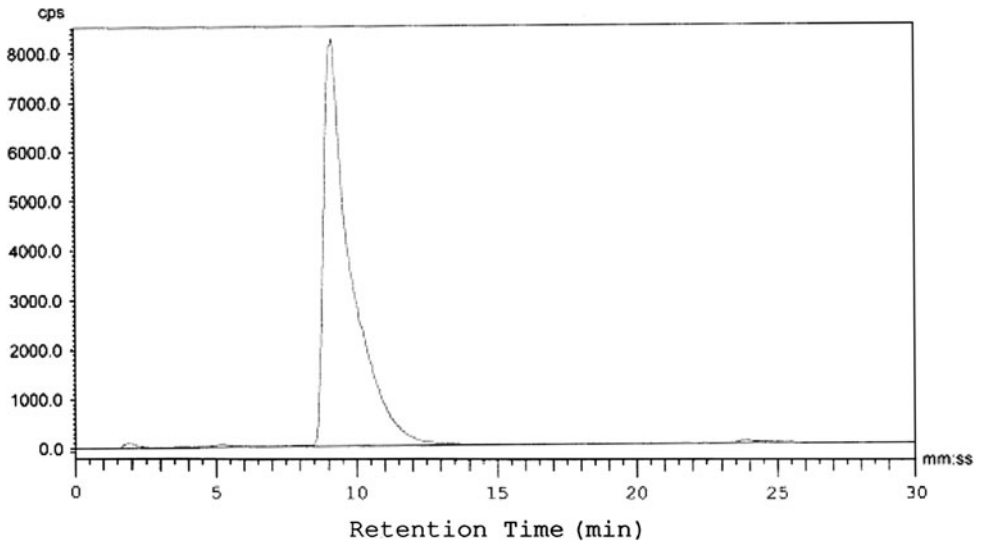

Fig. 8 HPLC analysis of ^{99m}Tc–DOX shows single specie of complex

the optimum amount of reducing agent was used. The complexation of ^{99m}Tc with DOX was not rapid and maximum labeling efficiency was achieved after 15 min. The resulting complex of ^{99m}Tc–DOX was quite stable and labeling of $\geq$ 92% was maintained for up to 5 h (Fig. 5). The electrophoresis result indicates that the complex of ^{99m}Tc–DOX is neutral in nature (Fig. 6). HPLC results indicates that the purity of ligand was >98% (Fig. 7). The retention time of inactive and labeled DOX was nearly 10 min. Figure 8 shows that the labeling efficiency was ~95% and complex was single specie. When the preparation was incubated with normal human serum at 37 °C, insignificant increase in free pertechnetate or reduced/hydrolyzed ^{99m}Tc up to 24 h was noticed. The total impurities were ~7% (Table 1).

The final formulation for the radiotracer ^{99m}Tc–DOX was DOX 200 µg, $SnCl_2{\cdot}2H_2O$ 12 µg, pH 6–7, ^{99m}Tc 370–450 MBq, reaction mixture volume ~1.5 mL, and incubation time 15 min at room temperature.

Conclusions

Methodology for radiolabeling of DOX with ^{99m}Tc has been developed and standardized. Radiolabeling efficiency of ^{99m}Tc–DOX monitored by paper and ITLC-SG was

Table 1 In vitro stability of ^{99m}Tc–DOX in normal human serum

Incubation time, h	^{99m}Tc–doxorubicin	Free pertechnetate	Colloid
1	93.5 ± 1.5	5.5 ± 0.2	1.0 ± 0.1
4	92.7 ± 1.4	5.7 ± 0.4	1.1 ± 0.4
24	92.5 ± 1.2	5.8 ± 0.5	1.2 ± 0.6

more than 92%. The resulting complex of ^{99m}Tc–DOX was quite stable and labeling of ≥92% was maintained for up to 5 h. The complex was stable in serum. HPLC shows single specie of ^{99m}Tc–DOX. No post-labeling purification is required for further studies.

Acknowledgments The authors were thankful to Rizwana Zahoor, Zafar Iqbal, Ibrar Haider, and Bashar Khan for useful discussions and to the Department of Chemistry, GC University Faisalabad.

References

1. Calame W, Welling MM (1993) Eur J Nucl Med 20:490
2. Edwards CL (1979) Sem Nucl Med 3:186
3. Pervez S, Mushtaq A, Arif M (2001) Appl Radiat Isotop 55:647
4. Roohi S, Mushtaq A, Salman AM (2005) Radiochim Acta 93:415
5. Roohi S, Mushtaq A, Jehangir M, Salman AM (2006) J Radioanal Nucl Chem 267(3):561
6. Roohi S, Mushtaq A, Jehangir M, Salman AM (2006) Radiochem Acta 94:147
7. Roohi S, Amir N, Mushtaq A, Paul BS, Salahuddin SM, Jehangir M (2009) Radiochim Acta 97(1):57
8. Amir N, Roohi S, Pervez S, Mushtaq A, Jehangir M, Miyashita Y, Okamoto K (2009) J Radioanal Nucl Chem 279(1):25
9. Qaiser SS, Khan AU, Khan MR (2010) J Radioanal Nucl Chem 284:189
10. Lumbrecht FY, Yilmaz O, Unak P, Seytigolu B, Durkan K, Baskan H (2008) J Radioanal Nucl Chem 277:491
11. El-Ghany EA, Amine AM, El-Sayed AS, El-Kolaly MT, Abdel-Gelil F (2005) J Radioanal Nucl Chem 266:125
12. Akhter M, Mushtaq A, Haider I, Aziz A, Karim HMA (1990) J Radioanal Nucl Chem Letters 146:415
13. Mushtaq A, Pervez S, Haider I, Mansur MS, Jehangir M (2000) J Radioanal Nucl Chem 243(3):827
14. Mansur MS, Mushtaq A, Jehangir M (2006) J Radioanal Nucl Chem 268(1):141
15. Pervez S, Mushtaq A (2009) J Radioanal Nucl Chem 28:371
16. Singh AK, Verma J, Bhatnagar A, Sen S, Bose M (2003) J Nucl Med 2:292
17. Fornari FA, Randolph JK, Yalowich JC, Ritke MK, Gewirtz DA (1994) Mol Pharmacol 45(4):649
18. Momparler RL, Karon M, Siegel SE, Avila F (1976) Cancer Res 36(8):2891
19. Vigevani A, Williamson MJ (1980) Doxorubicin. In: Florey K (ed) Analytical profiles of drugs substances, vol 9. Academic press, New York, p 245
20. Maruyama K, Unezaki S, Takahashi N, Lwatsurs M (1993) Biochem Biophys Acta 1149:206
21. Carr BI, Flickinger JC, Lotze MZ (1997) Cancer Treat Rep 72:1087
22. Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD, Colan SD (2005) J Clin Oncol 23:2629
23. Steinherz LJ, Steinherz PG, Tan C (1995) Med Pediatr Oncol 24:352
24. Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE, Sanders SP (1991) N Engl J Med 324:808