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# $^{99m}$Tc-labeled colchicine for tumor imaging using DTPA as bifunctional chelating agent

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**Abstract** For the purpose to develop novel $^{99m}$Tc-labeled tumor imaging agents with SPECT, colchicine (CHC) was directly labeled by $^{99m}$Tc using diethylene triamine pentacetate acid (DTPA) as bifunctional chelating agent. Trimethylcolchicinic acid, derivatived from colchicine, was conjugated to DTPA to get the new ligand DTPA-CHC, which was labeled by $^{99m}$Tc in the presence of $SnCl_2$ as reducing agent. The radiochemical purity of the $^{99m}$Tc-DTPA-CHC complex was over 90 %. It had good hydrophilicity and was stable at room temperature. The high initial tumor uptake with moderate retention, good tumor/muscle ratios and satisfactory scintigraphic images highlighted the potential of $^{99m}$Tc-DTPA-CHC for tumor imaging.

**Keywords** Colchicine · $^{99m}$Tc-labeled · Tumor imaging · DTPA · Bifunctional chelating agent

## Introduction

Cancer is one of the leading causes of death all over the world. The early imaging diagnosis is very valuable for treatment of tumor in clinic. For this purpose, [$^{18}$F]fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging provides high specificity and sensitivity in several kinds of cancer and has many applications [1–4]. [$^{18}$F]FDG is the most successful molecular probe in oncology available for routine use in the nuclear medicine clinics. However, its clinical application is heavily restricted by the short half-life, limited availability and high cost of production of $^{18}$F isotope by cyclotron [5]. Moreover, [$^{18}$F]FDG is not a "specific" radiotracer for imaging malignant disease [1, 6].

By comparison, $^{99m}$Tc is the most widely used radionuclide for diagnostic imaging with single photon emission computed tomography (SPECT) because of its favorable physical properties ($t_{1/2} = 6$ h, $E\gamma = 140$ keV), low cost, and widespread availability [7]. Therefore, the development of $^{99m}$Tc-labeled tumor imaging agent with SPECT would be of great value.

Colchicine (CHC), the major alkaloid of the meadow saffron, is one of the most prominent natural products and, like other tubulin-binding natural products (e.g. taxol and the epothilones), exhibits great pharmaceutical potential [8]. Colchicine is used for the treatment of acute gout. Colchicine binds to tubulin, thereby interfering with the polymerization of tubulin, interrupting microtubule dynamics, and arresting the mitosis in the metaphase stage. The cancer cells are dependent on the microtubule dynamics for their uncontrolled growth and division. Highly dynamic mitotic-spindle microtubules are among the most successful targets for anticancer therapy [9]. Colchicine was, therefore, selected as the biomolecule for preparing tumor-targeted radiopharmaceuticals for imaging or therapy purpose.

Colchicine, like many other cytotoxic drugs, enters the cell through the lipid bilayer by passive diffusion and binds reversibly to P-glycoprotein (Pgp) [8]. Pgp is a 170-kDa

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transmembrane drug efflux pump encoded by the MDR-1 gene in humans [10]. Pgp-mediated transport of chemotherapeutic drugs has been studied using single photon emission computed tomography (SPECT) and PET. It has been reported the feasibility of imaging Pgp functionality in tumours with [$^{11}$C]colchicine and PET [11, 12]. The potential of colchicine for tumor imaging and assessing antiangiogenic effect has been very well documented in the use of $^{99m}$Tc-ethylenedicysteine colchicines ([$^{99m}$Tc]EC-COL) [13]. Trimethylcolchicinic acid, derivatived from colchicine, has been labeled by $[^{99m}Tc(CO)_3]^+$ and $[^{99m}TcN]^{2+}$ core, both of which are used in targeting tumors and reported to exhibit good tumor uptake [14]. Colchicine has been labeled by $^{125}$I directly and found to be suitable for imaging of muscles [15]. Recently, we have reported a radioiodinated pegylated colchicine. It exhibits good clearance from background but poor tumor localization [16]. We have also explored two novel $^{99m}Tc(CO)_3$-labeled colchicine conjugates, the $^{99m}Tc(CO)_3$-AOPA colchicine conjugate and the $[^{99m}Tc(CO)_3$(PA-TZ-CHC)$]^+$. The former exhibits good uptake and retention in tumor with slow clearance from normal organs [17], while the latter exhibits fast clearance from background [18]. The latest progress of $^{99m}$Tc-radiolabeled colchicines for tumor imaging is the $^{99m}$Tc/HYNIC/colchicine conjugate, which shows accumulation in tumor with good uptake ($3.17 \pm 0.14$ % g/g at 1 h post-injection) and fast clearance from normal organs through urinary and hepatobiliary systems [19]. Desacetylcolchiceine has been conjugated with p-SCN-Bn-DOTA and p-SCN-Bn-NOTA for $^{68}$Ga labeling. The radiotracer $^{68}$Ga-NOTA-desacetylcolchiceine ($^{68}$Ga-2) has shown improved pharmacokinetic features over $^{68}$Ga-DOTA-desacetylcolchiceine ($^{68}$Ga-1) and the previously reported $^{99m}Tc(CO)_3$-colchicine radiotracer [14, 20]. Colchicine has also been labeled by therapeutic radioisotopes, including $^{90}$Y and $^{188}$Re [21, 22]. Both of the $^{90}$Y-DOTA-NCS-colchicine and $^{188}Re(CO)_3$-colchicine complexes exhibit good tumor uptake and retention, which suggest their potential for tumor therapy [21, 22].

Derivatization of colchicine to the suitable precursor was necessary for subsequent $^{99m}$Tc-labeling. DTPA is an aminopolycarboxylic acid consisting of a diethylenetriamine backbone with five carboxymethyl groups. The conjugate base of DTPA has a high affinity for metal cations. $^{99m}$Tc-DTPA has been used in kidney imaging for the measurement of glomerular filtration rate [23–25]. DTPA is also widely used in preparing $^{99m}$Tc-radiopharmaceuticals as a good bifunctional chelating agent (BFCA). Herein we report the synthesis, characterization, and radiochemistry of a $^{99m}$Tc-labeled colchicine conjugate based on DTPA and its bio-evaluation in tumor-bearing mice.

## Experimental

### Materials and methods

DTPA-bis(anhydride) was purchased from Sigma/Aldrich. Other reagents and solvents were from chemical companies in China. All the chemicals were analytically pure and used without further purification. Trimethylcolchicinic acid was synthesized according to the described method [14]. $^1$H NMR spectra were recorded with a 400 MHz spectrometer by using TMS as the internal standard. Mass spectrometer was performed on a Bruker Daltonics esquire 6000 mass spectrometer with electrospray-ionisation prob. A $^{99}$Mo/$^{99m}$Tc generator was obtained from the China Institute of Atomic Energy (CIAE).

### Synthesis of the ligand (Fig. 1)

The DTPA-colchicine conjugate (DTPA-CHC) was synthesized by reacting DTPA-bis(anhydride) with an equivalent amount of trimethylcolchicinic acid. The reaction is schematically shown in Fig. 1. To 1 mL of *N*,*N*-dimethyl formamidine (DMF) were added DTPA-bis(anhydride) (35.7 mg, 0.1 mmol) and trimethylcolchicinic acid (34.3 mg, 0.1 mmol), followed by addition of 20 μL of *N*,*N*-Diisopropylethylamine (DIPEA). The reaction mixture was stirred at room temperature for 1 h. Then 1 mL of pure water was added. The white precipitate was filtered. The final product was purified by high performance liquid chromatography (HPLC). HPLC purification was carried out by using a Hitachi LC2000 System equipped with a UV/vis detector ($\lambda = 254$ nm). The column (Kromasil 100-5C18, 250 × 100 mm) was eluted at a flow rate of 3.0 mL/min. Water with 0.1 % trifluoroacetic acid (A) and methonal with 0.1 % trifluoroacetic acid (B) mixtures were used as the mobile phase and the following gradient elution technique was adopted for the preparation (0 min 35 % B, 15 min 90 % B, 20 min 90 % B, 25 min 35 % B). Fractions at ~11.3 min were collected and lyophilized to give a white powder. The yield was 48.1 mg (67.0 %). $^1$H NMR (400 MHz, DMSO): $\delta = 2.00$–$2.36$ (m, 5 H), 2.56–2.63 (m, 2 H), 2.97–3.02 (m, 2 H), 3.30–3.34 (m, 2 H), 3.46–3.86 (m, 17 H), 4.27–4.41 (m, 4 H), 4.52 (bs, 1 H), 6.80 (s, 1 H), 7.17 (d, 1 H), 7.29 (s, 1 H), 7.35 (d, 1 H), 8.71 (d, 1 H), 11–13 (brs, 4 H), ppm. ESI–MS: 719.26 ($MH^+$), 741.25 ($M + Na^+$).

### Radiosynthesis of the $^{99m}$Tc-labeled DTPA-colchicine conjugate

The $^{99m}$Tc-labeled DTPA-colchicine conjugate was prepared very effectively through a simple method. DTPA-CHC (1 mg) was dissolved in 0.5 mL of pure water in a

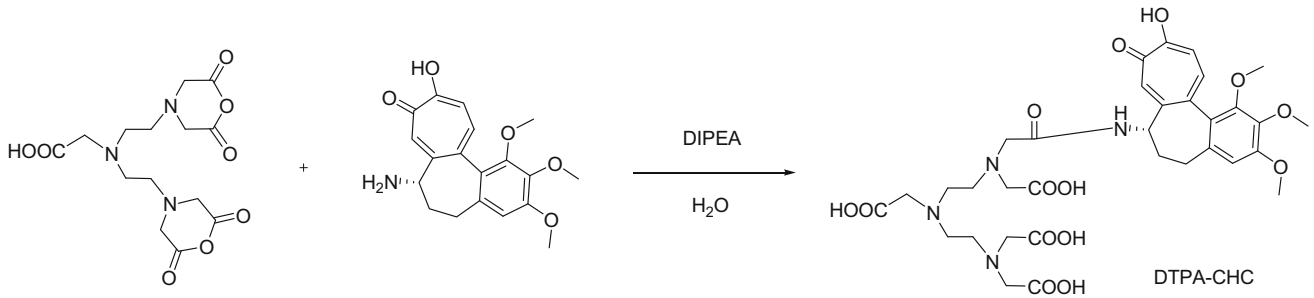


**Fig. 1** Synthesis of the DTPA-colchicine conjugate (DTPA-CHC)

shielded vial. Stannous chloride (50 μL, 1 mg/mL, pH 2) was added followed by addition of freshly eluted $^{99m}$Technetium pertechnetate (111 MBq; 100 μL). The pH of the reaction mixture was adjusted to 6.0–6.5 with 0.4 mL of PBS. The reaction mixture was shaken well and allowed to stand for 20 min at room temperature.

The radiochemical purity (RCP) of the complex was routinely checked by HPLC. HPLC analysis was carried out by using a Hitachi LC2000 System with Bioscan FC3200 radio-detector. The column (Kromasil 100-5C18, 250 × 4.6 mm) was eluted at a flow rate of 1.0 mL/min. Water with 0.1 % trifluoroacetic acid (A) and methonal with 0.1 % trifluoroacetic acid (B) mixtures were used as the mobile phase and the following gradient elution technique was adopted for the preparation (0 min 30 % B, 15 min 90 % B, 20 min 90 % B, 25 min 30 % B).

## Determination of the partition coefficient

The partition coefficient was determined by mixing the complex with an equal volume of 1-octanol and phosphate buffer (25 mM, pH 7.4) in a centrifuge tube. The mixture was vigorously stirred for 10 min at room temperature, and was then transferred to an Eppendorf microcentrifuge tube. The tube was centrifuged at 8000 rpm for 10 min. Samples in triplets from n-octanol and aqueous layer were obtained, and were counted in a well γ-counter. The partition coefficients were calculated using the following equation: P = (activity concentration in n-octanol)/(activity concentration in aqueous layer). The final partition coefficient value was expressed as log P.

## Stability studies

The stability of the complex was determined by measuring the RCP at room temperature (25 °C) at different time points (0, 1, 2, 4, 6 h) after preparation.

## Biodistribution studies in tumor-bearing mice

Biodistribution characteristics of $^{99m}$Tc-DTPA-CHC were evaluated using ICR mice bearing S180 cancer xenografts. The animal models were prepared according the published method [26]. In vivo growth was initiated by hypodermic injection of approximately $10^6$ S180 cells into the left front leg of female ICR mice. Seven-eight days after inoculation, the tumor size was in the range of 0.5–0.8 g, and animals

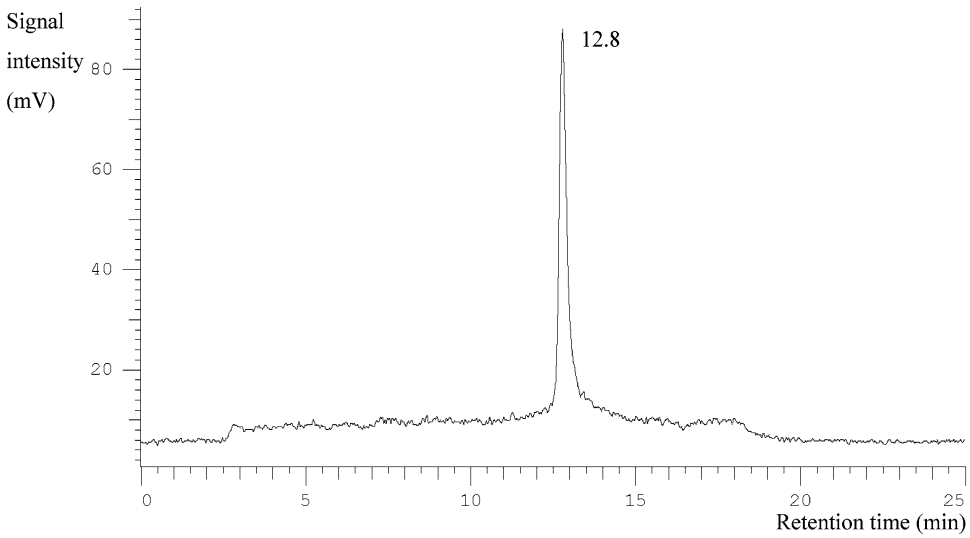


**Fig. 2** HPLC pattern of $^{99m}$Tc-DTPA-CHC

were used for biodistribution studies. A solution of the $^{99m}$Tc-DTPA-CHC (100 μL, 185 KBq) was injected into the tumor-bearing mice via the tail vein. The mice were sacrificed at 30, 60, 120 and 240 min post-injection (p.i.). The organs of interest and blood were collected, weighed and measured for radioactivity. The accumulated radioactivity in the tissue of organs was calculated in terms of percentage of injected dose per gram organ (%ID/g). The biodistribution data and tumor/non-tumor (T/NT) ratios are reported as an average plus the standard variation. All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

### Imaging studies

The imaging studies of $^{99m}$Tc-DTPA-CHC was performed using ICR mice bearing S180 cancer xenografts with Eplus-166 small animal SPECT/CT system (Institute of High Energy Physics, Chinese Academy of Sciences). A solution of the $^{99m}$Tc-DTPA-CHC (100 μL, 14.8 MBq) was injected into the tumor-bearing mice via the tail vein. Multiple static scans were obtained at 10, 30, 60, 120 and 240 min p.i.

## Results and discussion

### Synthesis and radiosynthesis

In order to get the DTPA-colchicine conjugate, trimethyl-colchicinic acid, derivated from colchicines, was directly reacted with DTPA-bis(anhydride) in the presence of DIPEA, followed by hydrolysis to get the crude product. Semi-preparative HPLC was used for purification to get the pure ligand. The DTPA-CHC ligand could be synthesized successfully with 67.0 % overall yield after HPLC purification.

The DTPA-colchicine conjugate (DTPA-CHC) could be directly labeled with $^{99m}$Tc very effectively through a simple method. It was found the pH value had serious impact on the labeling process. And weak acid condition (pH 6.0–6.5) would lead to good labeling results. The HPLC pattern of $^{99m}$Tc-DTPA-CHC is shown in Fig. 2. It was observed that the retention time (RT) of $^{99m}$Tc-DTPA-CHC was found to be 12.8 min. The mean radiochemical purity (RCP) of the complex was over 90 % after the preparation.

### Partition coefficient (log P)

The partition coefficient (Log P) of $^{99m}$Tc-DTPA-CHC was obtained to be $-2.66 \pm 0.02$, suggesting that it was hydrophilic. The hydrophilicity of the complex was mainly because of the $^{99m}$Tc-DTPA chelate.

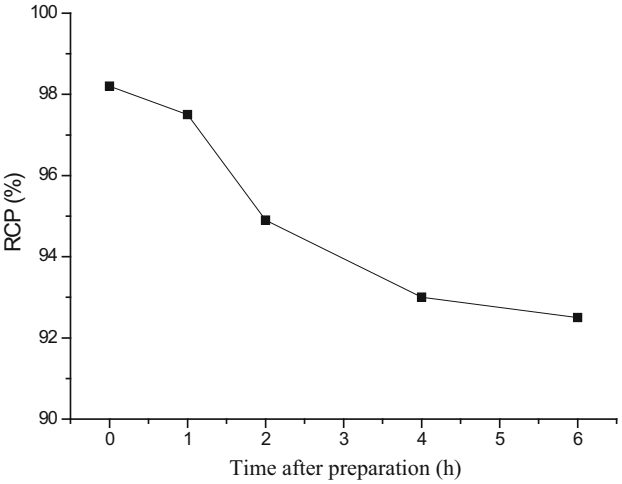


**Fig. 3** The RCP curve of $^{99m}$Tc-DTPA-CHC

**Table 1** Biodistribution study results of $^{99m}$Tc-DTPA-CHC in tumor-bearing mice (ID %/g, $x \pm s$, $n = 5$)

| Tissue | 30 min | 60 min | 120 min | 240 min |
|---|---|---|---|---|
| Tumor | 2.18 ± 0.22 | 1.64 ± 0.26 | 1.27 ± 0.16 | 1.09 ± 0.19 |
| Blood | 4.15 ± 0.72 | 2.69 ± 0.30 | 1.60 ± 0.20 | 1.17 ± 0.19 |
| Heart | 1.35 ± 0.12 | 0.92 ± 0.09 | 0.63 ± 0.07 | 0.54 ± 0.11 |
| Liver | 4.70 ± 0.23 | 3.33 ± 0.66 | 1.95 ± 0.33 | 1.72 ± 0.32 |
| Spleen | 1.50 ± 0.20 | 1.00 ± 0.17 | 0.79 ± 0.14 | 0.53 ± 0.12 |
| Lung | 3.42 ± 0.43 | 2.16 ± 0.15 | 1.34 ± 0.16 | 1.05 ± 0.22 |
| Kidneys | 10.68 ± 1.01 | 7.63 ± 0.92 | 7.85 ± 0.68 | 8.55 ± 1.16 |
| Brain | 0.18 ± 0.04 | 0.12 ± 0.04 | 0.07 ± 0.01 | 0.06 ± 0.01 |
| Muscle | 0.80 ± 0.26 | 0.67 ± 0.12 | 0.47 ± 0.10 | 0.37 ± 0.07 |
| Bone | 1.75 ± 0.55 | 1.08 ± 0.16 | 0.74 ± 0.13 | 0.63 ± 0.21 |
| T/M ratio | 3.15 ± 1.38 | 2.35 ± 0.20 | 2.96 ± 0.27 | 3.00 ± 0.53 |
| T/B ratio | 0.53 ± 0.10 | 0.60 ± 0.05 | 0.85 ± 0.13 | 0.93 ± 0.12 |

### Stability of the complex

The RCP of the product was >90 % during the observed period of 6 h (Fig. 3), suggesting that the complex possessed a great stability in the reaction mixture at room temperature.

### Biodistribution studies

The data of biodistribution are summarized in Table 1. $^{99m}$Tc-DTPA-CHC did exhibit tumor affinity with moderate accumulation and retention (30 min: 2.18 ± 0.22, 60 min: 1.64 ± 0.26, 120 min: 1.27 ± 0.16, 240 min: 1.09 ± 0.19 ID %/g). The maximum uptake time point in

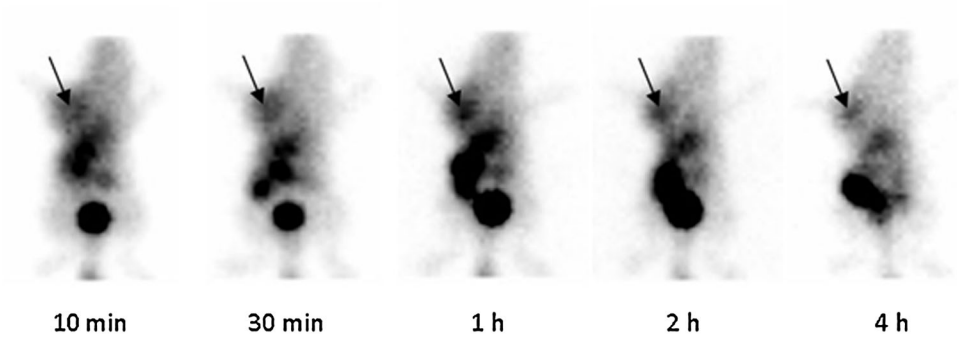


**Fig. 4** Scintigraphic images with tumor-bearing mice after injection of $^{99m}$Tc-DTPA-CHC

tumor was 30 min p.i. The complex was excreted via renal and especially hepatobiliary route. However, the clearance from normal organs was not so fast, leading to still high background at 4 h p.i. (blood: 1.17 ± 0.19, muscle: 0.37 ± 0.07, liver: 1.72 ± 0.32, kidneys: 8.55 ± 1.16 ID %/g). It had favorite tumor/muscle (T/M) ratios (30 min: 3.15 ± 1.38, 60 min: 2.35 ± 0.20, 120 min: 2.96 ± 0.27, 240 min: 3.00 ± 0.53) during the observation period. As the $^{99m}$Tc-labeled colchicine conjugates, $^{99m}$Tc-DTPA-CHC exhibited better tumor uptake than [$^{99m}$Tc]EC-COL [13], $^{99m}$TcN-colchicine-DTC [14] and [$^{99m}$Tc(CO)$_3$(PA-TZ-CHC)]$^+$ [18] as reported in literatures. The major deficiency lied in slow background clearance, especially the liver and abdomen uptake, which would limit the potential value of $^{99m}$Tc-DTPA-CHC for tumor imaging.

### Imaging studies

In imaging studies, the tumor (indicated by arrows) was clearly visualized during the whole experiment with very high tumor-to-muscle contrast (Fig. 4), which was consistent with the results from ex vivo biodistribution studies.

The development of $^{99m}$Tc-labeled tumor imaging agent with SPECT would be of great value, for the purpose of early imaging diagnosis of tumor in clinic. The biodistribution and imaging studies had proved the potential of $^{99m}$Tc-DTPA-CHC for tumor imaging. However, its clearance from normal organs was not fast. Thus further modification on the linker and/or $^{99m}$Tc-chelate will be necessary to improve tumor-targeting efficacy and pharmacokinetic profile. Moreover, we will explore novel $^{99m}$Tc-DTPA-conjugate by introducing double colchicine biomolecules.

## Conclusion

In this report, we have synthesized a new imaging agent $^{99m}$Tc-DTPA-CHC showing substantial promise for tumor scintigraphy, as significant accumulation is observed in S180 tumor bearing mice. The RCP of the complex was over 90 %. It had good hydrophilicity and was stable at room temperature. $^{99m}$Tc-DTPA-CHC showed obvious tumor uptake and retention, good tumor/muscle ratios and satisfactory scintigraphic images, suggesting it would be a promising candidate for tumor imaging.

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