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^{99m}Tc-labeling of colchicine using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $[^{99m}Tc{\equiv}N]^{2+}$ core for the preparation of potential tumor-targeting agents

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Abstract—Multidrug resistance (MDR) mediated by over-expression of P-glycoprotein (Pgp) is one of the major causes of failure of chemotherapy in cancer treatment. Colchicine, a naturally occurring alkaloid, is a Pgp substrate and acts as an antimitotic agent by binding to microtubules. Hence, Colchicine and its analogues radiolabeled with ^{99m}Tc may have potential for visualization of MDR in tumors. Here we report ^{99m}Tc-labeling of colchicine derivatives using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $[^{99m}Tc{\equiv}N]^{2+}$ cores. Trimethylcolchicinic acid synthesized from colchicine was used as the precursor to prepare iminodiacetic acid and dithiocarbamate derivatives which were then radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $[^{99m}Tc{\equiv}N]^{2+}$ cores, respectively. Radiolabeling yield for both the complexes was >98% as observed by HPLC and TLC patterns. In vitro studies in tumor cell lines showed significant uptake for ^{99m}Tc-carbonyl as well as for ^{99m}Tc-nitrido colchicine complexes. Biodistribution studies in Swiss mice bearing fibrosarcoma tumor showed $4.1 \pm 1.2\%$ ID/g of uptake at 30 min pi for $^{99m}Tc(CO)_3$-complex as against $0.42 \pm 0.24\%$ ID/g for the ^{99m}TcN-complex. $^{99m}Tc(CO)_3$–colchicine complex exhibited better pharmacokinetics with lower liver accumulation as compared to the ^{99m}TcN-complex. Thus, colchicine radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core is more promising with respect to in vivo distribution characteristics in tumor model.

1. Introduction

Colchicine is the main bioactive alkaloid of *Colchicum autmnale* and is used in diagnosis and treatment of gout. It acts as a potent inhibitor of cellular mitosis by binding to microtubules. Colchicine, like many other cytotoxic drugs, enters the cell through the lipid bilayer by passive diffusion and binds reversibly to P-glycoprotein (Pgp).[1] Pgp is one of the mechanisms involved in multidrug resistance (MDR) for chemotherapeutic drugs. Pgp-mediated transport of chemotherapeutic drugs has been studied using single photon emission computed tomography (SPECT) and positron emission tomography (PET). [^{11}C]Colchicine has been reported to be a feasible substrate for imaging Pgp functions in tumors.[2,3] Trimethylcolchicinic acid, a colchicine derivative, has also been used for preparation of ^{99m}Tc–EC(ethylenedicysteine)–colchicine in targeting tumors and reported to exhibit good tumor uptake.[4] Bearing in mind the potential of trimethylcolchicinic acid to exhibit tumor affinity, we report herein labeling of trimethylcolchicinic acid derivatives with two different novel cores of ^{99m}Tc viz. $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $[^{99m}Tc{\equiv}N]^{2+}$ cores and the evaluation of the resultant complexes for tumor imaging.

Commonly used technetium complexes based on ^{99m}Tc(V) oxo core suffer from low specific activity due to high concentration of chelating agents required for stabilization of +5 oxidation state. Advent of novel cores of technetium has opened fresh avenues for preparation of stable complexes with high specific activity.[5,6]

Keywords: Colchicine; $[^{99m}Tc(CO)_3(H_2O)_3]^+$; $[^{99m}Tc{\equiv}N]^{2+}$; Iminodiacetic acid; Dithiocarbamate; Tumor.

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Alberto et al. have developed and demonstrated the merits of using the new organometallic ^{99m}Technetium carbonyl precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ containing technetium in +1 oxidation state which is less sensitive to oxidation and hence does not require high concentration of ligands for stabilization.[7] Low ligand concentration is a specific feature aimed at the designing of ^{99m}Tc-labeled targeted radiopharmaceuticals. The tricarbonyl core $[^{99m}Tc(CO)_3(H_2O)_3]^+$ possesses a low spin d^6 Tc(I) center which is kinetically inert and hence offers more flexibility toward choosing ligands for designing complexes of desirable size, charge, and lipophilicity suitable for the specific study. The three substitutionally labile aqua ligands facilitate the formation of stable complexes with a tridentate ligand. The N-containing ligands such as histidine, histamine, imidazole, iminodiacetic acid, diethylenetriamine, and Schiff's bases which readily form stable complexes with the tricarbonyl precursor have been functionalized for the development of site-specific radiopharmaceuticals using varied biologically avid molecules.[8,9] Another novel nitrido core $[^{99m}Tc{\equiv}N]^{2+}$ isoelectronic with the $[^{99m}TcO]^{3+}$ core used earlier was synthesized by Baldas and Bonnyman, and found to exhibit a very high stability over a wide range of experimental conditions. It shows high affinity toward chelating ligands containing sulfur and phosphorus atoms. Dithiocarbamate ligand forms complexes of high radiochemical purity with the $[^{99m}Tc{\equiv}N]^{2+}$ synthon.[10,11] Colchicine has been functionalized to iminodiacetic acid (IDA) and dithiocarbamate (DTC) capable of complexation with the respective cores $[^{99m}Tc(CO)_3]^+$ and $[^{99m}Tc{\equiv}N]^{2+}$ in near-quantitative yields. In vitro binding studies with tumor cell lines as well as in vivo studies in tumor-bearing animals were carried out to explore the potential of these radiolabeled species to localize in tumors.

2. Results and discussion

2.1. Chemistry

Derivatization of colchicine to the suitable precursor was necessary for subsequent complexation with the ^{99m}Tc-cores. Hence, introduction of amino group to yield the precursor, trimethylcolchicinic acid, was attempted by acid hydrolysis of the parent colchicine **1** using 30% concentrated sulfuric acid.[12] The amino group of trimethylcolchicinic acid **2** was further derivatized to the iminodiacetic acid **3** and dithiocarbamate **4** for radiolabeling with $[^{99m}Tc(CO)_3]^+$ and $[^{99m}Tc{\equiv}N]^{2+}$ cores, respectively. The IDA of trimethylcolchicinic acid was prepared by reaction with 1.6 equiv of bromoacetic acid under alkaline conditions. The DTC of colchicine was prepared by reaction with carbon disulfide in the presence of KOH (Fig. 1). Hydrolysis of the $-NHCOCH_3$ group as well as the concomitant demethylation of $-OCH_3$ group of **1** could be ascertained by the ^1H NMR of **2** wherein the two 3H singlets corresponding to $-OCH_3$ and $-NHCOCH_3$ observed in **1** were found to be absent in **2**. The formation of the IDA **3** was confirmed by the appearance of a 4H singlet at δ 3.36 corresponding to the N–CH_2–COOH group. The dithiocarbamate **4** could be characterized with the help of elemental analysis and mass spectra.

To prepare the technetium-99m complexes of **3** and **4**, ^{99m}Tc-carbonyl and ^{99m}Tc-nitrido cores were first prepared. The ^{99m}Tc-tricarbonyl synthon was prepared in situ according to the reported procedure and then used for complexation.[7] It could be prepared in >98% yield as determined by C-18 reverse-phase HPLC system. The $^{99m}Tc(CO)_3$–colchicine–IDA complex could be obtained by incubation of IDA **3** with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ synthon. While the retention time of the synthon was 13.7 min, that of the radiolabeled complex, eluted as a single species in HPLC, was observed to be 18 min. The percentage complexation as obtained by HPLC analyses was >98% (Fig. 2). Complexation yields were studied at different pH by adjusting the pH of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ synthon prepared at pH ~ 10 using PO_4^{3-} buffer (pH 7.5, 0.5 M): HCl (1.0 M) (1:3, v/v), prior to addition of the ligand colchicine–IDA. Standardization and optimization studies for obtaining maximum complexation showed that the radiolabeling yield depends on the reaction pH and concentration of the ligand. The reaction was favorable at acidic pH (4.0) leading to 98% complexation. Increasing the pH led to a fall in the complexation yield which decreased to 70% and 40% at pH 7 and 9, respectively. In paper electrophoresis studies, it was observed that the $^{99m}Tc(CO)_3$–colchicine–IDA complex **5** does not show any movement. Lipophilicity of the radiolabeled complex was determined by distribution in octanol and water, and the partition coefficient (log *P*) was found to be 1.3. In vitro serum stability of the complex was studied by 1 h incubation of **5** at 37 °C. Observation of a single peak at 18 min, on analysis by HPLC, indicated serum stability of the complex.

^{99m}Tc-nitrido core was prepared by addition of $^{99m}TcO_4^-$ to the kit vial followed by preparation of the complex by incubation of **4** with $[^{99m}Tc{\equiv}N]^{2+}$ precursor. Formation of ^{99m}Tc-nitrido complex in good yields (>98%) was confirmed by TLC and electrophoresis. TLC was carried out in ethanol/chloroform/toluene: 0.5 M ammonium acetate mixture (6:3:3:0.5 v/v) as well as in saline. In the former solvent system, ^{99m}TcN intermediate as well as reduced technetium remained at the point of spotting (R_f = 0–0.1), whereas $^{99m}TcO_4^-$ moved at R_f 0.4–0.6. However, in saline, both $^{99m}TcO_4^-$ and ^{99m}TcN intermediate moved with the solvent front (R_f = 0.8–1.0) with reduced technetium at the point of spotting. Radiolabeling yield of the complex (>98%) could be determined by TLC in saline wherein the complex remained at the point of spotting (R_f = 0). In paper electrophoresis, while the ^{99m}TcN intermediate showed a movement of 5 cm toward the anode, the complex did not show any movement indicating neutrality of the complex.

2.2. In vitro cell binding studies

$^{99m}Tc(CO)_3$–colchicine complex as well as $[^{99m}TcN]$–colchicine complex corresponding to 1 nmol of colchicine were studied for in vitro binding with fibrosarcoma

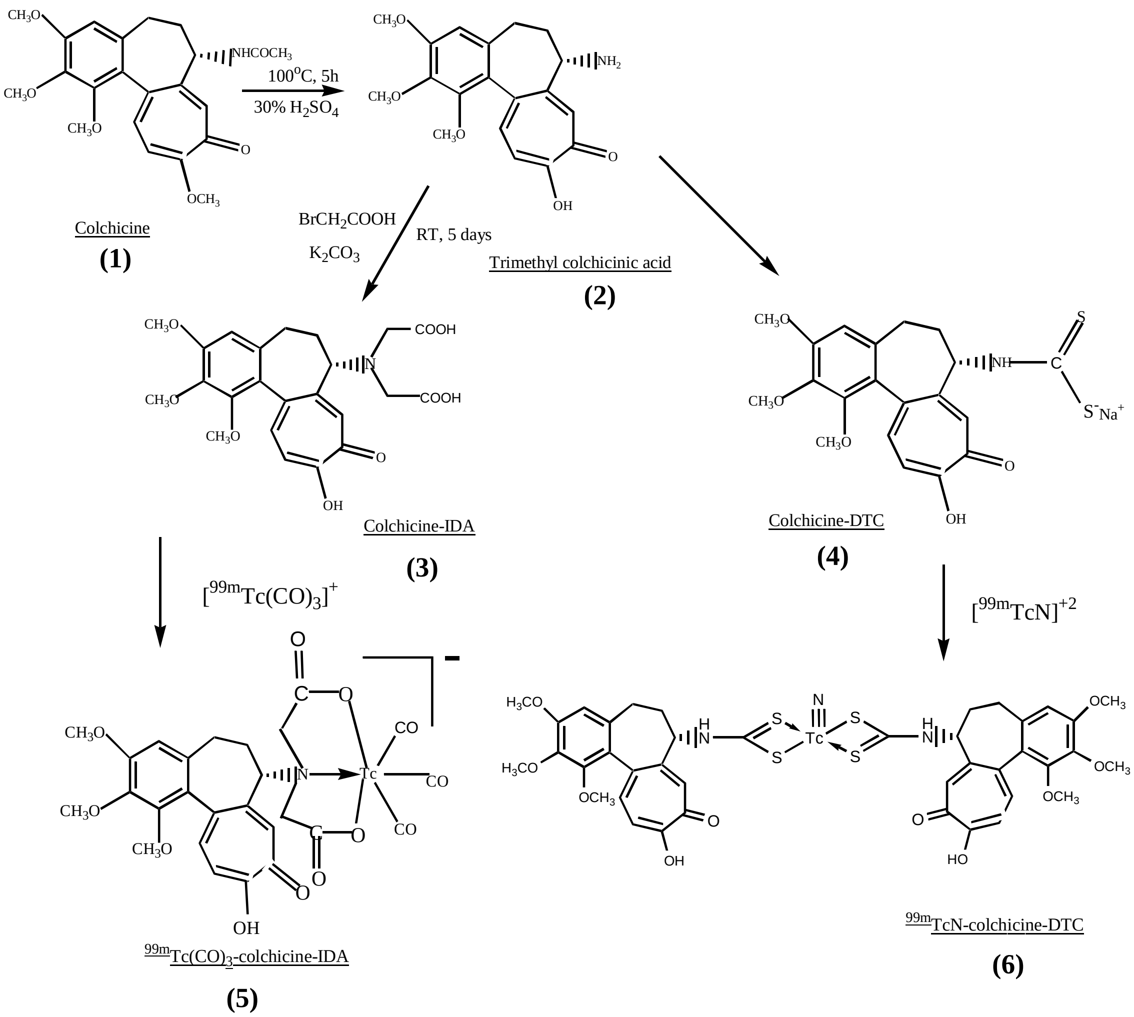


Figure 1. Synthesis of ^{99m}Tc(CO)$_3$–colchicine–IDA and ^{99m}TcN–colchicine–DTC complexes.

and melanoma tumor cells. Compound **5** exhibited significant binding of 50.6 ± 2.2% and 54.2 ± 2.3% with fibrosarcoma and melanoma cells, respectively. Similarly **6** also showed binding of 54.3 ± 1.7% and 57.4 ± 2.9% with respective tumor cells. Since tumor cells have more percentage of dividing cells as compared to normal spleen, normal murine spleen cells showed comparatively much less binding (15%) with both the tracers. Blank experiments were carried out using $[^{99m}Tc(H_2O)_3(CO)_3]^+$ synthon and $[^{99m}Tc{\equiv}N]^{2+}$ intermediate under similar experimental conditions. In this case, activity associated with the cell pellet was only around 10% and 6%, respectively. The in vitro results (Table 1) clearly indicate the affinity of both the products for tumor cells.

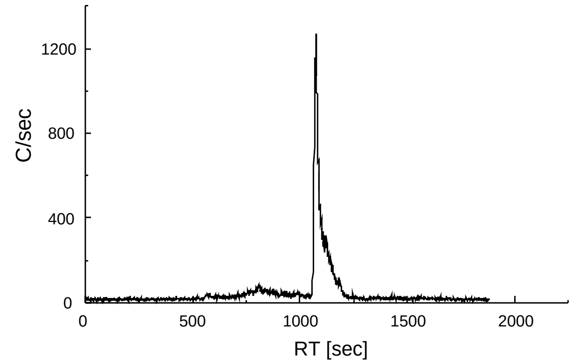
2.3. Biodistribution studies

In biodistribution studies in mice bearing fibrosarcoma tumor, [^{99m}TcN]–colchicine complex showed significant liver accumulation (~60% ID/organ) within 30 min with slow hepatic clearance. There was also very high uptake in lungs and spleen. Although tumor/blood and tumor/muscle ratios were found to increase from 0.52 to 2.4 and 0.68 to 1.16 between 30 min and 4 h, the actual tumor uptake was not very significant (0.42% ID/g at 1 h pi) (Table 2). On the contrary, ^{99m}Tc(CO)$_3$–colchicine–IDA complex exhibited greater tumor accumulation (4.1 ± 1.2% ID/g at 30 min pi) and better pharmacokinetics. The compound partly excreted via the hepatobiliary route and around 25% ID/g was retained in the liver at 4 h pi. Figure 3 shows the detailed results of the biodistribution study with ^{99m}Tc(CO)$_3$–colchicine–IDA complex.

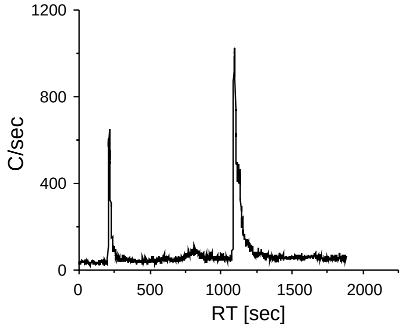
2.4. Imaging studies

^{99m}Tc(CO)$_3$–colchicine–IDA complex was injected in nude mice bearing MCF-7 tumors and scintigraphic images (Fig. 4) were obtained at 1 h pi and 3 h pi. The uptake in tumor is observable, more predominantly at 3 h pi, albeit with significant amount of radioactivity associated with the liver. The activity observed in the bladder was found to be cleared at 3 h pi in concurrence with the results obtained from biodistribution studies as well.

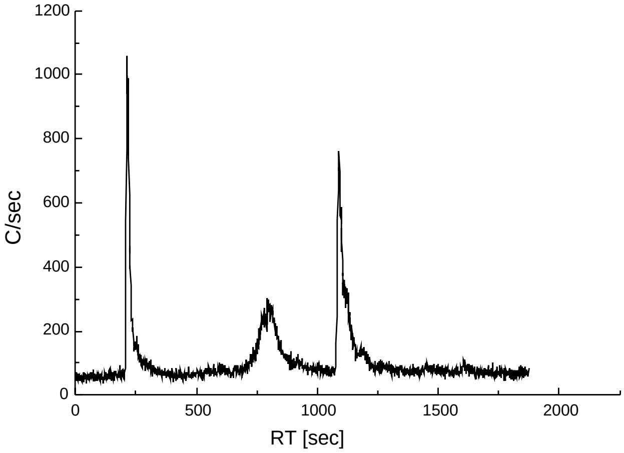
The complexation of tropolone with a number of metallic ions has been reported indicating a possibility of the tropolone moiety complexing with ^{99m}Tc.[13] However, in the present case since the colchicine molecule has been



HPLC pattern of $^{99m}Tc(CO)_3$-colIDA at pH4.0



HPLC pattern of $^{99m}Tc(CO)_3$-colIDA at pH 7



HPLC pattern of $^{99m}Tc(CO)_3$-colIDA at pH 9

Figure 2. HPLC pattern of $^{99m}Tc(CO)_3$–colchicine–IDA at different pH.

Table 2. Biodistribution studies of ^{99m}TcN–colchicine–DTC complex

Organ	% injected dose per organ ($n = 3$)		
	30 min	1 h	4 h
Blood	1.2 (0.16)	0.84 (0.23)	0.29 (0.06)
Liver	61.7 (1.28)	63.4 (0.85)	62.6 (4.08)
Int + GB	2.57 (0.27)	5.29 (0.46)	11.25 (0.7)
Lungs	13.5 (0.69)	11.4 (1.21)	10.69 (3.3)
Spleen	2.68 (0.33)	3.21 (0.12)	2.65 (0.65)
Tumor/gm	0.3 (0.08)	0.42 (0.24)	0.36 (0.1)
Tumor/blood	0.52	0.95	2.4
Tumor/muscle	0.68	0.97	1.16

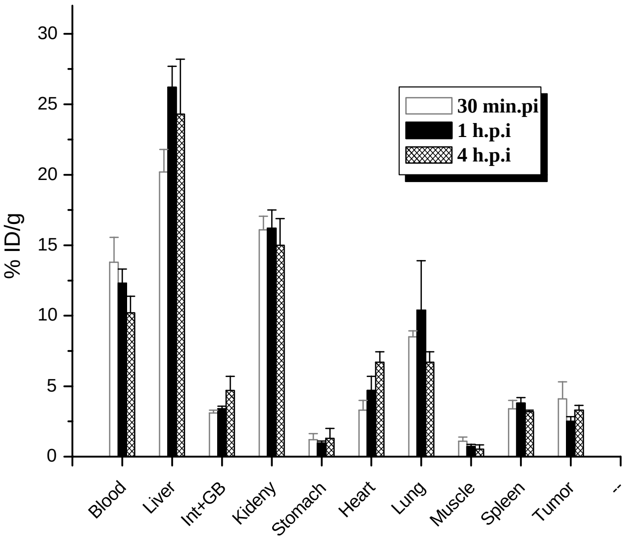


Figure 3. Biodistribution studies of $^{99m}Tc(CO)_3$–colchicine–IDA in a murine fibrosarcoma tumor model ($n = 3$ for each time point).

derivatized to the iminodiacetate substituent and the radiolabeling precursor is not $^{99m}TcO_4^-$ but $[^{99m}Tc(CO)_3(H_2O)_3]^+$ which is known to form stable complexes with the iminodiacetate group (a tridentate chelator) at a low concentration, the complexation with the iminodiacetate derivative is expected to be by far a greater possibility in comparison with that of the chelating groups of tropolone. This has been confirmed by carrying out a blank complexation of trimethylcolchicinic acid **2** with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ wherein no complexation was observed.

Since the [^{99m}TcN]–colchicine complex was found to be neutral, a possible structure which can be proposed indicates the probable involvement of two DTC molecules. Such a speculation could be further corroborated by the observation of Abram et al.[14] wherein formation of an analogous complex structure has been confirmed. However, $^{99m}Tc(CO)_3$–colchicine–IDA complex with nitrogen and carboxyl as coordinating groups of the

Table 1. In vitro studies in tumor cells

Tracer	Fibrosarcoma	Melanoma	Normal spleen cells
$^{99m}Tc(CO)_3$–colchicine—IDA	50.6 ± 2.2	54.2 ± 2.3	12 ± 1.6
$[^{99m}Tc{\equiv}N]$–colchicine– dithiocarbamate	54.3 ± 1.7	57.4 ± 2.9	15 ± 2.1
$^{99m}TcO_4^-$	1.9 ± 0.8	2.2 ± 1.1	—
$[^{99m}Tc{\equiv}N]^{2+}$ intermediate	6.0 ± 1.2	5.8 ± 2.5	—
$[^{99m}Tc(H_2O)_3(CO)_3]^+$	10.4 ± 0.7	9.5 ± 1.6	—

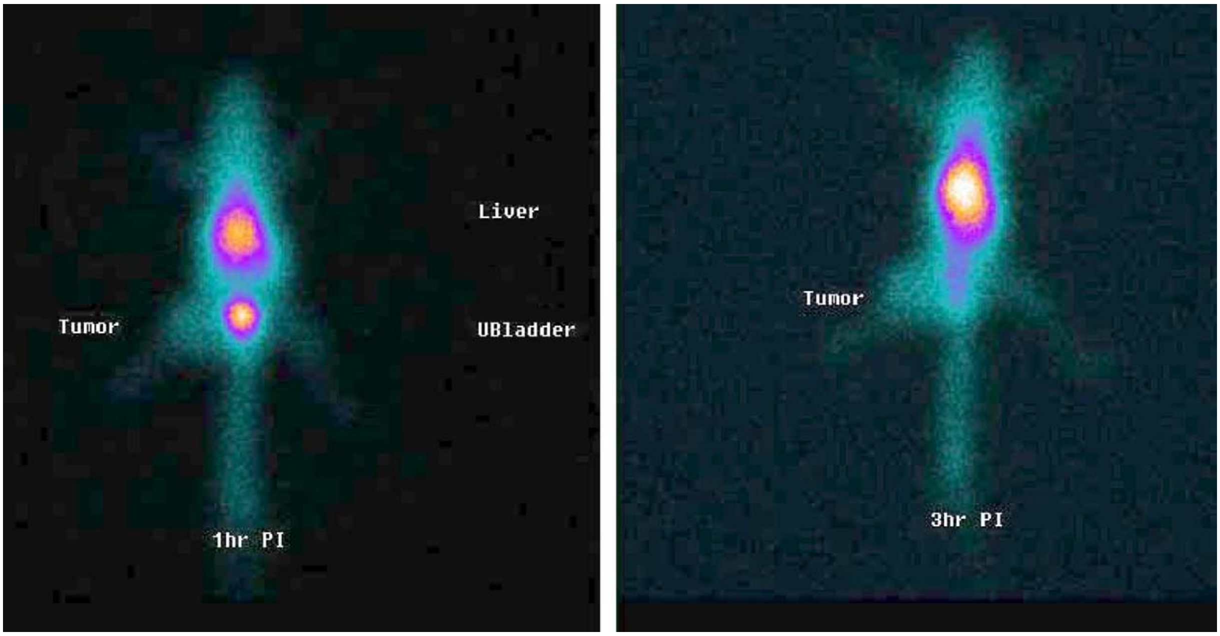


Figure 4. Scintigraphic images of $^{99m}Tc(CO)_3$–colchicine–IDA images in nude mice bearing MCF-7 tumor.

IDA moiety is expected to be uni-negative. ^{99m}Tc-carbonyl complex bearing polar IDA group has shown lower liver accumulation compared to the ^{99m}Tc-nitrido complex bearing two lipophilic DTC molecules. Although both the radiolabeled complexes exhibited significant binding in vitro with tumor cells, the difference in tumor uptake and pharmacokinetic patterns in vivo clearly indicates the influence of lipophilicity of ligands used for complexation. Scintigraphic images also support the results obtained by biodistribution studies.

3. Conclusion

Colchicine derivatives could be successfully radiolabeled with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and $[^{99m}Tc\equiv N]^{2+}$ in high yield and purity. Both the products showed good in vitro tumor cell uptake. However, $^{99m}Tc(CO)_3$–colchicine complex showed more promising results in vivo with respect to reduced liver accumulation and higher uptake in tumor. The tumor-specificity of this agent provides further insight toward its possible utility for imaging multi-drug resistant states.

4. Experimental

4.1. General

All reagents were of commercial grade. Carbon monoxide in 0.5 L refillable canisters was obtained from M/s Alchemie Gases & Chemicals, Mumbai, India. $^{99m}TcO_4^-$ was eluted from an in-house ^{99}Mo/^{99m}Tc column generator using normal saline. Commercial kit for preparation of ^{99m}Tc-nitrido species was obtained from CIS Bio International. Electrophoresis experiments were carried out using 0.025 M phosphate buffer (pH 7.5) at 300 V/cm for 1 h. HPLC analyses were performed on a Jasco PU 1580 system with a Jasco 1575 tunable absorption detector and a radiometric detector system. A C-18 reversed-phase HiQ Sil (5 μM, 250 × 4 mm) column has been used. About 25 μL of the test solution was injected into the column and the elution was monitored by observing the radioactivity profile. The flow rate was maintained at 1 mL/min. The gradient system consisting of eluting solvents H_2O (solvent A) and acetonitrile (solvent B) with 0.1% trifluoroacetic acid was used (0–28 min, 90% A–10% A; 28–30 min, 10% A; 30–32 min, 10% A–90% A). Proton NMR spectra were recorded on a 300 MHz Varian VXR 300 S spectrophotometer. Mass spectra were recorded on a QTOF Micromass Instrument using electron spray ionization (ESI) in positive mode. Elemental analyses were performed on C, H, N, and S elemental analyzer, Thermofinnigan, Flash EA 1112 series. Melanoma and Fibrosarcoma cell lines were procured from 'National Centre of Cell Sciences,' Pune, India. Fibrosarcoma tumors were raised in Swiss mice by subcutaneous injection of $\sim 10^6$ cells/animal. MCF-7 (human breast cancer) tumor-bearing animals were maintained at the nude mice housing facility of the 'Laboratory & Nuclear Medicine Section.' All the animal experiments were carried out in compliance with the relevant national laws as approved by the Local Committee on the Conduct and Ethics of Animal Experimentation.

4.2. Chemical synthesis

4.2.1. Trimethylcolchicinic acid 2. A mixture of **1** (150 mg, 0.37 mmol) and 4.5 mL of 30% concd. sulfuric acid was heated for 5 h at 100 °C in an oil bath. After 5 h, while hot, neutralization of the reaction mixture to pH 7–7.5 was effected with solid sodium carbonate. The resulting frothy mass was filtered and washed with cold water. The filtrate was extracted in chloroform (3× 10 mL), the combined extracts were washed with water and dried over anhydrous sodium sulfate. TLC (silica): 5% glacial acetic acid/acetonitrile. R_f (colchicine) = 0.4, R_f (product) = 0. MS (ESI): mass calcd for $C_{19}H_{21}O_5N$, 343; *m*/*z* (obsd): 344 $[M+H]^+$. ^1H NMR (CD_3OD, δ ppm) 3.6 (s, 3H, $-OCH_3$); 3.86 (s, 3H, $-OCH_3$); 3.9 (s, 3H, $-OCH_3$) 6.7 (s, 1H, 8H); 7.16 (d, 11H); 7.42 (d, 12H); 7.9 (s, 1H, Ar).

4.2.2. Colchicine–IDA derivative (3). Compound **2** (15 mg, 43.4 μmol) was dissolved in methanol and bromoacetic acid (10 mg, 71.96 μmol) was added to it along with potassium carbonate (3 mg, 21.7 μmol) and stirred at room temperature for 5 days. The solvent was removed under vacuum. The product was characterized by TLC in 30% glacial acetic acid/acetonitrile. R_f (trimethylcolchicinic acid) = 0.1, R_f (product) = 0.4. MS (ESI): mass calcd for $C_{23}H_{25}O_9N$, 459; *m*/*z* found, 413, 414 (M−COOH). ^1H NMR (CD_3OD, δ ppm) 3.36 (s, 4H, NCH_2); 3.55 (s, 3H, $-OCH_3$); 3.84 (s, 6H, $-OCH_3$).

4.2.3. Colchicine–dithiocarbamate derivative (4). Compound **2** (20 mg, 58 μmol) was dissolved in diethyl ether and 1:1 equivalent of carbon disulfide (3.5 μL) was added to it along with crushed NaOH (20 mg). The reaction mixture was stirred at room temperature for 2 h when the formation of a yellow precipitate was observed. After removal of the solvent, the precipitate was repeatedly washed with diethyl ether to remove any unreacted trimethylcolchicinic acid. The product was characterized by elemental analyses. C, H, N, S: obsd (calcd) 53.95 (54.16), 5.45 (5.00), 3.12 (3.16), 14.35 (14.46). MS (ESI): mass calcd for $C_{20}H_{20}O_5NS_2Na$, 441; *m*/*z* found, 419 [(M+H)−Na].

4.2.4. $[^{99m}Tc(H_2O)_3(CO)_3]^+$ synthon. The synthon was prepared by a modified procedure reported by Alberto et al.[7] A typical procedure consists of preparation of a mixture of 0.5 mL aqueous solution of $NaBH_4$ (5.5 mg), Na_2CO_3 (4 mg), and Na/K tartrate (15 mg), and purging of carbon monoxide through it for 5 min. After the addition of 1 mL $^{99m}TcO_4^-$ (37 MBq) to the solution, it was heated at 70 °C for 20 min. The reaction mixture was cooled and the pH was adjusted to 8 with 300 μL of 1:3 mixture of 0.5 M phosphate buffer (pH 7.5): 1 M HCl.

4.2.5. $^{99m}Tc(CO)_3$–colchicine IDA. 0.1 mL of methanolic solution of the colchicine IDA derivative (800 μg, 3.4 mM) was added to 0.4 mL $[^{99m}Tc(H_2O)_3(CO)_3]^+$ synthon, adjusted to pH 4.0, and heated at 70 °C for 20 min. The complex was characterized by HPLC.

4.2.6. $[^{99m}Tc{\equiv}N]^{2+}$ core. The commercially available kit vial, containing succinic dihydrazide (5.0 mg), stannous chloride dihydrate (100 μg), 1,2-diaminopropane–*N*,*N*,*N*′,*N*′-tetraacetic acid (5 mg), sodium dihydrogen phosphate (0.5 mg), and disodium hydrogen phosphate (5.8 mg) in freeze-dried form, was used for preparing the precursor. The kit vial stored at 4 °C was allowed to attain ambient temperature. One milliliter of freshly eluted $^{99m}TcO_4^-$ (1 mCi, 37 MBq) was added to the vial, vortexed, and allowed to stand at room temperature for 20 min. The ^{99m}Tc-nitrido intermediate thus prepared was characterized by TLC.

4.2.7. ^{99m}TcN–colchicine–dithiocarbamate. To 0.5 mL solution of colchicine–dithiocarbamate (1 mg, 4.34 mM), 0.5 mL of freshly prepared $[^{99m}Tc{\equiv}N]^{2+}$ precursor was added, vortexed, and incubated for 30 min at room temperature. The final ^{99m}TcN-complex formed herein was characterized by TLC and paper electrophoresis.

4.3. In vitro studies in tumor cells

Melanoma and fibrosarcoma cells were isolated under aseptic conditions from tumor-bearing mice. The cells were washed and cell suspension of 5×10^6 cells/mL was prepared in plain Dulbecco's modified Eagle medium (DMEM). The radiolabeled products namely $^{99m}Tc(CO)_3$–colchicine–IDA as well as ^{99m}TcN–colchicine–dithiocarbamate corresponding to 1 nmol colchicine were incubated at 37 °C for 1 h with 5×10^5 cells. After incubation, the cells were separated by centrifugation. The cell pellet was washed twice and was counted to determine the percentage of activity associated with cells. Nonspecific binding of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ synthon as well as of $[^{99m}Tc{\equiv}N]^{2+}$ intermediate was studied similarly following identical experimental conditions. Uptake in normal spleen cells was also studied for comparison to determine specific binding to tumor cells.

4.4. Biodistribution studies

Biodistribution studies for both the products were carried out in Swiss mice bearing fibrosarcoma tumor. 0.1 mL of the product containing 3–7 MBq of activity was injected via the tail vein. The studies were carried out in triplicate at 30 min, 1 h and 4 h post injection. All major organs as well as tumor were excised, rinsed, weighed, and counted in a NaI(Tl) flat geometry detector in order to estimate the percent of injected dose per gm of the tissue. Blood, muscle, and bone were taken as 7%, 40%, and 10% of total body weight.

4.5. Imaging studies

A Single Head Digital SPECT Gamma Camera (MPS GE, USA) with a parallel hole LEAP collimator was used for imaging studies. $^{99m}Tc(CO)_3$–colchicine–IDA complex (~15 MBq) was injected intravenously through tail vein in nude mice bearing MCF-7 tumors. Static images were acquired using 256 × 256 matrix with 125 KCounts at 1 and 3 h post injection.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.09.006.

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