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Preparation and preliminary evaluation of a tris-metronidazole-$^{99m}Tc(CO)_3$ complex for targeting tumor hypoxia

Madhava B. Mallia[1] · Anupam Mathur[2] · Rohit Sharma[1] · Chandan Kumar[1] · H. D. Sarma[3] · Sharmila Banerjee[4] · Ashutosh Dash[1]

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Abstract

Conventional ^{99m}Tc-radiopharmaceuticals for the detection of tumor hypoxia generally possess a single nitroimidazole moiety. Herein, we report the synthesis and evaluation of a ^{99m}Tc-complex with three-nitroimidazole moieties in an attempt to improve hypoxic cell detection. Isocyanide derivative of metronidazole (MetroNC) was synthesized and subsequently radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex, wherein the three labile water molecules were replaced with MetroNC ligand to form a pseudo-octahedral $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex. Analysis of corresponding $Re(CO)_3$-analog prepared in macroscopic scale confirmed the formation of expected complex. Cyclic voltammetric studies of $[Re(CO)_3(MetroNC)_3]^+$ complex showed no significant change in single-electron reduction potential (SERP) of MetroNC ligand (− 0.96 V) upon forming the $[Re(CO)_3(MetroNC)_3]^+$ complex (− 0.90 V). In vitro studies in Chinese hamster ovary (CHO) cells showed three-fold preferential accumulation of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in hypoxic cells over normoxic cells. Biodistribution studies of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in Swiss mice bearing fibrosarcoma tumor showed tumor uptake and steady retention till 60 min post injection. Present study constitutes a novel design approach towards development of a ^{99m}Tc-radiopharmaceutical for hypoxia imaging application, which could be extended to other potential nitroimidazole ligands.

Keywords Nitroimidazole · Metronidazole · Isocyanide · $^{99m}Tc(CO)_3$ complex · Hypoxia · Pseudo-octahedral complex

Introduction

The negative influence of hypoxia in the clinical management of cancer is well documented [1] and the possible reasons for the formation of hypoxic cancerous lesions are thoroughly understood [2, 3]. Poor prognosis has been associated with the presence of hypoxia in several types cancers such as advanced cancer of the uterine cervix, advanced squamous cell carcinoma of cervix [3–5], head and neck cancers [6–8], adenocarcinoma of pancreas [9] etc. Determination of hypoxic status in cancerous lesions can help in modifying therapeutic strategy for a better clinical outcome. Information on hypoxic status of cancer lesions can also help in selecting patients for hypoxia-directed radiotherapy [10, 11]. Invasive procedures to determine hypoxic status of cancerous lesions, which are also predictive of response to therapy, are available [12]. However, routine use of invasive procedures in a clinical set-up is severely limited due to their technical complexity, inconvenience and the inability to obtain repetitive measurements in target tissue. In addition, due to heterogeneous nature of hypoxia, results obtained through invasive methods are often inconsistent [12]. These factors favor non-invasive techniques for detecting tissue hypoxia.

Nitroimidazoles have been extensively used for non-invasive detection of tissue hypoxia [13, 14]. Due to their

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✉ Madhava B. Mallia
mallia@barc.gov.in

[1] Radiopharmaceuticals Division, Bhabha Atomic Research Centre, Mumbai 400085, India

[2] Medical and Biological Products Program, Board of Radiation and Isotope Technology, Mumbai 400705, India

[3] Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai 400085, India

[4] Radiation Medicine Centre, Parel, Mumbai 400012, India

selective, oxygen-dependent, reduction and trapping in hypoxic cells, these molecules are suitable biological vectors for developing hypoxia detecting radiopharmaceuticals. At present, [^{18}F]Fluoromisonidazole, a PET radiopharmaceutical, is used for clinical imaging of tissue hypoxia [15]. However, SPECT facilities being more common in developing countries, a SPECT-radiopharmaceutical for this application may find wider applicability. Among different SPECT-radioisotopes, owing to favourable nuclear characteristics and easy availability, ^{99m}Tc is often the preferred choice for developing SPECT-radiopharmaceuticals. Therefore, significant efforts have been directed towards the development of a ^{99m}Tc-radiopharmaceutical for hypoxia imaging.

Most of the ^{99m}Tc-radiopharmaceuticals evaluated for the detection of hypoxia contain a single nitroimidazole molecule [16–22]. Our group had extensively evaluated several nitroimidazole-^{99m}Tc(CO)$_3$ complexes with different overall charge, single-electron reduction potential and lipophilicity [20]. Although, misonidazole-^{99m}Tc(CO)$_3$ complex, an analogue of clinically used hypoxia imaging agent [^{18}F]fluromisonidazole, showed encouraging results in animal experiments, overall pharmacokinetics was not at par.

There are few reports on synthesis and evaluation of bis-nitroimidazole-radiometal complex for targeting tissue hypoxia [23–26]. Our group had synthesized and evaluated a ^{99m}TcN(PNP)-complex containing two metronidazole moieties for targeting hypoxic cells [26]. The purpose of introducing more than one nitroimidazole groups in a radiotracer is to improve its chances of oxygen dependent reduction in hypoxic cells, in vivo. We realized that the [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor complex introduced by Alberto et al. offered an excellent route for the preparation of such a complex [27], wherein, the substitution labile water molecules could be easily replaced by suitable nitroimidazole ligands to obtain a stable pseudo-octahedral complex. Recognizing that isocyanides are excellent monodentate ligands suitable for replacing labile water molecules in [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor complex [27], we synthesized isocyanide derivative of metronidazole, a 5-nitroimidazole, which is known to target hypoxic cells. Present work describes the preparation of a ^{99m}Tc(CO)$_3$ complex containing three metronidazole moieties and their preliminary biological evaluation in vitro as well as in vivo.

Materials and methods

General

The compound 1-(2-isocyanoethyl)-2-methyl-5-nitro-1H-imidazole (MetroNC) was synthesized following a procedure reported earlier [26]. Bis-tetraethylammonium Rheniumtricarbonyl tribromide ([NEt$_4$]$_2$[Re(CO)$_3$(Br$_3$)]) was also synthesized following a reported procedure [28]. All reagents were of analytical grade and used without additional purification. Sodium pertechnetate was obtained from ^{99}Mo/^{99m}Tc alumina column generator, constructed in-house. The [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor was prepared using Isolink® carbonyl kit vial obtained as a gift from Mallinckrodt Medical B. V. HPLC analyses were performed on a JASCO PU 2080 Plus dual pump HPLC system, Japan, with a JASCO 2075 Plus tunable absorption detector and a Gina Star radiometric detector system, using a C18 reversed phase HiQ Sil (5 µm, 4 × 250 mm) column. Aqueous 0.05 M triethylammonium phosphate (TEAP) buffer, pH = 2.5 (Solvent A) and methanol (Solvent B) were used as the mobile phase for HPLC. The HPLC solvents were filtered through 0.22 µ filter before use. The elution started with 100% A from 0 to 6 min. At 6 min the eluent switched to 75% A and 25% B and at 9 min to 66% A and 34% B followed by a linear gradient 66% A/34% B to 100% B from 9 to 20 min. Up to 30 min, the eluent remained at 100% B before switching back to the initial conditions. Flow rate was maintained at 1 mL/min. Infrared spectra were recorded on a JASCO-FT/IR-420 spectrophotometer, Japan. ^{1}H-NMR spectrum was recorded on a 300 MHz Bruker AvanceII NMR spectrometer. High-resolution mass spectra of the Re(CO)$_3$(MetroNC)$_3$ complex was recorded on a Bruker MicroTOFQ-2, Germany, by ESI. Cyclic voltammograms of MetroNC ligand and Re(CO)$_3$(MetroNC)$_3$ complex were recorded on a CH instrument (CHI760D), USA. The CHO cells used for in vitro studies were obtained from National Centre for Cell Science (NCCS), Pune.

Synthesis

Synthesis of Re(CO)$_3$ complex of 1-(2-isocyanoethyl)-2-methyl-5-nitro-1H-imidazole

MetroNC ligand (16 mg, 0.09 mmol) was refluxed with 0.3 eq of [NEt$_4$]$_2$[Re(CO)$_3$(Br$_3$)] (19 mg, 0.03 mmol) in acetonitrile for 4 h. Subsequently, solvent from the reaction mixture was removed, water was added and extracted with ethyl acetate (3 × 7 mL). Organic extracts were pooled together, washed with saline and dried. Upon removal of the organic solvent, complex **2** could be obtained in practically pure form (21 mg, 91%). R_f (ethyl acetate) = 0.4. IR (neat, cm^{-1}) 3127 (w); 2958 (w); 2923 (w); 2853 (w); 2221 (m); 2197 (m); 2037 (s); 1977 (s); 1923 (s); 1531 (m); 1470 (m); 1427 (m); 1364 (m); 1260 (m); 1189 (m); 1148 (w); 1011 (m); 824 (w); 743 (w); 679(w). ^{1}H-NMR (δ ppm, CDCl$_3$) 2.63 (s, 3H, –CH$_3$), 4.39 (m, 2H, –C$\underline{H}_2$CH$_2$NC), 4.72 (m, 2H, –CH$_2$C$\underline{H}_2$NC), 8.01

(s, 1H, metronidazole-C4-H). HRMS (ESI^+) m/z: 811.1802 $(M+H)^+$.

Radiolabeling

Preparation of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex

The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex was prepared using Isolink® kit vial by adding ~ 1 mL (~ 37 MBq) of freshly eluted $Na^{99m}TcO_4$ from $^{99}Mo/^{99m}Tc$ alumina column generator and heating the vial at 95 °C for 20 min. Thereafter, the vial was cooled, re-equilibrated to atmospheric pressure and the pH was adjusted to 7 using 1:3 mixture of 0.5 M phosphate buffer (pH 7.4):1 M HCl.

Radiolabeling of MetroNC with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex

Under optimized conditions, 900 µL of 10^{-3}M 5% aqueous ethanolic solution of MetroNC was mixed with 100 µL (~ 3 MBq) of freshly prepared $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex and the reaction mixture was incubated at 75 °C for 45 min. Thereafter, the preparation was cooled to room temperature and analyzed by HPLC.

Physicochemical studies

Electrochemical studies

Cyclic voltammogram of relevant nitroimidazole ligands and $[Re(CO)_3(MetroNC)_3]^+$ complex were obtained using a glassy carbon working electrode, platinum counter electrode and Ag/Ag^+ as reference electrode, following a procedure reported earlier [24]. Before recording the voltammograms, the test solutions were thoroughly purged with high purity argon to remove tracers of dissolved oxygen. The voltammograms was recorded within the potential range of 0 to − 2.5 volts. Scan rate was maintained at 50 mV/s.

HPLC

The radiochemical purity (RCP) of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex as well as the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex were analyzed by HPLC. About 15 µL of the radioactive preparation was injected into the HPLC column and the UV/radioactivity profile was monitored as a function of time.

Determination of octanol–water partition coefficient ($LogP_{o/w}$)

Octanol–water partition coefficients of the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex was determined following a reported procedure [29]. The study was carried out in triplicate and average $LogP_{o/w}$ was reported along with standard deviation.

Serum stability studies

Stability of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in human serum was evaluated by incubating the radiolabeled complex (~ 0.37 MBq) in human serum (500 µL) at 37 °C. A 100 µL aliquot was withdrawn after 3 h and serum proteins are precipitated by adding equal volume of ethanol. The mixture was centrifuged and the supernatant was analyzed by HPLC to assess stability of the complex in serum.

In vitro cell accumulation study under normoxic and hypoxic conditions

Accumulation of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in CHO cells under normoxic and hypoxic conditions were studied following a reported procedure [30, 31]. About 15 mL aliquot of CHO cells (1×10^6 cells/mL) in suspension culture at 37 °C in RPMI medium, supplemented with 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum, was kept stirring in a glass vial. The glass vial was flushed with a gentle, continuous flow of warm, humidified gas mixture of 95% air/5% carbon dioxide (aerobic exposure) or 95% nitrogen/5% carbon dioxide (hypoxic exposure). After equilibration for 45 min, about 0.1 mL of freshly prepared $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex was added to the vial such that final activity of 0.3 MBq/mL was obtained. Under these conditions, concentration of nitroimidazole in the vial was approximately 1 µg/mL. At 0.5, 1 and 3 h post incubation, 1 mL aliquots in triplicate was withdrawn from the vial. Subsequently, the ratio of activity in cells to that of supernatant was determined following a reported procedure [32].

Biodistribution studies

The procedures performed herein were in strict compliance with the national laws governing the conduct of animal experiments. Solid tumor models were developed in Swiss mice by implantation of HSDM1C1 murine fibrosarcoma cells obtained from National Centre for Cell Science (NCCS), Pune, India. About 10^6 cells were injected subcutaneously on the dorsum of the Swiss mice. The tumors were grown to a size of 10 mm in diameter before the animals were used for the experiment. Individual sets of

animals (n = 3) were administered with the HPLC purified radioactive preparation (~ 1.1 MBq per animal in 100 μL volume) through the lateral tail vein and incubated for different time points (30, 60, and 180 min). At the end of the respective time intervals, the animals were sacrificed and the relevant organs excised for measurement of retained activity. The organs were weighed and the activity associated with them was measured in a flat-bed type NaI(Tl) counter with energy window adjusted for ^{99m}Tc. The activity associated with each organ/tissue was expressed as percentage injected dose per gram (%ID/g).

Results and discussion

Mediated by nitroreductase enzymes, nitroimidazoles undergo a series of one-electron reduction in hypoxic cells leading to permanent or transient trapping inside hypoxic cells. The presence of oxygen, however, prevents such reduction, which explains non-accumulation of nitroimidazole in normoxic cells. One of our previous studies had shown that the time spend by the radiotracer inside hypoxic cell is an important parameter that decides its efficacy to target hypoxic cells. To be an effective agent for hypoxic cell detection, the radiotracer should undergo reduction within the limited time that it spends in hypoxic cells. To increase the chances of hypoxia specific reduction we envisaged a radiometal complex with multiple nitroimidazole moieties. We recognized that the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex offered a simple and attractive route to achieve this objective [27]. This precursor complex allowed substitution of the three labile water molecules with suitable monodentate ligands, such as isocyanide, resulting in a pseudo-octahedral $^{99m}Tc(CO)_3$ complex of the type $[^{99m}Tc(CO)_3(nitroimidazoleNC)_3]^+$. A similar complex was reported by Kothari et al., wherein the three labile water molecules in $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex were replaced by three *tert*-butyl isocyanide ligands [33]. We envisaged preparation of a $^{99m}Tc(CO)_3$ complex with three metronidazole isocyanide molecules from $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex and MetroNC ligand.

The synthesis of MetroNC ligand was carried out following a procedure reported by our group earlier (Fig. 1) [26]. Radiolabeling of the MetroNC ligand was carried out using freshly prepared $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex as well as the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex were analyzed by HPLC. Typical radioactivity elution profile of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex with retention time 3.7 ± 0.2 min was reported earlier [22]. Under similar conditions, the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex appeared at 15.4 ± 0.3 min (n = 3) in the HPLC chromatogram (Fig. 2a). From the peak area measurements radiochemical purity of the complex was found to be > 95%.

We prepared $[Re(CO)_3(MetroNC)_3]^+$ complex in macroscopic level for structural characterization. The precursor complex, $[NEt_4]_2[Re(CO)_3(Br_3)]$, prepared following a reported procedure, was used as the source for $Re(CO)_3$ core [27]. Figure 2b shows the UV-elution profile of $[Re(CO)_3(MetroNC)_3]^+$ complex, which matched with the radioactivity profile of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex [Fig. 2a]. This expected result indicated the formation of similar type of complexes both at the no carrier added level as well as macroscopic level.

The IR spectra of $[Re(CO)_3(MetroNC)_3]^+$ complex showed absence of peak at 2150 cm^{-1} representing –NC stretching in the free MetroNC ligand. Instead, two peaks (2220 and 2196 cm^{-1}) appeared at higher frequency, consistent with the coordination of isocyanide group to $Re(CO)_3$ core. Similar observations were made with alkyl isocyanide-$Cr(CO)_3$ complexes reported earlier [34]. Three strong IR peaks in the range 2034–1916 cm^{-1} marked the presence of *fac*-$Re(CO)_3$ core. The ^{1}H-NMR of the $[Re(CO)_3(MetroNC)_3]^+$ complexes also had signatures indicative of coordination of –NC group with the $Re(CO)_3$ core. The methylene protons linking the nitroimidazole group and $Re(CO)_3$ core in the complex showed a downfield shift compared to the corresponding methylene protons in the free MetroNC ligand. This could be attributed to the decreased electron density on N-atom of the –NC group, consequent to its coordination to Re-metal centre. Observable changes in the ^{1}H-NMR peak splitting is evidence indicating ligand–metal coordination in the complex. The two methylene protons, which appeared as triplets in free MetroNC ligand, were seen as multiplets in the $[Re(CO)_3(MetroNC)_3]^+$ complex. This could be attributed to the hindrance in free rotation over the C–C bond of the linker, which rendered the methylene protons diastereotopic. A four peak isotopic cluster observed in the high resolution mass spectrum of $[^{185/187}Re(CO)_3(MetroNC)_3]^+$ complex with ion peaks at m/z 809.1761, 810.1791, 811.1802 and 812.1815 provided the confirmatory evidence for the formation of expected, pseudo-octahedral complex containing three MetroNC molecules (Fig. 3).

The single electron reduction potential (SERP) of nitroimidazole is an important molecular parameter, which decides its efficiency of reduction in hypoxic cells [35–38]. Any modification to the nitroimidazole ring or its electronic environment can potentially alter its SERP and consequently, its efficiency of reduction in hypoxic cells. In the present work, metronidazole was synthetically modified to an isocyanide derivative for radiolabeling with $^{99m}Tc(CO)_3$ core. Though Adams et al. [36, 38] had shown that any synthetic modifications two or more carbon–

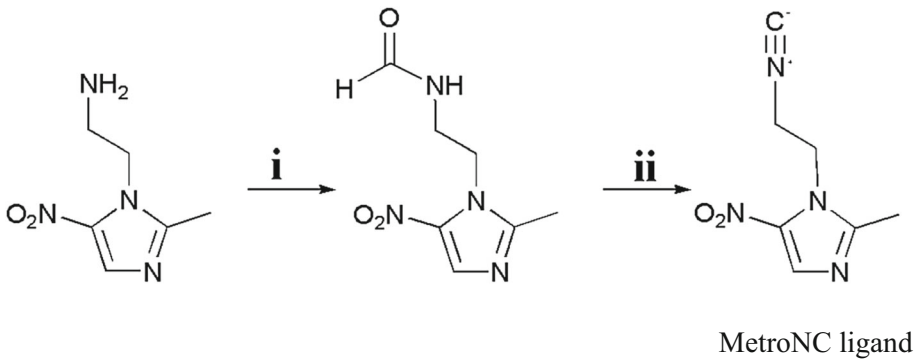

Fig. 1 Synthesis of nitroimidazole isocyanide ligands (**i**) Triethylamine (4 eq), Ethyl formate, Reflux (**ii**) Anhyd. pyridine (2 eq), p-TsCl (1.2 eq), anhydr. Dichloromethane, Room temperature

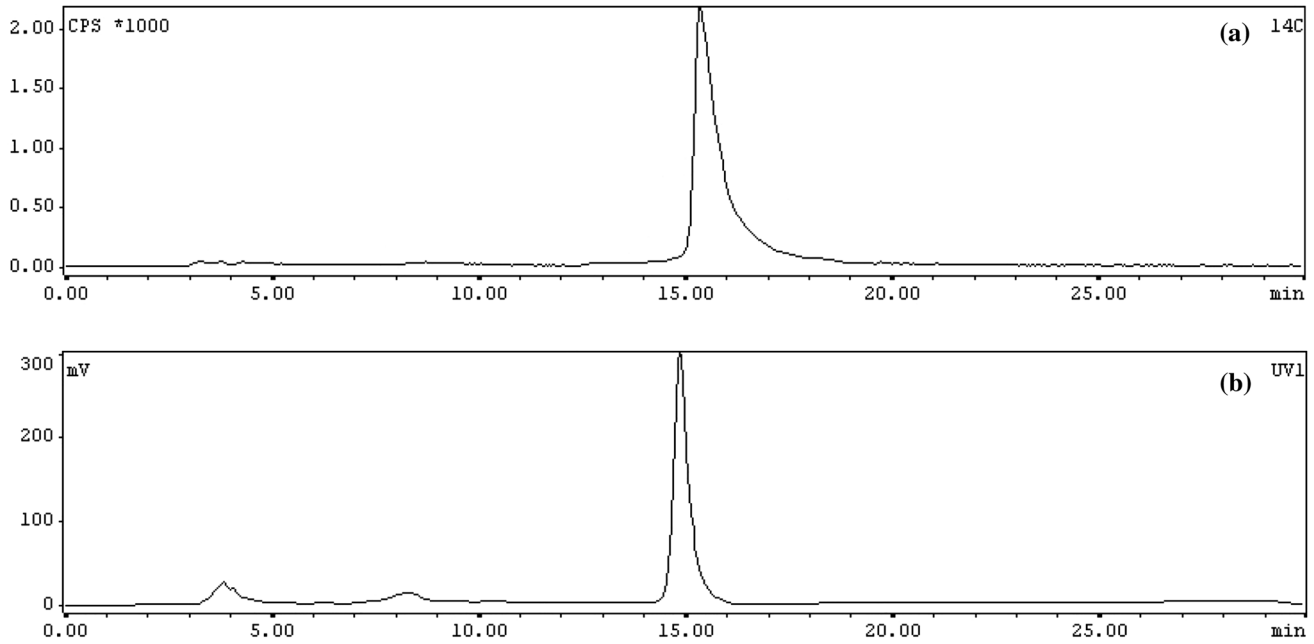

Fig. 2 Typical HPLC elution profile of **a** $(MetroNC)_3^{99m}Tc(CO)_3$ complex, **b** $(MetroNC)_3Re(CO)_3$ complex

carbon bonds away from the nitroimidazole ring had insignificant effect on its SERP, we carried out cyclic voltammetric studies of MetroNC as well as $[Re(CO)_3(MetroNC)_3]^+$ complex for confirmation. A single-electron reduction peak at − 0.99 V was observed in the cyclic voltammogram (CV) of unmodified metronidazole, while MetroNC ligand showed single electron reduction peak at − 0.96 V. These observations are completely in agreement with the observations of Adams et al. [36, 38].

Formation of a complex is another process which can potentially alter the electronic environment of the coordinating nitroimidazole ligand and consequently, its SERP. The Fig. 4 shows the cyclic voltammogram of MetroNC ligand and corresponding $[Re(CO)_3(MetroNC)_3]^+$ complex. It could be noted that the $[Re(CO)_3(MetroNC)_3]^+$ complex showed a single-electron reduction peak at − 0.90 V, compared to − 0.96 V shown by free MetroNC ligand. The small shift in SERP value of $Re(CO)_3(MetroNC)_3$ complex indicates possible influence of the metal centre on the nitroimidazole moiety.

The $LogP_{o/w}$ value of the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex determined following a reported procedure [29] was 0.46 (0.04). Stability of the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in human serum was analyzed following a reported protocol and it was found to the stable in human serum during the period of study (3 h).

Accumulation of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in CHO cells under normoxic and hypoxic conditions were studied to evaluated hypoxic cell selectivity of the radiotracer. Figure 5 shows the accumulation of activity in CHO cells as a function of time under normoxic and hypoxic conditions. It could be noted that accumulation of

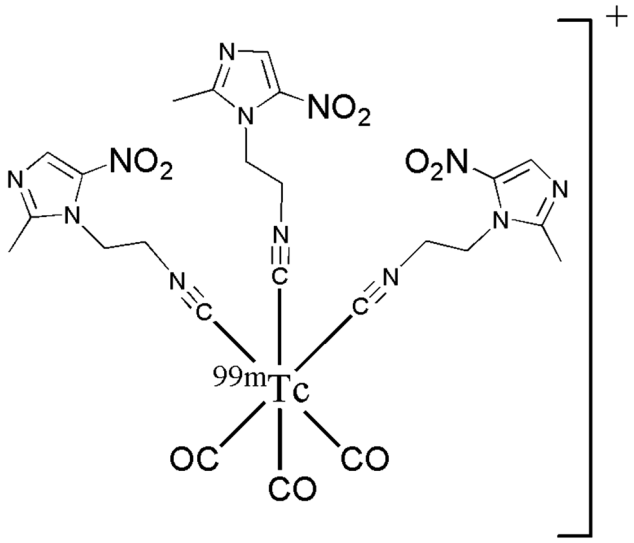

Fig. 3 Probable structure of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex

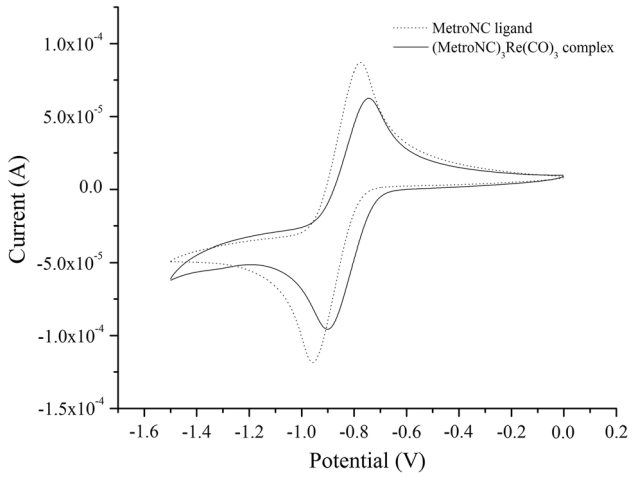

Fig. 4 Cyclic voltammograms of MetroNC ligand and $[Re(CO)_3(MetroNC)_3]^+$ complex

$[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex consistently increases under hypoxic conditions. The C_{in}/C_{out} ratio, the ratio of activity associated with the cell pellet to that of supernatant, under hypoxic conditions was more than twice the C_{in}/C_{out} ratio under normoxic condition, which is indicative of the hypoxia selectivity of the radiotracer.

Biodistribution of the $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex was carried out in Swiss mice bearing fibrosarcoma tumor. Table 1 shows the distribution of the complex in tumor bearing Swiss mice at different time points. The $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex showed a steady retention of activity in tumor ($\sim$ 0.67%ID/g) till 1 h p.i., before decreasing to 0.31%ID/g at 3 h p.i. Major clearance of the activity from the body was through hepatobiliary route. This is evident from the presence of significant levels of activity in liver and gastrointestinal tract. The

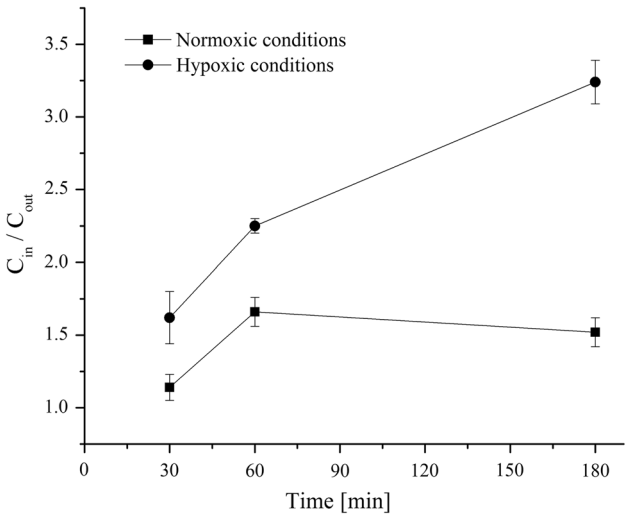

Fig. 5 Accumulation of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in CHO cells under normoxic and hypoxic conditions

Table 1 Distribution of $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex in Swiss mice bearing fibrosarcoma tumor

Organs	% ID/g (s.d.)[a] (n = 3)		
	$^{99m}Tc(CO)_3(MetroNC)_3$ complex		
	30 min	60 min	180 min
Liver	12.84 (5.26)	10.26 (1.59)	10.24 (3.26)
Intestine	19.24 (6.80)	20.64 (0.18)	24.89 (3.34)
Stomach	1.21 (0.27)	0.60 (0.11)	0.38 (0.12)
Kidney	4.80 (2.80)	1.93 (0.39)	1.65 (0.36)
Heart	0.97 (0.55)	0.27 (0.05)	0.49 (0.14)
Lungs	1.52 (0.44)	1.18 (0.08)	0.89 (0.19)
Blood	1.03 (0.15)	0.69 (0.12)	0.35 (0.08)
Muscle	0.48 (0.03)	0.16 (0.05)	0.14 (0.03)
Tumor	0.67 (0.10)	0.67 (0.17)	0.31 (0.09)
T/B	0.65 (0.09)	1.00 (0.27)	0.91 (0.22)
T/M	1.38 (0.11)	4.38 (0.51)	2.23 (0.16)

[a]*s.d.* standard deviation

maximum tumor/blood ratio obtained for $[^{99m}Tc(CO)_3(MetroNC)_3]^+$ complex was 1, at 60 min p.i. Similarly, peak tumor to muscle ratio of the complex, 4.38 (0.51), was also observed at 60 min p.i.

Conclusions

Present study reports the synthesis and evaluation of a ^{99m}Tc-complex with three-nitroimidazole groups to detect tumor hypoxia. The $^{99m}Tc(CO)_3$ chemistry was

effectively utilized to achieve this objective. In vitro studies showed consistent increase in accumulation of the radiotracer in CHO cells under hypoxic conditions, indicating its ability to target hypoxic cells. Present study also constitutes a novel design approach towards development of a ^{99m}Tc-radiopharmaceutical for hypoxia imaging application.

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