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A study on nitroimidazole-$^{99m}Tc(CO)_3$ complexes as hypoxia marker: Some observations towards possible improvement in in vivo efficacy

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ABSTRACT

Introduction: Hypoxia plays a negative role in the clinical management of cancer. Detection of hypoxic status of a cancer is important for selecting patients for hypoxia directed therapy. Though [^{18}F]fluoromisonidazole ([^{18}F]FMISO), a PET radiopharmaceutical, is presently being used in the clinic for the detection of hypoxia, considering the logistical advantages of ^{99m}Tc and wider availability of SPECT scanners, a radiopharmaceutical based on this isotope may find wider applicability.
Methods: Nine nitroimidazole (2-, 4- and 5-nitroimidazole) ligands were synthesized and radiolabeled using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor to obtain a group of complexes possessing different single electron reduction potential (SERP), overall charge and lipophilicity, the three attributes which decide the efficacy of the complex to detect hypoxic cells in vivo. The nitroimidazole-$^{99m}Tc(CO)_3$ complexes as well as [^{18}F]FMISO were evaluated in fibrosarcoma tumor bearing mice.
Results: The $^{99m}Tc(CO)_3$ complexes of nitroimidazole iminodiacetic acid (IDA) showed better tumor uptake and retention than nitroimidazole diethylenetriamine (DETA) and nitroimidazole aminoethylglycine (AEG) complexes. Tumor uptake observed with [^{18}F]FMISO was higher than any of the nitroimidazole-IDA-$^{99m}Tc(CO)_3$ complexes. However, [^{18}F]FMISO clearance from tumor was found to be faster compared to 2-nitroimidazole-IDA-$^{99m}Tc(CO)_3$ complex. Observed tumor uptake and retention of the radiotracers evaluated could be correlated to its blood clearance pattern and SERP.
Conclusions: Results of the present study indicated that uptake of the radiotracer in tumor is closely associated with its rate of clearance from blood. The study also indicated that along with SERP, clearance of radiotracer from blood (net effect of charge and lipophilicity) is a critical factor which decides the in vivo efficacy of the hypoxia detecting radiopharmaceutical.

1. Introduction

Tissue hypoxia is a condition where cells have inadequate oxygen supply leading to compromise in their biological functions. Hypoxia can occur due to several reasons including pathological conditions such as cancer, cardio/cerebrovascular disease, diabetes, infection/wound healing etc [1]. Hypoxia in cancerous lesions is a serious problem as it has direct implication on the prognosis and therapeutic outcome of the disease. The events leading to the formation of hypoxic regions in cancerous tissue are well documented [2,3].

Several experimental and clinical data had indicated a deleterious influence of hypoxia in tumor propagation, malignant progression and resistance to radiation therapy and chemotherapy [4]. Tumor hypoxia was associated with poor prognosis, especially in the case of advanced squamous cell carcinoma of cervix, advanced cancer of the uterine cervix [3,5,6], head and neck [7–9], adenocarcinoma of pancreas [10], soft tissue sarcoma etc [11]. Other potential problems associated with hypoxia have been reviewed [12]. Appropriate assessment of the hypoxic status of cancerous lesions can assist clinical oncologist to modify treatment strategy for a better clinical outcome. It can also help clinicians in selecting patients who will benefit from hypoxia directed therapy, thereby relieving other patients from the unnecessary therapeutic burden on their already deteriorated health condition.

Nitroimidazoles are widely explored compounds that can undergo oxygen dependent enzymatic reductions resulting in selective accumulation in hypoxic cells [13,14]. Several PET and SPECT agents having nitroimidazole pharmacophore have been evaluated for the detection of hypoxia [15–33]. At present, [^{18}F]FMISO is the most widely used radiopharmaceutical for the clinical assessment of tissue hypoxia [34]. Other PET radiopharmaceuticals that are clinically evaluated for imaging hypoxia, albeit to a lesser extent, include [^{18}F]FETA [17], [^{18}F]EF1 [35], [^{18}F]EF5 [36–38], [^{18}F]FAZA [39,40] and [^{18}F]FETNIM [41]. Though [^{18}F]FMISO is being used, its pharmacokinetics is far from ideal and has limitations owing to high uptake and slow clearance from non-target organs [42,43]. However, studies have established that 2 h images

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obtained using [^{18} F]FMISO clearly reflected the distribution of clinically significant hypoxic regions in the cancerous tissue [14].

Considering the favorable nuclear characteristics of ^{99m}Tc and availability of relatively larger number of SPECT scanners, a ^{99m}Tc-radiopharmaceutical which can provide clinical information equivalent to that of [^{18} F]FMISO, or superior, may find wider application. One of the possible reasons for the unsuccessful attempts to develop a radiopharmaceutical superior to [^{18} F]FMISO may be the lack of clear understanding of the optimal combination of physico-chemical parameters necessary for the radiotracer to achieve this objective. This is especially true for SPECT radiopharmaceuticals incorporating a transition metal radionuclide like ^{99m}Tc, which may form charged complexes. A charge on the complex can significantly alter its pharmacokinetics compared to neutral compounds. However, this apparent 'complication' may as well be looked upon as a tool to fine tune the pharmacokinetics of the complex in a favorable manner. To the best of our knowledge, there is no report on the comprehensive evaluation of the influence of different molecular properties that ultimately decides the overall efficacy and clinical utility of an agent indented for use in detecting tissue hypoxia.

There are evidences indicative of the role of SERP in trapping of nitroimidazole in hypoxic cells. For example, metronidazole, a 5-nitroimidazole, which has an SERP of −486 mV has been observed to undergo reduction in anaerobes but not in aerobes [44]. However, another compound misonidazole, a 2-nitroimidazole derivative having an SERP of −389 mV, has shown reduction in aerobes [45]. This indicates that the more positive the SERP value of a nitroimidazole, the better the chances of its reduction in cells having limited oxygen supply (hypoxic cells). Zhang et al. had reported an in vitro study on the effect of lipophilicity on the accumulation of 2-nitroimidazole complexes of ^{99m}Tc in CHO cells. The authors observed that anaerobic cell accumulation of the complex was related to P (partition coefficient), but within a very narrow range [46]. To the best of our knowledge in vivo evaluation of nitroimidazole ^{99m}Tc-complexes bearing different overall charge is not reported. In the present study, three series of 2-, 4- and 5-nitroimidazole ligands having tridentate ligands, viz. IDA, DETA and AEG, were envisaged and synthesized. These ligands upon radiolabeling with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor formed complexes with different overall charge, SERP and lipophilicity. The biological evaluation of these complexes was carried out in Swiss mice bearing fibrosarcoma tumor, and the results obtained were carefully analyzed to understand the influence of these molecular properties on overall pharmacokinetics of the radiotracer. [^{18} F]FMISO was also evaluated in the same animal model, and the results are compared with other nitroimidazole-$^{99m}Tc(CO)_3$ complexes.

2. Materials and methods

2.1. Synthesis

2.1.1. General

2-Nitroimidazole, 4-nitroimidazole and Boc-aminoethylamine were purchased from Aldrich, USA. Diethylenetriamine, 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON), N-ethyl-diisopropylamine (DIEA), 1, 3-dibromopropane and anhydrous potassium carbonate were purchased from Fluka, Germany. All reagents were of analytical grade and used as such without additional purification unless otherwise mentioned. Silica gel plates (silica gel 60 F_{254}) used for thin layer chromatography (TLC) as well as silica gel (60–120 mesh) used for column chromatography were obtained from Merck, India. [^{18} F]FMISO was obtained from Radiation Medicine Centre, Mumbai, India. Sodium pertechnetate was eluted from ^{99}Mo/^{99m}Tc column generator using normal saline. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex was prepared using Isolink® carbonyl kit vial obtained as a gift from Mallinckrodt Medical B. V. The HPLC analyses were performed on a JASCO PU 2080 Plus dual pump HPLC system, Japan, with a JASCO 2075 Plus tunable UV detector and a Gina Star radiometric detector system. A C18 reversed phase HiQ Sil (5 μm, 4 × 250 mm) column is used to effect separation of various components in the radioactive preparation. IR spectra were recorded on a JASCO-FT/IR-420 spectrophotometer, Japan. ^{1}H-NMR spectra were recorded either on a 300 MHz Varian VXR 300S spectrophotometer or 300 MHz Bruker AvanceII spectrophotometer. Mass spectra were recorded on a Varian 500MS Ion Trap mass spectrometer, USA. Cyclic voltammograms were recorded on a CH instrument (CHI760D), USA.

2.1.2. IDA derivatives of 2-, 4- and 5-nitroimidaole (1, 2 and 3)

The synthesis and characterization of 2-, 4- and 5-nitroimidazole-IDA derivatives were reported earlier [29].

2.1.3. DETA derivatives of 2-, 4- and 5-nitroimidaole

2.1.3.1. Synthesis of tert-butyl 2-(2-(tert-butoxycarbonyl)aminoethyl) aminoethylcarbamate (4). Selective protection of primary amine groups of diethylenetriamine was carried out using BOC-ON [47]. Diethylenetriamine (0.95 g, 9.2 mmol) in dry tetrahydrofuran (20 mL) was cooled to 0 °C in an ice bath. BOC-ON dissolved in dry tetrahydrofuran (10 mL) was added drop-wise to this solution over a period of 3 h with continuous stirring. On completion of the addition, the reaction mixture was brought to room temperature, and the stirring was continued for another 24 h. The solvent was removed using rotary evaporator. The pale yellow residue was dissolved in chloroform (100 mL) and washed with 10% NaOH (50 mL portions) till organic layer became colorless. Thereafter, organic layer was washed with brine (50 mL) and dried over anhydrous sodium sulphate. The solvent was removed using rotary evaporator, and the residue was purified by silica gel column chromatography eluting with ethyl acetate (2.34 g, 84%). R_f (methanol) = 0.70. IR (neat, cm^{-1}) 3342(w); 2976 (m); 2932(w); 2854(w); 2816(w); 1695(s); 1528(m); 1455(w); 1391(w); 1366(m); 1774(m); 1750(m); 1172(s); 781(w). ^{1}H-NMR ($CDCl_3$, δ ppm) 1.41 (s, 18H, $((\underline{CH_3})_3CO(CO)NHCH_2CH_2)_2NH$); 2.71 (t, 4H, J = 5.9 Hz, $((CH_3)_3CO(CO)NHCH_2\underline{CH_2})_2NH$); 3.19 (m, 4H, $((CH_3)_3CO(CO)NH\underline{CH_2}CH_2)_2NH$). MS ($ESI^+$): 304.4 $(M + H)^+$.

2.1.3.2. Synthesis of tert-butyl 2-((2-(tert-butoxycarbonyl)aminoethyl) (3-bromopropyl)amino) ethylcarbamate (5). To compound 4 (0.55 g, 1.8 mmol) in acetonitrile (5 mL), DIEA (0.35 g, 2.7 mmol) and 1,3-dibromopropane (3.6 g, 18 mmol) were added and the mixture refluxed for 24 h with continuous stirring. The solvent was removed using rotary evaporator and further work-up was carried out following the general procedure described below. Pure compound 5 was obtained by silica gel column chromatography eluting with diethyl ether (0.35 g, 45%). R_f (diethyl ether) = 0.66, IR (neat, cm^{-1}) 3345(w); 2975 (m); 2926(w); 2850(w); 2814(w); 1694(s); 1517(m); 1455(w); 1391(w); 1365(m); 1774(m); 1749(m); 1171(s); 783(w). ^{1}H-NMR ($CDCl_3$, δ ppm) 1.45 (s, 18H, $((\underline{CH_3})_3CO(CO)NHCH_2CH_2)_2$ N-); 1.98 (quintet, 2H, J = 6.9 Hz, $BrCH_2\underline{CH_2}CH_2$N-); 2.56 (t, 4H, J = 5.4 Hz, $((CH_3)_3CO(CO)NHCH_2\underline{CH_2})_2$ -N-); 2.62 (t, 2H, J = 6.9 Hz, $BrCH_2CH_2\underline{CH_2}$N-); 3.19 (m, 4H, $((CH_3)_3CO(CO)NHC\underline{H_2}CH_2)_2$ N-); 3.47 (t, 2H, J = 6.9 Hz, $Br\underline{CH_2}CH_2CH_2$N-). MS ($ESI^+$): 426.4 $(M + H)^+$.

2.1.3.3. Synthesis of tert-butyl 2-((2-(tert-butoxycarbonyl)aminoethyl) (3-(2-nitro-1H-imidazol-1-yl)propyl)amino)ethylcarbamate (6). To compound 5 (0.1 g, 0.24 mmol) in acetonitrile (5 mL), DIEA (0.07 g, 0.54 mmol) and 2-nitroimidazole (0.04 g, 0.36 mmol) were added and the reaction mixture refluxed for 12 h with continuous stirring. The solvent was removed using rotary evaporator, and further work-up was carried out following the general procedure described below. Pure compound 6 was obtained by silica gel column chromatography eluting with ethyl acetate (0.1 g, 87 %). R_f (ethyl acetate) = 0.56, IR (neat, cm^{-1}) 3345 (m); 3115(w); 2972 (m); 2931 (m); 2853(w); 2818(w); 1699 (s); 1530 (s); 1471 (m); 1369 (m); 1251 (m); 1173 (s); 1122(m); 1062 (w); 972 (m); 917 (w); 859 (w); 825 (w); 745 (w); 656 (w). ^{1}H-NMR ($CDCl_3$, δ ppm) 1.43 (s, 18H, $((\underline{CH_3})_3CO(CO)NHCH_2CH_2)_2$ N-); 2.05 (quintet, 2H,