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On the structural modification of 2-nitroimidazole-$^{99m}Tc(CO)_3$ complex, a hypoxia marker, for improving in vivo pharmacokinetics

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ABSTRACT

Introduction: A 2-nitroimidazole-$^{99m}Tc(CO)_3$ complex reported earlier showed promise with respect to its uptake and retention in hypoxic tumor. However, significant uptake and slow clearance from liver imposed severe limitations towards advocating its possible practical utility. In an attempt to improving its liver clearance, an ether linkage, which is known to help in liver clearance, was introduced in the molecule.
Methods: The 2-nitroimidazole iminodiacetic acid (IDA) derivative containing an ether linkage was synthesized in a five step procedure from 2-nitroimidazole. This derivative was radiolabeled using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex. The corresponding $Re(CO)_3$ analogue was also synthesized in the macroscopic level for structural characterization. The $^{99m}Tc(CO)_3$ complex was evaluated in an animal model bearing fibrosarcoma tumor.
Results: The in vivo evaluation of the complex indicated that, as envisaged, introduction of the ether linkage has improved clearance from the liver. The complex also showed higher retention in tumor compared to the 2-nitroimidazole-IDA-$^{99m}Tc(CO)_3$ complex reported earlier. Though the tumor to muscle ratio improved with time, the tumor to blood ratio did not show any significant improvement. Despite improved liver clearance, there was significant liver activity present even at 3 h p.i. attributable to gradual accumulation of activity cleared from muscle and blood.
Conclusions: Though the introduction of ether linkage improved liver clearance of the modified 2-nitroimidazole complex, it was found that a single ether linkage was not sufficient to achieve the desirable level of clearance. Probably, a linker with multiple ether groups, such as a di- or tri-ethylene glycol spacer, may be a possible solution to this issue.

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1. Introduction

Knowing the hypoxic status of a cancer is important in its clinical management. Several studies have amply demonstrated the intimate correlation existing between hypoxia and tumor propagation, malignant progression and resistance to radiation therapy and chemotherapy. Especially with advanced squamous cell carcinoma of cervix, advanced cancer of the uterine cervix [1–3], head and neck cancer [4–6], adenocarcinoma of pancreas [7], soft tissue sarcoma etc. [8] presence of hypoxia has been correlated with poor prognosis. Other problems associated with hypoxia in cancerous tissue have also been thoroughly reviewed [9].

There are several invasive techniques, like use of polarographic oxygen electrodes, immunohistochemical markers etc., which are available to detect and quantify hypoxia in tumors [10–14]. Though these techniques have proved their efficacy to predict the response to therapy, none of them are in routine clinical practice because of their technical complexity, inconvenience and inability to enable repetitive measurements. Also, due to heterogeneous nature of hypoxia, these 'limited sampling' techniques more often failed to present the actual hypoxia distribution in the tissue. Hence, non-invasive procedures such as MRI and imaging using radiopharmaceuticals have gained popularity and more clinical applicability. Though MRI has proved its clinical utility in the detection of hypoxia [15–18], the influence of temperature, pH, presence of other metal ions etc. on the relaxation time of the contrast agents made it difficult to draw a direct correlation with tissue oxygen concentration and limited its wider applicability.

Nitroimidazole-based radiopharmaceuticals are probably the most widely explored agents for the non-invasive detection of tissue hypoxia. Nitroimidazoles undergo oxygen dependent reduction in cells that result in selective accumulation in hypoxic cells [19]. The $[^{18}F]$Fluoromisonidazole (^{18}F-FMISO), a 2-nitroimidazole derivative, is probably the most widely used PET-radiopharmaceutical for clinical imaging of hypoxia [20]. Other clinically evaluated, PET-based 2-nitroimidazole radiopharmaceuticals include $[^{18}F]$FAZA ($[^{18}F]$Fluoroazomycin-arabinofuranoside) [21,22] and $[^{18}F]$FETNIM ($[^{18}F]$Fluoroerythronitroimidazole) [23]. Despite a few drawbacks [19], FMISO

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still continues to be the radiopharmaceutical of choice for imaging hypoxia, largely because of the absence of an alternate agent. The optimal decay characteristics, easy availability and low cost of ^{99m}Tc make it the obvious and first choice for envisaging new agents for this specific application. Identification of a ^{99m}Tc based radiopharmaceutical that can provide diagnostic information equivalent to ^{18}F-FMISO, or with improved properties, therefore, constitutes a relevant area of research. It is this factor that provides the impetus to the continued development of new ^{99m}Tc based agents to target hypoxia.

Among several SPECT-based radiopharmaceuticals evaluated to target hypoxia [24–26], 2-nitroimidazole-based radiopharmaceuticals showed promising potential owing to their more favorable single electron reduction potential (SERP) compared to that of the 4- and 5-nitroimidazoles [27]. We have recently reported the synthesis and evaluation of three $^{99m}Tc(CO)_3$-complexes prepared from corresponding iminodiacetic acid (IDA) derivatives of 2-, 4- and 5-nitroimidazole [28]. Though, both 2- and 5-nitroimidazole complex showed uptake and retention in tumor, in terms of absolute uptake, 2-nitroimidazole complex was better which was as per expectation. However, slow clearance of 2-nitroimidazole complex from the liver does not constitute a desirable feature as it may put limitation to its utility to image tumors in abdominal region. Structural modification of the radiotracer to enhance hydrophilicity which would reduce liver uptake could be a possible alternative. However, lipophilicity is essential for efficient distribution of the radiotracer in tumor by passive diffusion and too much reduction in lipophilicity may compromise the tumor uptake. A possible solution would be to incorporate appropriate functional groups, for example ether group, in the radiotracer that will accelerate its liver clearance. This strategy has been utilized in the development of ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin [29,30] and several other myocardial imaging agents [31].

Working in similar lines, the 2-nitroimidazole-IDA-$^{99m}Tc(CO)_3$ complex that has previously shown uptake and retention in tumor [28] was synthetically modified to incorporate an ether linkage between the 2-nitroimidazole and IDA group. The modified ligand was radiolabeled using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex and the resulting $^{99m}Tc(CO)_3$-complex was evaluated in animal model bearing fibrosarcoma tumor.

2. Synthesis

2.1. General

2-nitroimidazole, dibromoethyl ether, phthalimide, hydrazine hydrate, diisopropyl ethyl amine (DIEA) and potassium carbonate were purchased from Aldrich, USA. Sodium pertechnetate was eluted using normal saline from a ^{99}Mo/^{99m}Tc column generator. 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. Silica gel plates (silica gel 60 F_{254}) used for TLC as well as silica gel (60–120 mesh) used for column chromatography were obtained from Merck, India. The 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 column (5μm, 4×250 mm). IR spectra of the samples were recorded on JASCO-FT/IR-420 spectrophotometer, Japan. ^{1}H NMR spectra were recorded either on a 300 MHz Varian VXR 300 S spectrophotometer or 300 MHz Bruker AvanceII spectrophotometer. Mass spectrum was recorded on a Varian 500MS Ion trap mass spectrometer, USA.

*2.1.1. Synthesis of 1-(2-(2-bromoethoxy)ethyl)-2-nitro-1H-imidazole (**1**)*

To 2-nitroimidazole (0.20 g, 1.77 mmol) in acetonitrile (10 mL), crushed potassium carbonate (0.25 g, 1.77 mmol) and dibromoethyl ether (2 g, 8.85 mmol) were added and the reaction mixture was refluxed for 12 h. Upon completion of the reaction, the solvent was removed using rotary evaporator. The residue obtained was dissolved in water (30 mL) and extracted with chloroform (15 mL×3). The combined organic layer was washed with brine, dried and the solvent was evaporated to obtain the crude product as thick oil. The target compound **1** was obtained (0.40 g, 87 %) by silica gel column chromatography using diethyl ether as eluant. R_f (diethyl ether) = 0.4; IR (neat, cm^{-1}) 3139(m), 3118(m), 2963(m), 2920(m), 2872(m), 1537(s), 1485(s), 1438(w), 1360(s), 1286(s), 1219(w), 1184(w), 1162(s), 1119(s), 1019(w), 997(w), 943(w), 916(m), 889(w), 835(s), 781(s), 665(w), 649(m), 630(w); ^{1}H NMR ($CDCl_3$, δ ppm) 3.40 (t, 2H, J = 6.0 Hz, 2-nitroimidazole-CH_2CH_2-$OCH_2\underline{CH_2}Br$), 3.74 (t, 2H, J = 6.0 Hz, 2-nitroimidazole-$CH_2CH_2O\underline{CH_2}CH_2$-Br), 3.86 (t, 2H, J = 4.5 Hz, 2-nitroimidazole-($CH_2\underline{CH_2}OCH_2CH_2Br$), 4.63 (t, 2H, J = 4.5 Hz, 2-nitroimidazole-$\underline{CH_2}CH_2OCH_2CH_2Br$), 7.13 (s, 1H, 2-nitroimidazole-C5-H), 7.24 (s, 1H, 2-nitroimidazole-C4-H).

*2.1.2. Synthesis of 2-(2-(2-(2-nitro-1H-imidazol-1-yl)ethoxy)ethyl) isoindoline-1,3-dione (**2**)*

To compound **1** (0.20 g, 0.76 mmol) in acetonitrile (10 mL), crushed potassium carbonate (0.25 g, 1.77 mmol) and phthalimide (0.13 g, 0.91 mmol) were added and the mixture was refluxed for 48 h. Thereafter, the solvent was removed using rotary evaporator and the residue obtained was dissolved in 0.1 N NaOH (20 mL). The basic aqueous layer was extracted with chloroform (15 mL×3). The combined organic layer was washed with brine, dried and the solvent was evaporated to obtain the crude product. Pure compound **2** was obtained by silica gel column chromatography using diethyl ether as eluant (0.15 g, 60%). R_f (diethyl ether) = 0.3; IR (neat, cm^{-1}) 3117(w), 2922(m), 2870(m), 1771(m), 1710(s), 1536(s), 1485(s), 1359(s), 1286(m), 1160(m), 1117(m), 1025(w), 916(w), 834(m), 782(w), 721(m), 648(w); ^{1}H NMR ($CDCl_3$, δ ppm) 3.67 (t, 2H, J = 5.3 Hz, 2-nitroimidazole-$CH_2CH_2OCH_2\underline{CH_2}$-phthalimide), 3.80 (t, 2H, J = 4.8 Hz, 2-nitroimidazole-$CH_2\underline{CH_2}OCH_2CH_2$-phthalimide), 3.87 (t, 2H, J = 5.3 Hz, 2-nitroimidazole-$CH_2CH_2O\underline{CH_2}CH_2$-phthalimide), 4.55 (t, 2H, J = 4.8 Hz, 2-nitroimidazole-$\underline{CH_2}CH_2OCH_2CH_2$-phthalimide), 6.84 (s, 1H, 2-nitroimidazole-C5-H), 7.08 (s, 1H, 2-nitroimidazole-C4-H), 7.73- 7.84 (m, 4H, -$CH_2CH_2OCH_2CH_2$-phthalimide-$\underline{H}$).

*2.1.3. Synthesis of 1-(2-(2-aminoethoxy)ethyl)-2-nitro-1H-imidazole (**3**)*

To compound **2** (0.15 g, 0.45 mmol) in ethanol (10 mL), hydrazine hydrate (0.15 g, 4.5 mmol) was added and the reaction mixture refluxed overnight. A white precipitate formed was filtered, washed with cold ethanol (2 mL) and dried. The precipitate was heated with 2 N HCl (5 mL) at 50 °C for 2 h and the residue obtained was filtered off. The filtrate on evaporation gave the target compound as hydrochloride salt (**3**). The product was further purified by recrystallization from ethanol/ether mixture (0.07 g, 79%). IR (KBr, cm^{-1}) 3369(w), 3144(w), 3117(w), 2921(s), 2852(m), 1509(w), 1474(s), 1437(w), 1392(m), 1362(m), 1278(m), 1125(s), 912(w), 745(m), 667(m), 628(w).

*2.1.4. Synthesis of 1-(N,N-bis(tert-butoxycarbonylmethyl)-2-(2-(2-nitroimidazolyl)ethoxy)ethane (**4**)*

To compound **3** (0.07 g, 0.36 mmol) in acetonitrile (5 mL), DIEA (0.16 g, 1.26 mmol) was added and the solution was stirred for 5 min. To this solution tert-butyl bromoacetate (0.18 g, 0.9 mmol) was added and the reaction mixture refluxed for 12 h. The solvent was removed using rotary evaporator. The residue obtained was dissolved in water (15 mL) and extracted with chloroform (10 mL×3). The chloroform layers were combined, washed with brine and dried over anhydrous sodium sulphate. The crude product after the removal of chloroform was purified by silica gel column chromatography in ether to yield the target compound **4** (0.09 g, 57%). R_f (diethyl ether) = 0.6; IR (Neat, cm^{-1}) 3116(w), 2976(m), 2928(m), 2870(m), 1732(s), 1651(w), 1539(w), 1474(s), 1435(w), 1392(w), 1366(s), 1278(m), 1255(w), 1220(m), 1147(s), 1123(s), 974(w), 913(w), 835(w), 743(m), 666(m), 623(w); ^{1}H NMR ($CDCl_3$, δ ppm) 1.45 (s, 18H, -$CH_2CH_2OCH_2CH_2N$ ($CH_2CO_2C(\underline{CH_3})_3)_2$), 2.92 (t, 2H, J = 5.5 Hz, 2-nitroimidazole-$CH_2CH_2OCH_2\underline{CH_2}N$-), 3.48 (s, 4H, 2-nitroimidazole-

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