P025 PREPARATION AND BIODISTRIBUTION OF [61CU] DIACETYL-BIS-(N4-METHYLTHIOSEMICARBAZONE) AS A POSSIBLE PET RADIOPHARMACEUTICAL

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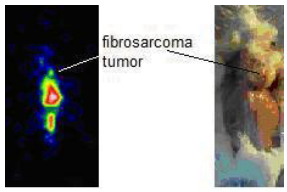
Objectives: Hypoxia is an important determinant for biological behavior of malignant solid tumors. In vitro and in vivo studies have shown that the presence of hypoxia in a tumor is closely related to resistance to radiotherapy and chemotherapy. Therefore, the detection of tumor hypoxia is important to predict tumor malignancy and to determine a medical treatment plan. Preclinical studies have shown that some copper-bis-thiosemicarbazones especially, Cu-ATSM accumulates avidly in hypoxic cells, but washes out rapidly from normoxic cell. Copper-61 is a positron emitter ($T_{1/2}$=3.33 h, ß$^+$: 62%, E.C: 38%), with excellent potentials for application in positron emission tomography (PET) method and molecular imaging. Based on the interesting properties of ^{61}Cu and the possibility of its production via ^{nat}Zn(p,x)^{61}Cu, we were interested in the production and purification of this radionuclide and its ultimate use in radiolabeling of ATSM as a possible PET tracer.

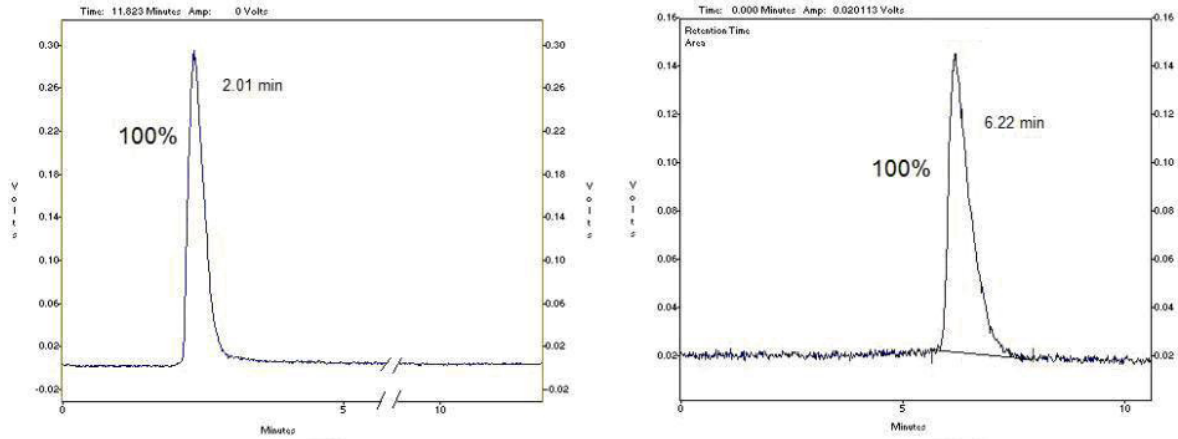
Methods: The production of ^{61}Cu was performed at the Nuclear Research Center for Agriculture and Medicine (NRCAM) 30 MeV cyclotron (Cyclone-30, IBA). [^{61}Cu]diacetyl-bis-(N4-methylthiosemicarbazone) ([^{61}Cu]ATSM) was prepared using in house made ATSM ligand and [^{61}Cu]$CuCl_2$ produced via the ^{nat}Zn(p,x)^{61}Cu (180¼A proton irradiation, 22MeV, 3.2h) and purified by a ion chromatography method. [^{61}Cu]ATSM was administered into normal and tumor bearing rodents up to 210 minutes followed by biodistribution and co-incidence imaging studies.

Results: [^{61}Cu]ATSM radiochemical purity was >97% shown by HPLC and RTLC methods. A significant tumor/non-tumor accumulation was observed by either animal scarification or imaging method.

Conclusions: The method used in this research for the production and chemical separation of ^{61}Cu was quite simple and cost effective. Total labeling and formulation of [^{61}Cu]ATSM took about 5 minutes, with a yield of higher than 98%. [^{61}Cu]ATSM is PET radiopharmaceutical for hypoxia imaging with an intermediate half-life, and our experiments on this radiopharmaceutical have shown satisfactory quality, suitable for future PET studies in human.



Co-incidence images for [61Cu] ATSM uptake of a fibrosarcoma bearing rat 60 minutes post injection. Thus, 60-120 min was confirmed to be the best acquisition time post tracer injection



HPLC diagram for [61Cu]Cu2+ (in acetate and chloride form, left) and [61Cu]ATSM (right) using a reverse phase column with a mixture of H2O:CH3CN (1:1) as eluent

P026 PREPARATION AND BIOLOGICAL EVALUATION OF [61CU]BLEOMYCIN COMPLEX AS A POSSIBLE PET RADIOPHARMACEUTICAL FOR FIBROSARCOMA TUMOR IMAGING

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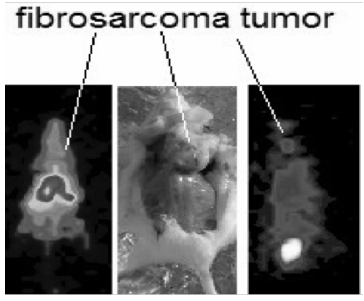
Objectives: In this study, we investigated the possibility of incorporating ^{61}Cu as a positron emitter with an antineoplastic compound, bleomycin, for use in tumor imaging. We optimized ^{61}Cu complex formation conditions with bleomycin, to develop [^{61}Cu]BLM. We hereby report production and biodistribution of this complex in tumor bearing mice.

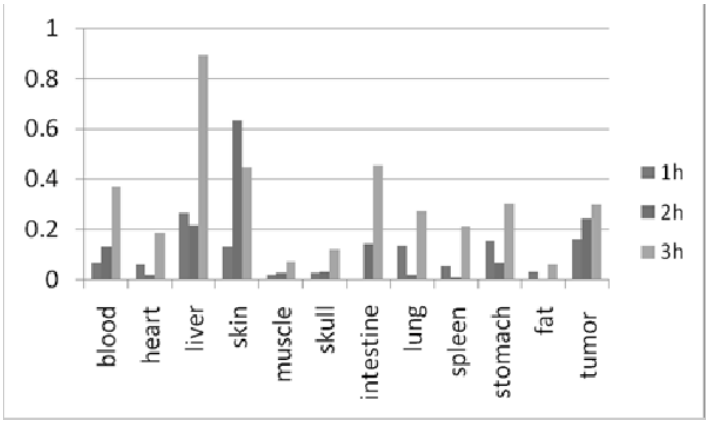
Methods: Copper-61 (T1/2=3.33h) was produced via the ^{nat}Zn(p,x)^{61}Cu nuclear reaction in a 30 MeV cyclotron. The product was used for preparation of [^{61}Cu]bleomycin ([^{61}Cu]BLM). the compound was injected to normal and fibrosarcoma-bearing mice for the biodisribution and co-incidence imaging.

Results: Radio-thin layer chromatography showed an overall radiochemical purity of >90% at optimized conditions after labeling. HPLC showed a radiochemical purity more than 95% (specific activity=400-450 GBq/M). The stability of the radioconjugate was tested in presence of human serum at 37ºC.

Conclusions: The biodistribution of tracer was checked in normal and tumor-bearing animals using scarification and co-incudence studies up to 3 hours and a significant accumulation took place in liver and kidneys, while significant fibrosarcoma uptake was observed in all animals after 3 hours. [^{61}Cu]BLM is PET radiopharmaceuical with an intermediate half life, and our experiments on this radiopharmaceutical have shown satisfactory quality, suitable for future PET studies.

Figure: coincidence imaging in fibrosarcoma bearing mice (left) and Biodistribution of 61 Cu in fibrosarcoma mice (right)





P027 IN VITRO AND IN VIVO EVALUATION OF HYDROPHILIC 64CU-BIS(THIOSEMICARBAZONE) BIOCONJUGATES FOR PET IMAGING OF HYPOXIA

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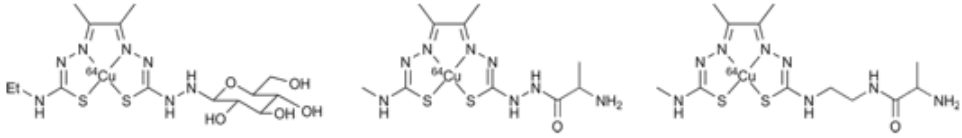
Objectives: The ^{64}Cu complex of diacetyl-bis(N-methylthiosemicarbazone) (ATSM) has been demonstrated to be an effective agent for the imaging and treatment of hypoxic tumours. However the pharmacokinetic properties of ^{64}Cu-ATSM are not ideal, with a particular problem being high liver uptake. Improved imaging/therapeutic properties could potentially be achieved by the development of a strategy for the derivatisation of ^{64}Cu-ATSM which allows control over its biodistribution without impeding its selectivity for hypoxic tissue. Liver uptake might be reduced by conjugation to hydrophilic biomolecules such as carbohydrates and amino acids.



Methods: A route to the exocyclic functionalisation of bis(N-methylthiosemicarbazone) ligands via hydrazine or alkyl amine linker groups was established, and conjugates with glucose and alanine were synthesised and radiolabelled. LogP and serum stability studies were carried out. Uptake of the ^{64}Cu-ATSM bioconjugates in vitro into HeLa cells was investigated at various dissolved oxygen concentrations. MicroPET imaging of the tracers was carried out in male BDIX rats implanted with a P22 syngeneic carcino-sarcoma. Images of ^{64}Cu-ATSM and $^{64}CuCl_2$ were also obtained in the same model were used for comparison.

Results: The ^{64}Cu-ATSM bioconjugates showed oxygen concentration dependent uptake in vitro, and under anoxic conditions gave comparable cellular uptake to ^{64}Cu-ATSM. In all cases normoxic uptake was lower than ^{64}Cu-ATSM, leading to higher anoxic/normoxic ratios. PET imaging revealed the tracers to show broadly similar patterns of biodistribution to ^{64}Cu-ATSM (and $^{64}CuCl_2$), including significant tumor uptake. Only ^{64}Cu-ATSM showed substantial brain uptake, whereas the hydrophilic bioconjugtes were the only tracers to undergo renal clearance and accumulation in the bladder. Liver uptake was not reduced in comparison to the controls.

Conclusions: In vitro the ^{64}Cu-ATSM bioconjugates show enhanced hypoxia selectivity compared to ^{64}Cu-ATSM in HeLa cells. The in vivo PET results indicate that the renal excretion pathway is active for the bioconjugates but not ^{64}Cu-ATSM and $^{64}CuCl_2$. Contrary to expectation the hydrophilic conjugates do not show reduced liver retention in comparison to the controls and it is believed that this is the result of degradation of the ^{64}Cu-ATSM core in the liver itself.

Research Support: Siemens Molecular Imaging, Technology Strategy Board, CRUK (C5255/A8591)

P028 RADIOSYNTHESIS OF [I-131]IAZGP VIA NUCLEOPHILIC SUBSTITUTION AND ITS BIOLOGICAL EVALUATION AS A HYPOXIA MARKER IN COMPARISON WITH CONVENTIONALLY-PRODUCED [I-131] IAZGP

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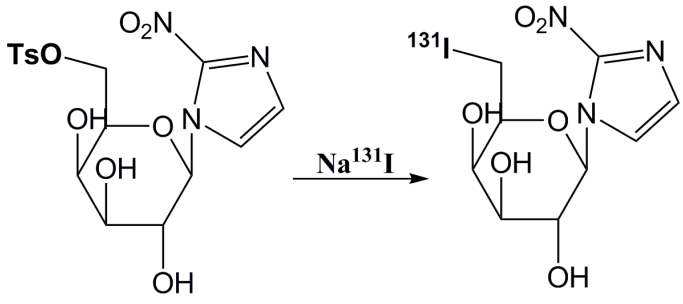
Objectives: a) To label the hypoxia marker IAZGP with radioiodine via nucleophilic substitution, producing the tracer in specific activity 500-3000 times higher (high SA IAZGP) than that of conventionally-labeled IAZGP via iodine-radioiodine exchange (low SA IAZGP) and b) to determine if the difference in specific activity influences the hypoxia-mapping ability of the radiotracers by comparing their biological behavior.

Methods: High SA [^{131}I]IAZGP was prepared by substitution of the tosyl functionality with [^{131}I]iodide. Low SA [^{131}I]IAZGP was prepared according to the literature procedure[1]. In-vitro uptake of high and low SA [^{131}I]IAZGP by HCT8 and HT29 cells was assessed in normoxic and hypoxic conditions. Their biodistribution and intra-tumor localization were also studied by injection of radiotracer into HT29 tumor-bearing mice.

Results: The nucleophilic substitution reaction proceeded efficiently in acetonitrile at 150°C. In 15 minutes, 70-80% of ^{131}I was incorporated into IAZGP molecules. The synthesis time including HPLC purification and formulation was approximately 90 min. The labeling efficiency depended upon the molar ratio between precursor and NaOH present in the reaction medium, temperature and the solvent in which the reaction took place. [^{131}I]IAZGP was produced in the highest efficiency when the precursor-to-base ratio was greater than approximately 0.5, while as the ratio approached 0.1, the yield became close to zero. At 100°C only 10% of the radioactivity was associated with IAZGP at 15 min. The reaction in DMF and 2-pentanone appeared to be affected by side-reactions which altered the chemical form of [^{131}I]iodide and/or decomposed the precursor. The average specific activity of high SA [^{131}I]IAZGP was 30GBq/μmol, whereas that of low SA [^{131}I]IAZGP was 34MBq/μmol. In vitro, the tracer was incorporated into cells depending on the oxygen levels the cells were exposed to, giving ratios between extreme hypoxia and normoxia of 5-6 in HT29 and 3-4 in HCT8 cells at 3 hours. Comparable rates of uptake were observed with low SA [^{131}I]IAZGP. In HT29 tumor-bearing mice, high and low SA [^{131}I]IAZGP tracers were distributed similarly with a correlation coefficient greater than 0.9, giving tumor-to-blood ratios of 5-6 at 3 hrs and 8-9 at 24 hrs. Ex vivo autoradiography revealed heterogeneous intra-tumor localization of high SA [^{131}I]IAZGP corresponding closely to distributions of other exogenous and endogenous hypoxia markers. In contrast, well-oxygenated tumor regions exhibited low accumulation. There were no observable differences between high and low SA IAZGP in the microregional distribution patterns.

Conclusions: a) Radioiodine-labeled IAZGP can be produced via nucleophilic substitution in high yields and in specific activity thousands-fold higher than that of the same tracer conventionally-produced and is validated as an exogenous hypoxia radiotracer for in vivo imaging of hypoxia. b) Specific activity does not appear to be a factor influencing the hypoxia-labeling ability of the radiotracer.

References: 1) Schneider et al. J Labelled Compd Radiopharm (1997) 39:541-557



[^{131}I]IAZGP

P029 PHOSPHONATE-COMPLEXES OF GALLIUM-68 FOR BONE TUMOUR IMAGING

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Objectives: As ^{99m}Tc-phosphonates are well established tracers for the diagnoses of bone metastases using SPECT, analogue attempts for PET using the ^{68}Ge/^{68}Ga generator based ^{68}Ga tracers would be potentially useful. Therefore molecules containing phosphonate structure with binding affinities to apatite and being adequate complexing agents for trivalent Gallium could be considered as interesting vectors for the synthesis of generator-based PET-tracers for skeletal imaging. EDTMP, different triazacyclononane- (n=1-3 phosphonates) and DOTA-derivatives (tetraphosphonate) as well as new phosphonate structures were investigated.

Methods: Germanium-68 provides the positron emitter Gallium-68 as an easily available and inexpensive source of a PET nuclide. With the published concentration and purification method by Zhernosekov et al. [1] ^{68}Ga is obtained in 400 μL acetone/HCl mixture.The first series of phosphonates (EDTMP, DOTP [2], NOTA-derivatives [3] and DO3AP-ABn [4]) were labelled in 400 μL 0.12 M Na-HEPES buffer by adding the ^{68}Ga fraction. Through variation of reaction time, temperature, pH and different amounts of the ligands, optimum reaction parameters for complex formation were tested. Analyses of radiochemical yield were carried out by TLC on cellulose. Binding studies on synthetic apatite were applied to simulate the binding of the ^{68}Ga-phosphonates to bone structures. A second generation of bis-phosphonates (BPAMD, BPAPD and BPPED) was labelled, assayed concerning binding to apatite and investigated in vivo as well.

Results: Labelling proceeds at temperatures between 25 and 75°C within 2 to 10 min in a total volume of 800 μL. Ligands are used in nanomole amounts only and the radiochemical yields are 50 to 95%. Strong and fast binding was observed for DOTP & EDTMP. Within the series of ^{68}Ga-triazacyclononanes with n=1-3 phosphonates, an increasing binding to apatite was observed. The radiochemical yield of ^{68}Ga-DOTP was only 50%. In vivo experiments showed a relatively low stability of ^{68}Ga-EDTMP whereby large amounts of the ligand (>1.5 mg/kg body weight) has to be used. The bis-phosphonates showed also high binding to apatite and furthermore high stability in vivo in rats. Only nanomole amounts of these ligands are necessary for bone imaging.

Conclusions: Syntheses of ^{68}Ga complexes are performed within 20 min after elution of the generator. Evaluations with synthetic apatite show high binding in a short time for ^{68}Ga-EDTMP and the ^{68}Ga-DOTP as well as the three new DOTA-derivatives BPAMD, BPAPD and BPPED. Preliminary μ-PET imaging on rats demonstrated bone uptake in vivo for ^{68}Ga-EDTMP and ^{68}Ga-DOTP. Due to the low stability of Ga-EDTMP and the low labelling yield of ^{68}Ga-DOTP the new ligands BPAMD, BPAPD and BPPED seem to be of more interest. μ-PET imaging of ^{68}Ga-BPAMD showed significant uptake in bone and high in vivo stability.

References: [1] Zhernosekov et al, J Nucl Med 48: 1741-8[2] Sherry et al, Inorg Chem 35: 460412[3] Geraldes et al, Magn Reson Med 9: 94104[4] Rudovsky et al, Org Biomol Chem 3: 1127

P030 pH LOW INSERTION PEPTIDE: RADIOIODINATION AND pH-DEPENDENT TUMOR CELL ACCUMULATION

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Objectives: The important role of extracellular pH (pH_e) in tumor biology and therapy response argues strongly for the development of a novel technique for the noninvasive imaging assessment of pH_e. pH (Low) Insertion Peptide (pHLIP) was recently discovered to possess the unique property of targeting high extracellular acidity in vivo [Andreev O.A., Dupuy A.D., Segala M., Sandugu S., Serra D.A., Chichester C.O.,Engelman D.M., Reshetnyak Y.K. PNAS, 2007, 104(19), 7893–7898]. At physiological pH 7.4 pHLIP exists in a free-form conformation, but upon a 1-unit drop in pH it assumes a strict helical conformation and inserts across the lipid bilayer of cellular membranes. Fluorescently-labeled pHLIP has been successfully applied for tumor imaging in vivo with a 5-fold image contrast between tumor and surrounding tissue. In order to readily translate this agent into the clinical arena we are developing this agent for both MRI and nuclear medicine applications. In this current work, we introduced ^{131}I or ^{124}I into the peptide backbone and performed preliminary in vitro and in vivo studies.

Methods: The oxidative iodination of pHLIP was the most straightforward way to introduce radioiodine into the tyrosine-containing peptide. We employed mild labeling conditions with Iodo-Gene© as the oxidation reagent. Peptide solubility was a key factor in the iodination procedure, and we therefore performed the reaction in a MeOH/AcOH/H_2O mixture. It was also found that a high concentration of iodine (> 10 mCi/ml) was also required. In vitro studies were undertaken in PC-3 prostate cancer cells. Briefly, $2x10^5$ cells in 6-well plates were incubated in PBS at the required pH (pH 5.5, 6.5, 7.0 or 7.4) in the presence of 1 microCi of the iodinated peptide for 45 min. Following isolation of the cells, the amount of associated activity with the cells was measured. In vivo studies were performed in mice bearing LNCaP prostate tumors where ~5 microCi of I-pHLIP was administered intravenously. Mice (n = 4 per group) were sacrificed at 4 and 24 hours post injection and selected tissues were harvested.

Results: The in vitro results demonstrated a significant difference in cell-associated activity at each pH measured. There was ~50% higher accumulation in cells at pH 5.5 than those at pH 7.4. Acute biodistribution in prostate tumor-bearing mice showed significant accumulation within the tumor at 4 h post injection but poor blood clearance. The blood and non-target tissue accumulation reduced and improved over 24 hours.

Conclusions: We have successfully iodinated pHLIP for in vitro and in vivo study. The iodinated peptide exhibited the expected pH-dependent accumulation in vitro. Initial animal studies demonstrated tumor accumulation, but in vivo deiodination reduced contrast. Additional studies are justified to further study this iodinated peptide in modulated in vivo biological systems.

Research Support: NIH R01 (GM073857) to DME; NIH RO1 133890 to OAA, DME and YRK; DOD (PC050351) to YKR; DOD (BC061356) and NIH R21(CA125280) to OAA

P031 FLUORINE-18 LABELING OF A NEW MELANIN-TARGETING TRACER FOR MELANOMA IMAGING WITH PET

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Objectives: Malignant melanoma is a serious type of skin cancer because of its aggressiveness, spreading potential and chemoresistance. In spite of decades of research, no treatment has demonstrated until now significant efficacy. Targeting the ubiquitous dissemination of melanoma with selectivity and specificity became challenging for many purposes such as early detection and staging of tumours and metastasis with imaging techniques, follow up of chemotherapies and internal curative radiotherapy. Several classes of agents capable of achieving these purposes have already been investigated. Benzamide-based melanin-targeting radiopharmaceuticals is a class of compounds whose several members have been evaluated pre-clinically but also clinically in melanoma patients. Among them, [^{123}I]N-2-diethylaminoethyl-2/4-iodobenzamides ([^{123}I]BZAs) have demonstrated a real potential as radiopharmaceuticals for melanoma imaging with SPECT. In a recent SAR study, new aromatic and heteroaromatic analogues of BZAs have been designed, synthesized, labeled with iodine-125 and evaluated in B16 melanoma bearing mice. Within this series, N-2-diethylaminoethyl-6-iodoquinoline-2-carboxamide as well as fluorine-containing analogues showed interesting pharmacological properties [1] and were selected for fluorine-18-labeling. We herein report the radiosynthesis of a first candidate, N-2-diethylaminoethyl-4-[^{18}F]fluoro-6-iodoquinoline-2-carboxamide ([^{18}F]ICF02110, [^{18}F]-1).

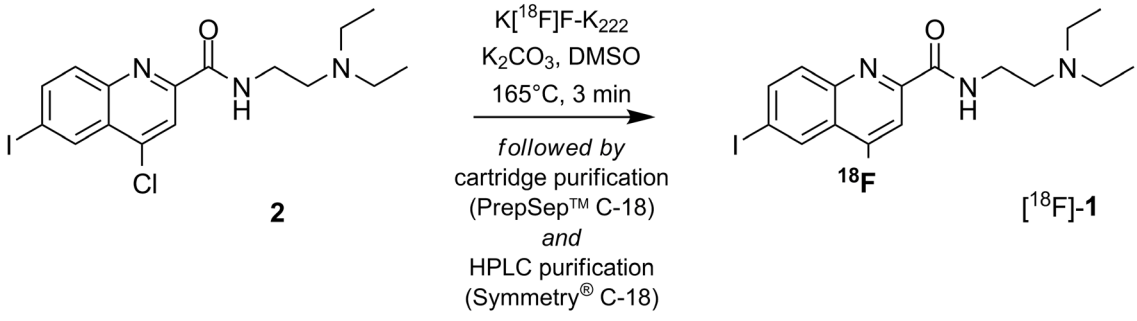
Methods: The labeling procedure has been automated on our Zymate-XP robotic system and involves four successive stages. First, fluorine-18 was incorporated into the precursor for labeling (2) via a chloro-for-fluoro substitution using K[^{18}F]F-Kryptofix®222 at 165°C for 3 min in DMSO (0.6 mL). Secondly, the reaction mixture was diluted with water (5 mL) and pre-purified on a PrepSep C-18 cartridge. Thirdly, [^{18}F]ICF02110 ([^{18}F]-1), was isolated using semi-preparative reversed-phase HPLC (column Waters Symmetry® C-18, isocratic elution performed using H_2O / CH_3CN / aq. 28% NH_4OH : 35/65/0.1 (v/v/v), flow rate : 5 mL/min, t_R : 13-13.5 min) and finally, formulated for i.v. injection. Chemical and radiochemical purities were assessed on an aliquot of the ready-for-injection preparation by TLC and HPLC, with a sample of authentic non labeled ICF02110 (1) as reference.

Results: [^{18}F]ICF02110, ([^{18}F]-1) has been prepared in one single radiochemical step starting from the corresponding 4-chloroquinoline-2-carboxamide (2) with chemical and radiochemical purities greater than 95% after HPLC purification. Typically, starting from 15 GBq of [^{18}F]fluorine-18 up to 3 GBq of [^{18}F]-1 can be produced (RCY: 20%, non-decay-corrected) in about 60 minutes and with specific radioactivities ranging from 150 to 260 GBq/μmol.

Conclusions: [^{18}F]ICF02110 has been prepared with decay-corrected radiochemical yields reaching 30%. Pharmacological evaluation in tumour-bearing mice with PET is currently in progress.

Research Support: Supported by ANR (Agence Nationale de la Recherche) and EMIL (European Molecular Imaging Laboratories) EU contract LSH-2004-503569.

References: [1] Chezal et al. J. Med. Chem (2008), 51, 3133-3144.



P032 SYNTHESIS AND EVALUATION OF 99mTc COMPLEXES OF 4-NITROIMIDAZOLE AS POTENTIAL AGENTS FOR TARGETING TUMOR HYPOXIA

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Objectives: The purpose of this study is to conjugate 4-nitroimidazole with 1,4,8,11-tetraazacyclotetradecane (cyclam) and evaluate the feasibility of ^{99m}Tc-labeled 4-nitroimidazole analogs as candidates for targeting tumor hypoxia.

Methods: [2-(4-Nitroimidazol-1-yl)ethyl]cyclam (NIEC) was prepared by combining cyclam with 4-nitroimidazole. ^{99m}TcO-NIEC, ^{99m}TcN-NIEC and ^{99m}Tc(CO)$_3$-NIEC were obtained by incubating NIEC ligand with the ^{99m}Tc-GH, ^{99m}Tc-nitrido and ^{99m}Tc-carbonyl intermediates, respectively. Radiochemical purity (RCP) and in vitro stability of these complexes were determined by TLC and HPLC. Lipophilicity of those ^{99m}Tc-complexes was characterized by distribution in 1-octanol and phosphate buffer at pH 7.4 and their charge was determined by paper electrophoresis. Biodistribution studies of three complexes were performed in Kunming mice (20-25g) bearing S180 tumor at 1, 2 and 4 h pi following the principles of laboratory animal care and the China Law on the protection of animals.

Results: ^{99m}TcO-NIEC, ^{99m}TcN-NIEC and ^{99m}Tc(CO)$_3$-NIEC were prepared in good yields (>95%). The RCP of these complexes had no significant difference over 4 h after preparation, which suggested that they possessed a good stability in vitro. The partition coefficient of the complexes showed they are hydrophilic complexes. The paper electrophoresis experiment exhibited ^{99m}TcO-NIEC and ^{99m}TcN-NIEC were neutrality and ^{99m}Tc(CO)$_3$-NIEC was cationic. Biodistribution studies indicated that these complexes had different biological behaviors due to their different ^{99m}Tc cores, although they had the same ligand. The tumor uptakes of ^{99m}TcO-NIEC and ^{99m}TcN-NIEC were 3.54±0.04%ID/g and 3.42±0.97%ID/g at 2 h pi, respectively, which are obviously higher than that of ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21 (0.55±0.08%ID/g and 0.37±0.14%ID/g at 2 h pi, respectively) without consideration of the tumor type [1.2]. However, ^{99m}Tc(CO)$_3$-NIEC exhibited lower tumor uptake (1.03±0.01%ID/g at 2 h pi). The ratios of tumor/muscle for ^{99m}TcO-NIEC and ^{99m}TcN-NIEC were 4.21 and 3.53 at 2 h pi, respectively, these are comparable to that of ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21 (2.63 and 3.84 at 2 h pi, respectively). Since an important consideration in the development of a ^{99m}Tc-based hypoxic agent is to decrease the hepatobiliary [2], the lower liver uptake (4.00±0.35%ID/g at 2 h pi) observed with ^{99m}TcO-NIEC deserves attention, but ^{99m}Tc(CO)$_3$-NIEC exhibited higher liver uptake (12.72±0.10%ID/g at 2 h pi). The injected radioactivity of these three complexes was found to excrete mainly through the hepatobiliary and kidney.

Conclusions: The cyclam derivative of 4-nitroimidazole was synthesized and labeled with ^{99m}Tc-oxo, ^{99m}Tc-nitrido and ^{99m}Tc-carbonyl cores successfully, respectively. ^{99m}TcO-NIEC and ^{99m}TcN-NIEC showed high tumor uptake with high tumor/muscle and moderate tumor/blood ratios. The liver uptake of ^{99m}TcO-NIEC was significantly lower and that of ^{99m}TcN-NIEC also compares well with them, comparing with ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21. They could be further studied as potential tumor hypoxia imaging agents.

Research Support: The authors wish to acknowledge the support of Beijing Shihong Pharmaceutical Center for the donation of SDH kits.

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P033 SYNTHESIS AND EVALUATION OF 99mTc COMPLEXES OF 2-METHYL-5-NITROIMIDAZOLE AS POTENTIAL AGENTS FOR TUMOR HYPOXIA IMAGING

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Objectives: The design, synthesis, characterization and evaluation of novel ^{99m}Tc-labeled 5-nitroimidazole complexes were reported in this paper for tumor hypoxia imaging.

Methods: [2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl]cyclam (MNIEC) was prepared by conjugate 2-methyl-5-nitroimidazole with 1,4,8,11-tetraazacyclotetradecane (cyclam). MNIEC was labeled with ^{99m}Tc-GH, ^{99m}Tc-nitrido and ^{99m}Tc-carbonyl intermediates, respectively, and ^{99m}TcO-MNIEC, ^{99m}TcN-MNIEC and ^{99m}Tc(CO)$_3$-MNIEC were obtained. Radiochemical purity (RCP) and in vitro stability of three complexes were determined by TLC and HPLC. Their lipophilicity was characterized by distribution in 1-octanol and phosphate buffer at pH 7.4 and their charge was determined by paper electrophoresis. Biodistribution studies of the complexes were performed in Kunming mice (20-25g) bearing S180 tumor at 1, 2 and 4 h pi following the principles of laboratory animal care and the China Law on the protection of animals.

Results: ^{99m}TcO-MNIEC, ^{99m}TcN-MNIEC and ^{99m}Tc(CO)$_3$-MNIEC were prepared in good yields (>95%). Their RCP was still over 95% after 4 h, which suggested that they possessed a good stability in vitro. The partition coefficient of these complexes showed they are hydrophilic complexes. The paper electrophoresis experiments exhibited ^{99m}TcO-MNIEC and ^{99m}TcN-MNIEC were neutrality and ^{99m}Tc(CO)$_3$-MNIEC was cationic. Biodistribution studies indicated that these three complexes showed different biological behaviors due to different cores of ^{99m}Tc. The tumor uptakes of ^{99m}TcO-MNIEC, ^{99m}TcN-MNIEC and ^{99m}Tc(CO)$_3$-MNIEC were 2.12±0.45%ID/g, 2.52±0.69 %ID/g and 1.09±0.17%ID/g at 2 h pi, respectively. Without consideration of the tumor type, their tumor uptakes were all obviously higher than that of ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21 (0.55±0.08%ID/g and 0.37±0.14%ID/g at 2 h pi, respectively) [1,2]. Their tumor/muscle ratios were 4.33, 3.72 and 1.95 at 2 h pi, respectively. Since an important consideration in the development of ^{99m}Tc-based hypoxic agent is to decrease the hepatobiliary uptake [2], the lower uptakes in the liver (2.29±0.37%ID/g and 2.26±0.22 at 2 h pi, respectively) observed with ^{99m}TcO-MNIEC and ^{99m}TcN-MNIEC deserve attention. On the contrary, the liver uptake of ^{99m}Tc(CO)$_3$-MNIEC (9.46±1.04) was higher than that of ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21 (8.79±3.05%ID/g and 8.37±0.87%ID/g, respectively) at 2 h pi. The injected radioactivity of these three complexes was found to excrete mainly through the hepatobiliary path along with the renal pathway.

Conclusions: The cyclam derivative of 2-methyl-5-nitroimidazole was synthesized and labeled with ^{99m}Tc-oxo, ^{99m}Tc-nitrido and ^{99m}Tc-carbonyl cores successfully, respectively. ^{99m}TcO-MNIEC and ^{99m}TcN-MNIEC showed high tumor uptake with high tumor/muscle ratio and low liver uptake. In comparison with the above complexes, ^{99m}Tc(CO)$_3$-MNIEC exhibited lower tumor uptake and tumor/muscle ratio and higher liver uptake. Among them, ^{99m}TcO-MNIEC and ^{99m}TcN-MNIEC could be further studied as potential tumor hypoxia imaging agents.

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P034 DISCOVERY AND EVALUATION OF A POTENTIAL MOLECULAR IMAGING AGENT FOR CATHEPSIN B

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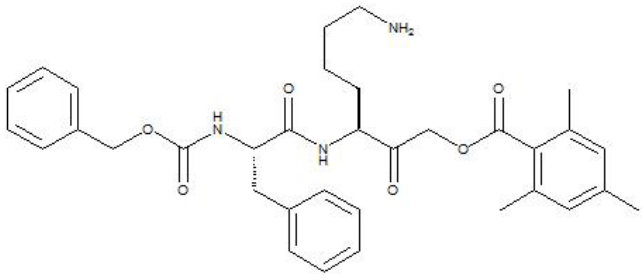
Objectives: Cathepsin B, a cysteine protease overexpressed in aggressive forms of breast cancer tumours, has been linked to the overall aggressiveness of tumour cells. Dipeptidyl (acyloxy)methyl ketones (AOMKs) are a class of compounds that have shown selective and effective inhibition of cathepsin B. The current objective was to establish a platform for the development of molecular imaging (MI) probes which target cathepsin B. The lead compound is in the form Z-Phe-Lys-CH_2OCOAr (Figure 1). By modification at the epsilon nitrogen of the lysine group, the aim is to develop and evaluate various derivatives incorporating radioisotopes that can be used in SPECT and PET imaging.

Methods: The lead was synthesized by conversion of the N-terminus protected dipeptide to the corresponding bromomethyl ketone via the diazomethyl ketone intermediate. The bromine was then displaced by the carboxylate mediated by potassium fluoride, resulting in the formation of the AOMK. Haloaryl derivatives were prepared via an EDC coupling of the free amine with a series of halogenated benzoic acid derivatives.

Results: Conversions of the methyl ketones were successfully prepared in 50 – 80% yields. The coupling reactions were successful carried out between the free amine and the benzoic acid derivatives in 30% yield. The uniqueness of this approach is that it is suited for both PET and SPECT agents.

Conclusions: Synthesizing derivatives of a known cathepsin B inhibitor of the type Z-Phe-Lys-CH_2OCOAr provides a means for delivering radionuclides that target the protease. These molecules have potential to be used as molecular imaging agents for SPECT and PET imaging.



P035 SYNTHESIS AND BIOLOGICAL EVALUATION OF A NEGATIVELY CHARGED 99mTc-LABELLED AMINOETHYLBENZENE SULFONAMIDE CONJUGATE FOR IN VIVO VISUALISATION OF CARBONIC ANHYDRASE IX (CA IX) EXPRESSION AS A SURROGATE MARKER OF TUMOR HYPOXIA

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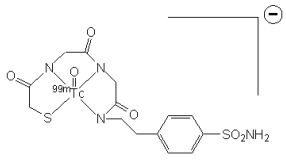
Objectives: In vivo visualization of an endogenous tumor hypoxia-related marker such as the transmembrane protein carbonic anhydrase IX (CA IX), may lead to novel therapeutic and diagnostic applications in treatment and management of solid tumors. The sulfonamide derivative 4-(2-aminoethyl)benzenesulfonamide (AEBS) having a Ki value of 33nM for CAIX[1] was conjugated with S-benzylmercaptoacetylglycylglycine, and the conjugate (S-Bn-MAG_2 1) was labelled with ^{99m}Tc (compound 2). We report here the synthesis and preliminary biological evaluation of this complex in HT-29 cell line and xenograft bearing mice.

Methods: Compound 1 was synthesized by conjugation of AEBS with S-Bn-MAG_2 in presence of DMAP and PyBOP at RT for 16 h. Compound 2 was obtained by a one pot deprotection and exchange labelling method (NaK tartrate, $SnCl_2.2H_2O$, $^{99m}TcO_4^-$, 15 min, 100°C). The corresponding rhenium analogue (compound 3) was synthesized using oxo rhenium (V) citrate. Compound 3 was evaluated in vitro by assessing the inhibition of CA IX mediated extracellular acidification in colorectal HT-29 cell line. Biodistribution study was carried out in NMRI-nu (nu/nu) mice bearing CA IX expressing HT-29 tumors at 2, 45, 60, 120 and 240 min p.i.



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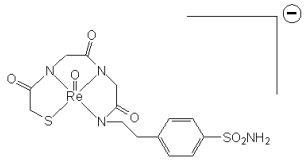

(3)

Results: Compound 2 was synthesized with a radio chemical yield of 25% and a radio chemical purity >99%. The reference Re-analogue 3 was prepared in a similar way with a chemical yield of 57%. Identity of Compound 2 was confirmed by co-injection with the rhenium congener 3. In vitro affinity studies showed that the rhenium congener 3 efficiently reduced CA IX induced extracellular pH acidification at 1mM concentration. Biodistribution studies in CAIX expressing HT-29 tumor bearing mice showed that the highest uptake of the tracer in tumor tissue was observed at 45 min p.i., but with only 0.5% of ID/g. The plasma clearance of the tracer was mainly through hepatobiliary pathway (62% of ID in intestines, 12% of ID in liver at 4h p.i.) and to some extent through the renal pathway (19% of ID in urine at 4h p.i.).

Conclusions: A negatively charged ^{99m}Tc- labelled 4-(2-aminoethyl)benzenesulfonamide conjugate and its rhenium congener were successfully synthesized. In vitro studies showed that the rhenium analogue efficiently reduced CA IX induced extracellular acidification. Despite of these encouraging in vitro studies results, in vivo studies revealed that the tumor uptake of the tracer is minimal. However, the new tracer can be explored for its potential in in vitro studies to understand the behavior of CA IX in tumor hypoxia.

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