P302 EVALUATION OF THE ALPHA 1I T-TYPE CALCIUM CHANNEL PET TRACER [18F]TTA-A4 IN AN ANESTHETIZED OR CONSCIOUS MONKEY

S. SANABRIA-BOHORQUEZ[1], W. LI[1], W. ENG[1], C. RYAN[1], K. RIFFEL[1], Z. ZENG[1], V. UEBELE[3], T. REGER[2], J. BARROW[2], D. WILLIAMS JR[1], R. J. VOLL[4], L. HOWELL[5], J. VOTAW[4], M. M. GOODMAN[4] and T. G. HAMILL[*1]

1. Merck Research Laboratories, Imaging Research, West Point, PA; 2. Merck Research Laboratories, Medicinal Chemistry, West Point, PA; 3. Merck Research Laboratories, Depression and Circadian Disorders, West Point, PA; 4. Emory University School of Medicine, Department of Radiology, Atlanta, GA; 5. Emory University School of Medicine, Yerkes Primate Center, Atlanta, GA

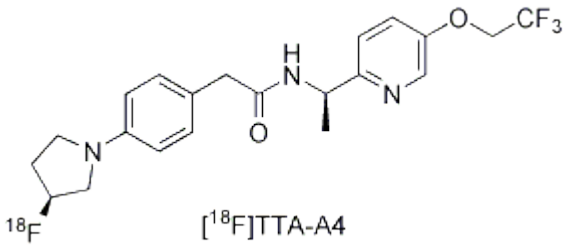
Objectives: T-type calcium channels are low voltage-activated channels that regulate intracellular calcium levels and membrane depolarization and play a role in signaling cascades. These channels are important in sleep, epilepsy and pain. A clinical T-type calcium channel PET tracer would be useful for determining the occupancy of therapeutic compounds and supporting the selection of clinical doses for proof of concept studies.[1]

Methods: [^{18}F]TTA-A4 was synthesized from the corresponding mesylate with a radiochemical yield of 11.4 ± 3.9% and a specific activity of 1990 ± 435 Ci/mmol (n=15, EOS) and was evaluated in in vitro autoradiographic and tissue homogenate binding studies. In vivo imaging studies were carried out in anesthetized (baseline and block) or conscious (baseline) rhesus monkeys.

Results: [^{18}F]TTA-A4 is a high affinity T-type calcium channel ligand (Kd 0.53 nM, human).[2] Autoradiography showed that across species (rat, rhesus and human) this ligand has low nondisplaceable binding, with highest binding densities in the cerebellum and thalamus. PET studies in rhesus monkeys showed good brain penetration with rapid washout and a specific signal too small to accurately measure occupancy. Baseline scans in conscious animals were very similar to what was seen in anesthetized animals.

Conclusions: Though [^{18}F]TTA-A4 has good in vitro characteristics for imaging T-type calcium channels and demonstrated specific binding in vitro, the resulting in vivo signal in conscious or anesthetized rhesus monkeys is not large enough to accurately measure occupancy. In a functional calcium flux assay, TTA-A4 exhibits a greater potency for the channel in depolarized conditions compared to hyperpolarized conditions. One explanation for the observed results is that under in vitro conditions (depolarized) a specific signal is observed and under in vivo conditions (hyperpolarized) the concentration of channel in the inactivated state is low, resulting in a small specific signal.

References: 1. Nuss CE, Barrow JC, Yang Z-Q, Schlegel K-AS, Rittle KE, Reger TS, Renger JJ, Uebele VN. Society for Neuroscience 2008, poster 32.10, D53. 2. Zeng Z, Miller PJ, O'Malley SS, Hamill T, Li W, Nuss CE, Uebele VN, Renger JJ, Shu Y, Yang Z-Q, Reger T, Barrow JC, Sur C, Williams Jr DL. Society for Neuroscience 2008, poster 32.15, D58.



[^{18}F]TTA-A4

P303 PREPARATION AND EVALUATION OF [61Cu]-THIOPHENE-2-ALDEHYDE THIOSEMICARBAZONE FOR PET STUDIES

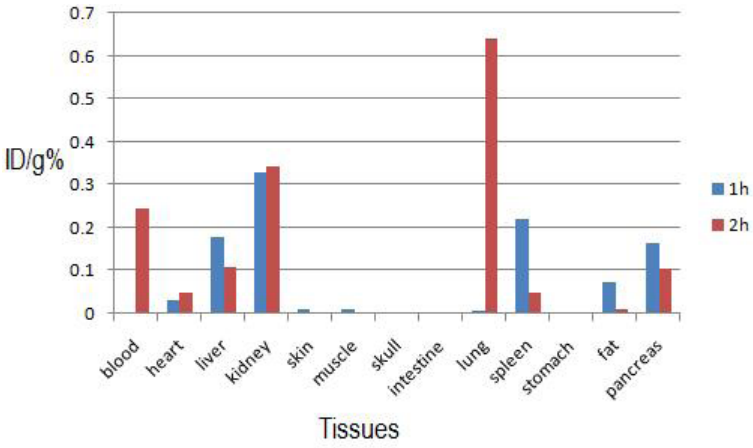
A. R. JALILIAN*[1], M. NIKZAD[2], H. ZANDI[1], F. NEMATI[2], P. ROWSHANFARZAD[1], M. ABEDINI[2] and M. AKHLAGHI[1]
1. Nuclear Medicine Research Group, Agricultural, Medical and Industrial Research School (AMIRS), Karaj, Iran; 2. Inorganic Chemistry Department, Faculty of Sciences, Tehran University, Tehran, Iran

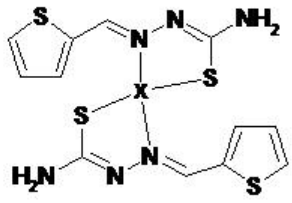
Objectives: [^{61}Cu]Thiophene-2-Aldehyde Thio-Semicarbazone ([^{61}Cu]TATS) (4) was prepared according to analogy of carrier copper compound with antitumor activity for ultimate use in PET.

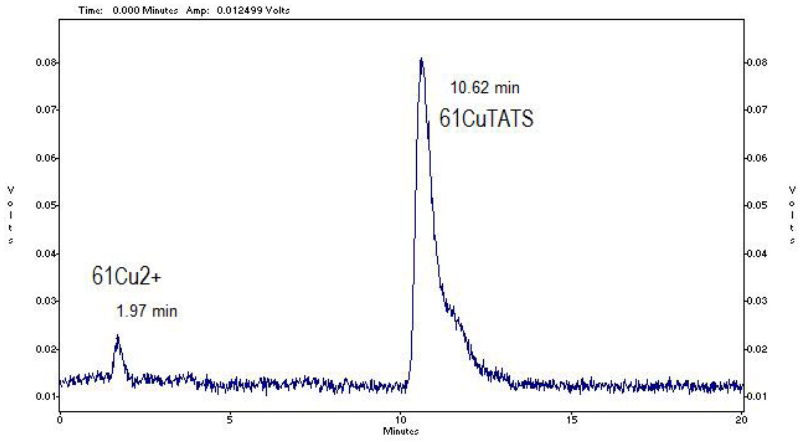
Methods: [^{61}Cu]TATS was prepared using copper-61 acetate and in house made ligand (TATS) in one step. ^{61}Cu was produced via the ^{nat}Zn(p,x)^{61}Cu nuclear reaction (180μA, 22MeV, 3.2h) followed by a two-step chromatography method (222 GBq of $^{61}Cu^{2+}$). [^{61}Cu]TATS preparation was optimized for reaction conditions (buffer concentration and temperature). The tracer was finally administerd to normal rats for biodistribution studies

Results: Total radiolabeling of the tracer took 30 min with a radiochemical purity of better than 90% (using HPLC and RTLC) and specific activity of about 250-300 Ci/mmol. The complex was stable in presence of human serum for an hour. The biodistribution of copper cation and the tracer was checked in normal rats up to 2 hours with significant spleen and lung uptake of the tracer.

Conclusions: The production of ^{61}Cu via the ^{nat}Zn(p,x)^{61}Cu is an efficient and reproducible method with high specific activity leading to the production and preliminary evaluation of [^{61}Cu]TATS, a potential PET tracer was reported.







P304 RADIOSYNTHESIS AND PRECLINICAL EVALUATION OF [61Cu]-9,10-PHENANTHRENEQUINONE THIOSEMICARBAZONE IN FIBROSARCOMA-BEARING ANIMALS FOR PET IMAGING

A. R. JALILIAN*[1], A. EMAMI[2], M. AKHLAGHI[1] and K. SHAFAII[1]

1. Nuclear Medicine Research Group, Agricultural, Medical and Industrial Research School (AMIRS), Karaj, Iran; 2. Faculty of Pharmacy, Islamic Azad University, Tehran, Iran

Objectives: Due to the importance of thiosemicarbazone compounds for inhibiting the DNA synthesis/maintanance enzymes, [^{61}Cu]-9,10-Phenanthrenequinone thiosemicarbazone (^{61}Cu-PQTS), a radiolabeled anticancer compound was prepared for malignant tissue imaging studies.

Methods: Copper-61 was produced via the ^{nat}Zn(p,x)^{61}Cu nuclear reaction in a 30 MeV cyclotron according to our routine method by a two-step column chromatography method (150 μA irradiation for 76 min, radionuclidic purity>99%). ^{61}Cu-PQTS was prepared using an optimized method with in-house synthesized PQTS ligand for radiolabeling following quality control procedures using RTLC. The tracer is mostly incorporated in heart, kidneys and brain compared to free copper cation as a control in normal animal studies. The tracer was then administered to fibrosarcoma-bearing mice for biodistribution and co-incidendence imaging studies.

Results: The radiochemical purity of the [^{61}Cu]PQTS was higher than 90%. The partition coefficient of the tracer showed high water solubility which can be explained by the ionic property of the tracer. The tracer was administerd to normal and tumor bearing mice and the biodistribution of the tracer was calculated among the critical organs and tumor parts. The tumor:blood ratio was 5 while the best tumor uptake was observed 3 hours p.i.. finally the tracer was injected into tumor-bearing mice veins for imaging studies using co-incidence camera and significant tumor uptake was also observed after 3 hours p.i.

Conclusions: [^{61}Cu]PQTS, can be a possible PET radiotracer with an intermediate half life, and the high tumor accumulation of this tracer makes it an interesting candidate for further preclinical studies

P305 CHELTATE CONJUGATION AND Cu-64 LABELING OF hu14.18K322A, AN ANTIBODY TARGETING NEUROBLASTOMA

A. L. VAVERE*[1], J. L. DEARLING[2], A. B. PACKARD[2], F. NAVID[3], B. L. SHULKIN[1], R. C. BARFIELD[4] and S. E. SNYDER[1]
1. St. Jude Children's Research Hospital, Department of Radiological Sciences, Memphis, TN; 2. Children's Hospital Boston, Division of Nuclear Medicine, Department of Radiology & Harvard Medical School, Boston, MA; 3. St. Jude Children's Research Hospital, Department of Oncology, Memphis, TN; 4. Duke University Medical Center, Division of Hematology-Oncology, Department of Pediatrics, Durham, NC

Objectives: Several monoclonal antibodies targeting disialoganglioside GD2 have been investigated in clinical trials for treating neuroblastoma, a pediatric tumor derived from primordial neural crest cells. One of these antibodies, hu14.18K322A, contains a single, point mutation shown to decrease complement activation in an attempt to minimize dose-limiting side effects. A phase I clinical trial using this antibody is being initiated at our institution. Our objective is to effectively label this antibody with ^{64}Cu, a positron-emitting nuclide with a 12.7 hour half-life, enabling PET imaging at the late time-points needed for antibody localization. Such an imaging moiety would be useful in following response to therapy or possibly in identifying potential responders to treatment.

Methods: The hu14.18K322A construct was provided by Merck KGaA and the antibody produced for clinical use by Children's GMP, LLC. Two methods were used for chelate conjugation to the antibody. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(N-hydroxysuccinimide ester (DOTA-NHS-ester) in 500-1000 fold excess was reacted with the antibody in 0.1 M sodium phosphate, pH 7.5, overnight at 4°C. Unconjugated DOTA was removed from the solution by buffer washings using a spin-column filter. Conjugation was also accomplished with S-2-(4-Aminobenzyl)-1,4,7,10-tetraazacyclododecane tetraacetic acid (p-NH_2-Bn-DOTA) at 500:1 in conjunction with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (250:1) at pH 5 in 0.1 M sodium acetate for 30 min at 37°C. Excess p-NH_2-Bn-DOTA was eliminated from solution by SE-HPLC. ^{64}Cu purchased from Isotrace as a 0.05 M HCl solution was diluted in 0.1 M sodium acetate, pH 5.0. 10-500 μCi ^{64}Cu was added to a solution of 5-10 μg of DOTA-hu14.18K322A or p-NH_2-Bn-DOTA-hu14.18K322A and incubated for 30 min to 2 h at 25-40°C. Labeling efficiency was determined by ITLC and HPLC. Number of chelates per antibody was approximated by isotopic dilution.

Results: ^{64}Cu-DOTA-hu14.18K322A and ^{64}Cu-p-NH_2-Bn-DOTA-hu14.18K322A were labeled successfully in high radiochemical purity (≥95% in both cases). Optimal labeling conditions were 1 hour at 37° and a ratio of 10 μCi/μg Ab. Specific activities of 0.010 mCi/μg for the p-NH_2-Bn-DOTA complex and 0.019 mCi/μg for the DOTA complex were achieved. Isotopic dilution experiments estimated an average of 0.5 chelates per antibody molecule for the DOTA complex and 4.3 for the p-NH_2-Bn-DOTA complex. In vitro and in vivo studies are ongoing.

Conclusions: ^{64}Cu-DOTA-hu14.18K322A and ^{64}Cu-p-NH_2-Bn-DOTA-hu14.18K322A represent novel PET imaging agents to complement other imaging modalities in the diagnosis and treatment of neuroblastoma.

P306 IMAGING OF THE CELL CYCLE: SYNTHESIS AND RADIOPHARMACOLOGICAL EVALUATION OF 124I-LABELED CDK4 INHIBITORS

L. KOEHLER*[1], F. GRAF[1], R. BERGMANN[1], J. STEINBACH[1], J. PIETZSCH[1] and F. WUEST[2]
1. Forschungszentrum Dresden-Rossendorf, Institute of Radiopharmacy, Dresden, Germany; 2. University of Alberta, Cross Cancer Institute, Edmonton, Canada

Objectives: Tumor cells are characterized by their loss of growth control resulting from alterations in regulating pathways of the cell cycle, e.g., a deregulated cyclin dependent kinase (Cdk) activity and/or expression. In 80% of tumors the cell cycle relevant Cdk4-cyclin D1/retinoblastoma (pRb) cascade is altered. Hence, appropriately radiolabeled Cdk4 inhibitors are discussed as promising molecular probes for imaging cell proliferation processes and tumor visualization by PET. This work describes the synthesis and radiopharmacological evaluation of two ^{124}I-labeled Cdk4 inhibitors as potential radiotracers for imaging of Cdk4 in vivo.

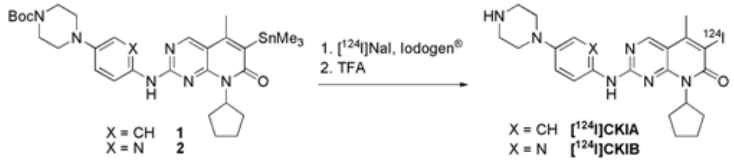


Fig. 1: Radiosynthesis of ^{124}I-labeled Cdk4 inhibitors

Methods: Reference substances and labeling precursors 1 and 2 were prepared with minor modifications according to literature procedures. Radioiodination was performed through regioselective destannylation reaction under mild conditions using [^{124}I]NaI and Iodogen® as the oxidizing agents (Fig. 1). ^{124}I-labeled radiotracers [^{124}I]CKIA and [^{124}I]CKIB were used in cell uptake studies. Biodistribution and small animal PET studies were carried out.

Results: Treatment of a solution containing labeling precursors 1 or 2 (5 mg/ml) with [^{124}I]NaI (29.0 to 275.0 MBq) in Iodogen® precoated tubes gave radiolabeled Cdk4 inhibitors [^{124}I]CKIA and [^{124}I]CKIB in radiochemical yields of up to 35% after removal of Boc protecting group and final HPLC purification. Both compounds were isolated in high radiochemical purity exceeding 95%. The specific radioactivity (A_s) was 25-35 GBq/μmol at the end of synthesis. The lipophilicity (logP) was determined to be 2.77±0.13 for [^{124}I]CKIA and 1.99±0.03 for [^{124}I]CKIB, respectively. Both compounds remained stable in buffer (PBS, pH 7.4) and ethanol after 24 h at 37°C. In vitro radiotracer uptake studies in human tumor cells using [^{124}I]CKIA showed substantial uptake in adenocarcinoma HT-29 (1264±84 %ID/mg protein) and squamous cell carcinoma FaDu cells (1429±229 %ID/mg protein) after 2 h at 37°C (A_s:25 GBq/μmol). A significantly lower uptake was detected at 4°C (HT-29: 383±38 %ID/mg protein, FaDu: 437±25 %ID/mg protein). Biodistribution studies of [^{124}I]CKIA and [^{124}I]CKIB showed rapid blood clearance in rats. [^{124}I]CKIA and [^{124}I]CKIB were mainly accumulated and metabolized by liver. Both radiotracers were administered intravenously to mouse FaDu xenograft tumor model and imaging studies were performed on a small-animal PET scanner confirming a high radioactivity concentration in the liver and intestine for both compounds. No radioactivity was found in the brain. PET showed only little uptake of both radiotracers in the tumor.

Conclusions: The radiosynthesis of two ^{124}I-labeled Cdk4 inhibitors has been developed. Both radiotracers were obtained in reproducible radiochemical yields and purity enabling detailed radiopharmacological characterization. Small-animal PET and autoradiography studies showed only low tumor uptake. Further studies are needed to search for more suitable derivatives of these substances as radiotracers for imaging Cdk4 by means of PET.

P307 EFFECTS OF CYCLOSPORIN A ON BRAIN UPTAKE AND METABOLISM OF [18F]FBAU 3',5'-DIBENZOATE

C. KAO*[1], H. XIE[1] and Y. WEI[2]
1. Buddhist Tzu Chi General Hospital, Dept Nuclear Medicine, Hualien, Taiwan; 2. Tzu Chi College of Technology, Dept Radiological Technology, Hualien, Taiwan

Objectives: [^{18}F]FBAU 3',5'-dibenzoate (FBAU-db) is a lipophilic prodrug of DNA incorporating [^{18}F]FBAU (FBAU). Previously, we have demonstrated higher brain uptake by using FBAU-db in rats, and shown its potential in monitoring brain cell proliferation. Nonetheless, the uptake was still less than desired. In the attempt of further uptake enhancement, we modulated P-glycoprotein (P-gp) with cyclosporin A (CsA) and examined its effect on brain uptake of the tracer and on metabolism of FBAU-db.

Methods: Two groups of rat (9 each) were injected either FBAU-db or CsA plus FBAU-db. CsA was injected 30 min prior to the tracer injection. At 15, 30 and 60 min after injection, rats were sacrificed and blood samples were collected. After saline perfusion, brains were removed. Blood samples and brains were counted for their activity before radioactive contents were extracted with acetonitrile and phosphate buffer mixture. The extracted species was analyzed by TLC (20:80, hexane:ethyl acetate). The blood and brain uptakes were expressed as differential uptake ratios (DUR: (%ID/g×body weight)/100), and the amounts of each radioactive species were the products of brain DUR and fraction of that species in total radioactivity extracted. Student t-tests were applied for data comparison.

Results: Blood DURs of CsA treated rats were higher than those of untreated ones by 100%, 210% and 257% at 15, 30 and 60 min, respectively. Similarly, brain DURs of treated rats were higher by 320%, 233% and 100%. All comparisons are statistically significant with P≦0.05. The radioactive metabolites extraction efficiency from plasma was 92.9±2.4% and that from brain was 88.4±6.4%. CsA treatment had no effect on FBAU-db metabolic profile in plasma, all showed quick breakdown of FBAU-db into FBAU (80% in 15 min; >90% in 1 h). In brain, the breakdown was slower than in plasma, and CsA treatment seemed to further impede the process. At 15 min, already 25.6±10.5% activity was FBAU in brain of untreated rats, while only 7.2±3.6% in that of treated ones; at 60 min, FBAU reached 89±7% in brain of untreated rats, but only 54.5±13.2% in the other group. Though CsA greatly enhanced brain uptake of injected dose, the benefit were offset by higher FBAU-db fraction in brain. The actual contents of FBAU (the active form for DNA incorporation) in brain showed only slight increase of 28%, 95% and 33% at 15, 30 and 60 min, respectively, and only the difference at 30 min was statistically significant (P=0.018).

Conclusions: When using FBAU-db as lipophilic prodrug to deliver FBAU into brain for potential proliferation imaging, attempt to further improve the delivery by modulating P-gp with CsA resulted in only slight increase of FBAU in brain. Nevertheless, CsA modulation suggested that FBAU-db is a P-gp substrate, and by blocking it, the brain uptake of the radioactive prodrug increased more than 4 times. The subsequent diminished FBAU enhancement was the result of smaller fraction of FBAU in total radioactivity in brain.

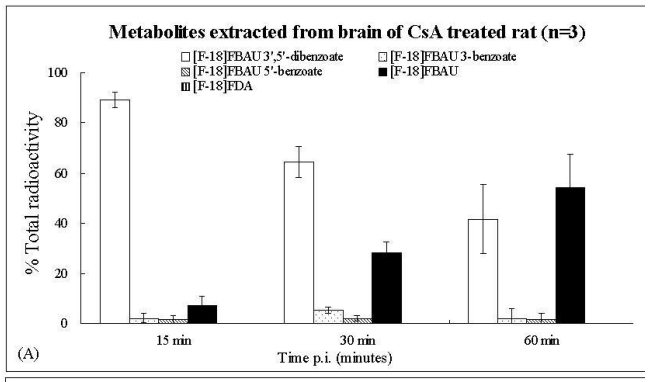
References: Kao C-HK, Xie H-L, Liao C-H, Chen W-M, Kao P-F. [^{18}F]FBAU 3',5'-dibenzoate, a lipophilic prodrug, enhances brain uptake of the cell proliferation tracer [^{18}F]FBAU. Nucl Med Biol 2008;35:635-643.

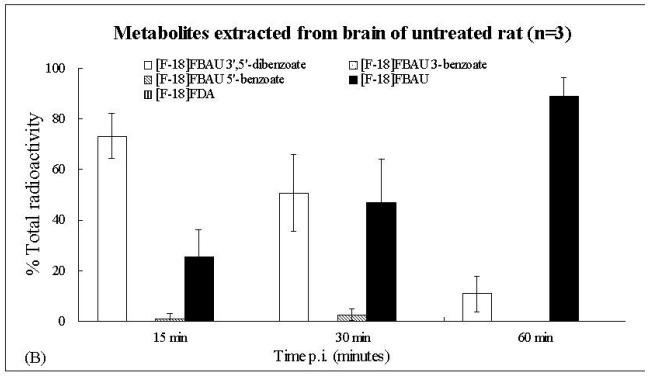
* Blood and brain differential uptake ratio (DUR) of injected activity. (All data n=3)

DUR		Untreated	CsA treated
Blood	15 min	0.51±0.17	1.02±0.03
	30 min	0.31±0.02	0.96±0.13
	60 min	0.14±0.03	0.50±0.17
Brain	15 min	0.05±0.02	0.21±0.02
	30 min	0.03±0.01	0.10±0.01
	60 min	0.02±0.01	0.04±0.01

* FBAU and FBAU-db contents in brain expressed as: Brain DUR × fraction of total activity. (All data n=3)

		Untreated	CsA treated
FBAU	15 min	0.012±0.002	0.016±0.008
	30 min	0.015±0.003	0.030±0.006
	60 min	0.017±0.004	0.022±0.011
FBAU-db	15 min	0.039±0.020	0.190±0.010
	30 min	0.018±0.009	0.067±0.009
	60 min	0.002±0.002	0.015±0.001





P308 SYNTHESIS OF A NEW PET RADIOTRACER TARGETING CARBONIC ANHYDRASE IX

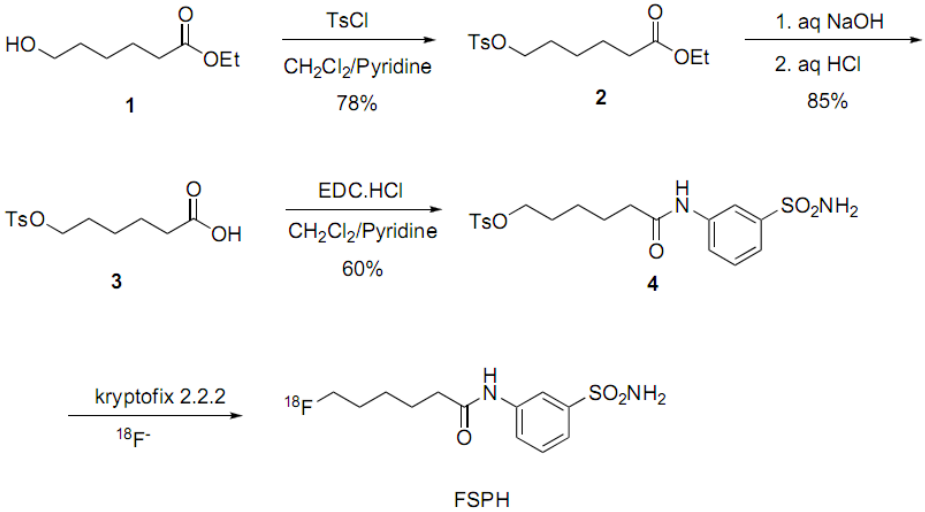
S. D. APTE*[1], F. T. CHIN[2] and E. E. GRAVES[1]
1. Stanford University School of Medicine, Department of Radiation Oncology, Stanford, CA; 2. Stanford University School of Medicine, Department of Radiology, Stanford, CA

Objectives: Hypoxia is a key modulator of tumor aggressiveness and therapeutic sensitivity. Lack of oxygen induces a host of molecular responses including stabilization of the transcription factor HIF-1 and expression of downstream genes. Carbonic anhydrase IX (CAIX) is a transmembrane protein that is regulated by HIF-1 and overexpressed under hypoxic conditions, and its expression has been shown to strongly correlate with clinical outcome. We sought to develop a radiotracer for positron emission tomography (PET) using a sulfonamide group that has been shown previously to bind specifically to CAIX. Such an imaging agent allows noninvasive monitoring of tumor CAIX status. We chose to synthesize 6-fluoro-N-(3-sulfamoylphenyl)hexanamide (FSPH) as it can be produced from a tosylated precursor via a fluorine exchange reaction, resulting in a product with high specific activity as is needed in this application.

Methods: Commercially available ethyl 6-hydroxyhexanoate (1) was converted to its tosylate (2). Base hydrolysis of the ester gave the tosylated carboxylic acid (3). The acid was coupled to 3-aminobenzenesulfonamide to obtain the amide (4), which is the precursor for the labeling reaction to produce FSPH. Radiochemical labeling was performed in commercial GE TracerLab FX_{FN} synthesis module. ^{18}F-fluoride containing ^{18}O-water was passed through a SepPak QMA cartridge. The fluoride was eluted from the cartridge with Kryptofix-222 and K_2CO_3 in acetonitrile. The eluate was dried under vacuum with heating. The solution of precursor (4) in dimethyl sulfoxide (DMSO) was added to the dry ^{18}F-fluoride and heated at 110°C for 15 minutes. The resulting mixture was separated on HPLC using Gemini 5 μ C6-phenyl column (110Aº, 250 X 10.0 mm) with water-ethanol (9:1) as eluant. The retention time was 35 minutes with the flow rate of 5 mL/min. To obtain cold ^{19}F-FSPH, the precursor (4) was heated at 120°C with excess KF in DMF for 1 h. The product was purified by preparative TLC using 100% ethyl acetate.

Results: The ^{1}H- and ^{13}C-NMR spectra of the ^{19}F–FSPH indicate the formation of the desired product. The coinjection of the ^{18}F-FSPH reaction mixture along with the authentic ^{19}F-FSPH indicates that the radiolabeled product is chemically identical as FSPH. Radiochemical yield was approximately 10% at the end of synthesis.

Conclusions: The proposed synthetic pathway has been shown to produce FSPH with acceptable radiochemical yield. Previous work in our group has focused on labeling CAIX imaging probes with ^{18}F-^{19}F substitution reactions, resulting in products with low specific activities that cannot be effectively quantitated due to inherent competition for CAIX targets between hot and cold probe molecules within the formulated dose. FSPH has the potential to overcome this limitation and offer a clinically-viable strategy for detecting and quantifying CAIX expression in vivo. This agent is currently undergoing in vitro and in vivo testing to evaluate its specificity for CAIX as well as its biodistribution and pharmacokinetics.



EDC.HCl = N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride

Scheme 1. Synthesis of 6-fluoro-*N*-(3-sulfamoylphenyl)hexanamide (^{18}F-FSPH)

P309 SYNTHESIS, LABELING AND EVALUATION OF A NEW NEUROPEPTIDE Y ANALOGUE AS A BREAST TUMORS DIAGNOSTIC AGENT

M. GANDOMKAR*, R. NAJAFI, M. MAZIDI, M. GOUDARZI and S. MIRFALLAH
Atomic Energy Organization of Iran, Nuclear Science Research School, Nuclear Science & Technology Research Institute (NSTRI), Tehran, Iran

Objectives: Selected peptide receptors in human tumors have been shown to represent clinically relevant targets for cancer diagnosis and therapy. Neuropeptide Y (NPY) is a 36-amino acid peptide of the pancreatic polypeptide family. The aim of this work was to investigate this peptide as a new radiopharmaceutical for diagnosis of breast cancer.

Methods: [Phe^{7}, Pro^{34}] NPY with Y_1 receptor preference and agonistic properties was synthesized by solid phase method. After conjugation with Hydrazino nicotinic acid (HYNIC) labeling with ^{99m}Tc was performed. For labeled peptide, yield of labeling, stability in human serum, receptor binding in cell surface with internalization in SK-N-MC cells, and biodistribution in normal rat were determined.

Results: Peptide was synthesized and labeled with more than 95% purity. Radiolabeled peptide was stable in human serum and specifically binds and internalized in SK-N-MC cells (4 h = 8%). A rapid clearance from blood pool with fast and high accumulation of the ^{99m}Tc-HYNIC-NPY in the kidney followed by urinary excretion and low activity in liver and spleen was observed.

Conclusions: Results showed that this radiolabeled peptide is a potential candidate for future applications in tumor diagnosis.

P310 REGIOSPECIFICITY IMPLICATIONS OF α- AND γ-[18F]FE-FOLATE FOR THE PET IMAGING OF FOLATE RECEPTOR POSITIVE TUMOURS

P. Y. LAM*[1], T. L. ROSS[1], M. HONER[1], V. GROEHN[2], P. SCHUBIGER[1] and S. M. AMETAMEY[1]
1. ETH Zurich, Center for Radiopharmaceutical Science, Zurich, Switzerland; 2. Merck Eprova AG, Schaffhausen, Switzerland

Objectives: Hitherto the potential of folic acid derivatives as specific and effective Folate Receptor (FR) positive tumour imaging agents has been shown, despite a lack of folate-based routine PET radiopharmaceutical. Folic acid (K_d~1nM) consists of a dicarboxylate moiety that can be derivatised on the α- or γ- position. With few exceptions, chemical derivitisation was always performed at either, but not both, of the carboxylates. What governs binding affinity retention is not clearly known. α- and γ-2-(fluoroethylamino)folic acid (α-and γ-FEF) have been separately synthesised and found to have favourable binding affinities, with that of α–FEF being 7-fold higher than γ-FEF. ^{18}F-labelling showed promising results. The significant next step would thus be to compare the in vivo performances of α-[^{18}F]FEF and γ-[^{18}F]FEF in an attempt to address the issue of regiospecificity and its ultimate implications.

Methods: The reference compounds α- and γ-FEF were synthesised by first coupling the aliphatic starting material 2-fluoroethylamine to selectively protected glutamic acid for regiospecific derivatisation. The glutamate derivatives were then coupled to selectively protected pteroic acid. Upon final hydrolysis, the reference compounds were obtained and tested in an in vitro assay using KB cells for binding affinity determination. ^{18}F-labelling was achieved by means of n.c.a. [^{18}F]fluoride either in a direct nucleophilic substitution on the appropriate tosylate precursor, or via prosthetic group labeling using n.c.a. 2-[^{18}F] fluoroethylamine and subsequent coupling to folic acid. Plasma stability studies were carried out on the radiolabelled final products.

Results: The α-FEF and γ-FEF reference compounds were separately synthesised in 4 steps with high overall yields. In vitro binding assays revealed that α-FEF (IC_{50} = 2.57 ± 0.99nM) has 7-fold higher binding to the FR than γ-FEF (IC_{50} = 17.3 ± 7.9nM). Both are in the same range as native folic acid (IC_{50} = 1.12 ± 0.12nM). The corresponding radiolabelling precursors were also synthesised in 4 steps with high overall yields. Direct, nucleophilic ^{18}F-labelling of the α-FEF precursor gave α-[^{18}F]FEF in 2 steps with a RCY of 30%, while indirect labelling via the 2-[^{18}F]fluoroethylamine prosthetic group gave a mixture of α-/γ-[^{18}F]FEF in 4 steps with a total RCY of 33%. α-[^{18}F]FEF was stable in human plasma over a period of 90min.

Conclusions: Our results have shown that α-[^{18}F]FEF is a promising candidate to become the first folate-based routine PET radiopharmaceutical. The difference in binding affinity between α- and γ-FEF has proven the necessity of synthesising the regioisomers separately. γ-[^{18}F]FEF is expected to be obtained with similar ease to α-[^{18}F]FEF. In vivo performances of both regioisomers will be compared.

P311 RELEVANCE OF NONINVASIVE IMAGING OF DEAD AND DYING CELLS IN ADVERSE CARDIOVASCULAR AND ONCOLOGICAL SITUATIONS

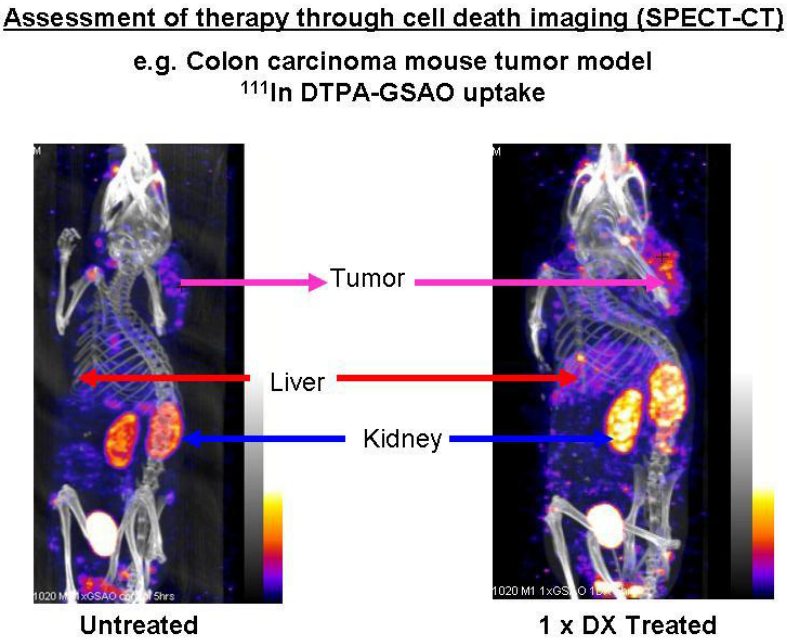
R. PANDURANGI* **and M. DYSLEWSZKI**
Covidien, Hazelwood, MO

Objectives: The objective of the present study is to summerise and compare radioimaging agents that image dead and dying cells in adverse clinical situations.

Methods: Tripeptide organic arsenical 4-(N-(S-glutathionylacetyl)amino)p-phenyl arsene oxide (GSAO, positive marker) and the corresponding acid (GSCA, negative control) were functionalized with DTPA, DOTA, Cy5.5 and pyrazine using standard conjugation techniques. DTPA derivatives were labeled with $^{111}InCl_3$. For in vitro studies, cultured Jurkat A3 cells were treated with100 ng/mL Fas-Ab either at 4 or 24 hrs and were stained with 10 μM GSAO-pyrazine or GSCA-pyrazine for flow cytometry analysis (Becton Dickinson). For in vitro cell binding assays, Jurkat A3 cells were incubated with either staurosporine or Fas antibody to induce cell death, followed by incubation with radiolabeled compounds, washed and counted for cell associated radioactivity. For in vivo imaging studies, Lewis Lung carcinoma (LLC) cells were implanted in C57BL/6 mice that were treated with cyclophosphamide (CPP, 150mg/kg) on days 10, 11 and 12 post implantation. Both treated and untreated mice were imaged with ^{111}in-DTPA-GSAO, ^{111}in-DTPA-GSCA and ^{99m}Tc Annexin V.

Results: Dysregulation of apoptosis is thought to have lead to pathological conditions such as cancer, autoimmune diseases which are often monitored though biopsies. A non-invasive imaging of cell death in real time using SPECT, PET or optical probes in adverse clinical situations can potentially change the patient management through the assessment of therapy. Current cell death imaging agents includes Annexin, Aposense and organo arsenical being developed in Covidien. Adverse cardiovascular situations include myocardial infarction (MI), myocardial ischemia-reperfusion, chronic heart failure, atherosclerosis and transplant rejection. Radiolabeled annexin V is very successful in imaging the cell death in above pathological situations. For example, salvaging of cardiomyocytes from reperfusion injury can have a clinical benefit for MI patients who can be assessed through the apoptosis imaging before and after inhibition of apoptosis using caspase inhibitors. Similarly, examples including 99mTc-Annexin V will be taken for the assessment of heart failure syndromes based on the upregulation of apoptosis of cardiomyocytes remote to MI. In adverse oncological clinical scenario, total cell death in cancer tissues correlates well with the regression of tumor. Optical probes of Annexin have shown the upregulation of cell death following chemotherapy is feasible. Radiolabeled organic arsenicals studied in our laboratory for the assessment of cell death will be discussed. Hence, the assessment of the efficacy of chemotherapy through total cell death quantification correlated to tumor regression and the role of cell death imaging in rational drug design will also be discussed.

Conclusions: Specific apoptosis imaging may have a clinical relavance in adverse cardiovascular situations, especially for salvaging apoptotic cells from death while in oncology it is the total cell death assessment can play a significant role for assessing the efficacy of chemotherapy



P312 STEREOSPECIFIC SYNTHESIS AND IN VITRO EVALUATION OF (S)/(R)-[123I]IVAIB FOR TUMOR IMAGING WITH SPECT

W. YU*[1], L. WILLIAMS[1], V. M. CAMP[1], E. MALVEAUX[1], Z. ZHANG[2], J. OLSON[2] and M. M. GOODMAN[1]
1. Emory University, Radiology, Atlanta, GA; 2. Emory University, Neurosurgery, Atlanta, GA

Objectives: Amino acids (S)- and (R)-2-amino-2-methyl-4-iodo-3-(E)-butenoic acid ((S)- and (R)-IVAIB) were synthesized, [^{123}I] radiolabeled and evaluated in vitro as potential SPECT tumor imaging agents in 5 human cancer cell lines.

Methods: The required (S)- and (R)-IVAIB precursors for radiolabeling were prepared from commercially available (S)- and (R)-α-methyl serine, respectively, according to the method developed in our lab (Yu, W., et al, J. Med. Chem., 2007, 50, 6718-6721). Briefly, (S)- and (R)-α-methyl serine were treated with di-tert-butyl dicarbonate followed by (trimethylsilyl)diazomethane to afford (R)- and (S)-N-Boc α-methyl-serine methyl ester. (S)- and (R)-alcohol were transformed to the corresponding aldehyde by Swern oxidation followed by reaction with iodoform/chromium(II) chloride then hexamethylditin/palladium(0) to afford (S)- and (R)-2-[N-(tert-butoxycarbonyl)amino]-2-methyl-4-trimethylstannyl-3-(E)-butenoic acid methyl ester as the radiolabeling precursors. Radioiodination was carried out with no-carrier-added [^{123}I]NaI-H_2O_2/H^+. (S)- and (R)- [^{123}I]IVAIB were obtained by hydrolysis with 4N hydrochloric acid and chromatographic purification. The in vitro cell study was performed in A549 (lung), H125 (lung), DU145 (prostate), MDA MB468 (breast) and U87 (brain) human cancer cells in amino acid-free Dulbecco's Modified Eagle's Medium incubated for 30 minutes at 37 °C to evaluate the compound tumor cell uptake and transport mechanism. 10 mM 2-Amino-bicyclo[2.2.1]-heptane-2-carboxylic acid (BCH) and 10 mM N-methyl-α-aminoisobutyric acid (MeAIB) were used as L- and A- amino acid transporter inhibitors, respectively.

Results: Radiolabeling yields were 72% (n=9, (S)-[^{123}I]IVAIB) and 67% (n=8, (R)-[^{123}I]IVAIB), with radiochemical purity over 99% as measured by radiometric TLC for both isomers. The results of cell assays were reported in the Table 1, with cell uptake from 0.17-1.1 ((S)-IVAIB) and 0.13-0.49 ((R)-IVAIB) percent initial dose per million cells (%ID /10^6 cells), respectively. The inhibition of uptake of (S)-IVAIB by BCH was 19-38%, vs. 68-88% by MeAIB, while uptake of (R)-IVAIB was inhibited 41-91% by BCH vs. 1-27% by MeAIB.

Conclusions: We found that the stereochemistry influenced substrate specificity of these amino acids. These findings suggested that (S)-IVAIB entered these cells in vitro primarily via A-type amino acid transport but (R)-IVAIB via L-type amino acid transport. The in vivo study will further validate these amino acids' property and the usefulness of tumor imaging with SPECT.

Research Support: Research supported by Nihon Medi-Physics Co., Ltd., and NIH.

Table 1: Cell uptake of (S)- and (R)-[123I]IVAIB (ID% /106 cells).

	A549	A549	H125	H125	DU145	DU145	MDA MB468	MDA MB468	U87	U87
[^{123}I]IVAIB	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-
Control	0.766	0.439	0.459	0.134	1.076	0.491	0.299	0.419	0.173	0.125
BCH	0.621	0.041	0.361	0.054	0.669	0.050	0.198	0.102	0.125	0.074
MeAIB	0.210	0.408	0.054	0.133	0.197	0.466	0.067	0.306	0.056	0.104

P313 GA-68 LABELLED RGD PEPTIDES FOR MONITORING ANGIOGENESIS

P. KNETSCH[1], M. PETRIK[1], C. RANGGER[1], M. FANI[2], A. HELBOK[1], E. VON GUGGENBERG*[1], C. DECRISTOFORO[1] and R. HAUBNER[1]

1. Medical University Innsbruck, Department of Nuclear Medicine, Innsbruck, Austria; 2. University Hospital Basel, Divisions of Radiological Chemistry, Basel, Switzerland

Objectives: Angiogenesis is a complex multistep process involved in a variety of diseases including tumor growth and development. One molecular target important there is the alpha(v)beta3 integrin. Thus, monitoring of its expression would be of benefit for planning and controlling of corresponding therapies. Here we present the synthesis and evaluation of a new Ga-68-labelled RGD-peptide targeting this receptor and compare it's tracer characteristics with [Ga-68]DOTA-RGD and [F-18]Galacto-RGD.

Methods: Fmoc-chemistry on a solid support was used for the synthesis of the peptides. Conjugation of the partially deprotected peptide c(-Arg(Pbf)-Gly-Asp(OtBu)-dPhe-Lys-) with the tert.Butyl-protected chelator NODAGA or DOTA, respectively, was carried out via in situ activation. Ga-68-Labelling of c(-Arg-Gly-Asp-dPhe-Lys(NODAGA)-) (NODAGA-RGD) was carried out at room temperature using 10μg peptide within 15min. Labelling of c(-Arg-Gly-Asp-dPhe-Lys(DOTA)-) (DOTA-RGD) followed similar protocols at 80°C. Stability was studied by incubating the compounds in Fe(III)-solution and human plasma. Hydrophilic properties were investigated by determining PBS/octanol partition coefficients. The protein binding characteristics were studied using human plasma and size exclusion spin columns. For in vitro uptake and in vivo biodistribution studies human melanoma M21 (alpha(v)beta3-positive) and M21-L (alpha(v)beta3-negative) cells were used. Melanoma-bearing nude mice were sacrificed 1h p.i. and activity distribution was measured using a gamma-counter.

Results: Labeling of DOTA-RGD and NODAGA-RGD can be carried out in almost quantitative radiochemical yields (>95%) for both tracers. Partition coefficients were -3.9 and -3.6, respectively, demonstrating the hydrophilic character of the tracers. Protein binding after 180 min was below 2% for [Ga-68]NODAGA-RGD. In contrast, for [Ga-68]DOTA-RGD this value was higher than 20%. HPLC-analysis of the supernatant revealed >96% intact [Ga-68]NODAGA-RGD. In vitro internalization assays showed significantly higher uptake in M21 as in M21-L cells which could be blocked only for the receptor positive cells. An initial biodistribution study 1h p.i. confirmed receptor specific uptake in the tumor (1.5±0.4% ID/g (positive) vs. 0.3±0.1% ID/g (negative) and improved blood clearance compared with [Ga-68]DOTA-RGD (0.18±0.03% ID/g vs. 0.72±0.05% ID/g).

Conclusions: Our data indicate that [Ga-68]NODAGA-RGD allows selective monitoring of alpha(v)beta3 expression and demonstrate that the NODAGA chelator system is superior to the DOTA-system for labeling of RGD with Ga-68. Thus, this compound may be an easily accessible alternative to the very complex to produce [F-18]Galacto-RGD in imaging alpha(v)beta3 expression.

P314 PHOSPHORYLATION AND UPTAKE OF 3-DEOXY-3-[18F]FLUOROTHYMIDINE IN COLON CANCER CELL LYSATES

N. GUO*[1], J. XIE[1], H. C. MANNING[1], N. G. DEANE[1], M. S. ANSARI[1], R. J. COFFEY[2], J. GORE[2], R. R. PRICE[2], R. M. BALDWIN[3] and J. O. MCINTYRE[3]

1. Vanderbilt University School of Medicine, Department of Radiology & Radiological Sciences, Vanderbilt University Institute of Imaging Science (VUIIS), Nashville, TN; 2. Vanderbilt University School of Medicine, Department of Cancer Biology, Nashville, TN; 3. Vanderbilt University School of Medicine, Department of Medicine, Nashville, TN

Objectives: Molecular imaging with positron emission tomography (PET) is a unique, noninvasive imaging approach for cancer diagnosis. [^{18}F]FLT has been developed as a tracer targeted to the thymidine kinase 1 (TK-1) for imaging tumor cellular proliferation. .Appropriate use of [^{18}F]FLT tracer requires validation of the TK1 activity and cell uptake. Here, we report a study on the phosphorylation and uptake of [^{18}F]FLT in cancer cells/or cell lysates by using novel phosphoryl-transfer assay developed in our lab. The intrinsic ^{18}F radioactivity was measured to quantify both substrate and phosphorylated products, separated using a rapid thin layer chromatography (TLC) method.

Methods: Cell lysates were prepared from actively growing SW480 human colon cancer cells in culture. Phosphorylation of [^{18}F]FLT by cell lysate, initiated by addition of ATP was followed for up to 2 h at 37° C. Reactions, terminated by addition of SDS (to 1%) and cooling to 4° C, were then analyzed by a rapid TLC (< 15 min elution time) to resolve phosphorylated products from the substrate. The ^{18}F-radio-spots were quantified using a Bioscan AR-2000 TLC Scanner.

Results: Initial phosphorylation of [^{18}F]FLT appears to be mediated by TK1 activity yielding monophophate (FLTMP). After 40 min incubation of [^{18}F]FLT in cell lysate, products are FLTMP, diphosphate (FLTDP) and/or triphosphate (FLTTP) with about 30% [^{18}F]FLT remaining with essentially complete conversion of [^{18}F]FLT to FLTDP and/or FLTTP after 2 h. The apparent K_m of [^{18}F]FLT measured is 4.8±0.3 μM and a maximum velocity (V_{max}) is 7.4 pmol•min^{-1} per 1×10^6 cells. Uptake kinetics analyzed by Eadie-Hofstee plots of [^{18}F]FLT in the absence and presence of known inhibitors of nucleoside transporters (NTs) in SW480 tumor cells yielded a value of K_t = 26.9 ± 2.4 μM, with maximum uptake velocity V_{max} = 97±6 pmol min^{-1} per 1×10^6 cells and consistent with a low-affinity and high-capacity system. The uptake of [^{18}F]FLT in SW480 cancer cells, 0.8 and 1.7 μCi per 10^6 cells at 30 and 60 min, respectively, was almost completely inhibited by thymidine, or dipyridamole and partly inhibited by adenine, uridine, or NBMPR.

Conclusions: The apparent K_m of [^{18}F]FLT was comparable to the value reported with purified recombinant TK-1. The uptake of [^{18}F]FLT by SW480 cells is inhibited by nitrobenzylthioinosine or dipyridamole indicating that it is mediated predominantly by the equilibrative nucleoside transporters in these tumor cells. The method developed here provides a fast and simple method to measure TK-1 kinetic parameters that can be used to test the phosphorylation of new analog tracers mediated by TK-1.

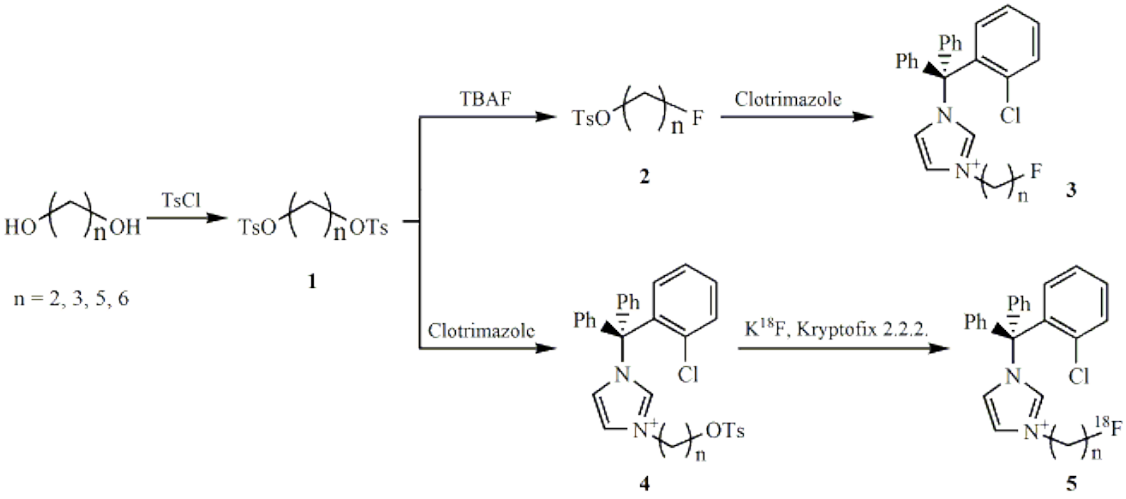
Research Support: NIH/NCI R25T-CA092043, ICMIC (P50CA128323), Vanderbilt Dept. of Radiology

P315 SYNTHESIS OF F-18 LABELED CLOTRIMAZOLE DERIVATIVES FOR TUMOR IMAGING

S. JUNG*[1], J. PARK[2], S. KIM[2], M. HUR[2], S. YANG[2] and K. YU[1]
1. Dongguk University, Dept of Chemistry, Seoul, Korea; 2. Korea Atomic Energy Research Institute, Radiation Research Division for Industry & Environment, Jeongeup, Korea

Objectives: Clotrimazole [1-{(2-chlorophenyl)-diphenylmethyl}-1H-imidazole] inhibits tumor cell proliferation and angiogenesis. Antitumor abilities of clotrimazole are caused by blocking the synthesis of G_1 cyclin, which is associated with CDK (cyclin dependent kinase) activity required for progression from G_1 into S phase and inhibiting the proliferation of vascular endothelial cells. The radioisotope labeled clotrimazole derivative can be utilized to monitor the physiologic processes of cancer. In this study, we synthesized the F-18 labeled clotrimazole derivatives as a new tumor imaging agent for PET.

Methods: α,ω-alkanediols were reacted with tosyl chloride in pyridine to give 1. 1 were converted to 2through the reaction with TBAF. The references 3 were prepared by refluxing with clotrimazole and excess of 2 in acetonitrile for 48 h and clotrimazole reacted with 1 to give 4. F-18 labeled reaction was performed with 4 in Kryptofix[2.2.2]/K_2CO_3 for 10 min at 80 ℃. The radiolabeling mixture was passed through a silica Sep-Pak cartridge to remove ^{18}F. The [^{18}F]F-clotrimazole derivatives 5 were purified by preparative HPLC method.



Scheme 1. The synthesis of fluoroclotrimazole derivatives and [^{18}F]F-clotrimazole derivatives.

Results: The references 3 were synthesized in 35-46% yield and the [^{18}F]F-clotrimazole derivatives 5 were synthesized in 20-25% yield. In the radiofluorination step, we used acetonitrile and DMSO as a solvent and observed higher yield at acetonitrile (25%) reaction compared with DMSO reaction (5%).

Conclusions: F-18 labeled clotrimazole derivatives were carried out as a novel potential PET radiotracer for cancer imaging agent. Further pharmacological investigation of [^{18}F]F-clotrimazole derivative is in progress.

References: [1] R. A. Al-Qawasmeh, Y. Lee, M. Cao, X. Gu, A. Vassilakos, J. A. Wright, A. Young, Bioorg. Med. Chem. Lett. 2004, 14, 347-350. [2] S. Takei, T. Iseda, M. Yokoyama, Int. J. Urology. 2003, 10, 78-85. [3] K. H. Yu, Y. S. Kim, S. W. Kim, J. H. Park, S. D. Yang, W. Herdering, A. Knoechel, J. Label. Compd. Radiopharm. 2003, 46, 1151-1160.

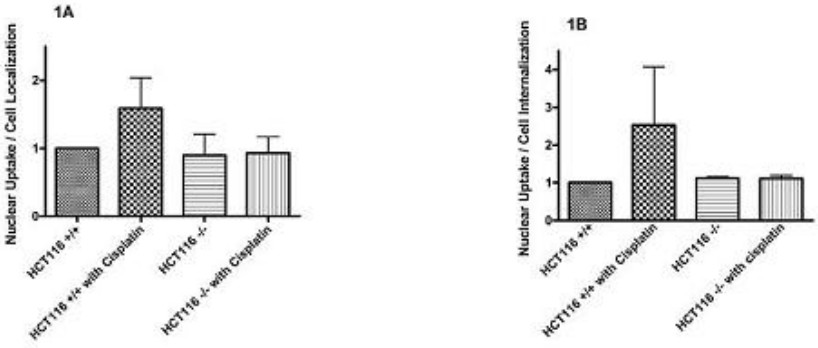
P316 RELATIONSHIP BETWEEN CISPLATIN, COPPER-64 RADIOPHARMACEUTICALS AND p53 IN THE TRAFFICKING OF 64Cu TO THE NUCLEI OF TUMOR CELLS

Y. GUO*, A. ZHELENZNYAK and C. J. ANDERSON
Mallinckrodt Institute of Radiology, Washington University School of Medicine, St.Louis, MO

Objectives: Copper-64 ($T_{1/2}$ =12.7 h) emits β^+ and β^- particles for applications in both PET imaging and cancer therapy. Cisplatin is an effective antineoplastic agent that is used for the treatment of cancer. Its cytotoxicity is mediated mainly through interaction with DNA and inhibition of DNA synthesis and replication. We previously reported that the tumor suppressor protein p53 was involved in copper trafficking to tumor cell nuclei, as ^{64}Cu was delivered to nuclei in a p53 positive HCT116 colorectal cancer cells in significantly greater amounts than in p53 negative HCT116 cells.[1] Recently it was demonstrated that p53 may be a functional link of the differential effect of copper on cisplatin mediated cell death.[2] We are investigating the mechanism(s) of how ^{64}Cu and cisplatin may affect each other's trafficking to tumor cell nuclei, and their effects on cancer therapy.

Methods: Two human colorectal HCT116 cell lines that are positive and negative for p53 were used to compare internalization and nuclear localization of ^{64}Cu-acetate under the influence of cisplatin. HCT116 cells were incubated with or without cisplatin for a 16 h time course. Two different time points for the addition of ^{64}Cu were applied: 1) ^{64}Cu was added 1 h before cisplatin; and 2) ^{64}Cu was added 12 h after cisplatin.

Results: In HCT116+/+ cell line, cisplatin enhanced ^{64}Cu nuclear localization (HCT116+/+ w/ cisplatin versus wo/ cisplatin = 1.60±0.45 in Figure 1A, 2.53±1.55, in Figure 1B). However, cisplatin did not affect ^{64}Cu nuclear uptake in p53 negative cells (HCT116-/- w/ C-DDP versus wo/ C-DDP = 1.06±0.15 in Figure 1A, 0.99±0.07 in Figure 1B). Western blot showed that the expression of p53 was notably up-regulated by cisplatin, and the accumulation of p53 was more prevalent in the nucleus rather than in the cytosol.



Conclusions: These data demonstrate that p53 plays an important role in ^{64}Cu trafficking to cell nucleus as the upregulation of p53 expression enhances the nuclear uptake of ^{64}Cu. The data suggest that a synergistic therapeutic effect will result in combining cisplatin chemotherapy with targeted radiotherapy using ^{64}Cu radiopharmaceuticals in p53 positive tumors, in part due to the delivery of a higher radiation dose to the cell nucleus.

References: 1.Eiblmaier et al., Cancer Biol Ther. 2008; 7: 63-69.2.Kabolizadeh et al., Biochem Pharmacol. 2007; 73: 1270 1279.

P317 SYNTHESIS AND IN VITRO EVALUATION OF 2-[C-11]METHOXYESTRADIOL BISSULFAMATE

C. M. KANG*, Y. S. CHOE, I. LEE, J. Y. CHOI, Y. CHOI, K. H. LEE and B. T. KIM
Samsung Medical Center, Sungkyunkwan University School of Medicine, Dept of Nuclear Medicine, Seoul, South Korea

Objectives: Angiogenesis represents the formation of new capillaries by outgrowth from existing microvessels. 2-Methoxyestradiol has shown potent inhibitory effects on proliferating cells and angiogenesis in vitro and in vivo. Our preliminary studies showed low uptake of 2-[C-11]methoxyestradiol in mice bearing cancer cells, probably due to its low bioavailability. Therefore, in the present study, we synthesized 2-[C-11]methoxyestradiol bissulfamate ([C-11]1), which is known to inhibit tumor growth in vivo and to have high bioavailability, and evaluated its biological properties in vitro.

Methods: [C-11]CH_4 was produced using a PETtrace cyclotron (GE Healthcare). Radiotracer [C-11]1 was prepared by labeling of N,N-bistritylated 2-hydroxyestradiol bissulfamate with [C-11]CH_3I in the presence of NaOH (80 °C, 5 min), followed by detritylation under acidic conditions (80 °C, 5 min) using a TRACERlab FXc module. After neutralization, [C-11]1 was purified by reverse phase HPLC (45:55 ethanol-water) and formulated in 10% ethanol in saline. N,N-Bistritylated 2-hydroxyestradiol bissulfamate was synthesized from estradiol in 10 steps. Non-radiolabeled standard, 1 was synthesized by bissulfamoylation of 2-methoxyestradiol. For in vitro evaluation, [C-11]1 (555 kBq) was incubated with HUVE cells at 37 °C for 5, 15, 30, and 60 min (n=3). Cells were then washed twice with PBS containing BSA (0.1%) and counted. For blocking studies, HUVE cells were incubated with [C-11]1 in the presence of 1 (10 μM) at 37 °C for 60 min. Cells were then washed as described above and counted. Partition coefficient of [C-11]1 was measured using octanol and water, and expressed as log Po/w.

Results: Synthesis of N,N-bistritylated 2-hydroxyestradiol bissulfamate required 10 reaction steps, because of the presence of two sulfamoyl groups. Radiotracer [C-11]1 was synthesized in 22-26% decay-corrected radiochemical yield (EOB). Synthesis time including HPLC purification was 42 min (EOB). In vitro studies showed that [C-11]1 was taken up by HUVE cells (0.5-1.0% ID) and this uptake increased slightly over time (i.e., 128% at 15 min, 151% at 30 min, and 156% at 60 min, relative to a value of 100% at 5 min). In addition, [C-11]1 uptake by HUVE cells at 60 min was inhibited in the presence of 1 by 33%, indicating that [C-11]1 binds specifically to HUVE cells. Radiotracer [C-11]1 had log Po/w value of 3.0.

Conclusions: Relatively low uptake of [C-11]1 by HUVE cells suggests its low permeability through the cell membranes, despite its moderate partition coefficient. Further studies are warranted to investigate the pharmacokinetics of [C-11]1 in mice bearing cancer cells.

Research Support: KOSEF grant funded by MEST, Korea (M208B090053010).