DUAL-MODALITY NANOPARTICLES FOR PET/MR MOLECULAR IMAGING

C. GLAUS*[1], A. HAGOOLY[2], M. J. WELCH[2] and G. BAO[1]
1. Georgia Institute of Technology, Dept. of Biomedical Engineering, Atlanta, GA; 2. Washington University School of Medicine, Mallinckrodt Institute of Radiology, St. Louis, MO

Objectives: Combining PET with the high resolution, contrast-enhanced abilities of MRI would produce a breakthrough in the detection and monitoring of disease. Interest in combining PET with MRI has surged, and structured nanomaterials have emerged as a platform for dual PET/MR imaging agents. To this end, we report the development of nanoparticles for dual PET/MR imaging of αvβ3 integrin expression in a model of angiogenesis.

Methods: Iron oxide cores coated with amine-modified, crosslinked dextran served as the magnetic nanoparticle (MNP) platform. DOTA was conjugated to the MNP coating to allow radiolabeling with ^{64}Cu. DOTA-MNPs were reacted with NHS-polyethylene glycol-maleimide and multiple c(RGDyC) peptides were conjugated to the particles. Labeled nanoparticles (^{64}Cu-MNP-RGD) were characterized by radio-FPLC, TLC, and zeta-DLS. The number of peptides per particle was measured and stability in mouse serum was determined (24h at 37°C). A 1% agar imaging phantom was used for PET and MRI characterization. Cell imaging was performed using U87MG cells (αvβ3 positive) treated with dye-labeled MNP-RGD or MNP, with and without free-peptide blocking. Uptake assays were performed using M21 (αvβ3 positive) and M21L (αvβ3 negative) cell lines treated with ^{64}Cu-MNP-RGD or ^{64}Cu-MNP, with and without blocking. U87MG tumor-bearing mice were injected with ^{64}Cu-MNP-RGD or ^{64}Cu-MNP and PET/CT imaging was performed, followed by organ biodistribution. MRI at 7T was performed before injection and at 24h p.i., tumor contrast was quantified, and colormaps were generated for T2-weighted images.

Results: Radio-FPLC and TLC analysis showed high yield, radiochemical purity, specific activity, and particle monodispersity. The particles had a diameter of 42±6nm, a surface potential of 4mV, contained 43±9 peptides, and were stable in serum. Imaging phantoms confirmed the labeled particles produced both strong PET signal and MRI contrast. Cell imaging and uptake studies verified specific targeting of ^{64}Cu-MNP-RGD to αvβ3. Peptide-functionalized ^{64}Cu-MNP-RGD showed rapid blood clearance and increased uptake by the reticuloendothelial system versus ^{64}Cu-MNP (blood: 1±0.3 vs. 16.7±1.5; liver: 50.4±5.1 vs. 22.4±11.5; spleen: 61.1±14.7 vs. 13.7±7.1%ID/g at 24h p.i., respectively). While both ^{64}Cu-MNP-RGD and ^{64}Cu-MNP was present in tumors, the tumor-to-tissue ratios for ^{64}Cu-MNP-RGD were significantly greater than those of ^{64}Cu-MNP (blood: 3.4±1.6 vs. 0.4±0.1; muscle: 6.4±2.2 vs. 2.9±0.8; fat: 8.9±4.7 vs. 3.4± at 24h p.i., respectively). PET images of ^{64}Cu-MNP-RGD show clear delineation of tumors, while ^{64}Cu-MNP has widespread, diffuse accumulation (Fig. 1). Both RGD-targeted and non-targeted particles produced contrast enhancement in MRIs, however accumulation of non-targeted particles is likely due to EPR effects.

Conclusions: Our studies assess the efficacy of the dual PET/MR nanoparticles for in vivo molecular imaging, and offer insight into opportunities for PET/MR in the detection and monitoring of disease.

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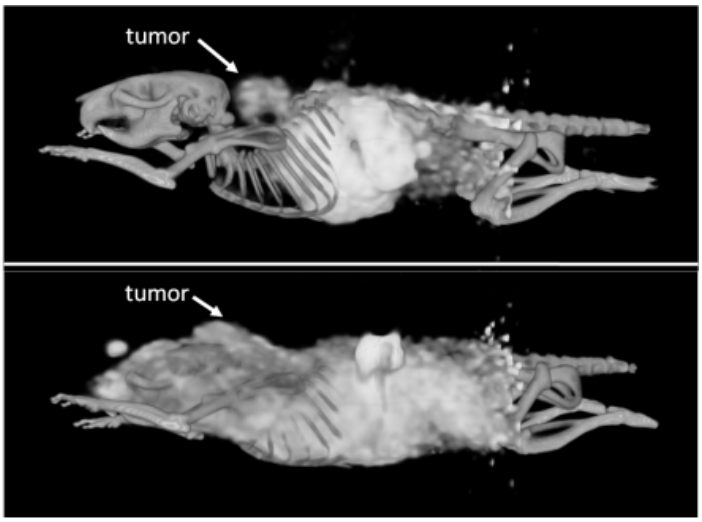
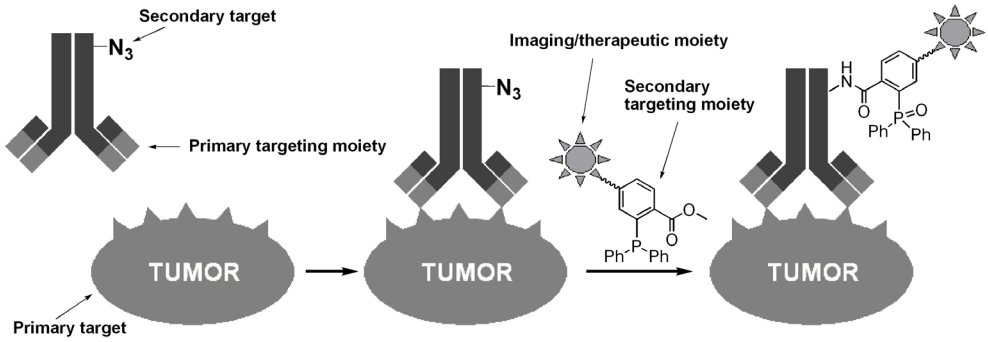


Figure 1: 3D MicroPET/CT imaging ^{64}Cu-MNP-RGD (top) and ^{64}Cu-MNP (bottom) in tumor-bearing mice at 24 hr post-injection. PET/CT images show clear tumor delineation using RGD-targeted nanoparticles (top), while untargeted nanoparticles (bottom) resulted in widespread uptake.

STAUDINGER LIGATION AS A CONJUGATION STRATEGY FOR MULTI-MODALITY IMAGING AND THERAPY WITH PRETARGETED ANTIBODIES

D. J. VUGTS*[1], A. VERVOORT[1], M. S. ROBILLARD[2], R. C. VULDERS[2], J. D. HERSCHEID[3] and G. A. VAN DONGEN[1]
1. VU University Medical Center, Dept of Otolaryngology/Head and Neck Surgery, Amsterdam, Netherlands; 2. Philips Research, Biomolecular Engineering, Eindhoven, Netherlands; 3. VU University Medical Center, Dept of Nuclear Medicine & PET Research, Amsterdam, Netherlands

Objectives: Monoclonal antibodies (MAbs) are gaining momentum for the use in diagnosis and disease-selective therapy. Presently, 21 MAbs have been approved by the FDA for therapy, most of them for systemic treatment of cancer. These are all intact MAbs, either in naked form or conjugated to a toxin or radionuclide. After administration of such intact MAbs, it takes 3-6 days before optimal tumor-to-normal tissue ratios are obtained. New strategies to prevent excessive exposure of normal tissues to the toxin or radionuclide include the use of pretargeting approaches, which involve tumor delivery of the targeting MAb in the first step, and subsequent binding of a fast-kinetic imaging/therapeutic probe to the tumor-localized MAb in the second step. In the present study we evaluated the possibility to use the Staudinger ligation as a conjugation strategy in such a pretargeting approach. A MAb (primary targeting moiety) is functionalized with an azide handle (secondary target). This azide can react covalently via a Staudinger ligation with a small phosphine probe, which contains an imaging/therapeutic moiety (secondary targeting moiety).



Methods: MAb-azide conjugates were synthesized via attachment of an NHS-triazide functionality to the lysine groups of the MAb. The integrity, immunoreactivity and in vivo biodistribution of the conjugates in tumor bearing nude mice were assessed. Phosphine probes containing the chelates (1) desferal for radiolabeling with e.g. Zr-89 or Ga-67/68 or (2) cubane moiety for radiolabeling with e.g. I-123/131, were synthesized, procedures for radiolabeling developed, and oxidation of the phosphine probe assessed. MAb-azide conjugates and phosphine probes were used for Staudinger ligation in PBS or PBS + human serum (HS). After 2 h ligation, the efficiency of conjugation was assessed either by PD10 purification or by SDS-PAGE analysis, while the integrity of the complex was evaluated.

Results: NHS-triazide moieties were coupled to the MAb with 50% efficiency. Up to 10 lysine groups can be modified (30 azides attached per MAb), without loss of MAb immunoreactivity or impairment of in vivo tumor targeting capacity. The yield of radiolabeling of the different phosphine probes containing (1) desferal was >95% and (2) cubane was 80%, while less than 15% oxidation was observed in the final product. The phosphine probes were stable in PBS as well as in 70% HS and in vitro Staudinger ligation under these conditions resulted in up to 30% efficiency in a 3.33 μM MAb solution with 1 equivalent of phosphine per azide molecule. The resulting MAb-phosphine complex was stable in PBS as well as in HS for at least 4 days.

Conclusions: Staudinger ligation has proven to occur efficiently in vitro for the coupling of small imaging/therapeutic probes to MAbs. Future research will be focused on extending this pretargeting approach to in vivo applications.

TRI-MODALITY IRON OXIDE NANOPARTICLES FOR TUMOR IMAGING

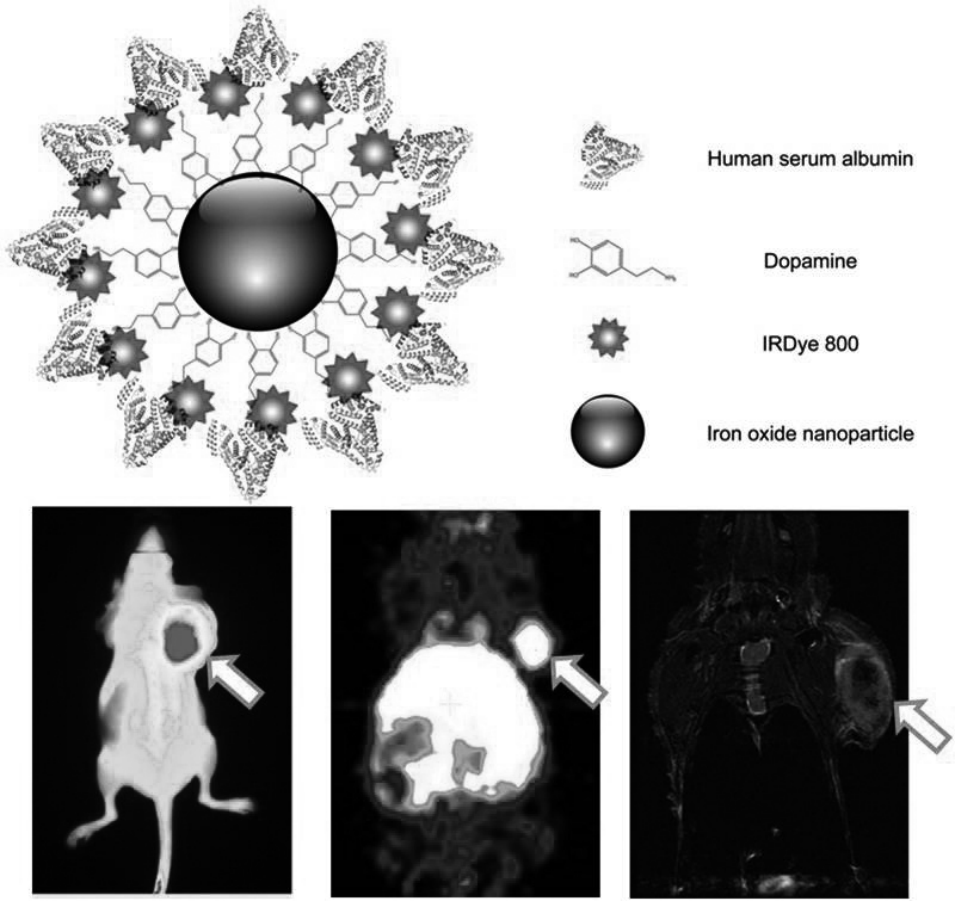
J. XIE*[1], K. CHEN[1], J. HUANG[2], X. LI[2] and X. CHEN[1]
1. Stanford University, The Molecular Imaging Program at Stanford (MIPS), Department of Radiology and Bio-X Program, Stanford University School of Medicine, Stanford, CA; 2. Peking University, Department of Chemistry, Beijing, China

Objectives: To prepare iron oxide nanoparticle based tri-modality probes for PET, MRI, and near-infrared fluorescence imaging.

Methods: Iron oxide nanoparticles with narrow size distribution were synthesized from pyrolysis, with single crystallinity and close-to-bulk magnetization. The hydrophobic coatings were replaced by dopamine or caffeic acid, and then further coated with human serum albumin. With multiple amine groups available from the HSA coating, the particles were coupled with IRDye 800 and DOTA, and then labeled with ^{64}Cu. The resulting trimodality imaging probe was i.v. injected into U87MG glioblastoma xenograft model. The tumor targeting ability and in vivo kinetics of the probes were monitored by PET, MRI and optical imaging.

Results: HSA-coated iron oxide particles (HSA-IONPs) are highly stable in water, various buffers and serum. In vitro cytotoxicity studies showed that HSA-IONPs are non-toxic. R2 relaxivity was measured to be about 200 $mmol^{-1}s^{-1}$, which is significant higher than that of FDA approved Feridex particles. When i.v. injected into U87MG tumor bearing mice, significant T2-weighted MR signal drop was observed at the tumor site, suggesting successful homing of the IONPs to the tumor site, which is most likely due to the enhanced permeability and retention (EPR) effect of the tumor. The MR result was further confirmed by PET and optical imaging as well as ex vivo histopathology.

Conclusions: To the best of our knowledge, we demonstrate here for the first time that iron oxide nanoparticle based tri-modality probes that are active in MRI, PET and fluorescence optical imaging. Such result is encouraging for allowing more accurate tumor detection and delineation, and potentially providing more anatomic, functional andmolecular information of the tumors under study.



A PET/MRI HYBRID NANO-PROBE FOR SENTINEL LYMPH NODE IMAGING

J. PARK[*1], J. CHOI[2], H. NAH[2], S. WOO[3], G. AN[4], K. KIM[4], G. J. CHEON[4], Y. CHANG[5], J. CHEON[2], S. LEE[6], J. LEE[6] and J. YOO[1]

1. Kyungpook National University School of Medicine, Department of Molecular Medicine, Daegu, South Korea; 2. Yonsei University, Department of Chemistry, Seoul, South Korea; 3. Kyungpook National University, Department of Biomedical Engineering, Daegu, South Korea; 4. Korea Institute of Radiological and Medical Sciences, Molecular Imaging Research Center, Research Institute of Radiological and Medical Sciences, Seoul, South Korea; 5. Kyungpook National University School of Medicine and Hospital, Department of Diagnostic Radiology, Daegu, South Korea; 6. Kyungpook National University School of Medicine, Department of Nuclear Medicine, Daegu, South Korea

Objectives: Many combinations of different imaging modalities, which benefit the complementary nature of the each modality, has been tested and applied for various biological research and early detection of diseases. Recently, simultaneous PET/MRI fusion scanner has been developed. In addition to the instrumental, the development of multimodality imaging probes is a prerequisite for more accurate and specific diagnostics. Herein, we report a magnetic nanoparticle-based PET/MRI hybrid probe and the evaluation of its usefulness as multimodality imaging probe.

Methods: Mn doped Magnetism Engineered Iron Oxide (MnMEIO, composition of $MnFe_2O_4$) was prepared and coated with serum albumin (SA). SA-MnMEIO was radio-labeled wih ^{124}I by using IODO-BEADS. The ^{124}I-SA-MnMEIO was purified by centrifugation to separate from free ^{124}I. Purified ^{124}I-SA-MnMEIO was injected into the paw of rat for lymph node imaging. Right after the PET scan, MR imaging was performed, then PET and MR images were co-registered using AMIDE program. After in vivo imaging, the lymph nodes were dissected at 40 min post injection of blue dye and ex vivo PET/MR imaging was performed. Long-term follow up study was carried out to study clearance pattern of the nano probe. Different amount of imaging probe was also tested to determine detection limit.

Results: SA-MnMEIO nanoparticles possess 32nm of hydrodynamic size. The radiochemical purity of ^{124}I-SA-MnMEIO after purification was higher than 92%. After injection of the hybrid probe, two bright spots were clearly identified in microPET imaging. However, only one dark spot could be assigned as lymph node unambiguously in MR imaging, but the assignment of another faint dark spot as lymph node could not be made without help of PET. Only when microPET image was overlaid with MR image by aid of four fiduciary markers, two different sentinel lymph nodes were clearly assigned as brachial and axillary lymph nodes thanks to the complementary nature of high sensitivity of PET and in detail anatomical information of MRI. The ex vivo PET/MR imaging of excised lymph nodes was consistent with in vivo images. Long-term follow-up studies revealed that the uptake of nano-probe was maximized 1d post-injection but lasted up to 3 weeks. Two lymph nodes could be visualized with only 10 mg injection of ^{124}I-SA-MnMEIO.

Conclusions: We have developed an iron oxide-based hybrid probe for PET/MR dual-modality imaging. Two different sentinel lymph nodes were clearly assigned by aid of fused PET/MR imaging. The differentiation studies of tumor-free from metastasized lymph node are undergoing using the developed hybrid PET/MR probe.

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SOMATOSTATIN TARGETED LIPOSOMES FOR MRI / SPECT DUAL IMAGING PROBES

D. ABOU*[1], D. SCHUHLE[2], J. PETERS[2] and H. WOLTERBEEK[1]
1. TUDelft, Radiation and Isotopes for Health, Delft, Netherlands; 2. TUDelft, Biocatalysis and Organic Chemistry, Delft, Netherlands

Objectives: MRI (Magnetic Resonance Imaging) and SPECT (Single Photon Emission Computarized Tomography) are two well-known imaging techniques widely used in the world of medical diagnosis of cancer. MRI gives a superb image resolution of tissue; however the results obtained with contrast agents are, in some case, not sensitive and selective enough towards tumors. On the other hand, SPECT is a highly sensitive imaging method but is giving a relatively poor spatial resolution. Combining both techniques in a nanocarrier such as a liposome could benefit from the advantages of both imaging systems. In the mean time, the surface modification of the liposomes allows the attachment of a protein or a peptide in order to confer the active tumor targeting property. The goal of this project is to develop liposomes (Fig.1) carrying Octreotide on their surface, formulated with Gd complexes within the membrane and enclosing radionuclides such as ^{99m}Tc in their cavity.

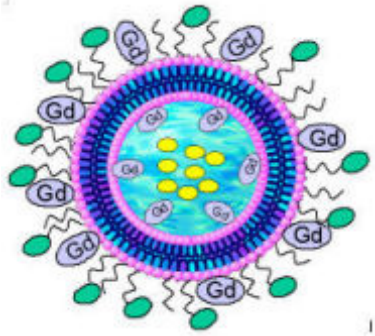
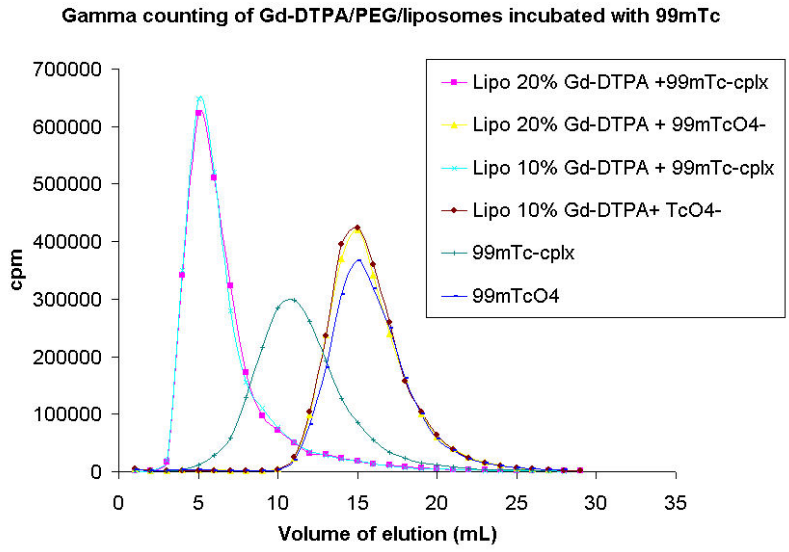


Fig1: Octreotide (green circles) liposome formulated with Gd contrast agent (blue circles) for MRI and loaded with ^{99m}Tc (yellow circles) for SPECT application. To prepare such a particle, a multidisciplinary approach is investigated, involving: •The coupling of Octreotide to PEGylated liposomes using EDC / S-NHS. •An exploration towards new paramagnetic liposomes formulated with a Gd-DOTA calixaren derivative. •The formulation and the stability study of ^{99m}Tc-liposomes combining Gd contrast agents.

Methods: The coupling efficiency of the peptide was defined using HPLC and LC-MS. The paramagnetic liposomes were characterized using relaxivity measurements with NMR. The relaxivity behavior of the liposomes was studied versus the concentration of the complex and the temperature. The formulation of ^{99m}Tc- liposomes and the stability of the radioactive liposomes were followed using a PD-10 gel permeation chromatography and gamma detection.

Results: Octreotide was attached to liposomes with a coupling efficiency of 35% and the product DSPE-PEG$_{2000}$-N-octreotide has been characterized using LC-MS. The formulations of Gd-DOTA calixaren liposomes showed impressive NMRD profiles with a high relaxivity r1, added to an amplified effect at 5C and 56C. Finally, the inclusion of ^{99m}Tc in liposomes containing Gd complexes was successfully performed with a relatively high labeling efficiency of 65% concentrated in the liposomes fractions. No trans-chelation occurred with the trans-membranar Gd complex.

Conclusions: Techniques to couple octreotide to liposomes have been developed. Incorporation of Gd-DOTA derivative of calixaren results in highly efficient MRI contrast agents, whereas the SPECT contrast agent ^{99m}Tc can be included conveniently into liposome cavities. Further work to the final assembly of a dual modality probe along these lines is in progress.



USE OF A BETA MICROPROBE SYSTEM AS AN AFFORDABLE TRANSLATIONAL TOOL COMPARED TO PET – EXAMPLES USING FDG AND 18F-FALLYPRIDE BINDING

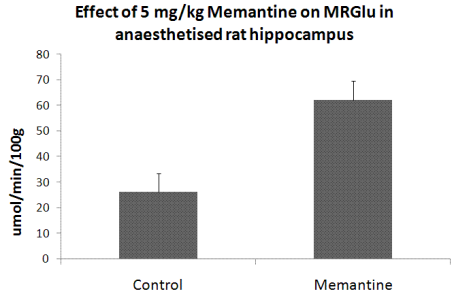
G. WARNOCK*[1], D. GOBLET[1], C. LEMAIRE[1], F. GIACOMELLI[1], M. BAHRI[1], X. LANGLOIS[2], A. LUXEN[1] and A. PLENEVAUX[1]

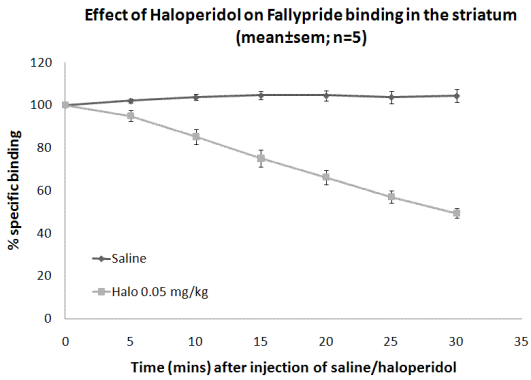
1. University of Liege, Cyclotron Research Centre, Liege, Belgium; 2. Johnson & Johnson Pharmaceutical Research & Development, A Division of Janssen Pharmaceutica N.V., Beerse, Belgium

Objectives: Small animal PET scanning allows the acquisition of functional data from the rodent brain using suitable radiotracers and radioligands. However, PET scanners themselves are prohibitively expensive for some labs. An increase in the affordability and availability of neuroimaging methods could benefit the understanding of brain disorders and the search for new therapies. Beta microprobe systems have recently become commercially available as an affordable alternative to PET. These systems utilise implantable probes consisting of a small (0.25-1mm diameter, 1mm length) scintillation crystal bonded to a fibre optic cable, which can be stereotactically located in a specific region of the rodent brain. Scintillation in the crystal is carried by fibre optic to a photomultiplier tube from which a time-activity curve is generated. We have used a beta microprobe system (Swisstrace) to measure glucose metabolism (MRGlu) using ^{18}F-fluorodeoxyglucose (FDG) and dopamine D2 receptor binding using ^{18}F-fallypride. To achieve quantitative analysis of FDG data an arterial input function was derived using an arteriovenous shunt/probe combination developed in our lab. For displacement of ^{18}F-fallypride, the antipsychotic haloperidol was chosen for its high affinity for D2 receptors.

Methods: All studies were carried out with the approval of the local ethical committee. For FDG studies catheters were implanted in the femoral artery and vein of anaesthetised rats for the connection of an arteriovenous shunt. A 0.5mm beta microprobe was implanted in the hippocampus for measurement of FDG uptake. A dual-injection protocol was used for within-subject control and treatment measurements. Prior to the second FDG injection 20 mg/kg memantine was administered IV. MRGlu was calculated using a 2 compartment model in PMOD. The beta microprobe system was used to measure ^{18}F-fallypride binding in the striatum of Sprague-Dawley rats, using the cerebellum as a reference region. ^{18}F-fallypride was injected intravenously through a catheter in the femoral vein and activity in the striatum and cerebellum measured for 90 minutes. After 30 minutes a bolus of saline was injected as a control before the injection of haloperidol (0.025-0.25mg/kg) 30 minutes later. Specific striatal binding was calculated as [total binding striatum]-[binding cerebellum].

Results: Memantine increased glucose metabolism in the hippocampus. Haloperidol displaced ^{18}F-fallypride binding in the striatum in a clear dose- and time-dependent manner.





Conclusions: This results confirm the usefulness of the beta microprobe system for the measurement of D2 binding using ^{18}F-fallypride, as an alternative to PET. Memantine may be of use in the NMDA antagonist model of psychosis, measured using FDG and the beta microprobe system.

Research Support: This research was supported by FRS-FNRS grant no. 3.4593.09 and by Johnson & Johnson Pharmaceutical Research & Development.

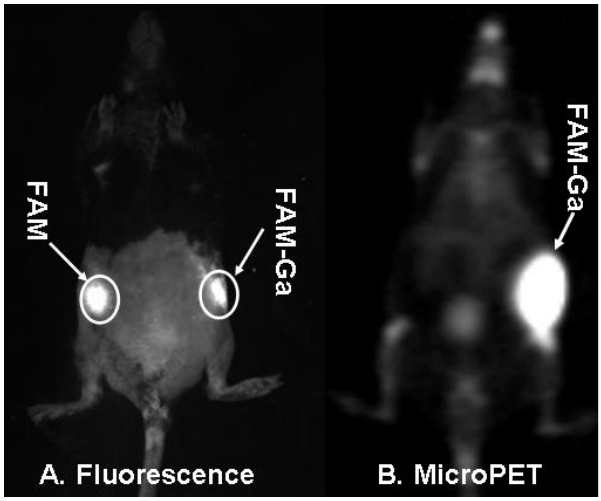
MULTIMODALITY IMAGING PROBE FOR MONITORING CELL TRANSPLANTATION AND MIGRATION

S. PANDEY*, A. SHAH, S. PANCHAL, B. EASWARAMOORTHY, C. CONSTANTINESCU, R. COLEMAN, R. KANT and J. MUKHERJEE
University of California-Irvine, Preclinical Imaging Facility, Department of Psychiatry and Human Behavior, Irvine, CA

Objectives: Our goal is to develop multimodality imaging agents for use in cell tracking studies by PET, SPECT, MRI and optical imaging (OI). For this purpose, bovine serum albumin (BSA) was complexed with biotin (Histological studies), 5(6)-carboxyfluorescein, succinimidyl ester (FAM) (OI studies), with diethylenetriamine pentaacetic acid (DTPA) for chelating gadolinium (MRI studies), gallium-68 (PET studies), or technetium-99m (SPECT studies). Thus, "BSA-Biotin-DTPA-FAM" may function as a multiprobe for PET, MRI, SPECT and OI. Previous studies with "BSA-Biotin-FAM-DTPA" have focused only on MRI and OI (Dafni et al., 2002; Granot et al., 2005).

Methods: For synthesis of BSA-Biotin-FAM-DTPA, 4g of BSA was coupled to 95mg of Biotin-NHSI. BSA-Biotin was treated with 3.6g of DTPA-anhydride and stirred for 2hr at 40 °C. Biotin-BSA-DTPA (100 mg) was reacted with fluorescin ((5(6)-carboxyfluorescein, succinimidyl ester (FAM) dissolved with dry DMF and added in stepwise (5-6 steps) to the protein solution at room temperature. The content was stirred for 90 min. The Biotin-BSA-DTPA-FAM (FAM conjugate) was further purified three times by centrifugal filtration using Amicon CentripepYM30 and $NaHCO_3$ buffer. Acidic gallium chloride 3 to 5 mCi eluted from the generator using 0.1 N HCl was passed through basic resin (AG® 11 A8) and 150μCi (100μL, pH = 7-8) was incubated with 0.1 mg of FAM conjugate (100μL) at room temp. for 15 min to give ^{68}Ga-BSA-Biotin-DTPA-FAM (FAM-^{68}Ga). Shaved C57 black mouse was injected (subcutaneously) with FAM conjugate (50μL) at one flank and FAM-^{68}Ga(50μL, 30μCi) at the other. Immediately after injection mouse was placed (prone, under isoflurane) in fluorescence imaging system (Kodak In-vivo F) and imaged (λ_{ex}: 465nm, λ_{em}: 535nm, time: 8 sec, Xenon light source). Same mouse was then placed (head first prone) under Inveon microPET scanner (Siemens Corp), injected (intravenously) with 25μCi of ^{18}F and after half hour (to allow sufficient bone uptake) was imaged for 30 minutes.

Results: Molecular weights were determined using MALDI at each step of conjugation. The mol. wt. of the BSA sample was found to be 66485, the Biotin-BSA sample was found to be 67116, indicating that 2 Biotin moieties are attached per BSA molecule. The mol. wt. of the Biotin-BSA-DTPA sample was found to be 81584 indicating that an average of 30 DTPA moieties per BSA molecule. The mol. wt. of FAM conjugate was found to be 82383 indicating that an average of 1.7 fluorescent moieties per BSA molecule. Fluorescence imaging clearly showed localization of FAM conjugate and FAM-^{68}Ga at respective flanks of the mouse, while only hot spot at the expected flank (FAM-^{68}Ga injection site) was observed in microPET imaging.



Conclusions: With the development of smart probes (ex. FAM-^{68}Ga) and using multimodality tools, better prognosis could me made for disease conditions. Experiments are currently under progress to demonstrate the cell tracking using both optical and nuclear imaging.

References: Dafni H, et al., Cancer Research, 62, 2002, 6731-6739. Granot D, et al., Magnetic Resonance Imaging, 54, 2005,789-797.