P125 PREPARATION AND BIOLOGICAL EVALUATION OF [67Ga]-LABELED-SUPERPARAMAGNETIC NANOPARTICLES IN NORMAL RATS

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Objectives: Considerable attention has recently been devoted to synthesizing of iron oxide nanoparticles due to their potential applications in pigments, magnetic drug targeting, genes, radionuclides, magnetic resonance imaging, recording materials and catalysts, because of their ultra-fine size, biocompatibility and superparamagnetic properties. In this report, a precise labeling strategy was employed using gallium-67 addition to the SPION (Super Paramagnetic Iron Oxide Nanoparticle) production stage resulting in an in situ radiolabeling/synthesis method. The possibility of usage of this alteration for targeting imaging/therapeutic radionuclides specifically to certain organs is shown.

Methods: Various methods were used in order to prepare radiolabeled nanoparticles and many of them using previously prepared SPION were not successful or with very low stability. The only possible way to produce radiolabeled-SPION with the lowest possible alteration in its structure seemed to be the incorporation of the radionuclide within the nanostructure of iron oxide network at the synthesis procedure. Production of ^{67}Ga was performed at the Agricultural, Medical and Industrial Research School (AMIRS) 30 MeV cyclotron (Cyclone-30, IBA). Enriched zinc-68 chloride with enrichment of >95% was obtained from Ion Beam Separation Department at NRCAM. Deionized (DI) water was utilized for preparation of the solutions after deoxygenating with nitrogen bubbles for 30 min. The ferric and ferrous chlorides (molar ratio of 2 : 1) were dissolved in DI water. The solution of ^{67}Ga chloride (in 0.2M HCl) was evaporated using N_2 gas followed by adding prepared iron salts solution to the ^{67}Ga vial. [^{67}Ga]-SPION was prepared by drop wise addition of the latest solution(^{67}Ga+iron salts) to the DI water. Chemical precipitation was achieved by adding 5molL−1 NaOH solution to DI water under N2 atmosphere and pH has been controlled on 8 using HCl in order to neutralize SPION solution. After treating for 30 min using high speed homogenization the particles were collected by magnetic field and re-dispersion in DI water. The solution is then used for biological studies.

Results: The SPION were synthesized with narrow size distribution (≈ 5 nm diameter). Total labeling and formulating of [^{67}Ga]-SPION took about 60 min, with a radiochemical purity >96% (using RTLC technique – Fig.1). [^{67}Ga]-SPION were stable in PBS for at least 4 days. The final preparation was administered to normal rats and biodistribution of the radiotracer was checked 1 and 24 hours later. The tracer were accumulated mostly in reticuluendothelial organs, especially liver, lung and spleen. The most significant difference between [^{67}Ga]-SPION and free ^{67}Ga uptake is observed in liver, which is at least 22-24 times higher.

Conclusions: The incorporation of therapeutic radioisotopes for reticuluendothelial system targeting is possible via this modality while possessing paramagnetic properties for usage in thermotherapy.

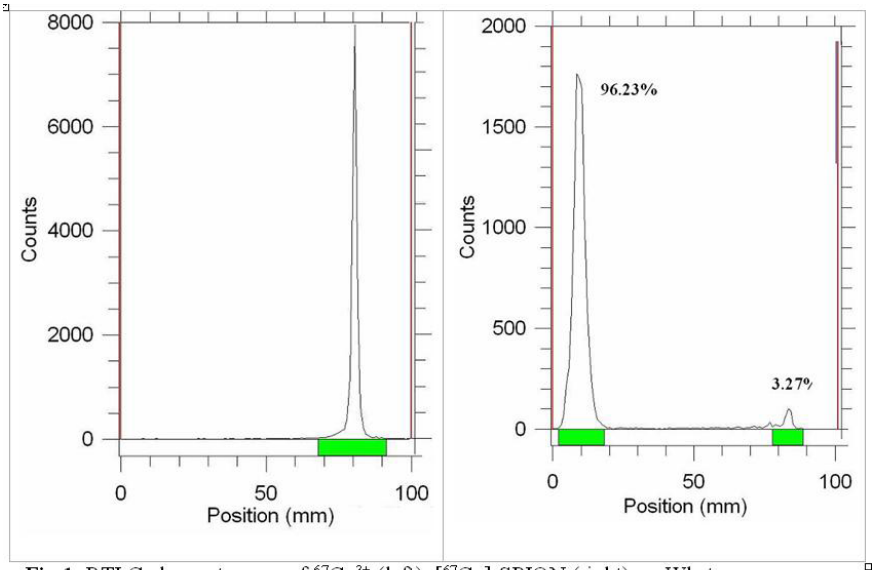

Fig 1. RTLC chromatogram of $^{67}Ga^{3+}$ (left), [^{67}Ga]-SPION (right) on Whatman paper

P126 SMALL ANIMAL PET IMAGING OF TUMORS WITH 64CU-LABELED RGD-BOMBESIN HETERODIMER

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Objectives: The overexpression of bombesin (BBN) family of receptors, especially the gastrin-releasing peptide receptor (GRPR) subtype in various tumor types suggests GRPR as an attractive target for tumor imaging and therapy with radiolabeled bombesin analogs. We recently reported the ability of ^{18}F-labeled RGD-BBN heterodimer for dual integrin $\alpha_v\beta_3$ and GRPR targeting. To further investigate the synergistic effects of the dual receptor recognition of peptide heterodimers, we evaluated ^{64}Cu-labeled RGD-BBN for positron emission tomography (PET) imaging of tumors.

Methods: RGD-BBN heterodimer was coupled with DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and labeled with ^{64}Cu. The in vitro dual receptor-binding affinity of NOTA-RGD-BBN and DOTA-RGD-BBN was determined by cell binding assay. The in vitro and in vivo characteristics of ^{64}Cu-NOTA-RGD-BBN were compared with ^{64}Cu-NOTA-RGD, ^{64}Cu-NOTA-BBN, and ^{64}Cu-DOTA-RGD-BBN.

Results: ^{64}Cu-NOTA-RGD-BBN and ^{64}Cu-DOTA-RGD-BBN had comparable integrin $\alpha_v\beta_3$ and GRPR-binding affinity in vitro, both of which were slightly lower than RGD for integrin binding and BBN for GRPR binding. ^{64}Cu-NOTA-RGD-BBN possessed significantly higher tumor uptake compared with ^{64}Cu-NOTA-RGD, ^{64}Cu-NOTA-BBN, the mixture of ^{64}Cu-NOTA-RGD and ^{64}Cu-NOTA-BBN, as well as ^{64}Cu-DOTA-RGD-BBN in PC-3 prostate tumor model (see table). ^{64}Cu-NOTA-RGD-BBN also showed improved in vivo kinetics such as lower liver and intestinal activity accumulation than that of ^{64}Cu-DOTA-RGD-BBN and ^{64}Cu-NOTA-BBN. For example, the liver uptake was 3.46±0.26, 2.80±1.15, 1.83±0.68, 0.98±0.40 %ID/g for ^{64}Cu-NOTA-RGD-BBN, 3.40±1.42, 3.05±1.07, 2.33±0.88, 1.74±0.90 %ID/g for ^{64}Cu-DOTA-RGD-BBN, and 10.45±2.27, 8.85±1.61, 6.35±0.57, 4.20±0.53 %ID/g for ^{64}Cu-NOTA-BBN at 0.5, 1, 4 and 20 h p.i, respectively (means ± SD, n=4). In 4T1 murine mammary carcinoma model which expresses integrin on the tumor vasculature but no GRPR in the tumor tissue, ^{64}Cu-NOTA-BBN had virtually no uptake in the 4T1 tumors, while both ^{64}Cu-NOTA-RGD and ^{64}Cu-NOTA-RGD-BBN showed clear tumor contrast due to the integrin $\alpha_v\beta_3$ recognition of RGD monomer and RGD-BBN heterodimer in vivo, respectively. At 2 h p.i, the 4T1 tumor uptakes of ^{64}Cu-NOTA-RGD, ^{64}Cu-NOTA-BBN and ^{64}Cu-NOTA-RGD-BBN were 0.65 ± 0.07, 0.33 ± 0.18 and 1.88 ± 0.28 %ID/g, respectively (means ± SD, n = 3).

Conclusions: ^{64}Cu-NOTA-RGD-BBN showed favorable in vivo kinetics and enhanced tumor uptake as compared with other tracers, which warrants its further investigation for targeting tumors that express integrin, GRPR, or co-express integrin and GRPR for imaging and therapeutic applications. The synergistic effect of RGD-BBN heterodimers observed in this study also encourages further investigations of novel heterodimers recognizing other cell surface receptors for tumor targeting.

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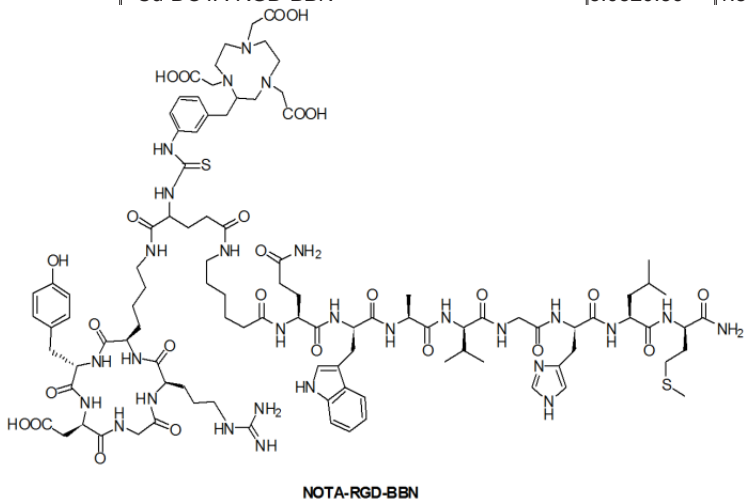
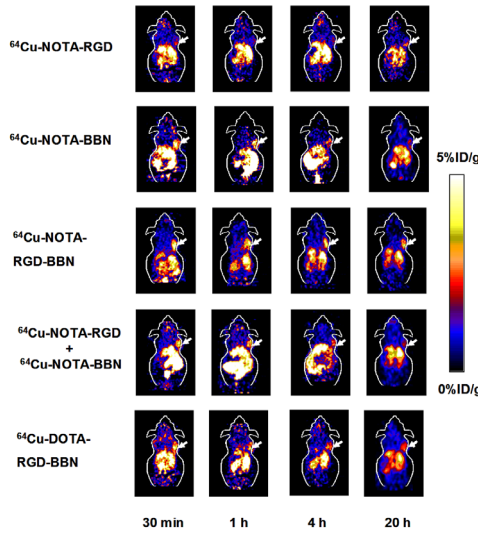
PC-3 tumor uptake of tracers (%ID/g±SD, n=4)

Tracers	0.5 h	1 h	4 h	20 h
^{64}Cu-NOTA-RGD	1.00±0.29	0.83±0.26	0.66±0.14	0.55±0.32
^{64}Cu-NOTA-BBN	2.28±0.35	1.25±1.03	0.56±0.39	0.44±0.21
^{64}Cu-NOTA-RGD + ^{64}Cu-NOTA-BBN	2.22±0.41	1.27±0.50	0.87±0.35	0.54±0.39
^{64}Cu-NOTA-RGD-BBN	3.06±0.11	2.78±0.56	2.21±0.49	2.04±0.35
^{64}Cu-DOTA-RGD-BBN	3.05±0.56	1.87±0.41	1.05±0.49	0.97±0.24



NOTA-RGD-BBN



P127 FLUORINE-18 LABELING OF QUANTUM DOTS FOR IN VIVO PET IMAGING

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Objectives: Quantum dots (QDs) are fluorescent semiconductor nanocrystals with exceptional intrinsic optical properties such as high quantum yields, excellent resistance to photobleaching, broad excitation spectra and narrow emission bands tunable from the UV to the NIR region. QDs have been used in laboratory animals for optical imaging of blood vessels as well as lymph node tracking. However, while optical imaging offers a tool for rapid qualitative assessment of the biodistribution of these nano-objects, its combination with a quantitative imaging technique such as positron emission tomography would give access to their complete pharmacokinetic and pharmacodynamic profile in vivo.

Methods: Core/shell CdSe/CdZnS nanocrystals (QDs) were synthesized using standard organometallic procedures and encapsulated into poly(ethyleneglycol) (PEG) phospholipid micelles (10%-thiol functionalized) [1]. QDs were labeled with the short-lived positron-emitter fluorine-18 (half-life : 109.8 min) using [^{18}F]FPyME (1-(3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl)pyrrole-2,5-dione), our [^{18}F]fluoropyridine-based maleimide reagent designed for prosthetic labeling of macromolecules via selective thiol-conjugation [2]. [^{18}F]FPyME was prepared using a three-step radiochemical pathway already reported [2] and purified by HPLC. HPLC-purified [^{18}F]FPyME was then conjugated with the QDs (4.75-6.25 nmol) in 1 mL of a 1/9 (v/v) mixture of DMSO and 0.1 M aq. PBS (pH 8) at room temperature for 15 minutes. [^{18}F]QDs were then separated from non-reacted [^{18}F]FPyME by gel filtration on a NAP-10 G25 Sephadex cartridge (Amersham Pharmacia Biotech) and formulated for i.v. injection. The whole process has been automated on our Zymate XP (Zymark) robotic system.

Results: 5.2-7.5 GBq batches of radiochemically pure [^{18}F]FPyME could be obtained in 110 minutes (semi-preparative HPLC included) starting from a 37-51 GBq cyclotron production batch of [^{18}F]fluoride (overall decay-corrected RCY: 23-30%). Conjugation of [^{18}F]FPyME with the QDs was achieved in good yields (>50%) according to radio-TLC. Typically, about 0.74-1.85 GBq of [^{18}F]QDs (SRA: 370-740 GBq/μmole @ EOB), ready to use (555-1110 MBq/mL in aq. 0.9% NaCl), could be obtained in 145 minutes starting from a cyclotron production batch of 37 GBq of [^{18}F]fluoride (overall radiochemical yields: 2-5% non-decay-corrected).

Conclusions: QDs have been successfully labeled with fluorine-18 using the thiol-selective reagent [^{18}F]FPyME, representing the first labeling of this class of nano-objets with a short-lived radioactive isotope of this kind. Combined PET and fluorescence imaging experiments have been performed to evaluate, from the whole-body to the cell level, the quantitative biodistribution of QDs in rodents.

Research Support: This work was supported by grants from the European Molecular Imaging Laboratory (EMIL) network (EU contract LSH-2004-503569), the Agence Nationale pour la Recherche (projects DotImager and ARTIC) and the Association pour la Recherche sur le Cancer (N°3527).

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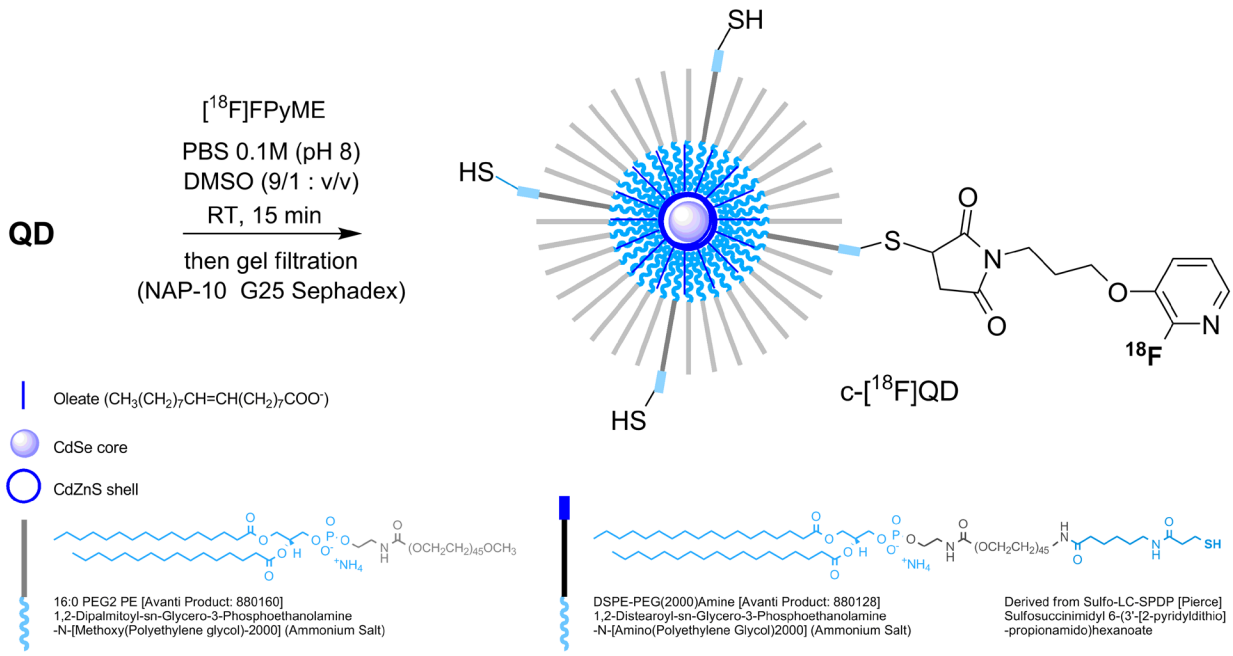

P128 FACILE FABRICATION OF ANIMAL-SPECIFIC POSITIONING MOLDS FOR MULTIMODALITY IMAGING

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Objectives: Recently, many researchers have tried to integrate various imaging modalities into fused images, such as PET/CT, SPECT/optical, MR/optical and PET/MRI, in order to overcome the weak points of each imaging modality and get benefits of its strong points. As part of efforts to obtain PET/MRI fused imaging, we tried to develop the fabrication of animal-specific positioning molds with two different materials. For successful multi-modality imaging, animal-specific positioning molds for immobilization and reproducible positioning of animal are prerequisite. Here, we demonstrated the fabrication of molds with foaming polymer and fiber clay for the reproducible positioning of small animals.

Methods: First, round bottomed-acrylic frame was prepared to fit into microPET gantry. The foaming mixtures (RT Cradle™) were poured into the wrapped acrylic frame. The anesthetized mouse was gently pressed into the pre-wrapped mixture for positioning. After removing the animal, the entirely hardened mold was used for fused microPET/MR imaging. In case of fiber clay (Angel Clay™), the anesthetized mouse was placed on flattened clay directly, and tenderly pushed down into the mold for positioning. After the mouse was removed, the mold was completely dried at 60℃ in oven overnight. The sealed pipet tip containing free F-18 activity was used as fiduciary markers. U87MG tumor cells were injected subcutaneously into the right hindlimb of nude mouse. The mouse was injected with [^{18}F]FDG for microPET imaging. Right after microPET imaging, T_2-weighted MR imaging was performed with fast gradient spin echo sequence in 0.8 mm of a slice thickness.

Results: Animal-specific positioning molds were successfully fabricated by using both foaming polymer and fiber clay for multimodality image. The fused PET/MRI images were reconstructed by using AMIDE fusion program with little discrepancy of two different images.

Conclusions: Small animal-specific positioning mold in addition to fiduciary markers was successfully fabricated by using easily available materials. And, the image fusion of microPET and MRI was also achieved by using freely available software AMIDE. This facile fabrication of animal-specific molds could be widely used for many different combination of multimodality imaging and many repeated imaging studies.

Research Support: This work was supported by the Brain Korea 21 Project in 2009.

P129 NEW IODINATED AND FLUORINATED RADIOTRACERS FOR PET/SPECT IMAGING AND TARGETED RADIONUCLIDE THERAPY OF MELANOMA

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Objectives: Melanoma is becoming a major problem of public health in industrial countries with a dramatic increase in incidence and mortality. This is a highly invasive cancer which disseminates in an unpredictable and silent fashion. For secondary lesions detection, [^{18}F]FDG PET imaging shows many contrast, resolution and quantization advantages, but suffers from a lack of specificity. As a consequence, there is a need for an early specific diagnosis of lesions allowing staging of patients. Moreover, development of specific therapies is required to overcome the lack of current efficient treatment for disseminated melanoma. A series of benzamides and analogues with a specific affinity for melanoma, by melanin binding, has already been developed in our team. We are currently investigating a new multi-modality approach: PET or SPECT imaging and targeted radionuclide therapy of melanoma with a single chemical structure. Designed compounds are iodinated and fluorinated analogues of previously developed radiotracers, and offer both diagnostic (^{123}I, ^{124}I or ^{18}F) and therapeutic (^{131}I) potentialities, depending on the type of radionuclide used.

Methods: Twenty compounds have been synthesized and thirteen of them have been evaluated in vivo, on melanoma B16F0 bearing mice model as follows: 1/ Initial pre-screening : labeling of each compound with ^{125}I, biodistribution study by gamma imaging, choice of tracers with the most appropriate kinetic profile. 2/ Labeling of selected compounds with ^{18}F, biodistribution study by PET imaging. 3/ Labeling with ^{131}I, targeted radionuclide therapy assay.

Results: The first pre-screening study allowed the selection of two compounds ([^{125}I]ICF02008, [^{125}I]ICF02110) due to their high, specific and long lasting tumoral uptake. Then, ICF02008 has been labeled with ^{18}F in order to perform PET imaging experiment. This assay has confirmed biodistribution and kinetic profile previously observed with [^{125}I]ICF02008, and the interest of combining such tracer specificity with PET technology performances. Finally, [^{131}I]ICF02008 administered after melanoma graft induced a significant slowing down in tumoral growth.

Conclusions: The present study gives a first validation of multi-modality concept feasibility with such radiotracers. Their specific affinity for melanin could allow PET (^{18}F) or SPECT (^{123}I) imaging and targeted radionuclide therapy (^{131}I) of melanoma.

Research Support: This project is supported by Laboratoires Cyclopharma SA (CIFRE grant, A. Maisonial), by CLARA (Cancéropôle Lyon Rhône Alpes Auvergne), by ANR (French Research Agency) and by EMIL (European Molecular Imaging Laboratories) EU contract LSH-2004-503569.

P130 SYNTHESIS AND EVALUATION OF A NOVEL GD-DOTA COMPLEX AS AN INTRACELLULAR MRI CONTRAST AGENT USING GD-153

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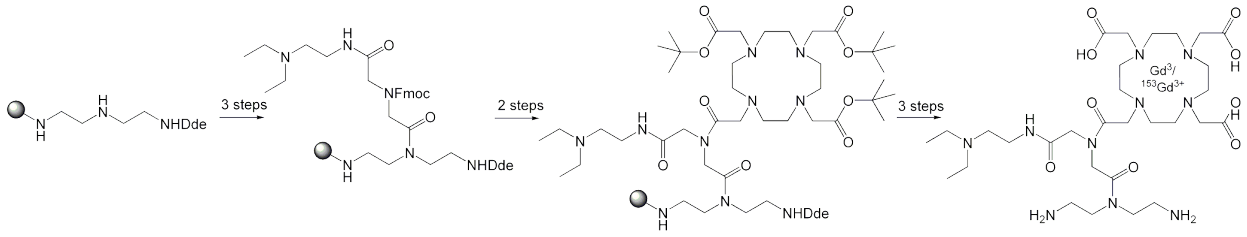
Objectives: Contrast agents in magnetic resonance imaging (MRI) are commonly based on gadolinium compounds. The problem of high toxicity of gadolinium ions is circumvented through complexation by strong chelators such as DTPA (diethylenetriamine-N,N,N',N'',N''-pentaacetic acid) or DOTA (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid). Increasing the tissue specificity of contrast agents by promoting their intracellular uptake is an important aim in the MRI contrast agent research. Endogenous polyamines are well-known for their tumor cell affinity[1,2]. The very efficient as well as tolerant polyamine transporter system leads to high uptake of polyamines and various derivatives in different types of cells. In previous works[3] we have shown high uptake of the polyamine substituted DTPA-Gd^{3+}-complex DTPA-AEA-DEA (AEA =bis(2-aminoethyl)amine; DEA = diethylaminoethylamine) into hepatoma cells.

Methods: In this work an analogous DOTA-Gd^{3+} complex was developed and compared to our previous DTPA-AEA-DEA complex. The amount of uptake was quantified using radioactive $^{153}Gd^{3+}$-complexes as tracer. The DOTA ligand has been derivatized withDEA and AEA. The synthesis was effected via a solid phase based strategy (figure 1). The respective Gd^{3+}/ $^{153}Gd^{3+}$ complexes were tested on hepatoma cells.



Results: Although the DOTA and DTPA ligands are carrying the same substituents they show large differences in their uptake into hepatoma cells. The DTPA-AEA-DEA complex reached uptake values of more than 0.7 fmol/ cell (10 mM incubation), whereas the DOTA-AEA-DEA complex remained slightly above 0.01 fmol/ cell.

Conclusions: Though very efficient for DTPA-AEA-DEA the polyamine transporter does not respond to DOTA-AEA-DEA. Defining the requirements for the polyamine transporter system will enable the development of tissue specific contrast agents.

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