EP527

Synthesis and evaluation of a ^{68}Ga-labeled affibody molecule for imaging carbonic anhydrase IX expression with positron emission tomography

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Objective: Affibody molecules are a class of small proteins (MW = ~ 7 k) that have been explored extensively for targeted therapy and imaging. Tolerant to high temperatures, extreme pH, and structural modifications, these scaffolds typically exhibit nano to picomolar affinities to their targets of interest. An oncogene that has garnered significant interest as both a therapeutic and diagnostic marker for cancer is carbonic anhydrase IX (CA-IX). CA-IX is a cell surface enzyme that promotes survival under low oxygen conditions and is overexpressed within the hypoxic milieu of solid tumours. Z09781, a potent CA-IX-targeting affibody molecule, was previously radiolabeled with ^{99m}Tc for SPECT imaging and showed excellent tumour-to-background contrast (Honarvar et al. Eur J Nucl Med Mol Imaging 2014; 41 (Suppl. 2): S176). To explore the use of Z09781 for imaging with PET, herein we present the synthesis and evaluation of a ^{68}Ga-labeled Z09781 derivative (Cys[^{68}Ga-NOTA]Z09781) for CA-IX targeted imaging. **Methods:** The affibody molecule Cys-Z09781 was obtained by standard Fmoc solid-phase peptide synthesis. A cysteine residue was introduced at the N-terminal domain of the affibody to facilitate site-specific conjugation of maleimido-mono-amide-NOTA. For radiolabeling, Cys[NOTA]Z09781 was labeled with $^{68}GaCl_3$ in 2 M HEPES buffer (pH 5.0) and 5% glycerol by microwave heating for 1 min. The radiolabeled product was subsequently purified by PD10 column before being used for in vivo evaluations. PET/CT imaging and biodistribution studies were performed at 2 h post-injection using HT-29 tumour xenograft-bearing immunodeficient mice. **Results:** Cys[^{68}Ga-NOTA]Z09781 was obtained in 64-73% decay-corrected radiochemical yields with 16.7 MBq/μg specific activity and > 99% radiochemical purity. Imaging and biodistribution studies showed that Cys[^{68}Ga-NOTA]Z09781 was predominantly excreted through the renal pathway, with the kidneys retaining majority of the radioactivity (96.1 ± 10.4 %ID/g at 2 h p.i.). Cys[^{68}Ga-NOTA]Z09781 allowed for clear delineation of HT-29 tumour xenografts in PET/CT images. With the exception of the kidneys and bladder, the highest uptake of activity was observed in tumour (1.05 ± 0.15 %ID/g at 2 h p.i.). The corresponding tumour-to-muscle and tumour-to-blood ratios were 11.8 ± 3.35 and 9.78 ± 1.59 respectively. **Conclusion:** We successfully synthesized and radiolabeled an affibody molecule with ^{68}Ga for CA-IX targeted imaging. Tracer pharmacokinetics and tracer uptake was assessed in a human hypoxia cancer model. Cys[^{68}Ga-NOTA]Z09781 enabled good visualization of HT-29 tumour xenografts with good contrast, and is promising for imaging CA-IX expression with positron emission tomography.

EP528

Physicochemical and biological characterization of SubstanceP fragments labeled with ^{177}Lu

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Aim: Gliomas, particularly WHO grade IV glioblastoma multiforme (GBM), is one of the most common and aggressive primary type of the cancer of the central nervous system. Despite of all current forms of treatment such as advanced surgery techniques, radiation therapy and chemotherapy, the life expectancy of patients diagnosed with GBM is only 12 to 15 months. The neuropeptide SubstanceP (SP) is the physiological ligand of the nerokinin1(NK1) receptor consistently overexpressed by glioblastoma cells. The aim of the work was to study physicochemical (lipophilicity, stability) and biological (K_D, IC_{50}) properties of different SP fragments labeled with β^- emitter - Lu-177. **Methods:** The ^{177}Lu-DOTA-SP fragments conjugates consist of central metal ion ^{177}Lu coordinated by macrocyclic ligand DOTA previously coupled with different SubstanceP fragments such as SP(1-11), [Thi^8,Met$(O_2)^{11}$]SP(1-11), SP(4-11), SP(5-11), [Thi^8,Met$(O_2)^{11}$]SP(5-11). The synthesized conjugates were characterized *in vitro* by partition coefficients (log P), in the *n*-octanol/PBS (pH 7.40) system. Stability of the complexes were investigated in different physiological solutions, namely phosphate buffered saline (PBS), histidine, cysteine, human serum (HS) and cerebral spinal fluid (CSF) using HPLC and ITLC methods. The biological properties (K_D, IC_{50}) were characterized using cell line T98G. **Results:** All studied conjugates are formed with good yield and high radiochemical purity. The obtained lipophilicity values of the ^{177}Lu-DOTA-SP fragments are in the range from -2.5 to -5.0. The shorter SP fragments are characterized by higher lipophilicity values, while the replacement of amino acids in positions 8 and 11, respectively Phe and Met by the Thi and Met(O_2) leads to lipophilicity decreasing. The studied conjugates are stable in PBS buffer, as well as in 10 mM histidine and/or cysteine solutions. Stability studies in HS showed in the case of shorter SP fragments quicker enzymatic biodegradation comparing to that of SP molecules containing all 11 amino acids. However, stability studies in CSF showed no enzymatic biodegradation of tested compounds. All studied conjugates bind specifically to NK1 receptors expressed on glioblastoma cells with affinity in the nanomolar range. **Conclusions:** To summarize, the conjugates containing shorter SP fragments are characterized with higher lipophilicity values what can allowed more effective migration into GBM tissue or into the walls of post-surgery cavity. The disadvantage of these conjugates is their poor stability in HS, however due to their total stability in CSF, they can be considered as potential radiopharmaceuticals for local administration directly to glioblastoma tumours. Support by Grant 011/01/M/ST406756 is acknowledged.

EP529

^{18}F-FDG-Aoe-LIKKP-Pyr-A: A new peptide radiotracer for apoptosis imaging

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Aim: Specific biochemical changes occur in cells undergoing apoptosis that provide potential targets for molecular imaging agents and imaging of apoptosis can provide a good way to predict effectiveness of cancer chemotherapy. Clinical information obtained from targeting of phosphatidyl serine (PS) would help to diagnosis and therapy of apoptosis related pathologies. A few LIKKPF peptides with acceptable affinity for PS have been already introduced for apoptosis imaging. We herein report the synthesis of a new LIKKPF peptide radiotracer with more affinity for PS for detecting apoptosis. Materials and Methods: The peptides were synthesized on solid phase using standard Fmoc strategy, conjugated with aminooxy (Aoe), and then was considered for the radiolabeling procedure with ^{18}F-FDG. Biological properties were determined in vitro using camptothecin treated Jurkat cells and in vivo using liver apoptosis mouse model induced via intraperitoneal (IP) injection of lipopolysaccharide (LPS). Also, Log P values determination, biodistribution studies and PET/CT imaging were performed. Results: The radiochemical purity

was >95% (100°C, 30 min, pH 5-5.5). Log P value for ^{18}FDG-Aoe-LIKKP-Pyr-A prepared by Fmoc-(4-pyridyl)-D-Ala-OH was -0.92. K_d of peptide was determined with value 0.52 μM. Biodistribution results showed 3 times liver uptake for apoptotic mice compared to normal mice. PET/CT images were in a good agreement with biodistribution results. The activity ratio of liver to kidney was 0.17 and 0.61 in normal and apoptosis mouse model, respectively. Conclusion: ^{18}FDG-Aoe-LIKKP-Pyr-A showed good affinity to PS and might be a potential radiotracer for the detection of apoptosis.

EP530

Preparation and biological evaluation of ^{99m}Tc-HYNIC-LIKKPF for the detection of apoptosis

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Aim: Radioligands that have affinity and bind to phosphatidyl serine (PS) are good candidates for noninvasive imaging of apoptosis. Because of limitations of Annexin V (as a most studied imaging agent for apoptosis), there is a need to develop radioligands with better biodistribution profile for this purpose. Materials and Methods: HYNIC-LIKKPF was successfully synthesized via Fmoc strategy and the compound was considered for the radiolabeling procedure with Tc-99m. Then, stability and Log P values of radiopeptide were determined. Finally, binding studies, biodistribution studies and SPECT/CT imaging were performed. Results: The radiochemical purity and Log P value were >95% and -0.92, respecteviely. ^{99m}Tc-HYNIC-LIKKPF was stable in human serum for at least 2 hr at 37°C. Although the affinity of radiolabeled LIKKPF was less than original phage peptide, the level of binding to apoptotic cells was 2.5 times higher than control cells. Biodistribution studies showed higher liver uptake of radiopeptide in apoptotic non-treated mouse model compare to normal and pre-treated mouse. The in vivo imaging results were consistent with biodistribution studies. Conclusion: ^{99m}Tc-HYNIC-LIKKPF has less affinity to PS compare to original phage peptide, but high enough for specific binding to apoptotic cells in vitro and in vivo. Less affinity of radiolabeled LIKKPF might be attributed to hydrophobicity of peptide. The future peptides should be more hydrophobic compare to LIKKPF.

EP531

Anti-VEGFR2 monoclonal antibody ramucirumab: comparison of selected radiolabeling methods

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Aim: Vascular endothelial growth factor (VEGF) is one of the most important regulator of angiogenesis, including tumor neovascularization. VEGF binds to two types of receptors with tyrosine-kinase activity of which VEGF receptor 2 (VEGFR2) is a key receptor in tumor neoangiogenesis. Ramucirumab (RAM) is a novel therapeutic anti-VEGFR2 monoclonal antibody (Mab) directed against the extracellular domain of VEGFR2 that inhibits binding of its natural ligand VEGF. Several types of cancer (e.g. gastric, pancreatic, lung or breast cancer) are known for their overexpression of VEGFR2. The MAb RAM labeled with appropriate radionuclides could be potentially used for scintigraphic imaging and targeted radiotherapy of oncological diseases. The aim of this work was to develop convenient radiolabeling method for selected radionuclides and to evaluate radiochemical purity and stability of radiolabeled products. Materials and methods: Two methods of RAM radiolabeling were evaluated in experiments. The first tested method was the optimization of the direct labeling with ^{99m}Tc based on the reduction of disulfide bridges in RAM molecule with 2-mercaptoethanol. The second method was radioiodination (^{131}I) according to chloramine-T protocol. The radiochemical purity of the prepared antibody was tested by instant thin layer chromatography on silica gel (ITLC) immediately after labeling. To evaluate the stability the samples were analyzed on various times by size-exclusion high-performance liquid chromatography (SE-HPLC) with radiometric detection. The preparations were incubated 8-24 h at 4°C. Results: The introduced methods enabled effective labeling of RAM with either ^{99m}Tc or ^{131}I. The obtained radiolabeled preparations exhibited sufficiently high stability. Radioiodination method provided slightly higher radiochemical purity after labeling. However, we observed higher stability provided by direct labeling method with ^{99m}Tc under the used conditions. Conclusion: The tested methods are convenient for radiolabeling of RAM. The developed radiolabeled preparations may be used in further preclinical studies to evaluate biological behavior of labeled RAM in vitro or in vivo. This project was supported by Charles University: GAUK(998216/C/2016), SVV(260293) and PRVOUK P40.

EP-26 – Sunday, October 16, 2016, during Exhibition hours, e-Poster Area

M2M: Miscellaneous

EP532

Development of a new multimodal diagnostic nanosystem based on inorganic nanoparticles as a potential radiopharmaceutical in SPECT-MRI or PET-MRI imaging

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Aim: Nanotechnology offers interesting tools that could help to overcome the handicaps of traditional radiopharmaceuticals: greater specificity through selective tropism to targeted tissues, and higher capacity of loading isotopes, to improve sensitivity and decrease toxicity and dosimetry in patients. The aim of this work is to develop a multimodal imaging system (PET - MRI or SPECT - MRI) based on inorganic nanoparticles (NPs) and assess their potential application as radiopharmaceutical. Material and Methods: Monodisperse NPs were synthesized, with a superparamagnetic iron oxide core (SPION) and silica coating, with different particle sizes (10-100 nm) and specific surface functionalization. These NPs were PEGylated to increase its circulation time and to provide a platform for subsequent functionalization with additional ligands that may improve their PK-PD and biodistribution. Magnetic properties were determined by measurements of magnetization and transverse relaxivity (T2), and its loading capacity of atoms of gallium, indium and zirconium was determined by two different methods: (1) cold, using chlorinated salts, determined by mass spectrometry with Inductively Coupled Plasma (ICP) and (2) labelling with radioactive isotopes Ga-67, In-111 and Zr-89, quantifying radiochemical purity (RCP) by thin layer chromatography (TLC) using a gamma-radioactivity single trace scanner. Loading kinetics was monitored at room temperature by performing measurements at different times (1, 3, 6, 24 and 48 hours). Moreover, the stability of said labelling was analyzed under physiological conditions, in PBS and fetal bovine serum (FBS). Results: Characterized NPs have shown good properties as