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^{68}Ga-labeling and in vivo evaluation of a uPAR binding DOTA- and NODAGA-conjugated peptide for PET imaging of invasive cancers

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Abstract

Introduction: The urokinase-type plasminogen activator receptor (uPAR) is a well-established biomarker for tumor aggressiveness and metastatic potential. DOTA-AE105 and DOTA-AE105-NH_2 labeled with ^{64}Cu have previously been demonstrated to be able to noninvasively monitor uPAR expression using positron emission tomography (PET) in human cancer xenograft mice models. Here we introduce ^{68}Ga-DOTA-AE105-NH_2 and ^{68}Ga-NODAGA-AE105-NH_2 and evaluate their imaging properties using small-animal PET.

Methods: Synthesis of DOTA-AE105-NH_2 and NODAGA-AE105-NH_2 was based on solid-phase peptide synthesis protocols using the Fmoc strategy. $^{68}GaCl_3$ was eluted from a ^{68}Ge/^{68}Ga generator. The eluate was either concentrated on a cation-exchange column or fractionated and used directly for labeling. For in vitro characterization of both tracers, partition coefficient, buffer and plasma stability, uPAR binding affinity and cell uptake were determined. To characterize the in vivo properties, dynamic microPET imaging was carried out in nude mice bearing human glioma U87MG tumor xenograft.

Results: In vitro experiments revealed uPAR binding affinities in the lower nM range for both conjugated peptides and identical to AE105. Labeling of DOTA-AE105-NH_2 and NODAGA-AE105-NH_2 with ^{68}Ga was done at 95°C and room temperature, respectively. The highest radiochemical yield and purity were obtained using fractionated elution, whereas a negative effect of acetone on labeling efficiency for NODAGA-AE105-NH_2 was observed. Good stability in phosphate-buffered saline and mouse plasma was observed. High cell uptake was found for both tracers in U87MG tumor cells. Dynamic microPET imaging demonstrated good tumor-to-background ratio for both tracers. Tumor uptake was 2.1% ID/g and 1.3% ID/g 30 min postinjection and 2.0% ID/g and 1.1% ID/g 60 min postinjection for ^{68}Ga-NODAGA-AE105-NH_2 and ^{68}Ga-DOTA-AE105-NH_2, respectively. A significantly higher tumor-to-muscle ratio ($P<.05$) was found for ^{68}Ga-NODAGA-AE105-NH_2 60 min postinjection.

Conclusions: The use of ^{68}Ga-DOTA-AE105-NH_2 and ^{68}Ga-NODAGA-AE105-NH_2 as the first gallium-68 labeled uPAR radiotracers for noninvasive PET imaging is reported, which combine versatility with good imaging properties. These new tracers thus constitute an interesting alternative to the ^{64}Cu-labeled version (^{64}Cu-DOTA-AE105 and 64Cu-DOTA-AE105-NH_2) for detecting uPAR expression in tumor tissue. In our hands, the fractionated elution approach was superior for labeling of peptides, and ^{68}Ga-NODAGA-AE105-NH_2 is the favored tracer as it provides the highest tumor-to-background ratio.

Keywords: uPAR; MicroPET; NODAGA; DOTA; AE105; Gallium-68; Cancer xenograft

1. Introduction

The urokinase-type plasminogen activator (uPA) and its receptor (uPAR) have been implicated in cancer as a marker for poor prognosis in a variety of human malignancies such as breast, colorectal and gastric cancer [1–4]. uPAR expression is particularly abundant at the invasive front of tumors or in

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the surrounding stroma cells. uPAR is therefore generally recognized as a molecular marker for tumor invasion and metastatic disease and is therefore also considered an important target in cancer research [3]. The ability to visualize and quantify uPAR expression noninvasively in vivo is thus attractive from a clinical perspective [5–8].

Based on an unbiased selection in a naive phage display library by cell lines expressing high levels of uPAR, a family of linear peptide antagonists of the uPA·uPAR interaction was developed after affinity maturation [9]. The resulting 9-mer lead peptide denoted AE105 [9] forms a tight 1:1 complex with purified human uPAR displaying a K_D of 0.4 nM with a k_{off} of 2×10^{-4} s^{-1} as measured by surface plasmon resonance. AE105 is a potent competitive inhibitor of the uPA·uPAR interaction, displaying an IC_{50} value of 11 nM in a purified system [9].

We have recently explored the use of this peptide for positron emission tomography (PET) imaging of uPAR expression [10, 11]. In both studies, DOTA was conjugated to the N-terminal of the targeting peptides (DOTA-AE105 and DOTA-AE105-NH_2), which were subsequently labeled with the long-lived PET isotope ^{64}Cu ($T_{1/2}$=12.7 hr, β^+=17.8%) (^{64}Cu-DOTA-AE105) and investigated in a human cancer xenograft mice model. A quantitative correlation between uPAR expression and the tumor uptake of ^{64}Cu-DOTA-AE105-NH_2 in several different human xenograft in mice was recently reported [10], thus illustrating the ability to noninvasively detect uPAR expression in vivo. Because of the limited availability due to the cyclotron-dependent production of ^{64}Cu, the use in medical centers worldwide is complicated logistically. With the increased use of the $^{68}Ge/^{68}Ga$ generator during the last decade, the advancement of ^{68}Ga-based PET imaging agents has begun offering a very cost-effective alternative to the on-site cyclotron [12, 13]. ^{68}Ga has some promising physical characteristics ($T_{1/2}$=68 min, β^+=89%) for imaging since the physical half-life more resembles the half-life of peptides in vivo and it has a higher positron abundance than ^{64}Cu.

Here we introduce the first ^{68}Ga-labeled peptides for PET imaging of uPAR. The amide form of the small linear peptide AE105 was conjugated with the macrocyclic chelators DOTA (DOTA-AE105-NH_2) and NODAGA [14] (NODAGA-AE105-NH_2) in the N-terminal. Both peptides were labeled with $^{68}GaCl_3$ eluate after either a cation-exchange column purification step or a fractionation of the eluate [15] in order to compare the two different approaches. Finally, the in vitro uPAR binding properties and stability were investigated together with dynamic in vivo PET imaging in nude mice bearing tumor xenograft of the uPAR-positive human glioblastoma cell line U87MG [10].

2. Materials and methods

2.1. Chemical and biological reagents

All commercial chemicals were of analytical grade. They were all used without further purification. 2-(4,7,10-tris(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclo-dodecan-1-yl)-acetic acid(DOTA-tris(tBu)ester and 4-(4,7-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7-triazacyclononan-1-yl)-5-(tert-butoxy)-5-oxopentanoic acid [NODAGA-tris(tBu)ester] were purchased from CheMatech (Dijon, France). $^{68}GaCl_3$ was eluted in 0.1 M HCl obtained from a $^{68}Ge/Ga^{68}$ generator (Eckert & Ziegler) at the Department of Nuclear Medicine & PET, Copenhagen University Hospital. $GaCl_3$ was purchased from Sigma-Aldrich.

2.2. Peptide synthesis of DOTA-AE105-NH_2 and NODAGA-AE105-NH_2

The amide form of the lead peptide [AE105-NH_2: Asp-Cha-Phe-(D)Ser-(D)Arg-Tyr-Leu-Trp-Ser-$CONH_2$] was synthesized on Tentagel S RAM resin (Rapp Polymere, Germany) using traditional Fmoc solid-phase peptide chemistry, and the macrocyclic chelators (DOTA or NODAGA) were conjugated to the N-terminal coupled as described previously [10]. Incubation of 20 nmol conjugated peptides with a twofold molar excess of the stable Ga^{3+} at 5 mM in water at room temperature led to complete complex formation of Ga-DOTA-AE105-NH_2 and Ga-NODAGA-AE105-NH_2 peptides, respectively, as revealed by analytical reverse phase high-performance liquid chromatography (HPLC) and Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) (data not shown).

2.3. In vitro affinity binding

The IC_{50} values of the synthetic peptides for inhibition of the uPA·uPAR interaction were measured by surface plasmon resonance using a Biacore3000 as recently described [10]. In brief, a high density of purified recombinant human pro-uPA was immobilized on a CM5 sensor chip by amine coupling (~6–-7000 RU). A low concentration of purified uPAR (0.5 nM) was preincubated with serial threefold dilutions of the synthetic peptides covering 0.1 nM to 300 μM.

2.4. Radiolabeling of DOTA-AE105-NH_2 and NODAGA-AE105-NH_2

Radiolabeling of DOTA-AE105-NH_2 and NODAGA-AE105-NH_2 with ^{68}Ga was performed by either a cation-exchange column approach or using fractionated elution [15] (Fig. 1A, B). For the cation-exchange column approach, the ^{68}Ga solution was eluted from the generator with 6 ml of 0.1 M HCl. The generator eluate was directly passed onto a Strata XC column, dried with air and eluted with 700 μl 0.02 M HCl/acetone (2:98). A total of 100 μl of this eluate was then added to 5 nmol conjugated peptide dissolved in 300 μl H_2O. For DOTA-AE105-NH_2, 100 μl 0.1 M sodium acetate (pH=3.5) was added to adjust the pH in the solution, whereas for NODAGA-AE105-NH_2, 100 μl 0.1 M sodium acetate (pH=5.0) was used. The complex formations were performed at 90°C for 10 min or at room temperature for 15 min for ^{68}Ga-DOTA-AE105-NH_2 and ^{68}Ga-NODAGA-