Nuclear Medicine and Biology 41 (2014) 51–57

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

^{68}Ga-NODAGA-VEGF$_{121}$ for in vivo imaging of VEGF receptor expression

Choong Mo Kang [a,b], Hyun-Jung Koo [a], Yearn Seong Choe [a,b,*], Joon Young Choi [a], Kyung-Han Lee [a,b], Byung-Tae Kim [a]

[a] Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul 135–710, Korea
[b] Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 135–710, Korea

ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form 12 September 2013
Accepted 13 September 2013

Keywords:
^{68}Ga-NODAGA-VEGF$_{121}$
VEGFR
Angiogenesis
MicroPET

ABSTRACT

Purpose: Vascular endothelial growth factor (VEGF) is a crucial regulator of angiogenesis. In this study, we labeled VEGF$_{121}$ with ^{68}Ga using a hydrophilic chelating agent, NODAGA and evaluated the resulting ^{68}Ga-NODAGA-VEGF$_{121}$ for in vivo imaging of VEGF receptor (VEGFR) expression.
Methods: NODAGA-VEGF$_{121}$ was prepared and its binding affinity for VEGFR2 was measured using ^{125}I-VEGF$_{121}$. ^{68}Ga-NODAGA-VEGF$_{121}$ was prepared by labeling NODAGA-VEGF$_{121}$ with $^{68}GaCl_3$ followed by purification using a PD-10 column. Human aortic endothelial cell (HAEC) binding studies of ^{68}Ga-NODAGA-VEGF$_{121}$ were performed at 37 °C for 4 h. MicroPET imaging followed by biodistribution studies were performed in U87MG tumor-bearing mice injected with ^{68}Ga-NODAGA-VEGF$_{121}$. Immunofluorescence staining of the tumor tissues was performed to verify VEGFR2 expression.
Results: Binding affinity of NODAGA-VEGF$_{121}$ for VEGFR2 was found to be comparable to that of VEGF$_{121}$. ^{68}Ga-NODAGA-VEGF$_{121}$ was prepared in 47.8% yield with specific activity of 3.4 GBq/mg. ^{68}Ga-NODAGA-VEGF$_{121}$ was avidly taken up by HAECs with a time-dependent increase from 9.88 %ID at 1 h to 20.86 %ID at 4 h. MicroPET imaging of mice demonstrated high liver and spleen uptake with clear visualization of tumor at 1 h after injection. ROI analysis of tumors revealed 2.53 ± 0.11 %ID/g at 4 h after injection. In the blocking study, tumor uptake was inhibited by 29% at 4 h. Subsequent biodistribution studies demonstrated tumor uptake of 2.38 ± 0.15 %ID/g. Immunofluorescence staining of the tumor tissues displayed high level of VEGFR2 expression.
Conclusions: These results demonstrate that ^{68}Ga-NODAGA-VEGF$_{121}$ led to VEGFR-specific distribution in U87MG tumor-bearing mice. This study also suggests that altered physicochemical properties of VEGF$_{121}$ after radiolabeling may affect biodistribution of the radiolabeled VEGF$_{121}$.

1. Introduction

Angiogenesis induced by tumors leads to the secretion of a host of growth factors, including vascular endothelial growth factor (VEGF), which stimulates tumor growth. A crucial regulator of angiogenesis, VEGF binds to VEGF receptor 1 (VEGFR1, Flt-1) and VEGFR2 (KDR, Flk-1), which activates endothelial cell proliferation, migration, and survival [1,2]. Moreover, VEGFRs are known to be over-expressed in tumor vasculatures, and thus, in vivo imaging of VEGFR expression may be useful for tumor diagnosis and monitoring of response to anti-VEGF/VEGFR therapy [3]. It is widely accepted that VEGFR2 mediates endothelial cell mitogenesis, angiogenesis, and increased vascular permeability [1,2]. Since VEGF$_{121}$ binds to VEGFR2 with high affinity, it is considered an excellent molecular candidate for PET imaging [4].

For imaging of VEGFR expression, VEGF$_{121}$ has been labeled with various radioisotopes, such as ^{125}I [5], ^{64}Cu via 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA) [6], and ^{68}Ga via 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)-benzyl group [7]. ^{125}I-VEGF$_{121}$ exhibits high tumor uptake of 9.12 ± 0.98 %ID/g at 2 h and 2.55 ± 0.28 %ID/g at 24 h after injection in LS180 tumor xenograft models; however, it suffers from in vivo deiodination (8.81 ± 1.87 %ID/g in the stomach at 2 h) [5]. ^{64}Cu-DOTA-VEGF$_{121}$ showed excellent tumor uptake in U87MG tumor-bearing mice [6]; it has also shown noninvasive visualization of VEGFR expression in other disease models, such as ischemia, myocardial infarction, and stroke [8–10].

^{68}Ga has wide applicability to the development of various molecular imaging probes because of its ready availability from a ^{68}Ge/^{68}Ga-generator, mild conditions for ^{68}Ga-labeling, and formation of a stable complex with NOTA due to its small ion radius [11,12]. NOTA is a bifunctional chelating agent to which ^{68}Ga is chelated and a biomolecule is conjugated via benzyl SCN. We previously prepared and evaluated ^{68}Ga-NOTA-benzyl-VEGF$_{121}$ for imaging of VEGFR expression. MicroPET imaging and biodistribution studies of U87MG tumor-bearing mice demonstrated significantly higher liver (53.26 ± 6.38 %ID/g) and spleen uptake (25.00 ± 9.80 %ID/g) than kidney uptake (2.39 ± 0.15 %ID/g) at 4 h after injection. Because of

* Corresponding author. Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul 135-710, Korea. Tel.: +82 2 3410 2623; fax: +82 2 3410 2667.
E-mail address: ysnm.choe@samsung.com (Y.S. Choe).

0969-8051/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nucmedbio.2013.09.005

the high stability of ^{68}Ga-NOTA-benzyl-VEGF$_{121}$ in serum and the known stability of the ^{68}Ga-NOTA complex (log stability constant 30.98) [13], we proposed that ^{68}Ga-NOTA-benzyl-VEGF$_{121}$ underwent hepatic clearance owing to a benzyl moiety in the linker. In order to reduce liver and spleen uptake of ^{68}Ga-labeled VEGF$_{121}$, more hydrophilic chelating agents may be required.

1,4,7-Triazacyclononane,1-glutaric acid-4,7-acetic acid (NODAGA) was first synthesized and used for synthesis of $^{67/68}$Ga- and ^{111}In-NODAGATOC, somatostatin receptor (sstr) ligands [14]. ^{67}Ga-NODAGATOC displayed higher tumor uptake (21.21 ± 3.55 %ID/g) than ^{111}In-NODAGATOC (12.50 ± 6.81 %ID/g) at 4 h after injection in biodistribution studies using sstr subtype 2-positive AR4-2 J tumor-bearing mice [14]. Several other studies have used NODAGA as a chelating agent for ^{68}Ga-labeling and have demonstrated that the resulting ^{68}Ga-NODAGA conjugates are stable in plasma or serum [14–17]. ^{68}Ga-NODAGA-TATE was prepared at room temperature within 10 min, and radiochemical purity was higher than 90%, which did not require purification [15]. ^{68}Ga-NODAGA-RGD also has favorable in vivo properties for imaging of integrin $\alpha_v\beta_3$ expression [16,17]. In biodistribution studies using $\alpha_v\beta_3$-positive human melanoma M21-bearing mice, ^{68}Ga-NODAGA-RGD showed comparable tumor uptake to ^{18}F-galacto-RGD (1.45 %ID/g and 1.35 %ID/g, respectively, at 90 min after injection), which is the most well-characterized RGD probe in animals and patients [17–19]. Although ^{68}Ga-NODAGA-RGD had lower tumor uptake than ^{68}Ga-DOTA-RGD, its tumor-to-blood uptake ratio was higher than ^{68}Ga-DOTA-RGD by 2.8-fold. MicroPET images also provided better properties with ^{68}Ga-NODAGA-RGD than with ^{68}Ga-DOTA-RGD. Considering the simple synthesis method, ^{68}Ga-NODAGA-RGD can be a favorable alternative to ^{18}F-galacto-RGD. ^{68}Ga-NODAGA-folic acid and ^{68}Ga-NODAGA-5,8-dideazafolic acid via 1,2-diaminoethane were developed for folate receptor imaging [20]. Biodistribution studies in KB tumor-bearing mice showed high tumor uptake of 16.56 ± 3.67 and 10.95 ± 2.12 %IA/g at 1 h after injection, respectively, and high uptake in the kidney (91.52 ± 21.05 and 62.26 ± 14.32 %IA/g), which is a folate receptor-positive organ. MicroPET imaging confirmed high tumor and kidney uptakes. ^{68}Ga-NODAGA-AE105-NH$_2$ and ^{68}Ga-DOTA-AE105-NH$_2$ were developed for imaging of urokinase-type plasminogen activator receptor [21]. MicroPET ROI analysis of U87MG tumor-bearing mice revealed tumor uptake of 1.1 %ID/g for ^{68}Ga-NODAGA-AE105-NH$_2$ and 2.0 %ID/g for ^{68}Ga-DOTA-AE105-NH$_2$ at 60 min after injection, but tumor-to-muscle uptake ratios were higher for ^{68}Ga-NODAGA-AE105-NH$_2$ (5.37 ± 0.7) than for ^{68}Ga-DOTA-AE105-NH$_2$ (3.34 ± 0.16).

In this study, VEGF$_{121}$ was conjugated with a hydrophilic chelating agent, NODAGA for ^{68}Ga-labeling and the resulting ^{68}Ga-NODAGA-VEGF$_{121}$ was then characterized in U87MG tumor-bearing mice.

2. Materials and methods

2.1. Materials and equipment

Obninsk ^{68}Ge/^{68}Ga-generator was purchased from Eckert & Ziegler (Berlin, Germany). Recombinant human VEGF$_{121}$ was purchased from PeproTech (Rocky Hill, NJ, USA), and NODAGA-NHS ester was from CheMatech (Dijon, France). Chelex 100 resin (50–100 mesh) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and BCA protein assay kits were from Pierce (Rockford, IL, USA). All buffers used for synthesis and radiolabeling were pretreated with Chelex 100 resin to make metal-free conditions. PD-10 columns were purchased from Amersham Biosciences (Piscataway, NJ, USA), and Amicon filters were from Millipore (Billerica, MA, USA). Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was performed on a UltrafleXtreme mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Paper chromatography was performed using Whatman No. 1 paper strips and the strips were analyzed on a Bioscan radio-TLC scanner (Washington, DC). Purification of NODAGA-VEGF$_{121}$ and analysis of ^{68}Ga-NODAGA-VEGF$_{121}$ were carried out on a HPLC (Thermo Scientific, Waltham, MA, USA) and the eluents were monitored using a UV (230 nm) detector and a NaI(Tl) radioactivity detector.

Radioactivity was measured using a dose calibrator (Biodex Medical Systems, Shirley, NY) and tissue radioactivity was counted using an automatic gamma counter (PerkinElmer, Waltham, MA, USA). MicroPET images were acquired at the Center for Molecular and Cellular Imaging, Samsung Biomedical Research Institute (Seoul, Korea) using an Inveon microPET/CT scanner (Siemens Medical Solutions, Malvern, PA, USA). All animal experiments were performed according to the guidelines issued by the Institutional Animal Care and Use Committee (IACUC), which are in accord with NIH guidelines.

2.2. Preparation of NODAGA-VEGF$_{121}$

VEGF$_{121}$ (300 μg, 10.6 nmol) was dissolved in 400 μL of 0.1 M sodium carbonate buffer (pH 8.5), to which NODAGA-NHS ester (155 μg, 211.3 nmol) was added. The mixture was stirred for 18 h at room temperature. At the end of the reaction, the reaction mixture was purified by HPLC using a C4 column (Vydac, 5 μm, 4.6 x 250 mm) and a gradient program from a 80:20 mixture to a 30:70 mixture of water-CH_3CN containing 0.1% trifluoroacetic acid (TFA) for 30 min at a flow rate of 1 mL/min. The desired fraction eluted between 9.5 and 11.2 min was collected and lyophilized. The purified NODAGA-VEGF$_{121}$ was analyzed by MALDI-TOF mass spectrometry and quantified using a BCA protein assay kit.

2.3. Preparation of ^{68}Ga-NODAGA-VEGF$_{121}$

$^{68}GaCl_3$ (277.69 ± 40.97 MBq/0.8 mL) was eluted from a ^{68}Ge-^{68}Ga generator using 0.1 N HCl, after which 0.5 M phosphate buffer (pH 7.4) was added. This solution was then added to NODAGA-VEGF$_{121}$ (20 μg); final pH of the mixture was 6.0. The reaction mixture was stirred at room temperature for 30 min with constant shaking using a Thermomixer (Eppendorf, Hamburg, Germany) and purified using a PD-10 column.

2.4. Radiochemical purity of ^{68}Ga-NODAGA-VEGF$_{121}$

Radiochemical purity of ^{68}Ga-NODAGA-VEGF$_{121}$ was measured using paper chromatography, size exclusion HPLC, reverse phase HPLC, and SDS-PAGE. For paper chromatography, bovine serum albumin (BSA; 5% in saline) was spotted at the origin of a paper chromatography strip and dried at ambient temperature for 20 min. ^{68}Ga-NODAGA-VEGF$_{121}$ was then spotted at the same position of the strip where BSA was previously spotted, and the strip was developed twice using saline [22]. ^{68}Ga-colloid was also prepared for comparison [22]. For size exclusion HPLC analysis, an aliquot of the product was injected onto a column (Shodex Protein KW-802.5, 8 x 300 mm) which was eluted with 0.05 M phosphate buffer (pH 7.0) containing 0.3 M NaCl at a flow rate of 1 mL/min. For reverse phase HPLC analysis, an aliquot of the product was injected onto an analytical HPLC column (Vydac C4, 5 μm, 4.6 x 250 mm) which was eluted with water containing 0.1% TFA for 10 min followed by a gradient program from water containing 0.1% TFA to CH_3CN containing 0.1% TFA for 30 min at a flow rate of 1 mL/min. Further analysis of ^{68}Ga-NODAGA-VEGF$_{121}$ (370 kBq) was performed using SDS-PAGE under non-reducing conditions and the gel was then exposed to an X-ray film.

2.5. Serum stability

^{68}Ga-NODAGA-VEGF$_{121}$ (23.68 MBq) in 1 mL of 0.01 M PBS (pH 7.4) was added to 50% fetal bovine serum (total volume of 2 mL) and incubated at 37 °C for 0, 1, 2, and 4 h. At the indicated