Nuclear Medicine and Biology 48 (2017) 1–8

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

Iminodiacetic acid as bifunctional linker for dimerization of cyclic RGD peptides

Dong Xu [a,1], Zuo-Quan Zhao [b,1], Shu-Ting Chen [c], Yong Yang [a,b], Wei Fang [b,*], Shuang Liu [d,**]

[a] International Medicine Center, International Medicine Division, Beijing Friendship Hospital, Capital Medical University, Beijing, China
[b] Department of Nuclear Medicine, Fuwai Hospital, the National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[c] Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, China
[d] School of Health Sciences, Purdue University, West Lafayette, IN, USA

ARTICLE INFO

Article history:
Received 11 October 2016
Received in revised form 3 January 2017
Accepted 17 January 2017

Keywords:
Integrin $\alpha_v\beta_3$
^{99m}Tc-labeling
Dimeric cyclic RGD peptides
Tumor imaging
SPECT

ABSTRACT

Introduction: In this study, I2P-RGD$_2$ was used as the example to illustrate a novel approach for dimerization of cyclic RGD peptides. The main objective of this study was to explore the impact of bifunctional linkers (glutamic acid vs. iminodiacetic acid) on tumor-targeting capability and excretion kinetics of the ^{99m}Tc-labeled dimeric cyclic RGD peptides.

Methods: HYNIC-I2P-RGD$_2$ was prepared by reacting I2P-RGD$_2$ with HYNIC-OSu in the presence of diisopropylethylamine, and was evaluated for its $\alpha_v\beta_3$ binding affinity against ^{125}I-echistatin bound to U87MG glioma cells. ^{99m}Tc-I2P-RGD$_2$ was prepared with high specific activity (~185 GBq/μmol). The athymic nude mice bearing U87MG glioma xenografts were used to evaluate its biodistribution properties and image quality in comparison with those of ^{99m}Tc-3P-RGD$_2$.

Results: The IC_{50} value for HYNIC-I2P-RGD$_2$ was determined to be 39 ± 6 nM, which was very close to that (IC_{50} = 33 ± 5 nM) of HYNIC-3P-RGD$_2$. Replacing glutamic acid with iminodiacetic acid had little impact on $\alpha_v\beta_3$ binding affinity of cyclic RGD peptides. ^{99m}Tc-I2P-RGD$_2$ and ^{99m}Tc-3P-RGD$_2$ shared similar tumor uptake values over the 2 h period, and its $\alpha_v\beta_3$-specificity was demonstrated by a blocking experiment. The uptake of ^{99m}Tc-I2P-RGD$_2$ was significantly lower than ^{99m}Tc-3P-RGD$_2$ in the liver and kidneys. The U87MG glioma tumors were visualized by SPECT with excellent contrast using both ^{99m}Tc-I2P-RGD$_2$ and ^{99m}Tc-3P-RGD$_2$.

Conclusion: Iminodiacetic acid is an excellent bifunctional linker for dimerization of cyclic RGD peptides. Bifunctional linkers have significant impact on the excretion kinetics of ^{99m}Tc radiotracers. Because of its lower liver uptake and better tumor/liver ratios, ^{99m}Tc-I2P-RGD$_2$ may have advantages over ^{99m}Tc-3P-RGD$_2$ for diagnosis of tumors in chest region.

1. Introduction

Integrin $\alpha_v\beta_3$ plays a significant role in tumor angiogenesis and metastasis [1–7]. It is a receptor for the extracellular matrix proteins (e.g., vitronectin, fibronectin, fibrinogen and laminin) with the exposed arginine–glycine–aspartic (RGD) tripeptide sequence [2,8–10]. It is expressed at low levels on epithelial cells and mature endothelial cells, but it is overexpressed on activated endothelial cells of neovasculature and tumor cells. The restricted $\alpha_v\beta_3$ expression during tumor growth and metastasis makes it an interesting target for development of $\alpha_v\beta_3$-targeted molecular imaging probes [11–20].

The RGD-containing cyclic pentapeptides (Fig. 1: c(RGDfK), RGD$_2$ (E[c(RGDfK)]$_2$); 2P-RGD$_2$ (E[PEG$_4$-c(RGDfK)]$_2$: PEG$_4$ = 15-amino-4,7,10,13-tetraoxapentadecanoic acid); and 3P-RGD$_2$ (PEG$_4$-E[PEG$_4$-

Abbreviations: ITLC, instant thin layer chromatography; MALDI, matrix-assisted laser desorption ionization; PET, positron emission tomography; RCP, radiochemical purity; SPECT, single photon emission computed tomography; HYNIC-OSu, sodium succinimidyl 6-(2-(2-sulfonatobenzaldehyde)hydrazono)-nicotinate); NOTA, 1,4,7-tritazacyclononane-1,4,7-triacetic acid; RGD$_2$: E[c(RGDfK)]$_2$, Glu[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$; RGD$_4$: E{E[c(RGDfK)]$_2$}$_2$, Glu{E[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$}$_2$; 2P-RGD$_2$: E[PEG$_4$-c(RGDfK)]$_2$, Glu[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_2$ (PEG$_4$ = 15-amino-4,7,10,13-tetraoxapentadecanoic acid); 3P-RGD$_2$: PEG$_4$-E[PEG$_4$-c(RGKfD)]$_2$, PEG$_4$-Glu[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_2$; I2P-RGD$_2$, *N*-(2-aminoethyl)iminodiacetyl-[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_2$; HYNIC-3P-RGD$_2$, HYNIC-PEG$_4$-E[PEG$_4$-c(RGKfD)]$_2$ (HYNIC = 6-(2-(2-sulfonatobenzaldehyde)hydrazono)nicotinyl); HYNIC-I2P-RGD$_2$, HYNIC-*N*-(2-aminoethyl)iminodiacetyl-[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_2$; ^{18}F-Alfatide-II, [^{18}F]AlF(NOTA-2P-RGD$_2$) (NOTA =1,4,7-tritazacyclononane-1,4,7-triacetic acid; ^{99m}Tc-3P-RGD$_2$, [^{99m}Tc(HYNIC-3P-RGD$_2$)(tricine)(TPPTS)] (TPPTS = trisodium triphenylphosphine-3,3′″,3″-trisulfonate); ^{99m}Tc-I2P-RGD$_2$, [^{99m}Tc(HYNIC-I2P-RGD$_2$)(tricine)(TPPTS)].

* Correspondence to: W. Fang, Department of Nuclear Medicine, Fuwai Hospital, the National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.
** Correspondence to: S. Liu, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907, USA. Tel.: +1 765 494 0236; fax: +1 765 496 1377.
E-mail addresses: nuclearfw@126.com (W. Fang), liu100@purdue.edu (S. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.nucmedbio.2017.01.007
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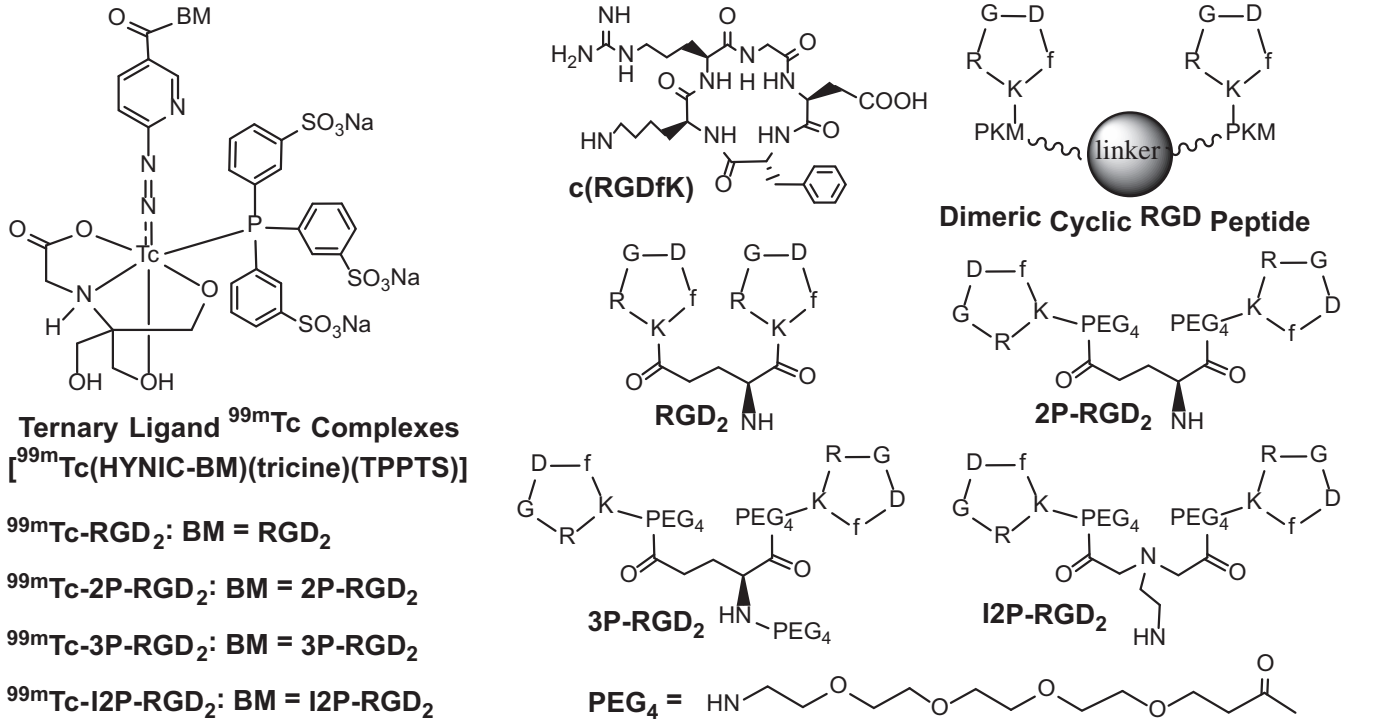

Fig. 1. The structures of dimeric cyclic RGD peptides (RGD_2, 2P-RGD_2, 3P-RGD_2 and I2P-RGD_2) and their ^{99m}Tc complexes [^{99m}Tc(HYNIC-BM)(tricine)(TPPTS)] (BM = biomolecule; ^{99m}Tc-RGD_2: BM = RGD_2; ^{99m}Tc-2P-RGD_2: BM = 2P-RGD_2; ^{99m}Tc-3P-RGD_2: BM = 3P-RGD_2; and ^{99m}Tc-I2P-RGD_2: BM = I2P-RGD_2). The RGD moiety is responsible for $\alpha_v\beta_3$-binding and tumor-targeting. PEG_4 groups are utilized to modify the excretion kinetics of ^{99m}Tc radiotracers from normal organs, and to keep the distance between two cyclic c(RGDfK) moieties in order to achieve bivalency. Glutamic and iminodiacetic acids are used as bifunctional linkers for dimerization of cyclic RGD peptides and attachment of the chelator, such as HYNIC.

c(RGDfK)$]_2$)) are potent $\alpha_v\beta_3$ antagonists [11,15,20]. We have been using dimeric, trimeric and tetrameric cyclic RGD peptides as targeting biomolecules (BM) to develop $\alpha_v\beta_3$-targeted radiotracers for tumor imaging by SPECT or PET [21–40]. Multiple cyclic RGD moieties are utilized to maximize the $\alpha_v\beta_3$-targeting capability and tumor uptake [11,15,20]. We found that radiolabeled (^{99m}Tc, ^{18}F, ^{64}Cu, ^{68}Ga and ^{111}In) multimeric cyclic RGD peptides all had higher tumor uptake with longer tumor retention time than their corresponding monomeric analogs [21–40], because of their higher $\alpha_v\beta_3$ binding affinity and their capability to target multiple integrins over-expressed in tumor tissues [20]. Among the dimeric cyclic RGD peptides evaluated in our laboratories, 2P-RGD_2 and 3P-RGD_2 show most promising results with respect to the tumor uptake and T/B ratios of their corresponding radiotracers [21–40]. Radiotracers ^{99m}Tc-3P-RGD_2 (Fig. 1: [^{99m}Tc(HYNIC-3P-RGD_2)(tricine)(TPPTS)]) and ^{18}F-Alfatide-II ([^{18}F]AlF(NOTA-2P-RGD_2)) are currently under clinical investigations as SPECT and PET radiotracers for tumor detection in cancer patients [41–49].

Dimeric cyclic RGD peptides are composed of two main components: c(RGDfK) moieties responsible for $\alpha_v\beta_3$-binding and a bifunctional linker (Fig. 1) for attachment of two c(RGDfK) moieties and conjugation of a chelator (e.g., HYNIC). Pharmacokinetic modifying groups (e.g., PEG_4 or Gly-Gly-Gly) are utilized to optimize the excretion kinetics of ^{99m}Tc radiotracers and to keep the distance between c(RGDfK) moieties in order to achieve bivalency. Over the last several years, we have been using glutamic acid for dimerization of cyclic RGD peptides [21–40]. In this study, we prepared a new dimeric cyclic RGD peptide conjugate HYNIC-2-(aminoethyl)iminodiacetyl-E[PEG_4-c(RGDfK)$]_2$ (HYNIC-I2P-RGD_2) using iminodiacetic acid as the bifunctional linker. Iminodiacetic acid is of our interest because it has similar length to glutamic acid. The $\alpha_v\beta_3$ binding affinity HYNIC-I2P-RGD_2 was determined in a displacement assay. ^{99m}Tc-I2P-RGD_2 ([^{99m}Tc(HYNIC-I2P-RGD_2)(tricine)(TPPTS)] (Fig. 1: ^{99m}Tc-I2P-RGD_2) was prepared and evaluated in athymic nude mice bearing U87MG glioma xenografts in comparison to ^{99m}Tc-3P-RGD_2. The main objectives of this study were to demonstrate the utility of iminodiacetic acid as a bifunctional linker for dimerization of cyclic RGD peptides, and explore the impact of bifunctional linkers (glutamic acid vs. iminodiacetic acid) on the tumor-targeting capability and excretion kinetics of their corresponding ^{99m}Tc radiotracer from normal organs (such as kidneys, liver and lungs).

2. Experimental section

2.1. Materials and instruments

Chemicals were purchased from Sigma/Aldrich (St. Louis, MO), and were used without further purification. The peptide *N*-(2-aminoethyl)iminodiacetyl-[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG_4)]]$_2$ (I2P-RGD_2) was custom-made by the Peptides International, Inc. (Louisville, KY). Sodium succinimidyl 6-(2-(2-sulfonatobenzaldehyde)hydrazono) nicotinate (HYNIC-OSu) was prepared according to literature method [50]. [^{99m}Tc(HYNIC-3P-RGD_2)(tricine)(TPPTS)] (^{99m}Tc-3P-RGD_2) was prepared using the procedure described in our previous reports [25]. $Na^{99m}TcO_4$ was obtained from Cardinal HealthCare (Chicago, IL). The MALDI (matrix-assisted laser desorption ionization) mass spectral data were collected on an Applied Biosystems Voyager DE PRO mass spectrometer (Framingham, MA), the Department of Chemistry, Purdue University.

2.2. HPLC methods

HPLC Method 1 used a LabAlliance HPLC system (Scientific Systems, Inc., State College, PA) equipped with a UV/vis detector (λ = 254 nm) and Zorbax C_{18} column (9.4 mm × 250 mm, 100 Å pore size; Agilent Technologies, Santa Clara, CA). The flow rate was 2.5 mL/min with a mobile phase being 90% A and 10% B at 0 min to 80%A and 20% B at 5 min, and to 50%A and 50% B at 20 min. The radio-HPLC (Method 2) used the LabAlliance HPLC system equipped with a β-ram IN/US detector