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ORIGINAL ARTICLE

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Preliminary evaluation of new ^{68}Ga-labeled cyclic RGD peptides by PET imaging

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ABSTRACT

Integrin $\alpha_v\beta_3$ plays an important role in the tumor metastases and angiogenesis. Arginine-glycine-aspartate (RGD) peptide motif binds to the integrin $\alpha_v\beta_3$. General ^{68}Ga-labeled cyclic RGD peptides was rapidly eliminated from the circulatory system by renal excretion because of its hydrophilic property. The purpose of this study was to develop a novel ^{68}Ga-labeled cyclic RGD peptides, which could acquire enhanced PET tumor images with improved pharmacokinetics by adopting biphenyl group between chelator and RGD peptides. ^{68}Ga-DOTA-2P-c(RGDyK) was demonstrated a 12% higher lipophilicity level than ^{68}Ga-DOTA-c(RGDyK) as a reference compound. In the animal PET, ^{68}Ga-DOTA-2P-c(RGDyK) represented relatively lower blood-clearance, and an increased signal to noise ratio compared to ^{68}Ga-DOTA-c(RGDyK). From these perspective, ^{68}Ga-DOTA-2P-c(RGDyK) could be a good candidate for in integrin $\alpha_v\beta_3$-expressed tumor imaging.

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Key Word: Radiometals, ^{68}Ga-DOTA-c(RGDyK), ^{68}Ga-DOTA-2P-c(RGDyK), Cyclic RGD peptides, PET imaging

Introduction

Cyclic Arg-Gly-Asp (RGD) is a one of the amino acid peptides capable of specific binding to integrin $\alpha_v\beta_3$, which plays a critical role in angiogenesis. A binding affinity for cyclic RGD tripeptide has been utilized for early diagnosis of integrin $\alpha_v\beta_3$ overexpressed tumor over the past several years(1).

To optimize uptake of integrin $\alpha_v\beta_3$ positive tumor, cyclic RGD peptides have been modified with various structure such as cyclic Arg-Gly-Asp-D-Phe-Lys peptides [c(RGDfK)] and cyclic Arg-Gly-Asp-D-Tyr-Lys peptides [c(RGDyK)](2). The cyclic RGD peptides have been also conjugated with bifunctional chelators (BFCs), which were used for labeling radiometals (^{68}Ga, ^{64}Cu, ^{111}In, etc.) such as 1,4,7,10-tetraazacyclodo-decane-N,N′,N′′,N′′′-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N′;N′′-triacetic acid (NOTA) and 1,4,7-triazacyclononane-1-glutaric acid-4,7-diacetic acid (NODAGA), and it has been actively utilized for nuclear medicine and molecular imaging(3-6). Based on these researches, some strategies were proposed to apply a peptide multiplicity or a hydrophilicity enhancement by conjugation with sugar residues for the higher tumor uptake of cyclic RGD tracer(7, 8).

Despite the attempts to improve the pharmacokinetics

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of the cyclic RGD peptide, previously developed cyclic RGD conjugates have identified rapid clearance from the blood-pool due to its intrinsic high hydrophilicity with low tumor-to-background ratio(9-11).

The aim of the present study, thus, was to develop and preliminary evaluate noble cyclic RGD peptides by inserting biphenyl group between BFC and cyclic ring to acquire better PET image of integrin $\alpha_v\beta_3$-expressed tumor.

Materials and Methods

Synthesis of cyclic RGD peptides conjugated phenyl group

The compound of DOTA-2P was prepared according to the literature methods(12). The synthesis of DOTA-2P-NCS was performed as follows: A solution of DOTA-2P (300 mg, 0.53 mmol) in 0.5 M HCl (2.5 mL) was added dropwise to a solution of thiophosgene (0.77 mL, 10.99 mmol) in $CHCl_3$ (2.5 mL). The biphasic reaction was vigorously stirred for 4 h at RT. The aqueous layer was concentrated in vacuo to give a white solid (300 mg, 93%). A solution of DO3A-2P-NCS (1.00 mg, 0.002 mmol) in 0.2 M diisopropylethylamine (4 μ L) was added c(RGDyK) (1.30 mg, 0.002 mmol). The reaction mixture was stirred at RT for 15 h and was evaporated to dryness. The formation of DOTA-2P-c(RGDyK) was confirmed by Waters HPLC system equipped with a C18 analytical column (5 μm, 3.0 × 150 mm, μBondapak™) with the following separation conditions: A mixture of an aqueous solution of trifluoroacetic acid (0.1%, v/v) an acetonitrile solution of trifluoroacetic acid (0.1%, v/v) with a 30 min linear gradient (form 5% to 65%) at a flow rate of 0.5 mL/min; retention time (Rt) = 17.5 min; MALDI-TOF MS (m/z): calcd. for $C_{56}H_{77}N_{15}O_{15}S$, 1232.4 [M +H]+. found: 1232.8 [M + H]+. The solution was lyophilized and purified by preparative C18 column (10μm, 4.6×300 mm, μBondapak™) with the same mobile phase above with a flow rate of 14 mL/min. The product was obtained as a white solid (yield: 90%).

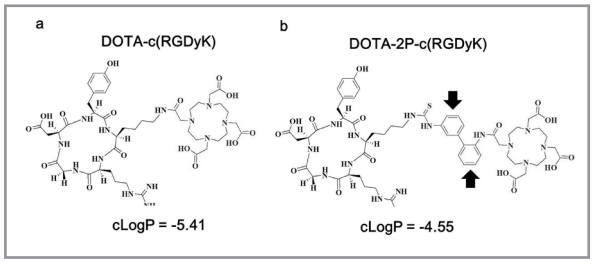

Figure 1. Chemical structures of DOTA-c(RGDyK) (a) and DOTA-2P-c(RGDyK) (b). The arrow indicated biphenyl group which was conjugated between BFC and c(RGDyK).

Synthesis of ^{68}Ga-DOTA-c(RGDyK) and ^{68}Ga-DOTA-2P-c(RGDyK)

^{68}Ga was produced from ^{68}Ge/^{68}Ga generator (^{68}Ge; $T_{1/2}$ = 280 days, ^{68}Ga; $T_{1/2}$ = 68 mins) made by ITG company. ^{68}Ga (~10 mCi) was eluted into V-vial (5 mL) using HCl solution (0.1 M, 1 mL), and the extracted ^{68}Ga solution was dried with a flow of nitrogen gas (99.9%) on a heating block at 100°C. The V-vial containing the completely dried ^{68}Ga, was added to the fabricated DOTA-2P-c(RGDyK) (0.5 mg/0.5 mL 1 M sodium acetate, pH 5-6), and then heated for 5 min at 80°C. The systhesis y

The radiochemical purity and yield were evaluated by instant thin-layer chromatography (ITLC) method with 0.1 M citric acid as mobile phase solvents.

Tumor xenograft model

Animal procedures were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of Korea Institute of Radiological and Medical Sciences (KIRAMS). Female BALB/c nude mice (SLC, Hamamatsu, Japan) at 4 - 6 weeks of age, were injected subcutaneously in the left shoulder with 5 × 10^6 U87MG glioblastoma cells suspended in 100 μL Dulbecco's modified eagle's medium (DMEM). The mice were subjected to PET studies when the tumor volume reached 5 - 7 mm in diameter (10 - 14 day after implant).

PET-CT acquisition and image analysis

Animals were anesthetized with 2.5% isoflurane, and radioligands were administrated to the tail vein (8.9 – 10.1 MBq). Under anesthetization, PET images were acquired in the list mode for 90 min (Inveon PET-CT, Siemens, Knoxville, TN, USA).

Raw PET data were reconstructed in user-defined time frames (1 min x 10 frames, 5 min x 16 frames) by a 2-dimensional order-subset expectation maximization (OSEM) algorithm. Regions of interests were tumor, heart, kidney, liver and muscle. Here, we used muscle as reference region for kinetic modeling. Regional time activity curves were normalized in units of percentage injected dose per gram (%ID/g). Non-displaceable binding potential

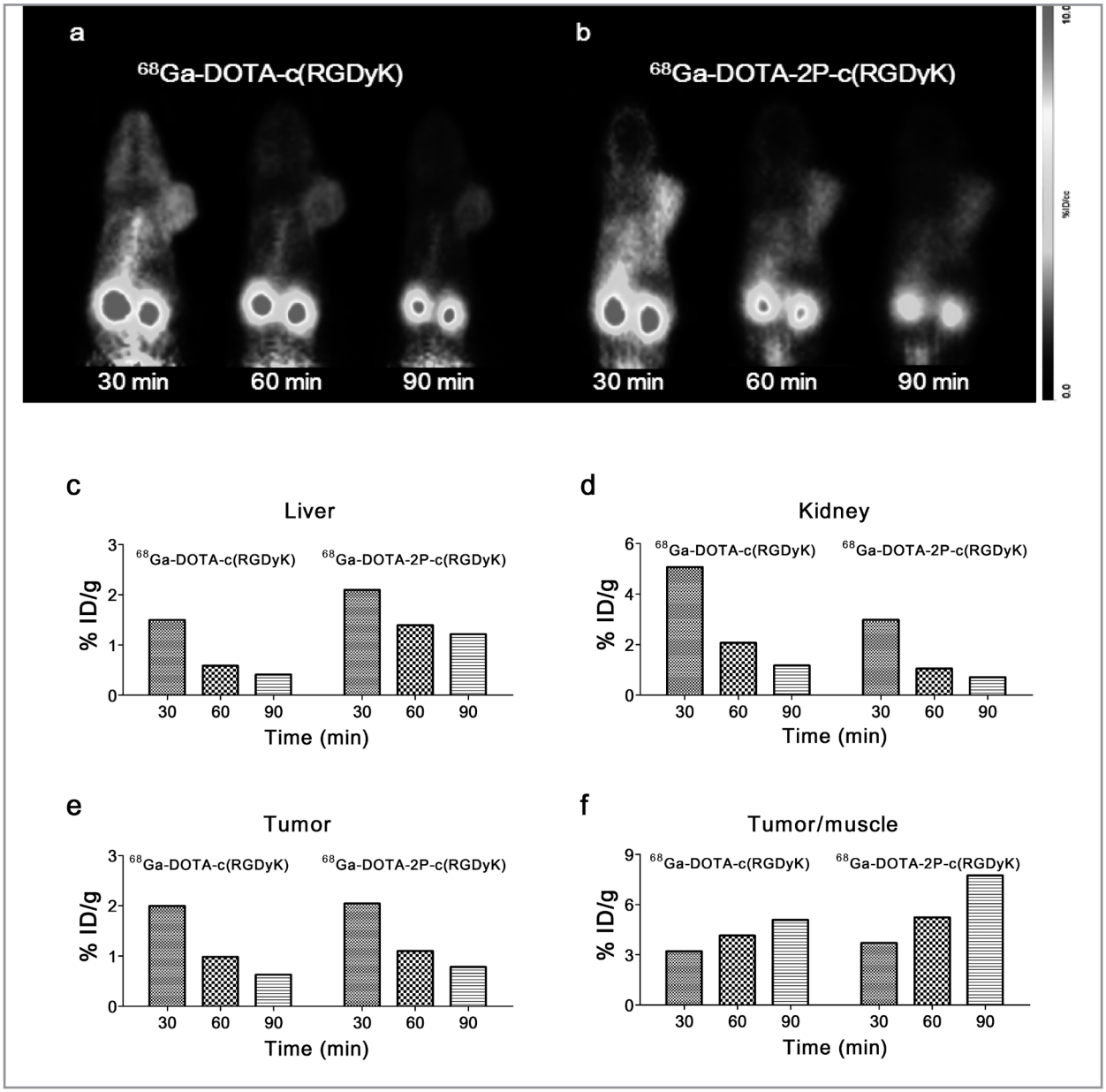

Figure 2. MicroPET images of U87MG tumor-bearing nude mice injection with ^{68}Ga-DOTA-c(RGDyK) (a) and ^{68}Ga-DOTA-2P-c(RGDyK) (b). Quantitative analysis of the PET images showing the time-course of accumulation of ^{68}Ga-DOTA-c(RGDyK) and ^{68}Ga-DOTA-2P-c(RGDyK) in liver (c), kidney(d), tumor (e) and tumor-muscle ratio (f).

(BP_{ND}) values were estimated from non-invasive Logan's graphical. BPND is the product of receptor density and the affinity to target.

Results and Discussion

The cyclic ring of RGD peptides, in this study, was conjugated with BFCs such as DOTA or DOTA-2P, and then each cyclic RGD conjugates was labeled with ^{68}Ga to evaluate pharmacokinetics using PET imaging. The chemical structures of ^{68}Ga-DOTA-c(RGDyK) and ^{68}Ga-DOTA-2P-c(RGDyK) were shown in Figure 1. Radiochemical yield and purity of ^{68}Ga-DOTA-2P-c(RGDyK) were 47.1% and over 98% (n = 10), respectively.

The integrin binding properties of the ^{68}Ga-DOTA-2P-c(RGDyK) revealed increased the renal clearance and tumor-to-background ratio. 30 min interval average PET images for ^{68}Ga-DOTA-c(RGDyK) and ^{68}Ga-DOTA-2P-c(RGDyK) were shown in Figure 2. Biodistribution patterns for both RGD ligands were similar. The tumor regions were clearly visualized for both radioligands (1.99 and 2.04%ID/g at 30 min p.i, for ^{68}Ga-DOTA-c(RGDyK) and ^{68}Ga-DOTA-2P-c(RGDyK) respectively). ^{68}Ga-DOTA-2P-c(RGDyK), showed comparable liver uptake to ^{68}Ga-DOTA-c(RGDyK) (2.04 and 1.99%ID/g at 30 min p.i, respectively), and the radiopeptide induced the increased radioactivity in the abdomen organs such as colon by hepatobiliary excretion, presumably due to its increased lipophilicity. Interestingly, however, ^{68}Ga-DOTA-2P-c(RGDyK) showed reduced in vivo accumulation in the kidneys compared to the ^{68}Ga-DOTA-c(RGDyK). The accumulation value of ^{68}Ga-DOTA-2P-c(RGDyK) in the kidneys is twice as low as that of ^{68}Ga-DOTA-c(RGDyK). As a results, tumor-to-muscle ratio of ^{68}Ga-DOTA-2P-c(RGDyK) increased as a function of time: 3.7%ID/g (30 min p.i.), 5.22%ID/g (60 min p.i.) and 7.33%ID/g (90 min p.i.). In case of ^{68}Ga-DOTA-c(RGDyK), the tumor-to-muscle ratio was 3.2%ID/g (30 min p.i.), 4.14%ID/g (60 min p.i.) and 5.09%ID/g (90 min p.i.).

We performed dynamic PET studies to determine and compare the effects of the structural modification on the early pharmacokinetics properties of the peptides. Figure 3

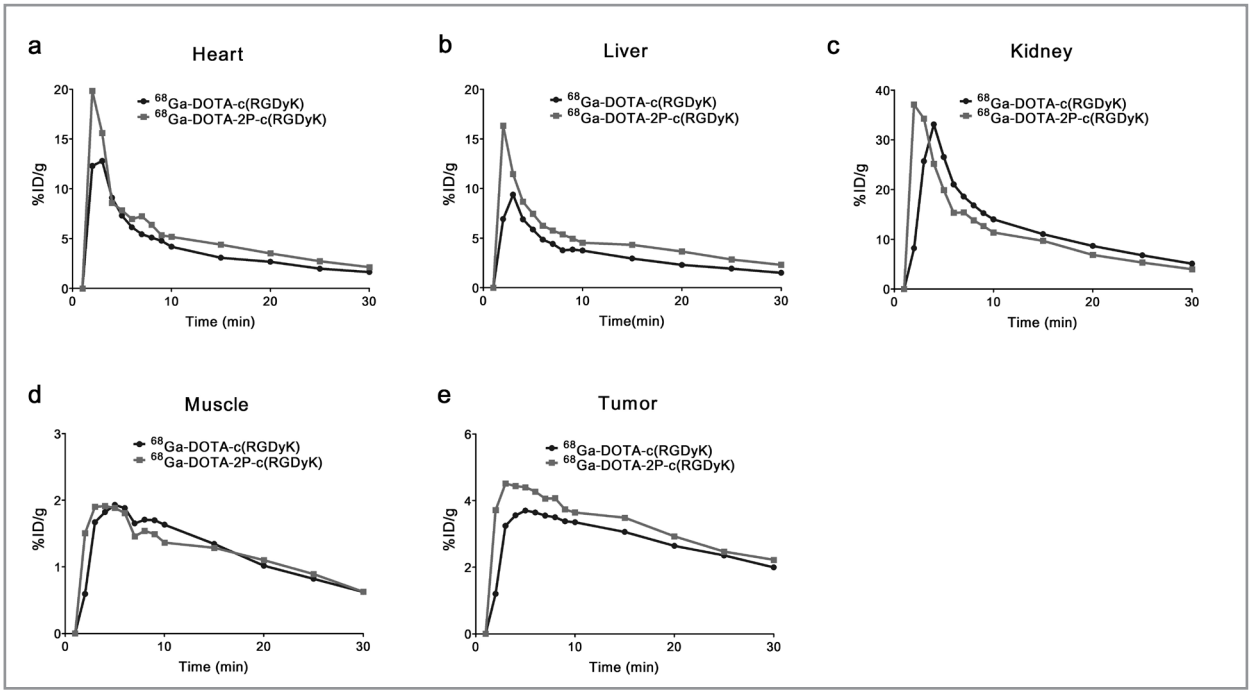

Figure 3. Dynamic PET-derived time-activity curve of ^{68}Ga-DOTA-c(RGDyK) and ^{68}Ga-DOTA-2P-c(RGDyK) in the heart (a), liver (b), kidney (c), muscle (d) and tumor (e) region during the first 30 min p.i.

shows dynamic PET-derived time-activity distribution of the heart, liver, kidneys, muscle and tumor. ^{68}Ga-DOTA-2P-c(RGDyK) showed higher uptake value compare to the ^{68}Ga-DOTA-c(RGDyK) in the heart and liver activity curves (Fig 3. a, b). In contrast, the time activity curve of ^{68}Ga-DOTA-2P-c(RGDyK) in the kidney showed more rapid renal clearance and lower activity accumulation than those of ^{68}Ga-DOTA-c(RGDyK) (Fig 3. c). Both compounds had similar muscle activity curves (Fig 3. d), but ^{68}Ga-DOTA-2P-c(RGDyK) exhibited higher tumor activity, resulting in the biphenyl conjugated compound (Fig 3. e). Binding value for ^{68}Ga-DOTA-2P-c(RGDyK) was 17% higher than that for ^{68}Ga-DOTA-c(RGDyK). ^{68}Ga-DOTA-2P-c(RGDyK), thus, could effectively detect a subtle change of receptors if being similar to $\alpha_v\beta_3$ integrin receptors expression in tumor cell of the animals.

Conclusion

This study developed a new cyclic RGD peptides which was conjugated to the biphenyl group to improve the pharmacokinetics, and the synthesized ^{68}Ga-DOTA-2P-c(RGDyK) was performed preliminary evaluation using PET imaging. This new cyclic RGD peptides demonstrated higher tumor uptake with enhanced retention as well as rapid renal clearance allowing high signal-to-noise ratio PET images. From these result, ^{68}Ga-DOTA-2P-c(RGDyK) is expected to utilize a potential radiopharmaceutical which is possible to acquire a good PET image of integrin $\alpha_v\beta_3$-expressed tumor.

Acknowledgments

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