Development of Diagnostic and Therapeutic Probes with Controlled Pharmacokinetics for Use in Radiotheranostics

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The word “theranostics,” a portmanteau word made by combining “therapeutics” and “diagnostics,” refers to a personalized medicine concept. Recently, the word, “radiotheranostics,” has also been used in nuclear medicine as a term that refers to the use of radioisotopes for combined imaging and therapy. For radiotheranostics, a diagnostic probe and a corresponding therapeutic probe can be prepared by introducing diagnostic and therapeutic radioisotopes into the same precursor. These diagnostic and therapeutic probes can be designed to show equivalent pharmacokinetics, which is important for radiotheranostics. As imaging can predict the absorbed radiation dose and thus the therapeutic and side effects, radiotheranostics can help achieve the goal of personalized medicine. In this review, I discuss the use of radiolabeled probes targeting bone metastases, sigma-1 receptor, and αVβ3 integrin for radiotheranostics.

Key words radiotheranostics; imaging; radionuclide therapy; cancer; bone metastasis; arginine-glycine-aspartic acid (RGD) peptide

1. Introduction

“Molecular imaging” refers to techniques of noninvasively recognition and determination of molecular processes caused by changes and interactions in the living body. This type of imaging greatly contributes to clinical diagnosis in nuclear medicine, drug discovery, and life sciences. Radiolabeled probes are useful in the molecular imaging. Diagnostic radiolabeled probes are injected into patients and accumulate in target lesions where the radioisotopes (RIs) in the radiolabeled probes decay with emission of radiation. The imaging data for diagnosis can be obtained by gamma scintigraphy, single photon emission computed tomography (SPECT), or positron emission tomography (PET). Quantitative imaging data shows the distribution of the radiolabeled probes within the patients’ bodies and provides information for diagnosis of lesions, such as the expression levels of target molecules.

In recent years, the word “theranostics,” a portmanteau of the words “therapeutics” and “diagnostics,” has been used in the field of oncology. “Theranostics” describes agents or techniques that couple diagnostic imaging with targeted therapy. The system of theranostics consists of an imaging component to investigate the lesions before treatment and a corresponding therapeutic component to treat the same lesions. Recently, the word “radiotheranostics” has also been used in nuclear medicine as a term that refers to the use of RIs in combined imaging and therapy.1,2) Radioisotopes can effectively be used to establish a theranostics system. A diagnostic probe and a corresponding therapeutic probe with equivalent pharmacokinetics for radiotheranostics can be prepared by introducing a diagnostic RI and a therapeutic RI into the same precursor. The equivalent pharmacokinetics of the two probes makes radiotheranostics possible. Because quantitative analyses based on diagnostic imaging data can strictly predict the absorbed radiation dose in the lesion sites and normal tissues, the therapeutic effects in the lesion namely and side effects in normal tissues can also be predicted. Radiotheranostics can greatly contribute to the realization of personalized medicine because it enables appropriate selection of patients and optimization of therapeutic doses.

In this review, I discuss my previous studies aimed at developing radiolabeled probes for use in radiotheranostics.

2. Bone-Seeking Radiolabeled Probes

Bone is a common tissue in which cancer metastases often appear because bone contains many growth factors and is a good environment for tumors, which is consistent with Paget’s “seed and soil” theory.3–5) Nuclear medicine imaging using radiopharmaceuticals has been used as the most sensitive diagnosis method of bone metastases because it can diagnose those bone metastases before detection by anatomical
X-ray imaging.\textsuperscript{6–9} As radiopharmaceuticals for diagnoses of bone metastases, [\textsuperscript{99m}Tc] Tc-bisphosphonate complexes, such as [\textsuperscript{99m}Tc] Tc-methylenediphosphonate (MDP, Fig. 1A) and [\textsuperscript{99m}Tc] Tc-hydroxymethylenediphosphonate (HMDP, Fig. 1B), have been used worldwide because of simple imaging methods using conventional gamma cameras or SPECT and the convenient physical characteristics of \textsuperscript{99m}Tc, such as suitable gamma energy for imaging, moderate half-life (6.01 h) for clinical usage, and in-house generator produced radionuclide.

Most patients with bone metastases suffer severe pain, which decreases their patients’ QOL.\textsuperscript{10} Thus, palliation of bone pain is important for these patients. To decrease cancer-induced bone pain, analgesic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, have been used to treat patients according to the WHO three-step ladder. Unfortunately, however, those drugs are often insufficient to effectively alleviate incidental pain. Localized radiation therapy and surgical removal of lesion sites are effective methods for treatment of metastatic bone pain, but, the majority of patients with severe bone pain have multiple metastatic sites. It therefore often makes application of these therapeutics difficult. On the other hand, targeted radionuclide therapy using bone-seeking radiopharmaceuticals has gathered attention because of their effectiveness for treatment of multiple metastatic sites and few side effects.

A classic bone-seeking radiopharmaceutical as an element for palliation therapy is \textsuperscript{[90]Sr}SrCl\textsubscript{2} (Metastron\textsuperscript{11}), and a newer one is \textsuperscript{[223]Ra}RaCl\textsubscript{2} (Xofigo\textsuperscript{6}), which was recently approved as the first alpha-particle emitting bone-seeking radiopharmaceutical for castration-resistant prostate cancer patients with bone metastases in many countries because it showed great treatment outcomes in a worldwide phase III study.\textsuperscript{12} Another bone-seeking radiopharmaceutical, \textsuperscript{[153]Sm}Sm-ethylene diaminetetramethylene phosphonic acid (EDTMP, Fig. 1C) complex, has also been approved by the Food and Drug Administration (FDA) as an agent for palliation of bone pain. These bone-seeking radiopharmaceuticals for palliation therapy and the above-mentioned [\textsuperscript{99m}Tc] Tc-bisphosphonate complexes for bone imaging highly accumulate in metastatic bone lesions. Therefore, the accumulation of therapeutic bone-seeking radiopharmaceuticals in lesion sites can be predicted by using bone scintigraphy.\textsuperscript{13} The combination of the above-mentioned agents therapeutic bone seeking radiopharmaceuticals and [\textsuperscript{99m}Tc] Tc-bisphosphonate complexes may be useful as “radiotheranostics,” however they do not always show the same complete biodistribution. [\textsuperscript{99m}Tc] Tc-MDP and [\textsuperscript{99m}Tc] Tc-HMDP have not yet been optimized from a chemical and pharmaceutical perspective because these complexes are not well-defined single-chemical species but rather mixtures of short-chain and long-chain oligomers.\textsuperscript{9,14} Moreover, the phosphonate groups in [\textsuperscript{99m}Tc] Tc-MDP and [\textsuperscript{99m}Tc] Tc-HMDP are used both as ligands for coordination and as carriers to hydroxyapatite (HA) in bone,\textsuperscript{15} which may decrease the inherent affinity of MDP and HMDP for bone. To overcome these drawbacks, a more logical design strategy has been proposed on the basis of the conjugation of a stable radionuclide with a carrier molecule to bone. To develop better tracers for radiotheranostics, I designed, synthesized, and evaluated radionuclide complex-conjugated bisphosphonate compounds.\textsuperscript{16–24} This drug design allows the ligand and carrier function to work independently and effectively.

In these radionuclide complex-conjugated bisphosphonate compounds, [\textsuperscript{186}Re] Re-mercaptopoacetylglycylglycylglycine (MAG3)-conjugated bisphosphonate, [\textsuperscript{186}Re] Re-MAG3-HBP (Fig. 1D), showed superior characteristics as a bone-seeking radiopharmaceutical.\textsuperscript{17} Rhenium, which has similar chemical

**Fig. 1.** Structures of Bisphosphonates Analogs (A) MDP, (B) HMDP, (C) EDTMP, (D) M-MAG3-HBP (M = \textsuperscript{99m}Tc or \textsuperscript{186}Re)

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**Biography**

Dr. Kazuma Ogawa was born in Shizuoka, Japan in 1973. He graduated Faculty of Pharmaceutical Sciences, Kyoto University in 1998. He became assistant professor of Kyoto Prefectural University of Medicine in 2002. He moved Advanced Science Research Center, Kanazawa University in 2004. He received his Ph.D. degree from Kyoto University in 2007 under the supervision of Prof. Hideo Saji. He became associate professor of School of Pharmaceutical Sciences, Kanazawa University in 2009. He moved to Institute for Frontier Science Initiative, Kanazawa University in 2015. He was honored by receiving the Best Paper Award for Young Researcher (The Japanese Society of Nuclear Medicine) in 2006 and the Pharmaceutical Society of Japan Award for Divisional Scientific Promotion in 2019. His major research field is development of radiolabeled probes for cancer imaging and therapy.
properties to technetium because technetium and rhenium are both members of group 7 elements of the periodic table, has two useful radionuclides, \(^{186}\text{Re} (T_{1/2} = 3.72 \text{ d}, \beta_{\text{max}} = 1.07 \text{ MeV}, \gamma = 137 \text{ keV})\) and \(^{188}\text{Re} (T_{1/2} = 17.0 \text{ h}, \beta_{\text{max}} = 2.12 \text{ MeV}, \gamma = 155 \text{ keV})\), for radionuclide therapy. These radionuclides were used for the preparation of radiolabeled bisphosphonates for therapy. The therapeutic effects of \(^{186}\text{Re}-\text{MAG3}-\text{HBP}\) for the palliation of metastatic bone pain in an animal model were evaluated. The results showed a significant palliation effect and inhibition of tumor growth in a bone metastasis rat model. Therefore, the combination of \(^{188}\text{Re}-\text{MAG3}-\text{HBP}\) for therapy could be one of the best candidates for radiotheranostics of bone metastases.

In the therapeutic experiments, \(^{186}\text{Re}-\text{MAG3}-\text{HBP}\) showed significant palliation effects and inhibition of tumor growth in a bone metastasis rat model. Therefore, the combination of \(^{186}\text{Re}-\text{MAG3}-\text{HBP}\) for therapy could be one of the best candidates for radiotheranostics of bone metastases.

It has been reported that major non-collagenous bone proteins, such as osteopontin and bone sialoprotein, contain many acidic amino acids (aspartic acid (Asp) or glutamic acid (Glu)) in their amino acid sequences, whose offers HA binding ability, and polyglutamic acid peptides and polyaspartic acid peptides could be carriers of drugs to bone because of their high affinity for HA.

To evaluate acidic amino acid peptides as carriers of radiometals to bone lesions, I designed \(^{67}\text{Ga}\)-DOTA-Dn, \(n = 2, 5, 8, 11, \text{ or } 14\), which have varying peptide lengths. \(^{67}\text{Ga}\)-DOTA-Dn conjugated to \(\text{RGDFK}\) showed equivalent biodistribution to that of \(^{67}\text{Ga}\)-DOTA-D11, which have varying peptide lengths. These results indicate that not only bisphosphonate molecules but also acidic amino acid peptides are useful as carriers of radiometals to proteins, such as osteopontin and bone sialoprotein, contain many acidic amino acids (aspartic acid (Asp) or glutamic acid (Glu)) in their amino acid sequences, whose offers HA binding ability, and polyglutamic acid peptides and polyaspartic acid peptides could be carriers of drugs to bone because of their high affinity for HA. To evaluate acidic amino acid peptides as carriers of radiometals to bone lesions, I designed \(^{67}\text{Ga}\)-DOTA-Dn, \(n = 2, 5, 8, 11, \text{ or } 14\), which have varying peptide lengths. \(^{67}\text{Ga}\)-DOTA-Dn conjugated to \(\text{RGDFK}\) showed equivalent biodistribution to that of \(^{67}\text{Ga}\)-DOTA-D11, which have varying peptide lengths. These results indicate that not only bisphosphonate molecules but also acidic amino acid peptides are useful as carriers of radiometals to proteins, such as osteopontin and bone sialoprotein, contain many acidic amino acids (aspartic acid (Asp) or glutamic acid (Glu)) in their amino acid sequences, whose offers HA binding ability, and polyglutamic acid peptides and polyaspartic acid peptides could be carriers of drugs to bone because of their high affinity for HA. To evaluate acidic amino acid peptides as carriers of radiometals to bone lesions, I designed \(^{67}\text{Ga}\)-DOTA-Dn, \(n = 2, 5, 8, 11, \text{ or } 14\), which have varying peptide lengths. \(^{67}\text{Ga}\)-DOTA-Dn conjugated to \(\text{RGDFK}\) showed equivalent biodistribution to that of \(^{67}\text{Ga}\)-DOTA-D11, which have varying peptide lengths. These results indicate that not only bisphosphonate molecules but also acidic amino acid peptides are useful as carriers of radiometals to proteins, such as osteopontin and bone sialoprotein, contain many acidic amino acids (aspartic acid (Asp) or glutamic acid (Glu)) in their amino acid sequences, whose offers HA binding ability, and polyglutamic acid peptides and polyaspartic acid peptides could be carriers of drugs to bone because of their high affinity for HA.

Since it is a \(^{68}\text{Ge}\)/\(^{68}\text{Ga}\) generator-produced radionuclide, an on-site cyclotron is not required. Although I am interested in \(^{68}\text{Ga}\), \(^{67}\text{Ga}\) (\(T_{1/2} = 3.26 \text{ d}\)) was used in these acidic amino acid peptide carrier studies as an alternative radionuclide because of its long half-life. An excellent chelator for radiotheranostics is the DOTA ligand because it forms stable complexes with trivalent metals, such as \(^{67}\text{Ga}\) and \(^{111}\text{In}\) for imaging and \(^{90}\text{Y}\), \(^{177}\text{Lu}\), and \(^{225}\text{Ac}\) for therapy. These studies showed that the binding affinities to HA of \(^{67}\text{Ga}\)-DOTA-Dn increased with increasing length of the aspartate peptide. In biodistribution experiments of normal mice, \(^{67}\text{Ga}\)-DOTA-D6 and \(^{67}\text{Ga}\)-DOTA-D11 (\(n = 11\) or \(14\)) selectively and highly accumulated in bone. These results indicate that not only bisphosphonate molecules but also acidic amino acid peptides are useful as carriers of radiometals to proteins, such as osteopontin and bone sialoprotein, contain many acidic amino acids (aspartic acid (Asp) or glutamic acid (Glu)) in their amino acid sequences, whose offers HA binding ability, and polyglutamic acid peptides and polyaspartic acid peptides could be carriers of drugs to bone because of their high affinity for HA.

Fig. 2. Curves Show Inhibition of Growth of MRMT-1 Tumor Cells on Therapy. Data are expressed as tumor volume relative to that on day of treatment (mean ± S.E.M. for 5–7 rats). Significance was determined using 1-way ANOVA followed by the Dunnett post hoc test (\(p < 0.05\) vs. no treatment). This research was originally published in JNM. Kazuma Ogawa et al. Therapeutic effects of a \(^{186}\text{Re}\)-complex-conjugated bisphosphonate for the palliation of metastatic bone pain in an animal model. J. Nucl. Med.; 48, 122–127, 2007 © SNMMI.

Fig. 3. Structures of Ga-DOTA Complex Conjugated Peptides (A) Ga-DOTA-D2, (B) Ga-DOTA-D5, (C) Ga-DOTA-D8, (D) Ga-DOTA-D11, (E) Ga-DOTA-D14, and (F) Ga-DOTA-D11-c(RGDFK).

Fig. 4. SPECT/CT Images (A, D, G, Axial Images; B, E, H, Sagittal Images; C, F, I, Coronal Images) of Tumor Bearing Mice at 2h after the Intravenous Injection of (A–C) \(^{67}\text{Ga}\)-DOTA-D11, (D–F) \(^{67}\text{Ga}\)-DOTA-c(RGDFK), or (G–I) \(^{67}\text{Ga}\)-DOTA-D11-c(RGDFK).

Arrows indicate the site where tumor cells were injected. Reprinted with permission from Ref. 36. Copyright © 2015 American Chemical Society. (Color figure can be accessed in the online version.)
bone lesions.

Not only clinically used $[^{99m}\text{Tc}]\text{Tc-bisphosphonate}$ complexes but also my synthesized probes can accumulate in metastatic osteoblastic lesions well, however it is difficult to accumulate in metastatic osteolytic lesions, because the bone accumulation mechanism of these probes is based on a high affinity for HA.\textsuperscript{35} To resolve the problem, I designed, synthesized, and evaluated Ga-DOTA-D$_1$-c(RGDfK)\textsuperscript{36} (Fig. 3C), which contains an aspartic acid peptide linker and c(RGDfK), could accumulate in primary tumors, osteoblastic bone metastases, and osteolytic bone metastases simultaneously because the aspartic acid peptide linker enables radioactivity localization in osteoblastic bone metastatic lesions and the arginine-glycine-aspartic acid (RGD) peptide site enables accumulation of radioactivity in primary tumor and osteolytic bone metastatic lesions.\textsuperscript{37–39} As expected, $[^{68}\text{Ga}]\text{Ga-DOTA-D}_1$-c(RGDfK) showed high binding affinity to both HA and $\alpha_\text{v}\beta_3$ integrin \textit{in vitro} and was highly accumulated in both bone and tumor in U87MG tumor-bearing mice (Fig. 4). $[^{68}\text{Ga}]\text{Ga-DOTA-D}_1$-c(RGDfK) could be the first PET probe to enable simultaneous diagnosis of primary tumors, osteoblastic bone metastatic, and osteoelastic bone metastatic lesions. The combination of $[^{68}\text{Ga}]\text{Ga-DOTA-D}_1$-c(RGDfK) and a therapeutic radiometal, such as $^{90}\text{Y}$ and $^{177}\text{Lu}$, labeled DOTATATE-c(RGDfK), is expected to be useful for radiotheranostics.

3. Sigma-1 Receptor Targeted Probes

Sigma receptors were reported as a new subtype of the opioid receptor in 1976;\textsuperscript{40} subsequently, they were reclassified as original receptors with at least two subtypes: sigma-1 and sigma-2.\textsuperscript{41} The sigma-1 receptor comprises 223 amino acids and its molecular size is 25.3 kDa. This receptor is mainly located on the endoplasmic reticulum membrane in the cell and works to maintain cellular homeostasis as a molecular chaperone.\textsuperscript{42,43} Since the sigma-1 receptor is related to functions of the central nervous system, such as signal transduction, memory, recognition, and emotion, determining the expression level of the sigma-1 receptor should be useful for diagnosis of neurodegenerative diseases, such as Alzheimer’s, Parkinson’s diseases, and amyotrophic lateral sclerosis.\textsuperscript{44–46} The sigma-1 receptor is also highly expressed in various cancer cells.\textsuperscript{47} Sigma-1 receptor agonists and antagonists have been candidates as drugs for the sigma-1 related diseases mentioned above.\textsuperscript{48,49} Therefore, imaging using radiolabeled probes to determine the sigma-1 receptor expression could become a companion diagnostic test of therapeutic agents targeting this receptor. In this review, I introduced some radiolabeled sigma-1 receptor targeting probes.

It has been found previously that vesamicol derivatives with high affinity for vesicular acetylcholine transporter also have high affinity for the sigma receptors. Vesamicol analogs containing iodine on the benzene ring were synthesized and evaluated.\textsuperscript{50,51} As a result, (+)-2-[4-(4-iodophenyl)piperidino] cyclohexanol \((+)-p\text{IV}, \text{Fig. 3A}\) showed higher affinity for the sigma-1 receptor than that of \((+)-\text{pentazocine} or \text{haloperidol as sigma-1 receptor ligands.}\textsuperscript{51}

$^{[125]}\text{I}p\text{IV}$ has been widely used clinically as a radionuclide for SPECT. Although I am interested in $^{[225]}\text{I}$ \((t_{\frac{1}{2}} = 9.7 \text{ h})\)-labeled probes for SPECT imaging,\textsuperscript{125} \((t_{\frac{1}{2}} = 59.4 \text{ d})\) was used as an alternative radionuclide because of its long half-life. (++)-$^{[225]}\text{I}p\text{IV}$ was prepared via the iododestannylation reaction and evaluated by using DU-145 tumor-bearing mice. DU-145 is a human prostate cancer cell line overexpressing the sigma-1 receptor. Testing showed that \((+)-[^{225}\text{I}]p\text{IV}\) was highly accumulated in the tumor, and showed high the tumor/blood and tumor/muscle ratios of radioactivity. In blocking experiments, co-administration of an excess amount of a sigma-1 receptor ligand significantly decreased the tumor accumulation of \((+)-[^{225}\text{I}]p\text{IV}\). This finding suggested that cancer accumulation of \((+)-[^{225}\text{I}]p\text{IV}\) was sigma-1 receptor specific.\textsuperscript{52} However, radioactivity accumulation in non-specific tissues, especially the liver, was also high, and the radioactivity was retained.

Thus, the purpose of the next study was to create a radioiodine labeled sigma-1 receptor ligand with better kinetic characteristics. I hypothesized that the high accumulation of \((+)-[^{225}\text{I}]p\text{IV}\) in the liver was derived from its high lipophility.\textsuperscript{53} \((+)-4-[2-(\text{Hydroxy}	ext{cyclohexyl})\text{piperidine-4-yl}]-2\text{-iodophenol ([+]-IV-OH, Fig. 3B]) was designed, synthesized, and evaluated.\textsuperscript{54} Lower lipophility of \((+)-[^{125}\text{I}]\text{IV-OH}\) was confirmed by measuring the 1-octanol/water partition coefficient. The log P value of \((+)-[^{225}\text{I}]\text{IV-OH}\) \((1.13 \pm 0.01)\) was lower than that of \((+)-[^{125}\text{I}]\text{IV}\) \((2.08 \pm 0.02)\). Although \((+)-\text{IV-OH}\) had lower affinity than \((+)-p\text{IV}\) in an \textit{in vitro} binding assay, biodistribution experiments using DU-145 tumor-bearing mice showed that \((+)-[^{125}\text{I}]\text{IV-OH}\) had comparable high uptake at 1h post-injection in tumor. In most tissues, \((+)-[^{125}\text{I}]\text{IV-OH}\) tended to be retained but \((+)-[^{225}\text{I}]\text{IV-OH}\) was cleared. As expected, the radioactivity in the liver after injection of \((+)-[^{125}\text{I}]\text{IV-OH}\) was significantly lower at all-time points relative to radioactivity of \((+)-[^{225}\text{I}]p\text{IV}\).

Bromine-76 \((^{76}\text{Br})\) is also a promising radioisotope because it is a positron emitter for PET, has a relatively long half-life \((t_{\frac{1}{2}} = 16.1 \text{ h})\), and has chemical properties similar to those of iodine. Thus, I prepared and evaluated the radiobromine-labeled vesamicol analogs, \((+)-[^{77}\text{Br}]p\text{BrV} (\text{Fig. 3A})\) and \((+)-[^{79}\text{Br}]\text{BrV-OH} (\text{Fig. 3B})\), by using \(^{77}\text{Br}\) which has a longer half-life \((57.0 \text{ h})\) instead of \(^{79}\text{Br}.\textsuperscript{54})\) These probes showed properties similar to those of the corresponding radioiodine-labeled probes.

Recently, receptor radionuclide therapy for somatostatin receptor-positive tumors has been performed clinically.\textsuperscript{56} I tried to apply the receptor radionuclide therapy to the sigma-1 receptor. $^{[131]}\text{I}$ is a therapeutic radionuclides for clinical use, and $^{[131]}\text{I}NaI has been used for therapy of thyroid cancer patients. Thus, \((+)-[^{131}\text{I}]p\text{IV}\) was prepared and its therapeutic efficacy was evaluated in DU-145-bearing cancer mice. The results showed that \((+)-[^{131}\text{I}]p\text{IV} (7.4 \text{ MBq single administration}) significantly inhibited tumor growth relative to tumor growth in the untreated control group.\textsuperscript{57} \(\alpha\)-Particle emitting radionuclides have gained much attention in radionuclide therapy because they have high linear...
energy transfer. Among alpha-emitting radionuclides, astatine-211 ($^{211}$At) is a candidate for clinical use in the future. $^{211}$At has an appropriate half-life for alpha therapy ($t_{1/2} = 7.2$ h) and emits high energy of α-particles (5.9, 7.4 MeV). Astatine has no stable isotopes. Even $^{210}$At, which has the longest life among astatine isotopes, has a half-life of 8.1 h. For this reason, the properties of astatine have not yet been fully characterized. However, because astatine is a halogen, it exhibits chemical properties similar to those of iodine. Thus, $^{211}$At might not be introduced into the tyrosine residue of c(RGDyK) via the chloramine-T method. As a result of biodistribution experiments in DU-145 tumor-bearing mice by the double tracer method using (+)-$^{211}$At-pAtV and (+)-$^{125}$I-pIV in DU-145 tumor-bearing mice showed biodistributions very similar to that of (+)-$^{125}$I-pIV. Tumor accumulations of both (+)-$^{211}$At-pAtV and (+)-$^{125}$I-pIV were significantly inhibited by co-injection of an excess amount of sigma-1 ligand, SAA4503 (10 µmol/kg).

These results indicate that radiotheranostics via coupling (+)-$^{125}$I-pIV for SPECT, (+)-$^{77}$Br-pIV for PET, and (+)-$^{131}$I-pIV and (+)-$^{211}$At-pAtV for radionuclide therapy could be useful. Moreover, further studies for development of radiohalogen labeled sigma-1 receptor ligands are now on going.58)

4. RGD Peptide

RGD peptides possess affinity for $\alpha_\beta_3$ integrin,59) a cell-adhesion molecule representative subtype present in heterodimeric transmembrane receptors that regulates angiogenesis.60) Since $\alpha_\beta_3$ integrin is overexpressed on angiogenic endothelial cells61) and some types of cancer cells,62) RGD peptides are used as carriers of RI to $\alpha_\beta_3$ integrin in tumors.63) Radiolabeled RGD peptides have been investigated for not only imaging and determination of $\alpha_\beta_3$ integrin expression but also for radionuclide therapy.64–67) However, $^{211}$At-labeled RGD peptides have never to date been reported. Thus, I aimed in this study to determine their radiotheranostics potential by coupling $^{211}$At-labeled and $^{125}$I-labeled RGD peptides.

For radioiodine labeling of RGD peptides, the simplest and most effective method is to introduce a radioiodine at the 3-position on the tyrosine residue in c(RGDfK) via the chloramine-T method, which typically results in high radiochemical yields.68) Although direct labeling of antibodies with radioiodine is also effective via the chloramine-T method,69) direct labeling of antibodies with $^{211}$At has been shown to be impractical.70) Thus, $^{211}$At might not be introduced into the tyrosine residue of c(RGDfK) via the normal chloramine-T method. As methods for $^{211}$At-labeling of antibodies, both N-succinimidyl $^{[211]}$At[astatobenzoate ($^{[211]}$At[SAB]-conjugated antibodies and N-succinimidyl 3-(tri-n-butylstannyl)benzoate (ATE)-conjugated antibodies followed by $^{211}$At labeling via the astatodestannylation reaction have been reported.71,72) These methods, namely $^{211}$At labeling via conjugation of $^{[211]}$AtSAB or ATE with the ε-amino group of the lysine residue or the N-terminus of amino acid sequences, are applicable to both proteins and peptides. Since the same strategy is applicable to RGD peptides, I tried to synthesize $^{125}$I- and $^{77}$Br-labeled RGD peptides via conjugation of N-succinimidyl $^{[125]}$Iidobenzoate ($^{[125]}$I[SIB]) or N-succinimidyl $^{[77]}$Brbromobenzoate ($^{[77]}$Br[SBrB]) with the ε-amino group of the lysine residue in the c(RGDfK) pep-
tide as a preliminary study of $^{211}$At-labeled RGD peptide.\(^{68}\) Radiolabeling with both $^{125}$I and $^{77}$Br was successful, but the biodistribution of the labeled RGD peptides was not favorable owing to low tumor uptake and high uptake in the intestine, which can be due to the increased lipophilicity of these peptides. The molecular sizes of peptides are much smaller than those of antibodies, so the biodistribution of labeled RGD peptides must be drastically affected by introduction of molecules for radiolabeling. Next, labeled RGD peptides containing a hydrophilic linker between the c(RGDfK) peptide and a radiolabeled site were prepared to decrease the lipophilicity of the labeled RGD peptides. Although these labeled RGD peptides containing a hydrophilic linker improved their biodistribution, the improvement was not sufficient. Therefore, I assumed that another strategy for preparing $^{211}$At-labeled RGD peptides would be necessary. As a new method for $^{211}$At-labeling of RGD peptides, I tried to introduce a tributylstannyl group (for subsequent halogen labeling) into the phenylalanine residue of the RGD peptide. Actually, an $^{211}$At-labeled RGD peptide, $^{[211]}$At[c(RGDf(4-At)K)], was prepared according to Chart 1.\(^{73}\) The $^{211}$At- and $^{125}$I-labeled RGD peptides, $^{[211]}$At[c(RGDf(4-I)K)] and $^{[125]}$I[c(RGDf(4-I)K)], showed very similar biodistributions based on their high stability in vivo and their high affinity for $\alpha_v\beta_3$ integrin. This result shows that their use in a radiotheranostics system is possible. Specifically, $^{[211]}$At-c[RGDf(4-At)K] SPECT imaging can predict the therapeutic effects and side effects of $^{[211]}$At-c[RGDf(4-A0)K] $\alpha$-targeted therapy as a radiotheranostics system.

5. Conclusion

The potential of radiotheranostics has been receiving increased attention and has been successfully used in nuclear medicine and clinical oncology. Further development is anticipated, and it is hoped that my research findings will contribute to future progress in radiotheranostics.

Conflict of Interest  The author declares no conflict of interest.

References

41. Quirion R., Bowen W. D., Itzhak Y., Junien J. L., Musacchio J. M., Rothman B. R., Su T. P., Tam S. W., Taylor D. P., Trends Pharma-