Int J Clin Exp Med 2017;10(2):2967-2973
www.ijcem.com /ISSN:1940-5901/IJCEM0038398

Original Article

Radiation dosimetry and bio-distribution of ^{99m}Tc-rgd-bombesin peptide in breast cancer patients

Qianqian Chen, Jie Chen, Shi Gao, Bin Chen, TiefengJi*, Qingjie Ma*

*Department of Nuclear Medicine, China-Japan Union Hospital of Jilin University, Changchun, China. *Equal contributors.*

Received August 22, 2016; Accepted November 14, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: ^{99m}Tc-Glu-c(RGDyK)-bombesin (^{99m}Tc-RGD-bombesin) was a novel radiopharmaceutical for cancer imaging. This study was to report the human data of ^{99m}Tc-RGD-bombesin in breast cancer patients. Six breast cancer patients were examined and the safety was assessed. Whole-body scans were acquired at 10, 30, 60, 120 and 1440 min post-injection. After injection, blood samples were collected at 1, 3, 5, 10, 15, 30, 60 and 120 min time point, respectively. Urine samples were obtained at thetime duration of 0-2, 2-4, 4-8, 8-12 and 12-24 h post injection, respectively. Regions of interest (ROI) were delineated for various source organs and the tumor. Tumor-background (T/B) ratios of all imaging time points were calculated. The OLINDA/EXM software was used to estimate the equivalent organ doses and the effective dose. No serious adverse events were reported during the study. ^{99m}Tc-RGD-bombesin showed rapid clearance from the blood and continuous increasing in urine. Bladder and kidney demonstrated predominant uptake, whilst uptake in heart and brain was low. The primary tumor was well visualized and the ideal time point was 120 min with highest T/B ratio. The highest absorbed radiation dose was in the kidneys (2.43×10^{-2} mGy/MBq) and the effective dose was 4.51×10^{-3} mSv/MBq. As a diagnostic breast cancer imaging agent, ^{99m}Tc-RGD-bombesin appears safe with acceptable dosimetric properties and bio-distribution.

Keywords: Radiation dosimetry, biodistribution, RGD, bombesin, breast cancer, ROI, T/B ratio, equivalent organ dose, effective dose, agent

Introduction

It is estimated that in 2016 there will be 246,660 new cases of breast cancer diagnosed and that 40,890 women will die of the disease [1]. Early diagnosis of breast cancer may lead to a higher 5-y survival rate [2, 3]. However, once breast cancer is locally invasive, lymphatic spread or distant metastasis, the survival rate fall dramatically.

Current diagnostic imaging modalities, such as X-ray mammography (XMM), ultrasonography (US) and magnetic resonance imaging (MRI), ^{99m}Tc-MIBI imaging, are suboptimal for early detection because of poor sensitivity and specificity. New molecular imaging approaches to assess the status of the disease more accurately may facilitate the early detection of breast cancer and improve the outcomes of the patients.

Integrin $\alpha_v\beta_3$ plays an important role in the regulation of tumor growth, angiogenesis, local invasiveness, and metastatic potential [4-6]. Integrin $\alpha_v\beta_3$ is up-regulated in the activated tumor endothelial cells and also highly expresseson various tumor cells such as lung, breast and esophageal cancers [7, 8]. Radiolabeled RGD (Arg-Gly-Asp) peptides and analogs that specifically target integrin $\alpha_v\beta_3$ have been wildly tested for tumor imaging in pre-clinical and clinical studies.

Bombesin is a gastrin-releasing peptide receptor (GRPR) analogue that is overexpressed on several human tumors including breast, prostate, lung, stomach, exocrine pancreatic, and colon. Bombesin-like peptides can be labeled various radioisotopes for cancer imaging [9, 10].

Recently, a dual integrin $\alpha_v\beta_3$ and GRPR targeted peptide Glu-c(RGDyK)-bombesin (RGD-bombesin) that contained dual RGD and bombesin motifs in one molecule was designed, synthesized and labeled with ^{99m}Tc in our labo-

Table 1. Patients' baseline characteristics and ^{99m}Tc-RGD-bombesin dose data

Patient no.	Diagnosis	Age (y)	Weight (kg)	Injected activity (MBq)
1	Invasive ductal carcinoma	43	72.1	810.6
2	Invasive ductal carcinoma	36	49.2	566.1
3	Invasive ductal carcinoma	53	57.4	627.1
4	Invasive ductal carcinoma	45	65.9	755.5
5	Invasive lobular carcinoma	55	60.7	677.3
6	Invasive ductal carcinoma	67	55.3	620.8
Mean ± SD		49.8 ± 10.9	60.1 ± 8.1	676.3 ± 91.6

ratory. Preclinical work evaluating the feasibility of ^{99m}Tc-RGD-bombesin imaging in tumor-bearing rodents has been previously published [11-14]. Tumor-to-background (T/B) ratios of ^{99m}Tc-RGD-bombesin were high in various tumors including breast cancer [15, 16]. Especially last year, we had an important discovery, that is, ^{99m}Tc-RGD-bombesin SPECT can reduce unnecessary biopsy of BI-RADS category 4, which had been published in the most authoritative journal of nuclear medicine [17]. This discovery made us see a dawn for the ability of changing clinical strategy of ^{99m}Tc-RGD-bombesin SPECT. Therefore, we must know the radiation dosimetry and biodistribution of ^{99m}Tc-RGD-bombesin in breast cancer patientsvery well, in order to lay foundation for the future clinical application.

Materials and methods

The protocol was approved by the institutional review board and independent ethic committees of China-Japan Union Hospital, Changchun, China. Written informed consent was obtained from all patients.

Radiopharmaceutical preparation

The RGD-bombesin freeze-dried form was generously provided by Medical isotopes research center of Peking University. $Na^{99m}TcO_4$ was obtained from a commercial $^{99}Mo/^{99m}Tc$ generator (Beijing Atom High Tech Co., Ltd.). The kit for preparation of ^{99m}Tc-RGD-bombesin was formulated by containing per mL, 20 μg of HYNIC-RGD- bombesin, 6 mg of TPPTS, 10 mg of tricine, 38.5 mg of disodium succinate hexahydrate, and 12.7 mg of succinic acid. 1-1.5 mL of $Na^{99m}TcO_4$ solution (1,110-1,850 MBq) in saline was added into each kit vial followed by 20-25 minutes incubation at 100°C. Then the vial was placed back into the lead pig and allowed to stand at room temperature.

A quality control by radioactive instant thin-layer chromatography (ra-dio-ITLC) and radioactive high-performance liquid chromatography (radio-HPLC) were performed before injection of the agent. A radio-chemical purity (RCP) of greater than 95% was required in this study.

Subjects

Six female patients with a pathologic diagnosis of breast cancer were included in the study (**Table 1**). There was no tumor stage restriction. None of the patients had received surgery or other treatment prior to evaluation for study participation. Exclusion criteria consisted of pregnancy, lactation, a history of renal insufficiency or hepatic insufficiency, and any other primary cancer.

Safety and sample collection

The ^{99m}Tc-RGD-bombesin was injected into the antecubital vein with a rapid bolus, followed by a 10 mL saline flush. Measurements of vital signs (body temperature, systolic and diastolic blood pressure and pulse rate), laboratory safety tests (renal and liver function chemistry, hematology, and blood coagulation parameters) and 12-lead electrocardiogram were recorded before and after tracer injection.

Blood samples were collected via an indwelling catheter at 1, 3, 5, 10, 15, 30, 60 and 120 min post-injection. Urine was collected and pooled at time intervals of 0-2, 2-4, 4-8, 8-12 and 12-24 h after dosing. Blood and urine samples were weighed and counted in a γ-counter (Wallac 1470-002, Perkin Elmer, Finland). Decay corrected time-activity curve was expressed as percentage of injected dose per gram (% ID/g).

SPECT procedure

Whole-body scans were performed via a SPECT scanner (Philips Healthcare) equipped with parallel-hole, low-energy and high-resolu-

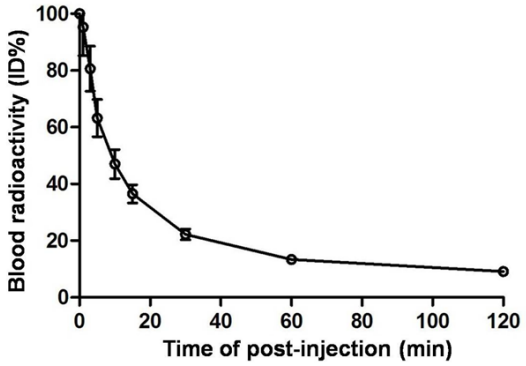

Figure 1. Averaged time-activity curve of ^{99m}Tc-RGD-bombesin in blood for all patients.

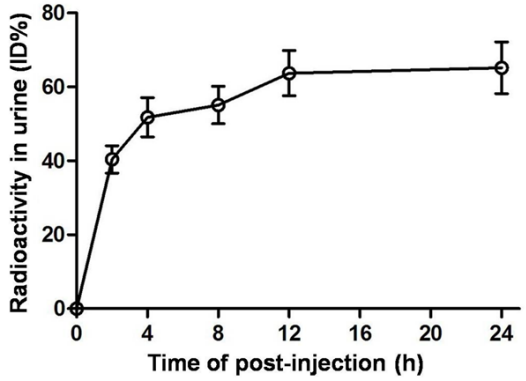

Figure 2. Averaged time-activity curve of ^{99m}Tc-RGD-bombesin in urine for all patients.

tion collimators. All images were acquired using ^{99m}Tc with a 20% energy window centered on 140 keV. Both anterior and posterior images were acquired at 10 min, 30 min, 1 h, 2 h and 4 h post-injection and were stored digitally in a 256×256 matrix. The velocity of scanning was 15 cm/min.

Biodistribution and dosimetry estimation

Visual analysis was applied to determine the integral biodistribution of the tracer and transient stability. Regions of interest (ROIs) were delineated over the identified organs including: brain, lung, heart, liver, kidneys, spleen, intestine, urinary bladder, and a background region near the body on the anterior image. The mirror ROIs were applied to the posterior images of each organ. The mean counts of each organ including anterior and posterior images were measured. In addition, a ROI was manually defined around the tumor, and then a duplicate in the contralateral normal breast was generated to serve as the background ROI. T/B ratios of all imaging time points were calculated.

Estimated human dosimetry was calculated from the biodistribution data on volunteers after administration of ^{99m}Tc-RGD-bombesin (av-erage injected activity 676.3 MBq). The number of disintegrations that occur in the source region per unit activity administered was calculated from the time-activity curves without decay correction. The radiation-absorbed doses in humans (mGy/MBq) were projected on the basis of the organ and the whole-body residence times and estimated by the use of the Organ Level Internal Dose Assessment/Exponential Modeling computer software (OLINDA/EXM, Vanderbilt, University, Nashville, TN).

Results

Safety

No serious adverse events or abnormal clinical chemistry were reported by the patients during the study.

Pharmacokinetics and excretion

An average blood time-activity curve was calculated from the blood sample data of all six patients (**Figure 1**). ^{99m}Tc-RGD-bombesin was rapidly cleared from the blood, dropping at 13.4 ± 1.2% of the initial dosage at 60 min respectively after injection.

The concentration of radioactivity in urine was shown in **Figure 2**. The radioactivities in urine kept increasing and a total cumulative recovery of 65.2 ± 7.0% of the original dose was present in the urine at 24 h.

Biodistribution

Figure 3 shows the typical biodistribution of ^{99m}Tc-RGD-bombesin at 10 min, 30 min, 60 min, 120 min, 240 min and 1440 min post-injection. Predominant uptake of ^{99m}Tc-RGD-bombesinwas visualized primarily in the bladder and kidneys. Moderate uptake was also seen secondarily in the liver and gastrointestinal tract. Low uptake was seen in the heart and brain. Apparent uptake was visualized in nasal cavity, salivary glands and the thyroid on

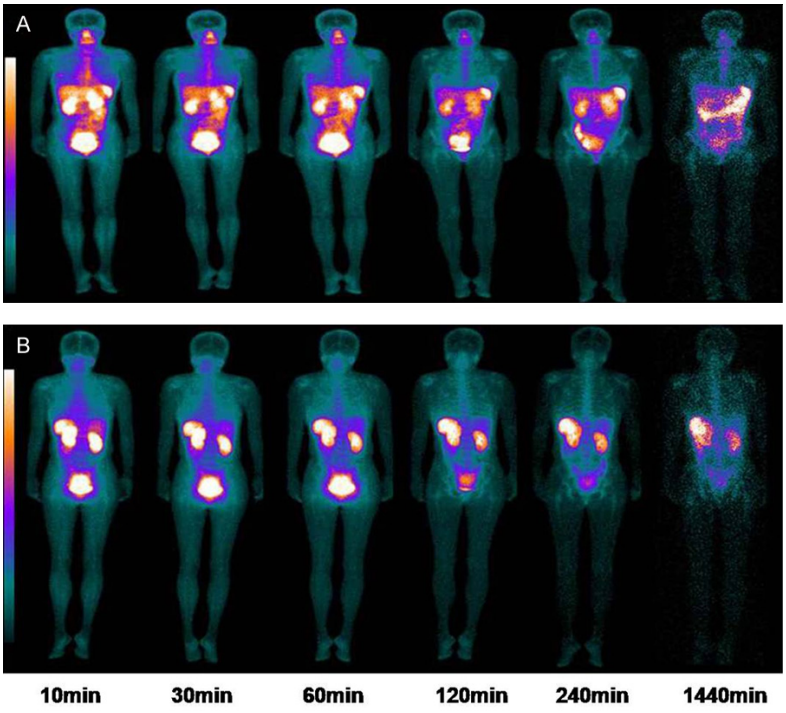

Figure 3. Whole-body planar images of ^{99m}Tc-RGD-bombesin (A: ant and B: post) at different times after injection showing biodistribution in a breast cancer patient.

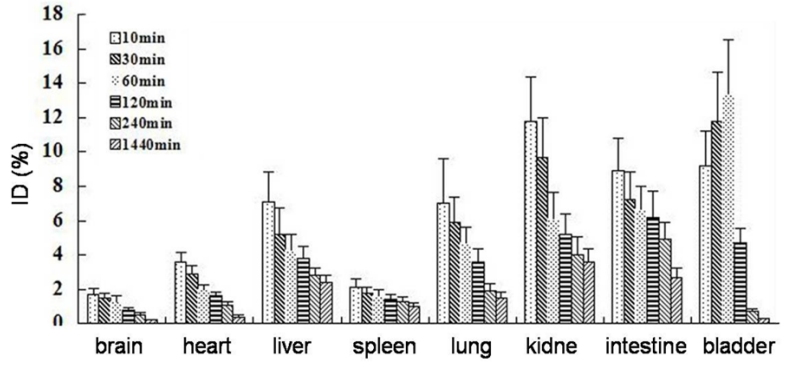

Figure 4. The quantified analysis of ^{99m}Tc-RGD-bombesin in major organs of patients calculated from the whole-body images obtained at 10 min, 30 min, 60 min, 120 min, 240 min and 1440 min after administration.

the early time points and almost undetectable at 4 h after injection.

The quantitative tracer uptakes in major organs were presented in **Figure 4**. Percentage ID was the highest in bladder which ascended from 9.2 ± 2.0% ID/organ (10 min p.i.) to 13.4 ± 3.1% ID/organ (60 min p.i.), followed by the kidneys, intestines and liver, which declined over time (10 min p.i. to 60 min p.i, 11.8 ± 2.6% to 6.1 ± 1.5% ID/organ, 8.9 ± 1.9% to 6.7% ± 1.3% ID/organ, 7.1 ± 1.7% to 4.3 ± 0.9% ID/organ, respectively). Low activities were remained in the heart, spleen and brain till 60 min after injection (2.0 ± 0.3%, 1.6 ± 0.4% and 1.3 ± 0.3% ID/organ, respectively).

Tumor uptake

^{99m}Tc-RGD-bombesinuptake was well visualized in tumors. As the blood and soft-tissue background cleared, the mean T/B ratio increased gradually at the initial 120 min (**Table 2**). The ideal time point was 120 min with the highest T/B ratio.

Radiation dosimetry

A summary of dosimetric parameters for various organs and whole body was given in **Table 3**. The mean effective dose was 0.0045 mSv/MBq. For a 740 MBq administered dose, the mean effective dose was about 3.33 mSv. The critical organ was the kidneys (2.43-E-02 mGy/MBq). The brain, skin and breast showed the relative low radiation-absorbed dose (7.90E-04, 8.52E-04 and 1.06E-03 mGy/MBq, respectively).

Discussion

The RGD-bombesin peptides can target integrin $\alpha_v\beta_3$ and GRPR which are the important roles during the process of tumor growth [18-21]. ^{99m}Tc-RGD-bombesin has been verified as a dual intergrin $\alpha_v\beta_3$ and GRPR selective tracer in previous study [18]. However, the associated radiation burden in patients has not been studied. In this paper, we described the biodistribution and dosimetry of ^{99m}Tc-RGD-bombesinin breast cancer patients for the first time.

In this study, the absence of adverse effects on vital signs after intravenous injection dem-

Table 2. ^{99m}Tc-RGD-bombesin T/B ratios at different times

Patients	Time					
	10 min	30 min	60 min	120 min	240 min	1440 min
1	1.68	1.76	1.83	2.14	1.94	1.03
2	1.62	1.81	3.01	3.34	2.66	1.1
3	2.37	2.69	3.08	3.77	2.36	1.08
4	1.55	1.79	1.97	2.33	2.03	1.03
5	2.19	2.36	3.88	4.22	3.17	1.01
6	1.57	1.95	2.21	2.49	1.97	1.01
Mean ± SD	1.83 ± 0.36	2.06 ± 0.38	2.66 ± 0.79	3.05 ± 0.85	2.36 ± 0.49	1.04 ± 0.04

Table 3. Radiation Dosimetry (n=6)

Target organ	mGy/MBq (SD)
Adrenal glands	3.71E-03 (0.97E-03)
Brain	7.90E-04 (2.07E-04)
Breasts	1.06E-03 (0.21E-03)
Gallbladder wall	5.03E-03 (1.20E-03)
Lower region of colon	8.26E-03 (0.28E-03)
Small intestine	3.41E-03 (0.67E-03)
Stomach wall	2.75E-03 (0.48E-03)
Upper colon	2.82E-03 (0.41E-03)
Heart wall	2.37E-03 (0.09E-03)
Kidneys	2.43E-02 (0.70E-02)
Liver	4.47E-03 (1.02E-03)
Lungs	2.80E-03 (0.81E-03)
Muscle	1.70E-03 (0.32E-03)
Ovaries	3.92E-03 (0.18E-03)
Pancreas	9.80E-03 (1.41E-03)
Red marrow	2.02E-03 (0.30E-03)
Osteogenic cells	3.64E-03 (0.72E-03)
Skin	8.52E-04 (2.31E-04)
Spleen	6.93E-03 (0.81E-03)
Thymus	1.04E-03 (0.28E-03)
Thyroid gland	9.96E-03 (0.76E-03)
Urinary bladder wall	1.45E-02 (0.55E-02)
Uterus	3.01E-03 (0.12E-03)
Whole body	2.28E-03 (0.59E-03)
Effective dose equivalent	6.40E-03 (0.21E-03)
Effective dose (mSv/MBq)	4.51E-03 (0.87E-03)

onstrated the ^{99m}Tc-RGD-bombesin is feasible and safe.

According to the pharmacokinetic data, there was a very sharp decline of radioactivity in the blood and continuous accumulation in the urine. The biodistribution data showed the primary route of clearance is renal, with high tracer accumulation in the kidneys and especially in the bladder. Due to the high activity in the urogenital tract and the moderate activity in abdomen organs, imaging quality in the abdomen and pelvis may be impaired and blurry. On the other hand, low accumulation of ^{99m}Tc-RGD-bombesin in background tissues, especially brain, heart, lungs and breast, would be an advantage for high-quality images and reliable quantification in these regions. In our study, the breast cancers were well visualized with increasing T/B ratios over time and attaching the maximum at 120 min after tracer injection. It verified the accumulation of main organs in chest had little influence on tumor imaging in this region and ^{99m}Tc-RGD-bombesin could facilitate the detection of breast cancer with significantly improved contrast.

In this study, imaging of breast cancer with 740 MBq of ^{99m}Tc-RGD-bombesin appeared to be feasible and safe. The effective dose equivalent for a single administration of a diagnostic dose was 3.33 mSv, which was lower than the radiation dose limit value set forth by the 2007 International Commission on Radiological Protection [22-24].

In conclusion, the novel radiopharmaceutical ^{99m}Tc-RGD-bombesin appears to be safe and have acceptable dosimetric and biodistribution properties as a diagnostic breast cancer imaging agent. This conclusion is based on the absence of adverse effects, a satisfactory biodistribution, acceptable dosimetry, and tumor uptake obvious higher than that in normal tissue. Larger studies are planned to further evaluate the diagnostic potential of this agent.

Acknowledgements

This research was supported by National Natural Science Foundation of China (NSFC)

projects (Grant number: 81271606), Norman Bethune Program of Jilin University (Grant number: [2015302], the Research Fund of Science and Technology Department of Jilin Province (Grant number: 20150520154JH), the Foundation of National Health and Family Planning Commission of Jilin Province (Grant number: 2015Q020) and the Hygiene Specific Subjects of Jilin Province (Grant number: SCZSY201508), the Department of Education of Jilin Province for Thirteen-Five Scientific Technique Rsearch (Grant number: [2016] 460 and 461).

Disclosure of conflict of interest

None.

Address correspondence to: Tiefeng Ji and Qingjie Ma, China-Japan Union Hospital of Jilin University, NO. 126 Xiantai Street, Economic and Technological Development Zone, Changchun, China. E-mail: jitiefeng460@163.com (TFJ); maqingjiejlu@163.com (QJM)

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[2] Huang ML, Rose S and Yang WT. Breast cancer screening: meeting the challenges of today and exploring the technologies of tomorrow. Semin Roentgenol 2015; 50: 88-100.

[3] Buscombe JR, Cwikla JB, Holloway B and Hilson AJ. Prediction of the usefulness of combined mammography and scintimammography in suspected primary breast cancer using ROC curves. J Nucl Med 2001; 42: 3-8.

[4] Niu G and Chen X. Why integrin as a primary target for imaging and therapy. Theranostics 2011; 1: 30-47.

[5] Cairns RA, Khokha R and Hill RP. Molecular mechanisms of tumor invasion and metastasis: an integrated view. Curr Mol Med 2003; 3: 659-671.

[6] Felding-Habermann B. Integrin adhesion receptors in tumor metastasis. Clin Exp Metastasis 2003; 20: 203-213.

[7] Ma Q, Ji B, Jia B, Gao S, Ji T, Wang X, Han Z and Zhao G. Differential diagnosis of solitary pulmonary nodules using (9)(9)mTc-3P(4)-RGD(2) scintigraphy. Eur J Nucl Med Mol Imaging 2011; 38: 2145-2152.

[8] Liu L, Song Y, Gao S, Ji T, Zhang H, Ji B, Chen B, Jia B, Wang F, Xu Z and Ma Q. (99)mTc-3PRGD2 scintimammography in palpable and nonpalpable breast lesions. Mol Imaging 2014; 13.

[9] Dimitrakopoulou-Strauss A, Hohenberger P, Haberkorn U, Macke HR, Eisenhut M and Strauss LG. 68Ga-labeled bombesin studies in patients with gastrointestinal stromal tumors: comparison with 18F-FDG. J Nucl Med 2007; 48: 1245-1250.

[10] Van de Wiele C, Phonteyne P, Pauwels P, Goethals I, Van den Broecke R, Cocquyt V and Dierckx RA. Gastrin-releasing peptide receptor imaging in human breast carcinoma versus immunohistochemistry. J Nucl Med 2008; 49: 260-264.

[11] Liu Z, Yan Y, Chin FT, Wang F and Chen X. Dual integrin and gastrin-releasing peptide receptor targeted tumor imaging using 18F-labeled PEGylated RGD-bombesin heterodimer 18F-FB-PEG3-Glu-RGD-BBN. J Med Chem 2009; 52: 425-432.

[12] Liu Z, Niu G, Wang F and Chen X. (68)Ga-labeled NOTA-RGD-BBN peptide for dual integrin and GRPR-targeted tumor imaging. Eur J Nucl Med Mol Imaging 2009; 36: 1483-1494.

[13] Liu Z, Li ZB, Cao Q, Liu S, Wang F and Chen X. Small-animal PET of tumors with (64)Cu-labeled RGD-bombesin heterodimer. J Nucl Med 2009; 50: 1168-1177.

[14] Liu Z and Wang F. Dual-targeted molecular probes for cancer imaging. Curr Pharm Biotechnol 2010; 11: 610-619.

[15] Chen Q, Ma Q, Chen M, Chen B, Wen Q, Jia B, Wang F, Sun B and Gao S. An exploratory study on ^{99m}Tc-RGD-BBN peptide scintimammography in the assessment of breast malignant lesions compared to ^{99m}Tc-3P4-RGD2. PLoS One 2015; 10: e0123401.

[16] Liu Z, Yan Y, Liu S, Wang F and Chen X. (18)F, (64)Cu, and (68)Ga labeled RGD-bombesin heterodimeric peptides for PET imaging of breast cancer. Bioconjug Chem 2009; 20: 1016-1025.

[17] Ji T, Gao S, Liu Z, Xing H, Zhao G, Ma Q. ^{99m}Tc-Glu-c(RGDyK)-bombesin SPECT Can Reduce Unnecessary Biopsy of BI-RADS Category 4 On Ultrasonography. J Nucl Med 2016; 57: 1196-1200.

[18] Cui L, Liu Z, Jin X, Jia B, Li F and Wang F. Evaluation of 188Re-MAG2-RGD-bombesin for potential prostate cancer therapy. Nucl Med Biol 2013; 40: 182-189.

[19] Liu Z, Huang J, Dong C, Cui L, Jin X, Jia B, Zhu Z, Li F and Wang F. ^{99m}Tc-labeled RGD-BBN peptide for small-animal SPECT/CT of lung carcinoma. Mol Pharm 2012; 9: 1409-1417.

[20] Yan Y, Chen K, Yang M, Sun X, Liu S and Chen X. A new 18F-labeled BBN-RGD peptide heterodimer with a symmetric linker for prostate cancer imaging. Amino Acids 2011; 41: 439-447.

[21] Jiang L, Miao Z, Liu H, Ren G, Bao A, Cutler CS, Shi H and Cheng Z. 177Lu-labeled RGD-BBN heterodimeric peptide for targeting prostate

carcinoma. Nucl Med Commun 2013; 34: 909-914.
[22] Boetticher H, Lachmund J, Looe HK, Hoffmann W and Poppe B. 2007 recommendations of the ICRP change basis for estimation of the effective dose: what is the impact on radiation dose assessment of patient and personnel? Rofo 2008; 180: 391-395.
[23] Kehoe T. International Commission on Radiological Protection publications 97 and 98: radiation protection can be fun! Clin Oncol (R Coll Radiol) 2007; 19: 539-541.
[24] Pradhan AS. Evolution of dosimetric quantities of International Commission on Radiological Protection (ICRP): Impact of the forthcoming recommendations. J Med Phys 2007; 32: 89-91.