Original Article

Human Dose Assessment of ^{68}Ga-NODAGA-RGD-BBN Heterodimer Peptide based on Animal Data

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Abstract

Aims: Calculation of the absorbed dose in human organs is one of the first steps for developing new radiopharmaceuticals. The aim of this study is to estimate the human absorbed dose of a newly developed ^{68}Ga-NODAGA-RGD-BBN radiolabeled compound. **Materials and Methods:** ^{68}Ga-NODAGA-RGD-BBN was prepared by varying different parameters at optimized conditions. The stability of the radiolabeled peptide in phosphate-buffered saline (PBS) and in human serum was evaluated for 120 min. Afterward, the biodistribution of the complex was assessed in normal and tumor-bearing mice, at least for 120 min postinjection. Finally, the human absorbed dose of ^{68}Ga-NODAGA-RGD-BBN was estimated based on mice data using Radiation Dose Assessment Resource and Spark method. **Results:** ^{68}Ga-NODAGA-RGD-BBN was produced with radiochemical purity of more than 98% (high-performance liquid chromatography/radio thin layer chromatography (RTLC)) with high stability in PBS buffer and in human serum at least for 2 h. The complex demonstrated high uptake in gastrin-releasing peptide receptor-expressing tumors compared to other nontarget organs. Furthermore, the dose assessment for the complex showed that the kidneys receive the highest absorbed dose in comparison with other organs. **Conclusion:** The result of this study showed that 68Ga-NODAGA-RGD-BBN is an effective and radiolabeled ligand for tumor detection, however more studies are still needed.

Keywords: ^{68}Ga, effective dose, NODAGA-RGD-BBN, positron emotion tomography scan, Radiation Dose Assessment Resource

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Introduction

The early diagnosis of prostate cancer, one of the most common types of cancer in men, is an important issue for the management of the disease.[1] The arginylglycylaspartic acid (RGD) is one of the most prevalent peptides in the human body with three amino acids which can target integrin proteins as one of the most key factors for regulating angiogenesis and tumor proliferation.[2-4] Bombesin (BBN) is another peptide in the body that consists of 14 amino acids, which target gastrin receptors.[5]

In developing new diagnostic and therapeutic radiolabeled agents, RGD and bombesin (BBN) showed excellent properties in labeling with diagnostic and therapeutic radionuclides.[6,7] This is due to various human cancers such as breast, prostate, glioma, pancreas overexpressed integrins, and gastrin receptors.[8,9] RGD-BBN heterodimer peptide can simultaneously target integrins and gastrin receptors. Besides, recent studies have shown that cancers can be diagnosed more effectively with peptide heterodimers compared with monomeric components alone.[10]

Nowadays, the positron emotion tomography (PET) method has many advantages such as detecting biochemical processes and expression of some proteins; therefore, PET can provide us with much molecular-level information just before any anatomic changes could be detectable.

Among the PET radioisotopes, ^{68}Ga has special physical and chemical characteristics. This radioisotope emits positron particles (89% branching ratio) with a physical half-life of 68 min. It also emits gamma radiations (511 and 1077 keV). ^{68}Ga availability in the form of ^{68}Ge/^{68}Ga generator makes it an excellent candidate for the development of new PET

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radiolabeled compounds. Recently, numerous ^{68}Ga-labeled pharmaceuticals have been developed and employed in preclinical and limited clinical trials.[11,12]

Radiation-absorbed dose quantity (energy deposited in the unit mass of organ) which is expressed in Gy (J/kg), should be considered not only for evaluating the risks associated with the administration of radiopharmaceuticals but also for the acceptable maximum amount of administrated activity.[13] For this purpose, medical internal radiation-absorbed dose as a basic and well-known method was considered for absorbed dose calculation. However, nowadays some resources such as Radiation Dose Assessment Resource (RADAR) are available for this purpose.[14]

In this study, ^{68}Ga-NODAGA-RGD-BBN was developed as a new agent for PET imaging. The human absorbed dose due to the radiolabeled heterodimer peptide was estimated based on biodistribution studies in mice by the RADAR method. For this purpose, a radiolabeled complex was prepared with high radiochemical purity at optimized conditions. The biodistribution of the radiolabeled compound was studied in normal and tumor-bearing mice at different time intervals postinjection. Finally, the human absorbed dose of ^{68}Ga-NODAGA-RGD-BBN was estimated based on the mice data.

Materials and Methods

The ^{68}Ge/^{68}Ga generator with the nominal activity of 1480 MBq was obtained from Pars Isotope Co. (Tehran, Iran). NODAGA-RGD-BBN heterodimer peptide with a purity of more than 99% was prepared by the International Atomic Energy Agency (IAEA) (Vienna, Austria) through F22067 Coordinated Research Project. To determine the biodistribution of radiolabeled complex, normal and tumor-bearing nude mice were also prepared from Royan Institute (Tehran, Iran). All chemicals were purchased from Sigma-Aldrich Co. (Germany) and were used without further purification. Radiochemical purity was checked by high-performance liquid chromatography (HPLC) (Knauer Co., Germany) equipped with an octadecylsilane column, ultraviolet, and gamma detectors. Radionuclide purity and activities were measured by an NIGC 4020 high-purity germanium (HPGe) detector (DSG Co., Germany) equipped with a multichannel analyzer (MCA). Chemical purity was studied by the inductively coupled plasma mass spectrometry (ICP-MS) technique. In the following, PET images were acquired with a dual-head single-photon emission computed tomography (SPECT) system (DST-XL, France). All animals were kept on a routine day/night standard diet.

Preparation and quality control of $^{68}GaCl_3$

^{68}Ga was prepared in the form of $^{68}GaCl_3$ from a 1480 MBq ^{68}Ge/^{68}Ga generator. The radiochemical purity of the eluted ^{68}Ga was studied using the instant thin-layer chromatography method by means of 10% ammonium acetate: methanol on silica gel sheets and in 10 mM diethylenetriaminepentaacetic acid (DTPA) solution (pH ~4) as a solvent and Whatman No. 2 paper as a stationary phase in accordance with the reported procedure.[15] Radionuclide purity of the elution was also assessed by HPGe detector. The chemical purity was investigated by the inductively coupled plasma (ICP-OES) method.

Preparation and quality control of ^{68}Ga-NODAGA-RGD-BBN

At first, NODAGA-RGD-BBN (5 mg) was dissolved in ultrapure water (5 mL) to prepare a stock solution of the peptide (1 mg/mL). Then, ^{68}Ga-NODAGA-RGD-BBN was prepared at optimized conditions by adding 1295 MBq $^{68}GaCl_3$ (2 mL) to the vial containing 90 mg of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 20 µg NODAGA-RGD-BBN while the reaction was continued at 95°C for 10 min. The final solution was purified by C18 Sep-Pak (waters) column preconditioned with 5 mL ethanol and 10 mL water, respectively. Finally, the radiochemical purity of the final solution was checked by both HPLC and RTLC (1 M ammonium acetate: methanol with volume ratio 1:1, and silica gel paper) methods.

Stability studies

The final radiolabeled peptide (about 18.5 MBq) was added to the phosphate-buffered saline (PBS) buffer and freshly prepared human serum while kept at 4°C and 37°C, respectively. The radiochemical purity of ^{68}Ga-NODAGA-RGD-BBN was determined using RTLC and HPLC methods, at least for 2 h.

Biodistribution of radiolabeled complex in mice

Two groups of mice were used to evaluate the biodistribution of radiolabeled complex: the PC3 tumor-bearing mice and the normal BALB/c mice. The radiolabeled complex was injected intravenously into mice through their vein tails. The tumor-bearing mice were sacrificed (using CO2 gas) at 30-, 60-, and 120-min postinjection. In parallel, the normal BALB/c mice were also sacrificed at different time intervals (15, 30, 60, and 120 min). For considering statistical analysis, three mice were sacrificed at each time interval ($n = 3$). Then, the 20 main organs were removed and washed in water. In the next step, the activity of each organ was measured using an HPGe detector and ID/g % of each organ was calculated. This quantity is defined as the net activity (corrected for background) of each organ divided by injected activity (total) and the organ mass (gr), as Equation 1:

$$ID/g\% = \left(\frac{Activity_{Organ}}{Activity_{Total}}\right) \times 100 \Big/ m_{Organ} \quad (1)$$

It is noteworthy that the entire animal experiments were conducted under the supervision of the Animal Care Committee of the Tarbiat Modares University.

^{68}Ga-NODAGA-RGD-BBN (3.7 MBq) was injected intravenously into normal and PC3 tumor-bearing nude mice. The mice were sacrificed at different time intervals up to 2 h postinjection. The mice organs including blood, liver, spleen, kidneys, stomach, small and large intestines, heart, lungs,

muscle, skin, and bone, and also the tumor were taken, rinsed with normal saline, weighted and their activity was measured by a P-type coaxial HPGe detector. The tissue activity (A) was calculated using Equation 2.[16]

$$A = \frac{N}{\varepsilon \gamma t_s k_1 k_2 k_3 k_4 k_5} \quad (2)$$

where, ε is the detector efficiency at photopeak (0.02 for 1077 eV), γ is the photopeak emission probability (=0.03), ts (=30 s) is the live time of the sample spectrum collection, k_1, k_2, k_3, k_4, and k_5, are the correction factors for the nuclide decay from the sample collection time to start the measurement (=1, instant counting after sacrifice), the nuclide decay during counting period (=1, no activity loss for short live time), self-attenuation in the measured sample (=1, a small mass of tissue), pulses loss due to random summing and the coincidence (=1, no high organ activity), respectively. N is the corrected net peak area of the corresponding photopeak given as Equation 3:

$$N = N_s - (t_s / t_b) N_b \quad (3)$$

where, N_s is the net peak area in the sample spectrum during the live time of the sample spectrum collection (t_s) and N_b is the corresponding net peak area in the background spectrum during the live time of the background spectrum collection (t_b), in seconds.

Accumulated activity calculation for animal organs

The accumulated activity versus time for different animal organs was plotted according to Equation 4:

$$\tilde{A} = \int_{t_1}^{\infty} A(t)\, dt \quad (4)$$

where, A (t) is the activity of each organ at time t.

The curves were extrapolated to infinity by fitting the tail of each curve to a monoexponential curve with the exponential coefficient equal to the physical decay constant of gallium-68 radionuclide. Whereas the activity of blood at t = 0 was considered the total amount of the injected activity, the activity of all other organs was assumed to be zero at that time.

Assessment of accumulated activity for human organs

Sparks method was used to extrapolate the cumulated activity for animal organs to the cumulated activity for human organs (Equation 5).[17]

$$\tilde{A}_{Human\ organ} = \tilde{A}_{Animal\ organ} \times \frac{Organ\ mass_{Human} / Body\ mass_{Human}}{Organ\ mass_{Animal} / Body\ mass_{Animal}} \quad (5)$$

The standard mean weights for each human organ were utilized for the extrapolation.[18]

Equivalent absorbed dose calculation

RADAR formalism (Equation 6) was used for the calculation of the absorbed dose in human organs based on biodistribution data in mice:[19]

$$D = \tilde{A} \times DF \quad (6)$$

where, $\tilde{A}$ is the accumulated activity for each human organ, and DF is the dose function which is defined as Equation 7:

$$DF = \frac{k \sum_i n_i E_i \varphi_i}{m_t} \quad (7)$$

In this equation, n_i is the radiation probability with energy E_i (MeV) emitted per nuclear transition, ϕ_i is the fraction of energy emitted that is absorbed in the target, m_t is the target organ mass (kg), and k is the normalization factor ($\frac{mGy.kg}{MBq.\ Sec.MeV}$).

In this research, DFs presented in OLINDA/EXM software[20] were employed for calculating the absorbed dose in human organs.

Calculation of effective dose

The effective dose (E) was calculated using Equation 8 as follows:

$$E = \sum_T W_T H_T \quad (8)$$

where, H_T is the equivalent dose for each organ and W_T is the tissue-weighting factor.[21] This factor is obtained from the reported value in ICRP 103. The effective dose is expressed in terms of Sievert (Sv).

$$H_T = \sum_R W_R D_{T,R} \quad (9)$$

An equivalent dose is a dose quantity calculated for individual organs (T). This quantity is account for the effectiveness of the type of radiation-weighting factor (W_R) and is expressed in terms of Sv, as the same as the effective dose. The radiation-weighting factor is considered 1 for photon and electron.

Results

The results of the existing research were presented in the following experimental steps: preparation and quality control of ^{68}GaCl3 and also ^{68}Ga-NODAGA-RGD-BBN, stability and biodistribution studies in normal and tumor-bearing mice, and finally, dose assessments, respectively.

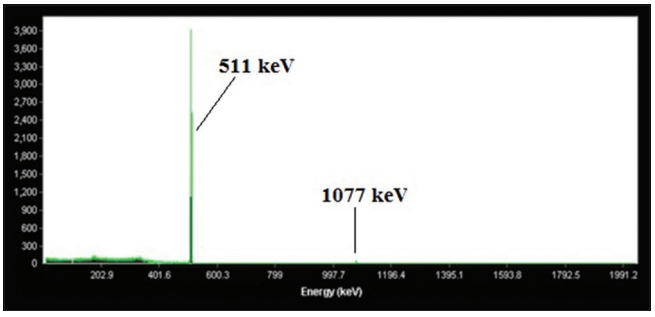

Figure 1: Gamma spectrum of $^{68}GaCl_3$ solution

Preparation and quality control of $^{68}GaCl_3$

After ligand formation, quality control and evaluation of radionuclide purity are the first steps. Radionuclide purity results are shown in Figure 1. Both 511 and 1077 keV peaks are clear in the $^{68}GaCl_3$ spectrum.

Figure 2 shows RTLC chromatogram of $^{68}GaCl_3$. The left side is related to ammonium acetate 1%: methanol (1:1) as a mobile phase and silica gel as a stationary phase. The right side is also related to 10 mM DTPA as a mobile phase and Whatman No. 2 as a stationary phase.

Preparation and quality control of ^{68}Ga-NODAGA-RGD-BBN

^{68}Ga-NODAGA-RGD-BBN was prepared with radiochemical purity of greater than 98% at optimized conditions. The radiochemical purity of the radiolabeled complex was studied by HPLC method [Figure 3].

As shown, the free gallium with a more polar nature is removed from the column in <1 min, while the labeled peptide is removed from the column after 4 min.

Stability studies

The stability of the radiolabeled compound was studied in PBS buffer (4 ºC), room temperature, and human serum (37 ºC). The results showed that at least for 2 h, the radiochemical purity of greater than 96%, 95%, and 90% in PBS buffer, room temperature, and human serum, respectively.

Biodistribution of ^{68}Ga-NODAGA-RGD-BBN in mice

Two groups of mice were used to assess the ^{68}Ga-NODAGA-RGD-BBN biodistribution: the PC3 tumor-bearing mice and the normal BALB/c mice. The percentage of the injected dose per gram in mice organs was determined up to 120 min postinjection of the complex. The ID/g % was driven using Equation 1 for each organ. In this part, statistical analysis ($n = 3$) was considered due to the statistical nature of the counts using Equation 1, as follows:

$$\sigma = \left(\frac{\sum_{i=1}^{3} (x_i - \bar{x})}{n-1} \right)^{0.5} \quad (10)$$

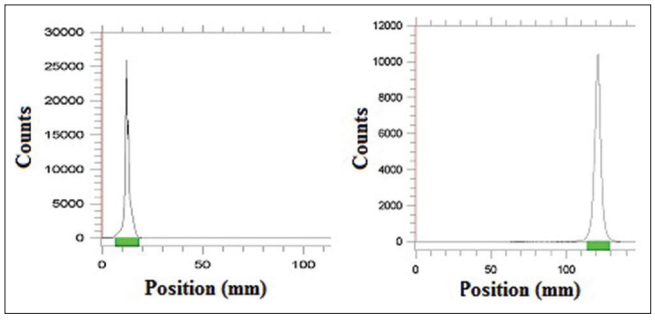

Figure 2: RTLC chromatogram of $^{68}GaCl_3$ using ammonium acetate 1%: methanol (1:1) as a mobile phase and silica gel as a stationary phase (left) and 10 mM DTPA as a mobile phase and Whatman No. 2 as a stationary phase (right). RTLC: Radio thin layer chromatography, DTPA: Diethylenetriaminepentaacetic acid

where x_i and $\bar{x}$ are the individual and average ID/g %, respectively.

The percentage of injected dose per gram of each tissue (ID/g %) is shown in Table 1. As is clear from the data, the results of ^{68}Ga-NODAGA-RGD-BBN biodistribution show that the radiolabeled complex had high concentrations both in tumor and kidney. The uptake of these two tissues at 2 h (120 min) postinjection was 1.200 ± 0.001 and 0.750 ± 0.001. The nondecay corrected clearance curves from the main organ sources of the mice for the radiolabeled peptide are also shown in Figure 4.

Equivalent and effective dose calculation

Human absorbed dose of ^{68}Ga-NODAGA-RGD-BBN was estimated using RADAR formalism based on mice biodistribution data [Table 2]. As it is seen, after the injection of the radiolabeled peptide, the highest absorbed dose is observed in the kidney tissue with 0.013 mGy/MBq.

Table 1: Percentage of injected dose per gram of each tissue

Tissue	Postinjection time (min)			
	15	30	60	120
Liver	0.535±0.002	0.566±0.003	0.108±0.002	0.165±0.002
Kidney	4.665±0.004	5.003±0.003	2.379±0.002	0.750±0.001
Big intestines	1.364±0.003	0.556±0.002	0.356±0.002	0.070±0.003
Small intestines	1.266±0.002	0.632±0.002	0.716±0.001	0.0862±0.001
Muscle	0.080±0.001	0.108±0.001	0.112±0.002	0.020±0.003
Stomach	0.936±0.002	0.508±0.001	0.408±0.003	0.051±0.002
Lung	0.671±0.003	0.380±0.002	0.195±0.003	0.063±0.002
Bone	0.171±0.001	0.056±0.003	0.087±0.002	0.030±0.002
Spleen	0.762±0.004	0.354±0.002	0.351±0.003	0.057±0.002
Skin	0.302±0.001	0.126±0.001	0.259±0.001	0.033±0.002
Heart	0.270±0.002	0.088±0.003	0.307±0.003	0.0197±0.002
Blood	0.358±0.001	0.298±0.001	0.066±0.002	0.021±0.003
Tumor	No data	4.011±0.003	2.600±0.002	1.200±0.001

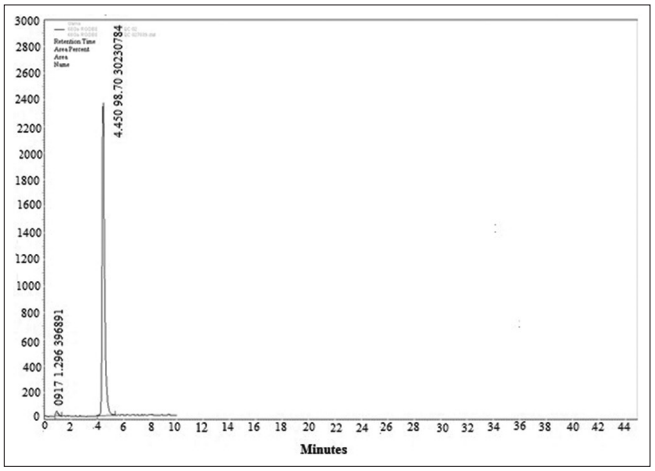

Figure 3: Radioactive HPLC chromatogram of the final radiolabeled compound. HPLC: High-performance liquid chromatography

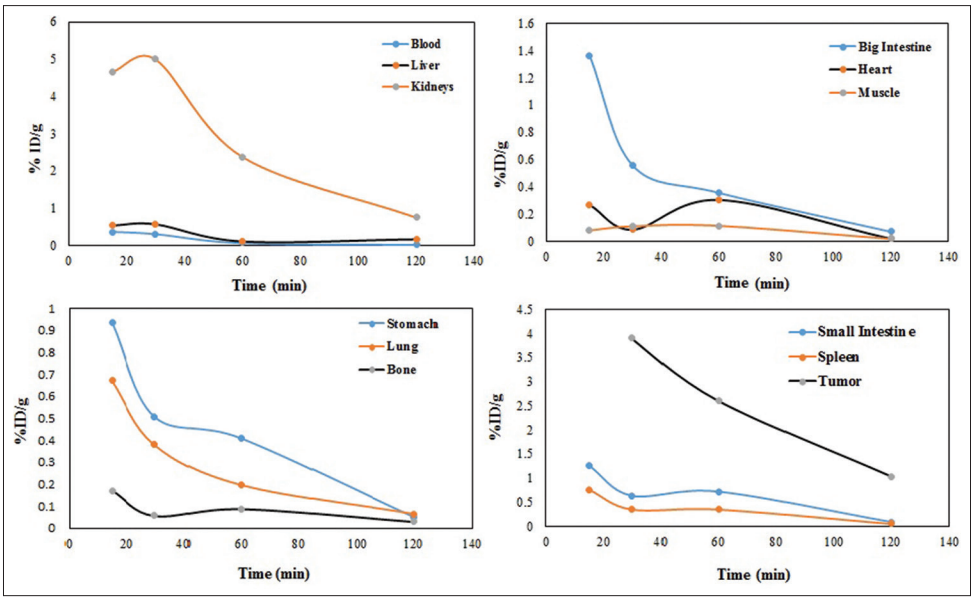

Figure 4: Nondecay corrected clearance curve of ^{68}Ga-NODAGA-RGD-BBN, BBN: Bombesin

Table 2: Effective dose delivered into human organs after injection of ^{68}Ga-NODAGA-RGD-BBN

Target organs	Absorbed dose in humans (mGy/MBq)	Wt[a]	Effective dose in humans (mSv/MBq)
Adrenals	8.32E-04	0.008	6.66E-06
Brain	1.92E-04	0.01	1.92E-06
GB wall	2.83E-04	0.008	2.26E-06
LLI wall	7.78E-04	0.12	9.34E-05
Small intestines	6.80E-04	0.008	5.44E-06
Stomach wall	1.03E-03	0.12	1.24E-04
ULI wall	6.92E-04	0.12	8.30E-05
Heart wall	1.22E-03	0.008	9.76E-06
Kidneys	1.28E-02	0.008	1.02E-04
Liver	1.98E-03	0.04	7.92E-05
Lungs	1.06E-03	0.12	1.27E-04
Muscle	3.44E-03	0.008	2.75E-05
Pancreas	8.09E-04	0.008	6.47E-06
Red marrow	2.42E-03	0.12	2.90E-04
Bone surf	2.57E-03	0.01	2.57E-05
Spleen	1.74E-03	0.008	1.39E-05
Testes	5.35E-04	0.08	4.28E-05
Thymus	5.75E-04	0.008	4.60E-06
Thyroid	6.06E-04	0.04	2.42E-05
UB wall	6.52E-04	0.04	2.61E-05
Total body	1.93E-03		1.10E-03

[a]Tissue weighting factors according to ICRP 103 (2007). ICRP: International Commission on Radiological Protection, GB: Gall bladder, LLI: Lower large intestine, ULI: Upper large intestine, UB: Urinary bladder

Discussion

Risk assessment associated with the administration of radiopharmaceuticals is an important issue which should be considered for determining the optimized amount of administrated activity. Human organ-absorbed dose assessment based on the animals' data is a prerequisite[22] and a common first step compatible with the ICRP 62 recommendations.[23]

^{68}Ga is an attractive PET radioisotope with interesting physical properties and availability in the form of a ^{68}Ge/^{68}Ga generator[24] that make it a favorable option for radiopharmaceutical research. Therefore, various radiolabeled compounds have been developed using this radionuclide such as ^{68}Ga-DOTATATE,[25] ^{68}Ga-citrate,[26] and ^{68}Ga-ECC[15] showing the usefulness of this radioisotope in the detection and diagnosis of infections and inflammatory lesions using PET.

As the quality control results [Figure 1] show the ^{68}GaCl3 radionuclide purity is greater than 99.9% and the activity of the eluted ^{68}Ge is <0.001% of the total activity. RTLC chromatogram of ^{68}GaCl3 is illustrated in Figure 2. The results show radiochemical purity of the ^{68}GaCl3 was higher than 99% which is an acceptable result. Furthermore, the stability of radiolabeled peptides in PBS buffer and also in human serum was investigated. The radiochemical purity of the complex remained >98% at least for 2 h [Figure 3].

Biological distribution investigation was performed for 120 minutes. According to the ^{68}Ga-NODAGA-RGD-BBN biological distribution data [Figure 4], with the increasing elapsed time after the radiopharmaceutical intravenous injection, the fraction of the total activity accumulated in the tissues (ID/g%) decreases. However, the data in different tissues show a peak at 60 min, especially for heart tissue. Indeed, gastrin-releasing peptide receptor-expressing tumors have a higher uptake compared to other nontarget organs. Furthermore, this agent demonstrates fast removal from the blood that the high uptake of the kidneys confirms the main excretion root of the radiolabeled compound is through the urinary tract.

From the absorbed dose point of view, the highest amount of absorbed dose is reported in the kidney (1.28E-2), muscle (3.44E-3), and bone surface (2.57E-3) tissues, respectively. Therefore, the dose assessment results demonstrate that the highest absorbed dose is in the kidneys and this tissue can be considered a dose-limiting organ. Furthermore, the absorbed dose and effective dose in human (whole-body) were estimated as 0.0019 mGy/MBq and 0.0011 mSv/MBq, respectively.

According to the ^{68}Ga-NODAGA-RGD-BBN biological distribution data [Figure 4], with the increasing elapsed time after the radiopharmaceutical injection, the fraction of the total activity accumulated in the tissues (ID/g %) decreases. However, the data in different tissues show a peak at 60 min, especially for heart tissue. From the absorbed dose point of view, the highest amount of absorbed dose is reported in the kidney, muscle, and bone surface tissues, respectively.

Shanehsazzadeh *et al*. reported that a 185-MBq injection of ^{67}Ga-BBN into humans would result in an estimated effective absorbed dose of 2.50 mSv (0.0135 mSv/MBq) whereas this value for ^{99m}Tc-BBN is 1.33 mSv (0.0072 mSv/MBq) in the whole body.[22] In another work, the whole-body distribution and radiation dosimetry of ^{68}Ga-NOTA-RGD in humans (10 cancer patients, 61.5 ± 7.4 kg) were investigated by Kim *et al*.[23] After an intravenous injection of 172.4 ± 20.5 MBq of ^{68}Ga-NOTA-RGD, whole-body PET scans were performed for 90 min. The results show the uptake of ^{68}Ga-NOTA-RGD was highest in the kidneys and urinary bladder. The mean effective doses were 0.0224 ± 0.0038 mSv/MBq using ICRP publication 103 weighting factor. Table 3 shows the comparison of the effective dose results for the present research with some relevant investigations.

As seen in Table 3, the kidneys received the highest absorbed dose after injection of different compounds based on BBN or RGD. This issue comes from the fact that the main route of excretion of peptide-based radioactive compounds is the urinary tract and therefore the kidneys will receive a high absorbed dose in peptide receptor radionuclide therapy (PRRT) and the kidneys are known as the dose-limiting organ in PRRT. Furthermore, it is necessary to mention that the effective half-life (including physical and biological half-life) of the radioactive compound is one of the most important parameters that effects on the absorbed dose. As demonstrated in Table 3, in the cases of animal studies with increasing the half-life (half-life of Ga-67 ~78 h, half-life of Tc-99m ~6 h, and half-life of Ga-68 ~68 min), the whole-body effective absorbed dose is increased. The difference between the whole-body effective absorbed dose for this study and ^{68}Ga-NOTA-RGD comes from the fact that the estimation of absorbed dose from animal to human will cause some overestimations and underestimations. On the other hand, the biodistribution and absorbed dose of a radiotracer may differ in different animal types and even from one person to another person.

Table 3: Comparison of the effective dose results of the present research with some similar investigation

Radiolabeled compound	Whole-body effective dose per activity injection (mSv/MBq)	Organ with highest absorbed dose	Reference
^{68}Ga-NODAGA-RGD-BBN	0.0011	Kidneys	Current study
^{67}Ga-BBN	0.0135	Kidneys	[22]
^{99}mTc-BBN	0.0072	Kidneys	[22]
^{68}Ga-NOTA-RGD	0.0224	Kidneys and UB	[23]

UB: Urinary bladder

Conclusion

^{68}Ga-NODAGA-RGD-BBN is a newly developed radiolabeled peptide that can simultaneously target integrin and gastrin receptors which makes it a high potential agent for PET imaging of prostate cancer. In this study, ^{68}Ga-NODAGA-RGD-BBN was prepared with radiochemical purity of more than 98%. The biodistribution results show that the radiolabeled complex had high concentrations both in tumor and kidney. High uptake of the complex in tumors compared to other nontarget organs shows that the radiolabeled peptide is an excellent agent for PET imaging of prostate cancer. The human effective dose was also estimated (120 min postinjection) based on mice data according to the RADAR and Spark methods. The data from this research show the fact that the highest amount of absorbed dose is reported in the kidney, muscle, and then bone surface tissues, respectively. Indeed, the highest absorbed dose per injected activity is reported in the kidneys tissue. Furthermore, the effective dose in the whole body was estimated as 0.0011 mSv/MBq.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Fischer R, Hill RM, Warshay D. Effects of psychodysleptic drug psilocybin on visual perception. Changes in brightness preference. Experientia 1969;25:166-9.
3. Tonini T, Rossi F, Claudio PP. Molecular basis of angiogenesis and cancer. Oncogene 2003;22:6549-56.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation.

Cell 2011;144:646-74.
5. Nagalla SR, Barry BJ, Falick AM, Gibson BW, Taylor JE, Dong JZ, *et al.* There are three distinct forms of bombesin. Identification of [Leu13]bombesin, [Phe13]bombesin, and [Ser3, Arg10, Phe13]bombesin in the frog Bombina orientalis. J Biol Chem 1996;271:7731-7.
6. Fournier P, Dumulon-Perreault V, Ait-Mohand S, Langlois R, Bénard F, Lecomte R, *et al.* Comparative study of 64Cu/NOTA-[D-Tyr6,βAla11, Thi13, Nle14]BBN (6-14) monomer and dimers for prostate cancer PET imaging. EJNMMI Res 2012;2:8.
7. Smith CJ, Volkert WA, Hoffman TJ. Gastrin releasing peptide (GRP) receptor targeted radiopharmaceuticals: A concise update. Nucl Med Biol 2003;30:861-8.
8. Moody TW, Lee L, Ramos-Alvarez I, Iordanskaia T, Mantey SA, Jensen RT. Bombesin receptor family activation and CNS/neural tumors: Review of evidence supporting possible role for novel targeted therapy. Front Endocrinol (Lausanne) 2021;12:728088.
9. Moreno P, Ramos-Álvarez I, Moody TW, Jensen RT. Bombesin related peptides/receptors and their promising therapeutic roles in cancer imaging, targeting and treatment. Expert Opin Ther Targets 2016;20:1055-73.
10. Li Q, Zhang C, Tan W, Gu G, Guo Z. Novel amino-pyridine functionalized chitosan quaternary ammonium derivatives: Design, synthesis, and antioxidant activity. Molecules 2017;22:E156.
11. Jalilian AR. An overview on Ga-68 radiopharmaceuticals for positron emission tomography applications. Iran J Nucl Med 2016;1:10-24.
12. Naderi M, Zolghadri S, Yousefnia H, Ramazani A, Jalilian AR. Preclinical study of ^{68}Ga-DOTATOC: Biodistribution assessment in Syrian rats and evaluation of absorbed dose in human organs. Asia Ocean J Nucl Med Biol 2016;4:19-29.
13. Stabin MG, Tagesson M, Thomas SR, Ljungberg M, Strand SE. Radiation dosimetry in nuclear medicine. Appl Radiat Isot 1999;50:73-87.
14. Stabin MG, Siegel JA. Physical models and dose factors for use in internal dose assessment. Health Phys 2003;85:294-310.
15. Mirzaei A, Jalilian AR, Aghanejad A, Mazidi M, Yousefnia H, Shabani G, *et al.* Preparation and Evaluation of (68) Ga-ECC as a PET Renal Imaging Agent. Nucl Med Mol Imaging 2015;49:208-16.
16. Yousefnia H, Zolghadri S, Shanehsazzadeh S. Estimated human absorbed dose of ^{177}Lu-BPAMD based on mice data: Comparison with ^{177}Lu-EDTMP. Appl Radiat Isot 2015;104:128-35.
17. Sparks R, Aydogan B. Comparison of the Effectiveness of Some Common Animal Data Scaling Techniques In Estimating Human Radiation Dose. TN (United States): Oak Ridge Associated Universities; 1999.
18. Yousefnia H, Zolghadri S, Jalilian AR, Tajik M, Ghannadi-Maragheh M. Preliminary dosimetric evaluation of (166) Ho-TTHMP for human based on biodistribution data in rats. Appl Radiat Isot 2014;94:260-5.
19. Shanehsazzadeh S, Yousefnia H, Jalilian AR, Zolghadri S, Lahooti A. Estimated human absorbed dose for (68) Ga-ECC based on mice data: Comparison with (67) Ga-ECC. Ann Nucl Med 2015;29:475-81.
20. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: The second-generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med 2005;46:1023-7.
21. Brenner DJ. Effective dose: A flawed concept that could and should be replaced. Br J Radiol 2008;81:521-3.
22. Shanehsazzadeh S, Lahooti A, Shirmardi SP, Erfani M. Comparison of estimated human effective dose of 67Ga- and 99mTc-labeled bombesin based on distribution data in mice. J Radioanal Nucl Chem 2015;305:513-20.
23. Radiological Protection in Biomedical Research. ICRP Publication 62. Ann ICRP 1992;22 (3).
24. Shanehsazzadeh S, Lahooti A, Yousefnia H, Geramifar P, Jalilian AR. Comparison of estimated human dose of (68) Ga-MAA with (99m) Tc-MAA based on rat data. Ann Nucl Med 2015;29:745-53.
25. Aghanejad A, Jalilian AR, Maus S, Yousefnia H, Geramifar P, Beiki D. Optimized production and quality control of 68Ga-DOTATATE. Iran J Nucl Med 2016;24:29-36.
26. Aghanejad A, Jalilian AR, Ardaneh K, Bolourinovin F, Yousefnia H, Samani AB. Preparation and quality control of (68) Ga-Citrate for PET applications. Asia Ocean J Nucl Med Biol 2015;3:99-106.