Applied Radiation and Isotopes 70 (2012) 2669–2676

Contents lists available at SciVerse ScienceDirect

Applied Radiation and Isotopes

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

^{44}Sc-DOTA-BN[2-14]NH_2 in comparison to ^{68}Ga-DOTA-BN[2-14]NH_2 in pre-clinical investigation. Is ^{44}Sc a potential radionuclide for PET?

E. Koumarianou [a,d,*], N.S. Loktionova [b], M. Fellner [b], F. Roesch [b], O. Thews [c], D. Pawlak [a], S.C. Archimandritis [d], R. Mikolajczak [a]

[a] National Center for Nuclear Research, Radioisotope Centre POLATOM, Otwock, Poland
[b] Institute of Nuclear Chemistry, University of Mainz, Germany
[c] Institute of Physiology University of Halle, Germany
[d] Institute R-RP, N.C.S.R DEMOKRITOS, Athens, Hellas

HIGHLIGHTS

- *In vitro* and *in vivo* evaluation of ^{44}Sc- and ^{68}Ga-DOTA-BN[2-14]NH_2 in reference to published data.
- Higher *in vitro* affinity to GRP receptors (PC-3 cells) for ^{nat}Ga-DOTA-BN[2-14]NH_2.
- Both showed similar internalization rates, however the efflux rate of the ^{44}Sc analog was lower.
- ^{68}Ga- and ^{44}Sc-DOTA-BN[2-14]NH_2 showed no differences in tumor accumulation.
- Hence the use of either ^{44}Sc or ^{68}Ga for detecting tumors with GRPR is equivalent.

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form
7 August 2012
Accepted 8 August 2012
Available online 23 August 2012

Keywords:
Scandium-44
Gallium-68
PET tracers
Bombesin analogue
GRP receptors affinity

ABSTRACT

Aim: In the present study we demonstrate the *in vitro* and *in vivo* comparison of the ^{44}Sc and ^{68}Ga labeled DOTA-BN[2-14]NH_2. ^{44}Sc is a positron emitter with a half life of 3.92 h. Hence it could be used for PET imaging with ligands requiring longer observation time than in the case of ^{68}Ga.
Methods: The binding affinity of ^{nat}Sc-DOTA-BN[2-14]NH_2 and ^{nat}Ga-DOTA-BN[2-14]NH_2 to GRP receptors was studied in competition to [^{125}I-Tyr^4]-Bombesin in the human prostate cancer cell line PC-3. A preliminary biodistribution in normal rats was performed, while first microPET images were assessed in male Copenhagen rats bearing the androgen-independent Dunning R-3327-AT-1 prostate cancer tumor.
Results: The affinity to GRP receptors in the PC-3 cell line was higher for ^{nat}Ga-DOTA-BN[2-14]NH_2 (IC_{50}(nM)$=0.85\pm0.06$) than that of ^{nat}Sc-DOTA-BN[2-14]NH_2 (IC_{50} (nM)$=6.49\pm0.13$). The internalization rate of ^{68}Ga labeled DOTA-BN[2-14]NH_2 was slower than that of ^{44}Sc, but their final internalization percents were comparable. ^{68}Ga-DOTA-BN[2-14]NH_2 was externalized faster than ^{44}Sc-DOTA-BN[2-14]NH_2. The biodistribution of ^{44}Sc-DOTA-BN[2-14]NH_2 and ^{68}Ga-DOTA-BN[2-14]NH_2 in normal rats revealed a higher uptake in target organs and tissues of the first one while both excreted mainly through urinary tract. In microPET images both tracers were accumulated in the tumor with similar uptake patterns.
Conclusions: Despite the differences in the receptor affinity both the ^{68}Ga- and the ^{44}Sc-labeled DOTA-BN[2-14]NH_2 tracers showed comparable distribution and similar time constants of uptake and elimination. Moreover no differences in tumor accumulation (neither in the overall uptake nor in the dynamics) were observed from the microPet imaging. From that perspective the use of either ^{44}Sc or ^{68}Ga for detecting tumors with GRP receptors is equivalent.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

^{44}Sc (Scandium-44) is a positron emitter ($E_{\beta+}$ 1475.4 keV (94.34%) with a gamma radiation component of 1157 keV (99.9%). Due to its half life ($T_{1/2}$=3.92 h), which is almost 4 times as long as the half life of ^{68}Ga (Gallium-68) ($T_{1/2}$=67.71 min),

* Correspondence to: Radiology Department, Duke University Medical Center, Box 3808 (Mail), Bryan Research Building, Room 161 A, 311 Research Drive (Courier), Durham, NC 27710, USA. Tel.: +1 91 96847886.
E-mail addresses: eftychia.koumarianou@duke.edu, e.koumarianou@polatom.pl (E. Koumarianou).

0969-8043/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.apradiso.2012.08.004

it may be considered as an interesting radionuclide for PET imaging. It can be produced by $^{44}Ca(p, n)^{44}Sc$ nuclear reaction in cyclotrons or from the decay of long lived ^{44}Ti ($T_{1/2}$=60.4 yr) (Welch and McCarthy, 2000). Although several generator systems have been described in literature (Welch and McCarthy, 2000; Rösch and (Russ) Knapp., 2003), including a $^{44}Ti/^{44}Sc$ generator, practically there were no sources of this radionuclide available so far. Recent developments by Filosofov et al. (2010) resulted in the high-performance, 5mCi$^{44}Ti/^{44}Sc$ radionuclide generator, which is currently available at the Institute of Nuclear Chemistry, University of Mainz. It has been shown by Pruszynski et al. (2010) and Loktionova et al. (2009), that the eluate of this generator can be effectively used for labeling of a somatostatin analogue, DOTATOC. Additionally, there is another radionuclide of scandium - ^{47}Sc ($T_{1/2}$=3.35 d) emitting γ radiation of 159.4 keV (63.3%) and β− radiation with maximum energy 0.600 MeV (31.6%) and 0.439 MeV (68.4%), which can be utilized in radiotherapy using the same vector molecules as for ^{44}Sc. Practical aspects of ^{47}Sc production both in the nuclear reactor and in cyclotron have been described by Mausner et al. (1998). The same group proved also the therapeutic potential of this radionuclide (Kolsky et al., 1998; Mausner et al., 1995). The combination of $^{44}Sc/^{47}Sc$ labeled radiopharmaceuticals for diagnostic PET imaging and for therapy, could provide a unique opportunity for patient qualification, dosimetry, therapy and therapy follow up. Both radionuclides create a matched pair and their clinical application may bring additional value, particularly in combination with ligands requiring longer observation time than the one which can be reached in the case of ^{18}F or ^{68}Ga labeled molecules. In recent years ^{68}Ga is experiencing its renaissance in PET imaging (Roesch and Riss, 2010). The ^{68}Ga labeled DOTA-chelated peptides (DOTA (1,4,7,10-teraazacyclododecane-N,N′,N″,N‴-teraacetic acid) such as somatostatin analogues DOTATOC, DOTATATE and DOTANOC have been proved to be useful in the imaging of neuroendocrine tumors. DOTA can also form stable complexes with Sc (Majkowska-Pilip and Bilewicz, 2011). The chemistry of Sc^{+3} is similar to that of Lanthanides. Due to its small ionic radius scandium is also chemically similar to aluminum and gallium. The thermodynamic stability of Sc complexes with DOTA is similar to that of Ga (Viola-Villegas and Doyle, 2009). However, there is no data published so far on the *in vitro* and *in vivo* receptor affinity and uptake of ^{44}Sc labeled DOTA-chelated peptides.

We have previously demonstrated that the affinity of a Bombesin (BN) analog, DOTA-BN[2-14]NH_2 (DOTA-QRLGNQWAV GHLMCONH$_2$) to the Gastrin Releasing Peptide Receptors (GRPR) in prostate cancer cell line PC3 varies, depending on the coupled radiometal (Koumarianou et al., 2009). Moreover these differences were reflected in the *in vivo* biodistribution of either ^{90}Y or ^{177}Lu labeled DOTA-BN[2-14]NH_2 in mice. Based on these findings we decided to extend our study using the same model peptide to assess the influence of Sc on the GRPR affinity in comparison to Ga, both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and quality control techniques

DOTA-BN[2-14]NH_2 was synthesized by standard Fmoc solid phase synthesis on Rink Amide Resin as described previously (Koumarianou et al., 2009; Gourni et al., 2006). Briefly, starting from α-fluorenyl-methoxycarbonyl (Fmoc) the amino acids [Met, Gln, Arg, Leu, Gly, Asn, Trp, Ala, Val, Gly, His, Leu] were coupled and then the terminal DOTA-tris (t-Bu-ester) (Macrocyclics) was conjugated. The purity and identity of the peptide was confirmed by HPLC and Electron Spray Ionization–Mass Spectroscopy (ESI–MS).

$^{68}GaCl_3$ was eluted from a commercially available $^{68}Ge/^{68}Ga$ generator (Cyclotron Co. Ltd.) using 0.1 M HCl and the post-elution purification with 0.05 M HCl/acetone (2:98) as eluent (Zhernosekov et al., 2007; Asti et al., 2008).

$^{44}ScCl_3$ was eluted with 3 mL of 0.25 M ammonium acetate pH 4 from a pilot $^{44}Ti/^{44}Sc$ generator working in reverse elution mode, as previously reported (Filosofov et al., 2010; Pruszynski et al., 2010). All chemicals and materials were used as supplied and were of analytical grade unless otherwise stated.

2.2. High Pressure Liquid Chromatography (HPLC)

C-18 reverse phase column (Macherey Nagel ET 125/4 Nucleosil 100-5 C18 AB) was used in HPLC system consisting of a pump (Dionex P680), a UV–Vis detector (Dionex UVD170U UV-Detector) and a well-type radioactivity detector (Gabi with NaI Detector, Raytest) connected in series. The solvents were A: 0.1% TFA/H_2O and B: acetonitrile, in isocratic elution of 75% solution A/25% solution B at 0.6 mL/min flow rate.

2.3. Solid Phase Extraction (SPE)

The purification of the radiolabeled compounds was performed using C-18 mini columns (Strata-X, 1 mL tube, 30 mg resin, Phenomenex) preconditioned with 1 mL ethanol followed by 1 mL H_2O. The sample was loaded on the cartridge followed by 2 mL H_2O (to elute non-bound radiometal) and by 400–500 μL pure ethanol (to collect radiolabeled peptide). The radioactivity of each fraction and the SPE cartridge, which retained colloidal residue, were measured in a well type γ-counter. C-18 mini columns (100 mg resin, Sep-Pak, Waters) were used for the purification of the cold metal complexes using 0.9% NaCl (non-bound fraction) and methanol (cold complex fraction) as eluents.

2.4. Thin Layer Chromatography (TLC)

Thin Layer Chromatography-Silica Gel strips (ITLC-SG, Pall) and 0.1 M sodium citrate as developing solution were used. Under these conditions the radiolabeled peptide remained at the spot (R_f=0.0), and non-bound $^{68}GaCl_3$ and $^{44}ScCl_3$ migrated with the solvent (R_f=0.9-1.0). Quantitative distribution of radioactivity on TLC plates was measured using an electronic autoradiography system (Instant Imager, Packard Canberra, USA)

For the *in vitro* binding affinity experiments the measurements were carried out using the LKB WALLAC 1272 CLINI GAMMA counter, while for the internalisation/externalisation studies the samples were measured using the 2470 Wizard^2 automatic gamma counter (PerkinElmer).

2.5. ^{68}Ga and ^{44}Sc labeling of DOTA-BN[2-14]NH_2

2.5.1. ^{68}Ga-DOTA-BN[2-14]NH_2

100–150 MBq of on line processed ^{68}Ga in 0.4 mL solution of 0.05 M HCl/acetone (Zhernosekov et al., 2007; Asti et al., 2008) was added to the reaction vial containing 50 mL H_2O and the peptide (50 μL of 1 mg/mL, 26.3 nmol), pH 2. The reaction mixture was incubated in an open vial at 95 °C for up to 25 min due to evaporation reaching a final volume of about 3 mL. To check the labeling yield 5 μL aliquots of reaction mixture were taken and analyzed by TLC at 1, 3, 10, 15, 20 and 25 min.

2.5.2. ^{44}Sc-DOTA-BN[2-14]NH$_2$

150–200 MBq ^{44}Sc in 3 mL of 0.25 M ammonium acetate, pH 4, was added to 26.3 nmol DOTA-BN[2-14]NH$_2$ (100 μL of 0.5 mg/mL in 0.25 M ammonium acetate, pH 4). The reaction mixture was incubated in an open vial at 95 °C for up to 25 min reaching a final volume of about 1.5 mL. 5 μL aliquots of reaction mixture were taken for quality control by TLC at 1, 3, 10, 15, 20 and 25 min.

After completing incubation the samples were purified by SPE and their radiochemical purity was checked by HPLC and TLC. Both ^{68}Ga- and ^{44}Sc-labeled DOTA-BN[2-14]NH$_2$ were used in further studies after SPE purification.

2.6. ^{nat}Ga and ^{nat}Sc non-radioactive metal complexes with DOTA-BN[2-14]NH$_2$

The non-radioactive complexes of ^{nat}Ga and ^{nat}Sc with DOTA-BN[2-14]NH$_2$ (here designated as cold complexes) were synthesized as described previously (Koumarianou et al., 2009; Zhang et al., 2007). Briefly, 100 μg of peptide was dissolved in 250 μL 0.4 M ammonium acetate, pH 5, followed by 250 μL of ascorbic acid (100 mg/mL). $ScCl_3$ or $GaCl_3$ (1 mg/mL) were dissolved in 0.05 M HCl and added to the peptide to obtain a peptide to metal molar ratio of 1:5. The samples were incubated at 95 °C for 25 min and left to cool down to room temperature. The cold complexes were analyzed by HPLC before and after purification and analyzed by Electron Spray Ionization–Mass Spectrum (ESI–MS).

2.7. Serum stability study

50 μL of ^{68}Ga-DOTA-BN[2-14]NH$_2$ or ^{44}Sc-DOTA-BN[2-14]NH$_2$ were added to 450 μL of freshly separated human serum and the mixture was incubated at 37 °C. Samples for TLC radiochemical purity assessment were taken after 30 min, 1 h and 2 h. At each time point a 50 μL aliquot of serum sample was added in 50 μL of ethanol and centrifuged for 3 min at 14000 rpm. 50 μL of the supernatant was diluted with 50 μL of water.

2.8. Cell culture

The human androgen-independent prostate carcinoma cell line PC-3 (ATCC, Cat. no.: CRL-1435) expressing the GRP receptor subtype known as BB2 (Smith et al., 2004) was used for the *in vitro* experiments. PC-3 cells were cultured in DMEM (Gibco Invitrogen) supplemented with 10% fetal calf serum, FCS (Gibco Invitrogen), a mixture of antibiotics (streptomycin 100 μg/mL, penicillin100 U/mL, Sigma Aldrich) and glutamax (Gibco Invitrogen). The cells were kept in a humidified atmosphere at 37 °C in 5% CO_2, fed every two days and sub-cultured by trypsinization (0.5%Trypsin-EDTA, Gibco Invitrogen) when the cells have covered about 80% of the culture flask surface.

2.9. In vitro binding studies

The *in vitro* GRP receptor binding affinity and specificity of ^{nat}Ga-DOTA-BN[2-14]NH$_2$ and ^{nat}Sc-DOTA-BN[2-14]NH$_2$ were determined by a competitive displacement cell-binding assay in the PC-3 cells and ^{125}I-[Tyr^4]-BN was used as the radiolabeled analog, as described previously (Koumarianou et al., 2009). Briefly, PC-3 cells were seeded in 24-well plates (8×10^4 cells/well) 48 h before the day of the experiment. At the day of the experiment the cells were incubated at 37 °C for 1 h in the presence of 30,000 to 35,000 cpm ^{125}I-[Tyr^4]-BN (Perkin-Elmer Life and Analytical Sciences) and increasing concentrations (10^{12}–10^6 M) of the nonradioactive DOTA-BN[2-14]NH$_2$ complexes with ^{nat}Ga and ^{nat}Sc. Upon completion of the incubation, the reaction medium was aspirated and the cells were washed twice with cold phosphate-buffered saline (PBS). Finally, the cells were treated with 1 N NaOH at 37 °C for 10 min to detach them from plates. The radioactivity of collected samples was measured in order to determine the IC_{50} value (inhibitory concentration, 50%). Three independent experiments were performed.

2.10. Internalization/efflux studies

The internalization/efflux experiments of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$were performed as described previously (Koumarianou et al., 2009). Briefly PC-3 cells were seeded in 6-well plates (8×10^5 cells/well) 48 h before the day of the experiment. The day of the experiment, the cells were incubated with fresh incubation medium and allowed to adjust to medium at 37 °C for 1 h. Approximately 200 fmol/100 μL of ^{44}Sc-DOTA-BN[2-14]NH$_2$ or ^{68}Ga-DOTA-BN[2-14]NH$_2$ so as to have a final concentration of 2×10^9 M, were added, and the cells were incubated at 37 °C in 5% CO_2. To determine nonspecific internalization, an excess of 1 μM DOTA-BN[2-14]NH$_2$ was added. The internalization was stopped at appropriate time points 5, 15, 30 and 60 min for ^{68}Ga and 5, 15, 30, 60 and 120 min for ^{44}Sc. The cells were washed twice with ice-cold PBS followed by washing twice with cold glycine buffer (0.05 M glycine solution, pH 2.8) for 5 min at 0 °C to distinguish between cell surface bound (acid releasable) and internalized (acid resistant) radioligand. Finally, cells were treated with 1 N NaOH at 37 °C for 10 min to detach them from the plates. The radioactivity of every fraction was measured on a γ-counter and expressed as the percent of specific internalized radiolabeled compound of total bound radioactivity after subtracting the percent of non specific uptake (% relative internalization, % r.i.).

The *in vitro* externalization rates were determined at the same time points as in the case of the internalization experiments. The results were presented as the percent of specific internalized (% relative internalization, % r.i.) and externalized tracer (% relative externalization, % r.e.) of total bound radioactivity after subtracting the percent of non specific uptake. Three independent experiments were performed for each study.

2.11. Statistical methods

IC_{50} values for the displacement of binding of ^{125}I-[Tyr^4]-BN by the different analogs and the internalization/efflux studies results were analyzed by non-linear regression analysis using the GraphPad Prism™ computer fitting program (GraphPad software, San Diego California).

2.12. Ex vivo organ distribution studies

Biodistribution studies were performed in male Sprague-Dawley rats (weight 190–230 g) under pentobarbital anesthesia (40 mg/kg body weight, Narcoren, Merial, Hallbergmoos, Germany), after intravenous injection (i.v.) of the radioactive sample into the jugular vein. Biodistribution of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ was studied at 1 h and 2 h post injection (p.i.). The injected dose was 11 MBq (7.4 MBq/nmol) for ^{68}Ga-DOTA-BN[2-14]NH$_2$ and 3 MBq (2.9 MBq/nmol) for ^{44}Sc-DOTA-BN[2-14]NH$_2$. Two rats per each time point were used. The radioactivity of the collected blood pool and samples of weighed tissues were measured using a dose calibrator. The results were calculated as percentage of the dose per gram of tissue (% I.D./g).

For receptor blocking native BN (100 μg/100 μL) was administered intravenously 15 min prior to administration of the ^{68}Ga or ^{44}Sc-labeled DOTA-BN[2-14]NH$_2$. Biodistribution was evaluated at 1 h p.i., in comparison to the control group, which was injected with the radiolabeled analog only.

During the experiments, the animals were housed in metabolic cages. All animal experiments were performed after approval and were carried out in accordance with the principals of Good Laboratory Practice (GLP).

2.13. Small animal PET imaging studies

The dynamic microPET imaging was performed in male Copenhagen rats bearing the androgen-independent Dunning R-3327-AT-1 prostate cancer tumor, which has been identified to express high affinity binding sites for GRP/BN analogs (Smith et al., 2004). Solid carcinomas were heterotopically induced by injection of R-3327-AT-1 cells (~0.4 mL, 10^4 cells/μL) subcutaneously into the dorsum of the hind foot. Tumors grew as flat, spherical segments and replaced the subcutis and corium completely. Tumors were used when they reached a volume of between 1.0 to 2.0 mL approximately, 10 to 14 days after tumor cell inoculation. The PET imaging was performed on a microPET Focus 120 small animal PET camera (Siemens/Concorde, Knoxville). During PET measurements the animals were placed in supine position and breathed room air spontaneously through a tracheal tube. After a 15 min transmission scan with an external ^{57}Co source, dynamic PET studies were acquired in 2D mode. The radiotracer was administered as a bolus injection of 0.4–0.7 mL via a catheter placed in the left jugular vein. The injected radioactivity was 30–50 MBq of the radiolabeled compound (^{68}Ga-DOTA-BN[2-14]NH$_2$: 8.2 MBq/nmol, ^{44}Sc-DOTA-BN[2-14]NH$_2$: 2.9 MBq/nmol).

3. Results

3.1. ^{68}Ge/^{68}Ga and ^{44}Ti/^{44}Sc generators processing

The eluted radioactivity of ^{68}Ga ranged from 100 to 150 MBq in 0.4 mL 0.05 M HCl/acetone (2:98), pH 2, the respective eluted radioactivity for ^{44}Sc varied from 150 to 200 MBq in 3 mL ammonium acetate 0.25 M, pH 4.

3.2. Radiolabeling of DOTA-BN[2-14]NH$_2$ with ^{68}Ga and ^{44}Sc

The radiolabeling yield was higher than 80% for both ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$. TLC and HPLC quality control were in good agreement. The HPLC analysis revealed one peak of non-bound ^{44}Sc at 2.82 min (6.0%) while the retention time for ^{44}Sc-DOTA-BN[2-14]NH$_2$ was about 5.55 min (94.0%) (confirmed in UV spectrum, data not shown) as shown in Fig. 1A. The equivalent TLC result is presented in Fig. 1B. The specific activity achieved for ^{68}Ga-DOTA-BN[2-14]NH$_2$ was in the range of 7.5 to 8.2 GBq/μmol DOTA-BN[2-14]NH$_2$ when incubated at 95 °C for 15 min. The maximum specific activity achieved for ^{44}Sc-DOTA-BN[2-14]NH$_2$ was 4.8 GBq/μmol DOTA-BN[2-14]NH$_2$, demanding longer incubation time of 20 min at 95 °C.

3.3. DOTA-BN[2-14]NH$_2$ cold complexes with ^{nat}Ga and ^{nat}Sc

The ESI-MS analysis of ^{nat}Ga-DOTA-BN[2-14]NH$_2$ confirmed the presence of a single main complex at 982.1 (m/z^{+2}), which was in agreement with the calculated value (MW=1964.173).

The respective ESI–MS analysis of ^{nat}Sc-DOTA-BN[2-14]NH$_2$ also confirmed the presence of single main peak at 967.3 (m/z^{+2}), with the doubled value c.a. 5 Da lower than the calculated one (MW=1939.406) which can be attributed to the loss of hydrogen ions during the spectrum acquisition.

3.4. Serum stability study

^{44}Sc-DOTA-BN[2-14]NH$_2$ and ^{68}Ga-DOTA-BN[2-14]NH$_2$ demonstrated similar stability (initially 90.2% and 85.8%, respectively) when incubated to human serum at 37 °C, as presented in Fig. 2 (the results are expressed as relative percent of intact radiolabeled compound), 89.6% of ^{44}Sc-DOTA-BN[2-14]NH$_2$ remained intact at 2 h post incubation.

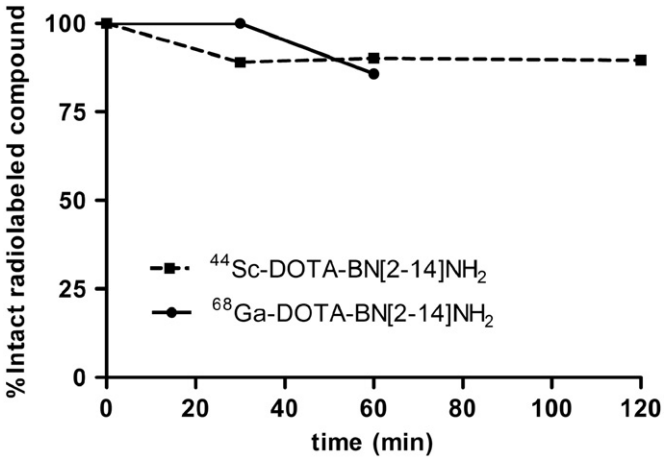

Fig. 2. Serum stability of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ when incubated to human serum for various time points at 37 °C, expressed as relative % of intact radiolabeled peptide.

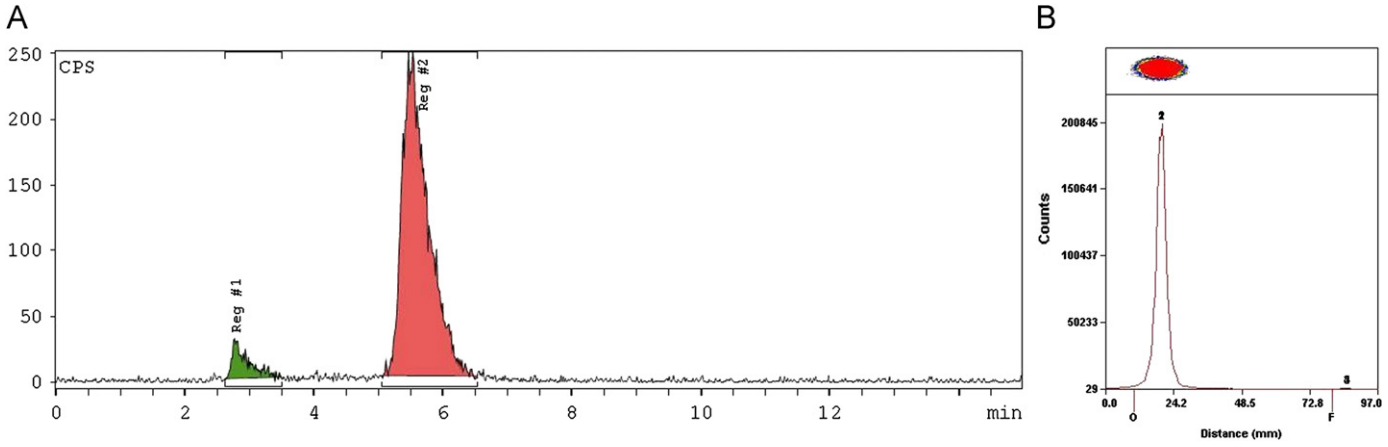

Fig. 1. ^{44}Sc-DOTA-BN[2-14]NH2 in (A) HPLC analysis (radioactivity detector) and (B) TLC analysis

3.5. In vitro binding studies

The displacement curves of ^{125}I-[Tyr^4]–BN with ^{nat}Ga- or ^{nat}Sc-DOTA-BN[2-14]NH$_2$ are presented in Fig. 3. The calculated IC$_{50}$ values were higher for ^{nat}Ga (0.85 ± 0.06 nM) than for ^{nat}Sc (6.49 ± 0.13 nM). In Table 1 these values are compared with IC$_{50}$ values obtained from our previous study (Koumarianou et al., 2009) for DOTA-BN[2-14]NH$_2$ and ^{nat}Y- and ^{nat}Lu-DOTA-BN[2-14]NH$_2$.

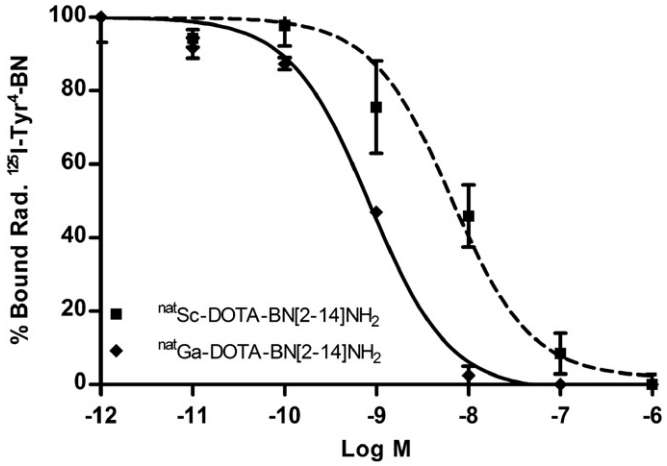

Fig. 3. Displacement curves of ^{125}I-[Tyr^4]-BN from the competitive binding studies for ^{nat}Ga-DOTA-BN[2-14]NH$_2$ and ^{nat}Sc-DOTA-BN[2-14]NH$_2$.

Table 1
IC$_{50}$ values vs. ^{125}I-[Tyr^4]-BN from competitive binding assays in PC-3 cells.

Derivative	IC$_{50}$ value (nM) (Mean ± S.D)
^{nat}Ga-DOTA-BN[2-14]NH$_2$	**0.85** ± 0.06
^{nat}Sc-DOTA-BN[2-14]NH$_2$	**6.49** ± 0.13
DOTA-BN[2-14]NH$_2$	**1.78** ± 0.12[a]
^{nat}Y-DOTA-BN[2-14]NH$_2$	**1.99** ± 0.06[a]
^{nat}Lu-DOTA-BN[2-14]NH$_2$	**1.34** ± 0.11[a]

[a] IC$_{50}$ values reported by Koumarianou et al.

3.6. Internalization/efflux studies

The maximum internalization rate for ^{44}Sc-DOTA-BN[2-14]NH$_2$ was observed after 30 min with 91.9 ± 10.1% of relative specific internalization while the maximum internalization rate for ^{68}Ga-DOTA-BN[2-14]NH$_2$ was 83.0 ± 3.4% at 60 min as shown in Fig. 4. The non specific uptake in both cases was $\leq 0.5 \pm 0.1$% at all time points, for both derivatives.

The relative externalization rate of ^{68}Ga-DOTA-BN[2-14]NH$_2$ was faster than that of ^{44}Sc-DOTA-BN[2-14]NH$_2$ at early time points. At 60 min post incubation the specific externalization of ^{68}Ga-DOTA-BN[2-14]NH$_2$ was 53.6 ± 7.9%, while for ^{44}Sc-DOTA-BN[2-14]NH$_2$ it was 41.0 ± 9.7% and 58.2 ± 12.0% at 120 min post incubation (Fig. 5).

3.7. Ex vivo organ distribution studies

The results of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ biodistribution in normal rats, expressed as %I.D/g ± S.D, are presented in Figs. 6A and B as well as in Table 2. Both radiolabeled derivatives were excreted mainly by urinary tract with rather high kidney retention (1.93 ± 0.40% I.D./g and 2.97 ± 0.82%.I.D./g at 2 h p.i. for ^{68}Ga- and ^{44}Sc-labeled peptide, respectively). The uptake of ^{68}Ga-DOTA-BN[2-14]NH$_2$ in pancreas was 0.64 ± 0.00% I.D./g at 1 h p.i. and 0.58 ± 0.05% I.D./g at 2 h p.i. (Fig. 6A). The respective values obtained for ^{44}Sc-DOTA-BN[2-14]NH$_2$ were 2.67 ± 0.53% I.D./g at 1 h p.i. and 1.51 ± 1.19% I.D./g at 2 h p.i. (Fig. 6B). The difference between the uptake in pancreas of ^{44}Sc-DOTA-BN[2-14]NH$_2$ in the animal group with non-blocked compared to blocked GRP receptors was significant, while it was not that prominent in the case of ^{68}Ga-DOTA-BN[2-14]NH$_2$. Both complexes showed fast blood clearance and mainly renal excretion.

3.8. Preliminary PET imaging

Images obtained in small animal PET of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ in male Copenhagen rats bearing the R-3327-AT-1 prostate cancer tumor are compared in Fig. 7. Both tracers were accumulated preferentially in the peripheral regions of the tumors whereas the more central part showed slightly lower concentration. The tumor uptake kinetics of both tracers showed a rapid increase within the first minutes after injection followed by a slow decrease over the whole observation period for ^{68}Ga-DOTA-BN[2-14]NH$_2$ and a stable but lower accumulation for ^{44}Sc-DOTA-

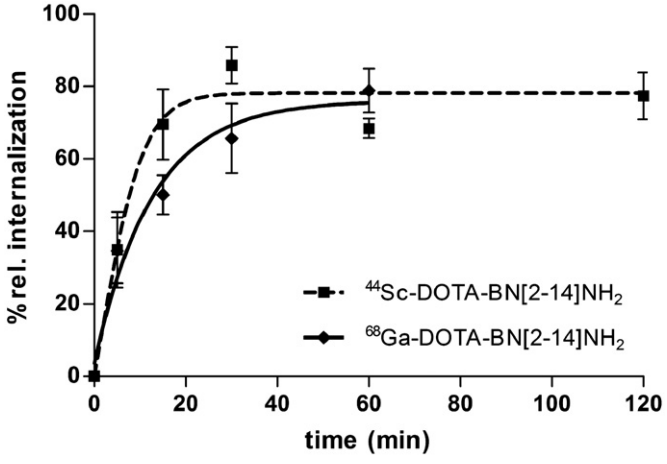

Fig. 4. Internalization yield of total bound radioactivity (% r.i.) of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ in PC-3 cells at 37 °C.

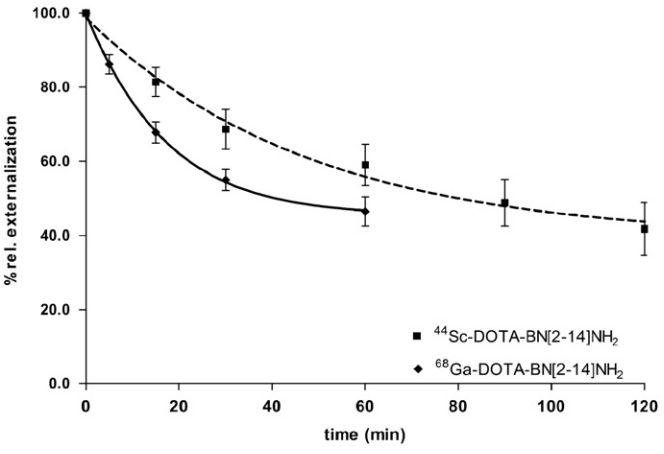

Fig. 5. Externalization yield of total bound radioactivity (% r.i.) of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ in PC-3 cells at 37 °C.

BN[2-14]NH_2 (Fig. 80A). However, the kinetics did not show profound differences between the ^{68}Ga and the ^{44}Sc labeled compound when relative tumor radioactivity normalized to an unspecific reference tissue (testis) was calculated (Fig. 8B).

4. Discussion

The increasing availability of new radionuclides with diagnostic and therapeutic properties offers new possibilities for individualized nuclear medicine options. This is especially relevant in case of matched pairs of radionuclides such as ^{44}Sc and ^{47}Sc. Grignon et al. (2007) reported that ^{44}Sc is the most interesting radionuclide for nuclear medicine imaging using β^+, γ coincidences. ^{44}Sc PET can be used for a pre-therapeutic imaging while the same ligand labeled with ^{47}Sc could be used for therapy.

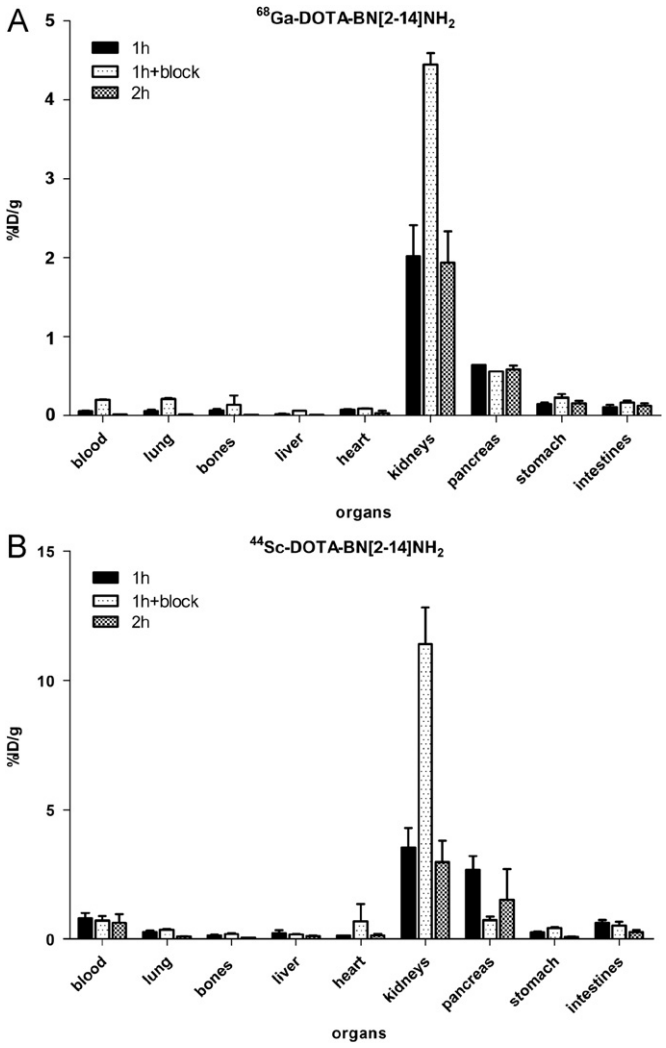

Fig. 6. *Ex vivo* organ distribution of ^{68}Ga-DOTA-BN[2-14]NH_2 (A) and ^{44}Sc-DOTA-BN[2-14]NH_2 (B) in male rats (%ID/g ± S.D; $n=2$).

Reubi et al. (2000) evaluated several somatostatin analogues *in vitro* and indicated that not only the peptide sequence and conjugated chelator, but to a large extent also, the metal involved in the complex formation influences the affinity of the molecule to the somatostatin receptor subtypes. Our previously published comparison of the radiolabelled DOTA-conjugated peptide, ^{90}Y-DOTA-BN[2-14]NH_2 and ^{177}Lu-DOTA-BN[2-14]NH_2 revealed differences in the receptor affinity of these two analogs (Koumarianou et al., 2009), both in terms of *in vitro* and *in vivo* behavior. Those differences may be attributed to the small structural changes in the radioligand molecule which influence the interaction with the receptor. The introduction of a certain metal or its replacement by another, may provoke considerable alterations in the *in vivo* binding affinity of a peptide to cell receptors and may have an important impact on the quality of the *in vivo* biodistribution of this radiopharmaceutical.

There was no published data on ^{44}Sc labeled peptides behavior *in vitro* and *in vivo* so far, therefore the main goal of this study was to evaluate the influence of this new radionuclide on the receptor affinity and uptake of DOTA-BN[2-14]NH_2 in reference to earlier published data on the same peptide labeled with ^{90}Y and ^{177}Lu, in order to preliminarily assess ^{44}Sc imaging potential. The ^{68}Ga-DOTA-BN[2-14]NH_2 was used in direct comparison, since ^{68}Ga complexes with DOTA chelated somatostatin analogues have demonstrated improved affinity to somatostatin receptor subtypes (Reubi et al., 2000). Considering the rather short half-life of ^{68}Ga, the ^{44}Sc can be an alternative for conjugation with biomolecules of longer metabolic half-life to allow prolonged PET imaging.

Both ^{68}Ga and ^{44}Sc complexes with DOTA-BN[2-14]NH_2 could be obtained in a fast and efficient way based on the labeling method established earlier for DOTA conjugated peptides (Asti et al., 2008; Pruszynski et al., 2009). The achieved radiosynthesis yields for ^{68}Ga-DOTA-BN[2-14]NH_2 and ^{44}Sc-DOTA-BN[2-14]NH_2 were higher than 80%. The specific activities and human serum stability were sufficient for further *in vitro* and animal studies.

^{nat}Ga-DOTA-BN[2-14]NH_2 showed superior binding affinity to GRP receptors compared to ^{44}Sc as well as to ^{90}Y or ^{177}Lu. The obtained IC_{50} values were 0.85 ± 0.06, 6.49 ± 0.13, 1.78 ± 0.12, 1.99 ± 0.06 and 1.34 ± 0.11 for ^{nat}Ga-DOTA-BN[2-14]NH_2, ^{nat}Sc- DOTA-BN[2-14]NH_2, DOTA-BN[2-14]NH_2, ^{nat}Y-DOTA-BN-[2-14]NH_2 and ^{nat}Lu-DOTA-BN[2-14]NH_2 complexes, respectively (see Table 2). Similar higher affinity of ^{nat}Ga-DOTATATE to somatostatin receptors in AR42J cells compared to ^{nat}Sc-DOTA-

Table 2
Ex vivo organ biodistribution of ^{44}Sc-DOTA-BN[2-14]NH_2 and ^{68}Ga-DOTA-BN[2-14]NH_2 in male Sprague-Dawley rats (%I.D./g ± S.D, $n=2$).

Organ	1h		1h block		2h	
	^{44}Sc-DOTA-BN [2-14]NH_2	^{68}Ga-DOTA-BN [2-14]NH_2	^{44}Sc-DOTA-BN [2-14]NH_2	^{68}Ga-DOTA-BN [2-14]NH_2	^{44}Sc-DOTA-BN [2-14]NH_2	^{68}Ga-DOTA-BN [2-14]NH_2
Blood	0.79 ± 0.21	0.05 ± 0.01	0.71 ± 0.17	0.20 ± 0.00	0.63 ± 0.32	0.01 ± 0.00
lung	0.26 ± 0.06	0.05 ± 0.02	0.35 ± 0.03	0.21 ± 0.01	0.08 ± 0.01	0.01 ± 0.00
bones	0.13 ± 0.04	0.06 ± 0.02	0.19 ± 0.03	0.13 ± 0.11	0.04 ± 0.00	0.01 ± 0.00
liver	0.22 ± 0.12	0.02 ± 0.01	0.17 ± 0.02	0.06 ± 0.00	0.10 ± 0.03	0.01 ± 0.00
Heart	0.12 ± 0.01	0.07 ± 0.01	0.69 ± 0.66	0.09 ± 0.00	0.13 ± 0.06	0.03 ± 0.03
Stomach	0.26 ± 0.03	0.15 ± 0.02	0.42 ± 0.04	0.22 ± 0.05	0.07 ± 0.02	0.16 ± 0.03
Intestines	0.63 ± 0.10	0.10 ± 0.04	0.52 ± 0.16	0.17 ± 0.02	0.26 ± 0.08	0.13 ± 0.03
pancreas	2.67 ± 0.53	0.64 ± 0.00	0.73 ± 0.13	0.56 ± 0.00	1.51 ± 1.19	0.58 ± 0.05
kidneys	3.53 ± 0.77	2.02 ± 0.39	11.40 ± 1.42	4.44 ± 0.15	2.97 ± 0.82	1.93 ± 0.40

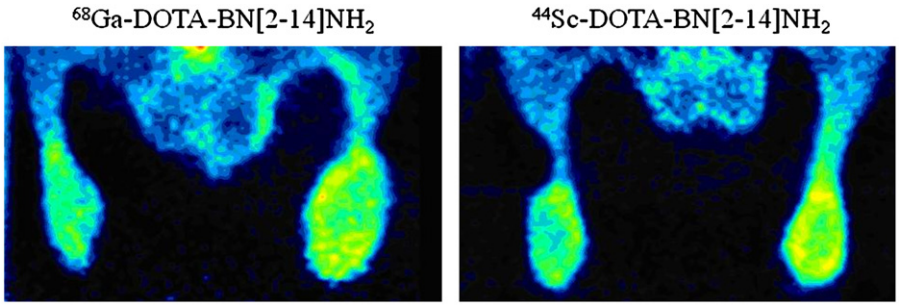

Fig. 7. Cumulative microPET images (15 to 60 min post injection) of subcutaneous R-3327-AT-1 tumors in male Copenhagen rats after injection of ^{68}Ga-DOTA-BN[2-14]NH$_2$ or ^{44}Sc-DOTA-BN[2-14]NH$_2$.

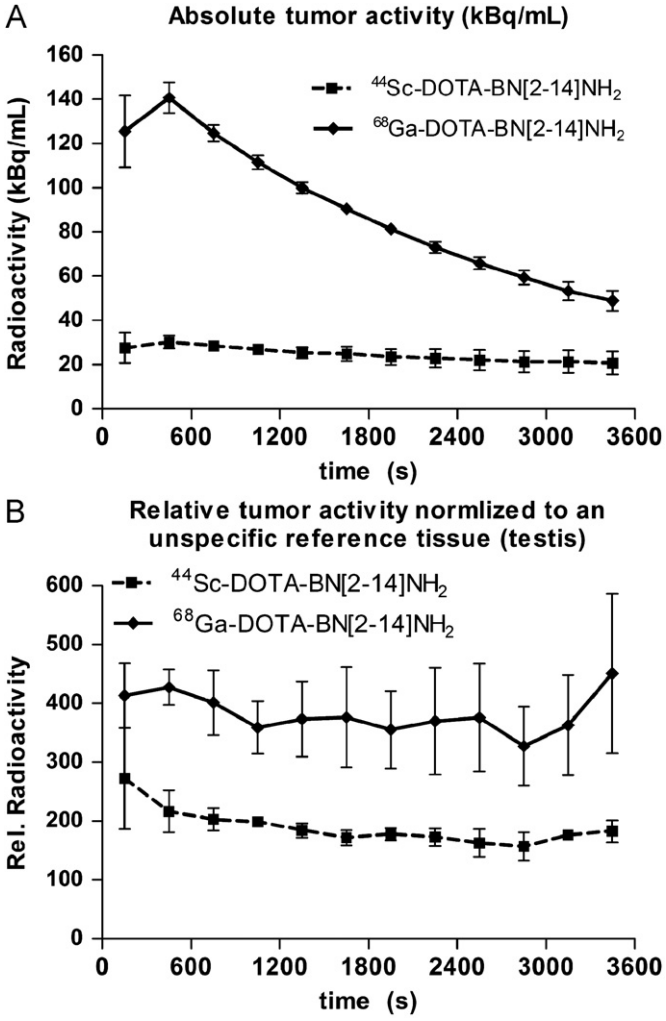

Fig. 8. (a) Absolute tumor activity [kBq/mL] and (b) relative tumor activity normalized to an unspecific reference tissue (testis), of ^{68}Ga-DOTA-BN[2-14]NH$_2$ and ^{44}Sc-DOTA-BN[2-14]NH$_2$ from the cumulative microPET images (15 to 60 min post injection) of subcutaneous R-3327-AT-1 tumors in male Copenhagen rats.

TATE was also reported (Koumarianou et al., 2011). These differences were not reflected in the internalization experiments, both ^{44}Sc- and ^{68}Ga-DOTA-BN[2-14]NH$_2$ revealed similar percentage of specific internalization within 1 h, however, the efflux rate of ^{44}Sc-labeled peptide was lower.

^{44}Sc-DOTA-BN[2-14]NH$_2$ and ^{68}Ga-DOTA-BN[2-14]NH$_2$ were rapidly cleared from the blood stream with high kidneys excretion. The bone uptake of ^{68}Ga-labeled peptide was as low as in the case of the ^{44}Sc-labeled, which indicated no dissociation of free ^{68}Ga or ^{44}Sc from the metallated conjugate and *in vivo* stability of the radiolabeled compounds. Both showed a significant uptake in pancreas, which is the organ naturally expressing GRPR but the uptake of ^{68}Ga-DOTA-BN[2-14]NH$_2$ was only at the level of 0.6%I.D/g and the specificity of its uptake was not confirmed. The specific uptake of ^{44}Sc-labeled peptide (73% blocking) was comparable to that reported for ^{177}Lu-DOTA-BN[2-14]NH$_2$ (81% blocking) and higher to that reported for ^{90}Y-DOTA-BN[2-14]NH$_2$ (53% blocking) at 1 h post injection (Koumarianou et al., 2009). However, for both studied preparations the kidney excretion of radioactivity at 1 h p.i. was higher in the receptor blocking experiments, as expected when the compound is not accumulated in the organs of interest and hence, more of radioactivity is excreted. Such phenomenon has been reported before (Panigone and Nunn, 2006; Barone et al., 2005) and it has been also addressed previously in our work (Koumarianou et al., 2009). The organ distribution pattern of both complexes was essentially similar to the previously reported for ^{177}Lu-DOTA-BN[2-14]NH$_2$ and ^{90}Y-DOTA-BN[2-14]NH$_2$ (Koumarianou et al., 2009), however the percent of uptake (% I.D./g) in organs expressing GRPR was almost 10 times lower. This observation is in accordance to the at least 10 times lower specific activities achieved for both studied compounds.

MicroPET imaging of tumors with high affinity binding sites for GRP/BN analogs showed accumulation of the tracer. The uptake was slightly higher in the peripheral regions of the tumor for both tracers. The regional differences might be the result of differences in GRP receptor expression within the tissue or differences in functional binding capacities due to differences in the metabolic microenvironment of the tumor.

Comparing the ^{68}Ga- and the ^{44}Sc-labeled peptide no differences in the tumor accumulation (neither in the overall uptake nor in the dynamics) were seen. Both tracers showed comparable distribution patterns and similar time constants of uptake and elimination. For these reasons the utility of either ^{68}Ga or ^{44}Sc preparation for detecting GPR-binding tumours is equivalent.

Acknowledgements

This work was supported by COST ACTION BM0607:Targeted Radionuclide therapy (TRNT) and COST ACTION D38:Metal Based Systems for Molecular Imaging. We are thankful to Dr. R. Postina and Dr. V. Metz from the Institute of Biochemistry at the Johannes Gutenberg University of Mainz, Germany for kindly provide the facilities for the cell culturing of PC-3 cells which were used for the *in vitro* studies and Dr. C. Zikos of the Institute R-RP, N.C.S.R "Demokritos", Athens, Hellas for the synthesis of DOTA-BN-[2-14]NH$_2$.

References

Asti, M., De Pietri, G., Fraternali, A., Grassi, E., Sghedoni, R., Fioroni, F., Roesch, F., Versari, A., Salvo, D., 2008. Validation of ^{68}Ge/^{68}Ga generator processing by chemical purification for routine clinical application of ^{68}Ga-DOTATOC. Nucl. Med. Biol. 35, 721–724.

Barone, R., Borson-Chazot, F., Valkema, R., Walrand, S., Chauvin, F., Gogou, L., et al., 2005. Patient-specific dosimetry in predicting renal toxicity with 90YDOTA-TOC: relevance of kidney volume and dose rate in finding a dose–effect relationship. J. Nucl. Med. 46 (Suppl. 11), 99S–106S.

Filosofov, D.V., Loktionova, N.S., Roesch, F., 2010. A ^{44}Ti/^{44}Sc radionuclide generator for potential application of ^{44}Sc-based PET-radiopharmaceuticals. Radiochim. Acta 98, 149–156.

Gourni, E., Paravatou, M., Bouziotis, P., Zikos, C., Fani, M., Xanthopoulos, S., Archimandritis, S.C., Livaniou, E., Varvarigou, A.D., 2006. Evaluation of a series of new $^{99\,m}$Tc-labeled bombesin-like peptides for early cancer detection. Anticancer Res. 26 (1A), 435–438.

Grignon, C., Barbet, J., Bardies, M., Carlier, T., Chatal, J.F., Couturier, O., Cssonneau, J.P., Faivre, A., Ferrer, L., Girault, S., Haruyama, T., LeRay, P., Luguin, L., Lupone, S., Metivier, V., Morteau, E., Servagent, N., Thers, D., 2007. Nuclear medical imaging using $\beta^+\gamma$ coincidences from ^{44}Sc radio-nuclide with liquid xenon as detection medium. Nucl. Instrum. Methods Phys. Res. A 571, 142–145.

Kolsky, K.L., Joshi, V., Mausner, L.F., Srivastava, S.C., 1998. Radiochemical purification of no-carrier added Scandium-47 for radioimmunotherapy. Appl. Radiat. Isot. 49, 1541–1549.

Koumarianou, E., Mikolajczak, R., Pawlak, D., Zikos, X., Bouziotis, P., Garnuszek, P., Karczmarczyk, U., Maurin, M., Archimandritis, S.C., 2009. Comparative study on DOTA-derivatized bombesin analog labeled with ^{90}Y and ^{177}Lu: *in vitro* and *in vivo* evaluation. Nucl. Med. Biol. 36, 591–603.

Koumarianou, E., Pawlak, D., Korsak, A., Mikolajczak, R., 2011. Comparison of receptor affinity of ^{nat}Sc-DOTA-TATE *versus* ^{nat}Ga-DOTA-TATE. Nucl. Med. Rev. 14 (2), 85–89.

Loktionova, N.S., Pruszynski, M., Majkowska, A., Riss, P., Roesch, F., 2009. Labelling and stability studies of ^{44}Sc-DOTATOC. J. Labelled Compds. Radiopharm. 52 (1), S490.

Mausner, L.F., Kolsky, K.L., Joshi, V., Srivastava, S.C., 1998. Radionuclide development at BNL for nuclear medicine therapy. Appl. Radiat. Isot. 49, 285–294.

Mausner, L.F., Joshi, V., Kolsky, K.L., Meinken, G.E., Mease, R.C., Sweet, M.P., Srivastava, S.C., 1995. Evaluation of chelating agents for radioimmunotherapy with scandium-47. J. Nucl. Med. 36, 104 (Suppl. 5), In: Proceeding of the meeting of the Society of Nuclear Medicine. Minneapolis, United States, pp. 104, 423.

Majkowska-Pilip, A., Bilewicz, A., 2011. Macrocyclic complexes of scandium radionuclides as precursors for diagnostic and therapeutic radiopharmaceuticals. J. Inorg. Biochem. 105, 313–320.

Pruszynski, M., Loktionova, N.S., Filosofov, D.V., Roesch, F., 2010. Post-elution processing of ^{44}Ti/^{44}Sc generator derived ^{44}Sc for clinical application. Appl. Radiat. Isot. 68, 1636–1641.

Pruszynski, M., Loktionova, N.S., Filosofov, D.V., Roesch, F., 2009. Processing of generator-produced ^{44}Sc for medical application–radiolabeling of DOTATOC with ^{44}Sc. J. Labeled Compds. Radiopharm. 52 (1), S490.

Panigone, S., Nunn, A.D., 2006. Lutetium-177 labeled gastrin releasing peptide receptor binding analogs: a novel approach to radionuclide therapy. Q J. Nucl. Med. Mol. Imaging 5 (4), 310–321.

Rösch, F., (Russ) Knapp, F.F., 2003. Radiochemistry and radiopharmaceutical chemistry in life science. In: Vértes, A., Nagy, S., Klencsár, Z. (Eds.), Handbook of Nuclear Chemistry, vol. 4. Kluver Academic Publishers, Dordrecht/Boston/London, pp. 81–118.

Roesch, Frank., Riss, Patrick J., 2010. The renaissance of the ^{68}Ge/^{68}Ga radionuclide generator initiates new developments in ^{68}Ga radiopharmaceutical Chemistry. Curr. Top. Med. Chem. 10, 1633–1668.

Reubi, J.C., Schar, J.C., Waser, B., Wenger, S., Heppeler, A., Schmitt, J.S., Maecke, H.R., 2000. Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. Eur. J. Nucl. Med. 27, 273–282.

Smith, C.J., Volkert, W.A., Hoffman, T.J., 2004. Gastrin releasing peptide (GRP) receptor targeted radiopharmaceuticals: a concise update. Nucl. Med. Biol. 30, 861–868.

Viola-Villegas, N., Doyle, R.P., 2009. The coordination chemistry of 1,4,7,10-tetraazacyclododecane-N,N′,N,N′-tetraacetic acid (H_4DOTA): Structural overview and analyses on structure-stability relationships. Coord. Chem. Rev. 253, 1906–1925.

Welch, M.J., McCarthy, T.J., 2000. The potential role of generator-produced radiopharmaceuticals in clinical PET. J. Nucl. Med. 41 (2), 315–317.

Zhernosekov, K.P., Filosofov, D.V., Baum, R.P., Aschoff, P., Bihl, H., Razbash, A.A., Jahn, M., Jennewein, M., Rösch, F., 2007. Processing of generator-produced ^{68}Ga for medical application. J. Nucl. Med. 48, 1741–1748.

Zhang, H., Schumacher, J., Waser, B., Wild, D., Eisenhut, M., Reubi, J.C., 2007. DOTA-PESIN, a DOTA conjugate bombesin derivative designed for the treatment of bombesin receptor – positive tumours. Eur. J. Nucl. Med. Mol. Imaging 34, 1198–1208.