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Radiolabeling of DOTATOC with the long-lived positron emitter ^{44}Sc

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ABSTRACT

The positron-emitting radionuclide ^{44}Sc with a half-life of 3.97 h and a β^+ branching of 94.3% is of potential interest for clinical PET. As so far it is available from a ^{44}Ti/^{44}Sc generator in Mainz, where long-lived ^{44}Ti decays to no-carrier-added (nca) ^{44}Sc. The ^{44}Sc is a trivalent metal cation and should be suitable for complexation with many well established bifunctional chelators conjugated to peptides or other molecular targeting vectors. Thus, the aim of this work was to investigate the potential of ^{44}Sc for labeling of DOTA-conjugated peptides. DOTA-D-Phe^1-Tyr^3-octreotide (DOTATOC) was used as a model molecule to study and optimize labeling procedure. Reaction parameters such as buffer conditions, concentration of peptide, pH range, reaction temperature and time were optimized. Addition of 21 nmol of DOTATOC to ^{44}Sc in ammonium acetate buffer pH 4.0 provided labeling yields $>98\%$ within 25 min of heating in an oil-bath at 95 °C. This time can be reduced to 3 min only by applying microwave supported heating. ^{44}Sc-DOTATOC was found to be stable in 0.9% NaCl, PBS pH 7.4, fetal calf and human serums, and also in the presence of competing metal cations (Fe^{3+}, Ca^{2+}, Cu^{2+}, Mg^{2+}), as well as other ligand competitors, like EDTA and DTPA, even after almost 25 h incubation at 37 °C. Present study shows that nca ^{44}Sc forms stable complexes with the macrocyclic ligand DOTA and that ^{44}Sc-DOTATOC and analog targeting vectors may be synthesized for further preclinical and clinical investigations.

1. Introduction

Positron emission tomography has become a powerful and widely used imaging technology in the last decade. While the basic principles of PET are similar to those of SPECT, PET systems are generally more sensitive than SPECT, have better spatial resolution and provide the possibility of more accurate attenuation correction (Lodge et al., 2005; Rahmim and Zaidi, 2008). In fact, recent PET imaging is dominated by the use of short-lived radionuclides such as ^{18}F, ^{11}C, ^{13}N and ^{15}O produced in on-site accelerators and mostly applied in the same medical centers. However, PET could expand beyond the large medical centers and become a truly routine clinical tool in the case additional sources of long-lived positron-emitting radionuclides are available. One possibility is to supply hospitals with "non-standard/unconventional" PET radionuclides directly produced at cyclotrons, e.g. ^{76}Br (16.1 h), ^{124}I (4.2 d), ^{89}Zr (78.4 h), ^{64}Cu (12.7 h) or ^{86}Y(14.7 h) (Haddad et al., 2008; Qaim, 2008; Hao et al., 2010).

Another source of positron-emitting radionuclides is a radionuclide-generator-system, similar to the ^{99}Mo/^{99m}Tc generator commonly used in SPECT. For PET, such a generator should consist of a long-lived parent radionuclide absorbed on a column from which a shorter-lived, positron-emitting decay product is eluted by passing a suitable solvent through the column. The interest on application of metallic radionuclides, especially generator-based, for PET diagnosis is increasing during the recent years. An excellent example of this trend is a ^{68}Ge/^{68}Ga generator which provides high (85%) β^+ branching ^{68}Ga ($T_{1/2}$=67.7 min) from long-lived ^{68}Ge ($T_{1/2}$=270.8 *d*) (cf. e.g. Roesch and Riss, 2010, for review). This generator is turning into an important source of new ^{68}Ga-labeled radiopharmaceuticals for routine use in clinical PET/CT. However, PET imaging with ^{68}Ga may be restricted by its rather short physical half-life, especially when proteins with long biological half-lives, like monoclonal antibodies or their fragments, are used. Therefore, intense studies focused on development of generators providing radionuclides with longer physical half-life, such as ^{72}As ($T_{1/2}$=26 h) from the ^{72}Se/^{72}As generator, or ^{44}Sc ($T_{1/2}$=3.97 h) from the ^{44}Ti/^{44}Sc generator (Rösch and Knapp, 2003). Especially, the latter radionuclide with its convenient physicochemical properties can find an application in PET. ^{44}Sc has almost 4-times longer half-life and higher β^+ branching than commonly used ^{68}Ga. Its decay product nuclide, ^{44}Ca, is stable and non-toxic. ^{44}Sc can be obtained from a ^{44}Ti/^{44}Sc generator. ^{44}Ti is obtained in reaction ^{45}Sc(p, $2n$)^{44}Ti and decays by electron

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capture into ^{44}Sc. The ^{44}Ti, with its long half-life of 59.2 ± 0.6 years, could provide a cyclotron-independent source of ^{44}Sc for several decades (Ahmad et al., 1998; Alenitzky et al., 2005; Filosofov et al., 2010). Several approaches have been investigated in the past to develop the chemistry of ^{44}Ti/^{44}Sc generators (Greene and Hillman, 1967; Mirza and Aziz, 1969; Seidl and Lieser, 1973; Schumann et al., 2007). Recently, Filosofov et al. (2010) described a 185 MBq (5 mCi) generator system with the relevant radiochemical parameters, such as >97% elution efficacy for ^{44}Sc and very low breakthrough of $<5\times10^{-5}$% of ^{44}Ti. This generator was further studied in the terms of post-processing of the eluate in order to provide high radiochemical purity and radionuclide concentration batches of ^{44}Sc in an aqueous system applicable to subsequent labeling reactions (Pruszyński et al., 2010).

Direct production of ^{44}Sc consists in bombarding enriched with ^{44}Ca target with proton beam (Haddad et al., 2008; Kamel et al., 2011). The production of this radionuclide by ARRONAX group is considered as a "great interest at short term" together with other PET radionuclides like ^{64}Cu, ^{68}Ga, ^{82}Rb or ^{124}I, and even before other positron emitters such as ^{86}Y, ^{89}Zr or ^{52}Fe (8.3 h) (Haddad et al., 2008).

The ^{44}Sc with its physicochemical properties of a trivalent rare earth metal appears to be appropriate candidate in PET/CT diagnosis. It may be used to synthesize radiopharmaceuticals based on bifunctional chelators (DOTA, DTPA, NOTA, etc) established to coordinate currently used trivalent radionuclides in diagnosis and therapy, such as ^{68}Ga and ^{111}In or ^{90}Y and ^{177}Lu, as well as non-radioactive Gd(III). As a relatively longer-lived β^+ emitter, it could be used for more accurate planning and dosimetric calculations in endoradiotherapy based on the radionuclides mentioned above, but also for direct matching β^- emitting ^{47}Sc radiopharmaceuticals (Mausner et al., 1995). Thus, ^{44}Sc/^{47}Sc can join the other matched-pairs of β^+/β^- radionuclides, which would permit coordinated dosimetric PET imaging and therapy.

Macrocyclic chelators are well known to form stable complexes with metal cations and are of great interest for radiopharmaceutical design. DOTA-conjugated peptides, such as octreotide and octreotate derivatives or substance P, as well as some small proteins, e.g. affibodies or nanobodies, are readily labeled with metal radionuclides e.g. ^{68}Ga, ^{90}Y, ^{177}Lu or ^{213}Bi (Breeman et al., 2003; Cordier et al., 2010; Tolmachev et al., 2010; de Blois et al., 2011).

The aim of this work was to determine optimal conditions for radiolabeling DOTA-conjugated octreotides using DOTATOC as a model molecule. Parameters that influence reaction kinetics, like incubation time and temperature, amount of chelate-peptide and pH of the reaction were investigated. Influence of microwave supported heating on time and completeness of complexation reaction was compared with the conventional heating method in an oil-bath. Stability of the formed conjugate was checked in 0.9% NaCl, phosphate buffered-saline (PBS, pH 7.4), fetal calf and human serums, and in the presence of different metal cations as well as other competing chelators, like EDTA and DTPA.

2. Materials and methods

2.1. Chemicals and reagents

DOTA-D-Phe^1-Tyr^3-octreotide (DOTATOC) was obtained as GMP-grade from piChem R&D (Graz, Austria) and an aqueous stock solution of 1 μg/μL was prepared. All chemicals were analytical or pure reagent grade and used as received unless otherwise specified. Deionized Milli-Q water (18.2 MΩ cm; Millipore) was used in all reactions. Fetal Calf Serum (FCS) and Human Serum Albumin (HSA) were purchased from Sigma-Aldrich (USA).

2.2. ^{44}Sc and the ^{44}Ti/^{44}Sc radionuclide generator

^{44}Sc was available from ^{44}Ti/^{44}Sc generator system developed in Mainz with ^{44}Ti adsorbed onto column filled with anion exchange resin Bio-Rad AG 1-X8 (200–400 mesh, Cl^--form). ^{44}Sc was eluted with 20 mL of 0.005 M $H_2C_2O_4$/0.07 M HCl mixture (Filosofov et al., 2010). The eluate was directly post-processed on miniaturized column filled with cation exchange resin AG 50 W-X8 (200–400 mesh, H^+-form, 53 mg) where ^{44}Sc was quantitatively adsorbed on-line and successively eluted using 2–3 mL of 0.25 M ammonium acetate buffer (pH 4.0) (Pruszyński et al., 2010). This ^{44}Sc solution of small volume and free of competing oxalates was used for further labeling studies.

Measurement of ^{44}Sc radioactivity was accomplished using a dose calibrator Aktivitätsmessgerät M2316 (Messelektronik, Dresden GmbH). The absolute radioactivity and purity of ^{44}Sc was measured by γ-ray spectrometry with a high-purity germanium (HPGe) well counter detector using both 1157.2 and 1499.4 keV γ-lines.

2.3. Labeling of DOTATOC with ^{44}Sc

Labeling of DOTATOC with ^{44}Sc was performed by mixing different volumes of DOTATOC stock-solution (1 μg/μL) with post-processed ^{44}Sc eluate in 0.25 M ammonium acetate buffer pH 4.0 and heating mixtures in an oil-bath. Optimization of labeling reaction was performed varying incubation time and temperature, changing concentration of DOTATOC and pH of reaction mixtures. Optimization of each parameter was repeated 3–4 times in separate experiments. Effect of DOTATOC concentration on labeling yields was evaluated with quantity of peptide being varied from 7 to 28 nmol. Solutions were heated for 30 min at 95 °C. Influence of temperature and incubation time on reaction yield was investigated by heating solutions containing Sc and 21 nmol of DOTATOC at 40, 80, 95 and 115 °C for up to 30 min. Effect of pH on ^{44}Sc-DOTATOC formation was determined by addition of 100–250 μL of concentrated HCl or NaOH to the mixture of 21 nmol DOTATOC with ^{44}Sc in ammonium acetate buffer (pH 4.0) up to desired pH. Solutions were heated again for 30 min at 95 °C. The pH of reaction mixture was measured before and after heating.

2.4. Quality control

Radiochemical analysis of ^{44}Sc-DOTATOC formation was performed mostly using thin layer chromatography (TLC). The TLC plates (Silica-gel 60, Merck) were developed by three different solutions: (a) 0.1 M sodium citrate pH 4.0; (b) mixture of 5% NaCl with MeOH (3:1); and (c) mixture of 5% NaCl with MeOH and 25% NH_3 (3:1:1). Quantitative distribution of radioactivity on TLC plates was measured using an electronic autoradiography system and associated software (Instant Imager, Packard Canberra). The R_f values for free ^{44}Sc and ^{44}Sc-DOTATOC conjugate were determined. If TLC plates were developed with 0.1 M sodium citrate pH 4.0, free uncomplexed ^{44}Sc moved almost with front of the developing solution with R_f=0.8, whereas ^{44}Sc-DOTATOC remained at the origin with R_f=0. Using 5% NaCl with MeOH (3:1) as solvent resulted in the following R_f values: 0 and 0.3 for free ^{44}Sc and ^{44}Sc-DOTATOC, respectively. Addition of 25% NH_3 aq. to 5% NaCl with MeOH (3:1:1) caused hydrolysis of unchelated free ^{44}Sc (R_f=0), whereas ^{44}Sc-DOTATOC moved with front of the developing solution (R_f=0.9). All three TLC's developing

solutions were used for analysis of each sample giving very good results of distinction between free ^{44}Sc and ^{44}Sc-DOTATOC.

RP-HPLC with Lichrosphere 100-RP18EC column (5 μm, 250 × 4 mm) was used to quantify the amount of ^{44}Sc-DOTATOC formation. HPLC was performed using Hitachi L-7100 pump system coupled with UV (Hitachi L-7400) and radiometric (Gamma Raytest) detectors. Solvents for HPLC were obtained as HPLC grade and degassed by ultrasonication for 15–20 min just before use. The gradient elution system utilized mobile phase A (deionized H_2O) and mobile phase B (100% acetonitrile) and flow rate of 0.8 ml/min, starting with 82%A/18%B for 2 min; then the gradient was increased to 30% B over the next 25 min and then held at 30% B for 6 min, after which gradient parameters returned to the initial conditions during next 2 min. Mobile phase A contained 0.01% TFA. The retention time of free ^{44}Sc was R_t=3.2 min, whereas for ^{44}Sc-DOTATOC it was 21.5 min (Fig. 1).

2.5. Microwave supported labeling of DOTATOC with ^{44}Sc

Influence of microwave heating on radiolabeling of DOTATOC with ^{44}Sc was determined under the optimal conditions found during optimization studies. Therefore, samples were prepared by mixing 21 nmol of DOTATOC with post-processed ^{44}Sc eluate and after exposed to microwave radiation. Commercially available LabMate microwave unit (CEM, Matthews, NC, USA) was used for these studies. This fully-automated device enables exposition of sample to microwave radiation and also provides process control according to time, radiation power, temperature and pressure in a hermetically sealed autoclave. Samples prepared in special pressurized glass vessels (delivered with LabMate) were sealed by Teflon coated cork and put into the apparatus single-mode cavity which were exposed to microwave radiation. Device was set to the PowerMax mode, with temperature of sample kept at fixed value of ~95 °C during the whole period of microwave heating. Stable temperature was achieved by continuous automatic temperature measurements and automatically-controlled changes in microwave power mode and simultaneous cooling of sample by compressed-air during heating. The yield of ^{44}Sc-DOTATOC formation was determined by TLC analysis at conditions mentioned above.

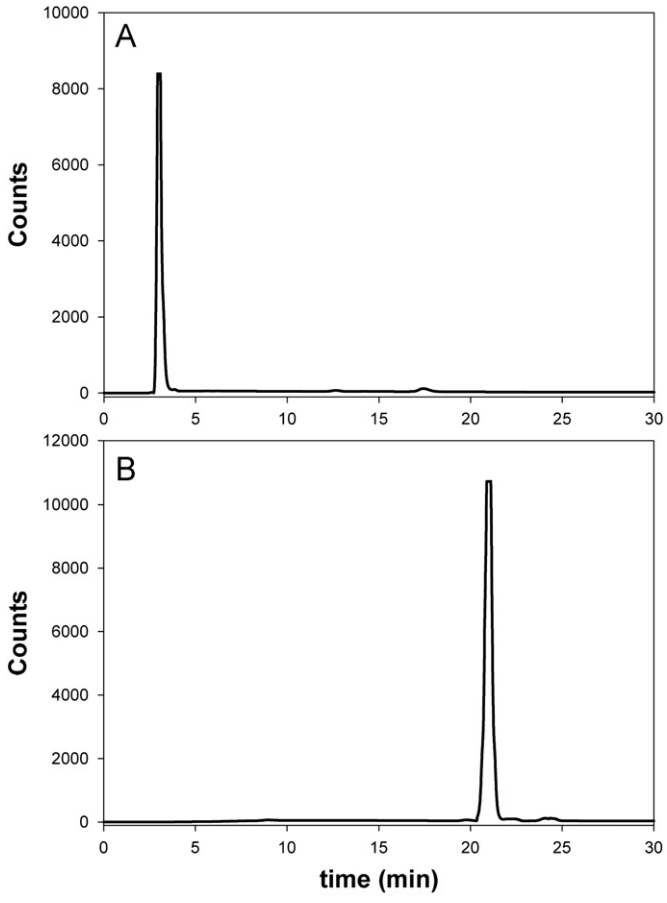

Fig. 1. Representative RP-HPLC radiochromatogram of free uncomplexed ^{44}Sc (A) and ^{44}Sc-DOTATOC (B).

2.6. Solid phase extraction (SPE)

^{44}Sc-labeled DOTATOC was purified from unreacted ^{44}Sc species by reversed-phase chromatography. Mini C-18 cartridges (Phenomenex Strata-X Tube, 30 mg) were first equilibrated with 2 mL ethanol and later rinsed with 10 mL water. Then, reaction mixture with ^{44}Sc-DOTATOC was passed through cartridge providing quantitative retention of both the chelated and non-chelated radionuclide. After washing cartridge with 5 mL water, ^{44}Sc-DOTATOC was recovered from column with 400 μL of pure ethanol.

2.7. Stability studies

^{44}Sc-DOTATOC of radiochemical purity >98% was used for stability studies. Every experiment was repeated 3 to 4 times. Stability studies of ^{44}Sc-DOTATOC in 0.9% NaCl and PBS (pH 7.4) were performed by addition 20 μL of purified ^{44}Sc-DOTATOC conjugate to 500 μL of pre-warmed 0.9% NaCl or PBS. Solutions were incubated for 22 h at 37 °C. Stability in fetal calf serum and human serum albumin were performed in similar way and incubated for 24 h at 37 °C.

Stability of ^{44}Sc-DOTATOC was also monitored in solutions containing different metal cations, e.g. Fe^{3+}, Ca^{2+}, Mg^{2+} and Cu^{2+} at concentration levels of 10^{-2} M each. The 20 μL purified fraction of ^{44}Sc-DOTATOC was added to 500 μL of an aqueous solution containing one of the metal cations. Solutions were incubated for 25 h at 37 °C. Studies with DTPA and EDTA were also performed to check the stability of synthesized ^{44}Sc-DOTATOC in the presence of competing chelating ligands. The appropriate aliquots of purified ^{44}Sc-DOTATOC were added to DTPA or EDTA solutions in 0.9% NaCl in such a way that the final molar ratio of DTPA or EDTA to conjugate was equal to 100:1. The final volume of all solutions was 500 μL. Solutions were incubated at 37 °C up to 25 h.

From all studied solutions adequate aliquots were taken at every time point and analyzed by TLC according to procedures described above. Also RP-HPLC quality control studies were done.

3. Results and discussion

Macrocyclic chelators are widely used in radiopharmacy for metallic radionuclides complexation, because they offer higher stability compare to acyclic ones, e.g. thermodynamic stability constant (log K) of Sc(III) with DOTA is 27.0, whereas for Sc(III)-DTPA it is 20.99 (Majkowska-Pilip and Bilewicz, 2011; Masuda et al., 1991). However, the complex formation with macrocyclic DOTA derivatives generally requires heating at elevated temperatures in contrast to open-chain analogs. Therefore, influence of temperature on ^{44}Sc-DOTATOC formation was evaluated and results are presented on Fig. 2. Incubation at 40 °C revealed around 5% yield of ^{44}Sc-DOTATOC complex even after 30 min of heating. Increasing temperature resulted in higher yields, e.g. yield approached 100% after 5 min at close to 100 °C (oil-bath temperature 115 °C). Because of an intensive bubbling of the aqueous solution and its high vaporing at this temperature, a temperature of 95 °C was selected for further studies. The overall

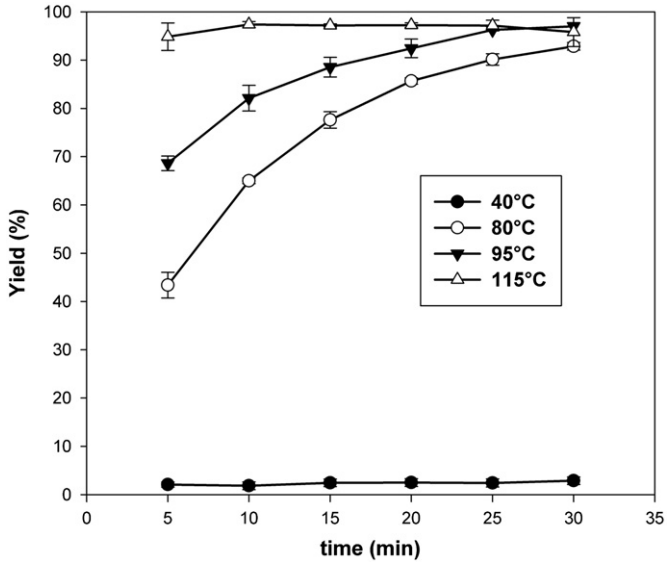

Fig. 2. Formation of ^{44}Sc-DOTATOC at pH 4.0 as a function of heating at different temperatures (21 nmol DOTATOC; $n=4$).

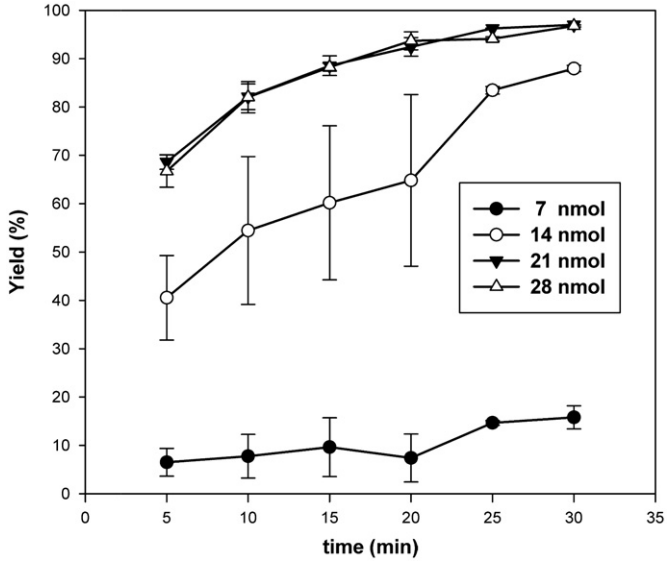

Fig. 3. Time course of ^{44}Sc complexation reaction forming ^{44}Sc-DOTATOC with different amounts of DOTATOC at pH 4.0 ($T=95$ °C; $n=4$).

radiolabeling yield for ^{44}Sc-DOTATOC was >98%, when 21 nmol of peptide was added to post-processed ^{44}Sc eluate (pH 4.0) and heated in an oil-bath for 25 min at 95 °C (Fig. 3). Increasing amount of peptide up to 28 nmol did not influence the reaction yield.

Scandium belongs to the transition metals group and its complex formation should strongly depend on pH of aqueous solution. Therefore, pH was considered to be an important parameter during optimization studies. The highest reaction yield was obtained, when pH was kept between 3 and 4 (Fig. 4). Increasing pH up to 5 caused slow decrease in labeling yield due to the hydrolysis of ^{44}Sc(III). Acidifying the solution to pH < 2 resulted in an extreme drop of labeling yields, probably due to the protonation of DOTA chelator. This study confirmed that previously developed method of post-elution processing of ^{44}Sc generator eluate and its final elution with acetate buffer (pH 4.0) gives not only high ^{44}Sc recovery, but also enables direct labeling of DOTA-conjugated tracers (Pruszyński et al., 2010).

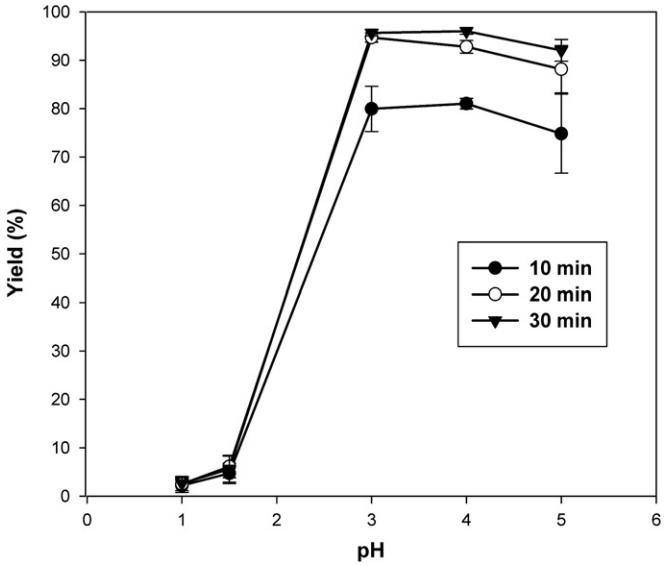

Fig. 4. Formation of ^{44}Sc-DOTATOC as a function of pH (21 nmol DOTATOC, $T=95$ °C, $n=4$).

Table 1
Influence of microwave heating on the yield of ^{44}Sc-DOTATOC (pH 4.0, 95 °C, 21 nmol peptide; $n=4$).

Time (min)	Yield (%)	SD (%) ($n=4$)
1	95.7	±2.0
3	97.1	±1.4
5	97.9	±0.9
10	97.6	±1.3
15	98.2	±0.8

Reaction time is a crucial factor when short-lived radionuclides are used for labeling. Microwave-assisted synthesis is commonly used in organic chemistry, because it enhances chemical yields by reducing reaction time without causing major degradation or introducing undesired by-products and it also improves reproducibility (Elander et al., 2000). This technique has been used for labeling organic molecules with ^{18}F (Hwang et al., 1989; Lemaire et al., 1989), but also in synthesis and complex formation with other radiohalogens such as ^{123}I, ^{131}I (Kumar et al., 2002; Pruszyński et al., 2008). Microwave heating has already shown its potential in increasing yield and shortening time of ^{68}Ga complexation with DOTA- and NOTA-conjugated oligonucleotides and peptides (Velikyan et al., 2004a; Velikyan et al., 2004b). The influence of microwave supported heating on ^{44}Sc-DOTATOC formation is demonstrated in Table 1. After 1 min of microwave heating, the reaction yield was >95% and increased up to 98% during next 2 min. Thus, it was confirmed that exposition of reaction mixture to microwave heating considerably shortened the reaction time.

In both cases of ^{44}Sc-DOTATOC formation, i.e. conventional and microwave supported heating, the radiochemical purity was mostly >98% and additional purification of the product was not necessary. However, to remove acetate ions, the purification was easily performed on RP C-18 mini-cartridge Strata-X and conjugate was recovered in 400 μL fraction of pure ethanol with ~94% efficacy and containing <0.9% free ^{44}Sc. Ethanol could be removed in the stream of nitrogen at room temperature and dried residue reconstituted in PBS.

Stability of ^{44}Sc-DOTATOC was analyzed first in 0.9% NaCl and PBS (pH 7.4). The results presented in Fig. 5 indicate high stability of the formed conjugate even after 22 h incubation at 37 °C.

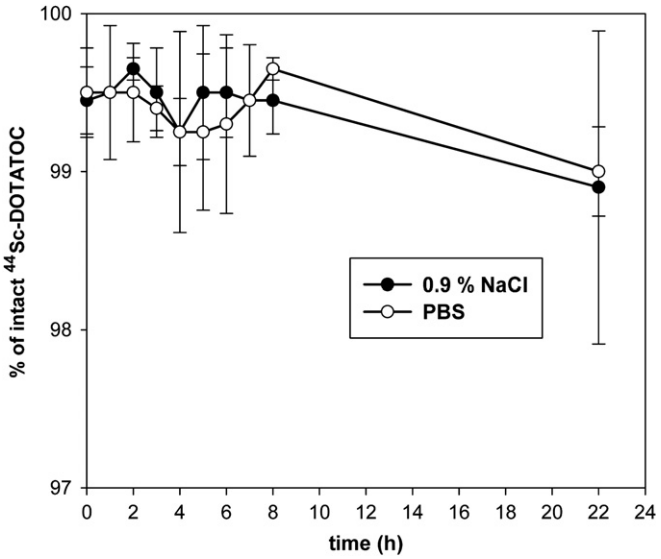

Fig. 5. Stability of ^{44}Sc-DOTATOC in 0.9% NaCl and PBS (pH 7.4) at 37 °C ($n=3$).

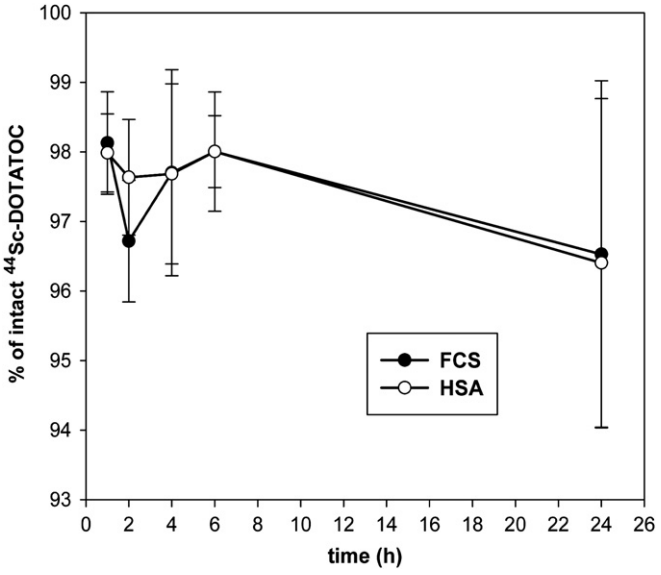

Fig. 6. Stability of ^{44}Sc-DOTATOC in calf (FCS) and human (HSA) serum at 37 °C ($n=3$).

Table 2
Stability of ^{44}Sc-DOTATOC at 37 °C in the presence of different metals cations at 10^{-2} M concentration ($n=3$).

Time (h)	% of intact ^{44}Sc-DOTATOC ± SD ($n=3$)			
	Fe^{3+}	Cu^{2+}	Ca^{2+}	Mg^{2+}
0	99.0 ± 0.3	99.5 ± 0.1	99.1 ± 0.3	99.6 ± 0.2
2	98.8 ± 0.1	99.3 ± 0.7	98.8 ± 0.2	99.3 ± 0.6
4	99.2 ± 0.1	99.6 ± 0.3	99.2 ± 0.2	99.6 ± 0.1
6	99.2 ± 0.3	99.5 ± 0.1	99.5 ± 0.2	99.7 ± 0.1
25	98.5 ± 0.7	99.2 ± 0.6	99.6 ± 0.1	99.6 ± 0.3

Also studies in calf (FCS) and human (HSA) serums confirmed high stability of ^{44}Sc-DOTATOC (Fig. 6).

Presence of different metal cations in final solution can cause transmetallation of radionuclide-conjugate and finally induce a release of the free radionuclide into solution. Therefore, it was interesting to determine whether ^{44}Sc-DOTATOC is stable in the presence of relevant metal cations. The addition of Fe^{3+}, Cu^{2+}, Ca^{2+} and Mg^{2+} did not induce transmetallation reaction (Table 2). ^{44}Sc-DOTATOC was stable even after 25 h incubation at 37 °C with metal cations at rather high concentration levels of 10^{-2} M. Similarly, stability of ^{44}Sc-DOTATOC in the presence of other complexing agents, like EDTA and DTPA was investigated to check if ^{44}Sc can follow transmetallation from DOTA-conjugate to competing ligand. This was not the case; experimental studies confirmed high stability of ^{44}Sc-DOTATOC even after 25 h incubation at 37 °C with EDTA or DTPA ligands at molar ratios of competing ligand to DOTA-peptide equal to 100:1 (Table 3).

Table 3
Stability of ^{44}Sc-DOTATOC at 37 °C in the presence of EDTA or DTPA at molar ratio 100:1 of competing ligand to DOTA-peptide ($n=3$).

Time (h)	% of intact ^{44}Sc-DOTATOC ± SD ($n=3$)	
	EDTA	DTPA
0	98.6 ± 0.1	98.6 ± 0.2
0.5	98.7 ± 0.4	99.1 ± 0.1
1	98.6 ± 0.6	99.1 ± 0.2
2	98.8 ± 0.1	98.7 ± 0.4
3	99.0 ± 0.1	98.9 ± 0.2
4	98.9 ± 0.2	99.0 ± 0.1
5	98.8 ± 0.6	99.0 ± 0.2
6	98.4 ± 0.6	99.1 ± 0.3
7	98.5 ± 0.1	99.1 ± 0.1
8	98.7 ± 0.1	99.0 ± 0.2
25	97.9 ± 1.6	97.6 ± 1.6

4. Conclusions

Synthesis of DOTATOC with the new generator-derived PET radionuclide ^{44}Sc was investigated in details. Incorporation of ^{44}Sc into DOTATOC was almost quantitative (>98%) at pH 4.0 after 25 min heating in an oil-bath at 95 °C. This time can be significantly reduced to 3 min only when microwave heating is adopted for synthesis. We also performed optimization studies with DOTA-D-Phe^1-Tyr^3-octreotate (DOTATATE, abx, Germany) and obtained the same results. Therefore, only experimental data for ^{44}Sc-DOTATOC are exemplified in this manuscript. Special efforts were focused on stability studies of ^{44}Sc-DOTATOC conjugate, which was found to be stable in 0.9% NaCl, PBS (pH 7.4), calf (FCS) and human (HSA) serum as well as in the presence of metal cations (Fe^{3+}, Cu^{2+}, Ca^{2+} and Mg^{2+}) and competing ligands, like EDTA and DTPA.

This study confirms the compatibility of ^{44}Sc radionuclide with established radiolabeling chemistry and may allow follow-up research on PET/CT imaging with this new trivalent metallic positron emitter. It can stimulate development of new metallorradiopharmaceuticals based on this longer-lived positron emitter ^{44}Sc in order to cover imaging periods of more than one day. A specific field might be application of diagnostic ^{44}Sc tracers for matching therapeutic analog compounds labeled with e.g. ^{90}Y, ^{177}Lu or with the β^- emitter ^{47}Sc. In addition, molecular imaging of ^{44}Sc-labeled tracers by means of a new PET/3G camera based on the β^+/γ emission of this radionuclide is discussed (Haddad et al., 2008).

Acknowledgments

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References

Ahmad, I., Bonino, G., Cini Castagnoli, G., Fischer, S.M., Kutschera, W., Paul, M., 1998. Three-laboratory measurement of the ^{44}Ti half-life. Phys. Rev. Lett. 80, 2550–2553.

Alenitzky, Yu.G., Novgorodov, A.F., Filosofov, D.V., Skripnik, A.V., Kaplun, V.G., Suzikov, A.G., Eliseev, I.A., Rösch, F., 2005. ^{44}Ti: Investigation of Target Preparation, Irradiation and Yields in the ^{45}Sc(p,2n) Process. Annual report. Institute of Nuclear Chemistry, University of Mainz.

de Blois, E., Chan, H.S., Naidoo, C., Prince, D., Krenning, E.P., Breeman, W.A.P., 2011. Characteristics of SnO_2-based ^{68}Ge/^{68}Ga generator and aspects of radiolabelling DOTA-peptides. Appl. Radiat. Isot. 69, 308–315.

Breeman, W.A.P., de Jong, M., Visser, T.J., Erion, J.L., Krenning, E.P., 2003. Optimising conditions for radiolabelling of DOTA-peptides with ^{90}Y, ^{111}In, and ^{177}Lu at high specific activities. Eur. J. Nucl. Med. 30, 917–920.

Cordier, D., Forrer, F., Bruchertseifer, F., Morgenstern, A., Apostolidis, C., Good, S., Müller-Brand, J., Mäcke, H., Reubi, J.C., Merlo, A., 2010. Targeted alpha-radionuclide therapy of functionally critically located gliomas with ^{213}Bi-DOTA-[Thi^{8},Met(O^{2})^{11}]-substance P: a pilot trial. Eur. J. Nucl. Med. Mol. Imaging 37, 1335–1344.

Elander, N., Jones, J.R., Lu, S.Y., Stone-Elander, S., 2000. Microwave-enhanced radiochemistry. Chem. Soc. Rev. 29, 239–249.

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

Greene, M.W., Hillman, M., 1967. A scandium generator. Int. J. Appl. Radiat. Isot. 18, 540–541.

Haddad, F., Ferrer, L., Guertin, A., Carlier, T., Michel, N., Barbet, J., Chatal, J.-F., 2008. ARRONAX, a high-energy and high-intensity cyclotron for nuclear medicine. Eur. J. Nucl. Med. Mol. Imaging 35, 1377–1387.

Hao, G., Singh, A.N., Liu, W., Sun, X., 2010. PET with non-standard nuclides. Curr. Top. Med. Chem. 10, 1096–1112.

Hwang, D.R., Moerlein, S.M., Dence, C.S., Welch, M.J., 1989. Microwave-facilitated synthesis of [^{18}F]-spiperone. J. Labelled Compd. Radiopharm. 26, 391–392.

Kamel, A., Cydzik, I., Federica, S., Krajewski, S., Kasperek, A., Bilewicz, A., 2011. Cyclotron production of ^{44}Sc – new radionuclide for PET technique. J. Labelled Compd. Radiopharm 54 (S1), S53.

Kumar, P., Wiebe, L.I., Asikoglu, M., Tandon, M., McEwan, A.J.B., 2002. Microwave-assisted (radio)halogenation of nitroimidazole-based hypoxia markers. Appl. Radiat. Isot. 57, 697–703.

Lemaire, C., Cantineau, R., Christiaens, L., Guillaume, M., 1989. Radiofluorination of altanserine a potential serotonin receptor binding radiopharmaceutical for positron emission tomography. J. Labelled Compd. Radiopharm. 36, 336–337.

Lodge, M.A., Braess, H., Mahmoud, F., Suh, J., Englar, N., Geyser-Stoops, S., Jenkins, J., Bacharach, S.L., Dilsizian, V., 2005. Developments in nuclear cardiology: transition from single photon emission computed tomography to positron emission tomography-computed tomography. J Invasive Cardiol. 17, 491–496.

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

Masuda, Y., Okada, Y., Murase, I., Sekido, E., 1991. Complex formation of scandium (III) with polyaminopolycarboxylic acids and isotachophoretic separation of scandium using complexation equilibria. Jpn. Anal. 40, 215–220.

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, 104P.

Mirza, M.Y., Aziz, A., 1969. A scandium generator. Radiochim. Acta 11, 43–44.

Pruszyński, M., Bilewicz, A., Zalutsky, M.R., 2008. Preparation of Rh[16aneS$_4$-diol]^{211}At and Ir[16aneS$_4$-diol]^{211}At complexes as potential precursors for astatine radiopharmaceuticals. Part I: Synthesis. Bioconjugate Chem. 19, 958–965.

Pruszyński, M., Loktionova, N.S., Filosofov, D.V., Rösch, F., 2010. Post-elution processing of ^{44}Ti/^{44}Sc generator-derived ^{44}Sc for medical application. Appl. Radiat. Isot. 68, 1630–1641.

Qaim, S.M., 2008. Decay data and production yields of some non-standard positron emitters used in PET. Q. J. Nucl. Med. Mol. Imaging 52, 111–120.

Rahmim, A., Zaidi, H., 2008. PET versus SPECT: strengths, limitations and challenges. Nucl. Med. Commun. 29, 193–207.

Roesch, F., Riss, P.J., 2010. The renaissance of the ^{68}Ge/^{68}Ga radionuclide generator initiates new developments in ^{68}Ga radiopharmaceutical chemistry. Curr. Top. Med. Chem. 16, 1633–1668.

Rösch, F., Knapp (Russ), F.F., 2003. Radionuclide generators. Handbook of Nuclear Chemistry. In: Vértes, A., Nagy, S., Klencsár, Z. (Eds.), Radiochemistry and Radiopharmaceutical Chemistry in Life Sciences, volume 4. Kluwer Academic Publishers, Dordrecht, pp. 89–97. Chapter 3.2.

Schumann, D., Horn, S., Neuhausen, J., 2007. Design of a ^{44}Ti/^{44}Sc generator system. Annual Report 2006, Paul Scherrer Institut & Universitaet Bern, January 2007, Villigen, Switzerland, p. 41.

Seidl, E., Lieser, K.H., 1973. Die Radionuklidgeneratoren ^{113}Sn/^{113m}In, ^{68}Ge/^{68}Ga und ^{44}Ti/^{44}Sc. Radiochim. Acta 19, 196–198.

Tolmachev, V., Velikyan, I., Sandström, M., Orlova, A., 2010. A HER2-binding Affibody molecule labelled with ^{68}Ga for PET imaging: direct in vivo comparison with the ^{111}In-labelled analogue. Eur. J. Nucl. Med. Mol. Imaging 37, 1356–1367.

Velikyan, I., Lendvai, G., Välilä, M., Roivainen, A., Yngve, U., Bergström, M., Långström, B., 2004a. Microwave accelerated ^{68}Ga-labeling of oligonucleotides. J. Labelled Compd. Radiopharm. 47, 79–89.

Velikyan, I., Beyer, G.J., Långström, B., 2004b. Microwave-supported preparation of ^{68}Ga bioconjugates with high specific radioactivity. Bioconjugate Chem. 15, 554–560.