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RESEARCH ARTICLE

Optimized labeling of NOTA-conjugated octreotide with F-18

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Abstract We recently reported a facile method based on the chelation of [^{18}F]aluminum fluoride (Al^{18}F) by NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid). Here, we present a further optimization of the ^{18}F labeling of NOTA-octreotide (IMP466). Octreotide was conjugated with the NOTA chelate and was labeled with ^{18}F in a two-step, one-pot method. The labeling procedure was optimized with regard to the labeling buffer, ionic strength, peptide concentration, and temperature. Radiochemical yield, specific activity, in vitro stability, and receptor affinity were determined. Biodistribution of ^{18}F-IMP466 was studied in AR42J tumor-bearing mice. In addition, microPET/CT images were acquired. IMP466 was labeled with Al^{18}F in a single step with 97% yield in the presence of 80% (*v*/*v*) acetonitrile or ethanol. The labeled product was purified by HPLC to remove unlabeled peptide and unbound Al^{18}F. The radiolabeling, including purification, was performed for 45 min. Specific activities of 48,000 GBq/mmol could be obtained. ^{18}F-IMP466 showed a high tumor uptake and excellent tumor-to-blood ratios at 2 h post-injection. In addition, the low bone uptake indicated that the Al^{18}F–NOTA complex was stable in vivo. PET/CT scans revealed excellent tumor delineation and specific accumulation in the tumor. Uptake in receptor-negative organs was low. NOTA-octreotide could be labeled with ^{18}F in quantitative yields using a rapid two-step, one-pot, method. The compound was stable in vivo and showed rapid accretion in $SSTR_2$-receptor-expressing AR42J tumors in nude mice. This method can be used to label other NOTA-conjugated compounds such as RGD peptides, GRPR-binding peptides, and Affibody molecules with ^{18}F.

Keywords Octreotide · Radiofluorination · NOTA · Peptide · PET · Aluminum fluoride

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Introduction

Radiolabeled receptor-binding peptides have emerged as an important class of radiopharmaceuticals that have changed radionuclide imaging. Peptides have been labeled with ^{111}In and ^{99m}Tc for SPECT imaging and with positron emitters such as ^{68}Ga, ^{64}Cu, ^{86}Y, and ^{18}F for PET imaging. ^{18}F is the most widely used radionuclide in PET and has excellent characteristics for peptide-based imaging since the half-life (110 min) matches the pharmacokinetics of most peptides. In addition, the low positron energy of 635 keV results in short ranges in tissue, which results in excellent preclinical imaging resolution (<2 mm). Various methods to label peptides with ^{18}F have been investigated. Usually, a nucleophilic substitution reaction is used to produce an ^{18}F-labeled synthon, which is then reacted with a (functionalized) peptide. One of the first generally applicable methods—and still most widely used—is based on conjugation of the synthon, N-succinimidyl-4-[^{18}F]fluorobenzoate, to a primary amino group of the peptide [1]. This

method requires a time-consuming and laborious multistep synthesis. Specific activities obtained with this method ranged from 57,900 to 147,000 GBq/mmol [2]. Searching for a faster method, Wester et al. developed an improved ^{18}F-labeling method. This procedure is based on the reaction of [^{18}F]fluorobenzaldehyde with an aminooxy-derivatized peptide, resulting in a stable oxime bond [3]. The specific activities of the radiolabeled peptides were not mentioned. Others showed that [^{18}F]fluorobenzaldehyde could also be reacted with hydrazino nicotinamide-conjugated peptides [4, 5]. The specific activity which could be achieved with an ^{18}F-labeled leukotriene B4 antagonist was 1,200 GBq/mmol [5]. To take advantage of the widespread availability of [^{18}F]FDG, two groups explored [^{18}F]FDG for labeling of aminooxy-derivatized peptides [6, 7]. Although it was shown that these functionalized peptides could be labeled with [^{18}F]FDG, these methods require the use of carrier-free [^{18}F]FDG, necessitating HPLC purification of [^{18}F]FDG before conjugation with the peptide. Specific activities were not reported. Additionally, methods based on the broadly used Huisgen cycloaddition of alkynes and azides were explored for the radiofluorination of peptides [8–12]. Specific activities varied considerably from 4,800–12,300 GBq/mmol [8] to 100,000–200,000 GBq/mmol [12]. In search for a kit-based radiofluorination method, silicon-based building blocks were used to fluorinate bombesin peptides. To improve the stability of the ^{18}F-labeled peptides, they required to be functionalized with two tertiary butyl groups. This resulted in a lipophilic ^{18}F-peptide and loss of tumor targeting [13, 14]. The maximal specific activity was 62,000 GBq/mmol. All of these methods require azeotropic drying of ^{18}F in the presence of a cryptand, such as Kryptofix (K_{222}).

We recently reported that NOTA-conjugated peptides could be labeled directly with ^{18}F using aluminum to bind ^{18}F [15–17]. With this two-step one-pot fluorination method, the peptide could be stably labeled with a 50% radiochemical yield at a high-specific activity within 45 min. Here, we present an optimization of the aluminum fluoride NOTA chelator labeling.

Materials and methods

Peptide synthesis

The octreotide peptide analog (IMP466), NOTA-D-Phe-*cyclo*[Cys-Phe-D-Trp-Lys-Thr-Cys]-Throl (MH^+ 1305), was synthesized using standard Fmoc-based solid phase peptide synthesis. After the peptide was cleaved from the resin, the peptide was cyclicized by overnight incubation with DMSO. The Throl resin and the protected amino acids were purchased from CreoSalus Inc. (Louisville, KY). The bis-*t*-butyl NOTA ligand was provided by Immunomedics, Inc. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). All buffers used for radiolabeling were metal-free.

Radiolabeling

^{18}F-labeling

A Chromafix PS-HCO_3 cartridge (ABX, Radeberg, Germany) with 4–6 GBq ^{18}F (BV Cyclotron VU, Amsterdam, The Netherlands) was washed with 3 mL of metal-free water. ^{18}F was eluted from the cartridge with 100 μL 0.9% NaCl. To the eluted Na^{18}F, 2 mM $AlCl_3$ in 0.1 M sodium acetate buffer, pH 4, was added (8.5 μl $AlCl_3$ per GBq ^{18}F). Then, 10–50 μL IMP466 (10 mg/mL) was added in 0.5 M sodium acetate (pH 4.1) and also 6 mg/mL gentisic acid. The reaction mixture was incubated at 100°C for 15 min unless stated otherwise. The radiolabeled peptide was purified on an RP-HPLC as described below. The ^{18}F-IMP466-containing fractions were collected and diluted twofold with H_2O and purified on an Oasis HLB cartridge (1 cc, 30 mg, Waters, Milford, MA) to remove acetonitrile and trifluoroacetic acid (TFA). In brief, the fraction was applied on the cartridge and the cartridge was washed with 3 mL H_2O. The radiolabeled peptide was then eluted with 2×200 μL 50% ethanol. Upon injection in mice, the peptide was diluted with 0.9% NaCl.

Effect of buffer

The effect of the buffer on the labeling efficiency of IMP466 with $^{18}F^-$ was investigated (n=3 for each buffer). IMP466 was dissolved at 10 mg/mL (7.7 mM) in sodium citrate buffer, sodium acetate buffer, 2-(N-morpholino) ethanesulfonic acid (MES), or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. The molarity of all buffers was 1 M and the pH was 4.1. To 153 nmol (200 μg) of IMP466, 100 μL Al^{18}F (pH 4.1) was added and incubated at 100°C for 15 min. Radiolabeling yield and specific activity were determined with RP-HPLC as described below.

Effect of hydrophilic organic solvent

The effect of the ionic strength on the labeling efficiency of IMP466 with ^{18}F was investigated (n=3 for each buffer). To IMP466 [100 μg (77 nmol) in 25 μL in sodium acetate buffer], 180 μL (unless stated otherwise) of acetonitrile, ethanol, dimethylformamide (DMF), or tetrahydrofuran (THF) was added [final concentration 80% (v/v)]. Finally, 20 μL Al^{18}F (pH 4) was added and the mixture was

incubated at 100°C for 15 min. Radiolabeling yield and specific activity were determined with RP-HPLC as described below.

Effect of temperature

The effect of the temperature on the labeling efficiency of IMP466 with ^{18}F was investigated (n=3 for each temperature). To IMP466 [77 nmol (100 μg) in 25 μL in sodium acetate buffer] 180 μL of acetonitrile and 20 μL "Al^{18}F" (pH 4) were added. The mixtures were incubated at 40°C, 50°C, 60°C, or 100°C for 15 min. Radiolabeling yield and specific activity were determined with RP-HPLC as described below.

HPLC analysis

The radiolabeled preparations were analyzed by RP-HPLC on an Agilent 1200 system (Agilent Technologies, Palo Alto, CA, USA). Samples containing organic solvents were diluted 50-fold before injection on HPLC. A C18 column (Onyx monolithic, 4.6×100 mm, Phenomenex, Torrance, CA, USA) was used at a flow rate of 2 mL/min with the following buffer system: buffer A, 0.1% *v*/*v* TFA in water; buffer B, 0.1% *v*/*v* TFA in acetonitrile; and gradient, 0–5 min 97% buffer A, 5–35 min 80% buffer A to 75% buffer A. The radioactivity of the eluate was monitored using an in-line NaI radiodetector (Raytest GmbH, Straubenhardt, Germany). Elution profiles were analyzed using Gina-star software (version 2.18, Raytest GmbH, Straubenhardt, Germany). Specific activity was determined by HPLC using calibration curves based on the UV signal.

Stability

Ten microliters of the ^{18}F-labeled IMP466 was incubated in 500 μL of freshly collected human serum and incubated for 4 h at 37°C. An equal volume of acetonitrile was added and the mixture was vortexed followed by centrifugation at 1,000×*g* for 5 min to pellet the precipitated serum proteins. The supernatant was analyzed on RP-HPLC as described above.

The in vivo stability of ^{18}F-IMP466 was examined by injecting 18.5 MBq of ^{18}F-IMP466 in a BALB/c nude mouse. After 30 min, the mouse was euthanized and blood and urine were collected and analyzed by HPLC.

Cell culture

The AR42J rat pancreatic tumor cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Life Technologies, Gaithersburg, MD, USA) supplemented with 4,500 mg/L D-glucose, 10% (*v*/*v*) fetal calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2.

IC_{50} determination

The apparent 50% inhibitory concentration (IC_{50}) for binding the somatostatin receptors on AR42J cells was determined in a competitive binding assay using ^{19}F-IMP466 and ^{115}In-DTPA-octreotide to compete for the binding of ^{111}In-DTPA-octreotide [16]. ^{19}F-IMP466 was formed by mixing an aluminum fluoride (AlF) solution (0.02 M $AlCl_3$ in 0.5 M NaAc, pH 4, with 0.1 M NaF in 0.5 M NaAc, pH 4) with IMP466 and heating at 100°C for 15 min. The reaction mixture was purified by RP-HPLC on a C-18 column (30×150 mm, Sunfire, Waters, Milford, MA), as described above.

^{115}In-DTPA-octreotide was made by mixing indium chloride (1×10^{-5} mol) with 10 μL DTPA-octreotide (1 mg/mL) in 50 mM NaAc, pH 5.5, and incubated at room temperature (RT) for 15 min. This sample was used without further purification. ^{111}In-DTPA-octreotide (OctreoScan®) was radiolabeled according to the manufacturer's protocol.

AR42J cells were grown to confluency in 12-well plates and washed twice with binding buffer (DMEM with 0.5% bovine serum albumin). After 10 min incubation at RT in binding buffer, ^{19}F-IMP466 or ^{115}In-DTPA-octreotide was added at a final concentration ranging from 0.1 to 1,000 nM, together with a trace amount (10,000 cpm) of ^{111}In-DTPA-octreotide (radiochemical purity >95%). After incubation at RT for 3 h, the cells were washed twice with ice-cold PBS. Cells were scraped and cell-associated radioactivity was determined. Under these conditions, some internalization may occur. We therefore describe the results of this competitive binding assay as "apparent IC_{50}" values rather than IC_{50}. The apparent IC_{50} was defined as the peptide concentration at which 50% of binding without competitor was reached. Apparent IC_{50} values were calculated using GraphPad Prism software (version 4.00 for Windows, GraphPad Software, San Diego, CA, USA).

Biodistribution studies

Male nude BALB/c mice (6–8 weeks old) were injected subcutaneously with 0.2 mL AR42J cell suspension of 1×10^7 cells/mL. When tumors were 5–8 mm in diameter, 370 kBq ^{18}F-labeled IMP466 (0.2 nmol) was administered intravenously (n=5). Separate groups of mice (n=5) were co-injected with a 1,000-fold molar excess of unlabeled IMP466. One group of three mice was injected with unchelated $(Al^{18}F)^{2+}$. All mice were killed by CO_2/O_2 asphyxiation 2 h post-injection (p.i.). Tissues of interest

were dissected, weighed, and counted in a gamma counter. The percentage of the injected dose per gram tissue was calculated. The animal experiments were approved by the local animal welfare committee and performed according to national regulations.

PET/CT imaging

Mice with s.c. AR42J tumors were injected intravenously with 10 MBq ^{18}F-IMP466 (0.7 nmol) per mouse. One and 2 h after the injection of peptide, mice were scanned on an animal PET/CT scanner (Inveon®, Siemens Preclinical Solutions, Knoxville, TN) with an intrinsic spatial resolution of 1.5 mm [18]. The animals were placed in a supine position in the scanner. PET emission scans were acquired over 15 min, followed by a CT scan for anatomical reference (spatial resolution 113 μm, 80 kV, 500 μA). Scans were reconstructed using Inveon Acquisition Workplace software version 1.5 (Siemens Preclinical Solutions, Knoxville, TN), using an ordered set expectation maximization-3D/maximum a posteriori (OSEM3D/MAP) algorithm with the following parameters: matrix 256×256×159, pixel size 0.43×0.43×0.8 mm^3, and a beta-value of 1.5.

Statistical analysis

All mean values are given ± standard deviation. Statistical analysis was performed using a Welch's corrected unpaired Student's *t* test or one-way analysis of variance using GraphPad InStat software (version 3.06, GraphPad Software). The level of significance was set at $P<0.05$.

Results

RP-HPLC analysis

As shown previously, HPLC analysis of the reaction mixture (Fig. 1) demonstrated the presence of unbound $(Al^{18}F)^{2+}$ (R_t 0.8 min) and two radioactive peptide peaks with retention times of 17.4 and 19.8 min [16]. Recent date revealed that these two peaks may be due to hindered rotation of the complex with F-18 in an axial position [19]. In addition, a UV peak of IMP466 is present (R_t 21.4 min). The radiolabeled ^{18}F-IMP66 could be obtained carrier-free after HPLC and HLB purification. This was confirmed by HPLC analysis: both the unbound $(Al^{18}F)^{2+}$ and the unlabeled IMP466 UV peaks disappeared (Fig. 1).

Effect of buffer

As reported previously, when the labeling procedure was performed using sodium acetate, MES, or HEPES, radiolabeling yields were 49±2%, 46±2%, and 48±3%, respectively (n=3 for each buffer) [16]. In sodium citrate, no radiolabeling was observed. Specific activities of the purified peptides were in the same range for all buffers used. In sodium acetate buffer, the specific activity was 32,000±17,000 GBq/mmol, whereas in MES and HEPES buffers, specific activities were 29,000±14,000 and 31,000±23,000 GBq/mmol, respectively.

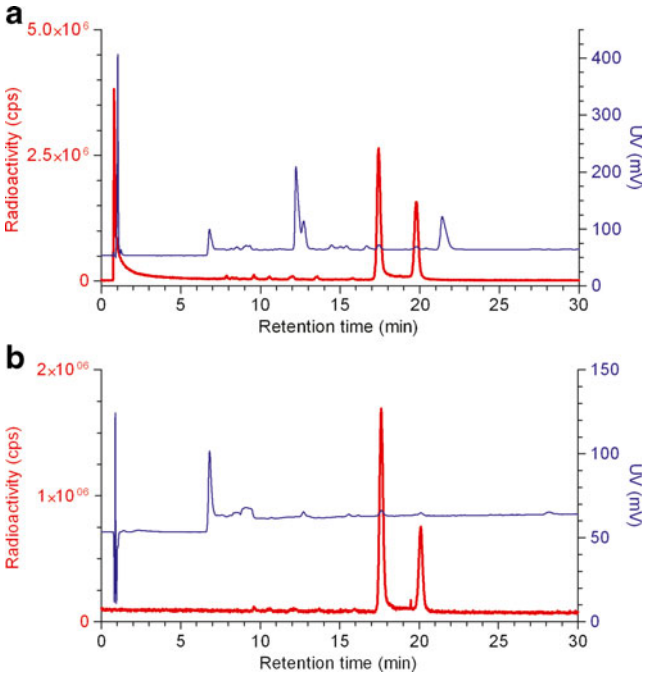

Fig. 1 RP-HPLC chromatograms of the IMP466 ^{18}F-labeling mix (**a**) and the purified ^{18}F-IMP466 (**b**). *Red traces* represent radioactivity (*left y*-axis) and *blue traces* represent UV signal (*right y*-axis). In the HPLC chromatogram of the crude mixture, unbound $Al^{18}F$ eluted with the void volume (R_t=0.8 min). Two radioactive peaks correspond to the stereoisomers of radiolabeled peptide (R_t=17.4 and R_t=19.8 min). Finally, the unlabeled IMP466 was present in the UV channel (R_t=21.4 min). After purification, only two radioactive peptide peaks are observed, indicating the formation of two stereoisomers

Effect of hydrophilic organic solvent

To investigate whether the labeling efficiency could be improved by lowering the ionic strength, the labeling reaction with $(Al^{18}F)^{2+}$ was performed in the presence of increasing concentrations of acetonitrile: 25%, 50%, 67%, or 80% (*v*/*v*) acetonitrile. Labeling efficiency at 25% was 40±5% and increased to 60±15% and 87±9% at 50% and 67%, respectively. Highest labeling efficiency was obtained at 80% (*v*/*v*) acetonitrile, 97±2%.

In addition, the effect of other organic solvents was investigated. Labeling efficiency in the presence of ethanol and DMF was 97±2% and 97±3%, respectively. Radiolabeling efficiency in THF was 92±7%. When the labeling

reaction was performed in the absence of organic solvent, the labeling efficiency was 46±7%.

Effect of temperature

The effect of the incubation temperature was studied using the optimal labeling condition described above, i.e., in the presence of 80% (*v*/*v*) acetonitrile. Labeling efficiency improved with increasing temperatures: at 40°C, the labeling efficiency was 30±21%, and at 50°C, the yield was 61±14%. At a temperature of 60°C, the labeling efficiency was 83±19%.

IC_{50} determination

We previously demonstrated that the IC_{50} was not affected by the radiofluorination [16]. Briefly, the apparent IC_{50} of $Al^{19}F$-labeled IMP466 was 3.6±0.6 nM. The apparent IC_{50} of the reference peptide, ^{115}In-DTPA-octeotride (Octreo-Scan®), was 6.3±0.9 nM. The affinity profiles are shown in Fig. 2.

Stability

In line with previous results [16], ^{18}F-labeled IMP466 did not release $(Al^{18}F)^{2+}$ after incubation in human serum at 37°C for 4 h, indicating excellent stability of the $Al^{18}F$-NOTA-octreotide.

Biodistribution studies

The biodistribution of ^{18}F-IMP466 in BALB/c nude mice with s.c. AR42J tumors at 2 h p.i. is summarized in Fig. 3 (data adapted from [16]). Unchelated $(Al^{18}F)^{2+}$ was included as a control. Tumor uptake of ^{18}F-IMP466 was

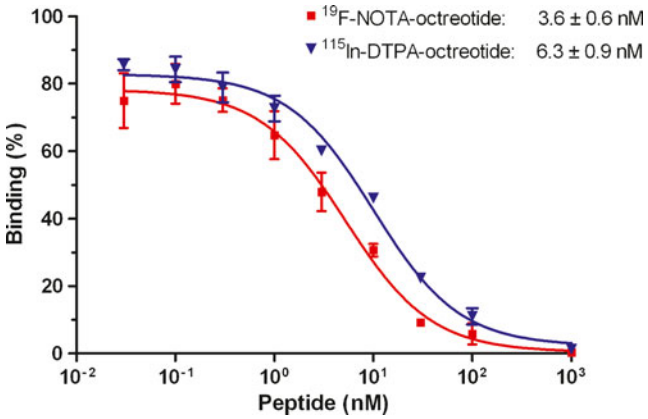

Fig. 2 Competitive binding assay (apparent IC_{50}) of ^{19}F-IMP466 and ^{115}In-DTPA-octreotide determined on AR42J tumor cells. Values on the *y*-axis represent binding expressed as a percentage of the binding without competitor

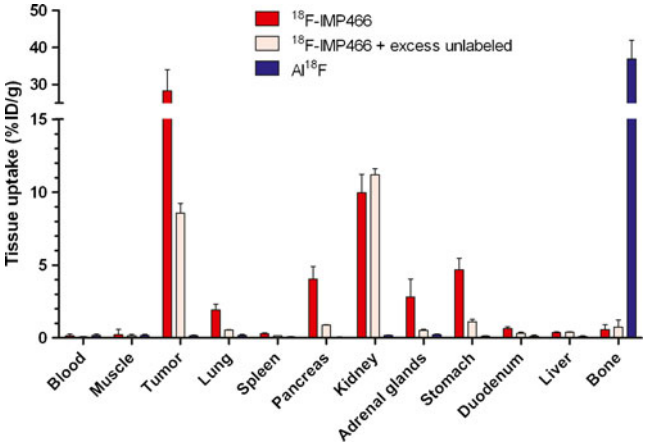

Fig. 3 Biodistribution of ^{18}F-IMP466 and unbound $Al^{18}F$ at 2 h p.i. in AR42J tumor-bearing mice (n=5/group). Tumors weighed 0.07–0.36 g

28.3±5.7% ID/g at 2 h p.i. Tumor uptake in the presence of an excess of unlabeled IMP466 was significantly decreased (8.6±0.7% ID/g, $P<0.002$), indicating that tumor uptake was receptor-mediated. Blood levels were low (0.10±0.07% ID/g, 2 h p.i.), which resulted in a tumor-to-blood ratio of 300±90. Uptake in normal tissues, except in the kidneys, was low. Receptor-mediated uptake was observed in SST_2 receptor-expressing tissues, such as adrenal glands, pancreas, and stomach. Bone uptake of ^{18}F-IMP466 was very low as compared to uptake after injection of non-chelated $(Al^{18}F)^{2+}$ (0.33±0.07 vs. 36.9±5.0% ID/g at 2 h p.i., respectively; $P<0.001$), indicating good in vivo stability of the ^{18}F-IMP466.

Fused PET and CT scans are shown in Fig. 4. ^{18}F-IMP466 showed high uptake in the tumor and high retention in the kidneys where the activity was localized mainly in the renal cortex. In addition, some intestinal uptake was observed. The PET/CT scans also demonstrated that the $(Al^{18}F)^{2+}$ was stably chelated by the NOTA chelator, since no bone uptake was observed.

Discussion

Radiolabeling of peptides with fluorine-18 generally involves laborious and time-consuming procedures and first requires the synthesis of an ^{18}F-labeled synthon. In our initial studies, we have shown that a NOTA-conjugated pretargeting peptide (IMP449) could be labeled with $Al^{18}F$ to yield a product with a good biodistribution profile [17]. These studies were performed with $^{18}F^-$ that was eluted from a QMA cartridge with $KHCO_3$ and required careful neutralization with acetic acid in an effort to control the pH. Subsequently, we found that the varying radiochemical yields arising during the neutralization could be avoided if the $^{18}F^-$ was eluted with 0.9% saline [15].

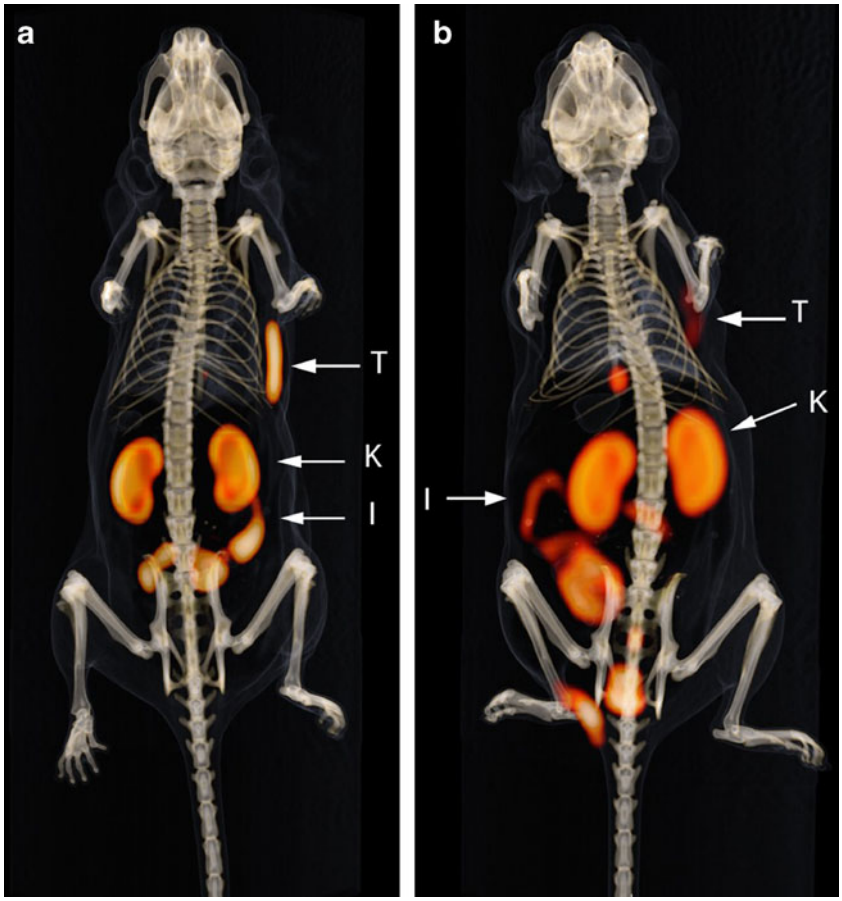

Fig. 4 Anterior 3D volume-rendering projections of fused PET and CT scans of mice with a s.c. AR42J tumor on the right flank injected with ^{18}F-IMP466 (**a**) and with ^{18}F-IMP466 in the presence of an excess of unlabeled IMP466 (**b**). "*T*" indicates tumor, "*K*" indicates kidneys, and "*I*" indicate intestine. Scans were recorded at 2 h p.i

In the present study, we used a NOTA which was covalently linked to the peptide, using one of the carboxylic groups. Recent findings indicated that this chelator performed equally well as the isothiocyanato-benzyl derivative of NOTA [16, 17]. We found that the labeling of NOTA-octreotide failed in the presence of sodium citrate buffer. This might be due to the high affinity of citric acid for Al (III), as described by Rajan et al. [20]. Recently, we obtained considerable improvements in radiochemical yields when the labeling was performed in the presence of an organic hydrophilic solvent [19]. Herein we describe the optimization of the ^{18}F-labeling of NOTA-octreotide (IMP466).

The labeling yield improved considerably when performing the reaction at a lower ionic strength using either acetonitrile, ethanol, or DMF. For subsequent experiments, we used acetonitrile which could easily be evaporated after the labeling reaction. Lowering the amount of acetonitrile resulted in lower labeling yields. Obviously, for future clinical studies, the use of ethanol is preferred over acetonitrile.

The peptide concentration plays a crucial role in the Al^{18}F-NOTA-labeling reaction. A labeling yield of 52% was obtained at a peptide concentration of 204 μM in the presence of 67% acetontirile. In a previous study [16], a peptide concentration of 1,815 μM was required to obtain a 50% labeling efficiency. This optimal concentration is approximately tenfold lower as reported for other peptide fluorination methods [1, 3, 5].

The radiolabeled peptide could be obtained carrier-free after preparative HPLC separation on a monolithic C-18 column. Purification on a cartridge was not feasible due to the small difference in organic solvent concentration required to separate the labeled from the unlabeled peptide on HPLC.

We demonstrated that the affinity of ^{18}F-NOTA-octreotide was at least as good as that of ^{111}In-DTPA-octreotide and was comparable with values reported in literature for DOTA-octreotate and DOTA-TOC [21].

The biodistribution of the ^{18}F-NOTA-octreotide was studied in AR42J tumor-bearing mice. ^{18}F-IMP466 showed a high tumor uptake at 2 h p.i., with lower uptake in all other organs. The in vivo studies also showed the excellent stability of the Al^{18}F–NOTA complex, since no significant bone uptake could be measured, and the intact product was isolated in the urine.

The current method can be performed in one pot, is fast (45 min), yields carrier-free fluorinated peptide in nearly quantitative yield, and does not affect the pharmacokinetics of octreotide. In most ^{18}F-labeling strategies for peptides

and proteins, a fluorinated synthon such as succinimidyl-[^{18}F]fluorobenzoate [1], 4-[^{18}F]fluorobenzaldehyde [3], and 2-[^{18}F]fluoropropionic acid 4-nitrophenyl ester [22] needs to be synthesized first. In general, these fluorination methods are based on a nucleophilic substitution which requires laborious azeotropic drying of the [^{18}F]fluoride/kryptofix complex. Subsequently, these synthons are reacted with the (functionalized) peptide, leading to longer synthesis times and lower overall yields.

More recently, a method based on Si-F has been published, in which the ^{18}F is bound to a silicon-containing building block in a single step [13, 14]. Although somewhat similar to our approach, the Si-^{18}F initially proved to be unstable, but could be stabilized by the addition of tertiary butyl groups. This, however, leads to a strong increase in lipohilicity (log *P*, 1.3±0.1).

Finally, "click" chemistry has been explored for the radiofluorination of peptides [9–11]. Although the yield of these click chemistry-based labeling procedures based on the alkyne-azide cycloaddition is excellent (>80%), the method starts with the fluorination of an azide or alkyne, such as fluoro(ethyl)azide or a fluoroalkyne. This requires azeotropic drying of the fluoride, resulting in a time-consuming multistep procedure. Recently, the radiosynthesis of a [^{18}F]fluoroethyl triazole-labeled [Tyr^3]octreotate has been described using a copper-catalyzed azide-alkyne cycloaddition reaction [8]. The [^{18}F]fluoroethyl azide was produced in 50% decay-corrected yields and the click reaction proceeded in 5 min at room temperature in 50–66% decay-corrected yields.

Compared to a ^{68}Ga labeling, the Al^{18}F method is easy and versatile, mainly due to the fact that both methods are based on a chelator-derivatized peptide. One of the advantages of the AlF method is the longer half-life of ^{18}F, allowing PET scanning at later timepoints after injection of the tracer.

Conclusion

In conclusion, our new approach combines the ease of chelator-based radiolabeling methods with the advantages of ^{18}F (i.e., half-life, availability, and positron energy). The Al^{18}F-labeled NOTA-octreotide could be synthesized carrier-free in quantitative yields in <45 min without the need to synthesize an ^{18}F synthon. Moreover, the fluorinated peptide was stable in vitro and in vivo and has excellent tumor-targeting properties. Therefore, this fluorination method is a promising facile and versatile fluorination procedure.

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Conflicts of interest WJM, CAD'S, and DMG are employed or have financial interest in Immunomedics, Inc.

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