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A novel facile method of labeling octreotide with ^{18}F-fluorine

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Abstract

Several methods have been developed to label peptides with fluorine-18. However, in general these are laborious and require a multistep synthesis. We present 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). The method is characterized by labeling NOTA-octreotide (IMP466) with ^{18}F.

Methods—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, peptide, and aluminum concentration. Radiochemical yield, specific activity, *in vitro* stability, and receptor affinity were determined. Biodistribution of ^{18}F-IMP466 was studied in AR42J tumor-bearing mice and compared to that of ^{68}Ga-labeled IMP466. In addition, microPET/CT images were acquired.

Results—IMP466 was labeled with "Al^{18}F" in a single step with 50% yield. The labeled product was purified by HPLC to remove unbound "Al^{18}F" and unlabeled peptide. The radiolabeling, including purification, was performed in 45 min. The specific activity was 45,000 GBq/mmol and the peptide was stable in serum for 4 h at 37° C. Labeling was performed at pH 4.1 in sodium citrate, sodium acetate, HEPES and MES buffer and was optimal in sodium acetate buffer. The apparent IC_{50} of the ^{19}F-labeled IMP466 determined on AR42J cells was 3.6 nM. Biodistribution studies at 2 h p.i. showed a high tumor uptake of ^{18}F-IMP466 (28.3 ± 5.2 %ID/g, tumor-to-blood ratio: 300 ± 90), which could be blocked by an excess of unlabeled peptide (8.6 ± 0.7%ID/g), indicating that the accumulation in the tumor was receptor-mediated. Biodistribution of ^{68}Ga-IMP466 was similar to that of ^{18}F-IMP466. ^{18}F-IMP466 was stable *in vivo*, since bone uptake was only 0.4 ± 0.2 %ID/g, whereas free "Al^{18}F" accumulated rapidly in the bone (36.9 ± 5.0 %ID/g at 2 h p.i.). MicroPET/CT scans showed excellent tumor delineation and high preferential accumulation in the tumor.

Conclusions—NOTA-octreotide could be labeled rapidly and efficiently with ^{18}F using a 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 with ^{18}F.

Keywords

octreotide; radiofluorination; NOTA; peptide; PET; aluminum fluoride

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INTRODUCTION

During the past decade, 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, more recently, with positron emitters like ^{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: the half-life (110 min) matches the pharmacokinetics of most peptides and the low positron energy of 635 keV results in short ranges in tissue and excellent preclinical imaging resolution (< 2 mm) (1). A wide range of methods to label peptides with ^{18}F have been investigated. In general, an ^{18}F-labeled synthon is produced by nucleophilic substitution, which is subsequently reacted with the functionalized peptide. One of the first generally applicable methods is based on conjugation of the synthon, N-succinimidyl-4-[^{18}F]fluorobenzoate to a primary amino group of the peptide (2). Although widely used, the method requires a multi-step synthesis and therefore is time-consuming and laborious. Wester et al. have developed an improved ^{18}F-labeling method by reacting [^{18}F]fluorobenzaldehyde with an aminooxy-derivatized peptide forming an oxime bond (3). Alternatively, [^{18}F]fluorobenzaldehyde was reacted with hydrazine-nicotinamide-conjugated peptides. However, this method resulted in increased lipophilicity of the peptide that may lead to unfavorable characteristics *in vivo* (4). Carbohydration of the peptide may counteract the enhanced lipophilicity (5). More recently [^{18}F]FDG was explored for labeling of aminooxy-derivatized peptides (6), (7). This approach requires the use of carrier-free [^{18}F]FDG, necessitating HPLC purification of [^{18}F]FDG before conjugation with the functionalized peptide. Furthermore, methods based on the Huisgen cycloaddition of alkynes and azides were explored for the fluorination of peptides (8), (9), (10), (11). Recently, silicon-based building blocks were used to fluorinate bombesin peptides functionalized with two tertiary butyl groups. However, this method also resulted in a lipophilic ^{18}F-peptide and loss of tumor targeting (12), (13).

We reported recently that a NOTA-conjugated pretargeting peptide could be labeled directly with ^{18}F (14). To demonstrate that this two-step, one-pot, method can be applied to other peptides, we report a new approach to label the somatostatin analogue, NOTA-octreotide with ^{18}F. Radiolabeled somatostatin analogues, such as DTPA-octreotide, DOTA-Tyr^3-octreotide, NOTA-octreotide and DOTA-Tyr^3-octreotate, can be used to image $SSTR_2$-receptor-expressing tumors. 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.

MATERIALS AND METHODS

General

The octreotide peptide analog, NOTA-D-Phe-*cyclo*[Cys-Phe-D-Trp-Lys-Thr-Cys]-Throl (MH^+ 1305), designated IMP466, was synthesized using standard Fmoc-based solid phase peptide synthesis. After cleavage from the resin, the peptide was cyclicized by incubation with DMSO overnight. 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 the radiolabeling procedures were metal-free.

Radiolabeling

^{18}F-labeling—The reaction is summarized in Figure 1. A QMA SepPak light cartridge (Waters, Milford, MA) with 2–6 GBq ^{18}F (BV Cyclotron VU, Amsterdam, The Netherlands) was washed with 3 mL metal-free water. ^{18}F was eluted from the cartridge with 0.4 M $KHCO_3$ and fractions of 200 μL were collected. The pH of the fractions was adjusted to pH 4, with 10 μL metal-free glacial acetic acid. Three μL of 2 mM $AlCl_3$ in 0.1 M sodium acetate buffer, pH 4 were added. Then, 10–50 μL IMP466 (10 mg/mL) were added in 0.5 M sodium acetate, pH 4.1. The reaction mixture was incubated at 100° C for 15 min unless stated otherwise. The radiolabeled peptide was purified on RP-HPLC as described below. The ^{18}F-IMP466-containing fractions were collected and diluted two-fold with H_2O and purified on a 1-cc Oasis HLB cartridge (Waters, Milford, MA) to remove acetonitrile and 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 in sodium citrate buffer, sodium acetate buffer, 2-(N-morpholino)ethanesulfonic acid (MES) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer at 10 mg/mL (7.7 mM). The molarity of all buffers was 1 M and the pH was 4.1. To 200 μg (153 nmol) of IMP466, 100 μL "Al^{18}F" (pH 4) were added and incubated at 100 °C for 15 min. Radiolabeling yield and specific activity were determined with RP-HPLC as described below.

^{68}Ga-labeling

IMP466 was labeled with $^{68}GaCl_3$ eluted from a TiO_2-based 1,110 MBq ^{68}Ge/^{68}Ga generator (Cyclotron Co. Ltd., Obninsk, Russia) using 0.1 M HCl (Ultrapure, J.T. Baker, Deventer, The Netherlands). Five 1-ml fractions were collected and an aliquot of the second fraction was used for labeling the peptide. IMP466 (4 μg) was dissolved in 2.5 M HEPES buffer, pH 7.0. ^{68}Ga eluate (120–240 MBq, 4 times the volume of the peptide) was added and the mixture was heated at 95° C for 20 min. Then 50 mM EDTA was added to a final concentration of 5 mM to complex the non-incorporated $^{68}Ga^{3+}$. The ^{68}Ga-labeled IMP466 was purified on a 1-cc Oasis HLB cartridge and eluted with 200 μL 50% ethanol. Specific activity of ^{68}Ga-IMP466 was 20,000 GBq/mmol at time of injection.

HPLC analysis

The radiolabeled preparations were analyzed by RP-HPLC on an Agilent 1200 system (Agilent Technologies, Palo Alto, CA, USA). 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; 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.

Octanol-water partition coefficient (log $P_{octanol/water}$)

To determine the lipophilicity of the radiolabeled peptides, approximately 50,000 cpm of the radiolabeled peptide were diluted in 0.5 mL phosphate-buffered saline (PBS). An equal volume of 1-octanol was added to obtain a binary phase system. After vortexing the mixture for 2 min, the two layers were separated by centrifugation (100 × g, 5 min). Three 100-μL

samples were taken from each layer and radioactivity was measured in a well-type gamma counter (Wallac Wizard 3", Perkin-Elmer, Waltham, MA).

Stability

Ten μL of the ^{18}F-labeled IMP466 were 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 1000 × 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, 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 4500 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, ^{69}Ga-IMP466 or ^{115}In-DTPA-octreotide to compete for the binding of ^{111}In-DTPA-octreotide.

^{19}F-IMP466 was formed by mixing an aluminium 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.

^{69}Ga-IMP466 was prepared by dissolving gallium nitrate (2.3×10^{-8} mol) in 30 μL mixed with 20 μL IMP466 (1 mg/mL) in 10 mM NaAc, pH 5.5, and heated at 90° C for 15 min. Samples of the mixture were used without further purification.

^{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, ^{69}Ga-IMP466 or ^{115}In-DTPA-octreotide was added at a final concentration ranging from 0.1–1000 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, a limited extent of internalization may occcur. 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 in the right flank with 0.2 mL AR42J cell suspension of 1×10^7 cells/mL. Approximately two weeks after inoculation, when tumors were 5–8 mm in diameter, 370 kBq ^{18}F-labeled or ^{68}Ga-labeled IMP466 (both 0.2 nmol) were 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". 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 (%ID/g) was calculated for each tissue based on a dilution of the product for injection. 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 or ^{68}Ga-IMP466 (both 0.7 nmol) per mouse. One and two hours 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 (1). 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 1.2 (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 0.1.

Statistical analysis

All mean values are given ± standard deviation (S.D.). 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

^{18}F-labeling procedure

HPLC analysis of the IMP466 labeling mixture (Figure 2) showed the presence of unbound "Al^{18}F" (R_t 0.8 min) and two radioactive peptide peaks (R_t 17.4 and 19.8 min), indicating the formation of two ^{18}F-IMP466 stereoisomers. Moreover, a UV peak of unlabeled IMP466 is present (R_t 21.4 min). After HPLC and HLB purification, both the unbound "Al^{18}F" and the unlabeled IMP466 UV peaks disappeared (Figure 2).

Effect of buffer—When using sodium acetate, MES, or HEPES, radiolabeling yield was 49 ± 2%, 46 ± 2%, and 48 ± 3%, respectively (n=3 for each buffer). In sodium citrate, no labeling was observed. When the labeling reaction was carried out in sodium acetate buffer, the specific activity was 32,000 ± 17,000 GBq/mmol, whereas in MES and HEPES buffers specific activities of 29,000 ± 14,000 and 31,000 ± 23,000 GBq/mmol respectively. were obtained.

Effect of peptide concentration—The effect of peptide concentration on the labeling efficiency also was investigated. IMP466 was dissolved in sodium acetate buffer, pH 4.1, at a concentration of 7.7 mM (10 mg/mL). Either 38, 153, or 363 nmol of IMP466 were added

to 200 μL "Al^{18}F" (581–603 MBq) to yield final IMP466 concentrations of 190, 765, and 1815 μM, respectively. The radiolabeling yield increased with increasing amounts of peptide. At a concentration of 190 μM, radiolabeling yield was 8 ± 1 %, at 765 μM, the yield increased to 42 ± 3 %, and at the highest concentration the radiolabeling yield was 50 ± 2%. Specific activity of the products obtained at each concentration was 48,000 GBq/mmol.

Effect of $AlCl_3$ concentration—Since $AlCl_3$ is used to form "Al^{18}F", the added amount of $AlCl_3$ is critical in the labeling procedure. Five stock solutions with various $AlCl_3$ concentrations were prepared: 0.2, 0.5, 1.0, 2.0 and 20 mM. From these solutions in sodium acetate, 3 μL was added to 200 μL of [^{18}F]-fluoride, pH 4 to form [^{18}F]aluminum fluoride, resulting in final amounts of $AlCl_3$ added of 0.6, 1.5, 3.0, 6.0 and 60 nmol, respectively. To these samples, 153 nmol of IMP466 (final concentration 765 μM) were added and incubated for 15 min at 100 °C. Radiolabeling yield was optimal (50 ± 2%, n=5) after incubation with 6 nmol $AlCl_3$. Lowering the $AlCl_3$ concentration resulted in reduced yields, ranging from 42% at 3 nmol to 10% at 0.6 nmol $AlCl_3$. Increasing the amount lead to a similar effect. Incubation with 60 nmol $AlCl_3$ resulted in a radiolabeling yield of only 6%.

Octanol-water partition coefficient

To determine the lipophilicity of the ^{18}F- and ^{68}Ga-labeled IMP466, the octanol-water partition coefficients were determined. The log $P_{octanol/water}$ value for the ^{18}F-IMP466 was −2.44 ± 0.12 and that of ^{68}Ga-IMP466 was −3.79 ± 0.07, indicating that the ^{18}F-IMP466 was slightly less hydrophilic than ^{68}Ga-IMP466.

IC_{50} determination

The affinity profiles are shown in Figure 3. The apparent IC_{50} of Al^{19}F-labeled IMP466 was 3.6 ± 0.6 nM, whereas that for ^{69}Ga-labeled IMP466 was 13 ± 3 nM. The apparent IC_{50} of the reference peptide, ^{115}In-DTPA-octeotride (OctreoScan®), was 6.3 ± 0.9 nM.

Stability

^{18}F-labeled IMP466 did not release "Al^{18}F" after incubation in human serum at 37° C for 4 h, indicating excellent stability of the "Al^{18}F"-NOTA-octreotide. These findings were confirmed *in vivo*. After 30 min, only intact radiolabeled peptide was found in urine (Figure 4) and no free fluorine-18. In addition, PET/CT scans did not reveal any bone uptake, indicating the absence of free ^{18}F-fluoride or "Al^{18}F".

Biodistribution studies

The biodistribution of both ^{18}F-IMP466 and ^{68}Ga-IMP466 in BALB/c nude mice with s.c. AR42J tumors at 2 h p.i. is summarized in Figures 5 and 6. Unchelated "Al^{18}F" was included as a control. Tumor uptake of ^{18}F-IMP466 was 28.3 ± 5.7% ID/g at 2 h p.i., and reduced in the presence of an excess of unlabeled IMP466 to 8.6 ± 0.7% ID/g, indicating that uptake was receptor-mediated. Non-specific uptake of ^{18}F-IMP466 was somewhat higher than that of ^{68}Ga-IMP466, as is illustrated by the less efficient blocking of the tumor uptake of ^{18}F-IMP466. Blood levels were very low (0.10 ± 0.07% ID/g, 2 h p.i.), resulting in a tumor-to-blood ratio of 300 ± 90. Uptake in normal tissues, except in the kidneys, was low, with specific uptake in SST_2 receptor-expressing tissues, such as adrenal glands, pancreas and stomach. Bone uptake of ^{18}F-IMP466 was negligible as compared to uptake after injection of non-chelated "Al^{18}F" (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.

Tumor uptake of ^{68}Ga-IMP466 (29.2 ± 0.5% ID/g, 2 h p.i.) was similar to that of ^{18}F-IMP466 (P = 0.7). Lung uptake of ^{68}Ga-IMP466 was two-fold higher than that of ^{18}F-IMP466 (4.0 ± 0.9% ID/g vs. 1.9 ± 0.4% ID/g, respectively). In addition, kidney retention of ^{68}Ga-IMP466 was significantly higher than that of ^{18}F-IMP466 (16.2 ± 2.86% ID/g vs. 10.0 ± 1.3% ID/g, respectively; P< 0.01).

Fused PET and CT scans are shown in Figure 7. PET scans largely corroborated the biodistribution data. Both ^{18}F-IMP466 and ^{68}Ga-IMP466 showed high uptake in the tumor and high retention in the kidneys. The activity in the kidneys was localized mainly in the renal cortex. Some intestinal uptake was observed on the scans of ^{18}F-IMP466. The PET/CT scans also demonstrated that the "Al^{18}F" was stably-chelated by the NOTA chelator, since no bone uptake was observed.

DISCUSSION

Radiolabeling of peptides with fluorine-18 involves laborious and time-consuming procedures and first requires the synthesis of a ^{18}F-labeled synthon. We showed recently that a NOTA-conjugated pretargeting peptide (IMP449) could be labeled with ^{18}F in a two-step, one-pot, reaction by creating [^{18}F]aluminumfluoride (14). Here, we describe the optimized ^{18}F-labeling of NOTA-conjugated octreotide (IMP466) using the same approach. The biodistribution of the ^{18}F-NOTA-octreotide was compared to that of ^{68}Ga-NOTA-octreotide. 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 (15).

In the present study, we used the directly-coupled NOTA derivative rather than the NCS-derivatized NOTA to diminish the effect of the phenyl group on the lipophilicity of the peptide. For ^{68}Ga labeling it has been demonstrated that this NOTA variant is as good as the NCS-activated NOTA (16). After preparing the "Al^{18}F" complex, the chelation of this complex by NOTA was investigated in various buffers. We showed that chelation of "Al^{18}F" could be carried out in sodium acetate, MES and HEPES buffer but, remarkably, the chelation failed in sodium citrate buffer. The labeling reaction requires fairly high NOTA-peptide concentrations. At the concentrations studied, the reaction was most efficient at an IMP466 concentration of 1.8 mM. This optimal concentration is in the same range as reported for other peptide fluorination methods (3), (4), but lower than the optimal concentration required in the 'classical' SFB method (2).

Subsequently, we investigated the effect of the amount of Al^{3+} on the radiolabeling yield. The Al^{3+} concentration greatly affects the labeling yield. First, the $^{18}F^{-}$ complexes with the Al^{3+}, then the "Al^{18}F" complex is chelated by NOTA. After incubation at elevated temperature, the reaction mixture is purified using HPLC to separate the "Al^{18}F"-labeled IMP466 from: i) unchelated "Al^{18}F", ii) Al^{3+}-labeled IMP466 and iii) unlabeled IMP466. We showed that the optimal concentration of $AlCl_3$ was 2 mM, whereas both at lower and at higher concentrations, the radiolabeling yield decreased. These data indicate that there is a delicate balance between $^{18}F^{-}$ and Al^{3+}. Lowering the amount of Al^{3+} will lead to less ^{18}F associated with Al^{3+}. In contrast, a higher amount will yield more undesired NOTA-peptide labeled with Al^{3+}, rather than labeled with "Al^{18}F". Since the formation of "Al^{18}F" is carried out at pH 4.1, we believe that the form of the Al in sodium acetate buffer is probably $Al^{3+}(AcO^{-})_3F^{-}$. In any case, the mole ratio of Al to F-18 is such that it is unlikely that any AlF_2 or AlF_3 is formed (17). We attempted to isolate the two isomers, but observed that there was a rapid equilibrium between them. Therefore, no further studies of separate isomers were possible.

The lipophilicity of both the ^{18}F-labeled and ^{68}Ga-labeled IMP466 was in the same range as reported for other ^{111}In-labeled octreotide analogues (18). Other ^{18}F-labeled octreotide analogues, such as the [^{18}F]fluoropropionyl octreotide (log P −0.07 ± 0.01) and N$^{\alpha}$-(1-deoxy-D-fructosyl)-N$^{\varepsilon}$-(2-[^{18}F]fluoropropionyl)-Lys^{0}-Tyr^{3}octreotate ([^{18}F]FP-Gluc-TOCA) (log P −1.70 ± 0.02), displayed a higher lipophilicity (19). Biodistribution of [^{18}F]FP-Gluc-TOCA was similar to the biodistribution of ^{18}F-IMP466, but tumor uptake of the latter compound was two-fold higher than that of the carbohydrate analogue.

The biodistribution of ^{18}F-IMP466 was very similar to that of ^{68}Ga-IMP466, indicating that the "Al^{18}F" complex did not affect the *in vivo* characteristics of octreotide. Both peptides showed an equally 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, and does not affect the pharmacokinetics of octreotide. In most ^{18}F-labeling strategies for peptides and proteins, a fluorinated synthon needs to be synthesized first. In general, this fluorination is based on a nucleophilic substitution which requires laborious azeotropic drying of the [^{18}F]fluoride/kryptofix complex. Examples of such a synthon are succinimidyl-[^{18}F]fluorobenzoate (2), 4-[^{18}F]fluorobenzaldehyde (3), and 2-[^{18}F]fluoropropionic acid 4-nitrophenyl ester (20). Subsequently, the synthon is reacted with the (functionalized) peptide, leading to longer synthesis times and lower overall yields. In addition, these techniques also lead to increased lipophilicity, because most synthons contain aromatic groups and this might affect the biodistribution profile. It has been reported that this effect can be counteracted by carbohydration (5). However, this requires considerable peptide modification.

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 (12), (13). 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, lead to a strong increase in lipohilicity (log P: 1.3 ± 0.1).

Finally, 'click' chemistry has been explored for the radiofluorination of peptides (8), (9), (10). 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.

Compared to a ^{68}Ga labeling, the "AlF" 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 time-points 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 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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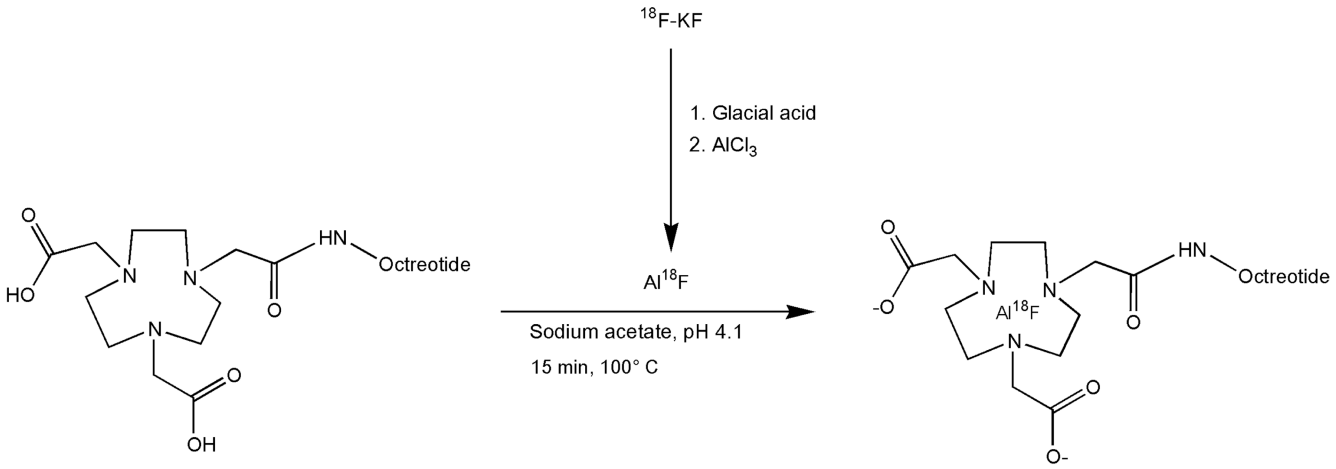

Figure 1.
Preparation of "Al^{18}F" and chelation with NOTA-octreotide.

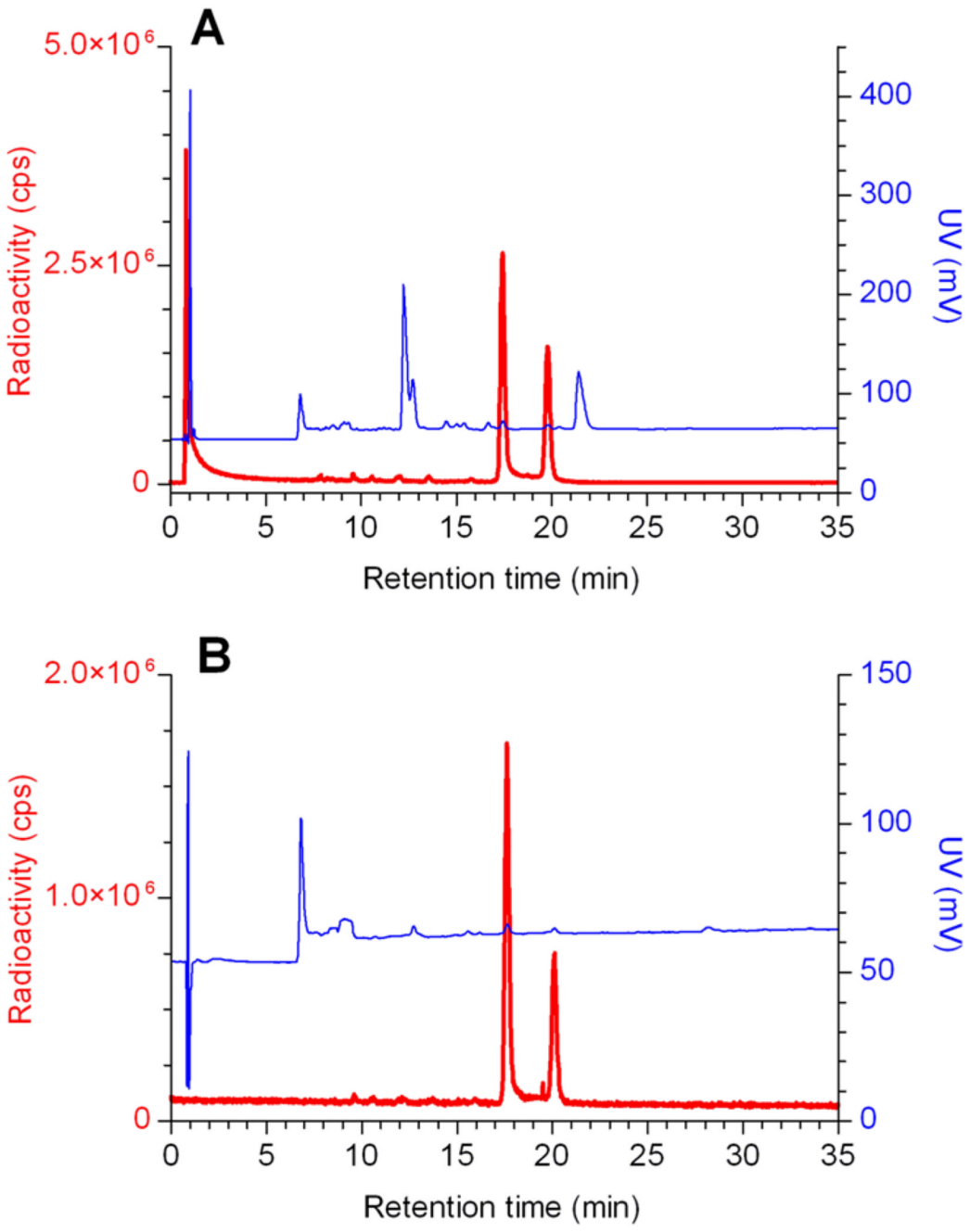

Figure 2.

RP-HPLC chromatograms of the IMP466 ^{18}F-labeling mix (panel A) and the purified ^{18}F-IMP466 (panel B). Red traces represents radioactivity(left y-axis) and blue traces represents 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 times (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.

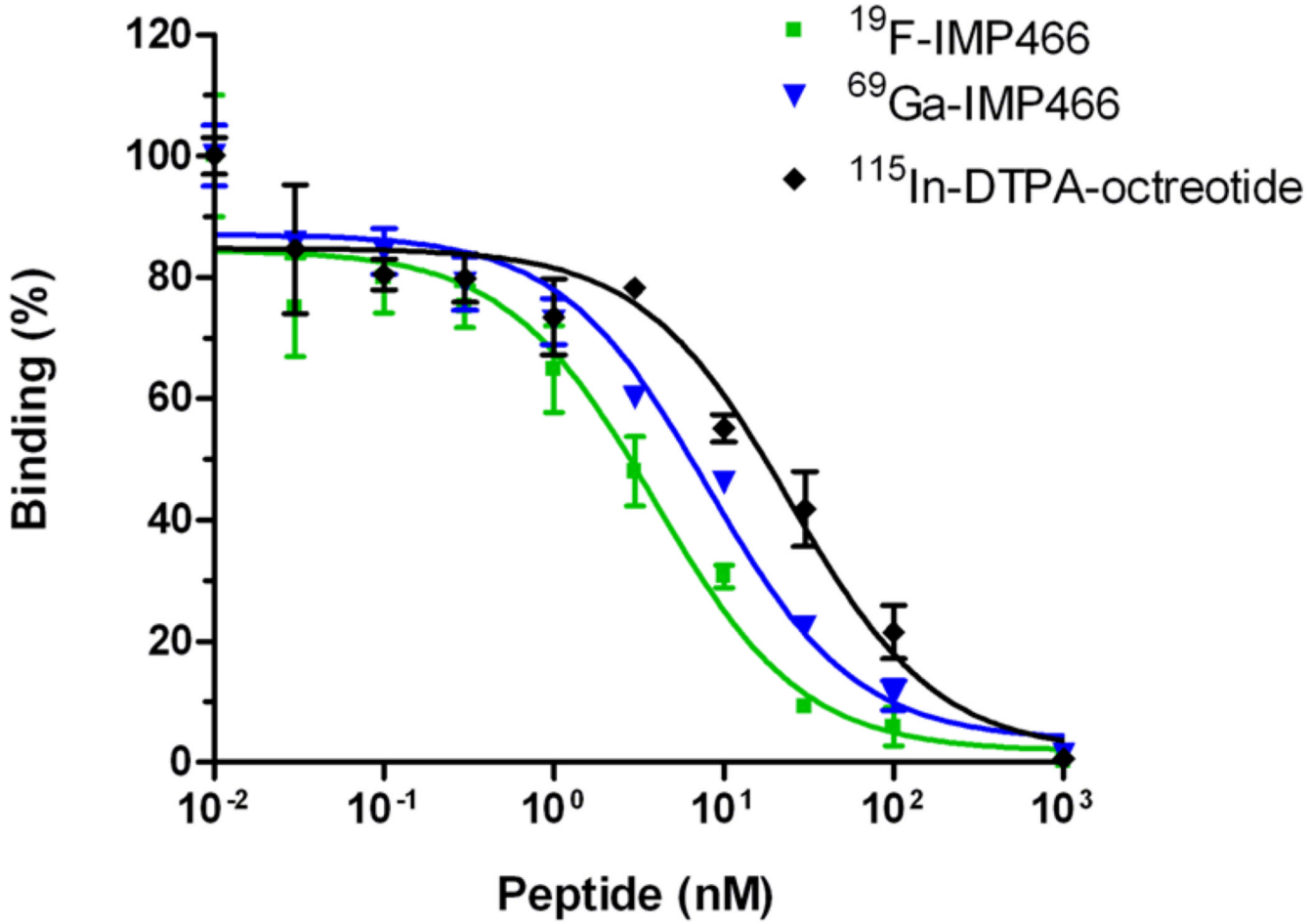

Figure 3.
Competitive binding assay (apparent IC_{50}) of ^{19}F-IMP466, ^{69}Ga-IMP466 and ^{115}In-DTPA-octreotide determined on AR42J tumor cells. Values on the y-axis represent binding expressed as a precentage of the binding without competitor.

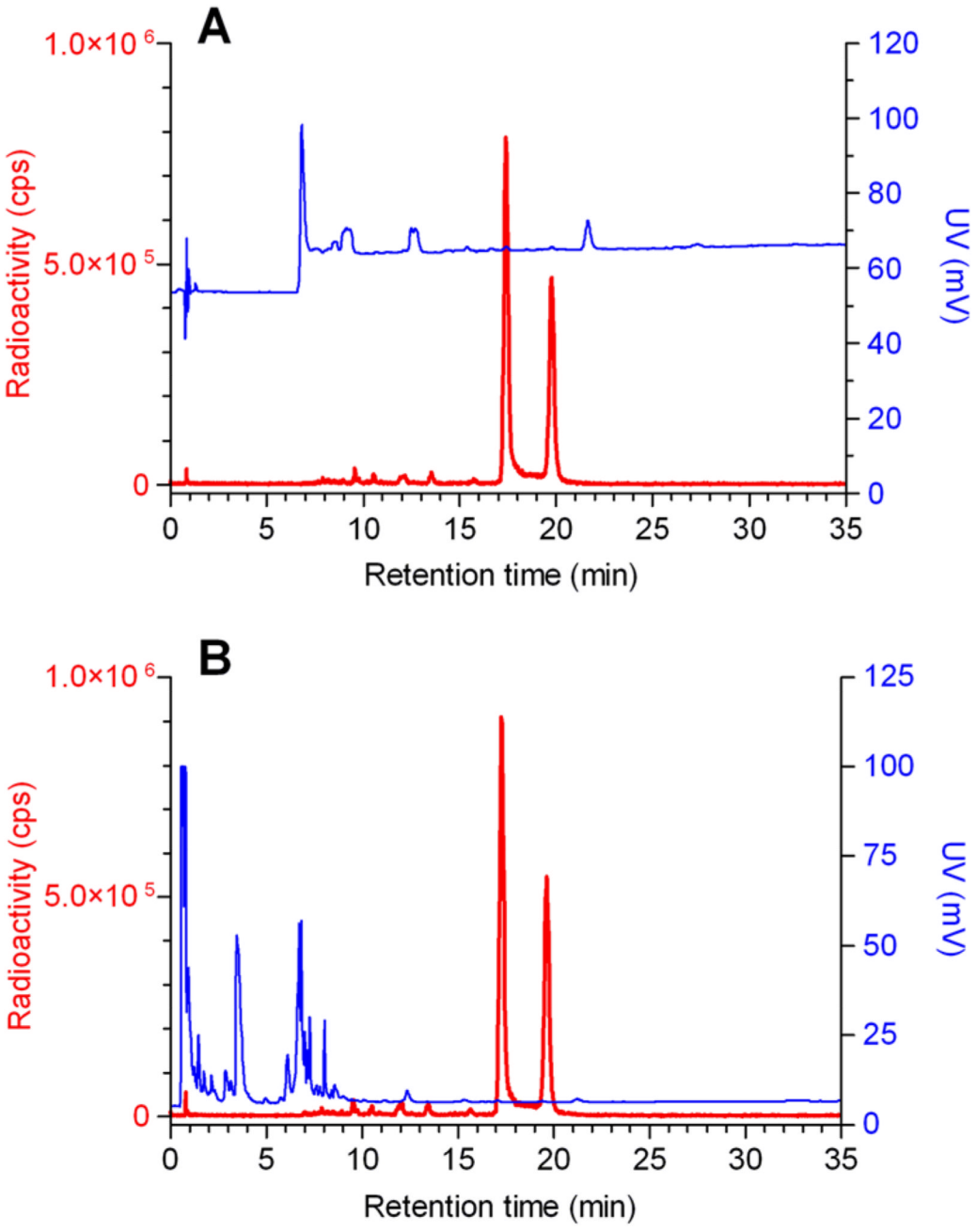

Figure 4.

HPLC chromatograms of purified ^{18}F-IMP466 before injection (panel A) and a urine sample 30 min p.i. (panel B). Red traces represents radioactivity(left y-axis) and blue traces represents UV signal (right y-axis). The HPLC traces of the two samples are very similar, indicating that the excreted product is intact ^{18}F-IMP466.

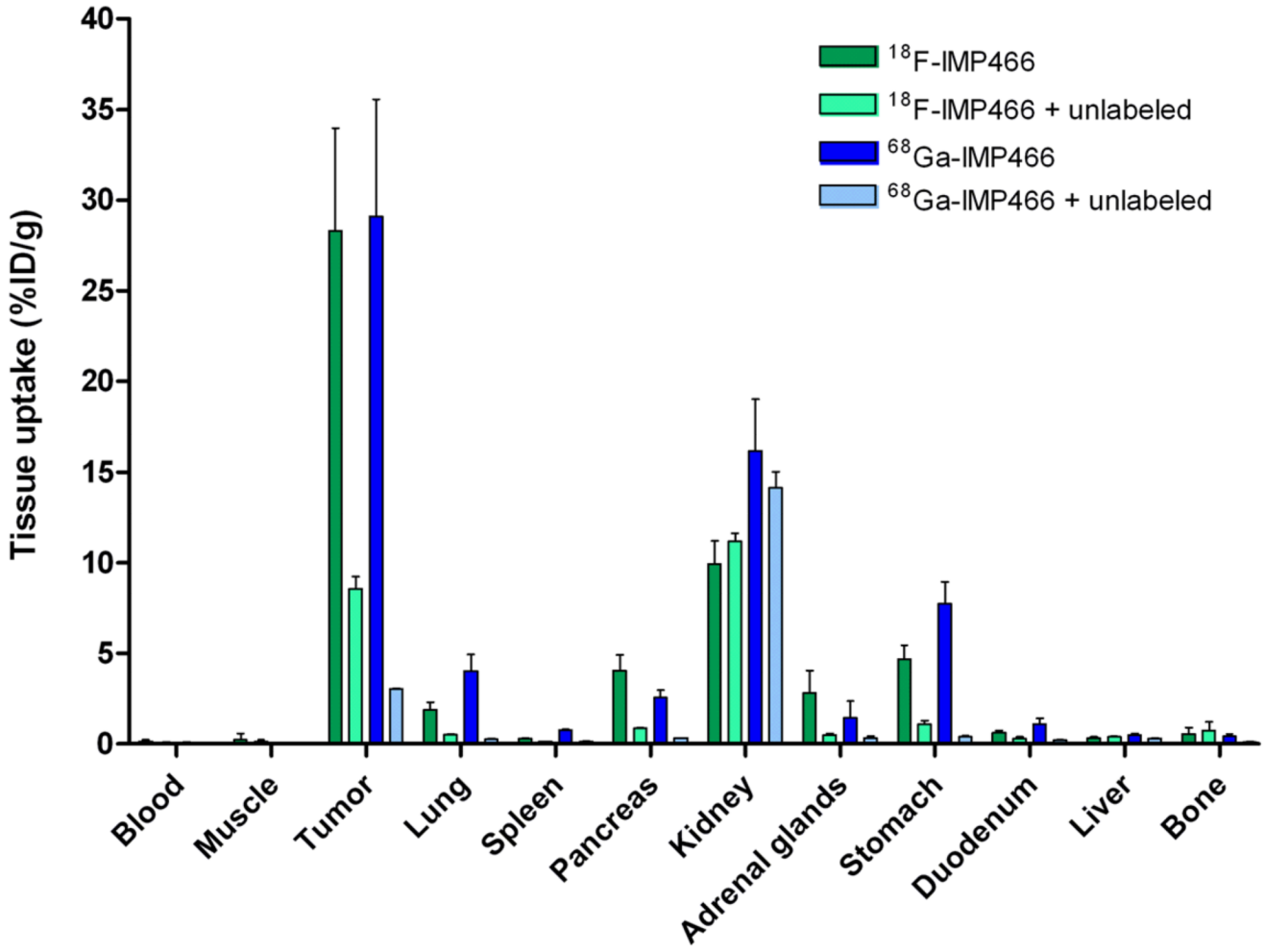

Figure 5.
Biodistribution of ^{18}F-IMP466 and ^{68}Ga-IMP466 at 2 h p.i. in AR42J tumor-bearing mice (n=5/group). As a control, mice in separate groups (n=3/group) received an excess of unlabeled octreotide to demonstrate receptor specificity. Tumors weighed 0.04 – 0.33 g.

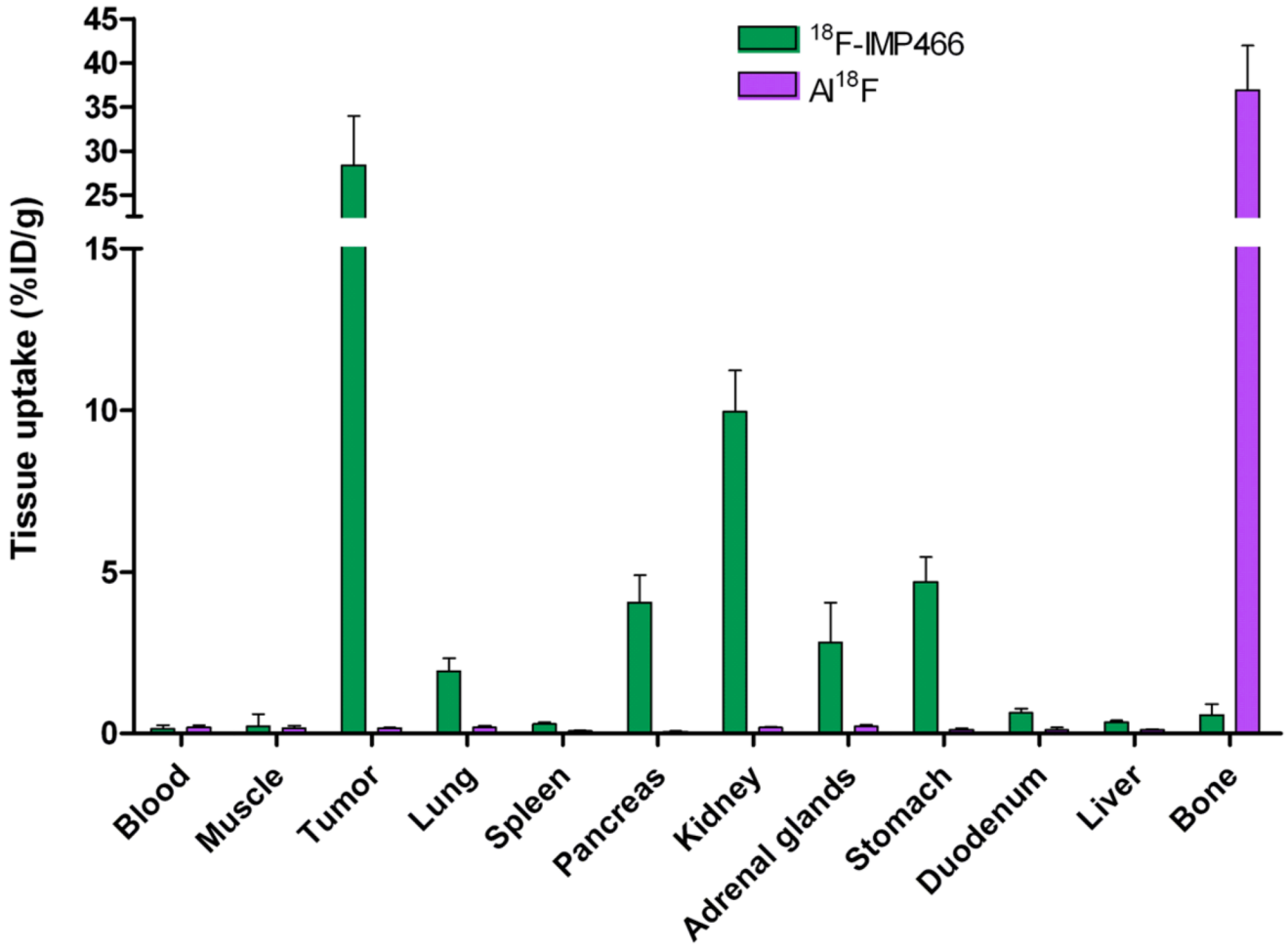

Figure 6.
Biodistribution of ^{18}F-IMP466 and unbound "Al^{18}F" at 2 h p.i. in AR42J tumor-bearing mice (n=5/group). Tumors weighed 0.07 – 0.36 g.

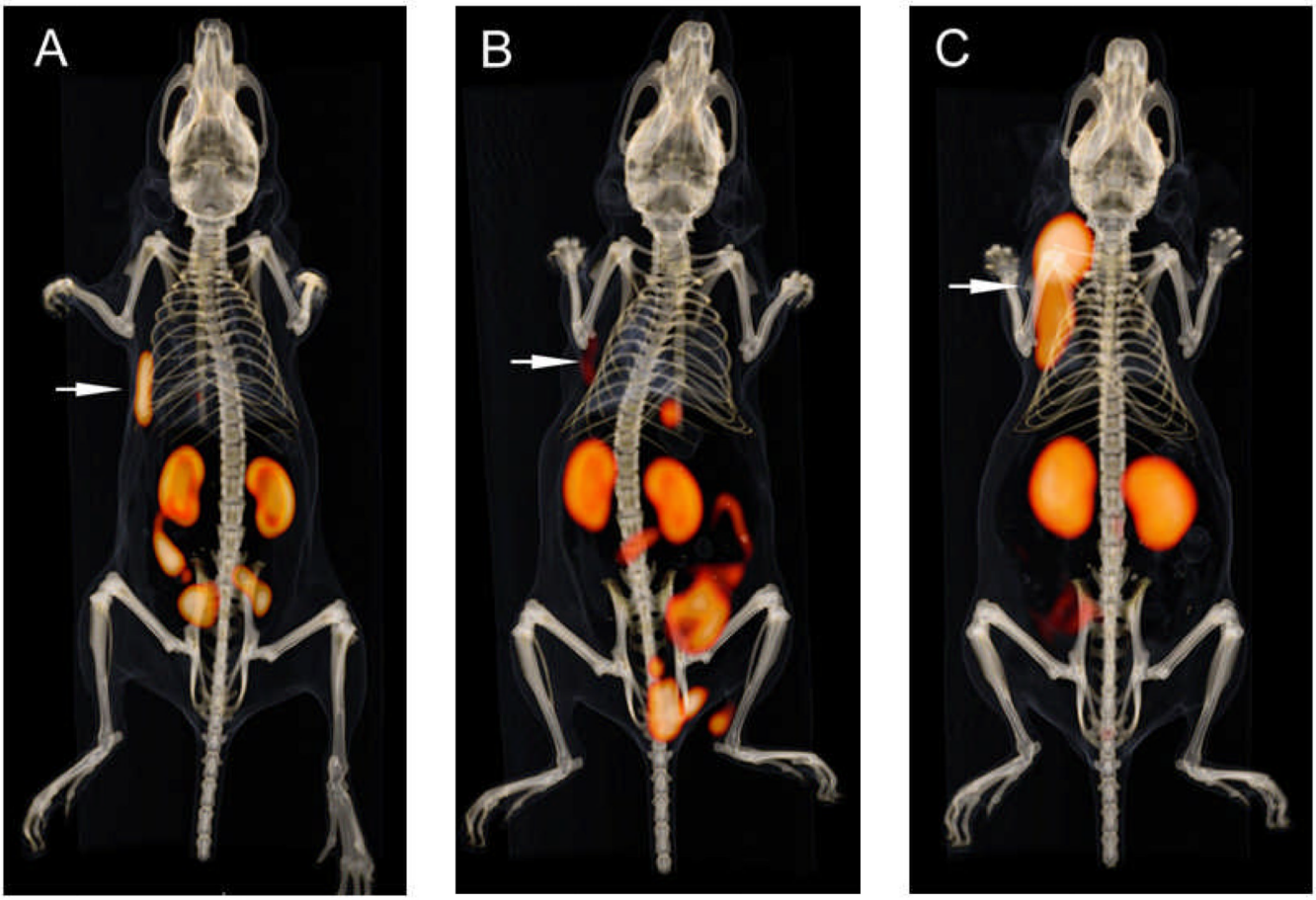

Figure 7.

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), ^{18}F-IMP466 in the presence of an excess of unlabeled IMP466 (B), and ^{68}Ga-IMP466 (C). Arrows indicate tumors. Scans were recorded at 2 h p.i.