Synthesis and Biological Evaluation of 18F-FB-NGA as a Hepatic Asialoglycoprotein Receptor PET Imaging Agent (Postprint)

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Abstract

Asialoglycoprotein receptor (ASGP-R) is a hepatic membrane receptor that uniquely exists on the surface of mammalian hepatocytes, and has been used as target of liver functional imaging agents for many years. We labeled the Galactosyl-neoglycoalbumin (NGA) with 18F to get a PET molecular probe 18F-FB-NGA and evaluated its ability as a liver functional PET imaging agent. The 18F-FB-NGA was prepared with NGA by conjugation with N- succinimidyl-4-18F-fluorobenzoate (18F-SFB) and purified with PD-10 desalting column. The radiolabeling yield and radiochemical purity of 18F-FB-NGA were determined by radio-HPLC. Starting with 18F-F– , the total time for 18F-FB -NGA was about 120$±10*min.Thedecay* − *correctedradiochemicalyieldisabout*25–30±3.42*and*12.12±$6.11%ID/g at 10 and 30 min after injection, respectively. Dynamic MicroPET images in mice were acquired with and without block after injection of the radiotracer, respectively. High liver activity accumulation was observed at 5 min after injection in normal group. On the contrary, the liver accumulation was significantly lower after block, indicating the specific binding to ASGP-R. 18F-FB-NGA is probably a potential PET liver imaging agent.

Full Text

Preamble

Synthesis and biological evaluation of ^{18}F-FB-NGA as a hepatic asialoglycoprotein receptor PET imaging agent

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Abstract

The asialoglycoprotein receptor (ASGP-R) is a hepatic membrane receptor uniquely expressed on the surface of mammalian hepatocytes and has served as a target for liver functional imaging agents for many years. We labeled galactosyl-neoglycoalbumin (NGA) with ^{18}F to obtain the PET molecular probe ^{18}F-FB-NGA and evaluated its potential as a liver functional PET imaging agent. The ^{18}F-FB-NGA was prepared by conjugating NGA with N-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB) and purified using a PD-10 desalting column. The radiolabeling yield and radiochemical purity of ^{18}F-FB-NGA were determined by radio-HPLC. Starting from ^{18}F-F^{-}, the total synthesis time for ^{18}F-FB-NGA was approximately 120$±$10 min, with a decay-corrected radiochemical yield of about 25–30%. The radiochemical purity of purified ^{18}F-FB-NGA exceeded 98%. When labeled with 185–1850 MBq of ^{18}F-SFB, the specific activity of ^{18}F-FB-NGA was estimated to be 7.83–78.3 TBq/mmol. Biodistribution studies in normal mice showed liver accumulation of 39.47$±3.42±$6.11%ID/g at 10 and 30 min post-injection, respectively. Dynamic microPET imaging in mice was performed with and without receptor blockade. High liver activity accumulation was observed at 5 min after injection in the normal group, whereas liver accumulation was significantly lower after blockade, indicating specific binding to ASGP-R. ^{18}F-FB-NGA demonstrates potential as a PET liver imaging agent.

Keywords: Asialoglycoprotein receptor, ^{18}F-FB-NGA, Biodistribution, PET Imaging

Introduction

Asialoglycoproteins are glycoproteins with terminal galactose or N-acetylgalactosamine residues that remain after removal of sialic acid residues. The asialoglycoprotein receptor (ASGP-R), located on the surface of hepatocyte membranes, can specifically recognize and bind to asialoglycoproteins. The number of ASGP-R molecules on hepatocytes is reduced in patients with liver disease, making it a specific indicator for evaluating liver function [1,2]. ASGP-R imaging agents can be used to assess liver function and directly reflect functional hepatocyte mass. Galactosyl-neoglycoalbumin (NGA) was synthesized as an analog ligand in 1976 and labeled with ^{99} Tc for ASGP-R single photon emission computed tomography (SPECT) imaging. In 1992, a DTPA-conjugate of NGA (diethylenetriaminepentaacetic acid-galactosyl human serum albumin, GSA) was introduced in Japan as a commercial product. ^{99} Tc-GSA was the first

commercially available receptor-binding radiopharmaceutical [3–5]. We have prepared several glycoprotein derivatives for ASGP-R imaging, including ^{99} Tc-NGA [6,7] and galactosyl-human serum albumin-interferon-$ $2b (G-HSA-IFN) [8,9].

Several ASGP receptor imaging agents have been reported for SPECT, but the image quality is inferior to that of positron emission tomography (PET). As a valuable quantitative imaging tool, PET is a rapidly expanding clinical molecular imaging modality worldwide, offering approximately two to three orders of magnitude higher sensitivity and superior resolution compared to SPECT. Fluorine-18 (^{18}F) is one of the most commonly used positron-emitting radionuclides for PET imaging and is widely employed for labeling peptides and proteins due to its low positron energy (0.64 MeV) and favorable half-life (109.8 min). Significant advances have been achieved in recent years with peptides and proteins labeled with ^{18}F using prosthetic groups [10,11]. Among the available prosthetic groups, the acylation agent N-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB) is the most widely and frequently used [12–15].

Here, we report the synthesis of ^{18}F-FB-NGA by labeling NGA with the prosthetic group ^{18}F-SFB. The synthetic strategy is illustrated in Scheme 1. Semi-automated radiosynthesis of ^{18}F-SFB was carried out on an ^{18}F-multifunction synthesizer (Beijing PET Technology Co. Ltd., PRC) with computer control. The quality was determined by radio-high performance liquid chromatography [16]. Factors affecting the labeling yields of ^{18}F-FB-NGA, biological distribution, and microPET imaging in mice were investigated.

Scheme 1. Radiolabeling of ^{18}F-FB-NGA via active ester intermediate ^{18}F-SFB coupling to the -amide of lysine residues of NGA.

2.1 Materials

All chemicals were obtained commercially and used without further purification. Methyl trifluoromethanesulfonate was purchased from Matrix Scientific. Ethyl 4-dimethylaminobenzoate was obtained from J & K Chemical LTD. Tetrapropylammonium hydroxide solution (1 mol/L in water) was purchased from Aladdin Chemistry Co., Ltd. N,N,N ,N -Tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TSTU) was purchased from Shanghai Medpep Co., Ltd. Ethyl 4-(trimethylammonium)benzoate triflate and unlabeled N-succinimidyl 4-fluorobenzoate (SFB) were synthesized according to the methods of Haka et al. [17] and Johnstrom et al. [18], respectively. Galactosyl-neoglycoalbumin (NGA) was prepared following the procedures of Zhang et al. [6], with 25 galactose units attached to each molecule.

The analytical HPLC system consisted of a binary HPLC pump (Waters 1525, USA), a UV detector (Waters 2487, USA), and a flow scintillation analyzer (Radiomatic 610TR, Perkin-Elmer, USA). A reversed-phase C-18 column (4.6 × 250 mm, 5 m particle size, Jiangsu Hanbon Science & Technology Co., Ltd) was eluted at a flow rate of 1 mL/min as described in the experimental section,

with absorbance monitored at 254 nm. The TSKgel G2000SWXL column (7.8 × 300 mm) was purchased from TOSOH Corp., Japan. The Sep-Pak Light Waters Accell Plus QMA cartridge (Waters, USA) was activated with $NaHCO_3$ and water before use. Reversed-phase extraction Sep-Pak C18 Plus cartridges (Waters, USA) were activated with methanol and water before use. A PD-10 column (filled with Sephadex G-25) was purchased from GE Healthcare. ICR mice were provided by the Comparative Medicine Center of Yangzhou University.

2.2 Synthesis of 4-^{18}F-fluorobenzoic acid (^{18}F-FBA)

Automated synthesis of ^{18}F-labeling was performed on an ^{18}F-multifunction synthesizer (Beijing PET Technology Co. Ltd., PRC) with a computer interface. The $^{18}F\text{-}F^-$ was transferred via a pneumatic transport system from the cyclotron to the radiopharmaceutical laboratory and separated from $^{18}O\text{-}H_2O$ using an anion exchange cartridge (Sep-Pak Light Waters Accell Plus QMA cartridge). The synthesis program began with elution of ^{18}F-fluoride from the anion exchange cartridge into reactor 1 using a solution of Kryptofix 222 (19.5 mg) and potassium carbonate (4.5 mg) in aqueous MeCN (1.5 mL, 90% MeCN) from vial 1. Reaction vessel 1 was heated to 116°C and the solvent evaporated with a stream of nitrogen. Anhydrous MeCN (2 mL) was added from vial 2 and evaporated to dryness at 116°C for approximately 5 min. A solution of ethyl 4-(trimethylammoniumtriflate)benzoate 1 (10 mg) in anhydrous MeCN (1 mL) was added from vial 3 to the ^{18}F-KF-K222 complex, and the mixture was heated five times to 90°C for 10 min with 5 s mixing between heating periods. Then 0.5 mL NaOH (0.5 M) was delivered from vial 4 and heated three times to 90°C for 5 min with 5 s mixing between heating periods. After cooling for 2 min and acidifying with 0.1 mol/L HCl (5 mL, vial 5), the solution was loaded onto a Sep-Pak C18 cartridge. The cartridge was dried using nitrogen, and the product was eluted with MeCN (3 mL, vial 6) into a second reaction vessel.

2.3 Synthesis of N-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB)

The 4-^{18}F-fluorobenzoic acid was eluted directly from the Sep-Pak C18 cartridge into reaction vessel 2, which previously contained 40 L tetrapropylammonium hydroxide solution (1 mol/L in water). The mixture was heated at 116°C and evaporated to dryness. After cooling, a solution of TSTU (12 mg) in anhydrous MeCN (0.8 mL) was delivered to the reaction vessel from vial 11, and the mixture was heated three times to 90°C for 5 min with 5 s mixing between heating periods. After cooling for 2 min and acidifying with 0.1 mol/L HCl (5 mL, vial 12), the solution was loaded onto a Sep-Pak C18 cartridge. Finally, ^{18}F-SFB was eluted with 3 mL MeCN (vial 13). The product was analyzed using radio-HPLC to evaluate radiochemical purity.

2.4 Conjugation of ^{18}F-SFB to NGA

The MeCN solution of ^{18}F-SFB was evaporated to dryness with a stream of nitrogen, and the residue was redissolved in 50 L MeCN. A solution of NGA (1 mg, 200 L, 0.014 mol) in 0.1 mol/L borate buffer (pH 9.0) was added and reacted for 30 min at room temperature. The crude product was loaded onto a PD-10 column and eluted with 0.05 mol/L phosphate buffer (pH 7.5). After purification, radiochemical purity was evaluated by radio-HPLC.

2.5 Radiochemical analysis

Analytical HPLC was performed using a Lichrospher C18 reversed-phase column (4.6 × 250 mm, 5 m). Column effluent was monitored using a UV detector (Waters 2487, USA) and a flow scintillation analyzer (Radiomatic 610TR, Perkin-Elmer, USA) for radioactivity. ^{18}F-SFB was analyzed using a mobile phase of H_2O and MeCN (v/v = 55:45) with 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 mL/min; UV detection was at λ = 254 nm. ^{18}F-FB-NGA was analyzed with a TSK-GEL G2000SWXL column (7.8 × 300 mm) using 0.1 mol/L phosphate buffer, 0.9% NaCl, and 0.05% NaN_3 as mobile phase at a flow rate of 1.0 mL/min; UV detection was at λ = 280 nm. ^{18}F-FB-NGA was incubated at room temperature for 4 h, and radiochemical purity (RCP) was evaluated by radio-HPLC at each hour.

2.6 Biodistribution

Biodistribution of ^{18}F-FB-NGA was studied in normal mice. ^{18}F-FB-NGA (200 L, approximately 0.37 MBq containing about 3 g NGA) was injected via the tail vein. At selected time points (10, 30, and 60 min), mice (n = 5 per time point) were sacrificed, and major organs and tissues were collected and weighed. Radioactivity in these tissues was measured using a gamma counter (1470 Automatic Gamma Counter, Perkin-Elmer, USA), and results were expressed as percentage injected dose per gram of tissue (%ID/g). For each mouse, tissue sample radioactivity was calibrated against a known aliquot of the injected activity, and mean uptake (%ID/g) for each animal group was calculated with standard deviations.

2.7 MicroPET imaging

PET scans and image analysis were performed using a microPET Inveon rodent model scanner (Siemens Medical Solutions USA, Inc.). Normal and blocking mice received tail-vein injections of approximately 200 L of ^{18}F-FB-NGA (about 3.7 MBq containing approximately 30 g NGA) under isoflurane anesthesia and underwent 30-min dynamic scans starting at the time of injection. Images were reconstructed using a three-dimensional ordered-subsets expectation maximization (OSEM) algorithm. Blocking mice were pre-injected with 0.2 mL NGA (3 mg) 30 min prior to tracer administration.

3.1 Radiosynthesis

^{18}F-fluorination of NGA was performed using ^{18}F-SFB (Scheme 1). ^{18}F-SFB was synthesized in a fully automatic multifunction module with a decay-corrected radiochemical yield of 42.7 ± 5.9% (n = 8) in 55–65 min, and its radiochemical purity exceeded 98% as determined by analytic HPLC. Starting from ^{18}F-F$^-$, the total radiolabeling time for ^{18}F-FB-NGA was 120 ± 10 min, including final purification. When labeled with 185–1850 MBq of ^{18}F-SFB, the specific activity of ^{18}F-FB-NGA was estimated to be 7.83–78.3 TBq/mmol. After purification with a PD-10 column, the radiochemical purity of ^{18}F-FB-NGA was above 98% as determined by radio-HPLC, and the tracer remained stable for up to 4 h. The overall decay-corrected radiochemical yield was 25–30%. NGA was conjugated with the activated ester ^{18}F-SFB through -amino groups of lysine residues under basic conditions; however, the coupling yield for NGA was relatively lower than that for HSA because many -amino groups of lysine in NGA had already been coupled with galactose groups.

Table 1 Dependency of the radiochemical yield on pH for radiolabeling of NGA with ^{18}F-SFB

Table 2 Dependency of the radiochemical yield on protein concentration for radiolabeling of NGA with ^{18}F-SFB

3.2 Radiochemical analysis

The HPLC chromatograms of ^{18}F-SFB and ^{19}F-SFB are presented in Fig. 1. The resulting ^{18}F-SFB exhibited a radiochemical purity of 98.2% at the same retention time as ^{19}F-SFB, with a retention time of 10.4 min in our gradient system. Yields for the reaction mixture and conjugation correlated with several reaction parameters, including pH value and initial protein concentration. As shown in Table 1, the radiochemical yield depended on pH value, with NGA labeling via coupling with ^{18}F-SFB occurring under basic conditions. The optimal labeling yield for ^{18}F-FB-NGA was achieved at pH = 9 after 30 min. The radiochemical yield of ^{18}F-FB-NGA increased from 4.3% to 58.1% as NGA concentrations increased from 0.1 to 5.0 mg/mL at pH = 9 (Table 2), with lower yields obtained when NGA concentration was below 5 mg/mL.

Starting from ^{18}F-F$^-$, ^{18}F-SFB was synthesized via a three-step reaction. NGA was then conjugated with the activated ester. Fig. 2 shows the HPLC characterization profiles of purified ^{18}F-FB-NGA and NGA. The resulting ^{18}F-FB-NGA exhibited a radiochemical purity of 98.6% at the same retention time as NGA, with a retention time of 6.8 min.

3.3 Biodistribution

To evaluate the tissue distribution characteristics of ^{18}F-FB-NGA, we performed biodistribution studies in ICR mice. Data are expressed as percentage administered activity (injected dose) per gram of tissue (%ID/g) (Table 3). ^{18}F-FB-

NGA demonstrated excellent liver accumulation, with liver uptake values of 39.47 ± 3.42 at 10 min, 12.12 ± 6.11 at 30 min, and 5.39 ± 0.92%ID/g at 60 min post-injection. Kidney uptake was 11.19 ± 6.28 and 29.96 ± 4.27%ID/g at 10 and 30 min post-injection, respectively. Radioactivity in blood decreased rapidly, with a concentration of only 1.69 ± 0.21%ID/g at 10 min after injection. These biodistribution results indicate that ^{18}F-FB-NGA has high affinity for the ASGP receptor and may enable high-quality imaging, making it possible to diagnose liver disease non-invasively. ^{18}F-FB-NGA could serve as a receptor-specific radiopharmaceutical with potential applications in liver imaging for evaluation of hepatocytic function.

Table 3 Biodistribution of ^{18}F-FB-NGA in ICR mice (%ID/g)

Tissue	10 min	30 min	60 min
Brain	0.16$±0.02|0.13±0.02|0.09±0.01||*Heart*|2.86±0.66|1.85±0.30|1.26±0.16||*Liver*|39.47±3.4		

3.4 MicroPET imaging

Dynamic microPET scans were performed on normal and blockade groups, with selected coronal images at different time points after ^{18}F-FB-NGA injection shown in Fig. 3. The normal and blockade groups showed significant differences, particularly in the heart and liver. The normal group exhibited low cardiac uptake and high liver uptake at 5 min after injection. The liver was clearly visible with high contrast to background, and no significant uptake was observed in other abdominal organs at 10 min after injection. After 30 min, the liver outline remained clearly defined.

In contrast, liver accumulation was significantly lower after blockade, with a vague liver outline and no significant hepatic uptake observed in the blockade group. The blockade group showed high cardiac uptake throughout the 30-min imaging period. These microPET imaging studies in normal and blockade groups clearly demonstrated the ASGP receptor-targeting avidity and specificity of the imaging agent ^{18}F-FB-NGA in vivo.

The microPET evaluation of ^{18}F-FB-NGA revealed high liver accumulation with certain retention. Liver uptake decreased significantly when free NGA was used for blockade, while the blockade group showed high cardiac uptake. The microPET results coincided with the biodistribution data, with both experiments demonstrating high affinity of ^{18}F-FB-NGA for the ASGP receptor.

Fig. 3 Coronal microPET images of mice. The upper panel shows normal group images at 5, 15, and 30 min after injection of ^{18}F-FB-NGA (3.7 MBq). The lower panel shows blockade group images at 5, 15, and 30 min after injection of ^{18}F-FB-NGA (3.7 MBq) following pre-administration of free NGA as blocking agent (3 mg per mouse).

4 Conclusion

NGA was successfully labeled with ^{18}F using the prosthetic labeling group ^{18}F-SFB. Purification with a PD-10 column yielded ^{18}F-FB-NGA with high radiochemical purity (>98%). In vivo biodistribution demonstrated high liver uptake with low uptake in other organs. Specific binding of this radiotracer to the ASGP receptor was confirmed by microPET imaging studies. ^{18}F-FB-NGA could serve as a hepatocyte-targeting agent for evaluating hepatic function. Future work will focus on improving labeling efficiency and examining hepatic function in animal models of liver injury.

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Note: Figure translations are in progress. See original paper for figures.