Bioorganic & Medicinal Chemistry 17 (2009) 7510–7516

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Fluorine-18 labeled galactosyl-neoglycoalbumin for imaging the hepatic asialoglycoprotein receptor

Wenjiang Yang [a], Tiantian Mou [a], Cheng Peng [b], Zhanhong Wu [c], Xianzhong Zhang [a,*], Fang Li [c], Yunchuan Ma [b]

[a] Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, PR China
[b] PET Center of Xuanwu Hospital, Capital Medical University, Beijing 100053, PR China
[c] Department of Nuclear Medicine, PUMC Hospital, CAMS and PUMC, Beijing 100730, PR China

ARTICLE INFO

Article history:
Received 24 July 2009
Revised 9 September 2009
Accepted 10 September 2009
Available online 15 September 2009

Keywords:
^{18}F
NGA
[^{18}F]FNGA
PET imaging
Asialoglycoprotein receptor

ABSTRACT

Asialoglycoprotein receptors (ASGP-R) are well known to exist on the mammalian liver, situate on the surface of hepatocyte membrane. Quantitative imaging of asialoglycoprotein receptors could estimate the function of the liver. ^{99m}Tc labeled galactosyl-neoglycoalbumin (NGA) and diethylenetriaminepentaacetic acid galactosyl human serum albumin (GSA) have been developed for SPECT imaging and clinical used in Japan. In this study, we labeled the NGA with ^{18}F to get a novel PET tracer [^{18}F]FNGA and evaluated its hepatic-targeting efficacy and pharmacokinetics. *Methods*: NGA was labeled with ^{18}F by conjugation with *N*-succinimidyl-4-^{18}F-fluorobenzoate ([^{18}F]SFB) under a slightly basic condition. The in vivo metabolic stability of [^{18}F]FNGA was determined. Ex vivo biodistribution of [^{18}F]FNGA and blocking experiment was investigated in normal mice. MicroPET images were acquired in rat with and without block at 5 min and 15 min after injection of the radiotracer (3.7 MBq/rat), respectively. *Results*: Starting with $^{18}F^-$ Kryptofix 2.2.2./K_2CO_3 solution, the total reaction time for [^{18}F]FNGA is about 150 min. Typical decay-corrected radiochemical yield is about 8–10%. After rapid purified with HiTrap desalting column, the radiochemical purity of [^{18}F]FNGA was more than 99% determined by radio-HPLC. [^{18}F]FNGA was metabolized to produce [^{18}F]FB-Lys in urine at 30 min. Ex vivo biodistribution in mice showed that the liver accumulated 79.18 ± 7.17% and 13.85 ± 3.10% of the injected dose per gram at 5 and 30 min after injection, respectively. In addition, the hepatic uptake of [^{18}F]FNGA was blocked by pre-injecting free NGA as blocking agent (18.55 ± 2.63%ID/g at 5 min pi), indicating the specific binding to ASGP receptor. MicroPET study obtained quality images of rat at 5 and 15 min post-injection. *Conclusion*: The novel ASGP receptor tracer [^{18}F]FNGA was synthesized with high radiochemical yield. The promising biological properties of [^{18}F]FNGA afford potential applications for assessment of hepatocyte function in the future. It may provide quantitative information and better resolution which particularly help to the liver surgery.

1. Introduction

Asialoglycoprotein receptors (ASGP-R) are well known to exist on the mammalian liver,[1] situated on the surface of hepatocyte membrane. Asialoglycoprotein receptors participate in the hepatic metabolism of serum proteins. They could recognize a glycoprotein having galactose residues on the terminal position of the saccharide chain, such as asialoglycoprotein. Quantitative imaging of asialoglycoprotein receptors could estimate the function of liver. This is a unique way to diagnose disease by a non-invasive method. ASGP receptor imaging agent can assess the anatomy and function of the liver, help the early diagnosis of hepatic diseases and accurate evaluation of functional status.[1,2] However, native ASGP receptor ligands require enzymatic preparation, and in vivo clinical experience has been quite scarce. A more clinically acceptable radiopharmaceutical neogalactosylalbumin (NGA) had been developed by attaching galactosyl units to human serum albumin (HSA) in 1980, and then labeled with ^{99m}Tc for imaging the liver.[3] Later, to make the labeling procedure simple, diethylenetriaminepentaacetic acid galactosyl human serum albumin (GSA) was obtained by added three to four DTPA molecules covalently attached to the albumin backbone, and developed as an instant kit.[4] This instant GSA kit is commercially available for routine clinical use in Japan now.[2,5]

The derivates of glycoprotein radiolabeled with technetium-99m, iodine-125/131,[6] and indium-111[7] have been reported for single photon emission computed tomography (SPECT) imaging applications. Positron emission tomography (PET) imaging is the gold standard for quantitative imaging in nuclear medicine. Vera reported deferoxamine modified NGA to labeled with positron emission nuclides gallium-67/68.[8] Biodistribution of ^{67}Ga-DF-NGA in rabbits was similar to ^{99m}Tc-NGA. ^{68}Ga-DF-NGA could be used for

* Corresponding author. Tel./fax: +86 10 62208126.
E-mail addresses: zhangxzh@gmail.com, zhangxzh@bnu.edu.cn (X. Zhang).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.09.017

hepatic ASGP receptor PET imaging. However ^{68}Ga has a high beta emission energy which results in diminished spatial resolution and less favorable dosimetry. Fluorine-18 is the most important positron-emitting isotope for PET imaging. It has low positron energy and optimal physical half-life of 110 min allows for multistep radiosynthesis, longer in vivo investigation. ^{18}F-Labeled prosthetic groups such as *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) have been developed, which can be attached to either N-terminal or lysine ε-amino groups with little or no loss of bioactivity of the protein or peptide ligand,[9] such as bombesin analogs[10] and RGD peptide.[11] Here we report the radiolabeling NGA with ^{18}F for hepatic asialoglycoprotein receptor imaging with PET.

2. Materials and methods

2.1. Materials

All chemicals obtained commercially were used without further purification. No-carrier-added [^{18}F]F$^-$ was trapped on Sep-Pak QMA cartridge (Waters) and then eluted with 0.5 mL K_2CO_3 (12 mg/mL in H_2O) combined with 1 mL Kryptofix 2.2.2. (Acros Organics) (11 mg/mL in acetonitrile). Ethyl 4-(dimethylamino)benzoate, methyl trifluoromethanesulfonate and tetrabutylammonium hydroxide (40 wt % solution in water) were purchased from Acros Organics. *N*,*N*,*N*′,*N*′-Tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) and L-lysine were purchased from Sigma–Aldrich. Ethyl 4-(trimethylammonium triflate)benzoate was prepared by the procedure of Guhlke et al.[9]

Instant thin-layer chromatography-silica gel (ITLC-sg) chromatographic strips were purchased from Pall Life Sciences. LabGEN 7 homogenizer was purchased from Cole-Parmer Ins, USA. Radio-high pressure liquid chromatography (Radio-HPLC) experiments were performed on a SHIMADZU system with SCL-10Avp HPLC pump system (SHIMADZU Corporation, Japan) and liquid scintillation analyzer (Packard BioScience Co., USA). The Reversed-phase Kromasil C-4 column (4.6 × 250 mm, 5 μm particle size, 300 Å, Eka Chemicals, Sweden) was eluted at a flow rate of 1 mL/min according to the procedure described in the experimental part. The absorbance was monitored at 220 nm. Reversed-phase extraction Sep-Pak C_{18} Plus cartridges (Waters) were activated with methanol and water before use. HiTrap Desalting column (filled with Sephadex G25) was purchased from GE Healthcare, eluted with 0.05 mol/L phosphate buffer (pH 7.5). Animal experiments were carried out in Kunming mice (average weight about 20 g) or SD rats (average weight about 300 g), obtained from the Animal Center of Peking University. All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

2.2. Synthesis of NGA

Galactosyl-neoglycoalbumin (NGA) was synthesized according to the procedure of Vera et al.[3] Briefly, 2-imino-2-methoxyethyl-thio-galactose was prepared, and reacted with HSA in borate buffer (pH 8.6, 0.2 mol/L). The conjugated number of galactose per molecule of human serum albumin was calculated by measuring galactose concentration from the absorbance at 490 nm using the phenol/sulfuric acid method.[12] Average 38 galactose units were attached to each HSA molecule.

2.3. Synthesis of 4-[^{18}F]fluorobenzoic acid, [^{18}F]FB

The aqueous solution of [^{18}F]F$^-$ was added to a 10 mL reaction vial with 6 mg K_2CO_3 and 11 mg Kryptofix 2.2.2.. The reaction vial was heated at 120 °C in oil bath and the solvent evaporated with a stream of nitrogen. Then 500 μL dry acetonitrile was added to dissolve the residue and concentrated again under a stream of nitrogen. The azeotropic drying process was repeated three times. To the residue was added a solution of 4 mg ethyl 4-(trimethylammonium triflate) benzoate dissolved in 1 mL dry dimethyl sulfoxide, the reaction vial was sealed and heated at 120 °C for 20 min with magnetic stirring. For hydrolysis 0.5 mL 0.2 mol/L NaOH solution was added and heated for additional 10 min at 120 °C. After cooling for 2 min and then acidified with 0.5 mL 1 mol/L HCl, the solution diluted with water to a final volume of 10 mL and loaded onto a Sep-Pak C_{18} cartridge. The cartridge was washed with 10 mL water and blown dry with a stream of nitrogen. The radioactivity was eluted with 3 mL acetonitrile (with 0.1% TFA) into a second reaction vial. The sample was evaluated by radio-HPLC.

2.4. Synthesis of *N*-succinimidyl-4-[^{18}F]fluorobenzoate, [^{18}F]SFB

Ten microliters of 40% solution of tetrabutylammonium hydroxide were added into the second vial and then evaporated the solvent in oil bath with a stream of nitrogen. The process was repeated three times by addition and evaporation of 0.5 mL dry acetonitrile. Finally, 15 mg TSTU in 1 mL dry acetonitrile was added to the residue. The vial was sealed and heated for 5 min at 80–90 °C, and cooled for 2 min. The solution was added 500 μL 50% acetic acid and diluted with water to a final volume of 10 mL and loaded onto a Sep-Pak C_{18} cartridge. The cartridge was washed with 10 mL water and blown dry with a stream of nitrogen. The [^{18}F]SFB was eluted with 2.5 mL methylene chloride into a third reaction vial. The sample was evaluated by radio-HPLC.

2.5. Conjugation of [^{18}F]SFB to NGA

The methylene chloride solution of [^{18}F]SFB was evaporated to dryness with a stream of nitrogen. The residue was taken with 50 μL dimethyl sulfoxide and added 200 μL of a solution of NGA (3 mg, 0.04 μmol) in 0.1 mol/L borate buffer, pH 8.6 and incubated for 30 min at room temperature. After reaction the crude product was passed through a 0.22 μm millipore filter and loaded onto a HiTrap desalting column, eluted with 0.05 mol/L phosphate buffer, pH 7.5. After purification the radiochemical purity was evaluated by ITLC chromatography and radio-HPLC.

2.6. Synthesis of [^{18}F]FB-Lys

The dried [^{18}F]SFB was then redissolved in dimethyl sulfoxide (50 μL) and added to the L-lysine (6 mg, 0.04 mmol) dissolved in borate buffer (400 μL, pH 8.6) and incubated for 30 min at room temperature. The [^{18}F]FB-Lys was evaluated by radio-HPLC without further purification.

2.7. Synthesis of [^{19}F]FNGA

N-Succinimidyl-4-[^{19}F]fluorobenzoate was synthesized from 4-[^{19}F]fluorobenzoic acid. To 1 mL of a 20 mg/mL solution of NGA in 0.2 mol/L borate buffer pH 8.6 was added dropwise 40 μL of a solution containing 3.5 mg of *N*-succinimidyl-4-[^{19}F]fluorobenzoate in DMSO. The solution was stirred gently for 1 h at room temperature. After reaction, the solution was passed through a 0.22 μm millipore filter and loaded onto a HiTrap desalting column, eluted with 0.05 mol/L phosphate buffer, pH 7.5. The collected [^{19}F]FNGA solution was dialyzed against distilled water for three days and lyophilized. ^{19}FNMR(D_2O) δ: −108.2 (*m*). The [^{19}F]FNGA was evaluated by HPLC with UV detection at 220 nm.

2.8. Radiochemical analysis

The chromatography analysis were performed on ITLC-sg strips with ACD (0.068 mol/L citrate, 0.074 mol/L glucose, pH 5.0) as mobile phase. [^{18}F]FNGA was remaining at the point of spotting (R_f = 0–0.1), while other radioactive impurities moved with the solvent front (R_f = 0.8–1.0). Radio-HPLC was performed on a SHIMADZU system with Reversed-phase Kromasil C-4 column (4.6 × 250 mm, 5 μm). The mobile phase was changed from 70% solvent A (0.1% trifluoroacetic acid [TFA] in water) and 30% solvent B (0.1% TFA in acetonitrile [ACN]) to 30% solvent A and 70% solvent B at 30 min. [^{19}F]FNGA was analyzed in same gradient and monitored at 220 nm.

After purified, [^{18}F]FNGA was incubated at room temperature for 4 h. The radiochemical purity (RCP) was evaluated by ITLC chromatography at every single hour. Plasma stability was determined by diluted [^{18}F]FNGA 20-fold with freshly prepared murine plasma, and the solutions were incubated at 37 °C for 4 h. The radiochemical purity (RCP) was evaluated by ITLC chromatography at every single hour. At the end of 4 h, the plasma was passed through a Sep-Pak C_{18} cartridge, and washed with 0.5 mL of water and eluted with 0.5 mL of acetonitrile containing 0.1% TFA. The combined aqueous and organic solutions passed through a 0.22 μm millipore filter and evaluated by radio-HPLC.

2.9. Metabolic stability

Mice were intravenously injected with 7.4 MBq of [^{18}F]FNGA. The animals were sacrificed at 5 and 30 min after tracer injection. Blood, liver and urine were collected. Blood samples were immediately centrifuged for 5 min at 13,200 rpm. After removal of the supernatants, the pellets were washed with 250 μL of PBS (pH 7.5). Supernatants of both centrifugation steps were combined and passed through a Sep-Pak C_{18} cartridge. The liver samples were washed with saline. After adding 4 mL PBS buffer (pH 7.5), the hepatic samples were homogenized by a polytronhomogenizer (LabGEN 7, Cole-Parmer Ins, USA) at full speed for 5 min. The resulting homogenate was centrifuged for 60 min at 14,000 rpm. Supernatants were passed through a Sep-Pak C_{18} cartridge. The urine samples were directly diluted with 0.5 mL of PBS and then passed through a Sep-Pak C_{18} cartridge. All the cartridges were washed with 0.5 mL of water and eluted with 0.5 mL of acetonitrile containing 0.1% TFA. The combined aqueous and organic solutions passed through a 0.22 μm millipore filter and then injected onto radio-HPLC using a flow rate of 1 mL/min and a gradient as described.

2.10. Ex vivo biodistribution

Ex vivo biodistribution study of [^{18}F]FNGA was carried out in normal mice. [^{18}F]FNGA (about 0.185 MBq in 100 μL solution contained about 2 μg NGA) was injected through the tail vein. At selected time points (5 and 30 min), mice (n = 5 at each time point) were sacrificed, and the tissues and organs of interest were collected, wet weighed and counted in a γ-counter. The percentage of injected dose per gram (%ID/g) for each sample was calculated by comparing its activity with appropriate standard of injected dose (ID), the values are expressed as mean ± SD.

In order to further confirm that the [^{18}F]FNGA had specific receptor-binding, blocking study was performed by conducting the biodistribution experiment in the presence of free NGA (10 mg/kg body weight) as blocking agent. Five minutes after the first injection of free NGA, [^{18}F]FNGA was intravenously injected (about 0.185 MBq in 100 μL solution). Mice were sacrificed at 5 min post-injection time (n = 5). Results were expressed as the percentage of the injected dose per gram tissue (%ID/g). Averages and standard deviations were calculated.

2.11. MicroPET imaging

PET imaging was performed using a Siemens Inveon dedicated microPET. The scanner has a computer-controlled bed and 10-cm transaxial active and 12.7-cm axial fields of view (FOVs). SD rat was placed near the center of the FOVs of the microPET scanner, where the highest image resolution and sensitivity are available. SD rat was deeply anesthetized by intraperitoneal injection of chloral hydrate. The microPET studies were performed by tail vein injection of 3.7 MBq (100 μCi) of [^{18}F]FNGA. For comparison one rat was coinjected 3.7 MBq (100 μCi) [^{18}F]FNGA with free NGA (10 mg/kg rats body weight) as blocking agent. MicroPET data acquisition was started at 5 min after radiotracer injection. Dynamic images at 5 and 15 min time points were acquired.

3. Results

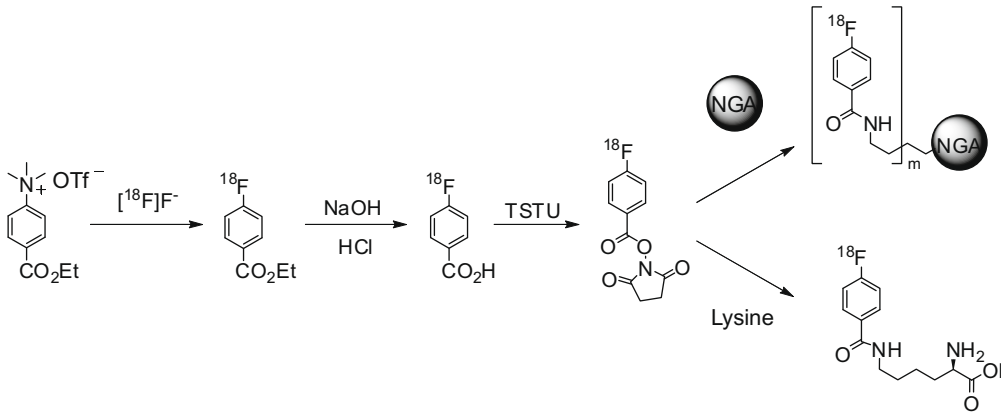
3.1. Radiosynthesis

^{18}F-fluorination of NGA was performed using [^{18}F]SFB (Scheme 1). Our decay corrected radiochemical yield of [^{18}F]SFB was 31.7 ± 8.9% (n = 8) in 100–110 min. [^{18}F]SFB was purified with simple cartridge desalting before coupling with NGA. The radiochemical purity of [^{18}F]FB and [^{18}F]SFB was investigated with radio-HPLC. The retention time of [^{18}F]FB and [^{18}F]SFB in our gradient system was 7.2 min and 10.2 min, respectively. NGA was labeled with ^{18}F by coupling with [^{18}F]SFB under a slightly basic condition. Yields for the conjugation correlate with initial protein concentration. We obtained lower radiochemical yields when the concentration of NGA is less than 5 mg/mL. Starting with [^{18}F]F^- in Kryptofix 2.2.2./K_2CO_3 solution, the total reaction time was about 150 ± 20 min. The overall radiochemical yield with decay correction was about 8–10% typical, including final purification. Starting with 370–1295 MBq [^{18}F]fluoride, the specific activity of [^{18}F]FNGA ranged from 1.92 TBq/mmol to 7.10 TBq/mmol. After rapid purified with HiTrap desalting column, the radiochemical purity of [^{18}F]FNGA was above 99% as determined by ITLC and radio-HPLC. The retention time of [^{18}F]FNGA was 12.3 min. [^{19}F]FNGA was analyzed in same gradient. The retention time of [^{19}F]FNGA was 12.1 min which is similar with [^{18}F]FNGA (Fig. 1). Lysine was coupled with [^{18}F]SFB to produce radiochemical yield of more than 98% after 30 min incubation at room temperature when determined by HPLC analyses. The retention time of [^{18}F]FB-Lys was 4.3 min in our gradient.

In vitro stability, the result measured by ITLC showed that [^{18}F]FNGA can be stable over 4 h in saline at room temperature, the radiochemical purity was above 95%. Stability in murine plasma was also very high, [^{18}F]FNGA was still intact after 4 h at 37 °C. HPLC analysis results of in vitro stability are shown in Figure 2.

3.2. Metabolic stability

The metabolic stability of [^{18}F]FNGA was determined in mouse blood, liver and urine samples. HPLC analysis results of blood, liver and urine samples are shown in Figure 2. After 5 min injection of the tracer, HPLC analysis results of blood indicated that a small amount of [^{18}F]FNGA which not uptake by liver were still stable in blood. After 30 min, most of [^{18}F]FNGA were degraded in lysosome, and the metabolites were eliminated from liver. When analyzed by HPLC, the liver supernatant at 30 min depicted a major radioactivity peak at a retention time close to that of [^{18}F]FB-Lys, while the peak of [^{18}F]FNGA was disappeared. The HPLC analysis results of urine sample showed same results. The retention time of urinary metabolite was 4.9 min. The results indicated that the major radiolabeled metabolite in liver coincided with chromatographic behaviors of [^{18}F]FB-Lys.



Scheme 1. Radiolabeling of [^{18}F]FNGA through active ester intermediate [^{18}F]SFB to coupling the ε-amide of lysine residue of NGA (*m* is less than 1). The [^{18}F]FB-Lys was radiosynthesised with same process. It can predict [^{18}F]FB-Lys mainly conjugate ε-amide due to excess of lysine.

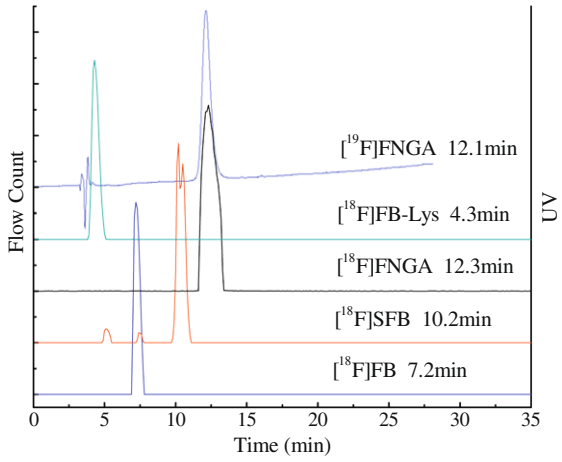


Figure 1. The radio-HPLC radiochromatograms of the [^{18}F]FB, [^{18}F]SFB, [^{18}F]FNGA and [^{18}F]FB-Lys. The retention time was 7.2, 10.2, 12.3 and 4.3 min, respectively, in our gradient system. [^{19}F]FNGA was analyzed in same gradient with UV detection at 220 nm. The retention time of [^{19}F]FNGA was 12.1 min which is similar with [^{18}F]FNGA.

3.3. Ex vivo biodistribution

To evaluate ex vivo tissue distribution characteristics of [^{18}F]FNGA, the biodistribution studies were performed using Kunming normal mice. The results were shown in Figure 3. For [^{18}F]FNGA, the liver uptake was 79.18 ± 7.17% of the injected dose per gram at 5 min after injection, which decreased to 18.55 ± 2.63%ID/g in the presence of a blocking dose of free NGA (10 mg/kg mice body weight). The different of kidney and blood accumulation between control and blocking groups were also statistically significant (P <0.01). The ratio of liver/blood was also decreased from 23.2 to 0.44 after blocking. In normal mice, the radioactivity was rapid eliminated from the liver by both hepatobiliary and renal excretion. At 30 min after injection, the radioactivity accumulation of liver was only 13.85 ± 3.10%ID/g. The radioactivity in kidney and small intestine was increased significantly.

3.4. MicroPET imaging

We also evaluated [^{18}F]FNGA in vivo with microPET scans. The representative microPET images were displayed in transaxial, coronal and sagittal orientation in Figure 4. High liver activity accumulation was observed at 5 min after injection. A small portion of radioactivity was eliminated from the liver after 15 min, the outline of liver was still clear. There were no significant uptakes in kidney, spleen and other organs in the abdomen. On the contrary, the liver accumulation was significant lower after inhibition, and the outline of liver was vague. The cardiac levels of activity were high and reduced at later time points when nonspecific activity accumulation in the blood after blocking had been gradually cleared. We can also find the significant uptake in the kidney, and gradually increase over time. After 15 min the high radioactivity concentration in kidney and urinary bladder indicated rapid clearance of the unbounded tracer through the kidney.

4. Discussion

It has been more than 17 years since the ^{99m}Tc-NGA/GSA clinical use as ASGP receptor-binding radiopharmaceutical. It provided a unique way to evaluate hepatic function and diagnose disease by a non-invasive method. There are over 10,000 ^{99m}Tc-GSA tests performed annually in Japan.[1] Positron emission tomography (PET) is one of the most sensitive molecular imaging techniques. Of all the positron nuclides employed in PET, fluorine-18 labeled PET radiopharmaceuticals have the most favorable physical properties,[13] and it is easily produced in the small biomedical cyclotrons. The increased sensitivity and unique spatial resolution that can be achieved with PET imaging allows for optimal quantitative imaging. The objective of this study was to get a novel ^{18}F labeled PET tracer for ASGP receptor imaging. *N*-Succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB), an agent widely used for labeling proteins and peptides with the positron-emitting radionuclide ^{18}F. It has good conjugation yields and metabolic stability.[11] We choose NGA as targeted molecular, and labeled ^{18}F via [^{18}F]SFB.

Starting with [^{18}F]F^{-}, the [^{18}F]SFB was synthesized by two-step reaction. The NGA was reacted with activated ester [^{18}F]SFB through ε-amino groups of lysine residues under basic condition. In Scheme 1, the 'm' in the structure of [^{18}F]FNGA is less than 1. That is because the amount of NGA is far excess compared with [^{18}F]SFB. Specific activity results also confirmed that. We choose HiTrap desalting column to purify [^{18}F]FNGA. The HiTrap desalting column is packed with Sephadex G25. The column is easy to perform separation of protein with low molecular weight materials. The time required for purification is less than using traditional size-exclusion chromatographic column. The radiochemical purity of [^{18}F]FNGA was above 99% after purification. The overall radiochemical yield with decay correction was about 8–10%, including final purification. The coupling yield for NGA is relatively lower than other proteins reported in the literature using [^{18}F]SFB. That

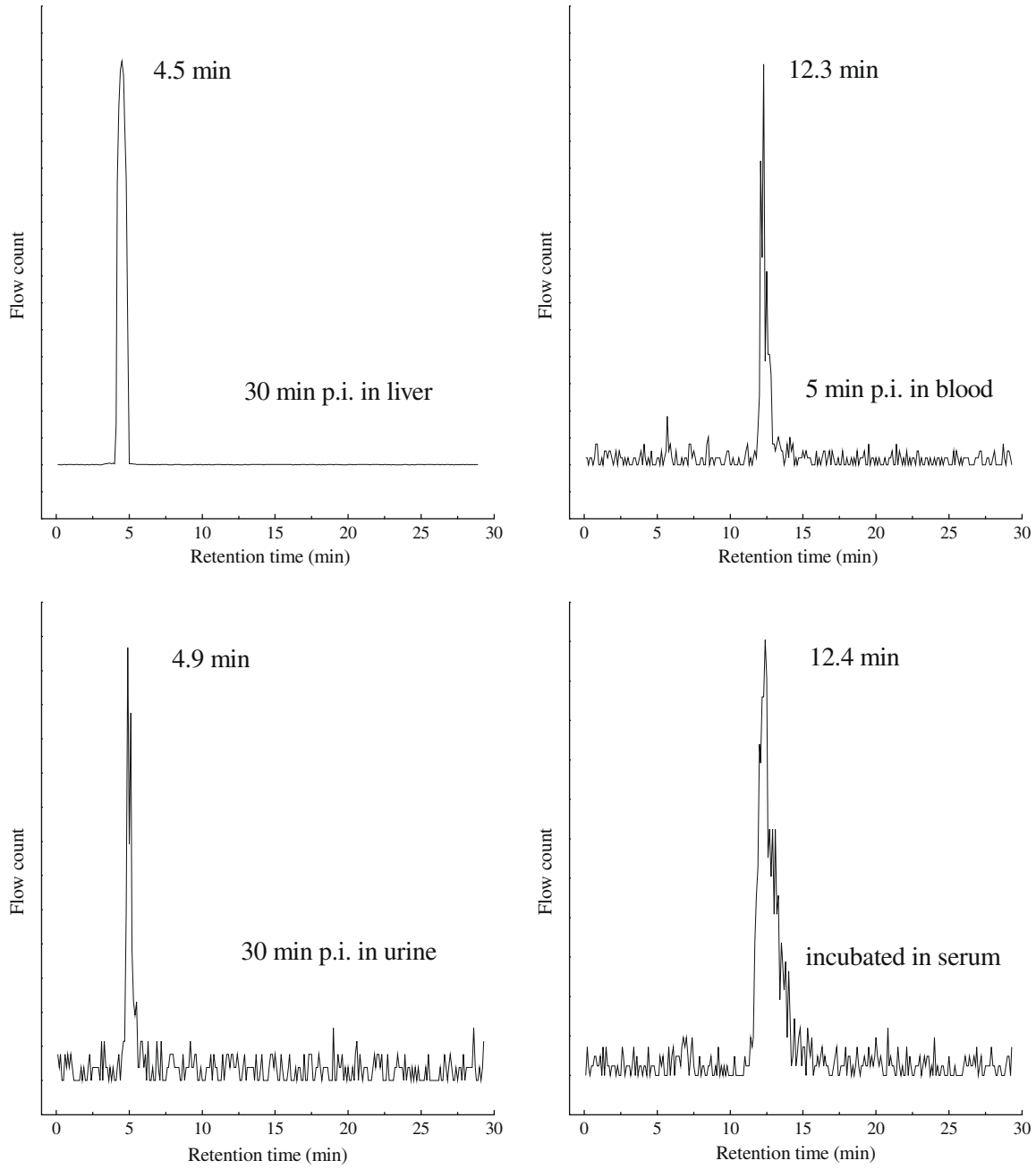


Figure 2. HPLC profiles of metabolic stability of [^{18}F]FNGA. Blood was collected at 5 min after injection of [^{18}F]FNGA to a female mice, liver and urine were collected at 30 min pi. The retention time of blood sample of 5 min pi was 12.3 min, and that of liver and urine sample of 30 min pi was 4.5 and 4.9 min, respectively. In vitro stability incubated in murine plasma at 37 °C for 4 h, the [^{18}F]FNGA still intact. The retention time was 12.4 min.

might be due to about 38 of 56 lysine groups in NGA molecule have been derivatized with galactose groups, which leads to more steric hindrance to acylate the residual ε-amino. When we use lysine to couple with [^{18}F]SFB, the labeling yield is high because the amount of ligand is far excess.

In the study of ex vivo biodistribution, [^{18}F]FNGA displayed a rapid and high uptake in the liver. While the liver accumulation decreased obviously by 30 min, the uptake in kidney and small intestine increased significantly (P <0.01). Higher radioactivity levels in the kidney indicated that radiometabolites excreted primarily through the kidneys, and increased radioactivity in small intestine indicated that small amount of radiometabolites excreted through the intestinal tract. [^{18}F]FNGA showed good plasma stability in vitro, and the result of in vivo metabolic stability analysis also confirmed the initial stability in blood after injection. The results of metabolic analysis of liver showed that the [^{18}F]FNGA was metabolized in the liver and generated to hydrophilic compounds after 30 min injection. According to the results of biodistribution and metabolic stability study, we speculate the intact [^{18}F]FNGA would be rapidly taken up by hepatocyte via receptor-mediated endocytosis and then degraded in lysosome. The metabolites excreted rapidly mainly through the kidney to the urinary bladder. That is quite different with ^{99m}Tc-NGA/GSA, which has a longer retention time in the liver and mainly eliminated through intestine. The difference in biodistribution between [^{18}F]FNGA and ^{99m}Tc-NGA/GSA can be explained by the different ways of chemical conjugation with the radionuclide. Gore et al.[14] found after injection of ^{99m}Tc-NGA, 40% of the radioactivity was secreted into the

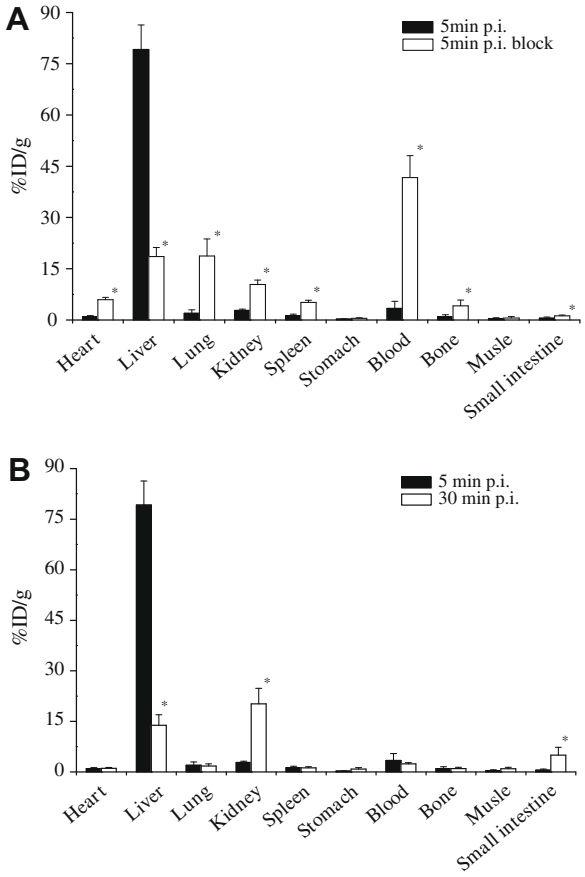


Figure 3. The biodistribution of the [^{18}F]FNGA in normal mice: (A) Mice were injected intravenously with 185 kBq of radiotracer with or without the pre-injecting of free NGA at 10 mg/kg mice body weight and executed at 5 min after injection. (B) The biodistribution at 5 and 30 min pi. Data are expressed as mean %ID/g ± SD, $n = 5$. *Statistically significant, $P < 0.01$, unpaired two-tail t-test.

bile within 1 h and postulated this may result from active transport of the $^{99m}TcO_4^-$. The ^{99m}Tc was electrostatic bonding to the NGA with low affinity. Rapid lysosomal degradation of ^{99m}Tc-NGA in hepatocyte would release as $^{99m}TcO_4^-$ which will persist in the liver for sufficient time. Duncan and Welch[15] reported DTPA conjugated polypeptides or proteins such as GSA (DTPA-NGA) were delivered to the lysosome when internalized by hepatocyte. The backbone of the GSA can be degraded to yield DTPA coupled lysine (DTPA-lysine) in lysosome. These metabolites remain within the lysosome and are only slowly released from the cell. Arano et al.[16] made similar findings in their study. The research of ^{111}In-DTPA-labeled NGA and NMA has indicated that lysine-DTPA-^{111}In is the final radiolabeled metabolite after lysosomal proteolysis in liver, and the metabolite is retained in the lysosomal fraction of murine liver for relatively long time. The biological characteristics of radiolabeled metabolites play a critical role in radioactivity elimination from liver cells.[7,16] The similar result was also found when using HYNIC as bifunctional coupling agent. The [^{99m}Tc](HYNIC-NGA)(tricine)$_2$ showed persistent localization of radioactivity in the liver, which was attributed to the slow elimination rate of the final radiometabolite, [^{99m}Tc](HYNIC-lysine)(tricine)$_2$ from the lysosomes.[17] In our study the ^{18}F was covalently substituted into the 4-fluorobenzoic acid and attached to the ε-amino groups of lysine residues in the NGA through activated ester intermediate. Since [^{18}F]FB was also conjugated to the lysine residues of protein backbone, we can surmise that after metabolizing in liver lysosome the protein backbone of [^{18}F]FNGA is degraded, but the amido linkage between [^{18}F]FB and lysine does not break, so the major radiometabolite is fluorine-18 conjugated lysine, [^{18}F]FB-Lys. [^{18}F]FB-Lys was prepared as a standard compound for analysis of the radioactivity metabolism. The in vivo metabolic stability analysis of the liver and urine sample 30 min after injection indicated the major metabolite was [^{18}F]FB-Lys (Fig. 2). The [^{18}F]FB-Lys has high hydrophilicity, which can be quickly cleared from liver and rapidly excreted to bladder through kidneys, and eliminated from the body finally. This result confirms our previous speculation.

The ex vivo receptor blocking studies were investigated by using 10 mg/kg of free NGA as the inhibitor. There have significant difference of biodistribution before and after inhibition, which was shown in Figure 3A. The most of radioactivity retained in the blood and the uptake in the kidney was also increased, while liver uptake declined significantly. The blocking and non-blocking results show significant difference in many other organs, such as heart, lung, spleen, bone and small intestine. That might be mainly due to the high radioactivity in blood which makes the increase of the radioactivity concentration in other non-target organs. When excess inhibitor was preinjected, most receptor situated on the

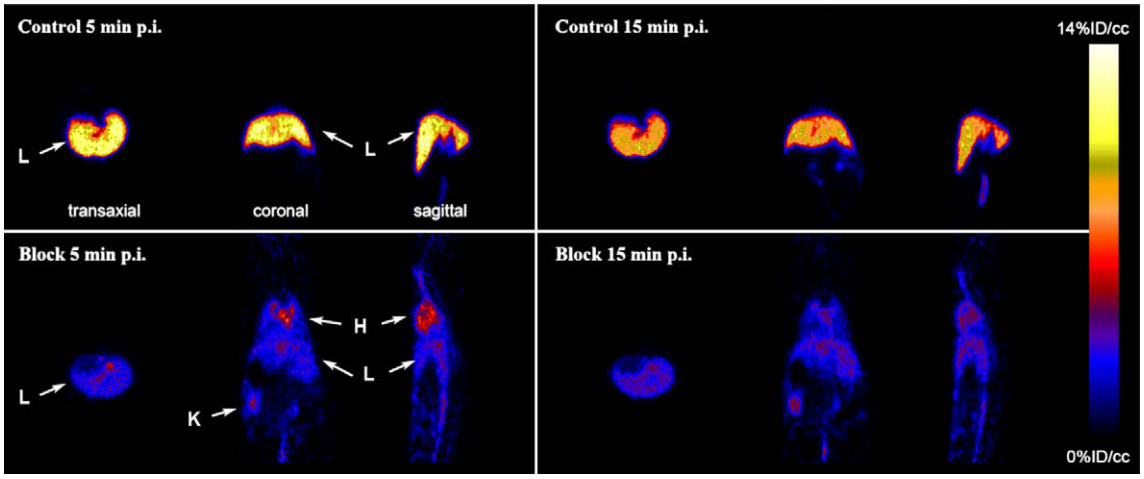


Figure 4. microPET images of rats. The upper is the control group: the liver is visualized as transaxial, coronal and sagittal sections at 5 and 15 min after injection of [^{18}F]FNGA (3.7 MBq [100 μCi]) (decay corrected to time of tracer injection). The lower is the blocking group: transaxial, coronal and sagittal images were collected at 5 and 15 min after injection of [^{18}F]FNGA (3.7 MBq [100 μCi]) with free NGA as blocking agent (10 mg/kg rats body weight). The liver (L) uptake decreased significantly comparing with control group. Radioactivity concentration was also seen in the heart (H) and kidney (K).

surface of hepatocyte membrane was occupied. The followed radiotracers would not uptake by the liver through receptor-mediated, and circulated in the blood. The tracers were absorbed by other organs slowly, metabolized and excreted eventually. The result of metabolic analysis and ex vivo biodistribution show that the [^{18}F]FNGA has high affinity with the ASGP receptor, and its uptake in the liver via receptor-mediated.

The in vivo microPET evaluation of [^{18}F]FNGA was observed with high liver accumulation and a certain retention. The liver uptake was decreased significantly after blocking with free NGA. The block group shows high kidney uptake, and the high radioactivity concentration in urinary bladder can also be seen after 15 min (not show in Fig. 4). The result of microPET coincides with ex vivo biodistribution. Both of the blocking experiments results indicated high affinity of [^{18}F]FNGA with the ASGP receptor.

It has been reported several models for quantitative evaluation of liver function using ^{99m}Tc-GSA. Recently ^{99m}Tc-GSA has been applied to quantitative evaluation of regional liver function and estimation of residual liver function in candidates for hepatectomy.[18–20] Regional evaluation requires more accuracy and finer resolution. Comparing with SPECT, PET has higher resolution, especially when combining with CT technologies which improves the reconstruction and visualization of data. The novel PET tracer [^{18}F]FNGA has good biological properties which may provide future applications for assessment of hepatocyte function with PET-CT. It is possible to functionally simulate the extension of hepatic resection and predict to some extent postoperative outcomes related to liver function.

5. Conclusion

This study demonstrates the successful coupling of galactosylneoglycoalbumin with positron-emitting radionuclide ^{18}F through the prosthetic labeling group [^{18}F]SFB. [^{18}F]FNGA has initial high activity accumulation in liver via ASGP receptor-mediated. The other tissues show low radioactivity accumulation. High liver/background ratio affords promising biological properties to get clear images. PET imaging with [^{18}F]FNGA may provide quantitative information and better resolution. That is the first report of ^{18}F labeled probe for ASGP receptor PET imaging so far as we know. That could be an important tool for quantificational evaluation of the risk of liver surgery and help determining the surgical procedures.

Acknowledgments

The Project was sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, and partly by the National Natural Science Foundation of China (20871020) and Beijing Natural Science Foundation (2092018). The authors will also thank Professor Boli Liu for his valuable discussion and guidance.

References and notes

1. Kokudo, N.; Vera, D. R.; Makuuchi, M. *Nucl. Med. Biol.* **2003**, *30*, 845.
2. Stadalnik, R. C.; Vera, D. R. *Nucl. Med. Biol.* **2001**, *28*, 499.
3. Vera, D. R.; Stadalnik, R. C.; Krohn, K. A. *J. Nucl. Med.* **1985**, *26*, 1157.
4. Kubota, Y.; Kojima, M.; Hazama, H.; Kawa, S.; Nakazawa, M.; Nishiyama, Y.; Nakagawa, S.; Murase, T.; Okuno, H.; Naitoh, Y., et al *Jpn. J. Nucl. Med.* **1986**, *23*, 899.
5. Torizuka, K.; Ha-Kawa, S. K.; Ikekubo, K.; Suga, Y.; Tanaka, Y.; Hino, M.; Ito, H.; Yamamoto, K.; Yonekura, Y. *Jpn. J. Nucl. Med.* **1991**, *28*, 1321.
6. Wakisaka, K.; Arano, Y.; Uezono, T.; Akizawa, H.; Ono, M.; Kawai, K.; Ohomomo, Y.; Nakayama, M.; Saji, H. *J. Med. Chem.* **1997**, *40*, 2643.
7. Arano, Y.; Mukai, T.; Akizawa, H.; Uezono, T.; Motonari, H.; Wakisaka, K.; Kairiyama, C.; Yokoyama, A. *Nucl. Med. Biol.* **1995**, *22*, 555.
8. Vera, D. R. *J. Nucl. Med.* **1992**, *33*, 1160.
9. Guhlke, S.; Coenen, H. H.; Stöcklin, G. *Appl. Radiat. Isot.* **1994**, *45*, 715.
10. Zhang, X.; Cai, W.; Cao, F.; Schreibmann, E.; Wu, Y.; Wu, J. C.; Xing, L.; Chen, X. *J. Nucl. Med.* **2006**, *47*, 492.
11. Zhang, X.; Xiong, Z.; Wu, Y.; Cai, W.; Tseng, J. R.; Gambhir, S. S.; Chen, X. *J. Nucl. Med.* **2006**, *47*, 113.
12. DuBois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.
13. Ametamey, S. M.; Honer, M.; Schubiger, P. A. *Chem. Rev.* **2008**, *108*, 1501.
14. Gore, S.; Morris, A. I.; Gilmore, I. T.; Maltby, P. J.; Thornback, J. R.; Billington, D. *J. Nucl. Med.* **1991**, *32*, 506.
15. Duncan, J. R.; Welch, M. J. *J. Nucl. Med.* **1993**, *34*, 1728.
16. Arano, Y.; Mukai, T.; Uezono, T.; Wakisaka, K.; Motonari, H.; Akizawa, H.; Taoka, Y.; Yokoyama, A. *J. Nucl. Med.* **1994**, *35*, 890.
17. Ono, M.; Arano, Y.; Uehara, T.; Fujioka, Y.; Ogawa, K.; Namba, S.; Mukai, T.; Nakayama, M.; Saji, H. *Bioconjugate Chem.* **1999**, *10*, 386.
18. Hwang, E.-. H.; Taki, J.; Shuke, N.; Nakajima, K.; Kinuya, S.; Konishi, S.; Michigishi, T.; Aburano, T.; Tonami, N. *J. Nucl. Med.* **1999**, *40*, 1644.
19. Sugai, Y.; Komatani, A.; Hosoya, T.; Yamaguchi, K. *J. Nucl. Med.* **2000**, *41*, 421.
20. Shuke, N.; Okizaki, A.; Kino, S.; Sato, J.; Ishikawa, Y.; Zhao, C.; Kinuya, S.; Watanabe, N.; Yokoyama, K.; Aburano, T. *J. Nucl. Med.* **2003**, *44*, 475.