Applied Radiation and Isotopes 74 (2013) 1–5

Contents lists available at SciVerse ScienceDirect

Applied Radiation and Isotopes

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

The preparation of ^{99m}Tc-DTPA–LSA and its instant lyophilized kit for hepatic receptor imaging

Wenjiang Yang [a,d], Zuoquan Zhao [d], Wei Fang [c], Xianzhong Zhang [b,d,*]

[a] Key Laboratory of Nuclear Analytical Techniques, Beijing Engineering Research Center of Radiographic Techniques and Equipment, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[b] Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen 361005, China
[c] Department of Nuclear Medicine, Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences, Beijing 100037, China
[d] Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, China

HIGHLIGHTS

- DPTA-LSA conjugate was prepared for ^{99m}Tc labeling and ASGP receptor targeting.
- A lyophilized DTPA–LSA kit was developed for instant preparation of ^{99m}Tc-compound.
- ^{99m}Tc-DTPA–LSA accumulated mainly in the liver (> 96 % ID/g at 5 min p.i.).
- High quality SPECT images were obtained in normal Japanese White rabbits.
- A potential alternative agent for assessment of hepatocyte function in the future.

ARTICLE INFO

Article history:
Received 8 March 2012
Received in revised form
26 September 2012
Accepted 17 December 2012
Available online 23 December 2012

Keywords:
DTPA–LSA
^{99m}Tc
ASGP
Instant lyophilized kit
SPECT imaging

ABSTRACT

Diethylenetriaminepentaacetic acid neolactosyl human serum albumin (DTPA–LSA) was prepared and labeled with technetium-99m. The labeling conditions of ^{99m}Tc-DTPA–LSA were optimized, and lyophilized kit was developed for instant preparing of ^{99m}Tc-DTPA–LSA. ^{99m}Tc-DTPA–LSA showed high liver uptake in normal mice (> 96% ID/g at 5 min after injection), and it could be blocked significantly by pre-injecting free neogalactosylalbumin (NGA). Single photon emission computed tomography (SPECT) study was performed in normal Japanese White rabbits and SPECT images with high quality were obtained at 15, 30, 60, and 120 min after injection of the radiotracer. The promising biological properties of ^{99m}Tc-DTPA–LSA combined with the development of reliable and instant lyophilized DTPA–LSA kit afford the opportunity of hepatic receptor imaging for routine clinical assessment of hepatic function.

1. Introduction

Asialoglycoprotein (ASGP) receptors exist on the surface of hepatocytes and play an important role in the hepatic metabolism of serum (Kokudo et al., 2003). Quantitative imaging of ASGP receptors could estimate the function of liver, help the early diagnosis of hepatic diseases and accurate evaluation of functional status (Stadalnik and Vera, 2001). ASGP receptors can recognize galactose, lactose or *N*-acetylgalactosamine residues of desialylated glycoproteins. Materials having these ligands can be transferred to the hepatocyte via receptor-mediated endocytosis and are affected only by hepatocyte function. That permits a non-invasive way to evaluate hepatic function and hepatic functional reserve directly and quantitatively. A novel NGA has been developed by attaching the galactosyl unit to human serum albumin (HSA), and then labeled with ^{99m}Tc for imaging the liver (Vera et al., 1985). Later, to simplify the labeling procedure, diethylenetriaminepentaacetic acid galactosyl human serum albumin (GSA) was obtained and developed as an instant kit (Kubota et al., 1986). The GSA kit can be labeled with ^{99m}Tc more easily. ^{99m}Tc-GSA has been clinically used as ASGP receptor-binding radiopharmaceutical in Japan since 1992. The derivates of NGA had been radio-labeled with different radioisotopes, such as technetium-99m (Ono et al., 1999; Yang et al., 2010b), iodine-125/131 (Wakisaka et al., 1997), indium-111 (Arano et al., 1995), gallium-67/68 (Vera, 1992) and fluorine-18 (Yang et al., 2009). Newly designed probes with nonalbumin backbone, such as

* Correspondence to: School of Public Health, Xiamen University, Xiang'an South Rd., Xiang'an District, Xiamen 361005, P.R. China.
Tel./fax: +86 5922880645.
E-mail addresses: zhangxzh@gmail.com, zhangxzh@xmu.edu.cn (X. Zhang).

0969-8043/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.apradiso.2012.12.009

dextran (Vera et al., 2005), chitosan (Kim et al., 2006; Kim et al., 2005; Yang et al., 2010a) or PVLA (Yang et al., 2011), could avoid the risk of viral breakthrough for the blood products of HSA. Considering the suitable affinity to ASGP receptor and potential clinical applications, the further improvement and research on derivates of NGA are still worth it.

The NGA was synthesized by conjugating 2-imino-2-methoxyethyl-1-thio-β-D-galactose to human serum albumin. The precursor requires long preliminary steps for synthesis and cannot be stored long-term due to instability. Recently, ^{99m}Tc-LACTAL was prepared by Philippe et al. (Chaumet-Riffaud et al., 2010). They prepared a DTPA–lactosyl albumin (LACTAL) with an alternate process. The thiol functions were introduced to albumin backbone by reaction of iminothiolane on the ε-amino groups of the protein lysines. Then the lactosyl ligand and chelate were covalently grafted on the protein simultaneously. The reaction time of LACTAL was considerably shorter than that of NGA and thus may reduce bacteria growth. However the synthesis process is still complicated, and is not conducive to large-scale production. Jeong et al. reported a simple-prepared probe, ^{99m}Tc labeled LSA which was reduced by β-mercaptoethanol to generate sulfhydryl groups for labeling (Jeong et al., 2004). The LSA was prepared by conjugating lactose to human serum albumin through reductive amination. The synthesis process is simple. And the precursor, lactose, is commercially available. In this paper, we introduced bifunction conjugate group DTPA to LSA instead of reduction of structural disulfide bonds for direct labeling. Thus, the impact of reduction of disulfide bonds on protein backbone could be avoided.

2. Experimental

2.1. Materials

All chemicals obtained commercially were used without further purification. Sodium cyanoborohydride was purchased from Acros Organics. Lactose and DTPA anhydride were purchased from Sigma-Aldrich.

Instant thin-layer chromatography-silica gel (ITLC-sg) chromatographic strips were purchased from Pall Life Sciences. Radio-high pressure liquid chromatography (Radio-HPLC) experiments were performed on a SHIMADZU system with the 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. Animal experiments were carried out in Kunming mice (average weight about 20 g) or Japanese White rabbits (female, average weight about 2.5 kg), 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 DTPA–LSA

LSA was prepared using the reductive lactosamination method (Jeong et al., 2004). In 5 mL of 0.2 mol/L potassium phosphate buffer (pH 8.0), human serum albumin (67.5 mg) was dissolved completely and 200 mg of α-lactose was dissolved successively. After slow stirring for 2 h, sodium cyanoborohydride (200 mg) was added, and the solution was filtered using a 0.22 μm filter. The filtered solution was incubated at 37 °C with slow stirring for 7 days. The collected LSA solution was dialyzed against distilled water for 3 days and lyophilized.

LSA (60 mg) was dissolved in 4 mL 0.1 mol/L HEPES buffer (pH 7.0), and DTPA anhydride (10 mg) was added while stirring for about 20 min. The solution was filtered using a 0.22 μm filter. The solution was dialyzed against distilled water for 3 days and lyophilized to give DTPA–LSA.

The conjugated number of lactose per molecule of human serum albumin was calculated by measuring lactose concentration from the absorbance at 490 nm using the phenol/sulfuric acid method (DuBois et al., 1956). The average number of DTPA units per protein was measured after complexation of the DTPA conjugate with gadolinium. Briefly, the DTPA–LSA was dissolved in tartaric acid buffer (pH 4.0). Excess of gadolinium was added and stirred for 2 h. After being purified by a HiTrap desalting column, the sample was assayed for gadolinium concentration by inductively coupled plasma spectrometry (ICP). The protein concentration of the same sample was measured using absorbance at 280 nm. The DTPA density was calculated by dividing the gadolinium concentration by the protein concentration.

2.3. Kit formulation and labeling with ^{99m}Tc

The kit formulation was prepared under aseptic conditions. One milliliter solution of sodium potassium tartrate buffer 0.2 mol/L with the final pH 3.5, containing 3 mg DTPA–LSA and 20 μg $SnCl_2$ (10 μL of 2 mg/mL $SnCl_2 \cdot 2\,H_2O$ in nitrogen purged 0.1 mol/L HCl), was filtrated into a glass vial. The vials were transferred to the freeze-dryer and the process continued for 24 h. The vials were closed under dry sterile nitrogen gas and stored at 2–8 °C.

To the above freeze-dried vial, 1–5 mL of generator-eluted ^{99m}Tc-pertechnetate was added and incubated at room temperature for 10 min to give ^{99m}Tc-DTPA–LSA. The ^{99m}Tc-DTPA–LSA was used directly without further purification in biodistribution and imaging studies.

2.4. Radiochemical analysis

The chromatography analysis was performed on ITLC-sg strips with ACD (0.068 mol/L citrate, 0.074 mol/L glucose, pH 5.0) as mobile phase. ^{99m}Tc labeled DTPA–LSA remained at the point of spotting (R_f=0–0.1), while hydrolyzed Tc-99m and other radioactive impurities moved with the solvent front (R_f=0.8–1.0). Radio-HPLC was performed on a SHIMADZU system with the Reversed-phase Kromasil C-4 column (4.6 × 250 mm, 5 μm). The column was eluted at a flow rate of 1 mL/min with mobile phase starting 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.

^{99m}Tc-DTPA–LSA was incubated in saline at room temperature (25 °C) for 4 h. The radiochemical purity (RCP) was evaluated by ITLC every single hour and radio-HPLC analysis at the end of 4 h.

2.5. Biodistribution study in mice

Biodistribution study of ^{99m}Tc-DTPA–LSA was carried out in normal mice. About 0.185 MBq (in 100 μL of solution) of ^{99m}Tc-DTPA–LSA ($\sim 1.2 \times 10^{-9}$ mol/kg of body weight) was injected through the tail vein. At selected time points (5, 30 and 120 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), and the values are expressed as mean ± SD.

The blocking study was performed by conducting the biodistribution experiment in the presence of free NGA (200 μg,