Preparation and preclinical studies of a hepatic receptor imaging agent

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Abstract Galactosyl neoglycoalbumin (NGA) was synthesized by the covalent coupling of a bifunctional reagent, 2-imino-2-ethyloxymethyl-1-thiogalactose, to human serum albumin. The average number of galactose groups per albumin molecule (Gal/HSA) of NGA was 30. NGA was labeled directly with $Na^{99m}TcO_4$. The radiochemical purity of ^{99m}Tc-NGA was over 90%. Consequences of the preclinical studies show that ^{99m}Tc-NGA is a good hepatic receptor imaging agent, uptaken by hepatic cells specifically, and excreted majorly from the biliary system and the gastrointestinal (GI) tract.

Keywords ^{99m}Tc-NGA, Hepatic receptor imaging agent, Bifunctional reagent , Glycoprotein

1 Introduction

Hepatic binding protein (HBP) is an asialoglycoprotein receptor, which resides only at the cell surface of mammalian hepatocytes, and binds with galactose-terminated glycoproteins specifically.[1] The model of action of ligand and receptor is receptor-mediated endocytosis and receptor recycling. The ligand is catabolized in hepatocellular lysosomes. The variation of HBP level reflects physiological and pathological change. So its application would show liver receptor function. We have obtained galactosyl neoglycoalbumin (NGA) by attaching galactosyl units to human serum albumin (HSA) with bifunctional reagent 2-imino-2-methoxyethy-1-thioglycosides (IME-thiogalactose). We have prepared the ^{99m}Tc-NGA, and carried out the preclinical studies in animals.

2 Materials and methods

2.1 Materials

$Na^{99m}TcO_4$ was from the First Institute of Chinese Nuclear Power Designing, cyanomethyl 2,3,4,6,-tetra-O-acetyl-1-thio-β-D-glucopyrianoside was prepared at our laboratory[2], human serum albumin was from Wuxi Blood Station, BSA-V and Sephadex G-50 from Pharmacia, other analytical reagents were made in China.

NIH mice, New Zealand white rabbits were provided by Jiangsu Wuxi Station of Quality Monitor for Laboratory Animals.

2.2 Synthesis of NGA[3]

Cyanomethyl 2,3,4,6-tetra-O- acetyl- 1-thio- β -D-glucopyrianoside 0.406 g(1.01 mmol), synthesized by our laboratory[2], was dissolved in dry methanol (10 ml), then mixed with 7.0 mg sodium methoxide (0.13 mmol) and stood overnight at room temperature. Under these conditions, the yield of the imidate was 56%. The mixture was evaporated to dryness in vacuo and then dissolved in 4 ml 100 mg HSA (0.15μmol) solution of 0.1 mol/L sodium borate buffer (pH8.5). After mixed over 4 h at 22°C, the reaction mixture was applied to a column of Sephadex G-50, and eluted NGA with 0.1 mol/L NaCl, collected the peak of protein by the measurement of UV (Econo, Bio-Rad) and stored in 0.1 mol/L NaCl at -20°C. The scheme for synthesis of NGA is shown in Fig.1.

2.3 Measurement of protein concentration

Protein concentration was measured by the method of Lowry[4] with BSA-V as standard (DU-650, Backman, USA).

2.4 Determination of sugars

A colorimetric analysis[5] was used to determine sugars of NGA with galactose as standard (Lambda 2S, Perkin-Elmer, UK).

2.5 Preparation of ^{99m}Tc-NGA

NGA was labeled with $Na^{99m}TcO_4$ by the method of Yoshitsugu Kubota.[6,7]

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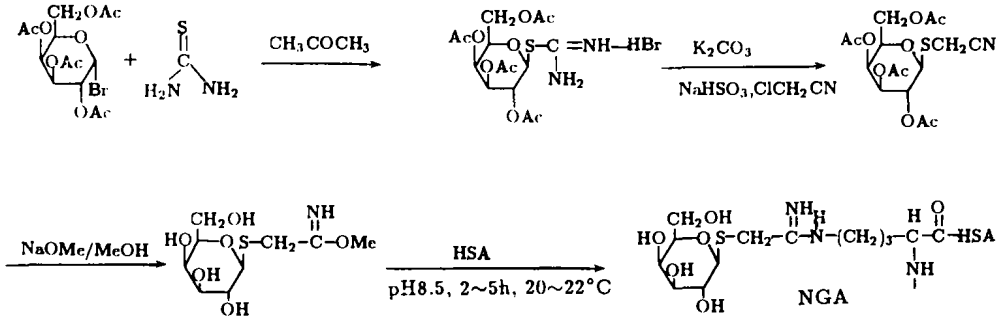

Fig.1 Synthesis scheme of NGA

3 Results

3.1 Analysis of average carbohydrate density of NGA

The protein concentration of NGA solution was 9.53 mg/ml, the sugar concentration of NGA solution was 0.768 mg/ml. Consequently, the NGA could be calculated by $(\frac{0.768/180}{9.53/67000})$ to be 30 galactose groups per albumin molecule (Gal/HSA).

3.2 Analysis of ^{99m}Tc-NGA

The labeling yields were determined to be 95% with the method of trichloroacetic acid precipitation (γ-counter, C5002, Parkard). The influences of labeling conditions on labeling yields of ^{99m}Tc-NGA were, respectively, studied as shown in Fig.2. The experimental results prove that labeling yields of ^{99m}Tc-NGA could be over 90% under the following labeling conditions: pH is below 3.5, the amount of NGA over 5 mg, the content of $SnCl_2$ over 30μg, and the amount of ascorbic acid over 0.8 mmol/L. The ^{99m}Tc-NGA is stable for at least 8 h at room temperature.

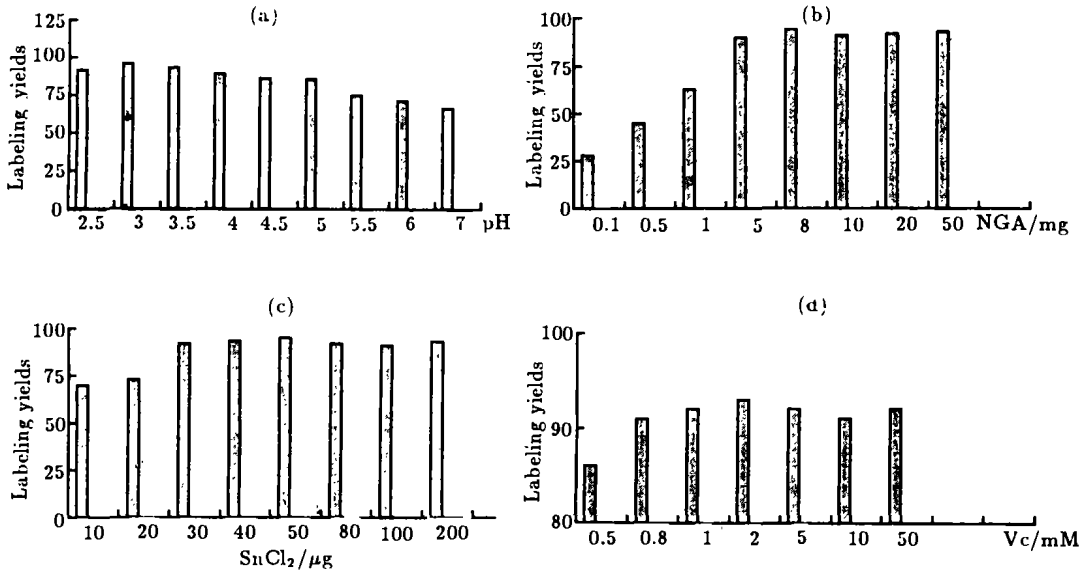

Fig.2 The labeling yield of ^{99m}Tc-NGA at different pH values (a); at different amounts of NGA (b); at different amounts of $SnCl_2$ (c); at different amounts of Vc (d)

3.3 Biodistribution in mice

30 NIH mice (~20 g) were used to measure the biodistribution of ^{99m}Tc-NGA. They were divided into 6 groups (5 per group). 0.2 ml (0.36 MBq) ^{99m}Tc-NGA solution containing 67μg NGA was injected to each animal through the tail vein. After injection, each group mice were decapitated at 5, 10, 15, 30, 60 and 120 min, respectively. The interested organs were excised, weighed, and counted in the automatic gamma counter (C5002, Parkard). The %ID/organ was determined by comparison of organ radioactivity levels with the injected dose (see Table 1).

Table 1 Distribution of ^{99m}Tc-NGA in mice (%ID/organ (n=5))

Organs	Time after injection/min					
	5	10	15	30	60	120
Blood[1]	28.78±6.58	12.49±1.19	5.04±1.95	5.04±1.91	3.43±0.77	1.94±0.19
Heart	0.39±0.12	0.31±0.02	0.25±0.04	0.20±0.02	0.13±0.04	0.09±0.04
Liver	25.53±1.16	26.40±1.05	28.56±2.60	23.38±2.25	21.10±0.21	20.79±0.67
Spleen	0.47±0.09	0.35±0.05	0.34±0.06	0.19±0.03	0.20±0.05	0.20±0.01
Lung	2.17±0.74	1.21±0.52	0.99±0.50	0.57±0.11	0.50±0.12	0.37±0.07
Kidney	4.59±0.59	6.53±0.61	7.43±0.72	8.23±1.28	8.20±2.54	8.17±3.55
Stomach[1]	0.32±0.08	0.26±0.03	0.23±0.06	0.21±0.04	0.20±0.06	0.20±0.07
Intestines[1]	1.70±0.36	1.85±0.54	2.81±0.51	4.26±1.02	7.43±2.02	9.47±2.18
Gallbladder	0.10±0.03	0.18±0.06	0.23±0.19	0.27±0.15	0.44±0.15	2.83±1.15
Brain	0.31±0.09	0.17±0.04	0.10±0.02	0.07±0.03	0.05±0.01	0.05±0.02

[1] %ID/g organ

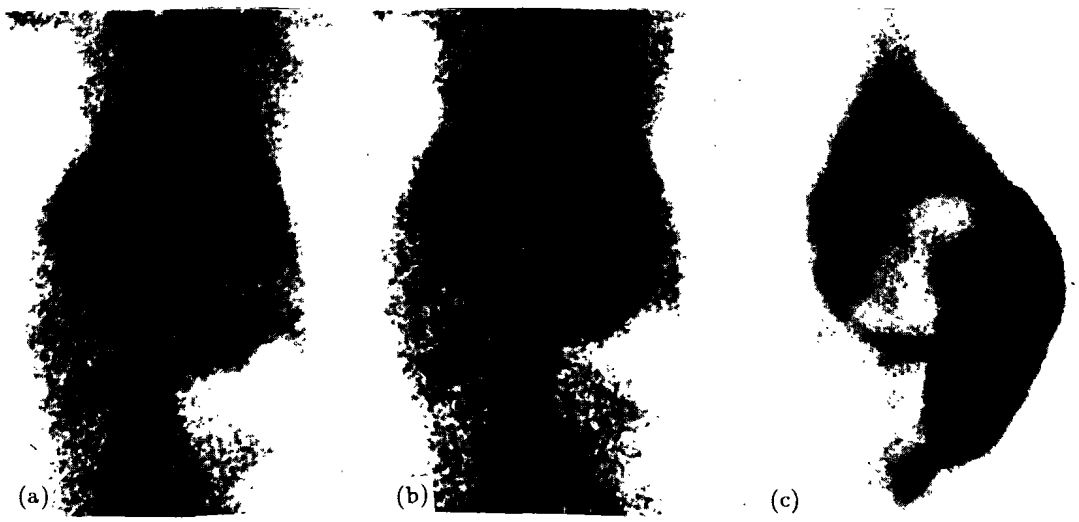

Fig.3 Serial imaging of ^{99m}Tc-NGA in the normal rabbit at 15min (a); 30min (b); 120min (c) after injection

Biodistribution in mice showed that the peak of liver uptake of ^{99m}Tc-NGA was reached within 15 min after injection, the radioactivity of blood decreased very fast, and that of intestinal tract increased with time.

3.4 Liver imaging in rabbit

After injected 18.5MBq ^{99m}Tc-NGA containing NGA 3.2×10^{-10} mol through the marginal ear vein, the rabbit (New Zealand white, ~2.5 kg) was scanned with SPECT (DAICOM, Siemens, Germany) at different time. Dynamic radioactivity in each ROI was measured and analysed. The results showed that the liver imaging was very clear, heart

imaging faded at 5 min after injection. The distribution of liver imaging was increased with the decrease of heart imaging at ten minutes. About 90 min later, the liver imaging was faded gradually while the biliary system and intestinal tract imaging was increased by degrees (see Fig.3).

4 Discussion

A number of techniques have been developed for attaching sugars to proteins. The better method is that imidates are used as bifunctional reagents for attaching carbohydrates to proteins. Imidates are water-soluble reagents that would react rapidly with primary amino groups of proteins to produce amidines. The reaction is very specific for primary amino groups because imidates donot react with phenolic, hydroxyl, imidazole or guanidino groups in proteins. The amidines are very stable in neutral and weak acidic pH. IME-thioglycosides have been prepared from cyanomethyl thioglycosides (CNM-thioglycosides), then it reacted with ε-amino groups or α-amino groups of HSA to produce NGA in weak base. Little or no change in HSA tertiary structure occurred after molecule was such modified. NGA can be distinguished by HBP and combined with it specifically, because galactosyl has been introduced . Affinity to HBP is related to carbohydrate density of NGA. Gla/HSA can be controlled by the molar ratio of IME-thioglycosides to HSA.

The number of galactose groups per albumin molecule can not be strictly equal so the carbohydrate density we have measured is an average value. After studied some major factors on labeling yields of ^{99m}Tc-NGA, we found the optimal labeling conditions as followings: pH2.5~3.5, NGA>5 mg, $SnCl_2$ >30μg, [ascorbic acid]>0.8 mmol/L. Under these conditions, the labeling yields of ^{99m}Tc-NGA could be over 90%, and the labeling compound could be stable over 8 h at room temperature, so it could be suitable for clinical application. Because of using high acidic condition, colloid technetium could be avoided on labeling. Ascorbic acid has advantages to increase the labeling yields. In this reaction, ascorbic acid was not only a reducing agent, but also a transfer ligand. ^{99m}Tc-ascorbate coordinate intermediates could prevent ^{99m}Tc-colloid from forming.

Biodistribution in mice and liver imaging in rabbit showed that ^{99m}Tc-NGA we prepared could be uptaken rapidly by liver cell specifically, and excreted mainly to the biliary system and the gastrointestinal (GI) tract. The weak imagings were found in kidney, urinary bladder, spleen, lung and marrow. The results showed that the resolution of liver receptor imaging was preferable and it could reflect well liver function. So ^{99m}Tc-NGA is a good liver receptor functional imaging agent.

References

1 Stockert R J, Morell A G. Hepatology, 1993, 3:750
2 Liang Gao-Lin, Hu Ming-Yang, Jin Jian. J Chi Phar Ind, 1997,28:37
3 Yuan Chuan Lee, Christopher P *et al.* Biochemistry, 1976, 15:3956
4 Lowry O H, Rosebrough N J, Farr A L *et al.* J Biol Chem, 1951, 193:265
5 Dubios M, Gilles K A, Hamilton J K *et al.* Anal Chem, 1956, 28:350
6 Yoshutsugu Kubota, Michimasa Kojima, Hiroshi Hazama *et al.* Jpn J Nucl Med, 1986, 23:899
7 Eckelman W C, Steigman J. Nucl Med Biol, 1991, 18:3