Preparation of N-(3-bromo 2,4,6-trimethylacetanilide) iminodiacetic acid and its ^{99m}Tc-complex for hepatobiliary imaging

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1. Introduction

In general, lipophilic compounds labeled with radionuclides are used for liver imaging to evaluate the functional status of the hepatocytes and the patency of the biliary duct. Most hephatobiliary agents labeled with ^{99m}Tc are iminodiacetic acid derivatives.
Among the IDA derivatives, ^{99m}Tc-mebrofenin®[N-(3-bromo 2,4,6-trimethylacetanilide) iminodiacetic acid] combines best the characteristics of high hepatic uptake, low urinary excretion, and fast blood clearance and hepatocellular transit. [1-4]

Furthermore, ^{99m}Tc-mebrofenin® has a lower renal clearance and the highest degree of resistance to the competitive effects of bilirubin. [5]
As for ^{99m}Tc-mebrofenin®, substitution of the three methyl groups at the ortho and para positions and bromine at the meta position increases the hepatic extraction, decreases the hepatocellular transit time, and impacts with a high degree on the resistance to the competitive effects of bilirubin and low urinary excretion. [6]

In this study, we synthesized BromoTIDA [N-(3-bromo 2,4,6-trimethylacetanilide) iminodiacetic acid] in which bromine is positioned at the meta site of 2,4,6-trimethyl aniline, then evaluated the ^{99m}Tc-complex as a hepatobiliary agent using animals.

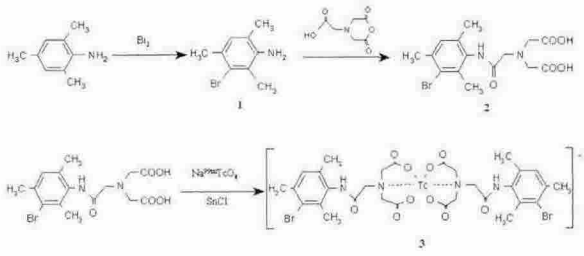
2. Methods and Results

2-1. Synthesis of N-(3-bromo 2,4,6-trimethylacetanilide) imino-diacetic acid [BromoTIDA]

BromoTIDA, an acetanilide inminodiacetate deriva- tive analogue, was synthesized by the reaction between 3-bromo 2,4,6-trimethylaniline and nitrilotriacetate anhy - dride prepared *in situ*.
The NMR in DMSO of BromoTIDA showed three distinct peaks of the three methyl groups in phenyl ring resulting from the substitution of bromine in *meta* position.

2-2. Preparation of ^{99m}Tc-IDA Complex

[^{99m}Tc] pertechnetate was reacted with IDA derivatives in the presence of stannous(II) chloride resulting in the formation of {^{99m}Tc(III)[IDA]$_2$}$^-$ ***3*** as given in Scheme 1.



Scheme 1. BromoTIDA [N-(3-bromo 2,4,6-trimethyl acetanilide) iminodiacetic acid] and its ^{99m}Tc-complex

The TLC pattern on silica alumina impregnated glass fiber sheets using a 20 % aqueous NaCl solution, ^{99m}Tc-BromoIDA complex and $^{99m}TcO_4$ was found at the origin (0.0~0.2) and at the solvent front (0.9~1.0), respectively. In contrast, when water was used, ^{99m}Tc-IDAs and $^{99m}TcO_4$ was found at the solvent front, $^{99m}TcO_2$ (colloid) was found at the origin.
The radiolabeling efficiency of ^{99m}Tc-BromoTIDA was maintained with high radiochemical purity (>95%) at room temperature for 6 h.

2-3. In Vivo Pharmacokinetics

2-3-1. Scintigraphic Imaging

A six week-old New Zealand white male rabbits (2.87 kg) were used for the imaging studies. The animal was kept in individual cages at 22 ± 1 °C with a relative humidity of 60 ± 10 % and a 12 h light/dark cycle, allowed free access to food and water, and used after acclimation for one week. It was anesthetized with ketamine and xylazine, and ^{99m}Tc-complex, 37 MBq/0.5 ml, was injected via the left ear vein.
Whole-body dynamic images for 30 min were obtained using a gamma camera (Orbiter, Simens, USA) fitted with a low-energy all-purpose collimator. Image data were analyzed using the dynamic procedure of the SCINTRON IV system (Medical imaging electronics, Germany).

The major excretion pathway of ^{99m}Tc-BromoTIDA was hepatocytic excretion.
Upon injection, ^{99m}Tc-BromoTIDA was quickly cleared from the blood by the hepatocytes and excreted into the gallbladder and intestine with negligible uptake by the kidneys and other organs.

The serial static image scans of the rabbits administered with ^{99m}Tc-BromoTIDA revealed that none of the tissues except the hepatobiliary system had taken up radioactivity.

2-3-2. Biodistribution Studies

^{99m}Tc-BromoTIDA with 7.4 ± 0.7 MBq/0.2 ml (0.2 ± 0.02 mCi) was injected into Sprague-Dawley male rats (SPF grade, 157.2 ± 6.3 g, n = 12; 4 for each time interval) through a lateral tail vein. To determine the radioactive concentration in the tissues and organs, the animals were sacrificed after being anesthetized at 10, 30 and 120 mins after administration. The tissues and organs were excised and weighed. The radioactivity in the samples was counted for 1 min using a well-type gamma counter (Canberra, USA). The measured counts were corrected along with the same radioactivity of a standard injected radiopharmaceutical. The distribution in each organ was calculated and expressed as a percent injected dose per gram tissue (%ID/g).

The results of the biodistribution studies of ^{99m}Tc-BromoTIDA in rats, which were administrated intravenously and sacrificed at 10, 30 and 120 min, are summarized in *Table 1*, as the percent of the injected dose to each selected organ of the SD rat (%ID/g). Fast clearance of the ^{99m}Tc-BromoTIDA from the blood, liver, and lung was observed within 10 min of injection. Furthermore, 92 % of injected dose of ^{99m}Tc-BromoTIDA was transported to the hepatocytes and cleared into gallbladder and intestine with minimal uptake by the other organs.

Table 1. Radioactivity concentrations in organs or tissues after intravenous injection of ^{99m}Tc-BromoTIDA in male rats at 10, 30 and 120 mins.

	Elapsed time after administration		
	10 min	30 min	120 min
Blood	0.17 ± 0.05	0.04 ± 0.02	0.09 ± 0.04
Heart	0.08 ± 0.03	0.03 ± 0.01	0.04 ± 0.02
Lung	0.15 ± 0.05	0.08 ± 0.03	0.06 ± 0.02
Liver	0.73 ± 0.21	0.61 ± 0.03	0.12 ± 0.03
Kidney	0.63 ± 0.18	0.45 ± 0.10	0.71 ± 0.12
Spleen	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
Stomach	0.09 ± 0.07	0.01 ± 0.01	0.03 ± 0.02
S. Intestine	2.36 ± 1.08	1.79 ± 1.34	0.93 ± 0.12
L. Intestine	0.57 ± 0.54	0.21 ± 0.34	0.03 ± 0.04

3. Conclusion

The prepared ^{99m}Tc-BromoTIDA showed the best charatericstics of high hepatic uptake, low urinary excretion, fast blood clearence and short hepatocellular transit. Therefore, it can be applied to evaluate various hepatobiliary diseases related to the functional status of the hepatocytes and the patency of the biliary duct.

Reference

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