〈 Technical Note 〉

PREPARATION OF *N*-(3-BROMO-2,4,6-TRIMETHYLACETANILIDE)IMINODIACETIC ACID AND ITS ^{99M}TC-COMPLEX FOR HEPATOBILIARY IMAGING

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N-(3-bromo-2,4,6-trimethylacetanilide)iminodiacetic acid (BrTIDA) was synthesized using nitrilotriacetic anhydride prepared *in situ*, and lyophilized vials were prepared which contained 20 mg of BrTIDA and 0.4 mg of $SnCl_2$. To evaluate the clinical efficacy of the in-house prepared lyophilized kit, a technetium-99m complex of BrTIDA was prepared; its in vivo pharmacokinetic behavior was evaluated via animal studies to assess the hepatocytic function and the functional status of the cystic duct and the gallbladder. Serial static image scans of rabbits and the biodistribution in mice injected with ^{99m}Tc-BrTIDA revealed that none of the tissues except for the hepatobiliary system showed radioactivity concentrations, and a rapid clearance from the organs was observed. In conclusion, a lyophilized kit and its prepared ^{99m}Tc-BrTIDA can be applied as a hepatobiliary imaging agent for the evaluation of the functional status of the hepatocytes and the patency of the biliary duct.

KEYWORDS : Cholescintigraphy, Hepatobiliary Imaging, BrTIDA, ^{99m}Tc, Nitrilotriacetic Anhydride

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 [1].

Most hepatobiliary agents labeled with ^{99m}Tc are iminodiacetic acid (IDA) derivatives, including ^{99m}Tc-disofenin® [*N*-(2,6-diisopropylacetanilide)iminodiacetic acid, DISIDA] [2], ^{99m}Tc-mebrofenin® [N-(3-bromo-2,4,6 -trimethylacetanilide)iminodiacetic acid] [3-7], ^{99m}Tc-EHIDA [*N*-(2,6 diethyl acetanilide)iminodiacetic acid] [2], ^{99m}Tc-lidofenin® [*N*-(2,6 dimethylacetanilide) iminodiacetic acid] [8-9], ^{99m}Tc-IODIDA [*N*-(3-iodo-2,4-diethyl acetanilide) iminodiacetic acid] [4], and ^{99m}Tc-IOTIDA [*N*-(3-iodo-2,4,6-trimethyl acetanilide)iminodiacetic acid] [10-11].

Among IDA derivatives, ^{99m}Tc-mebrofenin® best combines the characteristics of a high hepatic uptake, a low urinary excretion, and fast blood clearance, and it is a hepatocellular transit. Furthermore, ^{99m}Tc-mebrofenin® has a lower renal clearance and the highest degree of resistance to the competitive effects of bilirubin[12]. As for ^{99m}Tc-mebrofenin®, substitution of the three methyl groups at the ortho and para positions and of 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[7].

Although ^{99m}Tc-mebrofenin® shows excellent characteristics for use in cholescintigraphy, all of the kits used in Korea are imported from abroad. A synthetic procedure of the BrTIDA as compound to prepare ^{99m}Tc-mebrofenin® has not been previously reported in the literature.

In this study, we first synthesized BrTIDA [*N*-(3-bromo -2,4,6-trimethylacetanilide) iminodiacetic acid] using nitrilotriacetic acid; then, we prepared lyophilized kits and evaluated the ^{99m}Tc-complex as a hepatobiliary agent, using animals to evaluate the possibility of clinical application of the in-house prepared lyophilized kit.

2. MATERIAL AND METHODS

2,4,6-Trimethylaniline (97%), nitrilotriacetic acid, bromine(Br_2), and acetic anhydride were purchased from the Aldrich Chemical Co. (Milwaukee, USA). All other chemicals used in this study were of AR grade.

^{1}H and ^{13}C NMR were obtained at 300 MHz on a Bruker DRX300 instrument. Chemical shifts were reported in ppm at a scale relative to the solvent used. ^{1}H and ^{13}C NMR

spectra were obtained at the Korea Basic Science Institute (Daejeon, Korea). Melting points were determined using the Fischer Johns melting point apparatus.

Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained using a ^{99}Mo-^{99m}Tc generator (Samyoung Unitech Co. Ltd., Korea). The radiolabeling yield was determined by means of an instant thin layer chromatography (ITLC) scanner (EG & G Berthold linear Analyzer).

The thin layer chromatography (TLC) pattern of the ^{99m}Tc-IDA complex on silica alumina impregnated glass fiber sheets, using a 20% aqueous NaCl solution as a developing solvent, was investigated.

2.1 Synthesis of N-(3-bromo-2,4,6-trimethylacetanilide)Iminodiacetic Acid (BrTIDA)

2.1.1 3-Bromo-2,4,6-Trimethylaniline

To 50 ml of concentrated HCl continually stirred in an ice bath 12 ml of 2,4,6-trimethylaniline was added dropwise to obtain a yellowish suspension of 2,4,6-trimethylaniline hydrochloride salt. Then, 4.5 ml of bromine diluted in 60 ml of concentrated HCl was added dropwise to the suspension. After the addition of bromine was complete, the mixture was stirred gently at 40° C for 1 hr to avoid frothing. The resulting solid was filtered, washed with 1 N HCl solution, and dried under a vacuum. It was then dissolved in hot water, followed by an addition of liquid ammonia to adjust the pH to 10, and then extracted with diethyl ether. The organic layer was collected, dried with Na_2SO_4, and evaporated to yield a brown liquid. This brown liquid was used for the next reaction without any further purification.

2.1.2 N-(3-bromo-2,4,6-trimethylacetanilide) Iminodiacetic Acid

Nitrilotriacetic acid (5 g) was suspended in 70 ml of anhydrous pyridine. The mixture was heated at 50° C under a nitrogen atmosphere until the nitrilotriacetic acid was completely dissolved. Eight milliliters of acetic anhydride was rapidly introduced to the stirred solution, which was then heated to 100° C for 1 hr and left at room temperature overnight.

The solution was heated to 50° C and 3 ml of 3-bromo-2,4,6-trimethylaniline was added to the reaction mixture, and heated at 100° C for 1 hr. The solvent was evaporated off in a rotary evaporator. The yellowish oil residue was dissolved in methanol and treated with activated charcoal to decolorize the mixture; after which, the solvent was evaporated.

Double distilled water was added to the mixture, and the solution was adjusted to pH = 10 with a 1 N NaOH solution followed by an extraction with diethyl ether. The aqueous layer was acidified to a pH of 2 with 2 N HCl. The precipitate was filtered and recrystallized from 85% ethanol to yield 2.3 g of the desired product: m.p = 176 ~ 178° C (decomp.); ^{1}H NMR (DMSO-d_6; ref central line of DMSO set at 2.52 ppm) 7.11(s, Ar-H), 3.78(s, 4 H), 3.50(s, 2 H), 2.37(s, 3 H), 2.21(s, 3 H), 2.01(s, 3 H) ; ^{13}C NMR (DMSO-d_6; ref central line of DMSO set at 40.41 ppm) 175.22, 171.63, 140.23, 138.96, 132.78, 130.32, 106.06, 59.07, 56.82, 40.83, 30.19, 25.43, 17.96.

2.2 Preparation of 99mTc-BrTIDA Complex

In-house prepared lyophilized vials were used containing 20 mg of BrTIDA and 0.4 mg of $SnCl_2$ for each vial. The final pH was 5.5. ^{99m}Tc-complex was prepared by the mixing of the lyophilized compound with generator eluted $Na^{99m}TcO_4$ in saline (1 ml, 370 MBq) at room temperature for 10 min to permit a chelation with the IDA derivative molecules.

The labeling efficiency of the ^{99m}Tc-BrTIDA was determined by performing ITLC on a silica gel impregnated glass fiber sheets using a 20% aqueous NaCl solution and water as developing solvents. The labeling efficiency was calculated as follows: labeling efficiency (%) = 100 – [% of $^{99m}TcO_4^-$] - [% of $^{99m}TcO_2$].

2.3 In Vivo Pharmacokinetics

2.3.1 Scintigraphic Imaging Scan Using Animals

Six-week-old New Zealand white male rabbits (2.87 kg) were used for the imaging studies. The animals were 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 acclimatization for 1 week.

Each rabbit was anesthetized with ketamine and xylazine, and ^{99m}Tc- BrTIDA, 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, Siemens, USA) fitted with a low-energy all-purpose collimator. Image data was analyzed using the dynamic procedure of the SCINTRON IV system (Medical imaging electronics, Germany).

2.3.2 Biodistribution Studies.

^{99m}Tc-BrTIDA 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 min 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 of the injected dose per gram tissue (%ID/g).

3. RESULTS

3.1 Preparation of ^{99m}Tc-BrTIDA Complex

In the TLC pattern, ^{99m}Tc-BrTIDA 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- BrTIDA and $^{99m}TcO_4$ were found at the solvent front, and $^{99m}TcO_2$ (colloid) was found at the origin.

The radiolabeling efficiency of ^{99m}Tc-BrTIDA was maintained with a high radiochemical purity (> 95%) at room temperature for 6 hrs.

3.2 In Vivo Pharmacokinetics

3.2.1 Scintigraphic Imaging

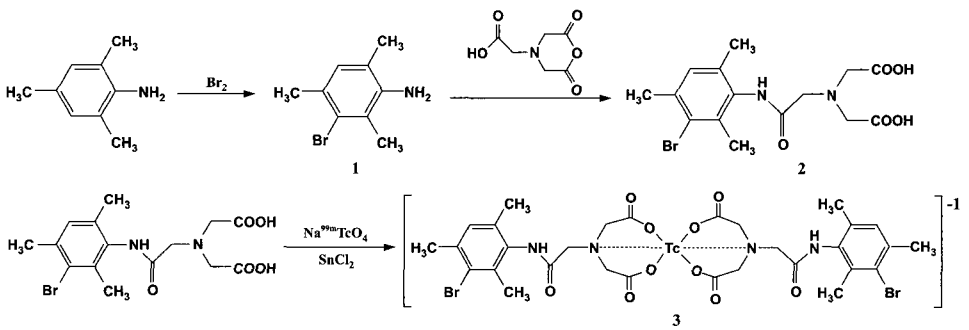
The major excretion pathway of ^{99m}Tc-BrTIDA was a hepatocytic excretion, but it was observed in a kidney in the first image acquired 0~2 min after administration. Upon injection, ^{99m}Tc-BrTIDA was quickly cleared from the blood by the hepatocytes and excreted into the gallbladder and intestine with a negligible uptake by the kidneys and the other organs. The serial static image scans of the rabbits administered with ^{99m}Tc-BrTIDA revealed that none of the tissues except the hepatobiliary system had taken up radioactivity. The whole-body images of rabbits after the intravenous administration of ^{99m}Tc-BrTIDA are shown in Fig. 1.

3.2.2 Biodistribution Studies

Table 1 summarizes the results of the biodistribution studies of ^{99m}Tc-BrTIDA in the test rats; results are shown as a percentage of the injected dose to each selected organ of the SD rat (%ID/g). Rapid clearance of the ^{99m}Tc-BrTIDA from the blood, liver, and lungs was observed within 10 min of an injection. Furthermore, 92% of the injected dose of ^{99m}Tc-BrTIDA was transported to the hepatocytes and cleared into the gallbladder and intestine with a minimal uptake by the other organs.

4. DISCUSSION AND CONCLUSION

A procedure to synthesize BrTIDA was successfully developed using nitrilotriacetic anhydride prepared *in situ* as a conjugator of aniline. The preparation of nitrilotriacetic anhydride was used for the development of new compound containing iminodiacetic acid by its conjugation with the



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

Table 1. Radioactivity Concentrations in Organs or Tissues After an Intravenous Injection of ^{99m}Tc-BrTIDA in Male Rats at 10, 30 and 120 Min

Organ	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

The radioactivity distribution in each organ was expressed as a percent injected dose per gram tissue (%ID/g)

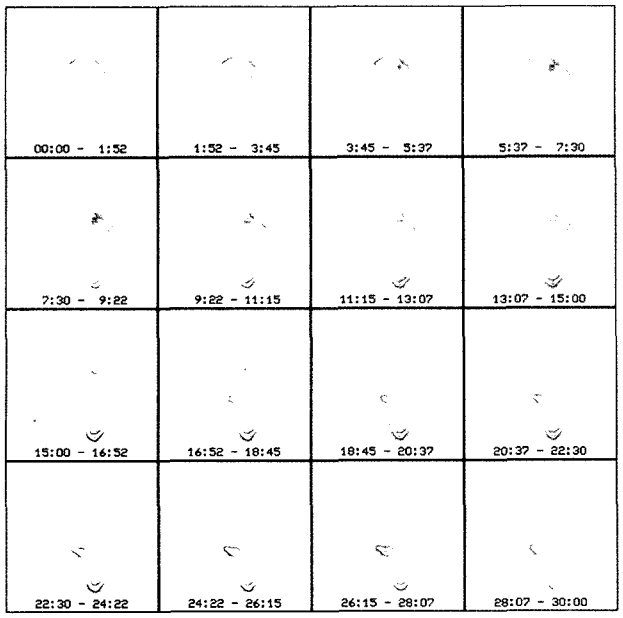


Fig. 1. The Whole-body Images of a Rabbit for 30 Min After an Intravenous Administration of ^{99m}Tc-BrTIDA

amine group of biomolecules (peptide, antibody, etc.). Furthermore, the radiolabeled compound formed a ^{99m}Tc(III) -complex and a ^{99m}Tc(I)-tricarbonyl[^{99m}Tc(CO)$_3$]-complex, and the electrical charge of the two complexes is -1.

Specifically, pertechnetate was reacted with BrTIDA in the presence of stannous (II) chloride, resulting in the formation of $\{^{99m}$Tc(III)[BrTIDA]$_2\}^{-1}$ ***3***, because ^{99m}Tc(III) has six coordination sites, as given in Scheme 1.

The prepared ^{99m}Tc-BrTIDA showed the desirable characteristics of a high hepatic uptake, a low urinary excretion, rapid blood clearance, and a short hepatocellular transit.

In conclusion, considering the results of the scintigraphic images and the biodistribution data using animals, a lyophilized vial containing only 20 mg of BrTIDA and 0.4 mg of $SnCl_2$ can be used in humans as a hepatobiliary imaging agent for an evaluation of the functional status of the hepatocytes and the patency of the biliary duct.

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