BIOLOGICAL EVALUATION OF ^{99m}Tc-N-(3-BROMO-TRIMETHYLACETANILIDE)-IMINODIACETIC ACID (^{99m}Tc-MEBROFENIN) AS HEPATOBILIARY RADIOPHARMACEUTICAL.

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ABSTRACT

^{99m}Tc-N-(3-bromo-2,4,6-trimethylacetanilide) iminodiacetic acid (^{99m}Tc-Mebrofenin) has been described as having optimal properties as hepatobiliary radiopharmaceutical. This paper describes the synthesis, radiopharmaceutical preparation and biological distribution of new labeled compound. The biodistribution study of ^{99m}Tc -Mebrofenin- was carried out in normal mice. The specificity for hepatobiliar excretion, blood clearance and cumulative biliary excretion were evaluated in normal and cirrhotic rats.

INTRODUCTION

Numerous ^{99m}Tc labeled IDA (iminodiacetic acid) derivatives have been successfully applied in the diagnosis of various hepatobiliary diseases in Nuclear Medicine.The biological properties of these agents have been influenced by the substituents introduced in the phenyl-carbamoyl- iminodiacetic acid, the basic structure of most ^{99m}Tc derivatives. In the past few years, a third generation of hepatobiliary agent, containing halogen substituted phenyl group has been reported. Among them, the 3-bromo-2,4,6-trimethyl-IDA, known as Mebrofenin, has been widely used and proved to be superior to other derivatives. Their application has been highly rated with regard to the clinical objective of rapid hepatobiliary excretion, particularly, for the diagnosis of anatomical abnormalities of the biliary tree. Thus, in order to respond to the demand of our Nuclear Medicine Community, preparation of Mebrofenin was proposed.

This paper reports the synthesis and describes a convenient procedure for preparing ^{99m}Tc-Mebrofenin on the basis of a single composition instant kit. The kit formulation was evaluated in healthy experimental animals and carbon tetrachloride-induced cirrhotic rats.

MATERIAL AND METHODS

Synthesis. The synthesis of 3-bromo-2,4,6-trimethyl-IDA was carried out in three steps using a modifed procedure reported by Nunn et al. [1] and Mitta et al. [2] . This involves reaction of the appropriate aniline derivative with chloroacetylchloride. The reaction scheme is shown in Figure 1.

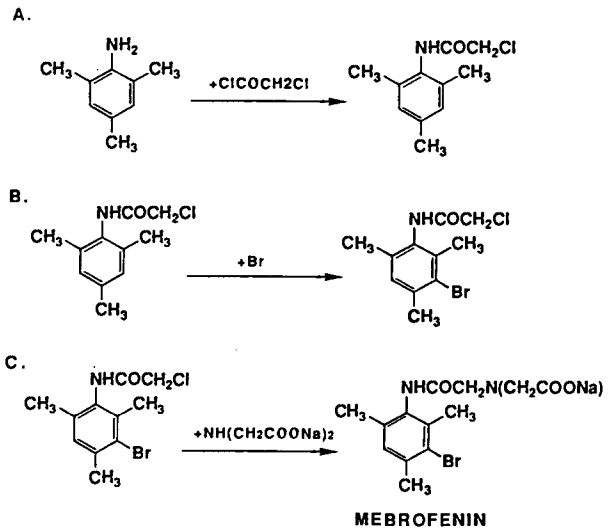
Formulation of kit. One ml of Mebrofenin was prepared by dissolving $2x10^{-6}$ mol Mebrofenin in 0.2N NaOH (0.5ml). Then, 8.8 x 10^{-7} mol freshly prepared stannous chloride solution was added; after the adjustament of the pH to 6.0, the solution was filtered through a 0.22 μm Millipore filter.

Radiolabeling and radiochemical quality control. The radiolabeling of Mebrofenin in the presence of ^{99m}Tc eluate was screened by thin layer chromatography (TLC) Merck-silica gel strip, using two sistems: (1) Methanol 85% and (2) NaCl 30%.

Samples of 5 μl were spotted and dried under nitrogen stream and immediately developed on solvent (1) and (2). The Rf of ^{99m}Tc-Mebrofenin was detected at Rf 1.0 in solvent (1) and at Rf 0.0 in solvent (2). Free ^{99m}Tc[pertechnetate] and reduced hydrolyzed technetium were observed at Rf 1.0 and Rf 0.0 respectively in both solvents.

Radioactivity was determined with a radiochromatographic scanner and the quantification carried out by cutting 0.5 cm section of the strip and counted in a gamma counter.

FIGURE 1
SYNTHESIS OF MEBROFENIN



MEBROFENIN

Biodistribution studies. Organs distribution studies were perfomed in healthy mice weighing 25-30g, the radiopharmaceuticals were injected through the tail vein. At specific times, the animals were killed and dissected, and their excised organs and tissues were weighed and counted . In order to screen, the cumulative biliary excretion studies were perfomed in normal rats, carbon tetrachloride-induced cirrhotic rats [3] and its age control group. The measurement of blood disappearance and the hepatobiliary clearance were carried out by cannulation of the carotid artery and the common bile duct, respectively. Also, a catheter placed in the femoral vein was used for the heparinization. Radiopharmaceutical was injected through the contralateral exposed femoral vein. Blood samples were taken at

1,2.3,5,6,7.15,45 and 65 min. postinjection. Bile samples were collected at 0 and 10 min. and then 10 min intervals to 80 min postinjection .

RESULTS AND DISCUSSION

The structure of synthesized Mebrofenin was identified by IR and NMR spectroscopy, also, melting point determination, as shown in Table 1. The elemental chemical analysis as shown in Table 2 indicated, that the spectral features and experimental parameters of the frequencies were in agreement with literature data.

Table 1. Physical data for Mebrofenin

M.P. °C	Infrared (IR) (cm^{-1})	(DMSO-d_6-TMS) (NMR) (ppm)
	3265 (N-H)	10.20 (2H.s.COOH)
210	1709-1686 (NH-CO)	7.08 (1H.s.H_5)
	1543 (COO^-)	3.50 (2H.s.CO-CH_2COOH)
		2.30 (3H.s.Me-4)
		2.07 (3H.s.Me-6)

Table 2. Elemental chemical analysis

$C_{15}H_{19}N_2BrO_5$ = 387.22 (MW)

Elements	Calculated(%)	Found (%)
C	46.52	46.56
H	4.91	5.05
N	7.23	7.40
Br	20.65	20.71

Thus, the synthetized Mebrofenin was used for the establishment of appropriate condition of kit formulation . The present study demonstrated that this complex can be readily prepared on instant freeze dried kit. Labeling was performed simply by adding pertechnetate as eluted from the generator . in the kit vial. Radiochemical purity of the formulation was evaluated by thin layer chromatography. More than 90% of the total activity was calculated as bound technetium .

The biodistribution study of ^{99m}Tc-Mebrofenin radiolabeled at the optimal conditions desirable was carried out in normal mice as shown in Table 3.

The complex was rapidly cleared from the blood and taken by tl liver. then it was excreted by the hepatobiliary system. After tl administration. approximately 50% dose organ was found in tl intestine after 5 min.. and after 60 min. was 85.2%. In this prelimina work. the biliary excretion of ^{99m}Tc-Mebrofenin showed son differences in the three studied groups as shown in Figures 2 and 3. Tl specifity of ^{99m}Tc-Mebrofenin in normal rats was 80.28% ± 5.45 in tl bile at 80 min . while in cirrhotic animals was 74.45% ±6.29. Bloc disappearance curves of ^{99m}Tc- Mebrofenin under normal cirrhot condiction were similar. The compound was rapidly cleared with < 1' of the injected dose. remaining in the blood at 15 min. In terms clinical significance these results demonstrated that ^{99m}Tc-Mebrofen has high specificity in the evaluation of hepatobiliary diseases.

Figure 2

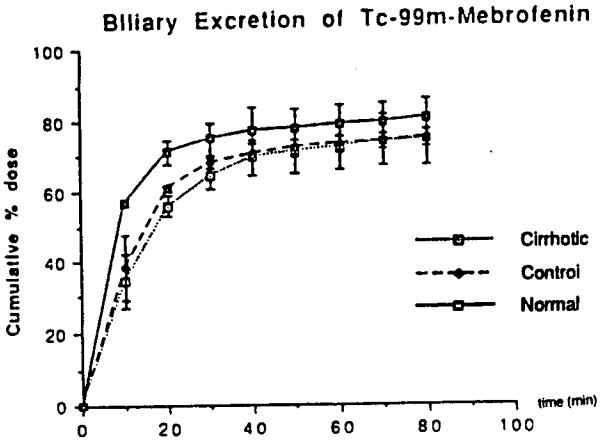
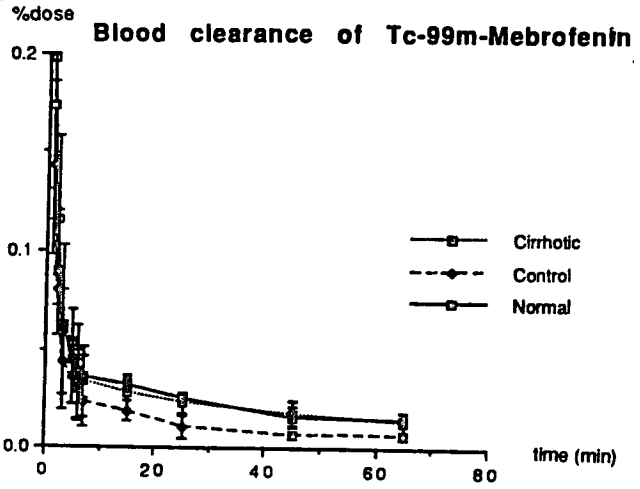


Figure 3



CONCLUSION

These studies indicated that the synthetized Mebrofenin could be radiolabeled with technetium. reaching very high labeling yield upon the determination of various labeling parameters . The optimization of the labeling condition offered very reproducible data.

The animals distributions studies offered good correletion with those from published papers [1.4.5]. Results of these studies suggest the potential use of this radiopharmaceutical in the evaluation of hepatobiliary diseases.

Table 3: Biodistribution data in mice

^{99m}Tc-Mebrofenin

		Time				
		5min	15min	30min	1hr	2hr
Blood	(a)	1.045(0.039)	0.415(0.058)	0.356(0.039)	0.284(0.044)	0.232(0.048)
	(b)	1.663(0.251)	0.915(0.154)	0.689(0.120)	0.521(0.086)	0.386(0.079)
Spleen	(a)	0.0062(0.011)	0.031(0.007)	0.025(0.009)	0.018(0.005)	0.018(0.003)
	(b)	0.481(0.088)	0.190(0.018)	0.182(0.059)	0.143(0.045)	0.129(0.023)
Liver	(a)	33.309(2.896)	12.942(3.292)	14.045(4.611)	11.527(6.501)	6.690(2.140)
	(b)	20.734(2.249)	6.395(2.078)	7.548(2.214)	7.255(3.740)	4.120(1.345)
Kidneys	(a)	1.993(0.272)	1.598(0.698)	1.302(0.399)	0.747(0.092)	0.675(0.180)
	(b)	4.734(0.484)	3.678(1.200)	2.564(0.760)	1.799(0.076)	1.436(0.336)
Stomach	(a)	0.387(0.275)	0.125(0.024)	0.721(0.578)	1.262(1.024)	1.080(0.607)
	(b)	0.731(0.505)	0.169(0.070)	1.890(1.702)	3.072(2.326)	2.422(1.424)
Intestines	(a)	49.406(19.520)	68.417(4.063)	71.385(11.841)	85.279(7.647)	79.485(2.880)
	(b)	18.786(7.166)	23.018(1.858)	26.888(4.835)	32.624(2.179)	30.576(0.911)

Each value represents the mean±S.D. of three mice

(a) % dose / organ

(b) % dose / gram

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