Environmental Mutagens & Carcinogens 21-1 : 1-8 (2001)

Differential Diagnosis of Chemical-induced Hepatobiliary Toxicities Using a New Hepatobiliary Imaging Agent in Mice

Chông-Kun Ryu[1], Jae-Eun Pie[2], Jae Gol Choe[3], Joon Cheon[4], Jeongwon Sohn[1], Jürgen Seidel[5], David S. Paik[5], Michael V. Green[5], Chang H. Paik[5], and Meyoung-Kon Kim[1*]

[1]*Department of Biochemistry, Korea University Medical College, Seoul,*
[2]*Department of Food and Nutrition Anyang university, Anyang,* [3]*Department of Nuclear Medicine, and*
[4]*Department of Urology, Korea University Medical College, Seoul, Korea, and*
[5]*Department of Nuclear Medicine, Warren G. Magnuson Clinical Center,*
National Institutes of Health, Bethesda, Maryland, U.S.A.

(Received, February 28, 2001 / Accepted, March 8, 2001)

ABSTRACT : **We have synthesized ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin as a new imaging agent for hepatobiliary scintigraphy. The aim of this study was to evaluate the usefulness of ^{99m}Tc-MAG3-biocytin scintigraphy in differentiating carbon tetrachloride (CCl_4)-induced hepatotoxicity from α-naphthylisothiocyanate (ANIT)-induced cholestasis in mice, which reflecting the differential diagnosis of neonatal jaundice caused by neonatal hepatitis from congenital biliary atresia in humans. Methods: Balb/c mice (female, 20 g, n = 4-6) were pretreated with CCl_4 (0.5 or 1.0 ml/kg) and ANIT (150 or 300 mg/kg) 18 h before scintigraphy. Biochemical and histopathological examinations showed a pattern of typical acute hepatitis (increase of transaminases and hepatocellular necrosis) in CCl_4-treated mice and cholestasis (increase of alkaline phosphatase and γ-glutamyltransferase, and biliary hyperplasia) in ANIT-treated mice, respectively. Mice were fasted at least 4 hr prior to the intravenous injection of ^{99m}Tc-MAG3-biocytin (18.5 MBq/ 20μg) in 2% human serum albumin in saline. Scintigraphy was performed with a γ-camera equipped with a 1-mm diameter pin-hole collimator for 30 min and images were acquired every 15 s. We compared the values of physical parameters, such as peak liver/heart ratio (r_{max}) and peak ratio time (t_{max}) for ^{99m}Tc-MAG3-biocytin scintigraphy. Results: Scintigraphic parameters of the CCl_4-pretreated (0.5 ml/kg) group showed a 81.9% decrease of r_{max}, and 42.2% decrease of t_{max}, whereas the ANIT-pretreated (150 mg/kg) group showed a 53% decrease of r_{max}, and 2.36-fold increase of t_{max}, (P<0.05). These results demonstrate that the decrease of r_{max} and the shortening of t_{max} are characteristic features for hepatotoxicity, in contrast to the increase of t_{max} and decrease of r_{max} for biliary hyperplasia. Conclusion: ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy can distinguish hepatitis from cholestasis in mice model and may be similarly useful in humans which differentiating the cause of neonatal jaundice in clinical study.**

Keywords : **Hepatobiliary, toxicity, ^{99m}Tc, biocytin, scintigraphy**

Introduction

We have found that ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin was a useful hepatobiliary imaging agent even in presence of coinjected bilirubin (Kim *et al.*, 1999) as well as in monitoring ethanol-induced cytochrome P450-mediated hepatotoxicity and its recovery by enzyme inhibitor (Kim *et al.*, 1997). In this study, we evaluate the usefulness of ^{99m}Tc-MAG3-biocytin scintigraphy in differentiating carbon tetrachloride (CCl_4)-induced hepatotoxicity from α-naphthylisothiocyanate (ANIT)-induced cholestasis in mice, which reflecting the differential diagnosis of neonatal jaundice cause by neonatal hepatitis from congenital biliary atresia in humans. The differential diagnosis between neonatal hepatitis and congenital biliary atresia is very important in deciding whether the cause of the neonatal jaundice requires medical or surgical treatment, especially when clinical laboratory studies, imaging studies, or even histological examinations do not provide definitive answers (Kim *et al.*, 1993: Heyman. 1994). To determine if pharmacokinetic parameters obtained from ^{99m}Tc-MAG3-biocytin scintigraphy could differentiate between liver damage versus biliary cholestasis, we used mice models for the CCl_4-induced hepatitis and ANIT-induced cholestasis. Biochemical and histopathological studies, i.e.,

*To whom correspondence should be addressed
126-1, Anam-dong 5-Ga, Sungbuk-Gu, Seoul 136-705, Korea
Tel: 82-2-920-6184, Fax: 82-2-923-0480
E-mail: jerrykim@mail.korea.ac.kr

conventional hepatic function tests, were performed to confirm that there was hepatic toxicity or biliary disorders in our animal models. Pharmacokinetic parameters of dynamic scintigraphy, for examples, peak liver/heart ratio (r_{max}) and peak ratio time (t_{max}), were compared with the region-of-interest (ROI) study over the heart and liver in these animal models.

In this study, we performed the ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy in mice models to test its applicability for differential diagnosis between hepatitis and cholestasis. Thus, this study could suggest the preliminary data for the use of several scintigraphic parameters in hepatobiliary imaging and for the applicability of ^{99m}Tc-MAG3-biocytin to differentiate the cause of neonatal jaundice in humans; neonatal hepatitis from congenital biliary atresia.

Materials and Methods

Synthesis of Radiopharmaceutical

^{99m}Tc-MAG3-biocytin was synthesized by the method illustrated in Fig. 1-A (Jeong *et al.*, 1993). This synthesis involved acylation of biocytin with *N*-hydroxylsuccinimide ester of benzoyl-MAG3 in dimethylformamide. The reaction resulted in a conjugate of benzoyl-MAG3 linked to biotin through a lysine spacer. The reaction mixture was precipitated from the crude product by the addition of tetrahydrofuran and washed with water. Benzoyl-MAG3-biocytin was labeled with ^{99m}Tc through a modification of the method of Fritzberg *et al.* (1996). Quality control was done by instant thin-layer chromatography with silica gel on glass fiber (ITLC-SG; Gelman Sciences, Ann Arbor, MI) developed with 10% ammonium acetate in water: methanol (1:1), and by reverse-phase thin-layer chromatography (RP-TLC; Uniplate, RPS-F, 5×20 cm, Analtech Inc., Newark, DE) developed with phosphate buffer (0.001 M, pH 6.7) in saline (PBS). This ITLC-SG system moved ^{99m}Tc-MAG3-biocytin and ^{99m}Tc-MAG3 toward the solvent front, whereas the hydrolyzed, reduced ^{99m}Tc colloids remained at origin. The Rf values of ^{99m}Tc-MAG3-biocytin, ^{99m}Tc-MAG3, and ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) on RP-TLC were 0.0, 0.6, and 0.9, respectively. The labeled compound was purified with a C_{18} Sep-Pak cartridge (Waters Corp., Milford, MA). A solvent mixture containing 5% ethanol and 95% PBS (0.01 M, pH 6.7) was run through the cartridge to eliminate polar ^{99m}Tc impurities, and the desired compound was then eluted out with ethanol. Next, the ethanol was evaporated off, and the dry product was dissolved in PBS (0.067 M, pH 6.7). The specific activity of ^{99m}Tc-MAG3-biocytin was 633 - 821 MBq/mg, and the radiochemical purity exceeded 95%, as determined by the same quality control method.

Animal Models for Hepatobiliary Toxicity

Animal studies were performed under a protocol approved by Institutional Animal Care and Use Committee of the National Institutes of Health. Female Balb/c mice, weighing 18-20g and 6-8 weeks old, were kept in cages and fed a standard laboratory diet with water *ad libitum*. The CCl_4-induced hepatotoxicity was followed by the method described previously (Zalatinai and Lapis. 1994) except olive oil was substituted for corn oil as the solvent. Briefly, CCl_4 (0.5 or 1.0 ml/kg of body weight, dissolved in olive

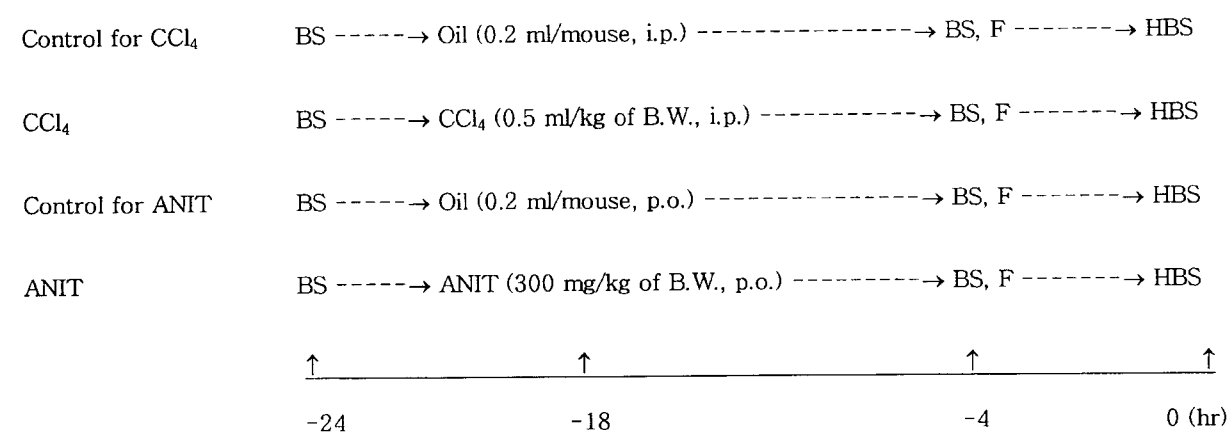

Fig. 1. Synthesis scheme for ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin (A). This chemical is composed of ^{99m}Tc-MAG3 and biotin with a lysine spacer. Scheme of pretreatment in experimental groups (B). Balb/c mice (female, 6-8 wk, 18-20 g of B.W.) were used for carbon tetrachloride (CCl_4)-induced hepatotoxicity and α-naphthylisothiocyanate (ANIT)-induced cholestasis. Blood samples (BS) were collected 48 h and again 4 h before the imaging. Intraperitoneal injection (i.p.) of corn oil (Oil) was used for the CCl_4-induced hepatotoxicity and the control for the ANIT-induced cholestasis by gavage (p.o.). All mice fasted (F) at least 4 h before the ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy (HBS).

oil) was administered intraperitoneally 18 hr subsequent to ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy (Fig. 1-B). Before the induction of hepatotoxicity, we sampled pretreatment serum for biochemical analysis and measured body weight to calculate CCl_4 dose and to estimate a gross indicator of acute toxicity. Alpha-naphthylisothiocyanate (ANIT: Sigma Chemical Co., St. Louis, MO) was dissolved in olive oil to produce 15 or 30 mg/mL solutions. Two-hundred microliters of the ANIT solutions were administered by gavage to ensure a dose of 150 and 300 mg/kg of body weight in accordance with the methods as described by Conolly *et al.* (1988) and Traiger *et al.* (1985) to induce biliary hyperplasia (Fig. 1-B).

Dynamic scintigraphy

All mice fasted at least 4 hr before the hepatobiliary studies. They were anesthetized with 0.6 mg of ketamine hydrochloride (Ketaset; Fort Dodge Laboratories, Inc., Fort Dodge, IA) and 0.1 mg of xylazine hydrochloride (Rompun; Miles, Inc., Shawnee Mission, KS) per 20 g of body weight. The animals were positioned 8.5 cm below a pin-hole collimator and injected via the dorsal tail vein with ^{99m}Tc-MAG3-Biocytin (20 to 40 μg, 14.8 to 22.2 MBq). Scintigraphic images were acquired at 15-s intervals for 30 min with a 38.1-cm (15-in) field-of-view gamma-camera (Dynamo, Picker International Co., Cleveland, OH) equipped with a pin-hole collimator 1 mm in diameter. Data acquisition and analysis were performed on a personal computer using the "NucLear Mac" hardware and software (Scientific Imaging, Littleton, CO).

As illustrated (Fig. 2-A), equally-sized regions-of-interest (ROIs) were drawn over the heart and the left upper lobe of the liver, and time-activity curves were generated from these ROIs. Special care was taken to avoid any overlap between the liver ROI and the gallbladder or other major organs. To obtain smooth replicates of the raw heart time-activity curve, we fit an exponentially decreasing model function H(t) to the heart time-activity curve,

$$H(t) = B \times e^{(-\ln 2 \bullet t/tB)} \quad (1)$$

where B is a scale parameter, t_B is the tracer half-clearance time from the bloodstream, and the fit region extends from time $t \cong 120$ to 1800 s, thus excluding the initial distribution phase of the tracer. The liver activity, L(t), was modeled as a two-compartment function:

$$L(t) = A \times [1\text{-}e^{(-\ln 2 \bullet t/t1)}] \times e^{(-\ln 2 \bullet t/t2)} + C \quad (2)$$

Here, $t1$ and $t2$ are the half time for total liver uptake and excretion, A is a scale parameter, and C is a constant background. The fit region for the liver time-activity curve included all time points. In general, equations (1) and (2) gave rise to excellent curve fits, as shown in Fig. 2-B.

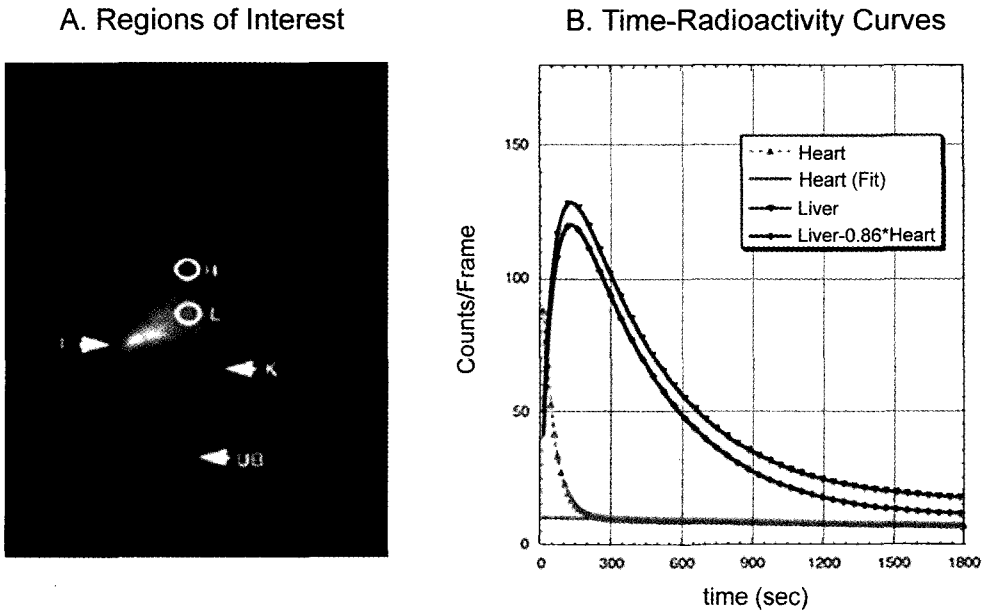

Fig. 2. Pharmacokinetic analysis of ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin hepatobiliary scintigraphy. Panel A shows the summed image from 0 to 30 min, while the regions of interest over the liver (L) and heart (H) used to derive the time-activity curves shown in panel B. Smooth replicates of the raw time-activity data were reconstructed by curve fitting and used to calculate the liver/heart activity ratio at each time point during acquisition. I, intestine; K, kidney; UB, urinary bladder.

The best-fit curves based on equations (1) and (2) were used to calculate various pharmacokinetic parameters. The next step consisted of creating two different physical parameters, i.e., peak liver/heart ratio (r_{max}) and peak ratio time (t_{max}) from the time-liver/heart ratio curve.

Biochemical Analysis

The sera of all mice were evaluated for biochemical markers of hepatotoxicity as analyzed by the experimental protocol (Table 2). We used commercially available diagnostic kits (Sigma Chemical Co., St. Louis, MO) to measure the concentration of four marker enzymes for hepatobiliary function, aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (GGT), and alkaline phosphatase (ALP). One International Unit (U) of enzyme is defined as the amount of each enzyme that will convert 1 μmol of substrate per minute under the specified conditions of the procedure.

Histopathological Analysis

Liver samples were taken from the anterior portion of left lateral lobe for histopathology (Bioulac-Sage *et al.*, 1984). Paraffin sections, measuring 5 to 6 μm, were prepared after they were fixed with 10% neutral formalin and stained with hematoxylin and eosin (Bioulac-Sage *et al.*, 1984). Dr. M. Anver in the National Cancer Institute Frederick Cancer Research and Development Center performed the interpretation of the data. To avoid bias, the slides were coded and graded in a blinded fashion. Toxicity to the liver was scored as 1+, minimal; 2+, mild; 3+, moderate; and 4+, severe on the basis of damage to (a) hepatocytes (Bioulac-Sage *et al.*, 1984: Lin and Satio. 1986: Bioulac *et al.*, 1980) for necrosis, vacuolation, ballooning, extramedullary hematopoiesis, and acute or subacute inflammation; (b) the interlobular bile duct (Desmet *et al.*, 1968: Desmet and Rees. 1958) for necrosis, desquamation, and acute or subacute inflammation; and (c) the endothelium of artery and vein for edema and acute or subacute inflammation.

Statistical analysis

Data were presented as mean±standard deviation. A one-way analysis of variance (ANOVA) was used to compare the control group with the experimental groups to determine the difference in mean value of all results. A probability value of $P<0.05$ was considered statistically significant.

Results

Hepatobiliary Scintigraphy

Dynamic hepatobiliary scintigraphy of the control groups, i.e., no pretreatment of either CCl_4 or ANIT, showed that ^{99m}Tc-MAG3-biocytin was taken up rapidly by the liver with r_{max} of 7.8-8.3 and t_{max} of 124-128 (Table 1). The pharmacokinetic studies in the CCl_4-treated group (Fig. 3, Table 1) showed a totally different pattern for the uptake and the clearance of ^{99m}Tc-MAG3-biocytin in the liver and the heart, when compared with the ANIT-treated group (Fig. 4, Table 1). The CCl_4-treated group showed severe decreases in r_{max} and t_{max}, whereas ANIT-treated group showed moderate increase of t_{max}, and moderate decreases

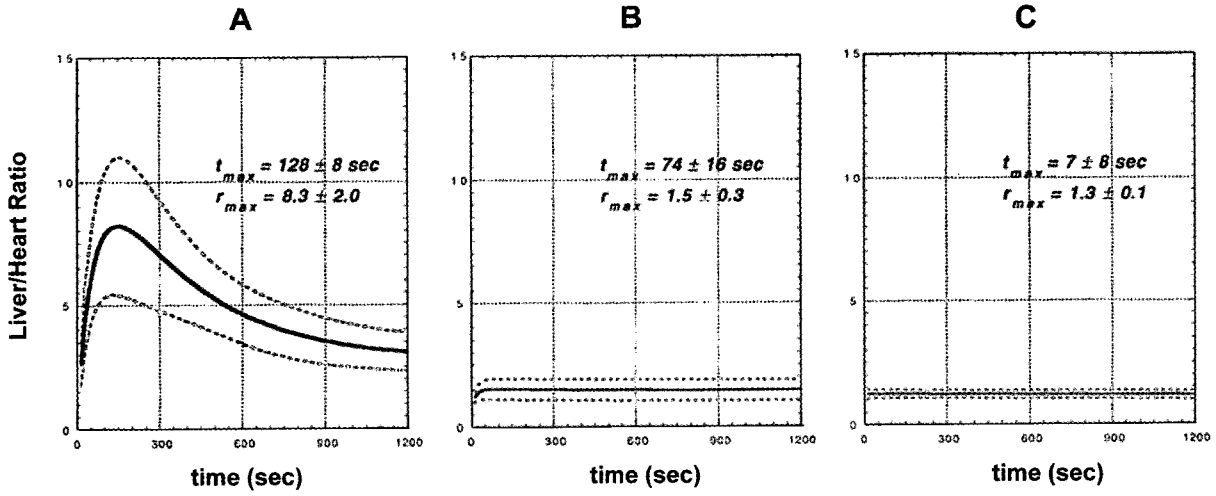

Fig. 3. Liver-to-heart ratio from ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin hepatobiliary scintigraphy without (A) and with pretreatment of 0.5 mL/kg (B) and 1.0 mL/kg (C) carbontetrachloride (CCl_4). Solid lines represent the mean values (n = 5) at each time point, dashed lines indicate the mean ± SD.

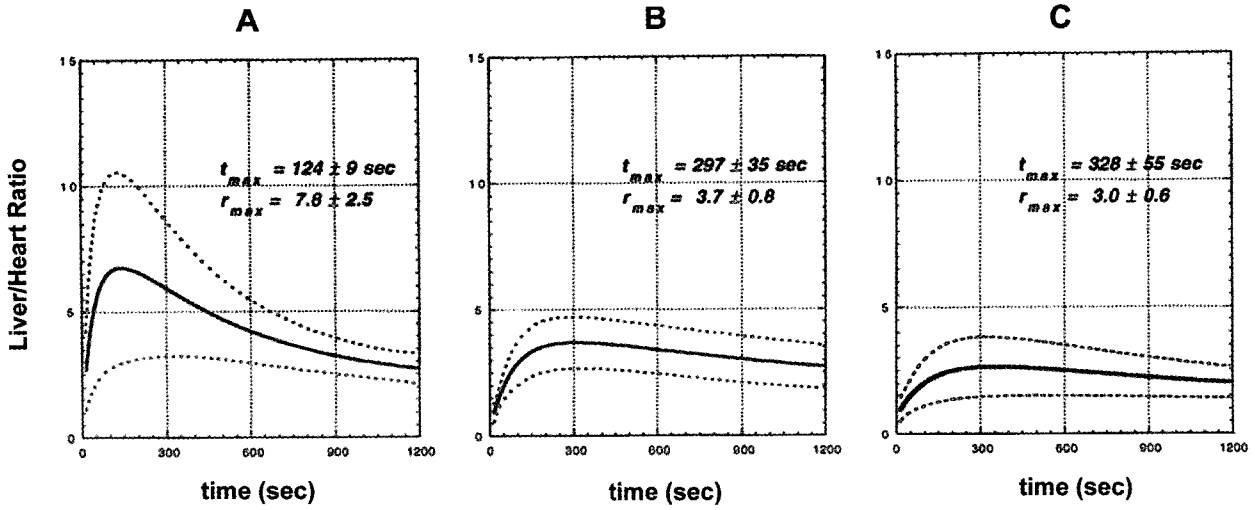

Fig. 4. Liver-to-heart ratio from ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin hepatobiliary scintigraphy without (A) and with pretreatment of 150 mg/kg (B) and 300 mg/kg (C) α-naphthylisothiocyanate (ANIT). Solid lines represent the mean values (n = 5) at each time point, dashed lines indicate the mean ± SD.

Table 1. Scintigraphic Parameters for ^{99m}Tc-MAG3-biocytin Hepatobiliary Imaging

Experiment		Scintigraphic Parameters[a]	
(/kg of BW)	N	r_{max} [b]	t_{max} (sec)[c]
Carbon tetrachloride-induced Hepatotoxicity			
0.0 ml	5	8.3±2.0	128±8.0
0.5 ml	5	1.5±0.3*	74±16*
1.0 ml	5	1.3±0.1*	7±8.0*
α-Naphthylisothiocyanate-induced Cholestasis			
0 mg	4	7.8±2.5	124±9.0
150 mg	5	3.7±0.8*	297±35*
300 mg	4	3.0±0.6*	328±55*

$P<0.05$, the one-way ANOVA is performed to determine whether any difference existed in mean value of all results, when compared control group versus acute hepatobiliary toxicities; a, data are expressed means±SD; b, peak liver/heart ratio (r_{max}) and c, peak liver uptake time (t_{max}).

Table 2. Biochemical Analysis of Marker Enzymes for Hepatobiliary Function

Experiment		Biochemical Analysis[a]			
(/kg of BW)	n	AST[b] (U/L)	ALT[c] (U/L)	GGT[d] (U/L)	ALP[e] (U/L)
Carbon tetrachloride-induced Hepatotoxicity					
0.0 ml	5	36± 9	36± 3	24± 1	63±32
0.5 ml	6	86±29*	121±16*	47± 1*	82±29
1.0 ml	5	82± 4*	124± 5*	47± 0*	81±43
α-Naphthylisothiocyanate-induced Cholestasis					
0 mg	5	43± 7	34± 4	23± 2	47±11
150 mg	7	47±10	56±22	60±11*	107±18*
300 mg	5	70±14*	78±33*	60±11*	94± 3*
Normal Range		<40	<35	<30	13-50

$P<0.05$, the one-way ANOVA is performed to determine whether any difference existed in mean value of all results, when compared control group versus acute hepatobiliary toxicities; a, data are expressed means ± SD; b-e, aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (GGT), and alkaline phosphatase (ALP).

of r_{max} (Table 1). Arbiturary scales for severe, moderate, and slight are: the values in the level of more than 3-fold, 2 to 3 -fold, and less than 2-fold higher or lower than the controls, respectively.

Biochemical Analysis

Biochemical examinations gave us some implications of serological findings such as acute hepatitis (i.e., increase of AST and ALT) in CCl_4-injected mice and acute biliary hyperplasia (i.e., increase of ALP) in ANIT-treated mice (Table 2). However, the biochemical data could not definitely distinguish the two different types of hepatobiliary toxicities, because the elevations of GGT in both CCl_4- and ANIT- treated groups appeared to be caused by the low specificity of it and the elevations of AST and ALT in the high-dose ANIT treatment by the excess of the dose.

Histopathology

Histopathological examinations showed a centrilobular hepatocellular necrosis in CCl_4-treated groups and a biliary hyperplasia and inflammation in ANIT-treated groups (Table 3). At 18 hr after CCl_4 induction, these groups showed classical lesions ascribed to this chemical (Meeks *et al.*, 1991: Rouiler. 1964): all of these samples had extensive necrosis of hepatocyte in the centrilobular zone sur-

Table 3. Histopathological Analysis for Hepatobiliary System[a]

Experiment		Hepatocyte															Biliary Tract							
(/kg)	n	Necrosis					Vacuolation					Ballooning					Necrosis				Inflammation			
		0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	0	1+	2+	3+
Carbon tetrachloride-induced Hepatotoxicity																								
0.0 ml	7	7	0	0	0	0	4	1	1	1	0	7	0	0	0	0	6	0	1	0	7	0	0	0
0.5 ml	6	0	0	0	0	6	1	1	1	3	0	0	2	2	2	0	6	0	0	0	6	0	0	0
P^b				<0.0001					<0.05					<0.001										
1.0 ml	14	0	2	0	0	12	0	2	3	8	1	0	7	2	5	0	14	0	0	0	14	0	0	0
P				<0.0001					<0.05					<0.001										
α-Naphthylisothiocyanate-induced Cholestasis																								
0 mg	7	7	0	0	0	0	4	0	2	1	0	5	0	2	0	0	7	0	0	0	7	0	0	0
150 mg	11	11	0	0	0	0	0	2	4	5	0	7	2	2	0	0	2	5	2	2	0	5	4	2
P																		<0.01				<0.001		
300 mg	8	8	0	0	0	0	2	2	4	0	0	5	2	1	0	0	0	4	3	1	1	4	3	0
P																		<0.01				<0.001		

a, data are interpreted with single blind fashion using an arbitrary portions and scales as follows: (a) hepatocyte (Bioulac P, 1980), necrosis, vacuolation, ballooning; (b) interlobular bile duct (Desmet V., 1968), necrosis and inflammation; (c) endothelium of artery and vein, edema and inflammation; 1+, minimal, 2+, mild, 3+, moderate, 4+, severe; b, Data are compared by Kruskal-Wallis One-way ANOVA on ranks to determine whether any difference existed in mean value of all results, when compared control group versus acute hepatobiliary toxicities.

rounded by some ballooned and vacuolated cells. In contrast, ANIT-pretreated groups showed a pattern similar to the previous report (Desmet *et al.*, 1968: Desmet and Rees. 1958): acute inflammation with necrosis (1+ to 3+) of the bile duct; some ballooned and vacuolated hepatocytes was apparent when compared with controls. Some mild changes associated with hepatocyte damage were seen in ANIT-treated group (e.g., vacuolation and ballooning), but necrosis, the major hallmark of the hepatocyte damage, was not seen with ANIT-treatment.

All of our data described above suggests that ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy using quantitative parameters, e.g., r_{max}, and t_{max}, can be a comparable methodology to biochemical and histopathological analyses as a hepatobiliary function test. Results from biochemical and histopathological tests showed almost identical patterns as previously reported in CCl_4-induced hepatocellular necrosis (Zalatinai and Lapis *et al.*, 1994; Meeks *et al.*, 1991; Rouiller, 1964) and ANIT-induced cholestasis (Desmet *et al.*, 1968: Desmet and Rees. 1958), thereby indicating the establishment of a murine model for hepatic toxicity and biliary toxicity. We tested two scintigraphic parameters including peak liver/heart activity ratio (r_{max}) and peak ratio time (t_{max}).

Discussion

The purpose of the current study was to evaluate whether ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy is applicable to differentiate CCl_4-induced hepatotoxicity from ANIT-induced biliary cholestasis in mice, when evaluated with pharmacokinetic parameters such as r_{max}, and t_{max}. One of the most definite applications of hepatobiliary scintigraphy in clinical study is to diagnose the cause of neonatal jaundice, which originated from neonatal hepatitis or congenital biliary atresia. Because it is very important in deciding whether the cause of the neonatal jaundice requires medical or surgical treatment, especially when clinical laboratory studies, imaging studies, or even histological examinations do not provide definitive answers (Kim *et al.*, 1993: Heyman. 1994). In this study, we tested the applicability of ^{99m}Tc-MAG3-biocytin, a newly-synthesized imaging agent, to evaluate the hepatobiliary function in mice model for acute hepatitis and cholestasis induced by CCl_4 and ANIT.

We employed modified Zalatinis method for CCl_4-induced hepatitis (Zalatinai and Lapis. 1994) and the modified methods ascribed by Conolly *et al.* (1988) and Traiger *et al.* (1985) for ANIT-induced cholestasis, because these methods were reproducible and had appropriate dose and duration for this experiment. A multitude of tests such as biochemical and histopathological analyses have been available to detect and diagnose hepatobiliary dysfunction in laboratory animals (Zalatinai and Lapis. 1994; Connolly *et al.*, 1988; Traiger *et al.*, 1985; Bioulac-Sage *et al.*, 1984; Bioulac *et al.*, 1980; Desmet *et al.*, 1968; McLean and Rees. 1958; Rouiller. 1964). Since CCl_4 and ANIT are the model compound associated with hepatocytic damage and

biliary cirrhosis, we speculated that these chemical-induced models for hepatobiliary toxicity have a number of advantages in nuclear imaging study. Besides replacing ANIT-induced biliary hyperplasia to the surgical intervention for bile-duct ligation, a quantitative estimate of liver uptake and clearance for a hepatobiliary imaging agent in each model can be achievable prior to applying it in patients. However, there has been no report regarding to the hepatobiliary scintigraphy in these animal models. Although biochemical and histopathological tests have been applied to evaluate the liver function for decades in laboratories and clinical fields, it is still recommended to investigate more reliable method which can be determined the dynamic change and the total integrity of the hepatobilary function with a noninvasive manner (Brunot *et al.*, 1994). Thus, we proposed that hepatobiliary scintigraphy has two outstanding advantages to evaluate the hepatobiliary function when compared with the biochemical or histopathological tests: (1) determination of dynamic alteration in hepatobiliary system, especially regarding to hepatic uptake and excretion; (2) noninvasive technique tracing a hepatobiliary system and quantitative assessment for hepatobiliary function.

For developing an ideal imaging agent for hepatobiliary scintigraphy, it has been tested several criteria of biological characteristics; including, rapid extraction from plasma by hepatocytes, rapid transit through these cells, high biliary concentration, little or no reabsorption from intestine, minimal concentration in urinary tract, rapid radiolabeling with high radiochemical purity and stability, and high resistance to competition from compounds such as bilirubin (Gerhold *et al.*, 1983: Nunn *et al.*, 1983: Wistow *et al.*, 1977). We previously reported that ^{99m}Tc-MAG3-biocytin entered the liver rapidly and was excreted through the biliary system and more than 80% of the injected activity was found in the intestine within 30 min (Jeong *et al.*, 1993). We also have found that ^{99m}Tc-MAG3-biocytin was a useful hepatobiliary imaging agent even in presence of bilirubin (Kim *et al.*, 1999) as well as in monitoring ethanol-induced cytochrome P450-mediated hepatotoxicity and its recovery by enzyme inhibitor (Kim *et al.*, 1998). In addition to these results of ours, this study confirms that ^{99m}Tc-MAG3-biocytin has a promising effect on differential diagnosis of hepatobiliary toxicity in mice model, which can be close to an ideal imaging agent for hepatobiliary scintigraphy.

Pharmacokinetic parameters for dynamic scintigraphy, such as r_{max}, and t_{max}, has been characterized in mice model and examined their pattern for hepatobiliary scan between CCl_4- and ANIT- treated mice. Physical parameters were shown to be valuable as quantitative parameters of liver function in humans (Dogan *et al.*, 1993; Juni and Reichle. 1990; Brunot *et al.*, 1994; Gerhold *et al.*, 1983). However, only simple quantitative methods are available with standard software packages and it is still suggested that subjective criteria of dynamic scintigraphy be used which is correlated with conventional liver function tests (Heyman. 1994: Juni and Reichle. 1990). In this study, we generated time vs liver/heart activity ratio curve and calculated r_{max}, thereby using it as a supported parameter to evaluate the hepatobiliary function. As a result, r_{max} on liver ROI gave consistent supporting data to show the different pattern of CCl_4-induced hepatocellular necrosis from that of ANIT-induced cholestasis. Then, we suggested that the combination of r_{max}, and t_{max}, is recommended because one of the most important factors in analyzing a hepatobiliary scintigraphic imaging is to correlate the physiology of bile synthesis and excretion with the imaging parameters.

In summary, hepatobiliary scintigraphy with pharmacokinetic parameter is a comparable and preferable methodology in detecting hepatobiliary functional abnormalities, when comparing with biochemical and histopathological analyses. ^{99m}Tc-MAG3-biocytin has been synthesized as a new hepatobiliary imaging agent, and is applicable to differentiate the CCl_4-induced hepatotoxicity from ANIT-induced biliary cholestasis in mice.

Conclusion

In conclusion, we have demonstrated that ^{99m}Tc-MAG3-biocytin hepatobiliary scintigraphy is applicable to differentiate CCl_4-induced hepatotoxicity from ANIT-induced cholestasis in mice and may be similarly useful in differentiating the cause of neonatal jaundice in clinical study. This is the first study to demonstrate that nuclear imaging can be used as a surrogate marker to evaluate the hepatobiliary function in mice models for chemical-induced toxicities.

Acknowledgment

The authors thank Dr. Mark Rotman, Dr. Clara Chen, and Miss Debby Drumm for review and editorial assistance. We are also grateful to Dr. Miriam R. Anver for histological evaluation of our specimens. This project is supported by a grant (#HMP-00-GN-01-002) of the Korea Health 21 R&D Project, Ministry of health & Welfare, Republic of Korea and a grant (#2001-01) from the Korea University Gene Therapy Center.

References

Bioulac, P., Despuyoos, L., Bedin, C., Iron, A., Saric, J. and Bala-

baud, C. (1980): Decreased acute hepatotoxicity of carbon tetrachloride and bromobenzene by cholestyramine in the rat. Gastroenterology, **81**(3): 520-576.

Bioulac-Sage, P., Lapostolle, V., More, N. and Balabaud, C. (1984): Acute hepatotoxicity of carbon tetrachloride: different liver lobes response in rats with portal branch ligation. Exp Pathol., **26**: 33-40.

Brunot, B., Petras, S., Germain, P., Vinee, P. and Constantinesco, A. (1994): Biopsy and quantitative hepatobiliary scintigraphy in the evaluation of liver transplantation, J. Nucl Med., **35**: 1321-1327.

Connolly, A.K., Price, S.C., Connelly, J.C. and Hinton, R.H. (1988): Early changes in bile duct lining cells and hepatocytes in rats treated with α-naphthylisothiocyanate. Toxicol Appl Pharmacol., **93**: 208-219.

Desmet, V., Krstulovic, B. and Van Damme, B. (1968): Histochemical study of rat liver in α-naphthylisothiocyanate. Toxicol Appl Pharmacol., **93**: 208-219.

Dogan, A.S., Conway, J.J. and Lloyd-Still, J.D. (1993): Hepatobiliary scintigraphy in children with cystic fibrosis and liver disease. J Nucl Med., **35**: 432-435.

Fritzberg, A.R., Kasina, S., Eshima, D. and Johnson, D.L. (1986): Synthesis and biological evaluation of ^{99m}Tc-MAG3 as a hippuran replacement. J Nucl Med., **27**: 111-116.

Gerhold, J.P., Klingensmith, W.C., Kuni, C.C., Lilly, J.R., Silverman, A., Fritzberg, A.R. and Nixt, T.L. (1983): Diagnosis of biliary atresia with radionuclide hepatobiliary imaging. Radiology, **146**: 499-504.

Heyman, S. (1994): Hepatobiliary scintigraphy as a liver function test. J Nucl Med., **35**: 436-437.

Juni, J.E. and Reichle, R. (1990): Measurement of hepatocellular function with deconvolutional analysis: application in the differential diagnosis of acute jaundice. Radiolog., **177**: 171-175.

Jeong, J.M., Kinuya, S., Paik, CH., Saga, T., Sood, V.K. and Neumann, R.C. (1993): Synthesis of a ^{99m}Tc-MAG3-biotin conjugate as a biliary agent. J. Labelled Cmpd Radiopham., **32**: 88-90.

Kim, E.E., Moon, T-Y, Delpassand, E.S., Delpassand, E.S., Podoloff, D.A. and Haynie, T.P. (1993): Nuclear hepatobiliary imaging. Radiologic Clinics of North America., **31**: 923-933.

Kim, M-k., Song, B.J., Seidel, J., Soh, Y., Jeong, K.S., Kim, I.S., Kobayashi, H., Green, M.V., Carrasquillo, J.A. and Paik, C.H. (1998): Use of [^{99m}Tc]MAG3-biocytin hepatobiliary scintigraphy to study protective effect of the synthetic enzyme inhibitor on acute hepatotoxicity in mice. *J. Nucl Med.*, **38**: 185.

Kim, M-k., Seidel, J., Le, N., Kim, I.S., Yoo, T.M., Barker, C., Kobayashi, H., Green, M.V., Carrasquilo, J.A. and Paik, C.H. (1999): Evaluation of [^{99m}Tc]MAG3-Biocytin as a new hepatobiliary imaging agent in bilirubin-coinjected mice. *J. Nucl Med.*, **38**: 50.

Lin, S. and Saito, H. (1986): Acute hepatotoxicity induced by hepatotoxins in Suncus murinus. *J. Toxicol Environ Health.*, **18**: 575-587.

McLean, M. and Rees, K. (1958): Hyperplasia of bile ducts induced by α-naphthylisothiocyanate: experimental biliary cirrhosis free from biliary obstruction. *J. Pathol Bacteriol.*, **76**: 175-188.

Meeks, R.G., Harrison, S.D. and Bull, R.J. (1991): Carbon tetrachloride-induced fatty liver. In Hepatotoxicology, CRC Press. 360-371.

Nunn, A.D., Loberg, M.D. and Conley, R.A. (1983): A structure-distribution-relationship approach leading to the development of ^{99m}Tc +Mebrofenin: An improved cholescintigraphic agent. *J. Nucl Med.*, **24**: 423-430.

Rouiller, C. (1964): Experimental toxic injury of the liver. In: The liver: morphology, biochemistry, physiology (Edited by Rouiller C.), New York: Academic Press. NY., **2**: 335-476.

Traiger, G.J., Vyas, K.P. and Hanzler, R.P. (1985): Effect of inhibitors of α-naphthylisothiocyanate-induced hepatotoxicity on the in vitro metabolism of α-naphthylisothiocyanate. Chem Biol Interactions, **52**: 335-345.

Wistow, B.W., Subramanian, G., Van Heertum, R.L., Henderson, R.W., Gagne, G.M., Hall, R.C. and McAffee, J.C. (1977): An evaluation of ^{99m}Tc-labeled hepatobiliary agents. *J. Nucl Med.*, **18**: 455-461.

Zalatinai, A. and Lapis, K. (1994): Simultaneous induction of liver cirrhosis and hepatocellular carcinomas in F-344 rats: establishment of a short hepatocarcinogenesis model. Exp Toxic Pathol., **46**: 215-222.