EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 33, No. 1, 23-28, March 2001

Arylamine *N*-methyltransferase and thiol methyltransferase activities in cholestatic rat liver induced by common bile duct ligation

You Hee Kim[1,2] and Il Joo[1]

[1] Department of Biochemistry, Keimyung University School of Medicine, Taegu 700-712, Korea
[2] Corresponding author: Tel, +82-53-250-7462; Fax, +82-53-252-1605; E-mail, noel@dsmc.or.kr

Accepted 2 March, 2001

Abstract

Methylation catalyzed by methyltransferases is a major metabolic pathway for an inactivation of some catecholamines, niacinamide as well as aliphatic sulfhydryl drugs and toxic hydrogen sulfides. To investigate the effects of obstructive jaundice in an animal model, common bile duct ligation (CBDL) was performed in the rat and enzyme activities of *S*-adenosyl-L-methionine-dependent arylamine *N*-methyltransferase and thiol methyltransferase were examined in liver cell fractions and serum for a period of 42 d after CBDL. Both mitochondrial and microsomal arylamine *N*-methyltransferase showed significant increases in their activities between the 1st through the 7th day ($P \leq 0.05$ to 0.001), and between the 1st through the 28th day ($P \leq 0.01$ to 0.001) post-ligation, although the cytosolic arylamine *N*-methyltransferase activity did not show a significant change compared to the activities from the sham-operated control. The mitochondrial as well as microsomal thiol methyltransferase showed significant increases in their activities between the 1st through the 28th day ($P \leq 0.05$ to 0.01 and $P \leq 0.01$ to 0.001, respectively) post-ligation, although the cytosolic thiol methyltransferase activity did not show a significant change compared to the activities from the sham-operated control. Arylamine *N*-methyltransferase and thiol methyltransferase in the serum from cholestatic rats also showed significant increases in their activities between the 1st through 28th day ($P \leq 0.01$ to 0.001), and between the 0.5th through the 42nd day ($P \leq 0.05$ to 0.001) post-ligation compared to the sham-operated control, respectively. Enzyme kinetic parameters (K_m and V_{max}) of hepatic membrane-bound arylamine *N*-methyltransferase and thiol methyltransferase were analyzed with the preparation from the 7th day post-ligation, using tryptamine or 4-chlorothiophenol as substrates and *S*-Adenosyl-L-[methyl-^{3}H]methionine as co-substrate. The results indicate that although the K_m values were about the same as the sham-operated control, the V_{max} values of both enzymes increased significantly ($P \leq 0.01$ and 0.001, respectively). These results suggest that the biosynthesis of arylamine *N*-methyltransferase and thiol methyltransferase have been induced in response to obstructive jaundice.

Keywords: arylamine *N*-methyltransferase, cholestatic rat liver, enzyme induction, thiol methyltransferase

Introduction

Methylation is a major metabolic pathway for an inactivation of some catecholamines, niacinamide as well as aliphatic sulfhydryl drugs and toxic hydrogen sulfides catalyzed by methyltransferases (*N*-methyltransferases and *S*-methyltransferases) using *S*-adenosylmethionine as the methyl donor (Lyon and Jacoby, 1982; Murray, 2000: Roediger and Babridge, 2000). *N*-methylation by arylamine, ethylamine (tryptamine) or *N*-methyltransferase (EC 2.1.1.49) is an important route of metabolism of the ring nitrogen of physiological tryptamine and pyrrole as well as a number of other arylamines including nonphysiological anilline and its derivatives (Lyon and Jacoby, 1982).

The enzyme was first extracted from a rabbit lung in 1962 (Axelrod). Arylamine *N*-methyltransferase was found to be widely distributed *i.e.* lung, liver, kidney, pineal gland, blood, and brain. In rat, the enzyme activity was also found in lung, heart, salivary gland, liver, stomach, small intestine, and brain (Borchardt, 1980).

Thiol methyltransferase (EC 2.1.1.9) is a membrane-bound enzyme that catalyzes the inactivation of aliphatic sulfhydryl drugs and sulfide detoxication of bacterial produced hydrogen sulfide (Weinshilboum *et al*., 1979; Weisiger and Jacoby, 1980; Roediger and Babridge, 2000). The thioester of cysteine, formed by conjugation with glutathione is a major known substrate (Weisiger and Jacoby, 1980; Drummer *et al*., 1983). The enzyme must be distinguished from cytosolic thiopurine methyltransferase (EC 2.1.1.67) of erythrocyte which catabolizes thiopurine drugs (Keith *et al*., 1984; Keith et *al*.,

1985; Otterness *et al*., 1986; Price *et al*., 1989). The enzyme activity is found in microsomal fractions of the liver (Weinshilboum *et al*., 1979), kidney, and lung of the rat as well as in the livers of other mammals (Weisiger and Jacoby, 1979; Otterness *et al*., 1986). In human, this activity is also present in erythrocyte membrane (Weinshilboum *et al*., 1979; Keith *et al*., 1983; Keith *et al*., 1984; Keith *et al*., 1985; Babridge *et al*., 1995), placenta and liver (Pacifici *et al*., 1991) as well as in colonocytes (Babridge *et al*., 1995). It has been detected in high concentration in the erythrocytes of patients with ulcerative colitis (Roediger and Babridge, 2000) and in low concentration in Parkinson's disease and rheumatoid arthritis (Babridge *et al*., 1995). Several diseases are reported to cause activity changes of thiol methyltransferase (Babridge *et al*., 1995; Roediger and Babridge, 2000), however, the possible changes of the arylamine *N*-methyltransferase and thiol methyltransferase activities under cholestasis have not been investigated yet.

Biliary obstruction (cholestasis, obstructive jaundice) is known to induce numerous pathophysiology (Moritz and Snodgrass, 1972). Bile flow may be impaired at any point from the liver cell canaliculus to the ampulla of Vater and induced by intrahepatic and extrahepatic causes. Cholestasis also accompany numerous changes of the hepatic xenobiotic enzymes (Mun and Kwak, 1989; Park *et al*., 1994; Ihm *et al*., 1995; Ihm and Kim; 1997; Kim and Kim; 1999) and striking metabolic imbalances such as glutathione conjugation in the liver.

Despite known clinical symptoms and signs of jaundice, the metabolic state of xenobiotic enzymes in acute and chronic cholestasis is not clearly understood. In order to investigate the effect of extrahepatic cholestasis on the major detoxification pathway enzymes for aromatic amines and sulfides, methyltrasferases were investigated using the common bile duct-ligated rats that have been widely used as an experimental model for human extrahepatic cholestasis (Kaplan and Righetti, 1970; Righetti and Kaplan, 1972; Kryszewski *et al*., 1973). For this study, we have systematically investigated the arylamine *N*-methyltransferase and thiol methyltransferase activities in serum and liver with subcellular fractions prepared from the cholestatic rat liver induced by common bile duct ligation for a period of 42 days. In addition, K_m and V_{max} values for these enzymes were also analyzed with the microsomal and mitochondrial preparations from the rat liver on the 7th day post-ligation.

Materials and Methods

Chemicals

Tryptamine hydrochloride, DL-dithiothreitol, 4-chlorothiophenol, sodium azide, potassium tetraborate tetrahydrate, ethylenediaminetetraacetic acid disodium salt: dihydrate, Triton X-100, potassium phosphate dibasic, potassium phosphate monobasic, and albumin from bovine serum (10 g/100 ml) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *S*-Adenosyl-L-[methyl-^{3}H]methionine were from New England Nuclear (Boston, MA, USA). 2, 5-Diphenyloxazole, *p*-bis-O-methylstyryl benzene and toluene of scintillation grade were purchased from Hewlet-Packard Co. (San Diego, CA, USA). Unless specified otherwise, additional reagents were of analytical grade.

Animals

Normal male rats of the Sprague-Dawley strain, weighing 320-350 g, were used for the experiments. All animals were maintained on a pellet diet obtained commercially (Sam Yang Food Co., Wonju, Korea) and tap water. During surgery, rats were anesthesized lightly with ether, and the abdomen was opened through a median line incision. The common bile duct was pulled out and then ligated doubly close to the liver and excised just below the confluence of the lobular ducts. Control animals were subjected to sham operation (midline laparectomy). Each experiment was carried out with a group of 5 rats. Rats were sacrificed at the 0.5th, 1st, 2nd, 3rd, 7th, 14th, 28th and 42nd day after operation. The livers were excised following perfusion (see below), and blood was collected from the abdominal aorta. The serum was separated by centrifugation and stored at -20^{o}C until use. All the animals had been fasted for 12 h prior to sacrifice or surgery.

Subcellular fractionation

The livers were perfused *via* portal vein with cold 0.25 M sucrose and then excised, blotted, weighed, minced, and homogenized in 9 vol. of 0.25 M sucrose. Each homogenate was subjected to cell fractionation. Cytosol, mitochondrial and microsomal fractions were isolated by sucrose linear density-gradient centrifugation method (Kwak and Kwak, 1986), and stored at -80^{o}C. All the isolation procedures were performed at 2 to 4^{o}C.

For arylamine *N*-methyltransferase assay, the 12 h-dialyzate of cytosolic fraction against 200 vol. of 10 mM potassium phosphate (cotaining 0.1 mM EDTA, 0.1 mM dithiothreitol, and 0.02% sodium azide, pH 7.8) was resuspended in 0.25 M sucrose to maintain protein concentration at 5 mg/ml and used. The mitochondrial and microsomal fractions were resuspended in 0.25 M sucrose to maintain protein concentration at 5 mg/ml, then diluted in 1 vol. of 1% Triton X-100 and used for enzyme assay.

For thiol methyltransferase assay, hepatic subcellular fractions were resuspended in 0.25 M sucrose to maintain protein concentration at 5 mg/ml. The mitochondrial and microsomal fractions were diluted in 1 vol. of 1%

Triton X-100 and used after 30 min at 4°C. The cytosolic fraction was used for enzyme assay without any processing.

Enzyme assays

Arylamine *N*-methyltransferase activity was assayed by measuring the formation of *N*-^{3}[H]methyltryptamine (Lyon and Jacoby, 1981) after incubation for 30 min at 37°C of 34 μM *S*-Adenosyl-L-[methyl-^{3}H]methionine (10 μCi/mol), 2 mM tryptamine, 0.1 M potassium phosphate pH 7.8, and enzyme, in a total volume of 200 μl. The reaction was stopped by the addition of 1 ml of 0.1 M potassium borate pH 9.5. The methylated product was extracted with 5 ml of toluene containing 3% (w/v) isoamylalcohol and the radioactivity was measured by scintillation spectrometry. Background levels were determined for a parallel reaction mixture in the absence of tryptamine; background was generally less than 10% of that obtained with a complete reaction mixture. Thiol methyltransferase activity was determined by measuring radioactive ^{3}[H]methyl 4-chlorophenyl sulfide formed by the enzyme action on thiol substrate (4-chlorothiophenol) and *S*-Adenosyl-L-[methyl-^{3}H]methionine according to the method of Weisiger and Jacoby (1979 and 1980). Reaction mixtures consisted of 0.1 M potassium phosphate pH 7.9, 1 mM EDTA, 1% Triton X-100, in final volume of 350 μl. The reaction was initiated by the addition of 20 μl of 0.1 M 4-chlorothiophenol (prepared fresh daily in 95% ethanol), 20 μl of 5 μM *S*-Adenosyl-L-[methyl-^{3}H]methionine (10 μCi/mol) in 10 mM sulfuric acid and 10 μl of enzyme solution (hepatic subcellular preparations and sera). The solution was mixed by vortexing for approximately 1 s. After incubation at 37°C for 10 min, the reaction was stopped with 1 ml of 2 M potassium borate pH 10. The resultant mixture was extracted with 6 ml of toluene by shaking vigorously for 10 s, separating the phases by brief centrifugation, then removing 5 ml of the toluene layer for determination of radioactivity. By single extraction, greater than 99% of the radioactive reaction product was obtained.

Specific activities of enzymes were expressed as ρmol of *N*-^{3}[H]methyltryptamine for arylamine *N*-methyltransferase and ^{3}[H]methyl 4-chlorophenyl sulfide for thiol methyltransferase formed per min per mg of protein of hepatic subcellular fractions or as per ml of serum.

Kinetic analysis

The reaction velocities of arylamine *N*-methyltarnsferase in hepatic mitochondrial and microsomal fractions of cholestatic rat liver at the 7th day post-ligation were determined using five concentration of tryptamine between 160 μM and 2 mM at the concentration of 10 μCi/μmol of *S*-Adenosyl-L-[methyl-^{3}H]methionine for arylamine *N*-methyltransferase and using various concentration of 4-chlorothiophenol (0.1-10 μM) at the concentration of 10 μCi/μmol of *S*-Adenosyl-L-[methyl-^{3}H]methionine for thiol methyltransferase. Apparent Michaelis (K_m) constants and maximal velocity (V_{max}) values were calculated from a Lineweaver-Burk transformation.

Protein assays

Protein concentrations in hepatic subcellular fractions and sera were measured by the biuret method (Gornal *et al.*, 1949). Bovine serum albumin was used as standard for assay.

Statistical analysis

Values are expressed as mean ± SD. Statistical evaluation of the experimental data was done by Student's *t*-test. *P* values of ≤ 0.05 were considered to be significant.

Results

The activities of mitochondrial and microsomal arylamine *N*-methyltransferase in cholestatic rat liver showed significant increases after common bile duct ligation compar-

Table 1. Activities of cytosolic, mitochondrial, and microsomal arylamine *N*-methyltransferases in cholestatic rat liver after common bile duct ligation

Day(s) following ligation	Arylamine *N*-methyltransferase activities (pmol *N*-^{3}[H]methyltryptamine min^{-1} mg protein^{-1})					
	Cytosol		Mitrochondria		Microsome	
	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver
0.5	1.27 ± 0.27	1.36 ± 0.24	2.49 ± 0.47	3.09 ± 0.56	2.05 ± 0.36	2.29 ± 0.32
1	1.29 ± 0.25	1.39 ± 0.33	2.46 ± 0.51	3.35 ± 0.67[a]	2.07 ± 0.33	4.22 ± 0.66[c]
2	1.32 ± 0.31	1.25 ± 0.26	2.48 ± 0.48	3.45 ± 0.59[a]	2.08 ± 0.36	3.26 ± 0.40[b]
3	1.28 ± 0.28	1.20 ± 0.23	2.42 ± 0.46	3.84 ± 0.55[b]	2.04 ± 0.38	3.15 ± 0.37[b]
7	1.26 ± 0.25	1.21 ± 0.30	2.37 ± 0.44	3.82 ± 0.43[c]	2.06 ± 0.35	3.02 ± 0.44[b]
14	1.27 ± 0.26	1.18 ± 0.28	2.39 ± 0.48	2.87 ± 0.46	2.04 ± 0.32	2.98 ± 0.30[b]
28	1.25 ± 0.30	1.13 ± 0.25	2.35 ± 0.43	2.84 ± 0.48	2.07 ± 0.35	2.92 ± 0.32[b]
42	1.26 ± 0.23	1.05 ± 0.22	2.37 ± 0.40	2.58 ± 0.45	2.08 ± 0.34	2.53 ± 0.35

The data are expressed as mean ± SD with 5 rats in each group; Liver of sham: sham-operated rat livers.
Significant differences from sham-operated rat livers (a, P ≤ 0.05; b, P ≤ 0.01; c, P ≤ 0.001).

Table 2. Activities of cytosolic, mitochondrial, and microsomal thiol methyltransferases in cholestatic rat liver after common bile duct ligation

Day(s) following ligation	Thiol methyltransferase activities (pmol ^{3}[H]methyl 4-chlorophenyl sulfide min^{-1} mg $protein^{-1}$)					
	Cytosol		Mitrochondria		Microsome	
	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver
0.5	6.09 ± 0.56	6.05 ± 0.49	5.26 ± 0.84	5.67 ± 1.01	5.10 ± 0.56	5.24 ± 0.78
1	6.11 ± 0.52	6.03 ± 0.65	5.27 ± 0.76	6.74 ± 0.97[a]	5.15 ± 0.63	6.48 ± 0.68[a]
2	6.13 ± 0.46	6.09 ± 0.59	5.24 ± 0.81	7.19 ± 0.78[b]	5.13 ± 0.58	8.43 ± 1.02[c]
3	6.10 ± 0.55	6.23 ± 0.43	5.28 ± 0.75	7.34 ± 0.82[b]	5.08 ± 0.67	7.45 ± 0.74[c]
7	6.08 ± 0.48	6.22 ± 0.59	5.21 ± 0.68	7.45 ± 0.74[b]	4.87 ± 0.60	7.06 ± 0.54[c]
14	6.06 ± 0.45	6.16 ± 0.64	5.17 ± 0.72	7.22 ± 0.67[b]	4.92 ± 0.65	6.47 ± 0.59[b]
28	6.07 ± 0.50	5.52 ± 0.49	5.06 ± 0.62	6.63 ± 0.59[b]	4.86 ± 0.71	6.21 ± 0.57[a]
42	6.06 ± 0.51	5.37 ± 0.60	4.97 ± 0.57	5.48 ± 0.70	4.78 ± 0.68	5.16 ± 0.61

The data are expressed as mean ± SD with 5 rats in each group; Liver of sham: sham-operated rat livers.
Significant differences from sham-operated rat livers (a; $P \leq 0.05$, b; $P \leq 0.01$, c; $P \leq 0.001$).

ed to the activities of sham-operated control between the 1st through the 7th day ($P \leq 0.05$ to 0.001), and between the 1st through the 28th day ($P \leq 0.01$ to 0.001), respectively, although the enzyme activity in cytosolic preparation did not change (Table 1). The activities of mitochondrial and microsomal thiol methyltransferase in cholestatic rat liver showed significant increases after common bile duct ligation compared to the activities of sham-operated control between the 1st through the 28th day ($P \leq 0.05$ to 0.01 and $P \leq 0.01$ to 0.001, respectively), although the enzyme activity in the cytosolic preparation did not change (Table 2). Arylamine *N*-methyltransferase and thiol methyltransferase in the serum from cholestatic rats also showed significant increases in their activities between the 1st through 28th day ($P \leq 0.01$ to 0.001), and between the 0.5th through the 42nd day ($P \leq 0.05$ to 0.001) after ligation compared to the sham-operated control, respectively (Tables 3 and 4). The V_{max} values for mitochondrial and microsomal arylamine *N*-methyltransferase and thiol methyltransferase from the cholestatic rat liver analyzed at the 7th day post-ligation increased significantly ($P \leq 0.01$ and 0.001, respectively), although the K_m values were about the same as the sham-operated control (Tables 5 and 6).

Table 3. Activities of serum arylamine *N*-methyltransferase after common bile duct ligation in rats

Day(s) following ligation	Arylamine *N*-methyltransferase activities (*p*mol *N*-^{3}[H]methyltryptamine $min^{-1}ml^{-1}$)	
	Sham	CBDL
0.5	9.32 ± 0.94	10.94 ± 1.55
1	9.48 ± 0.87	13.80 ± 1.52[c]
2	9.40 ± 0.83	13.62 ± 1.44[c]
3	9.42 ± 0.85	13.48 ± 1.66[b]
7	9.29 ± 0.88	12.54 ± 1.32[b]
14	9.24 ± 0.82	12.34 ± 1.29[b]
28	9.15 ± 0.84	11.44 ± 1.13[b]
42	9.18 ± 0.80	10.32 ± 1.06

The data are expressed as mean ± SD with 5 rats in each group; Sham: sham-operated rats, CBDL: common bile duct-ligated rats.
Significant differences from sham-operated control (b, $P \leq 0.01$; c, $P \leq 0.001$).

Table 4. Activities of serum thiol methyltransferase after common bile duct ligation in rats

Day(s) following ligation	Thiol methyltransferase activities (pmol ^{3}[H]methyl 4-chlorophenyl sulfide $min^{-1}ml^{-1}$)	
	Sham	CBDL
0.5	16.23 ± 0.89	18.87 ± 1.93[a]
1	16.27 ± 0.97	24.10 ± 1.27[c]
2	16.25 ± 0.93	25.42 ± 1.26[c]
3	16.18 ± 0.95	25.58 ± 1.74[c]
7	16.20 ± 0.86	24.78 ± 1.86[c]
14	16.02 ± 0.89	24.82 ± 1.77[c]
28	15.83 ± 0.94	20.60 ± 1.01[c]
42	15.67 ± 0.97	17.64 ± 1.03[a]

The data are expressed as mean ± SD with 5 rats in each group; Sham: shamoperated rats, CBDL: common bile duct-ligated rats.
Significant differences from sham-operated control (a, $P \leq 0.05$; c, $P \leq 0.001$).

Discussion

The effects of cholestasis on the activities of several xenobiotic biotransforming enzymes have been studied in cholestatic rat liver. We have previously reported that experimantal cholestasis affects the isoenzyme patterns of hepatic aryl sulfotransferase in rats (Ihm *et al.*, 1995). We have also reported that thiosulfate sulfurtransferase and UDP-glucuronosyltransferase activities are reduced during cholestasis induced by common bile duct ligation in rats (Ihm and Kim, 1997). In particular, arylamine *N*-methyltransferase and thiol methyltransferase are membrane-bound xenobiotic biotransforming enzymes (Lyon and Jacoby, 1982; Weishilboum *et al.*, 1979). Although they serve a role in various biosynthetic and regulatory

Table 5. Kinetic parameters of aryamine *N*-methyltransferase from cholestatic rat livers determined with tryptamine and *S*-adenosyl-L-[methyl^{3}H]methionine

cell fractions	K_m (mM)		V_{max} (pmol *N*-[^{3}H]methyltryptamine min^{-1} mg protein^{-1})	
	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver
Mitochondria	32.5 ± 3.9	28.3 ± 3.7	5.7 ± 1.2	9.8 ± 1.6[b]
Microsome	35.6 ± 3.3	34.1 ± 4.2	5.1 ± 1.0	8.2 ± 1.3[b]

Michaelis-Menten constants for arylamine *N*-methyltransferase were determined using tryptamine and [methyl-^{3}H] *S*-adenosyl-L-methionine at 37^{o}C for mitochondrial and micromal fractions of sham-operated rat livers and cholestatic rat livers at the 7th day post-ligation.
The data are expressed as mean ± SD with 5 rats in each group; Liver of sham; sham-operated rat livers.
Signicant differences from sham-operated rat livers (b, $P \leq 0.01$).

Table 6. Kinetic parameters of thiol methyltransferase from cholestatic rat livers determined with 4-chlorothiophenol and *S*-adenosyl-L-[methyl^{3}H]methionine

cell fractions	K_m (mM)		V_{max} (pmol [methyl^{3}H]4-chlorophenyl sulfide min^{-1} mg protein^{-1})	
	Liver of sham	Cholestatic liver	Liver of sham	Cholestatic liver
Mitochondria	75.8 ± 4.8	68.7 ± 7.6	14.2 ± 1.7	22.3 ± 2.1[c]
Microsome	71.4 ± 7.7	76.5 ± 6.3	13.1 ± 1.9	20.6 ± 2.7[c]

Michaelis-Menten constants for thiol methyltransferase were determined using 4-chlorothiophenol and [methyl-^{3}H] S-adenosyl-L-methionine at 37^{o}C for mitochondrial and microsomal fractions of sham-operated rat livers and cholestatic rat livers at 7th day post-ligation.
The data are expressed as mean ± SD with 5 rats in each group; Liver of sham; sham-operated rat livers.
Signicant differences from sham-operated rat livers (c, $P \leq 0.001$).

reactions (Borchardt, 1980; Weisiger and Jacoby, 1980), a little has been reported on their activity changes in disease states as well as under cholestasis except that activity of thiol methytransferase is reduced in Parkinson's disease and rheumatoid arthritis (Babridge *et al.,* 1995). In order to understand the effects of cholestasis on these enzyme activities, we have determined the activities of arylamine *N*-methyltransferase and thiol methyltransferase activities in rat liver (cytosolic, mitochondrial and microsomal preparations) and serum for a period of 42 days after common bile duct ligation. Enzyme kinetic parameters (K_m and V_{max}) of hepatic membrane-bound arylamine *N*-methyltransferase and thiol methyltransferase were analyzed with the preparation from the 7th day post-ligation, using tryptamine or 4-chlorothiophenol as substrates and *S*-Adenosyl-L-[methyl-^{3}H]methionine as cosubstrate.

In order to investigate whether the changes of the arylamine *N*-methyltransferase and thiol methyltransferase activities in the cholestatic rat liver and serum were due to an alteration in their catalytic activity or not, K_m and V_{max} values were determined with 7th day post-ligation preparations. As shown in Table 5, the K_m values of arylamine *N*-methyltransferase did not change significantly compared to those from the sham-operated liver in the preparations of mitochondria and microsome. However, V_{max} values significantly increased in both preparations; 5.7 pmol *N*-^{3}[H]methyltryptamine/min/mg protein *vs*. 9.8 for mitochondria and 5.1 pmol *N*-^{3}[H]-methyltryptamine/min/mg protein *vs*. 8.2 for microsomal fractions, respectively. Similarly, the V_{max} values of thiol methyltransferase also increased in mitochondrial and microsomal preparations compared to the control; 14.2 *p*mol ^{3}[H]methyl 4-chlorophenyl sulfide/min/mg protein *vs*. 22.3 for the mitochondria, and 13.1 *p*mol ^{3}[H]methyl 4-chlorophenyl sulfide/min/mg protein *vs*. 20.6 for the microsomal fractions, although the K_m values did not change (Table 6).

The fact that the unchanged activities of arylamine N-methyltyransferase and thiol methyltransferase in the cytosolic fractions of cholestatic livers, accompanied by the increased activities in the serum poses an interesting proposition in that these hepatic membrane-bound enzymes have been leaked easily into the blood stream due to an increased membrane permeability of hepatocytes caused by cholestasis as described previously (Ihm *et al*., 1995; Ihm and Kim, 1997).

It should be noted that the higher V_{max} values of mitochondrial and microsomal fractions of common bile duct-ligated livers might reflect the higher levels of activities in cholestatic liver. In addition, it is also conceivable that the increased enzyme levels of both arylamine *N*-methyltyransferase and thiol methyltransferase accompanied with higher V_{max} values in common bile duct-ligated livers. In addition, it is also conceivable that the increased enzyme levels of both arylamine *N*-methyltransferase and thiol methyltransferase accopmanied with higher V_{max} values in the mitochondrial and microsomal preparations of common bile duct-ligated livers might have been due to an increased biosynthetic capability of the cholestatic livers where funtional abnormalities of the liver are expected to develop. In spite of the previous and present findings, however, the mechanism of changes in activities of several biotransforming enzymes in cholestatic hepatobiliary disease is still unknown. Further investigations should eventually resolve this issue.

References

Axelrod, J. (1962) The enzymatic *N*-methylation of serotonin and other amines. *J. Pharmacol. Exp. Ther.* 138: 28-33

Babridge, W. J., Millard, S. H. and Roediger, W. E. W. (1995) Thiol methyltransferase activity in colonocytes and erythrocyte membranes. *J. Clin. Pathol.* 48: 641-644

Borchardt, R. T. (1980) *N*- and *O*-methylation. In *Enzymatic Basis of Detoxication* (Jacoby, W. B., eds.), vol II, pp. 43-62. Academic Press, New York

Drummer, O. H., Miach, P. and Jarrot, B. (1983) *S*-Methylation of captopril: demonstration of captopril thiol methyltransferase activity in human erythrocyte and enzyme distribution in rat tissues. *Biochem. Pharmacol.* 32: 1557-1562

Gornal, A. G., Bardawill, C. J. and David, M. M. (1949) Determination of serum protein by means of biuret reaction. *J. Biol. Chem.* 177: 751-766

Ihm, J. S., Kim, Y. H. and Kwak, C. S. (1995) Aryl Sulfotransferase Activity in cholestatic rat liver induced by common bile duct ligation. *Korean J. Biochem.* (presently *Exp. Mol. Med.*) 27: 141-147

Ihm, J. S. and Kim, Y. H. (1997) Thiosulfate sulfurtransferase and UDP-glucuronosyltransferase activities in cholestatic rat liver induced by common bile duct ligation. *Korean J. Biochem.* (presently *Exp. Mol. Med.*) 29: 197-201

Kaplan, M. M. and Righetti, A. B. (1970) Induction of rat liver alkaline phosphatase: the metabolism of the serum elevation in bile duct obstruction. *J. Clin. Invest.* 49: 508-516

Keith, R. A., Abraham, R. T., Pazmino, P. and Weishilboum, R. M. (1983) Correlation of low and high affinity thiol methyltransferase and phenol methyltransferase activities in human erythrocyte membranes. *Clin. Chim. Acta.* 131: 257-272

Keith, R. A., Jardine, I., Kerremans, A. and Weinshilboum, R. M. (1984) Human erythrocyte membrane thiol methyltransferase. *S*-methylation of captopril, *N*-acetylcysteine and 7-thiospironolactone. *Drug. Metab. Dispos.* 12: 717-724

Keith, R. A., Otterness, D. M., Kerremans, A. L. and Weinshilboum R. M. (1985) *S*-Methylation of D- and L-penicillamine by human erythrocyte membrane thiol methyltransferase. *Drug. Metab. Dispos.* 13: 669-676

Kim, Y. J. and Kim, Y. H. (1999) Benzoyltransferase and phenylacetyltransferase activities in cholestatic rat liver induced by common bile duct ligation. *J. Biochem. Mol. Biol.* 32: 67-71.

Kryszewski, A. J., Neale, G., Whilfield, J. B. and Moss, D. W. (1973) Enzyme changes in experimental biliary obstruction. *Clin. Chim. Acta.* 47: 175-182.

Kwak, C. S. and Kwak, J. S. (1986) Cell fractionation method of the rat liver. *Keimyung Univ. Med. J.* 5: 45-53

Lyon, E. S. and Jacoby, W, B. (1981) Arylamine *N*-methyltransferase. *Methods Enzymol.* 77: 263-266

Lyon, E. S. and Jacoby, W. B. (1982) Arylamine *N*-methyltransferase. Methylation of the indole ring. *J. Biol. Chem.* 257: 7531-7535

Moritz, M. and Snodgrass, P. J. (1972) Serun enzymes derived from liver cell fractions. Responses to bile duct ligation in the rats. *Gastroenterology* 62: 93-100

Mun, K. C. and Kwak, C. S. (1989) Monoamine oxidase activity in cholestatic rat liver. *Keimyung Univ. Med. J.* 8: 69-77.

Murray, R. K. (2000) Metabolism of xenobiotics. In *Harper's Biochemistry* (Murray, R. K., Granner, D. K., Mayes, P. A. and Rodwell, V. W. 25th eds.), pp. 780-784

Otterness, D. M., Keith, R. A. Kerreman, A. L. and Weishilboum, R. M. (1986) Mouse liver thiol methyltransferase. Assay conditions, biochemical properties, and strain variation. *Drug. Metabol. Disposit.* 14: 680-688

Pacifici, G. M., Santerini, S., Giuliani, L. and Rane, A. (1991) Thiol methyltransferase in humans: development and tissue distribution. *Dev. Pharmacol. Ther.* 17: 8-15

Park, E. M., Mun, K. C. and Kwak, C. S. (1994) α-D-mannosidase and β-D-mannosidase activities in cholestatic rat liver induced by bile duct ligation. *Korean J. Biochem.* (presently *Exp. Mol. Med.*) 26: 197-201.

Price, R. A., Keith, R. A., Spielman, R. S. and Weinshilboum, R. M. (1989) Major gene polymorphism for human erythrocyte (RBC) thiol methyltransferase (TMT). *Genet Epidemiol* 6: 651-662

Righetti, A. B. and Kaplan, M. M. (1972) Disparate response of serum and hepatic alkaline phosphatase and 5'-nucleotidase to bile duct obstruction in the rat. *Gastroenterology* 62: 1034-1039

Roediger W. E. and Babidge W. J. (2000) Thiol methyltransferase activity in inflammatory bowel disease. *Gut.* 47: 206-10.

Weinshilboum, R. M., Sladek, S. and Klumpp, S. (1979) Human erythrocyte methyltransferase: radiochemical microassay and biochemical properties. *Clin. Chim. Acta.* 97: 59-71

Weisiger, R. A. and Jacoby, W. B. (1979) Thiol *S*-methyltransferase from rat liver. *Arch. Biochem. Biophys.* 196: 631-637

Weisiger, R. A. and Jacoby, W. B. (1980) *S*-methylation: thiol *S*-methyltransferase. In *Enzymatic Basis of Detoxicatiobn* (Jacoby, W. B., eds.), vol II, pp. 131-140. Academic Press, New York

Weisiger, R. A. and Jacoby, W. B. (1981) Thiol *S*-methyltransferase. *Methods in Enzymology* 77: 257-262