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Biotransformation of Theophylline in Cirrhotic Rats Induced by Biliary Obstruction

Eun-Jeon Park, Geonil Ko, Jaebaek Kim and Dong Hwan Sohn

College of Pharmacy, Medicinal Resources Research Center, Wonkwang University, Iksan 570-749, Korea

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The object of this work was to study the pharmacokinetic differences and the cause of these differences in cirrhotic rats induced by biliary obstruction when aminophylline (8 mg/kg as theophylline, i.v.) was administered. The concentrations of theophylline and its major metabolite (1,3-dimethyluric acid) in plasma were determined by HPLC. In addition, formation of 1,3-dimethyluric acid from theophylline in microsomes and the changes in the activity of drug metabolizing enzymes, which are suggested to be involved in theophylline metabolism, were determined. In cirrhotic rats, the systemic clearance of theophylline was reduced to 30% of the control value while AUC (area under the plasma concentration-time curve) and $(t_{1/2})_\beta$ were increased 1.3 fold and 3.5 fold, respectively. The formation of 1,3-dimethyluric acid was decreased to 30% of the control value in microsomes of cirrhotic rat liver. In cirrhotic rat liver, activities of aniline hydroxylase (CYP2E1 related), erythromycin-*N*-demethylase (CYP3A related), and methoxyresorufin-*O*-demethylase (CYP1A2 related), which were reported to be related with theophylline metabolism, were decreased to 67%, 53%, and 76% that of normal rat liver, respectively. From the results, it can be concluded that in cirrhotic rats induced by biliary obstruction, the total body clearance of theophylline is markedly reduced and it may be due to decreased activity of drug metabolizing enzymes in liver.

Key words : Theophylline, Pharmacokinetics, Biliary obstruction, Liver cirrhosis

INTRODUCTION

The elimination of drug is frequently altered by hepatic diseases. Especially for drugs with narrow therapeutic range and cleared extensively by the liver, hepatic diseases affect the drug disposition (Mangione *et al.*, 1978). One of drugs that meets both of these criteria is theophylline. Theophylline (1,3-dimethylxanthine) is used widely as a bronchodilator in the treatment of reversible obstructive airway diseases. Therapeutic range of theophylline (8~20 μg/ml) is very narrow and if the plasma concentration of theophylline exceeds therapeutic range, nausea, vomiting, headache and some life-threatening conditions such as cardiac arrhythmia and cerebral seizure may occur (Evans *et al.*, 1986). To prevent these adverse effects and to obtain the optimal therapeutic effect, studies on altered pharmacokinetics of drug in liver disease state are essential.

In rat, rabbit and human, over 90% of the administered theophylline is metabolized into three metabolites by hepatic drug metabolizing enzymes (i.e., 1,3-dimethyluric acid (DMU), 1-methylxanthine (1MX) and 3-methylxanthine (3MX)) (Gaspari *et al.*, 1990; Mcmanus *et al.*, 1988; Oginie *et al.*, 1978; Sarkar *et al.*, 1990; Sarkar *et al.*, 1992). 1MX is metabolized subsequently to 1MU by xanthine oxidase. The major metabolite is DMU. In rat, rabbit and human it accounts for 94%, 77%, and 60%, respectively, among the total metabolites formed from theophylline (Mcmanus *et al.*, 1988). The metabolic pathway of theophylline is not certain at present but it is reported that CYP3A, CYP1A2 and CYP2E1 may be involved in the formation of DMU, CYP1A2 in formation of 1MX and CYP1A1 in formation of 3MX (Gu *et al.*, 1992; Lubet *et al.*, 1985; Mcmanus *et al.*, 1988; Sarkar *et al.*, 1992; Zhang *et al.*, 1995). Therefore, formation of DMU primarily determines the clearance of theophylline.

The hepatic elimination of a drug is mainly dependent on liver blood flow and the activity of drug metabolizing enzyme in liver (Callaghan *et al.*, 1993; Fenyves *et al.*, 1993; Wilkinson and Branch, 1984; Winter, 1988). The relative importance of each of these factors for a particular drug is dependent on its hepatic extraction ratio. For drugs with low extraction ratios, enzyme activity is the major factor determining its elimination. In contrast, the elimination of drugs with

Correspondence to: Dong Hwan Sohn , College of Pharmacy, Wonkwang Univ., 344-2 Shinyong-dong, Iksan, Cheonbuk 570-749, Korea

high extraction ratios is determined by liver blood flow. We have previously reported that theophylline clearance is reduced in early state of fibrosis (Han *et al.*, 1995). Since theophylline is a drug with low extraction ratio and is eliminated mostly from the body through biotransformation by hepatic microsomal oxidative enzymes, the reduced clearance in liver diseases may be related with alteration in drug metabolizing enzyme in liver (Cytochrome P450s) (Shargel and Yu, 1993; Winter, 1988). Although hepatic P450 is the key enzyme in drug metabolism but there is a dearth of information on its activity in disease states of liver.

In this study, the pharmacokinetics of theophylline and its major metabolite (1,3-dimethyluric acid) in normal rats and in cirrhotic rats induced by bile duct ligation and scission was studied after intravenous administration of the drug (6 mg/kg as theophylline). In addition, the extent of biotransformation of theophylline in liver microsomal incubation system and the activities of liver microsomal drug metabolizing enzymes which are known to be involved in metabolic pathway of theophylline are also studied in normal rats and in cirrhotic rats.

MATERIALS AND METHODS

Experimental hepatic cirrhosis

Male Sprague-Dawley rats (initial body weight: 200~240 g, Sam Yuk Laboratory Animal Co., Kyungki-Do, Korea) were used. They received normal chow and water ad libitum and maintained under 12-hour light-dark cycles throughout the experiment. Rats were anesthetized with ketamine/xylazin and the incision was made in the abdomen. Double ligatures were placed on the common bile duct and the scission was made into the bile duct between the two ligatures (Kountouras, 1984). In sham operated rats (control), only an incision was made in the abdomen and it was closed without any damage to bile duct.

Assessment of hepatic cirrhosis

Four weeks after bile duct ligation and scission (BDL/S) operation, serum and whole liver tissue was obtained from BDL/S and sham rats. Hydroxyproline concentration in liver was determined by spectrophotometry (Jamall *et al.*, 1981). Serum biochemical parameters (ALT, AST, ALP, total-bilirubin, albumin) were measured using commercial kits (Ciba-corning, USA) and clinical chemical analyzer (Gilford 400E). For morphological examination, sections of paraffin-embedded liver were stained with hematoxylin and eosin.

Pharmacokinetic study

Four weeks after operation, rats were fasted overnight and then cannulated in femoral vein and artery under light ether anesthesia. When rats were fully recovered from anesthesia, aminophylline (8 mg/kg as theophylline) was injected through cannula in femoral vein. Blood (about 200 μl) was obtained from femoral arterial cannula at 10, 25, 40, 60, 90, 120, 240 and 480 min after the injection. After each blood collection 200 μl of saline was injected to rats though cannula in femoral vein. Plasma was obtained by centrifugation at 2,000 g for 5 min and kept in -70°C until further treatment.

Determination of theophylline and DMU contents in plasma

Theophylline and DMU contents in plasma were measured by using HPLC (Daud *et al.*, 1986). In brief, plasma was mixed with equal volume of acetonitrile and centrifuged (16000 g, 10 min). The supernatant was dried under nitrogen gas at 40°C water bath. The mobile phase (50 μl) was added to the dried sample and injected into HPLC column. The recovery of theophylline and DMU were 85±7% and 92±5%, respectively. HPLC conditions are as follows: UV absorbance detector set at 280 nm; Lichrosorb RP-C_8 (5 μm) column; mobile phase, methanol/phosphate buffer (pH 6.0) (7:93 v/v) containing 7.8 mM N,N-dimethylbutylamine; flow rate, 1.2 ml/min; sensitivity, 0.002 aufs; internal standard, caffeine.

Pharmacokinetic analysis

The pharmacokinetics of theophylline was described by two-compartment open model. The plasma data was fitted to the equation of the model with the aid of the non-linear regression program MULTI (Yamaoka *et al.*, 1981). The AUC (area under the plasma concentration-time curve) was calculated by trapezoidal rule. The area from the last data point (C_t) to infinite time was estimated by the quotient of C_t and the beta disposition of constant (β).

Biotransformation of theophylline in microsomal incubation system

Microsomal fractions were prepared from liver of cirrhotic and sham operated rats by calcium aggregation method (Schenkman and Cinti, 1972). Protein concentration was determined according to the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as a standard. Ten mM theophylline was incubated with 4 mg protein of microsomes in phosphate buffer (pH 7.4) with NADPH generating system at 37°C for 30 min by the method of Robson *et al.* (Robson *et al.*, 1987). After the incubation, 2% zinc sulfate was added to the mixture and centrifuged at 16000 g for 10 min. DMU was extracted with dimethylchloro-

methane:isopropyl alcohol (80:20 v/v). The extract was dried under nitrogen gas at 40°C water bath and 100 μl of mobile phase was added to the dried sample. The concentration of DMU was measured by HPLC (Slusher *et al.*, 1987). Theobromine was used as an internal standard. HPLC conditions are as follows: UV absorbance detector, 280 nm; column, μ-Bondapak C_{18} (5 μm); mobile phase, 0.5% acetic acid/acetonitrile (97:3 v/v); flow rate, 1.0 ml/min; sensitivity, 0.002 aufs.

Enzyme activity in cirrhotic rat liver

Liver microsomal fractions were obtained from cirrhotic and sham operated rats by the calcium aggregation method. Protein concentration was determined according to the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as the standard. Activity of aniline hydroxylase (CYP2E1 related) in microsomal system was measured colorimetrically (Kim *et al.*, 1988). Methoxyresorufin-*O*-demethylase (CYP1A2 related) activity in microsomal system was measured by method of Lubet *et al.* (Lubet *et al.*, 1985; Nerurkar *et al.*, 1993). Activity of erythromycin-*N*-demethylase (CYP 3A related) in microsomal system was determined colorimetrically as described by Nash (Nash, 1953).

Statistical analysis

Data are expressed in the mean±S.D. Data were analyzed by the Student's *t*-test and the *p* value less than 0.05 was considered to be significant.

RESULTS

Assessment of cirrhotic state in BDL/S rats

The liver weight of BDL/S rats was enlarged to 250% of normal rats ($p<0.01$) (Table I) and liver was adhered to surrounding organs by 4 weeks after the bile duct ligation and scission. About 4~8 ml of bile juice was in the expanded bile duct, which had been obstructed. The changes in serum biochemical parameters are shown in Table II. ALT, AST, ALP and total bilirubin values in the sera of cirrhotic rats were significantly increased ($p<0.01$) and the albumin level was reduced but had no significant difference. The hydroxyproline content per gram of liver from BDL/S rats (568±83 mg/g liver) has increased to 314% compared with control rats (182±17.8 mg/g liver). Moreover, when hydroxyproline content in whole liver was calculated, hydroxyproline content in cirrhotic rats (11360 ±1667 mg/liver) was increased to 670% of that of normal rats (1688±166 mg/liver) (Fig. 1). In the liver of BDL/S rats, massive bile duct proliferation was seen due to bile duct obstruction and concentrated fibrosis was frequently observed around the interlobular and septal bile ducts resulting in distortion of the lobular architecture with increased deposition of inflammatory cells, showing the histological aspects of early stage of hepatic cirrhosis (Fig. 2).

Pharmacokinetics of theophylline and its major metabolite in cirrhotic rats

Fig. 3 shows the curves of theophylline serum levels in BDL/S and control rats. When aminophylline (8 mg/kg as theophylline, i.v.) is administered, the theophylline level in serum of BDL/S rats was markedly higher than that of control rats after 4 hours of injection. DMU (the major metabolite of theophylline) concentration in serum of control rats was shown in Fig. 3. DMU was not detected at all in sera of BDL/S rats. This result suggests that biotransformation of theophylline into

Table I. Body and liver weight changes in sham operated and bile duct scission and ligation (BDL/S) operated rats

Group	wk	*n*	Final liver weight (g)	Final body weight (g)	Liver/body weight ratio (%)
Sham	4	6	9.32±1.10	255.15±18.43	3.6±0.2
BDL/S	4	8	20.93±1.61**	260.10±20.94	8.1±0.4**

Each data represents the mean±S.D.
The significance of differences as compared with the sham group *$p<0.05$, **$p<0.01$.
wk: duration of study.
n: number of rats.

Table II. Serum biochemical values in sham operated and bile duct scission and ligation (BDL/S) operated rats

Group	*n*	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	t-Bilirubin (mg/dL)	Albumin (g/dL)
Sham	6	68±10	156±14	177±61	0.33±0.14	3.80±2.39
BDL/S	8	136±28**	570±70**	401±100*	12.95±3.97**	2.73±0.59

Each data represents the mean±S.D.
The significance of differences as compared with the sham group *$p<0.05$, **$p<0.01$.
n: number of rats.
ALT: alanine aminotransferase.
AST: aspartate aminotransferase.
ALP: alkaline phosphatase.

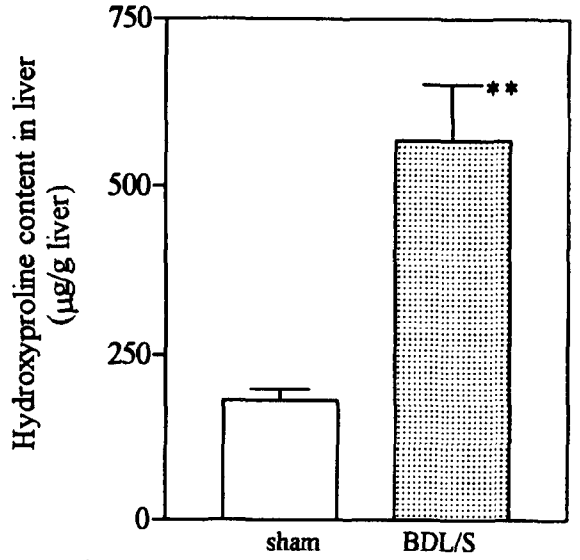

Fig. 1. Hydroxyproline in liver of cirrhotic rats induced by bile duct ligation and scission (BDL/S). Each data represents the mean±S.D. The significance of differences as compared with the sham group $^{**}p<0.01$. Duration of study: 4 week.

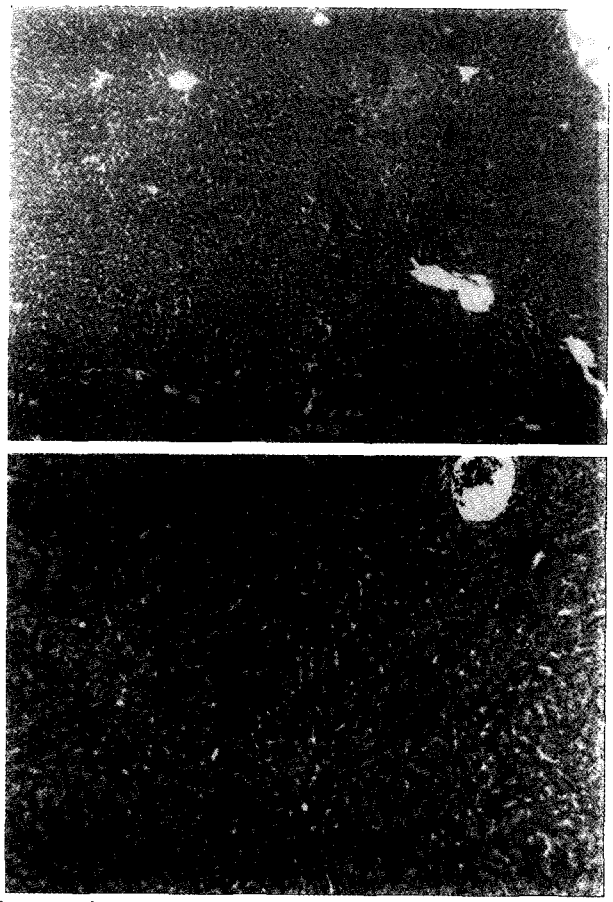

Fig. 2. Light microscopic appearance of liver of sham-operated rats (upper panel, ×100) and cirrhotic rats (lower panel, ×100) induced by bile duct ligation and scission. (H&E staining).

metabolites has been greatly decreased in cirrhotic rats. The pharmacokinetic parameters described by

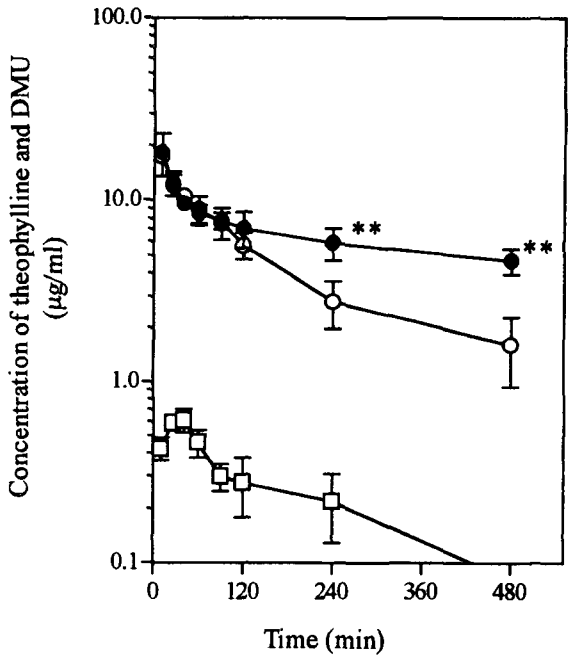

Fig. 3. Semilog plot of theophylline and 1,3-dimethyluric acid (DMU) concentration in plasma of the sham operated rats and cirrhotic rats induced by bile duct ligation and scission (BDL/S). ○—○, Concentration of theophylline in sham rats. ●—●, Concentration of theophylline BDL/S rats. □—□, Concentration of DMU in sham rats. DMU in plasma of BDL/S rats was not detectable. Each data represents the mean±S.D. The significance of differences as compared with the sham group $^{**}p<0.01$. Duration of study: 4 week.

Table III. Pharmacokinetic parameters of theophylline after intravenous injection (8 mg/kg as theophylline)

Parameters	Sham	Bile duct ligation and scission
$(t_{1/2})_{\alpha}$ (hr)	0.37±0.51	0.33±0.25
$(t_{1/2})_{\beta}$ (hr)	3.34±1.56	11.68±2.68**
k_{12} (hr^{-1})	2.00±2.37	1.68±0.85
k_{21} (hr^{-1})	1.70±0.95	0.97±0.28
k_{10} (hr^{-1})	0.59±0.18	0.18±0.08**
AUC (mg·hr/ml)	42.8±9.2	140.0±27.7**
Cl_s (ml/hr/kg)	187.0±9.6	9.00±12.5**
$(V_d)_{\beta}$ (ml/kg)	750.2±164.7	960.6±115.1

Each data represents the mean±S.D.
The significance of differences as compared with the sham group $^{*}p<0.05$, $^{**}p<0.01$.
K_{12}: Distribution rate constant from central compartment to peripheral compartment.
K_{21}: Distribution rate constant from peripheral compartment to central compartment.
K_{10}: Elimination rate constant from central compartment.

two-compartment model are summarized in Table III. The AUC and $(t_{1/2})_{\beta}$ (half-life of the elimination phase) values of theophylline in BDL/S rats were significantly increased ($p<0.01$). A drastic decrease in the systemic clearance (Cl_s) value was obtained for the BDL/S rats. These results indicate that the elimination of theophylline

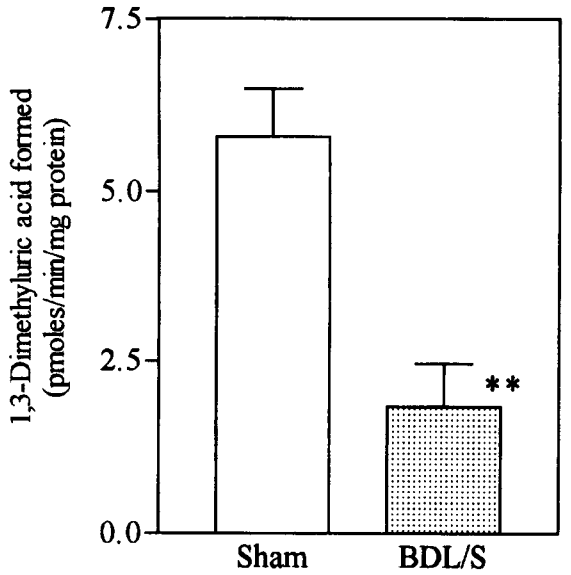

Fig. 4. Formation of 1,3-dimethyluric acid from 10 mM theophylline in rat liver microsomes of cirrhotic rats induced by bile duct ligation and scission (BDL/S). Each data represents the mean±S.D. The significance of differences as compared with the sham group $^{**}p<0.01$.

is markedly reduced in cirrhotic rats.

Biotransformation of theophylline to DMU in microsomal system

DMU content produced by the BDL/S rat liver microsomes was reduced to 32% of control rats (Fig. 4). This result supports the *in vivo* data that DMU was not detected in serum of cirrhotic rats. From this result, it is suggested that 8-hydroxylation of theophylline to DMU be significantly reduced in the cirrhotic rat liver induced by bile duct ligation and scission.

Enzyme activity in microsomes of cirrhotic rat liver

The changes in enzyme activities of microsomal system are shown in Table IV. Aniline hydroxylase (CYP2E1 related) activity in cirrhotic rats was reduced to 67% of that in sham rats ($p<0.01$). Activity of erythromycin-*N*-demethylase (CYP3A related) in liver of the cirrhotic rat was decreased to 53% that of sham rats ($p<0.01$). The activity of methoxyresorufin-*O*-demethylase (CYP1A2 related), which involves in biotransformation of theophylline into 1,3-dimethyluric acid, 1-methylxanthine and 3-methylxanthine, was also reduced to 76% of that in sham rats ($p<0.01$). From these results, the activities of drug metabolizing enzymes involved in biotransformation of theophylline into 1,3-dimethyluric acid have been decreased significantly.

Table IV. Activity of aniline hydroxylase, methoxyresorufin-O-demethylase and erythromycin-N-demethylase in hepatic microsomes from cirrhotic rats induced by bile duct ligation and scission (BDL/S)

Group	*n*	Aniline hydroxylase[a]	Erythromycin-*N*-demethylase[b]	Methoxy-resorufin-*O*-demethylase[c]
Sham	6	766±38	107.1±22.2	7.31±0.23
BDL/S	8	512±115**	56.4±6.1**	5.53±0.44**

Each data represents the mean±S.D.
The significance of differences as compared with sham group $^{*}p<0.05$, $^{**}p<0.01$.
n: number of rats.
[a]pmoles of hydroxyaniline formed/min/mg protein at 37°C.
[b]pmoles of formaldehyde formed/min/mg protein at 37°C.
[c]pmoles of resorufin formed/min/mg protein at 25°C.

DISCUSSION

The structural organism of the liver reflects its function that is to serve as a guardian situated between the digestive tract and the rest of the body (Sherlock, 1989). Because of its interposition, it receives not only large amounts of nutrients but also noxious substances entering the body, which make the liver the most likely organ to be damaged. However, the liver has such a large reserved capacity and it is hence hard to be diagnosed in early state of liver disease. When it develops chronically, it results in hepatic cirrhosis, whatever its etiology may be (Bickel *et al.*, 1991; Hogemann and Domske, 1993). Hepatic cirrhosis is defined anatomically as a diffuse process with fibrosis and nodule formation (Anthony *et al.*, 1978). It causes reduced hepatic blood flow and metabolizing enzyme activity, intrahepatic shunting and changes in bile juice flow resulting in hepatic dysfunction which may lead to altered drug elimination (Williams, 1984).

In this study, 4 weeks after the bile duct ligation and scission operation in rats, there were marked increases in liver weights, serum bilirubin, and the activities of serum ALT, AST and ALP as well as in hydroxyproline content in liver. The elevation of these biochemical parameters showed the major characteristics of biliary cirrhosis and the distortion of normal lobular architecture was also observed showing that cirrhosis has been induced in BDL/S rats. It had been well established that cirrhotic state is developed very well in 4 weeks after bile duct ligation and scission (Kountouras *et al.*, 1984), while only liver fibrosis is developed in bile duct ligated rats without scission (Han *et al.*, 1995).

Hepatic disease is well known to change the pharmacokinetics of a number of drugs. It is difficult to predict the pharmacokinetics of drugs because of various effects from hepatic disease. In this study, AUC and $(t_{1/2})_{\beta}$ have increased significantly when theophylline (8 mg/kg, i.v.) was administered to cirrhotic rats. These results are similar to those of previous reports. The

systemic clearance and constant K_{10} of theophylline have decreased significantly, indicating that the excretion of theophylline from central compartment has been markedly decreased in fibrotic rats induced by bile duct ligation only (duration of 3 weeks) (Han *et al.*, 1995) and in patients with decompensated cirrhosis (Cukier *et al.*, 1992; Jusko *et al.*, 1979; Mangione *et al.*, 1978; Piafsky *et al.*, 1977).

In addition to these results, DMU was not detectable in plasma of cirrhotic and DMU formation has been decreased to 30% of normal rat liver in microsomal incubation system of cirrhotic rat liver. It is thought that reason why DMU was not detectable in plasma of cirrhotic rats was due to the 70% inhibition of theophylline biotransformation in cirrhotic liver microsomal incubation system. Activities of aniline hydroxylase (CYP2E1 related), erythromycin-*N*-demethylase (CYP3A related) and methoxyresorufin-*O*-demethylase (CYP1A2 related), which exhibit catabolic activities for theophylline metabolism into its major metabolite, have been decreased significantly. But the activity of ethoxyresorufin-*O*-deethylase (CYP1A1 related) in liver of cirrhotic rats induced by BDL/S (duration of 4 weeks) which are known to be involved in metabolic pathway of theophylline into its minor metabolites, was same as in normal rats (unpublished data). There were no change in activity of CYP1A1 in liver of cirrhotic rats induced by carbon tetrachloride treatment (1 ml/kg, p. o., twice a week for 10 weeks) (unpublished data). It is reported that drug metabolism by male rats can reflect human metabolism when the compound of interest is metabolized by CYP1A and CYP2E because there is strong regulatory conservation of these isoforms between rodents and humans (Mugford and Kedderis, 1998). Therefore when patients with liver cirrhosis with bile duct obstruction are treated with drugs that are metabolized by CYP2E1 or CYP1A2 modification in drug dosage is recommended.

We have determined the immunoreactive protein levels of these decreased CYPs in cirrhotic rat livers induced by BDL/S, recently. The immunoreactive protein levels of CYP3A and CYP1A were decreased 4 weeks after the BDL/S operation, but immunoreactive protein level of CYP2E1 was not decreased (unpublished data). The significant decrease in the activity of CYP 2E1 is thought to be due to allosteric effect because there was no decrease in protein content of CYP2E1. It has been reported that the disease-specific alterations of drug-metabolizing enzymes among patients with cirrhosis is due, at least in part, to pre-translational mechanisms (George *et al.*, 1995). But further study is needed to investigate the exact regulation mechanism of the reduced activities and immunoreactive protein levels of CYP1A and CYP3A in cirrhotic rat liver induced by BDL/S.

The common factors that affect the elimination of drug in liver disease state are known to be hepatic blood flow, hepatic enzyme activity, and drug binding (Callaghan *et al.*, 1993; Wilkinson and Branch, 1984; Winter, 1988). The hepatic blood flow is a major factor for the drugs that are highly extracted by the liver (Fenyves *et al.*, 1993). The hepatic enzyme activity is a major factor for the drugs that are poorly extracted by the liver and have a low protein binding ratio. Protein binding is a major factor for the drugs that are poorly extracted by the liver and have a high protein binding percent (Wilkinson and Branch, 1984; Winter, 1988).

It was reported that hepatic extraction ratio of theophylline is low within the range of 0.0338~0.09 and its protein binding is 40~80% (Gaspari and Bonati, 1990; Shargel and Yu, 1993; Simons *et al*, 1979). The extent of theophylline binding to plasma protein is said to be negligible and is usually not considered when theophylline clearance rates are calculated. Since theophylline has a low hepatic extraction ratio and a low a protein binding ratio, significant decrease in theophylline clearance in cirrhotic state is due to metabolizing enzyme activity in liver. And hepatic blood flow and protein binding are minor factors that may change the elimination of theophylline in cirrhotic state.

Other factors that may change the elimination of theophylline are renal clearance and bile juice flow. Renal clearance of theophylline comprises only 8~12% of plasma clearance, which have little effect on systemic clearance (American Society of Hospital Pharmacists, 1994). It is reported that only small part of unchanged theophylline is excreted to bile juice and most of its metabolites are excreted to urine (Gaspari and Bonati, 1990). And molecular weight of many of the substances which are excreted to bile juice are known to be over 325 in rats (Rollins, 1984), but molecular weight of theophylline is only 180 so the amount of the theophylline excreted to bile juice is thought to be negligible.

It was suggested that insufficient oxygen supply to hepatocytes due to capillarization of the hepatic sinusoids in liver cirrhosis induced by carbon tetrachloride may reduce the oxidation of theophylline into DMU, 1MX or 3MX (Hickey *et al.*, 1995). But in this study, formation of DMU in cirrhotic liver microsomal incubation system was reduced to 30% of the normal rat liver. And the amount of oxygen supplemented to these cirrhotic liver and normal liver microsomal incubation systems was not different. If oxygen supplementation is the major limiting factor for the biotransformation of theophylline, the formation of DMU in the liver microsomal incubation system should be same whether the microsomes are obtained from cirrhotic liver or normal liver.

It was reported that in all types of liver disease groups compared to controls, the metabolite pattern

in urine was changed, i.e., increased excretion of 1-methyl uric acid and a concomitant decrease of 1,3-dimethyl uric acid and particularly of 3-methylxanthine (Staib *et al.*, 1980). These results show that the inhibition of one of the metabolic pathways is compensated by other metabolic pathways. Theophylline is 1-demethylated to 1-methylxanthine by CYP1A1 and 1-methylxanthine undergoes further oxidation by xanthine oxidase to 1-methyluric acid. As mentioned above, activity of CYP1A1 was not changed in cirrhotic rat liver induced by BDL/S or carbon tetrachloride. So, in hyperoxia, this shifted metabolic pathway which is intact in cirrhotic state may be more activated than in normoxia. But the precise mechanism for the effect of oxygen on the restoration of theophylline can be revealed by determining its metabolites in serum and urine of cirrhotic rats in hyperoxia.

From the results above, it may be concluded that the reduced clearance of theophylline in cirrhotic rats with bile duct ligation and scission may be due to the decreased activities of drug metabolizing enzymes in liver. In addition, when patients with liver cirrhosis with bile duct obstruction are treated with drugs that are metabolized by CYP2E1 or CYP1A2 modification in drug dosage is recommended.

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REFERENCES CITED

American Society of Hospital Pharmacists In *AHFS Drug Information*, American Society of Hospital Pharmacists, Inc., Bethesda, pp. 2026-2033, 1994.

Anthony, P. P., Ishack, K. G., Nayak, N. C., Poulson, H. E., Scheuer, P. J. and Sobin, L. H., The morphology of cirrhosis. *J. Clin. Pathol.*, 31, 395-414 (1978).

Bickel, M., Baader, E., Brocks, D. G., Engelbart, K., Gunzler, V., Schmidts, H. L. and Vagel, G. H., Beneficial effects of prolyl 4-hydroxylase in CCl_4-induced fibrosis of the liver in rats. *J. Hepatol.*, 13, s26-s34 (1991).

Callaghan, R., Desmond, P. V., Paull, P. and Mashford, M. L., Hepatic enzyme activity is the major factor determining elimination rate of high-clearance drugs in cirrhosis. *Hepatol.*, 18, 54-60 (1993).

Cukier, A., Strauss, E., Filho, M. T., Santos, S. R. C. J. and Vargas, F. S. Theophylline metabolism in patients with hepatosplenic mansoniasis and cirrhosis. *J. Hepatol.*, 15, 35-39 (1992).

Daoud, N., Arvidsson, T. and Wahlund, K. G., The liquid chromatographic determination of theophylline in untreated plasma by simple direct injection. *J. Pharm. Biomed. Anal.*, 4, 253-260 (1986).

Fenyves, D., Gariepy, L. and Villeneuve, J. P., Clearance by the liver in cirrhosis I. Relation between propranolol metabolism in vitro and its extraction by the perfused liver in the rat. *Hepatol.*, 17, 301-306 (1993).

Frei, A. and Zimmermann, A. and Weigand, K, The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. *Hepatol.*, 4, 830-834 (1984).

Gaspari, F. and Bonati, M., Interspecies metabolism and pharmacokinetic scaling of theophylline disposition. *Drug. Metab. Rev.*, 22, 179-207 (1990).

George, J., Liddle, C., Murray, M., Byth, K. and Farrell, G. C., Pre-translational regulation of cytochrome P 450 genes is responsible for disease-specific changes of individual P450 enzymes among patients with cirrhosis. *Biochem. Pharmacol.*, 49, 873-881 (1995).

Gu, L., Gonzalez, F. J., Kalow, W. and Tang, B. K., Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics.*, 2, 73-77 (1992).

Han, S. S., Kim, K. Y., Ham, S. H., Sohn, D. H. and Kim, J. B., Pharmacokinetics of theophylline: effects of hepatic fibrosis in rats induced bile duct ligation. *Biol. Pharm. Bull.*, 18, 470-473 (1995).

Hickey, P. L., Angus, P. W., McLean, A. J. and Morgan, D. J., Oxygen supplementation restores theophylline clearance to normal in cirrhotic rats. *Gastroenterol.*, 108, 1504-1509 (1995).

Hogemann, B. and Domschke, W., Hepatic fibrosis-current concepts of pathogenesis and therapy. *Gastroenterol. Jpn.*, 28, 570-579 (1993).

Jamall, I. S., Finelli, V. N. and Que Hee, S. S., A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Anal. Biochem.*, 112, 70-75 (1981).

Jusko, W. J., Gardner, M. J., Mangione, A., Schentag, J. J., Koup, J. R. and Vance, J. W., Factors affecting theophylline clearances: Age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates and ethanol., *J. Pharm. Sci.*, 68, 1358-1366 (1979).

Kim, S. G., Williams, P. E., Schuetz, E. G., Guzelian, P. S. and Novak, R. F., Pyridine induction of cytochrome P450 in the rat:Role of P450j(alcohol-inducible form) in pyridine N-oxidation. *J. Pharmacol. Exp. Ther.*, 246, 1175-1182 (1988).

Kountouras, J., Billing, B. H. and Scheuner, P. J., Prolonged bile duct obstruction:A new experimental model for cirrhosis in th rat. *Br. J. Exp. Pathol.* 65, 305-311 (1984).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 26-275 (1951).

Lubet, R. A., Mayer, R. T., Cameron, J. W., Nims, R. W., Burke, M. D., Wolff, T. and Guengerich, F. P.,

Dealkylation of pentoxyresorufin: A rapid and sensitive assay for induction of cytochrome P450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys.*, 238, 43-48 (1985).

Mangione, A., Imhoff, T. E., Lee, R. V., Shum, L. Y. and Jusko, W. J., Pharmacokinetics of theophylline in hepatic disease. *Chest*, 73, 616-622 (1978).

Mcmanus, M. E., Miners, J. O., Gregors, D., Stupans, I. and Birkett, D. J., Theophylline metabolism by human, rabbit and rat liver microsomes and by purified forms of cytochrome P450. *J. Pharm. Pharmacol.*, 40, 388-391 (1988).

Meeks, R. G., Harrison, S. D. and Bull, R. J., Xenobiotic Biotransformation *In Hepatotoxicology.*, CRC press, New York, pp. 139-180, 1991.

Mugford, C. A. and Kedderis, G. L., Sex-dependent metabolism of xenobiotics, *Drug Metab. Rev.*, 30, 441-498 (1998).

Nash, T., The colorimetrical estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.*, 55, 416-421 (1953).

Nerurkar, P. V., Park, S. S., Thomas, P. E., Raymond, T., Nims, R. W. and Lubet, R. A., Methoxyresorufin and benzyloxyresorufin: substrates preferentially metabolized by cytochromes P4501A2 and 2B, respectively, in the rat and mouse. *Biochem. Pharmacol.*, 46, 933-943 (1993).

Niemela, O., Risteli, L., Sotaniemi, E. A. and Risteli, J., Aminoterminal propeptide of type III procollagen in serum in alcoholic liver disease. *Gastroenterol.*, 85, 254-259 (1983).

Oginie, R. I., Clinical pharmacokinetics of theophylline. *Clin. Pharmacol.*, 3, 267-293 (1978).

Piafsky, K. M., Sitar, D. S., Rangno, R. E. and Ogilvie, R. I., Theophylline disposition in patients wth hepatosplenic mansoniasis and cirrhosis. *New Engl. J. Med.*, 296, 1495-1497 (1977).

Robson, R. A., Matthews, A. P., Miners, J. O., Mcmanus, M. E., Meyer, U. A., Hall, P. M. and Birkett, D. J., Characterization of theophylline metabolism in human liver microsomes. *Br. J. Clin. Pharmacol.*, 24, 293-300 (1987).

Rollins, D. E., Pharmacokinetics and Drug Excretion in Bile. *In Pharmacokinetic Basis of drug treatment*, Raven Press: New York, pp. 77-88, 1984.

Sarkar, M., Polk, R. E., Guzelian, P. S., Hunt, C. and Karnes, H. T., In vitro effect of fluoroquinolones on theophylline metabolism in human liver microsome. *Antimicrob. Agents Chemotherapy*, 34, 594-500 (1990).

Sarkar, M. A., Hunt, C., Guzelian, P. S. and Karns, H. T., Characterization of human liver cytochrome P 450 involved in theophylline metabolism. *Drug Metab. Dispos.*, 20, 31-37 (1992).

Shargel, L. and Yu, A. B. C., *Applied Biopharmaceutics and Pharmacokinetics*, 3rd Ed., Appletone & Lange, London, pp. 293-334, 1993.

Schenkman, J. B. and Cinti, D. L., Preparation of microsomes with Calcium. *Methods Enzymol.*, 52, 83-89 (1978).

Sherlock, S., *In Disease of the liver and biliary system* 8th ed., Blackwell Scientific Publications, London, pp. 1-18, 1989.

Simons, K. J., Simons, F. E. R., Briggs, C. J. and Lo, L., Theophylline protein binding in humans. *J. Pharm. Sci.*, 68, 252-263 (1979).

Slusher, L. B., Park, S. S., Gelboin, H. V. and Vesell, E. S., Studies on the metabolism in human liver microsomes. *Biochem. Pharmacol.*, 36, 2359-2367 (1987).

Staib, A. H., Schuppan, D., Lissner, R., Zilly, W., Bomhard, G. V. and Richter, E., Pharmacokinetics and metabolism of theophylline in patients with liver cirrhosis. *Intl. J. Clin. Pharmacol. Ther. Toxicol.*, 18, 500-502 (1980).

Wilkinson, G. R. and Branch, R. A. Effects of Hepatic Disease on Clinical Pharmacokinetics *In Pharmacokinetic basis for drug treatment*, Raven Press, New York, pp. 49-61, 1984.

Williams, R. L., *Pharmacokinetic Basis for Drug Treatment*, Raven press, New York, pp. 63-75, 1984.

Winter, M. E., Theophylline *In Basic Clinical Pharmacokinetics*, 2nd Ed., Applied Therapeutics, Inc.: Washington, pp. 125-151, 1988.

Yamaoka, K., Tanigawa, Y., Nakagawa, T. and Uno, T., A pharmacokinetic analysis program (multi) for microcomputer. *J. Pharmacobiodyn.*, 4, 879-885 (1981).

Zhang, Z. Y. and Kaminsk, L. S., Characterization of human cytochromes P450 involved in theophylline 8-hydroxylation. *Biochem. Pharmacol.*, 50, 205-211 (1995).