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Effect of Hepatic Cirrhosis on the Pharmacokinetics of Theophylline in Rats

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The experimental hepatic cirrhosis was induced either by bile duct ligation (BDL) or by pretreatment with dimethylnitrosamine (DMNA). The pharmacokinetics of theophylline were studied after a single intravenous or a single oral administration. Using the ultrafiltration method, protein-drug binding experiments were also carried out. The bilirubin level was several-fold increased by BDL, but not by DMNA treatment. The albumin content was decreased in both cirrhotic groups. The total clearance (Clt, ml/kg/hr) of theophylline in both hepatic cirrhosis groups significantly decreased and the terminal half-life ($t_{1/2}$) in the cirrhotic rats was increased about two-fold after intravenous and oral administration. The volume of distribution at steady state (Vdss, ml/kg) was increased slightly in the cirrhotic groups. Protein binding in BDL (8.67±4.85%) decreased about four-folds, but in DMNA (73.00±9.85%) similar result was observed as compared with the control. Increased free fraction of theophylline did not increase the volume of distribution in BDL. Therefore decreased total body clearance of theophylline was mainly due to decreased intrinsic clearance of theophylline in the liver. The absolute bioavailability of theophylline in these experiments was between 63.8 and 72.8%(66.1% in BDL, 63.8% in Sham operated and Control, 72.8% in DMNA). These results suggest that in the experimental hepatic cirrhosis model, administration route does not affect the disposition of theophylline.

Key words : Experimental hepatic cirrhosis, Theophylline pharmacokinetics, Protein binding, Disease state pharmacokinetics

INTRODUCTION

There are multiple diseases leading to the development of hepatic cirrhosis: viral infection (30%), ethanol abuse (30%), cholestatic disease (10%), and other conditions (30%, Bickel,1991). Chronic liver disease and cirrhosis is the third largest cause of death for Korean men between the age of 30 and 49, and the 4th cause of death for Korean (National Statistical Office, 1991, 1992, 1993, 1994). Because of the high morbidity and mortality related to hepatic cirrhosis, a reliable experimental animal model for understanding and overcoming this disease is difficult to find. An animal model of hepatic cirrhosis can be classified by the etiological factor (Tsukamoto *et al.*, 1990): (1) carbon tetrachloride (CCl_4), or dimethylnitrsamine (DMNA) (2) nutritional, (3) immunologic; (4) biliary; (5) alcoholic; and (6) genetic.

CCl_4 is one of the oldest and most widely used materials for experimental hepatic cirrhosis. However, several problems have been repeatedly observed in this model (Tamayo, 1983). Experimental hepatic cirrhosis induced by conventional bile duct ligation (BDL, Kountouras *et al.*, 1984) and by DMNA (Jezequel *et al.*, 1987) has been re-established for circumventing the problems induced by CCl_4. The BDL method has several advantages compared to CCl_4-induced hepatic cirrhosis: (1) hepatic cirrhosis with high yield in rats obstructed for 1 month or longer, (2) the morphological changes close to those in human, (3) the techniques are easy to perform, and (4) most animal survived for many weeks (Kountouras *et al.*, 1984). The hepatic cirrhosis induced by DMNA reproduces a number of characteristics of human disease (Tsukamoto *et al.*, 1990).

The liver disease has the potential for affecting drug binding to macromolecules by qualitative or quantitative changes, by alteration of other proteins, and by the accumulation of endogenous substances such as bilirubin and bile acids that may displace drugs, especially acidic drugs from binding sites (Wilkinson *et al.*, 1984). Especially in BDL, endogenous material such

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as bilirubin (several-fold increased, Kountouras *et al.*, 1984; Boker *et al.*, 1991; Han *et al.*, 1995) and albumin (decreased significantly, Han *et al.*, 1995) would cause a decrease in drug-protein binding. Reduced plasma binding will tend to increase the volume of distribution by making more drug available for equilibration with extravascular tissues (Shand, 1977).

The aim of this study was to elucidate the influence of biological parameters in two experimental hepatic cirrhosis models caused by BDL and DMNA pretreatment to the pharmacokinetics of theophylline.

MATERIALS AND METHODS

Materials

Theophylline was obtained from Wako Chem. Co. (Osaka, Japan). DMNA, chloramine T, β-hydroxyethyltheophylline, trans-4-hydroxy-L-proline, and p-dimethylaminobenzaldehyde were obtained from Sigma Chem. Co. (St.Louis, USA). Other chemicals were either generally available or purchased from E. Merck (Germany). Aminophylline (Daewon Pharm. Co., Korea), Rompun (Bayer Korea Ltd., Seoul, Korea), and Ketalar® (Yuhan Corp., Seoul, Korea) were also used.

Animals

Female Splague-Dawley rats (Samyuk Experimental Animal Co., Kimpo, Korea), of 12 weeks old, were used. Rats were divided into three groups; a pharmacokinetic experimental group after intravenous administration, a pharmacokinetic experimental group after oral administration and protein binding experimental group. Each group was divided into four subgroups; BDL-induced hepatic cirrhosis, and its control, DMNA induced hepatic cirrhosis, and its control.

Experimental hepatic cirrhosis

Hepatic cirrhosis induced by BDL (Boker *et al.*, 1991): Under anesthesia (Rompun®; 10 mg/kg, i.p., 2 ml/kg, Ketalar®; 20 mg/kg, i.m., 0.6 ml/kg), common bile duct was ligated (two tight ligatures around common bile duct and the bile duct cut between the ligatures). Four weeks after the ligation pharmacokinetic experiments were performed.

Hepatic cirrhosis induced by DMNA pretreatment (Ala-Kokko *et al.*, 1989): DMNA (10 μg/kg) was administered by i.p. (1.6 ml/kg) injection. Injections were given on the first 3 consecutive days of each week over a period of 4 weeks.

Pharmacokinetic experiment

Theophylline (10 mg/kg, as aminophylline) was administered i.v. (1.6 ml/kg) through the tail vein of rats under light ether anesthesia. The proximal part of the tail vein was incised (approx. 1 mm) and blood samples for the measurement of theophylline were collected in a capillary (70 μl, Chase Instr. Co., NY, USA) from the same part of the tail vein of animals at 10, 25, 40, 60, 90, 120, 240 and 480 min. after administration. When the drug was administered orally (10 mg/kg, as aminophylline), using the same method previously described blood samples were collected from the tail vein at 30, 60, 90, 120, 180, 300, and 480 min after administration. Blood samples were centrifuged at 3000 rpm for 10 min and serum was kept frozen at -20°C until analysis.

Theophylline analysis

The concentration of theophylline was determined by high performance liquid chromatography (Waters Associates, USA) according to the method described by Orcutt (1977). Briefly, serum was mixed with an equal volume of acetonitrile containing the internal standard, β-hydroxyethyltheophylline, to precipitate serum proteins. After centrifugation, a 20 μl of sample was injected. The HPLC conditions were as follows: UV detector set at 254 nm; μ-Bondapak C18 column; mobile phase, acetonitrile:10 mM acetate buffer (9:91, pH 4.0); flow rate, 2.0 ml/min.; sensitivity, 0.005 aufs.

Protein binding experiment (Lesko, 1981)

The experiments on the binding of theophylline to plasma protein were carried out *in vitro* by ultrafiltration with the micropartition system (MPS-1, Amicon Inc., MA. USA). Theophylline (10 mg/kg, as aminophylline) was administered i.v. through the tail vein of rats under light ether anesthesia. Blood samples were collected at 60 min. by direct heart puncture and the serum was obtained after centrifugation (3000 rpm for 10 min.)

Measurement of clinical biochemical parameters and hydroxyproline

The clinical biochemical parameters (alkaline phosphatase; ALP, alanine aminotransferase; ALT, aspartate aminotransferase; AST, total protein, albumin, total bilirubin) in serum were measured with a commercial kit (Gilford Co., USA). Also body weight in each week and liver weight were measured. In order to measure total collagen content, total hydroxyproline content in the liver was determined according to the method described by Jamall *et al.* (1981).

Microscopic examination of the liver

The liver was fixed in formalin and embedded in paraffin sections of 2 μm thickness, which were then stained with hematoxylin and eosin (H&E) and examined by light microscopy.

Pharmacokinetic analysis

Because all the serum concentration of theophylline versus time curves after i.v. administration were biphasic, they could be described by a two-compartment open model (Piafsky *et al.*, 1977; Shargel *et al.*, 1993) and those after oral administration by one compartment open model. The serum concentration data was fitted to the equation of the model with the aid of RSTRIP®, least-squares parameter optimization computer program. The pharmacokinetic parameters after i. v. administration were estimated by conventional methods as follows.

$$Cp=Ae^{-at}+Be^{-bt}$$

$$k=\frac{ab(A+B)}{Ab+Ba}$$

$$k_{12}=\frac{AB(b-a)^2}{(A+B)(Ab+Ba)}$$

$$k_{21}=\frac{Ab+Ba}{A+B}$$

$$Clt=k\cdot Vp$$

$$AUC_0^{\infty}=\frac{D}{k\cdot Vp}$$

$$(Vd)ss=Vp(1+\frac{k_{12}}{k_{12}})$$

where Cp is the concentration of drug in the central comparment, Vp is the volume of drug in the central comparment, the constant A and B are intercepts on the y axis for each exponetial segmenet of the curve, the constants a and b are the rate constants for the distribution phase and elimination phase, respectively, D is the dose of drug, and k, k_{12}, k_{21} are the first-order rate transfer constant for the movement of drug. AUC is the area under the drug conentration-time curve.

Statistical analysis

All data are presented as mean±standard deviation. Data were statistically analyzed by the unpaired Student's t test. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Clinical parameters (Table I)

During the postoperative period, both the BDL and SHAM groups showed a slight increase in the body weight, by 9.8% and 2.9%, respectively, but the body weight in DMNA groups decreased slightly. A significant increase in liver weights was observed in the BDL group (7.7 g/100 g body weight) compared with the SHAM group (4.2 g/100 g body weight), but liver weights in DMNA (3.8 g/100 g body weight) were similar to those of controls (3.8 g/100 g body weight). The common clinical parameters list impairment of liver function in liver cirrhosis; the ALP, AST, ALT values were increased significantly in BDL and DMNA groups (when compared with these in each control groups). The total protein values in the DMNA group were significantly decreased. The albumin values were significantly decreased in both cirrhotic groups, and the total bilirubin content was significantly increased in the BDL group. Hydroxyproline contents, which represent the liver fibrosis/cirrhosis, were significantly increased in both cirrhotic groups.

Microscopic examination of the liver (Fig. 1)

The impairment of liver function in both BDL and DMNA groups was also proven by microscopic examination of the liver. In BDL, the normal nodular architecture was destroyed and well defined nodules indicative of cirrhosis were observed, Narrow zone of edema and ductular proliferation were seen at the junction of parenchyma and septa. In the DMNA group, the pattern of micronodular cirrhosis was observed and thick intralobular septa was evident.

Pharmacokinetic parameters of theophylline

The pharmacokinetic parameters of theophylline are summarized in Tables II and III after an intravenous and an oral administration, respectively.

Pharmacokinetic parameters after intravenous administration: In cirrhotic group, $t_{1/2}$ was increased and

Table I. Clinical parameters in rat serum

Group	ALP (U/L)	AST (U/L)	ALT (U/L)	Total protein (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Hydroxyproline (μg/0.2 g liver)
BDL(n=14)	432.8±366.9**	560.8±194.0*	114.7±59.86**	8.85±1.73	3.21±0.36**	5.75±3.05***	86.12±21.1**
SHAM(n=12)	154.9± 59.70	136.6± 37.58	61.45±18.52	9.59±1.80	5.26±1.07	0.33±0.17	40.71± 7.20
DMNA(n=9)	333.3±115.6**	143.9± 62.34**	80.90±34.36	8.12±0.92*	4.10±0.61**	0.69±1.26	72.45± 9.17**
CNTL(n=12)	173.9± 70.50	123.6± 46.48	49.30±26.19	9.22±1.28	5.12±0.72	0.21±0.06	44.54±17.3

BDL: cirrhosis induced by bile duct ligation, SHAM: sham operated, CNTL: control, DMNA: cirrhosis induced by dimethylnitrosamine pretreatment

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, when compared BDL with SHAM groups, and DMNA with CNTL groups, respectively.

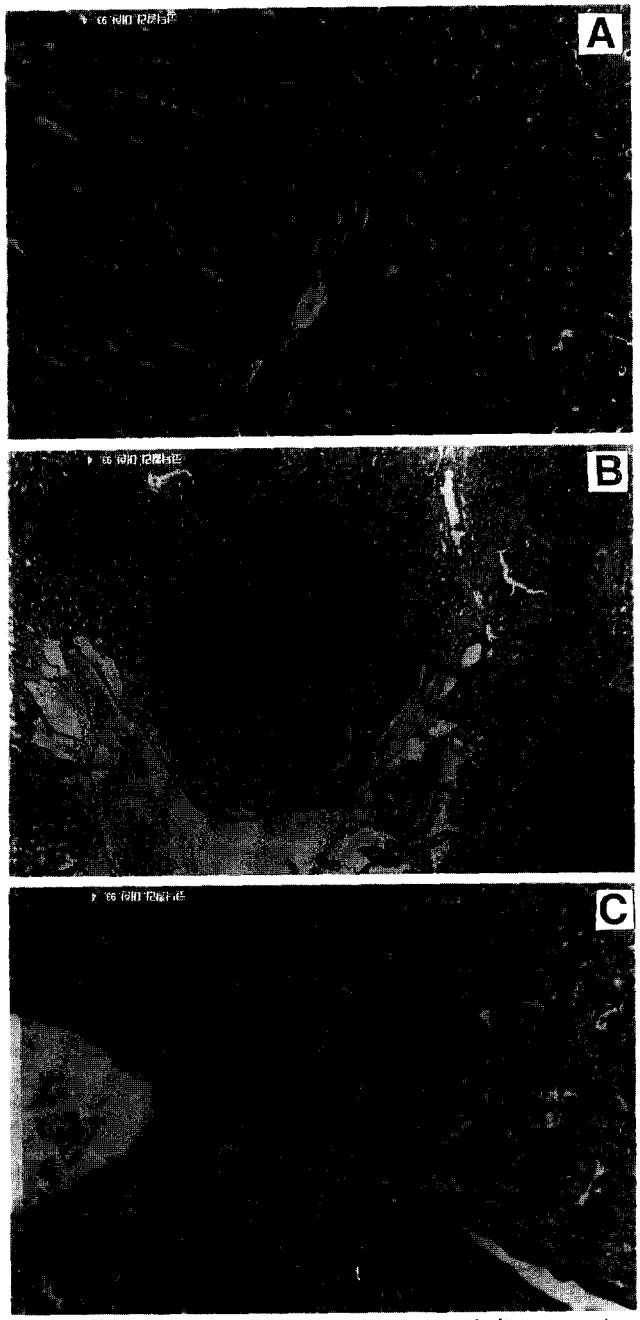

Fig. 1. Light microscopical appearance of liver sections from a female Splague-Dawley rats. (A) control (×200). (B) hepatic cirrhosis induced by bile duct ligaton (×100). (C) hepatic cirrhosis induced by dimethylnitrosamine (×200). H&E.

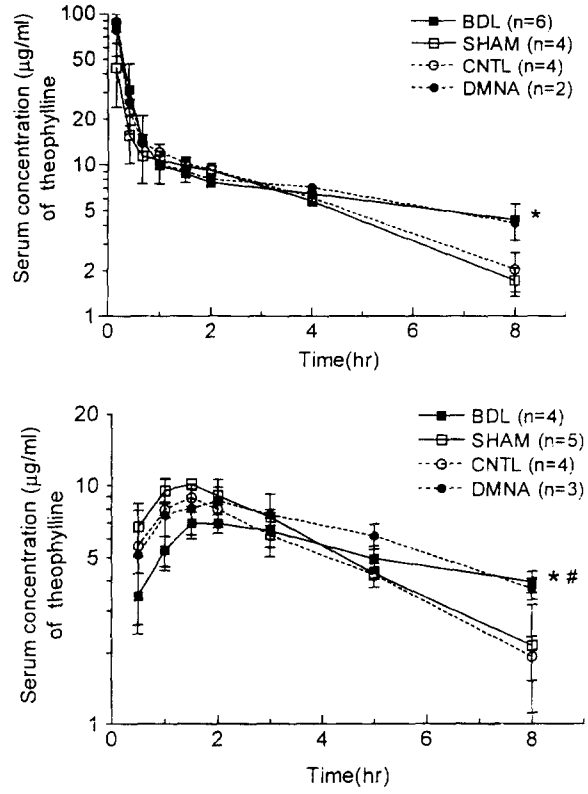

Fig. 2. Semilog plots of serum theophylline concentration-time curves after intravenous administration (upper) and oral administration (lower) in rats. BDL: cirrhosis induced by bile duct ligation, SHAM: sham operated, CNTL: control, DMNA: cirrhosis induced by dimethylnitrosamine pretreatment. Each value represents the mean±S.D. *: $p<0.05$, when compared BDL with SHAM groups, #: $p<0.05$, when compared DMNA with CNTL groups.

Table II. Pharmacokinetic parameters of theophylline after intravenous administration to rats

Groups	(Vd)ss (ml/kg)	Clt (ml/hr/kg)	$t_{1/2}$ (hr)
BDL (n=6)	447.7±212.7	53.9± 9.4**	8.36±3.95*
SHAM (n=4)	305.6± 82.43	92.8± 9.3	3.16±0.18
DMNA (n=2)	429.1	62.8	6.38
CNTL (n=4)	265.5±112.0	90.8±21.2	2.68±0.54

BDL: cirrhosis induced by bile duct ligation, SHAM: sham operated, DMNA: cirrhosis induced by dimethylnitrosamine pretreatment, CNTL: control (Vd)ss: steady-state volume of distribution, Clt: total body clearance, $t_{1/2}$: half life.
*: $p<0.05$, **: $p<0.01$, when compared BDL with SHAM groups, and DMNA with CNTL groups, respectively.

total clearance was significantly decreased when compared with those of each control group.

Pharmacokinetic parameters after oral administration: In cirrhotic groups, elimination rate constant k was significantly decreased, AUC was significantly increased, $t_{1/2}$ were increased.

Protein binding percent: In BDL it was significantly decreased.

DISCUSSION

In the hepatic disease states, the elimination of a drug is highly variable and frequently unpredictable because the hepatic blood flow, enterohepatic cycling, drug protein binding or intrahepatic functional processes may altered. The disposition of poorly ex-

Table III. Pharmacokinetic parameters of theophylline after oral administration in rats

Groups	k	AUC (μg · hr/ml)	Cmax (μg/ml)	Tmax (hr)	$t_{1/2}$ (hr)
BDL (n=4)	0.125±0.037*	73.01±6.30**	6.89±0.71**	2.02±0.43*	5.86±1.38**
SHAM (n=5)	0.283±0.113	53.50±4.43	10.0±0.80	1.31±0.22	2.69±0.76
DMNA (n=3)	0.136±0.051*	83.89±8.80**	8.72±2.21	1.84±0.84	5.71±2.52[a]
CNTL (n=4)	0.282±0.080	46.76±2.71	8.54±0.76	1.53±0.25	2.47±0.58

BDL: cirrhosis induced by bile duct ligation, SHAM: sham operated, DMNA: cirrhosis induced by dimethylnitrosamine pretreatment, CNTL: control k: elimination rate constant, AUC: area under the concentration-time curve, Cmax: maximum concentration of drug, Tmax: time of occurrence for maximum drug concentration, $t_{1/2}$: half life.
*: $p<0.05$, **: $p<0.01$, a: $p=0.051$, when compared BDL with SHAM groups, and DMNA with CNTL groups, respectively.

Table IV. Protein binding percent of theophylline after intravenous administration in rats

Group	BDL (n=4)	SHAM (n=3)	DMNA (n=4)	CNTL (n=4)
Protein binding (%)	8.67±4.85***	77.62±6.08	75.03±10.23	61.10±6.33
Serum theophylline concentration (μg/ml)	8.06±1.17	13.8 ±1.35	13.1 ±2.78	12.1±1.43

BDL: cirrhosis induced by bile duct ligation, SHAM: sham operated, DMNA: cirrhosis induced by dimethylnitrosamine pretreatment, CNTL: control.
***: $p<0.001$, when compared BDL with SHAM groups, and DMNA with CNTL groups, respectively.

tracted drugs is more sensitive to changes in the intrinsic ability of the liver to eliminate a drug and in binding of drug to blood constituents than it is to hepatic blood flow by well stirred model (Williams, 1983). Because numerous physiologic factors can influence both drug binding and biliary excretion, the influence of acute and chronic hepatic disease on the disposition of poorly extracted drugs is predictably more variable than their influence on the disposition of highly extracted drugs (Shand, 1977).

In the previous study (Han *et al.*, 1995), we obtained following results in BDL induced fibrosis model: half life ($t_{1/2}$) of theophylline was increased, total clearance was decreased, apparent volume of distribution (Vdβ) of theophylline showed a insignificant decrease.

Most of the administered dose of theophylline is biotransformed in the liver; less than 10% is eliminated unchanged in urine (Mangione *et al.*, 1978). The decrease in plasma theophylline clearance in cirrhotic patients was probably due to a combination of disordered hepatocyte function and drug-protein binding because of the hepatic extraction of theophylline is about 10% in normal subjects (Shand, 1977).

In this experiment, bilirubin, a potent inhibitor of drug-protein binding, was several-fold increased in BDL (5.75±3.05 mg/dl), but not in DMNA (0.69±1.26 mg/dl). Albumin which binds drugs especially acidic drug was decreased in both cirrhotic group (3.21±0.36 g/dl in BDL, 4.10±0.61 g/dl in DMNA). The total clearance of theophylline in hepatic cirrhosis group (53.9±9.4 ml/hr/kg in BDL, 62.8 ml/hr/kg in DMNA after i.v. administration) was decreased significantly and the half-life ($t_{1/2}$) in the cirrhotic rats (8.36±3.95 h in BDL, 6.38 h in DMNA after i.v. administration) was increased about two-folds after intravenous and oral administration, respectively. The volume of distribution at steady state (Vdss) was increased insignificantly in both cirrhotic group (447.7±212.7 ml/kg in BDL, 429.1 ml/kg in DMNA after i.v. administration). Protein binding percent in BDL (8.67±4.85%) decreased significantly, but in DMNA (73.00±9.85%) was similar to control group.

Regarding to the changes of these endogenous substances especially increased bilirubin level, which causes a decrease in drug-protein binding. Decreased plasma drug binding could increase the volume of distribution (ϕ ie *et al.*, 1979). But, in this experiment, increased bilirubin and decreased albumin contents had no effect on the disposition kinetics of theophylline.

Piafsky *et al.* (1977) have reported that protein binding percent was (36.8±4.1% in 3 patients with liver fibrosis, 52.6±3.8% in normal) decreased and these increased free fraction did not influence theophylline disposition. In clinical studies of Mangione *et al.*, (1978), total bilirubin level (2.49±2.12 mg/dl) was increased significantly and serum albumin level (3.18±0.52 g/dl) was significantly decreased and protein binding percent was decreased (29±16%; range 9~61) in cirrhotic patients, 65±6% in normal). Mangione *et al.* (1978) concluded that a large decrease in the fraction bound, which results in a doubling of the free fraction of theophylline (35 to 71%) have no effect on the volume of distribution of theophylline.

In this experiment, protein binding percent in BDL was 8.67±4.75% (77.62±6.08% in SHAM), thus free fraction of theophylline was about 4 folds increased (23 to 91%). But in DMNA, showed similar results as compared with control. Volume of distribution in cirrhotic group was increased insignificantly. From these

results, it might be suggested that increased free fraction of theophylline does not influence the volume of distribution in experimental hepatic cirrhosis model. In view of the result that total clearance of theophylline was significantly decreased without any significant change in Vd in hepatic cirrhosis, we might conclude that decreased elimination of theophylline might be due to a hepatic cellular dysfunction.

In experimental model, it is necessary to investigate whether the administration route affect the disposition kinetics of drug. Bioavailability of theophylline in human is about 100% (Hendeles *et al.*, 1977; Shargel *et al.*, 1993), that is, administration route does not affect its disposition in human. In this experiment, absolute bioavailability of theophylline is between 63.8 and 72.8%(66.1% in BDL, 63.8% in SHAM and CNTL, 72.8% in DMNA group). In the report of Gomita *et al.* (1991), AUC values of theophylline after p.o, i.p., i.v. administration in rats showed no marked differences. Therefore incomplete absorption of theophylline in this experiment might be due to its orally administered preparation. Thus in these experimental hepatic cirrhosis model, administration route did not change the kinetic disposition of drug such as theophylline.

Further experiment is needed to elucidate the role of hepatic cellular function to the kinetic disposition of theophylline in hepatic cirrhosis.

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