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Hepatobiliary Excretion of Tributylmethylamonium in Rats with Lipopolysaccharide-Induced Acute Inflammation

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The alteration in the pharmacokinetic behaviors of organic cations (OCs) in rats during acute inflammation (AI) was investigated. AI was induced by an intraperitoneal injection of lipopolysaccharide (LPS, 5 mg/kg) 24 hr prior to the start of pharmacokinetic studies. Tributylmethylammonium (TBuMA) was selected as a model OC since it is largely excreted into bile, and is neither metabolized nor binds to proteins in the body. When TBuMA was administered intravenously to AI rats at a dose of 6.6 μmole/kg, the AUC was increased, while biliary excretion (i.e., cumulative amount and apparent clearance) was decreased compared to normal rats. When TBuMA was administered intravenously to AI rats at a constant rate (i.e., a bolus injection at a dose of 1.5 μmole/kg followed by a constant infusion at a rate of 1.5 μmole/kg/hr for 165 min), steady-state concentrations of plasma and liver concentrations of TBuMA were increased significantly, while in vivo hepatic uptake (amount) and canalicular excretion (clearance) were decreased. These results are consistent with a hypothesis in which both the sinusoidal uptake of TBuMA into hepatocytes via the OCT1 and the canalicular excretion of the compound from hepatocytes via the P-gp are decreased by LPS-induced AI.

Key words: TBuMA, LPS, Acute inflammation, Hepatic uptake clearance, Canalicular excretion clearance

INTRODUCTION

It has been reported that pathophysiological changes during acute inflammation (AI) are associated with dramatic increases in the plasma concentrations of drugs (Belpaire *et al.*, 1989; Piquette-Miller and Jamali, 1993). AI induced by lipopolysaccharide (LPS) leads to the local and systemic release of inflammatory mediators (i.e., cytokines), that are thought to cause changes in the expression and activity of several liver derived proteins (Vos *et al.*, 1997; Aono *et al.*, 1997). Recently, the suppression of cytochrome P450 metabolic activity (Morgan, 1989; Saitoh *et al.*, 1999) and changes in the expression of membrane-bound transport proteins such as P-glycoprotein (Piquette-Miller *et al.*, 1998), bile acid transporter (Bolder *et al.*, 2002), and glucose transporter (Cidad *et al.*, 2001) have been reported. Therefore, AI would be expected to influence the hepatobiliary transport of xenobiotics that are substrates of these transporters. Our laboratory has demonstrated that the hepatobiliary transport of drugs is multiply altered as a consequence of CCl_4-induced acute hepatic failure (Hong *et al.*, 2000). In this model, the sinusoidal uptake of organic cations (OCs) via the organic cation transporter (OCT) is decreased, while the canalicular transport of OCs via P-glycoprotein (P-gp) is increased (Hong *et al.*, 2000; Nakasukasa *et al.*, 1993).

In the present study, we report on the effect of LPS-induced AI on the hepatobiliary excretion of organic cations (OCs). Tributylmethylammonium (TBuMA) was selected as a model OC, because it is neither metabolized nor protein-bound in the body and is largely excreted into the bile via a sinusoidal transporter (i.e., OCT) and a canalicular transporter (i.e., P-gp) in the liver (Hughes *et al.*, 1973; Han *et al.*, 1999; Song *et al.*, 1999). Therefore, the pharmacokinetics of TBuMA including its hepatobiliary excretion in LPS-induced AI rats would provide information on the effect of the AI on the functional activity of

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these transporters.

MATERIALS AND METHODS

Materials

[^{3}H]TBuMA (0.5 Ci/mmole) was synthesized as previously described (Song *et al.*, 1999). LPS (from *Escherichia coli*) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents employed here were of the highest grade commercially available.

Induction of acute inflammation by LPS

Male Sprague-Dawley rats (250-270g, Dae-Han Biolink, Taejon, Korea) were injected intraperitoneally with a single dose of LPS (5 mg/kg) 24 hr prior to pharmacokinetic studies, and were allowed access to food and water *ad libitum*. Experimental protocols were reviewed by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University according to the NIH guidelines (NIH publication number 86-23, revised 1985, Guide for the Care and Use of Laboratory Animals).

Systemic *in vivo* pharmacokinetic study

Normal and LPS-induced AI rats were anesthetized with ketamine (50 mg/kg, ip, Ketalar®, Yuhan Co., Kyounggi-do, Korea) and acepromazine (1.5 mg/kg, ip, Sedaject®, Samu Chemical Co., Kyounggi-do, Korea), and the femoral artery and vein of the rats were cannulated with a polyethylene tube (PE-50) for blood sampling and TBuMA administration, respectively. The common bile duct was cannulated with PE-10 in order to collect bile. After recovery from the surgery, the rats received [^{3}H]TBuMA in the form of a bolus dose of 6.6 μmole/kg (13.2 μCi) via the femoral vein, and blood samples (300 μl) and bile were collected at appropriate intervals over a 420 min period. Fluid loss was compensated by an injection of saline via the iv catheter, and body temperature was maintained using a heat lamp. The concentrations of [^{3}H]TBuMA in the plasma and bile were quantified using a Wallac 1409 liquid scintillation counter (Perkin Elmer Life Science Inc., Boston, MA). The area under the plasma concentration-time curve (AUC) from zero to 420 min was calculated by a trapezoidal rule. The apparent biliary clearance (CL_b) was obtained by dividing the amount of TBuMA excreted into the bile by the AUC up to 420 min.

Estimation of *in vivo* excretion clearance

Under identical anesthesia, the femoral artery and vein were cannulated with PE-50, and bile duct with PE-10, as described above. [^{3}H]TBuMA was infused to normal and LPS-induced AI rats at a rate of 1.5 μmole (1.32 μCi)/hr/kg after an iv bolus injection of 1.5 μmole (1.32 μCi)/kg in order to obtain a value for the steady state concentration of TBuMA in the liver. Blood samples (300 μl) and bile were collected at appropriate intervals over a 165 min period. Fluid loss was compensated by an injection of saline. Body temperature was maintained with a heat lamp. After the last sampling (i.e., at 165 min), the rats were sacrificed and the liver was dissected immediately. A 20% liver homogenate was prepared using normal saline, and the homogenate was centrifuged at 3000 rpm for 10 min, after which, an aliquot of the supernatant was collected for a determination of the liver concentration (C_{liver}) of TBuMA at steady state conditions. The radioactivity of TBuMA in plasma, liver and bile was determined by liquid scintillation counting, and in vivo uptake clearance (CL_{uptake}) and excretion clearance ($CL_{excretion}$) of TBuMA were calculated using following equations.

$$CL_{uptake} = \frac{CL_{excretion} \times C_{liver}}{C_{plasma}} \quad \text{(equation 1)}$$

$$CL_{excretion} = \frac{\text{Biliary excretion rate}}{C_{liver}} \quad \text{(equation 2)}$$

Data analysis

All data are expressed as means ± S.D. The Students unpaired t-test was used to test the difference between treatments. In all cases, $p<0.05$ was accepted as representing a statistical difference.

RESULTS AND DISCUSSION

Temporal profiles of the plasma concentrations of TBuMA following intravenous administration of the compound to rats at a dose of 6.6 μmole/kg are shown in Fig. 1A. Plasma concentration was increased significantly by LPS-induced AI. As a consequence, the value of AUC up to 420 min was increased by 1.8-fold in AI rats compared to control rats (i.e., from 262.4 ± 50.9 to 467.5 ± 46.8 μM·min). Cumulative biliary excretion (amount, Fig. 1B) and the biliary clearance (CL_b) of TBuMA were decreased significantly by LPS-induced AI (i.e., 26.47 ± 1.12 vs 20.47 ± 2.30% for the amount, and 6.188 ± 1.35 vs 2.340 ± 0.865 ml/min/kg for the clearance) (Table I).

TBuMA is a quaternary ammonium OC which does not undergo additional metabolism and protein binding in the body, and is eliminated from the body via hepatobiliary and/or renal excretion. Thus, an increase in the AUC of TBuMA (Table I) reflects a decrease in the excretion of the compound. In order to investigate the issues of whether or not and what unit process(es) of hepatobiliary excretion of TBuMA are decreased by LPS-induced AI, TBuMA was infused intravenously at a constant rate, and the sinusoidal uptake clearance and canalicular excretion clearance (i.e., CL_{uptake} and $CL_{excretion}$, respectively) for the compound

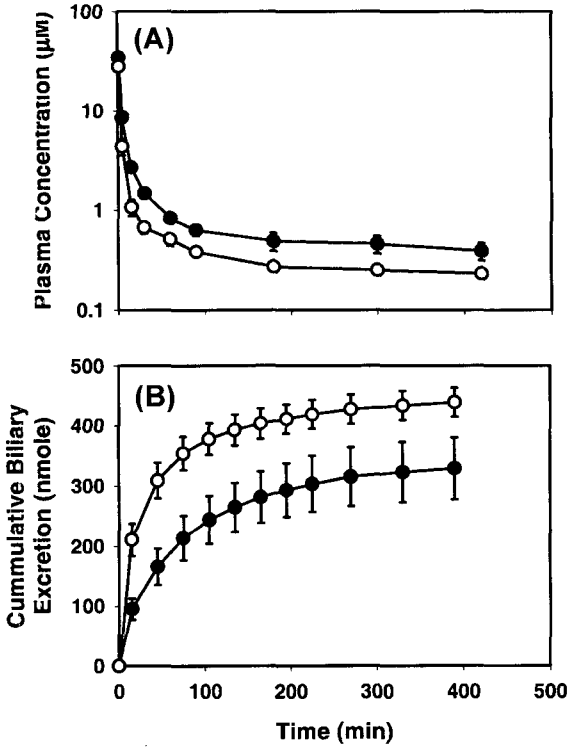

Fig. 1. Plasma concentration (A) and cumulative biliary excretion (B) of TBuMA in control (○) and LPS-induced acute inflammation (AI) rats (●). TBuMA was administered at a dose of 6.6 μmole/kg. Each data point represents the mean ± S.D. of five animals.

Table I. Effects of LPS-induced acute inflammation (AI) on the pharmacokinetic characteristics of TBuMA after i.v. administration (6.6 mmole/kg)[a]

Pharmacokinetic characteristics[b]	Control	AI
AUC (μM·min)	262.4 ± 50.9	467.5 ± 46.8**
Cumulative biliary excretion (% of dose)	26.47 ± 1.12	20.47 ± 2.30*
CL_b (ml/min/kg)	6.188 ± 1.35	2.340 ± 0.865 **

[a]Each data represents the mean ± S.D. of five separate experiments.
[b]Each characteristics was calculated based on data up to 420 min.
*p<0.05, **p<0.01.

were estimated according to equations 1-2. The plasma concentration (C_{plasma}) and biliary excretion rate of TBuMA reached a steady state in 60 min after the start of infusion in normal and AI rats (Fig. 2A, B). Thus, the steady-state values were expressed as the average of the values for the last four data points in Fig. 2. In AI rats, 1.7- and 2.3-fold increases in the steady-state were observed for the plasma (i.e., C_{plasma}, from 3.506 ± 0.472 to 5.876 ± 1.69 μM) and liver (i.e., C_{liver}, from 24.15 ± 4.15 to 55.06 ± 6.50 μM) concentrations of TBuMA compared to control rats (Table I). As a consequence, a 1.4-fold increase in the ratio of the concentrations between the liver and plasma (i.e., C_{liver}/C_{plasma}) was observed in the AI rats (i.e., 6.948 ±

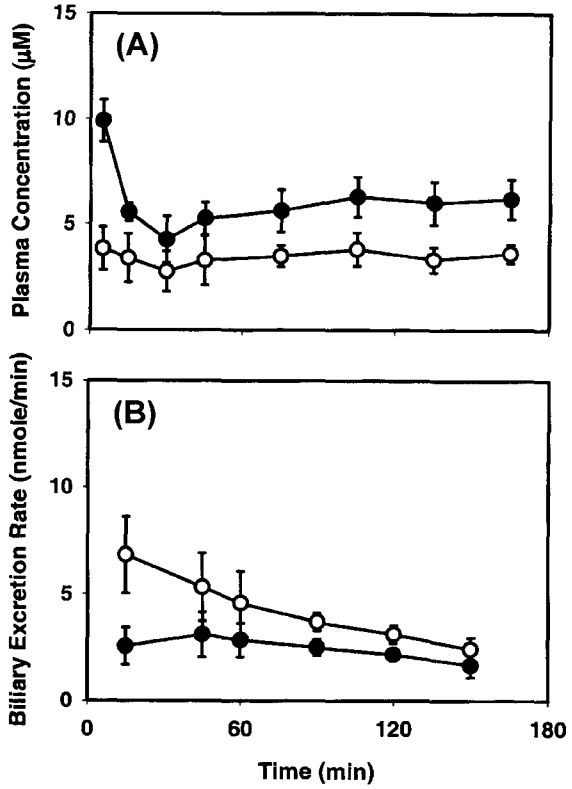

Fig. 2. Plasma concentration (A) and biliary excretion rate (B) of TBuMA in control (○) and LPS-induced acute inflammation (AI) rats (●). TBuMA was administered at a loading dose of 1.5 μmole/kg followed by an intravenous infusion of 1.5 μmole/hr/kg. Each data point represents the mean ± S.D. of six animals.

Table II. Effects of LPS-induced acute inflammation (AI) on the pharmacokinetic characteristics of TBuMA following an intravenous infusion of the compound (i.e., an iv bolus dose of 1.5 μmole/kg followed by an iv infusion of 1.5 μmole/hr/kg)[a]

Kinetic parameters	Control	AI
C_{plasma}(μM)	3.506 ± 0.472	5.876 ± 1.69*
C_{liver}(μM)	24.15 ± 4.15	55.06 ± 6.50***
C_{liver}/C_{plasma}	6.948 ± 1.27	9.867 ± 1.61*
Biliary excretion rate (nmole/min)	3.459 ± 0.143	2.295 ± 0.342**
CL_{uptake} (ml/min/kg)	3.597 ± 0.679	1.481 ± 0.409***
$CL_{excretion}$ (ml/min/kg)	0.5324 ± 0.143	0.1497 ± 0.007***

[a]Each data represents the mean±S.D. of five separate experiments.
*p<0.05, **p<0.01, ***p<0.001.

1.27 vs 9.867 ± 1.61) (Table II). This suggests that either hepatic uptake or canalicular excretion of TBuMA is altered in AI rats. Consistent with this suggestion, the biliary excretion rate, in vivo uptake clearance (CL_{uptake}, equation 1) and excretion clearance ($CL_{excretion}$, equation 2) of TBuMA at steady-state conditions were decreased by 1.5- (i.e., 3.459 ± 0.143 vs 2.295 ± 0.342 nmole/min), 2.4-(i.e.,

3.597 ± 0.679 vs 1.481 ± 0.409 ml/min/kg) and 3.6-fold (i.e., 0.5324 ± 0.143 vs 0.1497 ± 0.007 ml/min/kg), respectively, in the AI rats (Table II). The CL_{uptake} represents the balanced sum of hepatic uptake and hepatic efflux. Since the hepatic efflux of TBuMA is negligible compared to hepatic uptake (i.e., less than 1%, Han *et al.*, 1999), we conclude that the decrease in CL_{uptake} in the present study represents the decrease in hepatic uptake. TBuMA is transported into hepatocytes across the sinusoidal membrane via OCT1, which is expressed on the sinusoidal membrane, and is then excreted into the bile across the canalicular membrane via P-gp, which is expressed on the canalicular membrane (Han *et al.*, 1999; Song *et al.*, 1999). Therefore, the decrease in CL_{uptake} and $CL_{excretion}$ of TBuMA by LPS-induced AI suggests that the transport of TBuMA via OCT1 and P-gp is decreased in the case of AI. Either a decrease in the affinity of TBuMA to the responsible transporters or a decrease in the expression of the transporters might constitute the relevant mechanism(s). The involvement of a decreased expression of transporters is consistent with the down-regulated expression of P-gp in endotoxemic rats and mice (Piquette-Miller *et al.*, 1998; Georgy *et al.*, 2001).

In conclusion, LPS-induced AI led to a decrease in the sinusoidal uptake of TBuMA into hepatocytes and the canalicular excretion of the compound from hepatocytes. A decreased function of the responsible transporters on the sinusoidal (i.e., OCT1) and canalicular membranes (i.e., P-gp) appears to represent a relevant mechanism.

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