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Short Communication

TIME-DEPENDENT EFFECTS OF *KLEBSIELLA PNEUMONIA*E ENDOTOXIN (KPLPS) ON THE PHARMACOKINETICS OF THEOPHYLLINE IN RATS; RETURN OF THE PARAMETERS IN 96-HOUR KPLPS RATS TO THE CONTROL LEVELS

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Running title page

a) **TIME-DEPENDENT KPLPS EFFECTS ON THEOPHYLLINE PHARMACOKINETICS**

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c) 20 pages

2 Tables

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24 References

186 words in “Abstract” section

308 words in “Introduction” section

1260 words in “Results and Discussion” section

d) ABBREVIATIONS: KPLPS, lipopolysaccharide induced by *Klebsiella pneumoniae*; HPLC, high performance liquid chromatography; AUC, total area under the plasma concentration–time curve from time zero to time infinity; MTR, mean residence time; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged non-renal clearance; V_{ss}, apparent volume of distribution at a steady state; $Ae_{0-24\,h}$, percentage of the dose excreted in the 24-h urine; GI_{24h}, percentage of the dose recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h; V_{max}, maximum velocity; K_m, apparent Michaelis–Menten constant; CL_{int}, intrinsic clearance; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max} ; F, extent of absolute oral bioavailability.

Abstract:

It has been reported that theophylline is primarily metabolized via hepatic CYP1A1/2, 2B1/2, and 3A1/2, and 1,3-DMU is primarily formed via CYP1A1/2 in rats. Compared with control rats, the expression of CYP1A subfamily, 2B1/2, and 3A subfamily significantly decreased 24 h (24-h KPLPS rats) after intravenous administration of LPS derived from *Klebsiella pneumoniae* (KPLPS) to rats, but returned to that in control rats after 96 h (96-h KPLPS rats). After intravenous or oral administration of theophylline to 24-h KPLPS rats, the AUC values of theophylline and 1,3-DMU became significantly greater (46.5 and 34.0% increase after intravenous and oral administration, respectively) and smaller (36.3 and 21.6% decrease, respectively), respectively. Because theophylline is a low hepatic extraction ratio drug in rats, the above results could have been due to significantly slower CL_{int} for the disappearance of theophylline and for the formation of 1,3-DMU (37.1 and 60.6% decrease, respectively). However, in 96-h KPLPS rats, the pharmacokinetic parameters of theophylline and 1,3-DMU returned fully or partially to those in control rats. These findings indicate the existence of time-dependent effects of KPLPS on the pharmacokinetics of theophylline and 1,3-DMU in rats.

Theophylline, a bronchodilator, has widely been used for the management of acute bronchospasm associated with asthma or chronic obstructive pulmonary disease (COPD). It is metabolized to 1-methylxanthine (1-MX), 3-methylxanthine (3-MX), and 1,3-dimethyluric acid (1,3-DMU), and 1-MX is further metabolized to 1-methyluric acid (1-MU) via xanthine oxidase in rats (McManus et al., 1988). Recently, Yang et al. (2008) reported that theophylline is primarily metabolized via hepatic microsomal cytochrome P450 (CYP) 1A1/2, 2B1/2, and 3A1/2 (but not via CYP2C11, 2D6, and 2E1), and 1,3-DMU was primarily formed via CYP1A1/2 in male Sprague–Dawley rats.

Lipopolysaccharide (LPS) is an active component in the outer membrane of Gram-negative bacteria. There have been several studies on changes in the expression of hepatic CYP isozymes in rats pretreated with LPS derived from *Klebsiella pneumoniae*, KPLPS (Nadai et al., 1998; Ueyama et al., 2005). However, to our knowledge, no studies on changes in the expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily in rats pretreated with KPLPS after 24 h (24-h KPLPS) and 96 h (96-h KPLPS) based on Western blot analysis have yet been reported. It has been reported that inflammation is triggered in rats by a small dose of LPS (Deng et al., 2006), bacterial infection is an important factor in bronchial asthma (Oehling, 1999), and Gram-negative bacterial infections are common in the patients with COPD combined with pneumonia (Yi et al., 2003). Thus, we examined theophylline in this study.

The aim of this study was to investigate changes in pharmacokinetics of theophylline and 1,3-DMU after intravenous or oral administration of theophylline at a dose of 5 mg/kg, 24 h and 96 h after intravenous administration of KPLPS at a dose of 0.5 mg/kg to rats. Changes in the expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily in 24-h and 96-h KPLPS rats, based on Western blot analysis, were also

investigated.

Materials and Methods

Materials. Aminophylline® intravenous solution (10 ml ampoule; 25 mg/ml as aminophylline) was a product from Daewon Pharmaceutical Company (Seoul, South Korea). 1,3-DMU, β-hydroxyethyltheophylline [internal standard for the high-performance liquid chromatographic (HPLC) analysis of theophylline and 1,3-DMU], KPLPS (lipopolysaccharide derived from *Klebsiella pneumoniae*; purified by phenol extraction; protein < 3%), β–actin, primary monoclonal antibody for β-actin, ethylenediamine tetraacetic acid (EDTA; as a disodium salt), and Kodak X-OMAT film were purchased from Sigma–Aldrich Corporation (St. Louis, MO). Microsomes from baculovirus-infected insect cells expressing CYP1A1, 1A2, 2B1, 3A1, and 3A2 (Supersome™) were obtained from Gentest Corp. (Woburn, MA). Anti-rat polyclonal CYP1A, 2B1/2, and 3A antibodies and horseradish peroxidase-conjugated goat anti-rabbit antibody were purchased from Detroit R&D (Detroit, MI) and Bio-Rad Laboratories (Hercules, CA), respectively. Enhanced chemiluminescence reagents were products from Amersham Biosciences Corporation (Piscataway, NJ). Other chemicals were of reagent or HPLC grade.

Animals. The protocols for the animal studies were approved by the Animal Care and Use Committee of the College of Pharmacy of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (6–8 weeks old and weighing 265–325 g) were purchased from the Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). The procedures used for maintenance of the rats were similar to a reported method (Kim et

al., 1993).

Administration of KPLPS to Rats. Rats were randomly divided into three groups, 24-h KPLPS, 96-h KPLPS, and control rats. KPLPS (dissolved in 0.9% NaCl-injectable solution) at a dose of 0.5 mg (1 ml)/kg was administered via the tail vein to rats 24 h and 96 h before administration of theophylline. An equal volume of 0.9% NaCl-injectable solution was injected into control rats.

Western blot Analysis. The procedures used for the preparation of hepatic microsomal fractions of 24-h KPLPS, 96-h KPLPS, and control rats (n = 5, each) were similar to a reported method (Kim et al., 2003). The procedures used for Western blot analysis for 24-h KPLPS, 96-h KPLPS, and control rats (n = 3, each) were similar to a reported method (Kim et al., 2001).

Measurement of V_{max}, K_m, and CL_{int} for the Disappearance of Theophylline and for the Formation of 1,3-DMU in Hepatic Microsomal fractions. The procedures used for the measurement of V_{max} (the maximum velocity) and K_m (the apparent Michaelis−Menten constant, the concentration at which the rate is one-half of V_{max}) for the disappearance of theophylline and for the formation of 1,3-DMU were similar to a reported method (Kim et al., 2003). The concentrations of theophylline were 0.1, 0.2, 0.5, 1, 2, 5, 7.5, or 10 mM. The reaction was terminated by addition of 0.5 ml of acetonitrile containing 20 μg/ml of β-hydroxyethyltheophylline (internal standard) after 30 min incubation. The kinetic constants, V_{max} and K_m for the disappearance of theophylline and for the formation of 1,3-DMU, were calculated using a non-linear regression method (Duggleby, 1995). The intrinsic clearance (CL_{int}) for the disappearance of theophylline and for the formation of 1,3-DMU was calculated by dividing the V_{max} by the K_m.

Disappearance of Theophylline and Formation of 1,3-DMU after Incubation of Theophylline with CYP1A1, 1A2, 2B1, 3A1, and 3A2. The procedures used were similar to those described for the above V_{max} and K_m studies. Theophylline (5 mM) was incubated with microsomes from Baculovirus-infected insect cells expressing CYP1A1, 1A2, 2B1, 3A1, and 3A2 (final concentration of 60 pmol/ml, each). Acetonitrile, 300 μl, was added after 60 min incubation to terminate enzyme activity.

Intravenous or Oral Administration of Theophylline to Rats. The procedures used including the pretreatment and cannulation of the jugular vein (for drug administration in the intravenous study) and the carotid artery (for blood sampling) are similar to a reported method (Kim et al,. 1993). Theophylline (aminophylline injectable solution was diluted in 0.9% NaCl-injectable solution) at a dose of 5 mg/kg was administered intravenously over 1 min (total injection volume of approximately 0.6 ml) via the jugular vein to rats in each group (n = 7, 8, and 9 for 24-h KPLPS, 96-h KPLPS, and control rats, respectively). A blood sample (approximately 0.12 ml) was collected via the carotid artery at 0 (control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after the start of the intravenous infusion of theophylline. Blood samples were immediately centrifuged (16,000g, 5 min), and a 50-μl aliquot of each plasma sample was stored at –70^{o}C until used for the HPLC analysis of theophylline and 1,3-DMU (Yang et al., 2008). The procedures used for the preparation and handling of 24-h urine samples and the entire gastrointestinal tract contents samples (including its contents and feces) at 24 h were similar to a reported method (Kim et al., 1993).

The same dose of theophylline (the same solution used in the intravenous study) was administered orally (total oral volume of approximately 0.6 ml) using a feeding tube to

rats in each group (n = 7, 7, and 8 for 24-h KPLPS, 96-h KPLPS, and control rats, respectively). Blood samples were collected via the carotid artery at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 600 min after oral administration of theophylline. Other procedures were similar to those for the intravenous study.

HPLC Analysis of Theophylline and 1,3-DMU. Concentrations of theophylline and 1,3-DMU in the samples were determined by a slight modification (Yang et al., 2008) of a reported HPLC method (Kwaskatsu et al., 1989). The retention times of theophylline, 1,3-DMU, and internal standard in rat plasma samples were approximately 11.1, 6.3, and 13.2 min, respectively, and the corresponding values in rat urine samples were approximately 18.7, 9.9, and 23.5 min, respectively. The quantitation limits of theophylline in rat plasma and urine samples were 0.1 and 1 μg/ml, respectively, and the corresponding values of 1,3-DMU were 0.05 and 1 μg/ml, respectively.

Pharmacokinetic Analysis. The total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated using the trapezoidal rule–extrapolation method (Chiou, 1978). The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods (Gibaldi and Perrier, 1992) were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin®; Pharsight Corporation, Mountain View, CA); the time-averaged total body, renal, and non-renal clearances (CL, CL_R, and CL_{NR}, respectively), the terminal half-life, the first moment of AUC (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady state (V_{ss}), and the extent of absolute oral bioavailability (F) (Kim et al., 1993). The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were

directly read from the experimental data.

Statistical Analysis. A p-value < 0.05 was deemed to be statistically significant using a Duncan's multiple range test of Statistical Package for the Social Sciences (SPSS) *posteriori* analysis of variance (ANOVA) among the three means for the unpaired data. All data are expressed as mean ± S.D., except median (ranges) for T_{max}.

Results and Discussion

Treatment of KPLPS (both in 24-h and 96-h KPLPS rats) caused significant decrease in body weight gain compared with that in control rats (Tables 1 and 2). However, the liver and kidney function in 24-h KPLPS and 96-h KPLPS rats were not seriously impaired based on the plasma chemistry data and tissue histology (data not shown).

Many investigators reported dose-dependent metabolic disposition of theophylline in humans (Lesko, 1979; Massey et al., 1984) and in rats (Teunissen et al., 1985). Thus, theophylline at a dose of 5 mg/kg, which has been reported to be in the ranges of dose-independent metabolic disposition of theophylline in rats (for up to 10 mg/kg) (Teunissen et al., 1985), was administered intravenously or orally to rats.

The contribution of the gastrointestinal (including biliary) excretion of unchanged theophylline to the CL_{NR} of theophylline was almost negligible; theophylline was below the detection limit in the gastrointestinal tract at 24 h ($GI_{24\ h}$) for all groups of rats (Table 1). However, below the detection limit of $GI_{24\ h}$ was not likely due to chemical and enzymatic degradation of theophylline in rats' gastric juices, because theophylline was stable for up to 24 h incubation in various buffer solutions having pHs ranging from 1 to 13 and for up to 4 h incubation in four rats' gastric juices (pHs of 1.5, 1.5, 2.0, and

2.0, respectively); more than 91.8 and 94.5% of the spiked amounts of theophylline were recovered from various buffer solutions and rats' gastric juices, respectively, using a reported method (Yu et al., 2003). Kim et al. (2003) reported that the contribution of the biliary excretion of unchanged theophylline to the CL_{NR} of theophylline is almost negligible. Thus, the CL_{NR} of theophylline listed in Table 1 could represent the metabolic clearance of theophylline. Additionally, changes in the CL_{NR} of theophylline could represent changes in the metabolism of theophylline in rats.

Yang et al. (2008) reported that theophylline is primarily metabolized via hepatic CYP1A1/2, 2B1/2, and 3A1/2, and 1,3-DMU is primarily formed via CYP1A1/2 in rats. To confirm the CYP isozymes responsible for the disappearance of theophylline and for the formation of 1,3-DMU, microsomes expressing CYP1A1, 1A2, 2B1, 3A1, and 3A2 were incubated for 60 min with theophylline. The theophylline disappearance (primarily metabolism) rates were 125 ± 47.4, 224 ± 71.0, 153 ± 48.5, 229 ± 111, and 171 ± 52.1 nM/pmol CYP/min for CYP1A1, 1A2, 2B1, 3A1, and 3A2, respectively, and the corresponding values for the formation of 1,3-DMU were 11.1 ± 4.60, 25.4 ± 0.663, 0.0470 ± 0.0337, 2.98 ± 0.408, and 2.22 ± 0.419 nM/pmol CYP/min, respectively. The above data suggest that CYP1A1, 1A2, 2B1, 3A1, and 3A2 are responsible for the metabolism of theophylline and CYP1A1 and CYP1A2 are primarily responsible for the formation of 1,3-DMU in rats.

Compared with control rats, the expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily significantly decreased (77.3, 47.2, and 59.1% decrease, respectively) in 24-h KPLPS rats (Fig. 1). Thus, it could be expected that compared with control rats, the CL_{NR} of theophylline would be slower in 24-h KPLPS rats, because theophylline is a low hepatic extraction ratio drug in rats. The hepatic first-pass effect of theophylline in

this study was estimated using a reported equation (Lee and Chiou, 1983) based on the CL_{NR} of theophylline (Table 1) and the hepatic blood flow rate of 55.2 ml/min/kg (Davies and Morris, 1993) and the hematocrit of approximately 45% (Mitruka and Rawnsley, 1981) in rats; the value thus estimated was 4.41% in control rats. Thus, the hepatic clearance of theophylline depends more on the CL_{int} for the disappearance of theophylline rather than on the hepatic blood flow rate (Wilkinson and Shand, 1995). As expected, after intravenous administration of theophylline to 24-h KPLPS rats, the CL_{NR} of theophylline was significantly slower (41.9% decrease) than that in control rats (Table 1). The significantly slower CL_{NR} of theophylline in 24-h KPLPS rats could be supported by significantly slower (37.1% decrease) CL_{int} for the disappearance of theophylline (3.42×10^{-3} and 2.15×10^{-3} ml/min/mg protein for control and 24-h KPLPS rats, respectively) than that in control rats. The significantly slower CL_{int} could have been due to a decreased expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily in 24-h KPLPS rats (Fig 1). In 24-h KPLPS rats, the significantly smaller AUC of 1,3-DMU could also be supported by the significantly slower (60.6% decrease) CL_{int} for the formation of 1,3-DMU (0.0625×10^{-3} and 0.0246×10^{-3} ml/min/mg protein for control and 24-h KPLPS rats, respectively) than that in control rats. This could have been due to decreased expression of CYP1A compared to that in control rats (Fig. 1). Microsomal protein contents were 143 ± 16.0, 133 ± 20.3, and 129 ± 16.7 mg/whole liver for 24-h KPLPS, 96-h KPLPS, and control rats, respectively; they were not significantly different among three groups of rats.

After oral administration of theophylline to 24-h KPLPS rats, the AUC values of theophylline and 1,3-DMU were also significantly greater (34.0% increase) and smaller (21.6% decrease), respectively, than that in control rats (Table 2). However, this was not

likely due to increased gastrointestinal absorption of theophylline compared with that in control rats, because, the $GI_{24\ h}$ values were below the detection limit after oral administration of theophylline to all groups of rats (Table 2). Thus, theophylline is almost completely absorbed from all groups of rats. Haruta et al. (1998) also reported that theophylline is a highly absorbable drug without the first-pass elimination in rats. Changes in the AUC values of theophylline and 1,3-DMU after oral administration of theophylline to 24-h KPLPS rats could have also been due to the same reasons as explained in the intravenous study, since the first-pass effect of theophylline is almost negligible in rats (Haruta et al., 1998).

In 96-h KPLPS rats, some pharmacokinetic parameters of theophylline and 1,3-DMU are expected to return fully or partially to that in control rats, because the expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily (Fig 1) and the CL_{int} for the disappearance of theophylline (3.42×10^{-3} and 3.75×10^{-3} ml/min/mg protein for control and 96-h KPLPS rats, respectively) and for the formation of 1,3-DMU (0.0625×10^{-3} and 0.0539×10^{-3} ml/min/mg protein for control and 96-h KPLPS rats, respectively) returned to those in control rats. As expected, after intravenous or oral administration of theophylline to 96-h KPLPS rats, the AUC, terminal half-life, MRT, CL, CL_R, and CL_{NR} of theophylline, and AUC of 1,3-DMU returned fully or partially to those in control rats. Wang et al. (1993) reported that the pharmacokinetic parameters of 1-MX, 1,3-DMU, and theophylline are not changed after intravenous administration of theophylline at a dose of 10 mg/kg to male Wistar rats 2 h after 20–30 min intravenous infusion of KPLPS at a dose of 0.25 mg/kg. This could have been because the expression of CYP1A subfamily, 2B1/2, and 3A subfamily is not altered 2 h after KPLPS administration to rats.

In conclusion, significant changes in the pharmacokinetic parameters of theophylline and 1,3-DMU were observed in 24-h KPLPS rats, and this could have been due to decreased expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily compared with that in control rats. However, no such changes were observed in 96-h KPLPS rats, and this could have been due to return of the expression of hepatic CYP1A subfamily, 2B1/2, and 3A subfamily to that in control rats. These findings indicate the existence of time-dependent effects of KPLPS on the pharmacokinetics and metabolism of theophylline in rats.

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Footnotes

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Legends for Figures

Fig. 1. Hepatic CYP1A subfamily, 2B1/2, and 3A subfamily expression in control, 24-h KPLPS, and 96-h KPLPS rats was quantitated by immunoblotting and densitometry. (A) Immunoblot of gel loaded with 10 μg of microsomal proteins per lane. β-actin was used as a loading control. CYP1A subfamily, 2B1/2, and 3A subfamily were detected by enhanced chemluminescence on Kodak X-OMAT film. (B) Expression of CYP1A subfamily, 2B1/2, and 3A subfamily was quantitated by densitometry. Results are shown relative to CYP1A subfamily, 2B1/2, and 3A subfamily expressions in control rats (control = 100%). Bars represent S.D. *, $P < 0.05$ compared with control and 96-h KPLPS rats; control and 96-h KPLPS rats are not significantly different ($P < 0.05$).

Fig. 2. Mean arterial plasma concentration–time profiles of theophylline and 1,3-DMU after 1 min intravenous infusion [(A) and (B) for theophylline and 1,3-DMU, respectively] or oral administration [(C) and (D) for theophylline and 1,3-DMU, respectively] of theophylline at a dose of 5 mg/kg to control (□; $n = 9$ and 8 for i.v. and oral administration, respectively), 24-h KPLPS (●; $n = 7$ for both i.v. and oral administration), and 96-h KPLPS (○; $n = 8$ and 7 for i.v. and oral administration, respectively) rats. Data are expressed as mean ± S.D.

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TABLE 1

Pharmacokinetic parameters of theophylline and 1,3-DMU after 1 min intravenous infusion of theophylline at a dose of 5 mg/kg to control, 24-h KPLPS, and 96-h KPLPS rats.

Data are presented as mean ± S.D.

Parameter	Control (n = 9)	24-h KPLPS (n = 7)	96-h KPLPS (n = 8)
Initial body weight (g)	289 ± 16.7	296 ± 3.78	293 ± 12.5
Final body weight (g)	307 ± 19.4	293 ± 15.2	281 ± 18.9[*]
Theophylline			
AUC (μg min/ml)	2540 ± 813	3720 ± 650[**]	2380 ± 400
Terminal half-life (min)	172 ± 75.9	277 ± 66.9[**]	172 ± 51.8
MRT (min)	235 ± 107	377 ± 94.1[**]	236 ± 65.8
V_{ss} (ml/kg)	447 ± 72.1	501 ± 62.2	488 ± 60.8
CL (ml/min/kg)	2.15 ± 0.666	1.37 ± 0.278[**]	2.15 ± 0.329
CL_R (ml/min/kg)	0.808 ± 0.205	0.590 ± 0.129[**]	0.835 ± 0.258
CL_{NR} (ml/min/kg)	1.34 ± 0.601	0.779 ± 0.165[**]	1.31 ± 0.483
$Ae_{0-24\,h}$ (% of theophylline dose)	39.4 ± 10.3	43.1 ± 3.60	40.2 ± 14.5
$GI_{24\,h}$ (% of theophylline dose)	BD[a]	BD	BD
1,3-DMU			
AUC (μg min/ml)	76.8 ± 17.5	48.9 ± 5.52[**]	74.7 ± 16.5
Terminal half-life (min)	222 ± 179	266 ± 85.7	221 ± 144
CL_R (ml/min/kg)	22.3 ± 7.24	21.6 ± 3.83	24.2 ± 7.44
C_{max} (μg /ml)	0.233 ± 0.124	0.115 ± 0.0507[***]	0.189 ± 0.0835
T_{max} (min)	30 (15–180)	45 (15–180)	105 (15–180)
$Ae_{0-24\,h}$ (% of theophylline dose)	28.1 ± 5.96	19.6 ± 3.37[**]	31.6 ± 3.71
$GI_{24\,h}$ (% of theophylline dose)	BD	BD	BD
$AUC_{1,3\text{-DMU}}/AUC_{theophylline}$ (%)	3.11 ± 1.26	2.30 ± 0.942[**]	3.81 ± 1.07

[*]96-h KPLPS was significantly different ($P < 0.05$) from control.

[**]24-h KPLPS was significantly different ($P < 0.05$) from control and 96-h KPLPS.

[***]24-h KPLPS was significantly different ($P < 0.05$) from control.

[a]Below the detection limit.

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TABLE 2

Pharmacokinetic parameters of theophylline and 1,3-DMU after oral administration of theophylline at a dose of 5 mg/kg to control, 24-h KPLPS, and 96-h KPLPS rats.

Data are presented as mean ± S.D.

Parameter	Control (n = 8)	24 h KPLPS (n = 7)	96 h KPLPS (n = 7)
Initial body weight (g)	282 ± 5.94	285 ± 9.57	286 ± 8.02
Final body weight (g)	300 ± 8.45*	289 ± 9.32	284 ± 9.00
Theophylline			
AUC (μg min/ml)	2000 ± 537	2680 ± 438**	2120 ± 354
Terminal half-life (min)	204 ± 61.0	220 ± 52.1	169 ± 39.8
C_{max} (μg /ml)	6.26 ± 2.02	6.93 ± 1.62	7.98 ± 1.99
T_{max} (min)	30 (5–60)	45 (5–90)	15 (5–60)
CL_R (ml/min/kg)	0.605 ± 0.0891	0.631 ± 0.165	0.685 ± 0.148
$Ae_{0-24\,h}$ (% of theophylline dose)	24.3 ± 7.45	33.3 ± 8.67***	28.0 ± 4.60
$GI_{24\,h}$ (% of theophylline dose)	BD[a]	BD	BD
F (%)	78.7	71.9	89.1
1,3-DMU			
AUC (μg min/ml)	95.5 ± 16.7	74.9 ± 21.9***	85.5 ± 10.8
Terminal half-life (min)	223 ± 92.2	247 ± 82.3	197 ± 60.7
CL_R (ml/min/kg)	21.7 ± 5.13	19.1 ± 4.73	19.0 ± 3.58
C_{max} (μg /ml)	0.278 ± 0.155	0.151 ± 0.0732***	0.220 ± 0.0677
T_{max} (min)	90 (30–240)	240 (90–240)**	90 (30–180)
$Ae_{0-24\,h}$ (% of theophylline dose)	37.8 ± 10.2	25.4 ± 6.92***	29.2 ± 7.09
$GI_{24\,h}$(% of theophylline dose)	BD	BD	BD
$AUC_{1,3\text{-DMU}}/AUC_{theophylline}$ (%)	5.22 ± 2.06	2.89 ± 0.981***	4.12 ± 0.769

*Control was significantly different ($P < 0.05$) from 24-h KPLPS and 96-h KPLPS.

**24-h KPLPS was significantly different ($P < 0.05$) from control and 96-h KPLPS.

***24-h KPLPS was significantly different ($P < 0.05$) from control.

[a]Below the detection limit.

Figure. 1.

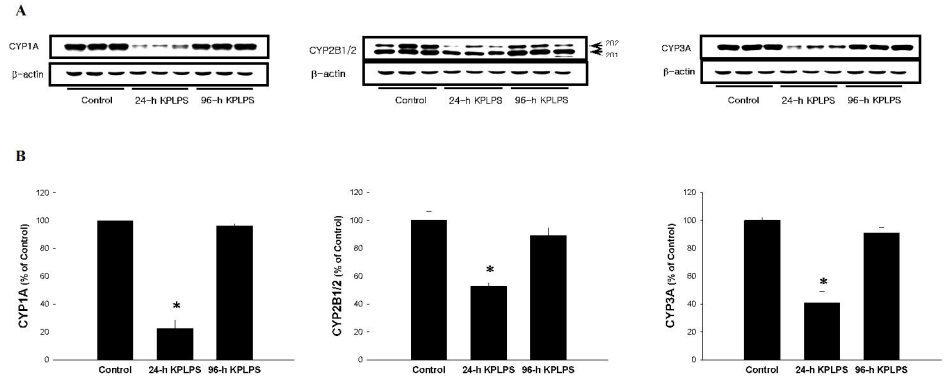
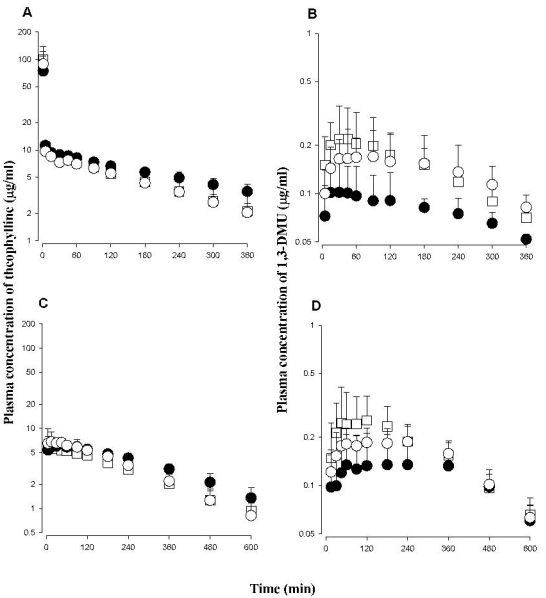

A
CYP1A
β-actin
Control
24-h KPLPS
96-h KPLPS
CYP2B1/2
β-actin
Control
24-h KPLPS
96-h KPLPS
CYP3A
β-actin
Control
24-h KPLPS
96-h KPLPS
B
CYP1A (% of Control)
120
100
80
60
40
20
0
*
Control
24-h KPLPS
96-h KPLPS
CYP2B1/2 (% of Control)
120
100
80
60
40
20
0
*
Control
24-h KPLPS
96-h KPLPS
CYP3A (% of Control)
120
100
80
60
40
20
0
*
Control
24-h KPLPS
96-h KPLPS

Figure. 2.


A
B
C
D
Plasma concentration of theophylline (μg/ml)
Plasma concentration of 1,3-DMU (μg/ml)
Time (min)