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# Effects of bacterial lipopolysaccharide on the pharmacokinetics of metformin in rats

Young H. Choi [a], Inchul Lee [b], Myung G. Lee [a,*]

[a] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea*
[b] *Department of Diagnostic Pathology, College of Medicine, University of Ulsan, Asan Foundation, Asan Medical Center, 388-1 Poongnap-Dong, Songpa-Gu, Seoul 138-736, South Korea*

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**Abstract**

It was reported that the hepatic microsomal cytochrome P450 (CYP) 2C11, 2D1, and 3A1 (not via the CYP1A2, 2B1/2, and 2E1) were involved in the metabolism of metformin in rats. It was also reported that the expressions of CYP2C11 and 3A2 decreased in rats pretreated with *Klebsiella pneumoniae* lipopolysaccharide (KPLPS). Therefore, the pharmacokinetic parameters of metformin could be changed in rats pretreated with KPLPS. Hence, the pharmacokinetic parameters of metformin were compared after both intravenous and oral administration of the drug at a dose of 100 mg/kg to control rats and rats pretreated with KPLPS. After intravenous administration of metformin to rats pretreated with KPLPS, the total area under the plasma concentration–time curve from time 0 to $\infty$ (AUC) of the drug was significantly greater (40.5% increase) than the controls due to significantly smaller CL value (27.7% decrease) than the controls. The significantly smaller CL value could be due to significantly smaller both the $CL_R$ and $CL_{NR}$ values (34.0% and 18.1% decrease, respectively) than the controls. The significantly smaller $CL_{NR}$ value could be due to decrease in the expressions of CYP2C11 and 3A2 in rats pretreated with KPLPS. After oral administration of metformin, the AUC of the drug was not significantly different between two groups of rats, and this may be at least partly due to decrease in absorption from the gastrointestinal tract compared with the controls.


*Keywords:* Metformin; Pharmacokinetics; *Klebsiella pneumoniae* lipopolysaccharide; CYP2C11 and 3A2; Rats

## 1. Introduction

Metformin, a biguanide antihyperglycemic agent, is widely used in the management of type 2 diabetes mellitus; it lowers the blood glucose concentration without causing hypoglycemia (Scheen, 1996). After intravenous (at doses of 0.25–1.0 g) and oral (at doses of 0.5–1.5 g) administration of metformin to four healthy volunteers, the terminal half-lives of the drug were 1.52–4.50 h, 78.9–99.9% of the dose were excreted in the urine via active renal tubular secretion, absorption of the drug was not complete (20–30% of the oral dose were recovered from the feces) possibly due to active, saturable absorption process, and extent of absolute oral bioavailability ($F$) was 33–55% (Scheen, 1996). Binding of metformin to human plasma proteins does not occur (Sirtori et al., 1978; Tucker et al., 1981). Metabolism of metformin was suggested in humans (Scheen, 1996) based on incomplete recovery of the drug in the urine after intravenous administration of the drug (Sirtori et al., 1978) in accordance with a further study (Tucker et al., 1981) in which 20% of the dose was not accounted for. Recently, it has been reported that metformin was mainly metabolized via the hepatic microsomal cytochrome P450 (CYP) 2C11, 2D1, and 3A1/2 (not via the CYP1A2, 2B1/2, and 2E1) in male Sprague–Dawley rats (Choi and Lee, 2006).

*Abbreviations:* KPLPS, *Klebsiella pneumoniae* lipopolysaccharide; AUC, total area under the plasma concentration–time curve from time 0 to $\infty$; CL, time-averaged total body clearance; $CL_R$, time-averaged renal clearance; $CL_{NR}$, time-averaged nonrenal clearance; $CL_{CR}$, time-averaged creatinine clearance; $V_{ss}$, apparent volume of distribution at steady state; MRT, mean residence time; $V_{max}$, maximum velocity; $K_m$, Michaelis–Menten constant; $CL_{int}$, intrinsic clearance; $C_{max}$, peak plasma concentration; $T_{max}$, time to reach a $C_{max}$; $Ae_{0-24\,h}$, percentage of dose excreted in 24 h urine; $GI_{24\,h}$, percentage of dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h; $F$, extent of absolute oral bioavailability

* Corresponding author. Tel.: +82 2 880 7855/7877; fax: +82 2 889 8693.
*E-mail address:* leemg@snu.ac.kr (M.G. Lee).

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Lipopolysaccharide (LPS) is an active component in the outer membrane of Gram-negative bacteria. Recently, it has been reported that after intravenous administration of *Klebsiella pneumoniae* lipopolysaccharide (KPLPS) at a dose of 0.5 mg/kg to 8-week-old male Wistar rats, the expressions of CYP2C11 and 3A2 decreased significantly compared with the controls (Ueyama et al., 2005). Therefore, it could be expected that the time-averaged nonrenal clearance [$CL_{NR}$; the $CL_{NR}$ of metformin could represent the metabolic clearance of the drug in rats (Choi and Lee, 2006)] of metformin could be changed in rats pretreated with KPLPS.

It has been reported that abscesses caused by *K. pneumoniae* were most strongly associated with diabetes mellitus (Yang et al., 2004). Also *K. pneumoniae* was the most common infective organism in patients with diabetes mellitus in deep neck infection (Huang et al., 2004) or meningitis (Chang et al., 2003). Although the pharmacokinetic changes of drugs (Bae et al., 2004 and references therein) and decrease in non-specific CYP isozymes (Nadai et al., 1998 and references therein) in rats pretreated with LPS have been reported, the pharmacokinetic changes of drugs with respect to specific CYP isozyme changes did not seem to be published except chlorozoxazone in rats pretreated with *Escherichia coli* LPS, ECLPS (Rockich and Blouin, 1999). For example, the significantly smaller both the time-averaged total body clearance (CL) and intrinsic clearance ($CL_{int}$) values of chlorozoxazone (which is metabolized to 6 hydroxychlorozoxazone via the CYP2E1 in rats) in male Sprague–Dawley rats pretreated with ECLPS were due to decrease in both the activity and concentration of CYP2E1 compared with the controls (Rockich and Blouin, 1999). However, the pharmacokinetic changes of metformin in rats pretreated with KPLPS with respect to specific CYP isozyme changes did not seem to be published to date, because the decrease in the expressions of CYP2C11 and 3A2 in rats pretreated with KPLPS has been reported recently in rats (Ueyama et al., 2005). Moreover, the CYP isozymes responsible for the metabolism of metformin were also reported recently (Choi and Lee, 2006). Hence, metformin was chosen in this study using rats pretreated with KPLPS as an animal model.

The aim of this study is to report the pharmacokinetic changes of metformin after intravenous and oral administration of the drug at a dose of 100 mg/kg to rats pretreated with KPLPS with respect to CYP isozymes changes (Ueyama et al., 2005).

## 2. Materials and methods

### 2.1. Chemicals

Metformin hydrochloride and ipriflavone (an internal standard of high-performance liquid chromatographic, HPLC, analysis of metformin) were supplied from Dalim Medical (Seoul, South Korea) and Research Laboratory of Dong-A Pharmaceutical Company (Yongin, South Korea), respectively. Reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), ethylendiamine tetraacetatic acid (EDTA), tri(hydroxymethyl)aminomethane (Tris®)-buffer, and KPLPS were purchased from Sigma–Aldrich Corporation (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade.

### 2.2. Animals

Male Sprague–Dawley rats of 6–8 weeks of age (weighing 200–320 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). The rats were randomly divided into two groups, control rats and rats pretreated with KPLPS. All rats were maintained in a light-controlled room (light: 07:00–19:00, dark: 19:00–07:00) kept at a temperature of between 20 and 23 °C and a relative humidity of 50% (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air, and with food (Samyang Company, Seoul, South Korea) and water ad libitum. This animal study protocol was approved by Animal Care and Use Committee of College of Pharmacy of Seoul National University.

### 2.3. Preliminary study

The following preliminary study was performed 2 h after the start of the infusion of KPLPS or 0.9% NaCl-injectable solution ($n = 5$; each) to measure the kidney and liver functions. The 24 h urine was collected for the measurement of the creatinine level. Blood was collected for the measurement of the hematocrit value (Readacroit Centrifuge; Clay Adams, Parsippany, NJ). Plasma was collected for the measurement of the total proteins, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and creatinine levels (analyzed by Green Cross Reference Lab., Seoul, South Korea), and protein binding of metformin using the equilibrium dialysis technique (Choi et al., 2006). The whole kidney and liver of each rat were excised 26 h after the start of the infusion of KPLPS, rinsed with 0.9% NaCl-injectable solution, blotted dry with tissue paper, and weighed. Small portions of each organ were fixed in a 10% neutral phosphate-buffered formalin and then processed for routine histological examination with hematoxylin–eosin staining.

### 2.4. Measurement of $V_{max}$, $K_m$, and $CL_{int}$ for the disappearance of metformin in hepatic microsomal fractions of control rats and rats pretreated with KPLPS

The procedures were similar to previously reported methods (Kim et al., 2005). The livers of control rats ($n = 5$) and rats pretreated with KPLPS ($n = 5$) were removed 2 h after the start of the infusion of KPLPS (or 0.9% NaCl-injectable solution), and were homogenized (Ultra-Turrax T25; Janke & Kunkel, IKA-Labortechnik, Staufeni, Germany) in an ice-cold buffer of 0.154 M KCl/50 mM Tris–HCl in 1 mM EDTA, pH 7.4. The homogenates were centrifuged at 10,000 × $g$ for 30 min and the supernatant fractions were further centrifuged at 100,000 × $g$ for 90 min. Protein content was measured using the reported method (Bradford, 1976). The $V_{max}$ (the maximum velocity) and $K_m$ (the Michaelis–Menten constant; the concentration at

which the rate is one half of $V_{max}$) for the disappearance of metformin were determined after incubating the above microsomal fractions (equivalent to 0.5 mg protein), a 5 μl aliquot of 0.9% NaCl-injectable solution that contained 1, 2.5, 5, 7.5, 10, 30, 50, 100, and 200 μM of metformin base, and a 50 μl aliquot of a 0.1 M phosphate buffer of pH 7.4 that contained 1 mM of NADPH in a final volume of 0.5 ml by adding a 0.1 M phosphate buffer of pH 7.4 in a water-bath shaker kept at 37 °C and at a rate of 500 oscillations per min (opm). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of 1 ml of acetonitrile after 15 min incubation. The kinetic constants ($K_m$ and $V_{max}$) for the disappearance of metformin were calculated using the nonlinear regression method (Duggleby, 1995). The intrinsic clearance ($CL_{int}$) for the disappearance of metformin was calculated by dividing the respective $V_{max}$ by the respective $K_m$.

### 2.5. Intravenous study

In the early morning, the jugular vein (for drug administration) and the carotid artery (for blood sampling) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) while each rat was under light ether anesthesia (Kim et al., 1993). Both cannulas were exteriorized to the dorsal side of the neck where each cannula was terminated with a long silastic tube (Dow Corning, Midland, MI). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed for 4–5 h to recover from anesthesia before the study began.

KPLPS at a dose of 250 μg/kg was infused via the jugular vein over a period of 30 min (KPLPS-treated group) with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, Southnatick, MA) as previously described (Bae et al., 2004 and references therein). KPLPS was dissolved in 0.9% NaCl-injectable solution to produce a concentration of 150 μg/ml. In control group, rats were pretreated with the same volume of 0.9% NaCl-injectable solution. Two hours after the start of the intravenous infusion of KPLPS or 0.9% NaCl-injectable solution, metformin (metformin hydrochloride was dissolved in 0.9% NaCl-injectable solution) at a dose of 100 mg/kg was infused (total infusion volume of approximately 0.6 ml) over 1 min via the jugular vein of control rats ($n = 9$) and rats pretreated with KPLPS ($n = 11$). An approximately 0.12 ml aliquot of blood sample was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, and 720 min after the start of the infusion of metformin. An approximately 0.3 ml aliquot of the heparinized 0.9% NaCl-injectable solution (20 units/ml) was used to flush each cannula immediately after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately and a 50 μl aliquot of each plasma sample was stored in a −70 °C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of metformin was conducted. After 24 h, each metabolic cage was rinsed with 20 ml of distilled water and the rinsings were combined with 24 h urine. After measuring the exact volume of the 24 h urine output and the combined urine samples, two 50 μl aliquots of the combined urine sample were stored in a −70 °C freezer until HPLC analysis of metformin was conducted. At the same time (24 h), each rat was exsanguinated via the carotid artery and sacrificed through cervical dislocation. And then, the entire gastrointestinal tract (including its contents and feces) of each rat was removed, transferred into a beaker that contained 100 ml of methanol (to facilitate the extraction of metformin), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 50 μl aliquots of the supernatant were collected from each beaker and stored in a −70 °C freezer until HPLC analysis of metformin was conducted.

### 2.6. Oral study

Two hours after the start of the intravenous infusion of KPLPS or 0.9% NaCl-injectable solution, metformin (the same solution that was used in the intravenous study) at a dose of 100 mg/kg was administrated orally (total oral volume of approximately 2 ml) to control rats ($n = 9$) and rats pretreated with KPLPS ($n = 9$) using a feeding tube. Blood samples were collected at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 960, 1200, and 1440 min after oral administration of metformin. Other procedures were similar as those in the intravenous study.

### 2.7. HPLC analysis of metformin

The concentrations of metformin in the aforementioned samples were determined by a slight modification of the reported HPLC method (Hale et al., 2002); ipriflavone instead of hydrocodeine was used as an internal standard. A 50 μl aliquot of biological sample was deproteinized with a 100 μl aliquot of acetonitrile, and a 50 μl aliquot of methanol that contained 10 μg/ml of ipriflavone (an internal standard) was added. After vortex-mixing and centrifugation at 16,000 × $g$ for 10 min, a 50 μl aliquot of the supernatant was injected directly onto a reversed-phase ($C_{18}$) HPLC column. The mobile phase (pH 6), 10 mM $KH_2PO_4$:acetonitrile (40:60; v/v) was run at a flow-rate of 1.5 ml/min, and the column effluent was monitored using an ultraviolet detector set at 235 nm. The retention times of metformin and the internal standard were approximately 4 and 6.5 min, respectively. The quantitation limits of metformin in rat plasma and urine were 0.05 and 1 μg/ml, respectively. The inter- and intra-day coefficients of variation were below 9.91% and 7.52% for plasma and urine samples, respectively, in the concentration ranges from 0.05 to 5000 μg/ml and from 1 to 1000 μg/ml for plasma and urine samples, respectively.

### 2.8. Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time 0 to ∞ infinity (AUC) was calculated using the trapezoidal rule–extrapolation method (Chiou, 1978).

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters using the noncompartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA); the time-averaged total body (CL), renal

($CL_R$), and nonrenal ($CL_{NR}$) clearances, terminal half-life, first moment of AUC (AUMC), mean residence time (MRT), apparent volume of distribution at steady state ($V_{ss}$), and $F$ (Kim et al., 1993). The peak plasma concentration ($C_{max}$) and time to reach a $C_{max}$ ($T_{max}$) were read directly from the experimental data.

Glomerular filtration rate (GFR) was estimated by measuring the creatinine clearance ($CL_{CR}$) assuming that kidney function was stable during the experimental period. The $CL_{CR}$ was measured by dividing the total amount of creatinine excreted in the urine over 24 h by the $AUC_{0-24\,h}$ of creatinine in plasma.

The mean values of $V_{ss}$ (Chiou, 1979), terminal half-life (Eatman et al., 1977), and each clearance (Chiou, 1980) were calculated using the harmonic mean method.

### 2.9. Statistical analysis

A $P < 0.05$ was considered to be statistically significant using the $t$-test between the two means for the unpaired data. All results are expressed as mean ± standard deviation (S.D.) except median (ranges) for $T_{max}$.

## 3. Results

### 3.1. Preliminary study

Body weight, hematocrit, 24 h urine output, plasma chemistry data, plasma protein binding of metformin, $CL_{CR}$, and relative liver and kidney weights in control rats and rats pretreated with KPLPS are listed in Table 1. The parameters listed in Table 1 were not significantly different between two groups of rats. Similar results have also been reported from other rat studies (Bae et al., 2004). The above data suggest that the liver and kidney functions in rats with KPLPS did not seem to be impaired compared with the controls. This could be supported based on the liver and kidney microscopy; there were no significant findings in the livers and kidneys of both groups of rats. No significant findings in the kidney of rats pretreated with KPLPS at a dose of 250 μg/kg have also been reported from other rat studies based on the kidney microscopy (Nadai et al., 1993, 1995; Hasegawa et al., 1994).

Table 1
Body weight, hematocrit, urine output, plasma chemistry data, plasma protein binding of metformin, creatinine clearance ($CL_{CR}$), and relative liver and kidney weights in control rats and rats pretreated with *Klebsiella pneumoniae* LPS (KPLPS)[a]

| Parameter | Control ($n=5$) | KPLPS ($n=5$) |
|---|---|---|
| Body weight (g) | 258 ± 5.70 | 242 ± 16.4 |
| Hematocrit (%) | 48.0 ± 2.48 | 47.7 ± 1.21 |
| Urine output (ml/24 h) | 28.2 ± 23.6 | 31.4 ± 10.4 |
| Plasma | | |
| Total proteins (g/dl) | 5.68 ± 0.110 | 5.74 ± 0.134 |
| Albumin (g/dl) | 3.68 ± 0.130 | 3.66 ± 0.114 |
| Urea nitrogen (mg/dl) | 12.7 ± 2.63 | 14.8 ± 2.23 |
| GOT (IU[b]/l) | 67.4 ± 22.4 | 56.2 ± 5.07 |
| GPT (IU/l) | 28.4 ± 13.8 | 21.2 ± 3.27 |
| Protein binding of metformin (%) | 11.9 ± 4.26 | 10.5 ± 6.53 |
| $CL_{CR}$ (ml/(min kg)) | 3.96 ± 0.490 | 4.14 ± 0.836 |
| Liver weight (% of body weight) | 3.44 ± 0.160 | 3.83 ± 0.388 |
| Kidney weight (% of body weight) | 0.776 ± 0.0445 | 0.827 ± 0.0685 |

[a] Each value represents the mean ± S.D.
[b] International unit.

Table 2
$V_{max}$, $K_m$, and $CL_{int}$ for the disappearance of metformin by hepatic microsomes of control rats and rats pretreated with *K. pneumoniae* LPS (KPLPS)[a]

| Parameter | Control ($n=5$) | KPLPS ($n=5$) |
|---|---|---|
| $V_{max}$ (nmol/(min mg) protein) | 1.94 ± 0.649 | 3.02 ± 1.65 |
| $K_m$ (μM) | 97.2 ± 22.2 | 252 ± 152 |
| $CL_{int}$ (ml/(min mg) protein) | 0.0203 ± 0.00601 | 0.0122 ± 0.00217[b] |

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P < 0.05$) from the control.

### 3.2. Measurement of $V_{max}$, $K_m$, and $CL_{int}$ for the disappearance of metformin in the hepatic microsomal fractions

The $V_{max}$, $K_m$, and $CL_{int}$ for the disappearance of metformin in the hepatic microsomal fractions of control rats and rats pretreated with KPLPS are listed in Table 2. In rats pretreated with KPLPS, the $V_{max}$ was comparable to the controls, suggesting that the maximum velocity for the disappearance (mainly due to metabolism) of metformin was not affected considerably by the treatment of KPLPS compared with the controls. However, in rats pretreated with KPLPS, the $K_m$ became considerably higher ($P = 0.0834$) compared with the controls, suggesting that the affinity of metformin to the enzyme(s) somewhat decreased compared with the controls. As a result, the $CL_{int}$ value for the disappearance of metformin in rats pretreated with KPLPS became significantly smaller (39.9% decrease) than the controls, suggesting that the metabolism of metformin decreased in rats pretreated with KPLPS compared with the controls.

### 3.3. Pharmacokinetics of metformin after intravenous administration of the drug to rats

After intravenous administration of metformin at a dose of 100 mg/kg to control rats and rats pretreated with KPLPS, the mean arterial plasma concentration–time profiles of the drug are shown in Fig. 1, and some relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration of metformin to rats pretreated with KPLPS, the changes in the pharmacokinetic parameters of the drug compared with the controls are as follows; the AUC became significantly greater (40.5% increase), CL, $CL_{NR}$, and $CL_R$ values became significantly smaller (27.7%, 34.0%, and 18.1% decrease, respectively), and percentage of intravenous dose of metformin excreted in 24 h urine as an unchanged drug ($Ae_{0-24\,h}$) became significantly smaller (7.93% decrease) than the controls.

### 3.4. Pharmacokinetics of metformin after oral administration of the drug to rats

After oral administration of metformin at a dose of 100 mg/kg to control rats and rats pretreated with KPLPS, the mean arte-

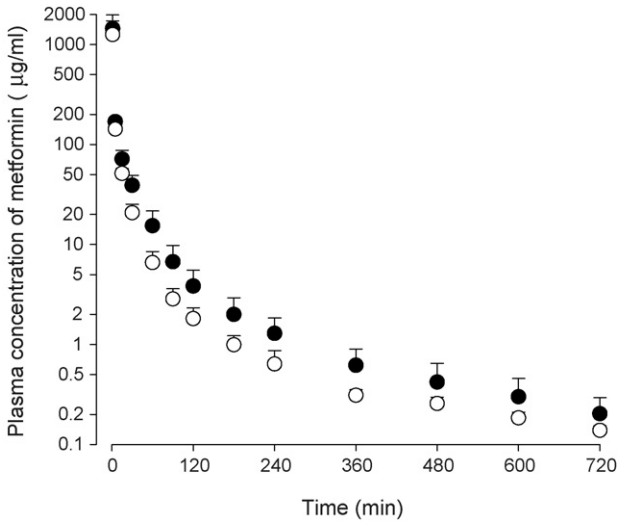


Fig. 1. Mean arterial plasma concentration–time profiles of metformin after 1 min intravenous infusion of the drug at a dose of 100 mg/kg to control rats ($n = 9$; ◯) and rats pretreated with *Klebsiella pneumoniae* LPS ($n = 11$; ●). Vertical bars represent standard deviation.

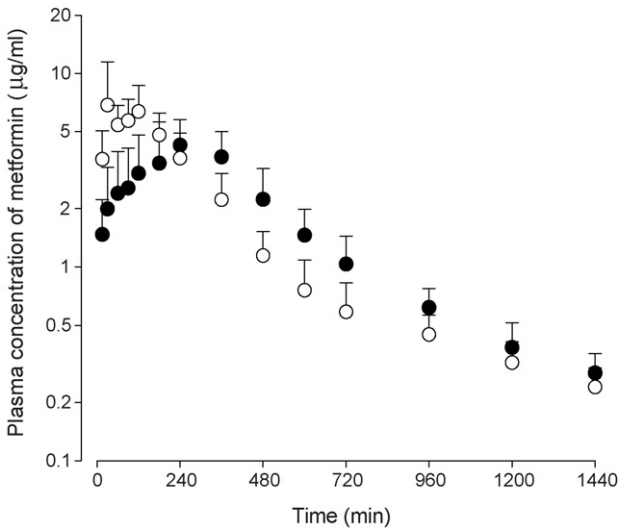


Fig. 2. Mean arterial plasma concentration–time profiles of metformin after oral administration of the drug at a dose of 100 mg/kg to control rats ($n = 9$; ◯) and rats pretreated with *K. pneumoniae* LPS ($n = 9$; ●). Vertical bars represent standard deviation.

rial plasma concentration–time profiles of the drug are shown in Fig. 2, and some relevant pharmacokinetic parameters are listed in Table 4. After oral administration of metformin, absorption of the drug from the rat gastrointestinal tract was rapid; metformin was detected in plasma from the first or second blood sampling time (15 or 30 min) for both groups of rats. After oral administration of metformin to rats pretreated with KPLPS, the changes in the pharmacokinetic parameters of the drug compared with the controls are as follows; the $C_{max}$ became significantly lower (43.4% decrease), $T_{max}$ became significantly longer (100% increase), $CL_R$ value became significantly smaller (16.7% decrease), percentage of oral dose of metformin recovered from the entire gastrointestinal tract (including its contents and feces) as an unchanged drug at 24 h ($GI_{24\,h}$) became significantly greater (420% increase), and $Ae_{0–24\,h}$ became considerably smaller (17.8% decrease; $P = 0.0796$) than the controls.

Table 3
Pharmacokinetic parameters of metformin after 1 min intravenous infusion of the drug at a dose of 100 mg/kg to control rats and rats pretreated with *K. pneumoniae* LPS (KPLPS)[a]

| Parameter | Control ($n = 9$) | KPLPS ($n = 11$) |
|---|---|---|
| Body weight (g) | 298 ± 20.3 | 300 ± 7.91 |
| Urine volume (ml/24 h) | 29.1 ± 2.88 | 26.5 ± 2.98 |
| AUC (μg min/ml) | 4940 ± 840 | 6940 ± 1280[b] |
| Terminal half-life (min) | 294 ± 56.0 | 259 ± 50.6 |
| MRT (min) | 41.9 ± 7.93 | 49.3 ± 11.7 |
| CL (ml/(min kg)) | 20.2 ± 4.21 | 14.6 ± 2.05[c] |
| $CL_R$ (ml/(min kg)) | 11.7 ± 2.65 | 7.72 ± 1.38[c] |
| $CL_{NR}$ (ml/(min kg)) | 8.31 ± 2.45 | 6.81 ± 0.933[d] |
| $V_{ss}$ (ml/kg) | 797 ± 343 | 686 ± 187 |
| $Ae_{0–24\,h}$ (% of dose) | 58.0 ± 5.81 | 53.4 ± 4.11[d] |
| $GI_{24\,h}$ (% of dose) | 1.86 ± 2.34 | 3.37 ± 1.71 |

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P < 0.001$) from the control.
[c] Significantly different ($P < 0.01$) from the control.
[d] Significantly different ($P < 0.05$) from the control.

Table 4
Pharmacokinetic parameters of metformin after oral administration of the drug at a dose of 100 mg/kg to control rats and rats pretreated with *K. pneumoniae* LPS (KPLPS)[a]

| Parameter | Control ($n = 9$) | KPLPS ($n = 9$) |
|---|---|---|
| Body weight (g) | 215 ± 9.01 | 216 ± 10.8 |
| Urine volume (ml/24 h) | 23.7 ± 4.56 | 28.6 ± 14.5 |
| AUC (μg min/ml) | 2400 ± 381 | 2540 ± 488 |
| Terminal half-life (min) | 509 ± 98.4 | 498 ± 193 |
| $C_{max}$ (μg/ml) | 8.41 ± 3.83 | 4.76 ± 1.58[b] |
| $T_{max}$[c] (min) | 90 (30–240) | 180 (60–360)[d] |
| $CL_R$ (ml/(min kg)) | 16.2 ± 3.06 | 13.5 ± 3.35[b] |
| $Ae_{0–24\,h}$ (% of dose) | 40.4 ± 9.83 | 33.2 ± 5.98[e] |
| $GI_{24\,h}$ (% of dose) | 2.02 ± 0.934 | 10.5 ± 8.81[b] |
| $F$ (%) | 48.6 | 36.6 |

[a] Each value represents the mean ± S.D. except median (ranges) for $T_{max}$.
[b] Significantly different ($P < 0.05$) from the control.
[c] Median (ranges).
[d] Significantly different ($P < 0.01$) from the control.
[e] Considerably different ($P = 0.0796$) from the control.

## 4. Discussion

In the pharmacokinetic studies in rats pretreated with LPS, the pharmacokinetic changes of drugs (compounds) seemed to be dependent on gender (male or female) and species (CD, Wistar, or Sprague–Dawley rats) of rats, sources (*E. coli* or *K. pneumoniae*) and doses (50, 250, 500, or 1000 μg/kg) of LPS, and starting time (2, 6, 10, 24, or 96 h) of experiment after LPS administration (Bae et al., 2004 and references therein). In most studies, KPLPS was administered at a dose of 250 μg/kg and the experiment was started 2 h after the start of the infusion of KPLPS in male rats (Bae et al., 2004 and references therein). Hence, in the present study, the same protocol was employed.

The contribution of gastrointestinal (including biliary) excretion of unchanged metformin to $CL_{NR}$ of metformin was almost negligible; the $GI_{24\,h}$ values were 1.86% and 3.37% of intra-

venous dose for control rats and rats pretreated with KPLPS, respectively (Table 3). The smaller values of $GI_{24\,h}$, 1.86% and 3.37%, were not due to chemical and enzymatic degradation of metformin in the rat gastric fluids. It has been reported that metformin was stable up to 48 h incubation in various buffer solutions having pHs ranging from 1 to 12 and up to 24 h incubation in two rat gastric juices (pHs of 2.5 and 4.5, respectively) (Choi et al., 2006). Moreover, it has been also reported that after intravenous administration of metformin at a dose of 100 mg/kg to six rats with bile duct cannulation, the biliary excretion of an unchanged drug was almost negligible; the percentage of the intravenous dose of metformin excreted in 24 h bile as an unchanged drug was only 0.343% (Choi et al., 2006). Hence, the $CL_{NR}$ of metformin listed in Table 3 could represent the metabolic clearance of the drug. Therefore, the changes in the $CL_{NR}$ of metformin could represent the changes in the metabolism of the drug in rats.

The AUC values of metformin were dose-proportional after both intravenous and oral administration of the drug (both at 50, 100, and 200 mg/kg) studied (Choi et al., 2006). Hence, a dose of 100 mg/kg of metformin was arbitrarily chosen in this study.

After intravenous administration of metformin to rats pretreated with KPLPS, the AUC of the drug was significantly greater than the controls, and this could be due to the significantly smaller both $CL_{NR}$ and $CL_R$ values than the controls (Table 3). The smaller $CL_{NR}$ value could be due to significant decrease in the expressions of the CYP2C11 and 3A2 in rats pretreated with KPLPS (Ueyama et al., 2005), since metformin was metabolized via the CYP2C11, 2D1, and 3A1/2 in rats (Choi and Lee, 2006). Although the aforementioned data suggest that the contribution of the CYP2D1 to the $CL_{NR}$ of metformin in rats pretreated with KPLPS did not seem to be considerable, more studies are required to find the role of the CYP2D1 in the metabolism of metformin in rats pretreated with KPLPS. The hepatic first-pass effect of metformin was 27.1% based on the AUC difference of the drug between intravenous and intraportal administration of the drug to control male Sprague–Dawley rats (Choi et al., 2006). Since metformin is a low hepatic extraction ratio drug, the hepatic clearance of metformin depends more on the $CL_{int}$ value and free (unbound to plasma proteins) fractions of metformin in plasma rather than on the hepatic blood flow rate (Wilkinson and Shand, 1975). The significantly smaller $CL_{NR}$ value of metformin in rats pretreated with KPLPS (Table 3) could be supported by significantly smaller $CL_{int}$ value than the controls (Table 2). The contribution of free fractions of metformin in plasma to the smaller $CL_{NR}$ value in rats pretreated with KPLPS (Table 3) did not seem to be considerable; the plasma protein binding values of metformin were comparable between two groups of rats (Table 1).

After intravenous and oral administration of metformin to rats pretreated with KPLPS, the $Ae_{0-24\,h}$ of the drug was significantly smaller than the controls, and this contributed the significantly smaller $CL_{NR}$ in the rats (Table 3). It was obtained (our unpublished data) that the timed-interval renal clearance of metformin depends on the urine flow rate in both control rats and rats with diabetes mellitus induced by streptozotocin before and after insulin injection; the more urine output, the urinary excretion of metformin decreased in male Sprague–Dawley rats. However, this factor seemed to be ruled out, since the 24 h urine output was comparable between two groups of rats (Table 3). It has been reported that metformin is a superior substrate for the renal organic cation transporter, OCT2 (Kimura et al., 2005a) and transported by the basolateral organic cation transporter hOCT2 in the human kidney (Kimura et al., 2005b). The significantly smaller $Ae_{0-24\,h}$ of metformin in rats pretreated with KPLPS (Table 3) may be due to the changes in OCT2 compared with the controls. The changes in OCT2 in rats pretreated with KPLPS do not seem to be published yet.

After intravenous administration of metformin to control rats and rats pretreated with KPLPS, the $CL_R$ of the drug was estimated as free fractions of metformin in plasma based on the $CL_R$ (Table 3) and plasma protein binding of metformin (Table 1). The values thus estimated were 13.3 and 8.63 ml/(min kg) for control rats and rats pretreated with KPLPS, respectively. The values, 13.3 and 8.63 ml/(min kg), were considerably greater than the GFR (as estimated by $CL_{CR}$) in control rats and rats pretreated with KPLPS (Table 1), indicating that metformin is secreted in the renal tubules for both groups of rats. Active renal tubular secretion of metformin was also reported in humans (Scheen, 1996).

After intravenous administration of metformin to rats pretreated with KPLPS, the AUC of the drug was significantly greater (Table 3) than the controls. However, after oral administration of metformin to rats pretreated with KPLPS, the AUC of the drug was not significantly different compared with the controls (Table 4). Although the exact reason is not clear, this could not be due to increased metabolism of metformin via the intestinal CYP3A in rats pretreated with KPLPS; it was reported that the intestinal epithelial CYP3A activity reduced by 41% in rats pretreated with ECLPS (Maezono et al., 2005). However, the changes in CYP3A in rats pretreated with KPLPS were not reported. The comparable AUC of metformin after oral administration of the drug may be at least partly due to decreased absorption of the drug from the gastrointestinal tract in rats pretreated with KPLPS. Based on the linear pharmacokinetics (Choi et al., 2006), the "mean" true fractions of oral dose of metformin unabsorbed ($F_{unabs}$) in this study could be estimated by the following equations (Lee and Chiou, 1983):

$$0.0202 = F_{unabs} + (0.486 \times 0.0186), \quad \text{for control rats} \tag{1}$$

$$0.105 = F_{unabs} + (0.366 \times 0.0337), \quad \text{for rats pretreated with KPLPS} \tag{2}$$

in which 0.0202 (0.105), 0.486 (0.366), and 0.0186 (0.0337) are the $GI_{24\,h}$ after oral administration of the drug to control rats (rats pretreated with KPLPS) (Table 4), $F$ (Table 3), and $GI_{24\,h}$ after intravenous administration of the drug to control rats (rats pretreated with KPLPS) (Table 4), respectively. The $F_{unabs}$ values thus estimated were 1.12% and 9.27% for control rats and rats pretreated with KPLPS, respectively. Hence, approximately 99% and 91% of the oral dose were absorbed from the entire gastrointestinal tract for control rats and rats pretreated with KPLPS, respectively. As a result, the $F$ in rats

pretreated with KPLPS was somewhat smaller (24.7% decrease) than the controls (Table 4). The decrease in the gastrointestinal absorption of metformin in rats pretreated with KPLPS could be supported by the followings. It has been reported (Wallace et al., 1987) that after intravenous administration of ECLPS to rats, extensive necrosis and vascular congestion in the stomach and small intestine (the duodenum and jejunum were the tissues most susceptible to damage), but not the distal colon, were produced. After intravenous administration of ECLPS to rats, gastric motility was also reported to be increased (Esplugues and Whittle, 1989).

Note that the mean terminal half-lives of metformin after oral administration of the drug (Table 4) were significantly longer than those after intravenous administration of the drug (Table 3). This could be due to different blood sampling time schedules between two routes of administration. For comparison, the half-lives of metformin after oral administration were estimated based on 720 min plasma data (the same time in the intravenous study; Fig. 1); the values were close to those after intravenous administration of the drug. The values were $266 \pm 29.4$ and $222 \pm 45.1$ min for control rats and rats pretreated with KPLPS, respectively.

In conclusion, after intravenous administration of metformin to the rats pretreated with KPLPS, the AUC was significantly greater than the controls due to significantly smaller CL value than the controls (Table 3). The smaller CL value could be due to significantly smaller both the $CL_{NR}$ and $CL_R$ values than the controls. After oral administration of metformin to rats pretreated with KPLPS, the AUC was not significantly different compared with the controls (Table 4), at least partly due to decrease in the gastrointestinal absorption of metformin in rats pretreated with KPLPS.

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