# Pharmacokinetic Parameters of Chlorzoxazone and its Main Metabolite, 6-Hydroxychlorzoxazone, after Intravenous and Oral Administration of Chlorzoxazone to Liver Cirrhotic Rats with Diabetes Mellitus

**Choong Y. Ahn, Soo K. Bae, Young S. Jung, Inchul Lee, Young C. Kim, Myung G. Lee, and Wan G. Shin**

*College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul [C.Y. A., S.K. B., Y.S. J., Y.C. K., M.G. L., W.G. S.]; Department of Bioequivalence, Korean Food & Drug Administration, Seoul [C.Y. A]; Department of Clinical Pharmacology, Busan Paik Hosptial, Inje University, Busan [S.K. B.]; and Department of Diagnostic Pathology, College of Medicine, University of Ulsan, Asan Foundation, Asan Medical Center, Seoul [I.L]; South Korea*

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**Running title page**

a)    Pharmacokinetics of CZX and OH-CZX in LCD rats

b)     Wan Gyoon Shin, Pharm. D., Ph. D.

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea.

Tel.; +822-880-9261

Fax; +822-874-4169

E-mail address; wgshin@snu.ac.kr

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d)   ABBREVIATIONS: LC, liver cirrhosis; DM, diabetes mellitus; LCD, liver cirrhosis with diabetes mellitus; HPLC, high-performance liquid chromatography, AUC, total area under the plasma concentration–time curve from time zero to time infinity; $t_{1/2}$, half-life; MRT, 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 steady state; $Ae_{0-24\,h}$, percentage of the dose excreted in the 24-h urine; $GI_{24\,h}$, 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

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**Abstract:**

Protein expression of the hepatic CYP2E1 has been reported to be increased in diabetic rats. This enzyme is the primary metabolizer of chlorozoxazone (CZX) to 6-hydroxychlorzoxazone (OH-CZX). Although patients with liver cirrhosis have a higher prevalence of diabetes mellitus, there have no reported studies on the protein expression of CYP2E1 in rats induced to have liver cirrhosis and diabetes mellitus by injection of N-dimethylnitrosamine followed by streptozotocin (LCD rats). Thus, in the present study, the pharmacokinetics of CZX and OH-CZX were evaluated in LCD rats. Compared with control rats, LCD rats had significantly decreased (by 62%) total liver protein and significantly increased (by 124%) protein expression of CYP2E1, but the intrinsic clearance ($Cl_{int}$; formation of OH-CZX per mg protein) was comparable in both groups of rats. As a result, the relative $Cl_{int}$ was also comparable for the two groups. Thus, OH-CZX formation in LCD and control rats was expected to be similar. As expected, after intravenous (20 mg/kg) and oral (50 mg/kg) administration of CZX, the AUC of OH-CZX was comparable in control and LCD rats (intravenous, $571 \pm 85.8$ and $578 \pm 413$ µg • min/ml, respectively; oral, $1540 \pm 338$ and $2170 \pm 1070$ µg • min/ml, respectively). In LCD rats, the $AUC_{OH\text{-}CZX} / AUC_{CZX}$ ratio was similar to value in control rats after intravenous and oral administration. These results indicate that OH-CZX can be used as a chemical probe to assess the activity of CYP2E1 in LCD rats.

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Chlorzoxazone [5-chloro-2(3H)-benzoxazolone; CZX], a skeletal muscle relaxant once used for the treatment of painful muscle spasms, is primarily metabolized to 6-hydroxychlorzoxazone (OH-CZX), which is subsequently glucuronidated and excreted in the urine (Conney and Burns, 1960; Desiraju et al., 1983). Formation of OH-CZX from CZX is primarily catalyzed by the hepatic microsomal cytochrome P450 (CYP) enzyme 2E1 in humans (Conney and Burns, 1960) and rats (Rockich and Blouin, 1999; Moon et al., 2003). OH-CZX formation has been used as a chemical probe to assess the activity of CYP2E1 *in vitro* and *in vivo* because of its good correlation with CYP2E1 activity in humans (Peter et al., 1990) and rats (Rockich and Blouin, 1999).

Kim et al. (2005) reported that induction of diabetes mellitus in male Sprague–Dawley rats by treatment with alloxan or streptozocin (DMIA or DMIS rats, respectively) also increased their protein expression and mRNA level of CYP2E1. Furthermore, Baek et al. (2006) reported that the increased protein expression of CYP2E1 caused a significant increase in the formation of OH-CZX in both DMIA and DMIS rats. The association between liver disease and diabetes mellitus is well known (Vidal et al., 1994; Kwon, 2003; Moscatiello et al., 2007). Thus, we examined CZX in this study.

Wang et al. (2003) orally administered CZX to diabetic patients and found that the total area under the plasma CZX concentration-time curve from time zero to infinity (AUC) was reduced by 25% in type 1 diabetics and by 70% in obese type II diabetics, as compared with that in 20 control volunteers. Furthermore, they found that protein expression of CYP2E1 in peripheral blood mononuclear cells increased in both types of diabetic patients. However, to our knowledge, no studies on the protein expression of CYP2E1 and the pharmacokinetics of CZX and OH-CZX in diabetic rats or humans with liver cirrhosis have yet been reported.

The objectives of the current studies were to evaluate, using a rat model, the effects of

diabetes and liver cirrhosis, alone and in combination, on the pharmacokinetics of CZX and OH-CZX. Changes in the protein expression of hepatic CYP2E1 in rats with liver cirrhosis with or without diabetes were also investigated.

**Materials and Methods**

**Chemicals.** CZX, OH-CZX, 3-aminophenyl sulfone (internal standard for the HPLC analysis of CZX and OH-CZX), monoclonal anti-$\beta$-actin antibody, NADPH (tetrasodium salt), tris(hydroxymethyl) aminomethane (Tris) buffer, EDTA (disodium salt), $\beta$-glucuronidase (Type H-1, from Helixa pomatia), streptozotocin, and Kodak X-OMAT film were purchased from Sigma-Aldrich Corporation (St. Louis, MO). N-dimethylnitrosamine was a product from Tokyo Kasei Kogyo Company (Tokyo, Japan), and ketamine hydrochloride was from Yuhan Corporation (Seoul, South Korea). Polyclonal anti-human CYP2E1 antibody was obtained from Detroit R&D (Detroit, MI), and horseradish peroxidase-conjugated goat anti-rabbit antibody was from Bio-Rad Laboratories (Hercules, CA). Enhanced chemiluminescence reagents were purchased from Amersham Biosciences Corporation (Piscataway, NJ). Other chemicals were of reagent or HPLC grade.

**Animals.** Protocols for the animal studies were approved by the Animal Center and Use Committee of the College of Pharmacy of Seoul National University, Seoul, South Korea. Male Sprague−Dawley rats (4–5 weeks old, weighing 180–200 g) were purchased from the Charles River Company Korea (Orient, Seoul, South Korea). The rats were randomly divided into three disease groups [liver cirrhosis (LC), diabetes mellitus (DM), and liver cirrhosis with diabetes mellitus (LCD)] and a control group. They were maintained in a clean-room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of $22 \pm 2°C$ with 12-h light (07:00-19:00) and dark (19:00-07:00) cycles and a relative humidity of

55 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Sam Yang Company, Pyungtaek, South Korea) and water available *ad libitum*.

**Induction of Liver Cirrhosis (LC) in Rats by *N*-Dimethylnitrosamine Injection.** Freshly prepared *N*-dimethylnitrosamine (diluted to 0.01 mg/ml in 0.9% NaCl injectable solution) was injected intraperitoneally at a dose of 0.01 mg/kg on three consecutive days per week for four weeks (Ohara and Kusano, 2002; Bae et al., 2006). On day 29, citrate buffer (pH 7.4; 1 ml/kg) was injected via the tail vein. On day 36, rats were treated with CZX.

Laboratory rats with *N*-dimethylnitrosamine-induced liver cirrhosis have clinical features similar to those of humans with liver cirrhosis, such as increased mortality, hepatic parenchymal cell destruction, formation of connective tissue, and nodular regeneration (Kang et al., 2002). Liver cirrhosis in the LC rats was evident by histological analysis, which revealed extensive micronodular cirrhosis with regenerative hepatocellular changes. Bile ductular proliferation was also detected (Bae et al., 2006). It has been reported that *N*-dimethylnitrosamine-induced liver cirrhosis in rats is reproducible (Jenkins et al., 1985; Jezequel et al., 1987).

**Induction of Diabetes Mellitus (DM) in Rats by Streptozotocin Injection**. A 0.9% NaCl injectable solution was injected intraperitoneally (1 ml/kg) on three consecutive days a week for four weeks. On day 29, one dose (45 mg/kg) of freshly prepared streptozotocin [dissolved in citrate buffer (pH 4.5) to 45 mg/ml] was administered via the tail vein (Kim et al., 2005). The rats were treated with CZX on day 36.

**Induction of Liver Cirrhosis with Diabetes Mellitus (LCD) in Rats with *N*-Dimethylnitrosamine and Streptozotocin Injections.** Liver cirrhosis was induced by intraperitoneal injection of *N*-dimethylnitrosamine as described above. Then, on day 29, diabetes mellitus was induced by injection of streptozotocin via the tail vein as described above. The rats

were treated with CZX on day 36.

**Control Rats.** Rats were injected intraperitoneally with 0.9% NaCl injectable solution (1 ml/kg) on three consecutive days a week for four weeks. On day 29, one dose (1 ml/kg) of citrate buffer (pH 4.5) was administered via the tail vein. The rats were treated with CZX on day 36.

During the pretreatment, food and water were available *ad libitum* to all rats. Immediately before the experiment, blood glucose levels in all rats were measured using the Medisense Optium kit (Abbott Laboratories, Bradford, MA), and rats with blood glucose levels greater than 250 mg/dl were selected as diabetic (DM and LCD rats).

**Measurement of Liver, Kidney, and Spleen Function.** To assess liver, kidney, and spleen function, a 24-h urine sample was collected on day 36 from LC, DM, LCD, and control rats ($n = 6$, each) for the measurement of creatinine levels. A blood sample was collected from the carotid artery for the measurement of the hematocrit (Microprocessor pH/°C Meter; Eutek Cybernetics, Singapore, Singapore). The plasma was measured for the total protein, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and creatinine by the Green Cross Reference Laboratory (Seoul, South Korea). Plasma protein binding of CZX was measured using equilibrium dialysis (Shim et al., 2000).

The whole liver, kidney, and spleen of each rat were excised, rinsed with 0.9% NaCl-injectable solution, blotted dry with tissue paper, and weighed. Small portions of each organ were fixed in 10% neutral phosphate-buffered formalin and then processed for routine histological examination with hematoxylin and eosin staining. Each rat was exsanguinated and sacrificed by cervical dislocation.

**Preparation of Hepatic Microsomes.** The procedures used were similar to those described by Baek et al. (2006). Livers from LC, DM, LCD, and control rats ($n = 5$, each) were

homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in ice-cold homogenization buffer (0.154 M KCl/50 mM Tris-HCl in 1 mM EDTA, pH 7.4). After the homogenate was centrifuged ($10,000 \times g$, 30 min), the supernatant fraction was removed and centrifuged at high speed ($100,000 \times g$, 90 min). The resulting microsomal pellet was resuspended in homogenization buffer and stored at –70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until used. Protein content was measured using the Bradford method (Bradford, 1976).

**Western immunoblot Analysis of CYP2E1.** The procedures used were similar to a reported method (Kim et al., 2001). Liver microsomes were resolved by SDS gel electrophoresis on a 7.5% polyacrylamide gel (10 µg protein per lane; $n = 3$, each). Proteins were transferred to a nitrocellulose membrane (Bio-Rad Laboratories) that was then blocked for 1 h in 5% milk powder in phosphate-buffered saline containing 0.05% (v/v) Tween 20 (PBS-T). For immunodetection, blots were incubated overnight at 4°C with rabbit anti-human CYP2E1 antibody (diluted 1:10,000 in PBS-T containing 5% bovine serum albumin), followed by incubation for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (diluted 1:10,000 in PBS-T containing 5% milk powder). Protein expression of CYP2E1 was detected by enhanced chemiluminescence on Kodak X-OMAT film and quantitated by densitometry with a microcomputer imaging device (model M1; Imaging Research, St. Catharines, Ontario, Canada). The $\beta$-actin band was used as a loading control.

**Measurement of $V_{max}$, $K_m$, and $Cl_{int}$ for the Formation of OH-CZX in Hepatic Microsomes.** Microsomal fractions (equivalent to 0.2 mg protein) were mixed with 50 µl of 0.1 M phosphate buffer (pH 7.4) containing 1 mM NADPH; 10 µl of CZX dissolved in a minimal amount of 10 N NaOH to make final concentrations of 2.5, 5, 10, 20, 50, 100, 200, 500, and 1000 mM CZX; and 0.1 M phosphate buffer (pH 7.4) sufficient to make a final volume of 0.5 ml. This reaction mixture was incubated in a water-bath shaker [37°C, 50 oscillations per minute (opm)]

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for 20 min, at which time the reaction was terminated by addition of 1 ml of ether. The formation of OH-CZX was determined using an HPLC method (Frye and Stiff, 1996). The kinetic parameters ($K_m$, $V_{max}$) for the formation of OH-CZX were determined by fitting the unweighted kinetic data from rat liver microsomes to a single-site Michaelis–Menten equation:

$$V = V_{max} \times [S] / (K_m + [S]),$$

where [S] is the substrate concentration. The best-fit model was selected based on the statistical goodness of fit (Yamaoka et al., 1978); the model with the lowest AIC (Akaike Information Criterion) was chosen. Calculations were performed using the WinNonlin software (Pharsight, Mountain View, CA). The intrinsic clearance ($Cl_{int}$) for the formation of OH-CZX per mg protein was calculated by dividing the $V_{max}$ by the $K_m$. The relative $Cl_{int}$ for the formation of OH-CZX based on the whole rat liver was estimated by the protein expression below and expressed as a percentage of the controls (100%):

Total liver protein (mg) $\times$ protein expression of CYP2E1 (% relative to controls) $\times$ $Cl_{int}$

(ml/min/mg protein).

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**Pretreatment of Rats for Intravenous or Oral Study.** Early in the morning on day 36, each rat was anesthetized by intramuscular injection of ketamine hydrochloride at a dose of 100 mg/kg. The jugular vein (for drug administration in the intravenous study) and the carotid artery (for blood sampling) were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ). 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. Then, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from

anesthesia for 4 to 5 h before beginning the experiment. Thus, the rats were not restrained in the present study. Ketamine was employed instead of ether to minimize the effect on CYP2E1, since Liu et al. (1993) reported that ether anesthesia alone increased the protein expression of CYP2E1 by 40%, as determined by assaying *p*-nitrophenol hydroxylase activity.

**Intravenous Study.** CZX (dissolved in a minimum amount of 10 N NaOH) at a dose of 20 mg/kg was infused (total infusion volume of 2 ml/kg) over 1 min via the jugular vein to rats in each group (*n* = 9, 7, 7, and 8 for LC, DM, LCD, 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, and 180 min after the start of the intravenous infusion of CZX. A heparinized 0.9% NaCl injectable solution (20 units/ml; 0.3 ml) was used to flush the cannula immediately after each blood sampling to prevent clotting.

Each blood sample was immediately centrifuged, and a 50-µl aliquot of plasma was stored at –70°C for later analysis of CZX and OH-CZX by HPLC (Frye and Stitt, 1996). At the end of the experiment (24 h after CZX treatment), each metabolic cage was rinsed with 20 ml of distilled water, and the rinse water was combined with the 24-h urine sample. The volume of the combined urine sample was determined, and two 50-$\mu$l aliquots were stored at –70°C for later analysis. At the same time (24 h), as much blood as possible was collected via the carotid artery, and each rat was sacrificed by cervical dislocation. Then, the abdomen was opened, and the entire gastrointestinal tract (including its contents and feces) of each rat was removed, transferred to a beaker containing 50 ml of 0.1 N NaOH (to facilitate the extraction of CZX and OH-CZX), and cut into small pieces with scissors. After stirring with a glass rod for 1 min, two 50-µl aliquots of the supernatant were collected from each beaker and stored at –70°C for later analysis.

**Oral Study.** CZX (the same solution used in the intravenous study) at a dose of 50 mg/kg was administered orally (total oral volume of 3 ml/kg) using a feeding tube to rats in each group (*n* =

8, 8, 7, and 7 for LC, DM, LCD, and controls, respectively). Blood samples were collected at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, and 480 min after oral administration of CZX. Other procedures were similar to those described above for the intravenous study.

**Measurement of Rat Plasma Protein Binding of CZX Using Equilibrium Dialysis.** Binding of CZX to protein in fresh plasma from LC, DM, LCD, and control rats ($n = 5$, each) was measured using equilibrium dialysis (Shim et al., 2000). Plasma (1 ml) was dialyzed against 1 ml of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran to minimize volume shift (Boudinot and Jusko, 1984) in a 1-ml dialysis cell (Fisher Scientific, Fair Lawn, NJ) fitted with a Spectra/Por 4 membrane (molecular weight cutoff of 12–14 kDa; Spectrum Medical Industries Inc., Los Angeles, CA). The initial concentrations of CZX spiked into the plasma compartment were 1, 10, and 50 µg/ml. After a 24-h incubation, two 50-µl aliquots were removed from each compartment and stored at –70°C for later HPLC analysis of CZX.

**HPLC Analysis of CZX and OH-CZX.** Concentrations of CZX and OH-CZX in the samples were determined using an HPLC method (Frye and Stiff, 1996). Briefly, a 0.1-ml aliquot of 0.2 M sodium acetate buffer (pH 4.75) and a 0.1-ml aliquot of isotonic Sørensen phosphate buffer (pH 7.4) containing 200 units of *β*-glucuronidase were added to 50 µl of sample. The mixture was mixed manually and incubated in a water-bath shaker (50 opm) for 2 h at 37°C. A 50-µl aliquot of methanol containing 10 mg/ml of 3-aminophenyl sulfone (internal standard) was then added. After the mixture was vortexed, 1 ml of diethyl ether was added, and the mixture was shaken for 10 min. After centrifugation (16,000 × $g$, 10 min), the upper organic layer was transferred to a clean tube and dried (Dry Thermobath; Eyela, Tokyo, Japan) under a gentle stream of nitrogen gas at 37°C.

The residue was reconstituted in 0.1 ml of mobile phase [0.1 M ammonium acetate : acetonitrile : tetrahydrofuran (72:22:5.5, v/v/v)], and a 50-µl aliquot was directly injected onto a

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reversed-phase ($C_{18}$) HPLC column. The mobile phase was run at a flow rate of 1.0 ml/min. An ultraviolet detector at 283 nm was used to monitor the column eluent. Unconjugated concentrations of OH-CZX were also measured in the urine samples, without incubation with $\beta$-glucuronidase. The retention times of OH-CZX, 3-aminophenyl sulfone (internal standard), and CZX were approximately 6, 10, and 18 min, respectively. The detection limit for CZX and OH-CZX in the rat plasma and urine samples was all 0.05 μg/ml. The coefficients of variation of the assay (within- and between-day) were less than 8.2%.

**Pharmacokinetic Analysis**. The 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, 1982) 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 ($t_{1/2}$), 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 ($F$). The peak plasma concentration ($C_{max}$) and the 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 an unpaired $t$-test or a Duncan's multiple range test, with the Statistical Package for the Social Sciences (SPSS) *posteriori* analysis of variance (ANOVA) among the four means for the unpaired data. All data are expressed as the mean ± standard deviation (S.D.), with the exception of $T_{max}$, which is expressed as the median (range).

# Results

**Liver, Kidney, and Spleen Function.** Body weight, blood glucose level, hematocrit, 24-h urine output, plasma chemistry data, $Cl_{cr}$, and relative organ weights for the four rat groups are listed in Table 1. For comparison, literature values from normal (albino) rats (Mitruka and Rawnsley, 1981; Davies and Morris, 1993) are also shown.

Compared with the control rats, the LC rats had significantly decreased 24-h urine volume and plasma levels of total protein and albumin; significantly increased plasma levels of GOT, GPT, total bilirubin, direct bilirubin, alkaline phosphatase, and total cholesterol; significantly decreased relative liver weight; and significantly increased relative spleen weight. In DM rats, the blood glucose level, the 24-h urine output, the plasma levels of GPT, alkaline phosphatase, urea nitrogen, and total cholesterol, and the relative kidney weight were significantly increased, compared with the control rats. In LCD rats, the blood glucose level, the plasma levels of total protein and albumin, the 24-h urine output, and the relative liver weight were significantly decreased compared with the control rats, whereas the plasma levels of GOT, GPT, total bilirubin, direct bilirubin, alkaline phosphatase, LDH, urea nitrogen, and total cholesterol and the relative kidney and spleen weights were significantly increased. However, the $Cl_{cr}$ did not differ significantly among the four groups.

These findings suggest that in LC and LCD rats, neither kidney nor spleen function was seriously impaired, whereas liver function was somewhat impaired. Consistent with this result, no significant histological findings were detected in the liver, kidney, or spleen in any rats, except that extensive hepatocellular degeneration with bridging fibrosis (pre-cirrhotic change) was detected in the livers of LC and LCD rats.

**Protein expression of CYP2E1.** Compared with the control rats, the protein expression of

CYP2E1 increased (by 258%) in the DM rats, decreased (by 22.0%) in the LC rats, and increased (by 124%) in the LCD rats (Fig. 1).

**$V_{max}$, $K_m$, and $Cl_{int}$ for Formation of OH-CZX in Hepatic Microsomes.** The rates of OH-CZX formation in liver microsomes from LC, DM, LCD, and control rats treated with varying concentrations of CZX are shown in Fig. 2, and the $V_{max}$, $K_m$, $Cl_{int}$, relative liver weight, total protein, and relative $Cl_{int}$ for each group are listed in Table 2. For the LCD rats, the $V_{max}$ for OH-CZX formation was significantly lower than that of the DM rats (by 39.7%) and higher than that of the LC rats (by 289%), but was similar to value observed in control rats. The changes oveserved in the $V_{max}$ reflect the changes observed in the amount of CYP2E1 (Table 2). This result suggests that in LCD rats, the maximum velocity for the formation of OH-CZX was similar to value observed in control rats. However, the $K_m$ for the formation of OH-CZX was comparable (not significantly different) among the four groups of rats, indicating that the affinity of the enzyme(s) for CZX was not changed. As a result, in LCD rats, the $Cl_{int}$ for the formation of OH-CZX per mg protein was significantly slower than in DM rats (by 53.6%) and faster than in LC rats (by 197%), but was similar to value observed in control rats. The total protein was significantly lower in LCD rats than in control or DM rats, but it was comparable to that in LC rats.

As discussed above (Fig. 1), the protein expression of CYP2E1 differed significantly among the four groups. Because the relative liver weight, total protein per whole liver, and protein contents of CYP2E1 were not comparable among the four groups, the relative $Cl_{int}$ for the formation of OH-CZX, based on total liver protein, was calculated; in LCD rats, the value was considerably higher (by 353%) than that in LC rats and lower (by 82.4%) than that in DM rats, but was similar to value observed in control rats. This result suggests that in LCD rats, the formation of OH-CZX, based on the whole liver, may be comparable to that in control rats and that the $V_{max}$,

$Cl_{int}$ per mg protein, and relative $Cl_{int}$, based on the whole liver, was similar to values observed in control rats.

Mizuno et al. (2000) reported a $K_m$ of $73 \pm 3.1$ µM and a $V_{max}$ of $1.09 \pm 0.38$ nmol/min/mg protein for the formation of OH-CZX in 10 control rats. These values differ somewhat from the present data (Table 2), possibly because of differences in the preparation of hepatic microsomes, in the concentrations of protein (0.1 vs. 0.2 mg) and substrate (0.01–1 vs. 2.5–1000 µM) used, or in the incubation time used (15 vs. 20 min).

**Rat Plasma Protein Binding of CZX.** The values for CZX binding of proteins in fresh plasma from the four groups of rats were $73.0 \pm 4.96\%$ (control), $62.5 \pm 8.53\%$ (LC), $67.1 \pm 2.44\%$ (DM), and $72.3 \pm 2.23\%$ (LCD), respectively; the value in LC rats was significantly lower than that in control and LCD rats. Protein binding of CZX to plasma from control rats ($n = 3$, each) was constant for CZX concentrations of 1, 10, and 50 µg/ml, which yielded values of $68.3 \pm 3.51$, $69.6 \pm 1.91$, and $67.3 \pm 5.44\%$, respectively. Thus, a CZX concentration of 10 µg/ml was arbitrarily chosen for the plasma protein binding studies.

**Pharmacokinetics of CZX and OH-CZX after Intravenous Administration of CZX.** The mean arterial plasma CZX concentration–time profiles for intravenous administration of CZX (20 mg/kg) to LC, DM, LCD, and control rats are shown in Fig. 3A, and the relevant pharmacokinetic parameters are listed in Table 3. In LC rats, the AUC of CZX was significantly greater, the terminal $t_{1/2}$ and MRT were significantly longer, and the Cl, $Cl_r$, and $Cl_{nr}$ were significantly lower than those in control rats. In DM rats, the AUC was significantly smaller, the terminal $t_{1/2}$ was significantly shorter, and the Cl, $Cl_r$, and $Cl_{nr}$ were significantly faster than those in control rats. Interestingly, the AUC, MRT, Cl, $Cl_r$, and $Cl_{nr}$ of CZX were similar between LCD and control rats. The contribution of the $Cl_r$ to the Cl of CZX was almost negligible; the values were less than 3.63% in all rats studied. However, the $V_{ss}$ of CZX and the percentage of the intravenous dose of

CZX excreted in the 24-h urine as unchanged drug ($Ae_{0-24\,h}$) were not significantly different among the four groups. CZX was undetectable (under the detection limit) in the gastrointestinal tract at 24 h ($GI_{24\,h}$) in all rats. Thus, the contribution of changes in the $Cl_r$ of CZX to other pharmacokinetic changes of CZX may also be almost negligible.

For the intravenous administration of CZX to DM, LC, LCD, and control rats, the mean arterial plasma OH-CZX concentration–time profiles are shown in Fig. 3B, and the relevant pharmacokinetic parameters are shown in Table 3. Formation of OH-CZX was rapid; for all four groups of rats, OH-CZX was detected in plasma at the first blood sampling time (1 min), and it rapidly reached $T_{max}$ within 5–60 min. In LC rats, the AUC of OH-CZX was significantly smaller, the $C_{max}$ was significantly lower, the $T_{max}$ was significantly longer, the $Ae_{0-24\,h}$ of total OH-CZX was significantly smaller, and $AUC_{OH\text{-}CZX}/AUC_{CZX}$ ratio was significantly smaller than those in controls. In DM rats, the AUC of OH-CZX was significantly greater, the terminal $t_{1/2}$ was significantly longer, the $C_{max}$ was significantly higher, the $Ae_{0-24\,h}$ of both total and free OH-CZX was significantly larger, and the $AUC_{OH\text{-}CZX}/AUC_{CZX}$ ratio was significantly greater than those in controls. Interestingly, in LCD rats, the AUC, $C_{max}$, $Ae_{0-24\,h}$ of total OH-CZX, and $AUC_{OH\text{-}CZX}/AUC_{CZX}$ ratio were similar to those in control rats. OH-CZX was also undetectable in $GI_{24\,h}$ for all rats studied.

The ratios of $Ae_{0-24\,h,\ conjugated\ OH\text{-}CZX}$ to $Ae_{0-24\,h,\ total\ OH\text{-}CZX}$ were 0.638, 0.439, 0.577, and 0.405 for the control, LC, DM, and LCD rats, respectively, suggesting that formation of conjugated OH-CZX decreased considerably in LC and LCD rats compared with control and DM rats. CZX was excreted in the 24-h urine samples as the free (unconjugated) form.

**Pharmacokinetics of CZX and OH-CZX after Oral Administration of CZX.** The mean arterial plasma CZX concentration–time profiles for the oral administration of CZX (50 mg/kg) are shown in Fig. 4A, and the relevant pharmacokinetic parameters are listed in Table 4. After oral

administration, CZX was rapidly absorbed; in all four groups of rats, it was detected in plasma at the first blood sampling time (5 min) and rapidly reached $T_{max}$ within 5–45 min. In LC rats, the AUC of CZX was significantly greater and the terminal $t_{1/2}$ was significantly longer than in the control rats. In DM rats, the AUC of CZX was significantly smaller, the terminal $t_{1/2}$ was significantly shorter, the $Cl_r$ was significantly faster, and the $Ae_{0-24\,h}$ was significantly greater than in the control rats. Interestingly, in LCD rats, the AUC, terminal $t_{1/2}$, $C_{max}$, and $Cl_r$ of CZX were similar to those in the controls.

The mean arterial plasma OH-CZX concentration–time profiles for the oral administration of CZX are shown in Fig. 4B, and the relevant pharmacokinetic parameters are listed in Table 4. OH-CZX formed rapidly after oral administration of CZX; in all four groups of rats, it was detected in plasma at the first blood sampling time (5 min) and rapidly reached $T_{max}$ within 5–90 min. In LC rats, the terminal $t_{1/2}$ of OH-CZX was significantly longer, the $C_{max}$ was significantly lower, the $T_{max}$ was significantly longer, the $Ae_{0-24\,h}$ of both total and free OH-CZX was significantly smaller, and the $AUC_{OH-CZX}/AUC_{CZX}$ ratio was significantly smaller than those in controls. In DM rats, the AUC of OH-CZX was significantly greater, the $C_{max}$ was significantly higher, the $Ae_{0-24\,h}$ of both total and free OH-CZX was significantly greater, and $AUC_{OH-CZX}/AUC_{CZX}$ ratio was significantly greater than those in controls. Interestingly, in LCD rats, the AUC, terminal $t_{1/2,}$ $C_{max}$, $T_{max}$, and $Ae_{0-24\,h}$ of total OH-CZX and the $AUC_{OH-CZX}/AUC_{CZX}$ ratio were similar to those in the control rats.

The ratios of $Ae_{0-24\,h,\,conjugated\,OH-CZX}$ to $Ae_{0-24\,h,\,total\,OH-CZX}$ were 0.561, 0.553, 0.562, and 0.458 for the control, LC, DM, and LCD rats, respectively, suggesting that the formation of conjugates of OH-CZX decreased considerably in LCD rats compared with the other rats. CZX was also excreted in the 24-h urine sample as the free (unconjugated) form.

## Discussion

The presence of liver cirrhosis and diabetes mellitus in LCD rats was apparent by their significantly decreased body weight gain, significantly higher blood glucose level, significantly larger 24-h urine output, significantly higher plasma levels of GOT, GPT, alkaline phosphatase, and LDH, and significantly lighter and heavier relative liver and kidney weights, respectively, compared with control rats (Tables 1, 3, and 4). Liver cirrhosis was also proven, based on histology, as explained in the **Results** section.

Baek et al., (2006) reported that the $Cl_{nr}$ of CZX could represent the metabolic clearance of the drug in rats. Additionally, the $Cl_{nr}$ of CZX listed in Table 3 could represent the hepatic metabolic clearance of CZX. Thus, changes in the $Cl_{nr}$ of CZX could represent changes in hepatic metabolism of CZX via CYP2E1 in rats.

Pathological conditions such as diabetes (Fig. 1) (Kim et al., 2005) and starvation (Johansson et al., 1990) induce CYP2E1. Pathological production of ketone bodies might be responsible for this induction of CYP2E1, presumably as the result of an adaptive response (Tu et al., 1983; Lieber, 1997). Nevertheless, ketone body production does not completely account for CYP2E1 induction. A previous study showed that, in the absence of insulin, ketone bodies at concentrations up to 10 mmol/L failed to affect or produced a decrease in mRNA levels of CYP2E1 (Woodcroft et al., 2002), which supports the concept that the induction of CYP2E1 in diabetes or during fasting is not the result of elevated circulating ketone bodies levels. Another study indicated that alterations in energy metabolism (e.g., mitochondrial dysfunction) were associated with induction of CYP2E1 (Chung et al., 2001). We found that the hepatic CYP2E1 level was moderately greater in LCD rats than in control rats (Fig. 1). However, the relative $Cl_{int}$ of CZX was comparable for the two groups (Table 2), which may be due to the accumulation of

extracellular matrix in combination with a decrease in liver parenchymal cells.

After intravenous administration of CZX, the AUC of CZX was significantly greater in LC rats (57.0% increase) than in the control rats, possibly as a result of the significantly slower Cl of CZX (38.2% decrease) in the LC rats (Table 3). The slower Cl was attributable to a significantly slower $Cl_{nr}$ of CZX (37.5% decrease) in the LC rats, because the two groups had comparable $Cl_r$ values (Table 3). The AUC of OH-CZX was significantly smaller (70.6% decrease) in LC rats than in controls (Table 3). These results could have been due to a significantly slower (96.9% decrease) relative $Cl_{int}$ for the formation of OH-CZX, based on total liver, because both the content and total liver protein of CYP2E1 were considerably decreased (by 78 and 67.6%, respectively, compared with the controls) (Table 2, Fig. 1). The significantly smaller formation (AUC) of OH-CZX in LC rats could also be supported by their smaller $AUC_{OH\text{-}CZX}$ / $AUC_{CZX}$ ratio (82.0% decrease) (Table 3).

In contrast to LC rats, DM rats exhibited a significant (57%) decrease in AUC of CZX after intravenous administration of CZX, compared with the controls, possibly because the Cl in LC rats was significantly faster (by 121%) than in the controls (Table 3). The faster Cl was attributable to a significantly faster (by 119%) $Cl_{nr}$ of CZX than in the controls (Table 3). Although $Cl_r$ of CZX was significantly faster (by 146%) in DM rats than in the control rats, the contribution of the $Cl_r$ to the Cl of CZX was almost negligible, constituting only 3.24% in DM rats (Table 3). The AUC of OH-CZX was significantly greater (by 75.1%) in DM rats than in the controls (Table 3). These results could have been caused by an increased (by 541%) relative $Cl_{int}$ for the formation of OH-CZX, based on total liver, which could have been the result of the significantly higher (by 258%) content of CYP2E1 in the DM rats, despite their significantly lower (by 35.9%) total liver protein, compared with the controls (Table 2, Fig. 1). Thus, the contribution of the increased content of CYP2E1 to CZX metabolism and OH-CZX formation

was greater than that of the decreased total liver protein. The significantly greater formation of OH-CZX (AUC) in DM rats could also be supported by the significantly greater (by 282%) $AUC_{OH\text{-}CZX}$ / $AUC_{CZX}$ ratio compared with that in the control rats (Table 3).

Similar results for the pharmacokinetics of CZX and OH-CZX and for $Cl_{int}$ per mg protein have been reported for other rat studies (Baek et al., 2006). Also, Wang et al. (2003) reported that following oral administration of 500 mg of CZX to patients with type I diabetes or obese, type II diabetes, the AUC of CZX was reduced by 25 and 70%, respectively, compared with that in healthy volunteers. However, the urinary recovery of CZX did not differ significantly between the three groups. Wang et al. (2003) also reported increased mRNA levels for CYP2E1 in peripheral blood mononuclear cells in both types of diabetes.

Protien expression of CYP2E1 was significantly increased in DM and LCD rats and was decreased in LC rats, compared with the controls (Fig. 1). Although the protein expression of hepatic CYP2E1 was increased (by 124%) in the LCD rats, compared with that in the control rats (Fig. 1), the total liver protein in the LCD rats was significantly reduced (by 61.7%) (Table 2). As a result, the relative $Cl_{int}$ for the formation of OH-CZX, based on whole liver, in LCD rats was similar to that of the control rats, with a difference of only 13% (Table 2). Thus, some pharmacokinetic parameters of CZX and OH-CZX would be expected to be similar in LCD and control rats. As expected, the AUC, MRT, Cl, $Cl_r$, and $Cl_{nr}$ of CZX, and the AUC and $C_{max}$ of OH-CZX, the $Ae_{0\text{-}24\ h}$ of total OH-CZX, and the $AUC_{OH\text{-}CZX}$ / $AUC_{CZX}$ ratio did not differ significantly between control and LCD rats (Table 3).

After oral administration of CZX, the AUC of CZX was significantly greater in LC rats and smaller in DM rats than in control or LCD rats (Table 4). However, this finding was not likely due to result from the increase or decrease in gastrointestinal absorption of CZX found in LC and DM rats, respectively, compared with the control and LCD rats, since the $GI_{24\ h}$ values were

undetectable for both groups after intravenous and oral administration of CZX (Tables 3 and 4). CZX was stable in rat gastric and intestinal fluids (Baek et al., 2006). Thus, CZX was almost completely absorbed in all groups of rats. Similar results were obtained from our intravenous studies (Table 3), especially for the AUC values of CZX and OH-CZX (Table 4). The $F$ of CZX in LC rats was considerably greater than in the control, DM, and LCD rats (by 45.3, 63.6, and 28.5%, respectively; Table 4). This result could have primarily been due to the decreased hepatic metabolism of CZX in LC rats.

Although CYP2E1 is the major enzyme that metabolizes CZX to OH-CZX, human CYP1A1/2 and/or CYP3A4 (Carriere et al., 1993; Shimada et al., 1993; Gorski et al., 1997; Ono et al., 1997) and rat CYP1A1 and CYP3A1/2 (Jayyosi et al., 1995) have also been reported to carry out CZX hydroxylation. The role of CYP3A2 in the formation of OH-CZX in rats was measured by treatment with DDT (an inducer of CYP3A2; Sierra-Santoyo et al., 2000). The $V_{max}$, $K_m$, and $Cl_{int}$ for the formation of OH-CZX were not significantly different for the DDT-treated vs. the control rats, indicating that the effect of CYP3A2 on the formation of OH-CZX was almost negligible (Sierra-Santoyo et al., 2000). Li et al. (1995) reported that treatment of rats with DDT had no effect on CYP2E1. We recently found (our unpublished data) that the protein expression of both CYP1A and CYP3A was increased in DM rats and decreased in LC rats, but in LCD rats, CYP1A was increased, whereas CYP3A was decreased.

In summary, after intravenous (Table 3) and oral (Table 4) administration of CZX, the AUC of OH-CZX was significantly smaller in LC rats than in control rats because protein expression of CYP2E1 and total liver protein were both decreased in the LC rats (Fig. 1). However, the AUC of OH-CZX was significantly greater in DM rats than in control rats because of the increased protein expression of CYP2E1 in DM rats (Fig. 1). The LCD and control rats had comparable values for AUC of OH-CZX, which may have resulted from the decrease in total liver protein in LCD rats

despite their increase in protein expression of CYP2E1; as a result, the relative $Cl_{int}$ for the

formation of OH-CZX, based on total liver, was comparable to that in the controls. These results

suggest that OH-CZX could be used as a chemical probe to assess the activity of hepatic CYP2E1

in LC, DM, and LCD rats.

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b) Wan Gyoon Shin, Pharm. D., Ph. D.

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul
National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South
Korea.

Tel.; +822-880-9261

Fax; +822-874-4169

E-mail address; wgshin@snu.ac.kr

**Figure Legends**

Fig. 1. Hepatic protein expression of CYP2E1 in LC, DM, LCD, and control rats was quantitated by Western immunoblotting and densitometry. (A) Immunoblot of gel loaded with 10 µg of microsomal protein per lane. $\beta$-actin was used as a loading control. CYP2E1 was detected by enhanced chemiluminescence on Kodak X-OMAT film. (B) Protein expression of CYP2E1 was quantitated by densitometry. Results are shown relative to protein expression CYP2E1of in the control rats (control = 100%). Error bars represent S.D. *, $p < 0.05$ compared with the controls; each value was significantly different.

Fig. 2. Kinetics for the formation rate of OH-CZX. CZX was incubated at the indicated concentrations (2.5–1000 µM) with liver microsomes from LC (■), DM (○), LCD (□), and control (●) rats ($n = 5$, each) at $37^{o}C$ for 20 min. The kinetic data were fit to a simple Michaelis–Menten equation. Error bars represent S.D.

Fig. 3. Mean arterial plasma concentration–time profiles of CZX (A) and OH-CZX (B) after intravenous infusion of CZX (20 mg/kg over 1 min) to LC (■; $n = 9$), DM (○; $n = 7$), LCD (□; $n = 7$), and control (●; $n = 8$) rats. Error bars represent S.D.

Fig. 4. Mean arterial plasma concentration–time profiles of CZX (A) and OH-CZX (B) after oral administration of CZX (50 mg/kg) to LC (■; $n = 7$), DM (○; $n = 8$), LCD (□; $n = 8$), and control (●; $n = 7$) rats. Error bars represent S.D.

TABLE 1

*Body weight, blood glucose, hematocrit, 24-h urine output, plasma chemistry data, Cl$_{cr}$, and relative organ weights in LC, DM, LCD, and control rats.*

*Literature values from normal (albino) rats are shown for comparison.*

| Parameter [a] | Control ($n = 6$) | LC ($n = 6$) | DM ($n = 6$) | LCD ($n = 6$) | Literature values from normal (albino) rats |
|---|---|---|---|---|---|
| Initial body weight (g) | 216 ± 17.4 | 208 ± 11.5 | 194 ± 24.5 | 204 ± 19.4 | |
| Final body weight (g)[b] | 348 ± 14.7 | 285 ± 12.6 | 263 ± 15.7 | 264 ± 17.6 | |
| Blood glucose (mg/dl)[c] | 134 ± 11.6 | 139 ± 13.7 | 516 ± 71.4 | 512 ± 59.3 | 50–135 |
| Hematocrit (%)[d] | 52.1 ± 1.15 | 34.9 ± 3.78 | 50.1 ± 1.58 | 41.2 ± 1.50 | 44.4–50.4 |
| Urine volume (ml/24-h)[e] | 25.2 ± 5.46 | 13.2 ± 7.60 | 83.5 ± 15.5 | 90.2 ± 7.14 | |
| Plasma | | | | | |
| Total protein (g/dl)[f] | 5.50 ± 0.141 | 4.17 ± 0.339 | 5.22 ± 0.527 | 4.55 ± 0.543 | 4.70–8.15 |
| Albumin (g/dl)[d] | 3.43 ± 0.121 | 2.50 ± 0.310 | 3.28 ± 0.293 | 2.85 ± 0.138 | 2.70–5.10 |
| GOT (IU/l)[f] | 48.7 ± 11.5 | 169 ± 29.8 | 90.0 ± 27.7 | 223 ± 10.2 | 45.7–80.8 |
| GPT (IU/l)[b] | 18.8 ± 6.74 | 65.5 ± 16.2 | 64.5 ± 23.5 | 73.3 ± 18.9 | 17.5–30.2 |
| Total bilirubin (mg/dl)[g] | BD [h] | 1.08 ± 0.685 | BD | 0.410 ± 0.313 | 0.00–0.55 |
| Direct bilirubin (mg/dl)[g] | BD | 0.872 ± 0.553 | BD | 0.260 ± 0.265 | |
| Alkaline phosphatase (IU/l)[i] | 213 ± 24.6 | 550 ± 161 | 942 ± 267 | 516 ± 139 | 56.8–128 |
| Lactate dehydrogenase (IU/l)[j] | 161 ± 47.0 | 409 ± 293 | 201 ± 83.8 | 580 ± 400 | 61.0–121 |
| Urea nitrogen (mg/dl)[k] | 17.6 ± 2.26 | 19.4 ± 2.27 | 28.4 ± 2.03 | 40.2 ± 6.79 | 5.0–29.0 |
| Total cholesterol (mg/dl)[b] | 49.0 ± 8.44 | 76.3 ± 13.0 | 75.2 ± 4.62 | 79.3 ± 8.68 | 10.0–54.0 |
| Cl$_{cr}$ (ml/min/kg) | 3.74 ± 0.619 | 3.46 ± 0.733 | 3.30 ± 0.589 | 2.79 ± 0.431 | 5.24 |

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(TABLE 1, continued)

| | | | | | |
|---|---|---|---|---|---|
| Liver weight (% of body weight)[d] | 3.84 ± 0.280 | 2.11 ± 0.419 | 3.50 ± 0.237 | 3.00 ± 0.296 | 4.00 |
| Kidney weight (% of body weight)[c] | 0.706 ± 0.0700 | 0.772 ± 0.143 | 1.02 ± 0.0586 | 1.06 ± 0.0642 | 0.80 |
| Spleen weight (% of body weight)[d] | 0.203 ± 0.0305 | 0.455 ± 0.0287 | 0.182 ± 0.0233 | 0.335 ± 0.115 | 0.30 |

[a] Data are expressed as mean ± S.D.

[b] Control group was significantly different ($p < 0.05$) from LC, DM, and LCD groups.

[c] Control and LC groups were significantly different ($p < 0.05$) from DM and LCD groups.

[d] Control and DM groups, LC group, and LCD group were significantly different ($p < 0.05$).

[e] Control group, LC group, and DM and LCD groups were significantly different ($p < 0.05$).

[f] Control and DM groups were significantly different ($p < 0.05$) from LC and LCD groups.

[g] LC group was significantly different ($p < 0.05$) from LCD group.

[h] Below the detection limit.

[i] Control group, LC and LCD groups, and DM group were significantly different ($p < 0.05$).

[j] Control and DM groups were significantly different ($p < 0.05$) from LCD group.

[k] Control and LC groups, DM group, and LCD group were significantly different ($p < 0.05$).

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TABLE 2

*$V_{max}$, $K_m$, and $Cl_{int}$ for the formation of OH-CZX in the hepatic microsomes of LC, DM, LCD, and control rats*

| Parameter[a] | Control ($n = 5$) | | LC ($n = 5$) | | DM ($n = 5$) | | LCD ($n = 5$) | |
|---|---|---|---|---|---|---|---|---|
| Final body weight (g)[b] | 384 | ± 18.5 | 292 | ± 25.6 | 309 | ± 37.3 | 253 | ± 18.2 |
| Liver weight (% of body weight)[c] | 3.25 | ± 0.418 | 2.55 | ± 0.568 | 3.17 | ± 0.246 | 3.52 | ± 0.263 |
| $V_{max}$ (nmol/min/mg protein)[d] | 1.63 | ± 0.139 | 0.612 | ± 0.270 | 3.95 | ± 0.995 | 2.38 | ± 0.785 |
| $K_m$ (μM) | 110 | ± 48.3 | 108 | ± 45.2 | 86.8 | ± 20.3 | 119 | ± 31.8 |
| $Cl_{int}$ (ml/min/mg protein)[e] | 0.0165 | ± 0.00520 | 0.00720 | ± 0.00509 | 0.0461 | ± 0.00970 | 0.0214 | ± 0.00866 |
| Total protein (mg/whole liver)[f] | 373 | ± 113 | 121 | ± 24.7 | 239 | ± 13.6 | 143 | ± 35.7 |
| Protein expression of CYP2E1 (%)[g, h] | 100 | ± 15.0 | 22 | ± 10 | 358 | ± 22.0 | 224 | ± 36 |
| Relative $Cl_{int}$ (%) | 100 | | 3.11 | | 641 | | 113 | |

[a] Data are expressed as mean ± S.D.

[b] Control group, LCD group, and LC and DM groups were significantly different ($p < 0.05$).

[c] Control, DM, and LCD groups were significantly different ($p < 0.05$) from LC group.

[d] Control and LCD groups, LC group, and DM group were significantly different ($p < 0.05$).

[e] Control, LC, and LCD groups were significantly different ($p < 0.05$) from DM group, and LC group was significantly different ($p < 0.05$) from LCD group.

[f] Control group, DM group, and LC and LCD groups were significantly different ($p < 0.05$).

[g] Each group was significantly different ($p < 0.05$).

[h] $n = 3$ per each group.

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TABLE 3

*Pharmacokinetic parameters of CZX and OH-CZX after intravenous administration of CZX at a dose of 20 mg/kg to LC, DM, LCD, and control rats*

| Parameter [a] | Control (n = 8) | | LC (n = 9) | | DM (n = 7) | | LCD (n = 7) | |
|---|---|---|---|---|---|---|---|---|
| Initial body weight (g) | 213 | ± 13.6 | 210 | ± 14.1 | 206 | ± 16.5 | 208 | ± 9.94 |
| Final body weight (g)[b] | 334 | ± 8.21 | 268 | ± 20.6 | 259 | ± 19.2 | 236 | ± 12.4 |
| Blood glucose (mg/dl)[c] | 117 | ± 9.90 | 102 | ± 20.3 | 397 | ± 90.2 | 455 | ± 33.6 |
| Urine volume (ml/24-h)[d] | 21.2 | ± 3.31 | 18.8 | ± 4.49 | 40.2 | ± 14.6 | 25.1 | ± 9.83 |
| Hematocrit (%)[e] | 50.3 | ± 3.95 | 34.5 | ± 7.50 | 49.6 | ± 3.07 | 35.4 | ± 7.60 |
| CZX | | | | | | | | |
| $\quad$ AUC ( µg • min/mL )[f] | 1720 | ± 442 | 2700 | ± 429 | 744 | ± 92.1 | 1740 | ± 809 |
| $\quad$ Terminal $t_{1/2}$(min)[g] | 28.9 | ± 2.97 | 65.1 | ± 25.0 | 24.2 | ± 7.13 | 38.3 | ± 19.6 |
| $\quad$ MRT (min)[h] | 31.9 | ± 5.98 | 71.5 | ± 30.6 | 16.7 | ± 6.75 | 42.2 | ± 24.4 |
| $\quad$ Cl (ml/min/kg)[f] | 12.3 | ± 3.21 | 7.60 | ± 1.47 | 27.2 | ± 3.27 | 14.4 | ± 8.29 |
| $\quad$ $Cl_r$ (ml/min/kg)[i] | 0.358 | ± 0.326 | 0.0989 | ± 0.154 | 0.881 | ± 0.534 | 0.523 | ± 0.443 |
| $\quad$ $Cl_{nr}$ (ml/min/kg)[f] | 12.0 | ± 3.38 | 7.50 | ± 1.32 | 26.3 | ± 3.68 | 13.8 | ± 7.92 |
| $\quad$ $V_{ss}$ (ml/kg) | 391 | ± 61.1 | 524 | ± 181 | 459 | ± 196 | 466 | ± 93.6 |
| $\quad$ $Ae_{0-24\,h}$ (% of CZX dose) | 3.31 | ± 3.12 | 1.06 | ± 1.05 | 3.44 | ± 2.44 | 3.42 | ± 2.25 |
| $\quad$ $GI_{24\,h}$ (% of CZX dose) | BD[j] | | BD | | BD | | BD | |

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(TABLE 3, continued)

OH-CZX

| | | | | |
|---|---|---|---|---|
| AUC ($\mu$g $\cdot$ min/mL)$^f$ | 571 $\pm$ 85.8 | 168 $\pm$ 153 | 1000 $\pm$ 176 | 578 $\pm$ 413 |
| Terminal $t_{1/2}$ (min)$^k$ | 32.3 $\pm$ 6.09 | 34.5 $\pm$ 8.70 | 45.5 $\pm$ 4.04 | 44.3 $\pm$ 14.6 |
| $C_{max}$ ($\mu$g/mL)$^f$ | 8.36 $\pm$ 1.50 | 2.38 $\pm$ 2.54 | 15.2 $\pm$ 3.24 | 6.55 $\pm$ 3.76 |
| $T_{max}$ (min)$^e$ | 15 (15–30) | 30 (15–60) | 15 (5–15) | 45 (15–60) |
| $Ae_{0–24\,h,\,total\,OH\text{-}CZX}$ (% of CZX dose)$^f$ | 42.3 $\pm$ 14.0 | 18.7 $\pm$ 14.5 | 78.0 $\pm$ 6.32 | 43.0 $\pm$ 13.6 |
| $Ae_{0–24\,h,\,free\,OH\text{-}CZX}$ (% of CZX dose)$^k$ | 15.3 $\pm$ 6.22 | 10.5 $\pm$ 10.1 | 33.0 $\pm$ 2.26 | 25.6 $\pm$ 11.9 |
| $GI_{24\,h}$ (% of CZX dose) | BD | BD | BD | BD |
| $AUC_{OH\text{-}CZX}$ / $AUC_{CZX}$ (%)$^f$ | 35.6 $\pm$ 13.0 | 6.41 $\pm$ 5.69 | 136 $\pm$ 25.6 | 39.1 $\pm$ 30.8 |

[a] Data are expressed as mean ± S.D.

[b] Control group, LC and DM groups, and LCD group were significantly different ($p < 0.05$).

[c] Control and LC groups, DM group, and LCD group were significantly different ($p < 0.05$).

[d] Control, LC, and LCD groups were significantly different ($p < 0.05$) from DM group.

[e] Control and DM groups were significantly different ($p < 0.05$) from LC and LCD groups.

[f] Control and LCD groups, DM group, and LC group were significantly different ($p < 0.05$).

[g] Control group, DM and LCD groups, and LC group were significantly different ($p < 0.05$).

[h] Control and DM groups, Control and LCD groups, and LC group were significantly different ($p < 0.05$).

[i] Control and LC groups, Control and LCD groups, and LCD and DM groups were significantly different ($p < 0.05$).

[j] Below the detection limit.

[k] Control and LC groups were significantly different ($p < 0.05$) from DM and LCD groups.

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TABLE 4

*Pharmacokinetic parameters of CZX and OH-CZX after oral administration of CZX at a dose of 50 mg/kg to LC, DM, LCD, and control rats*

| Parameter [a] | Control (*n* = 7) | | LC (*n* = 8) | | DM (*n* = 8) | | LCD (*n* = 7) | |
|---|---|---|---|---|---|---|---|---|
| Initial body weight (g) | 214 | ± 7.87 | 213 | ± 8.86 | 212 | ± 9.61 | 211 | ± 10.3 |
| Final body weight (g)[b] | 349 | ± 15.7 | 257 | ± 22.8 | 243 | ± 17.3 | 232 | ± 12.2 |
| Blood glucose (mg/dl)[c] | 95.4 | ± 9.61 | 103 | ± 29.2 | 470 | ± 25.8 | 474 | ± 25.9 |
| Hematocrit (%)[d] | 52.0 | ± 1.54 | 38.2 | ± 7.89 | 50.0 | ± 3.81 | 37.0 | ± 4.91 |
| CZX | | | | | | | | |
| AUC ($\mu$g $\cdot$ min/ml)[e] | 2500 | ± 509 | 5700 | ± 1280 | 960 | ± 386 | 2860 | ± 1750 |
| Terminal $t_{1/2}$ (min)[f] | 124 | ± 41.6 | 159 | ± 24.6 | 75.1 | ± 27.7 | 101 | ± 15.9 |
| $Cl_r$ (ml/min/kg)[g] | 0.0352 | ± 0.0375 | 0.112 | ± 0.0700 | 0.909 | ± 0.772 | 0.219 | ± 0.181 |
| $C_{max}$ ($\mu$g/ml)[h] | 23.1 | ± 8.59 | 35.3 | ± 16.8 | 19.8 | ± 11.1 | 25.6 | ± 11.5 |
| $T_{max}$ (min) | 15 (15–30) | | 15 (5–45) | | 5 (5–30) | | 15 (5–30) | |
| $Ae_{0-24 h}$ (% of CZX dose)[i] | 0.167 | ± 0.159 | 0.276 | ± 0.266 | 1.56 | ± 0.874 | 0.914 | ± 0.490 |
| $GI_{24 h}$ (% of CZX dose) | BD[j] | | BD | | BD | | BD | |
| F (%) | 58.1 | | 84.4 | | 51.6 | | 65.7 | |
| OH-CZX | | | | | | | | |
| AUC ( $\mu$g $\cdot$ min/ml)[k] | 1540 | ± 338 | 804 | ± 397 | 2860 | ± 982 | 2170 | ± 1070 |
| Terminal $t_{1/2}$ (min)[l] | 104 | ± 25.6 | 161 | ± 68.2 | 131 | ± 21.0 | 125 | ± 31.3 |
| $C_{max}$ ($\mu$g/ml)[e] | 12.6 | ± 4.64 | 3.85 | ± 2.88 | 27.1 | ± 11.3 | 12.3 | ± 4.95 |
| $T_{max}$ (min)[m] | 30 (15–90) | | 90 (30–90) | | 22.5 (5–30) | | 45 (5–90) | |

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(TABLE 4, continued)

| | | | |
|---|---|---|---|
| $Ae_{0-24 h, \text{ total OH-CZX}}$ (% of CZX dose)$^e$ | 32.1 ± 7.49 | 9.75 ± 3.22 | 62.6 ± 13.7 | 42.6 ± 21.2 |
| $Ae_{0-24 h, \text{ free OH-CZX}}$ (% of CZX dose)$^n$ | 14.1 ± 4.48 | 4.36 ± 1.59 | 27.4 ± 8.93 | 23.1 ± 13.1 |
| $GI_{24 h}$ (% of CZX dose) | BD | BD | BD | BD |
| $AUC_{\text{OH-CZX}}$ / $AUC_{\text{CZX}}$ (%)$^e$ | 63.9 ± 20.9 | 15.0 ± 7.78 | 323 ± 109 | 83.8 ± 40.5 |

$^a$ Data are expressed as mean ± S.D.

$^b$ Control group, DM and LC groups, and DM and LCD groups were significantly different ($p < 0.05$).

$^c$ Control and LC groups were significantly different ($p < 0.05$) from DM and LCD groups.

$^d$ Control and DM groups were significantly different ($p < 0.05$) from LC and LCD groups.

$^e$ Control and LCD groups, DM group, and LC group were significantly different ($p < 0.05$).

$^f$ Control and LCD groups, DM and LCD groups, and LC group were significantly different ($p < 0.05$).

$^g$ Control, LC, and LCD groups were significantly different ($p < 0.05$) from DM group.

$^h$ DM group was significantly different ($p < 0.05$) from LC group.

$^i$ Control and LC groups, LCD group, and DM group were significantly different ($p < 0.05$).

$^j$ Below the detection limit.

$^k$ Control and LC groups, Control and LCD groups, and LCD and DM groups were significantly different ($p < 0.05$).

$^l$ Control group was significantly different ($p < 0.05$) from LC group.

$^m$ Control, DM, and LCD groups were significantly different ($p < 0.05$) from LC group.

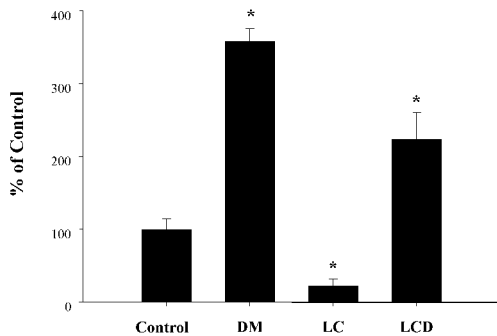$^n$ Control group, DM and LCD groups, and LC group were significantly different ($p < 0.05$).
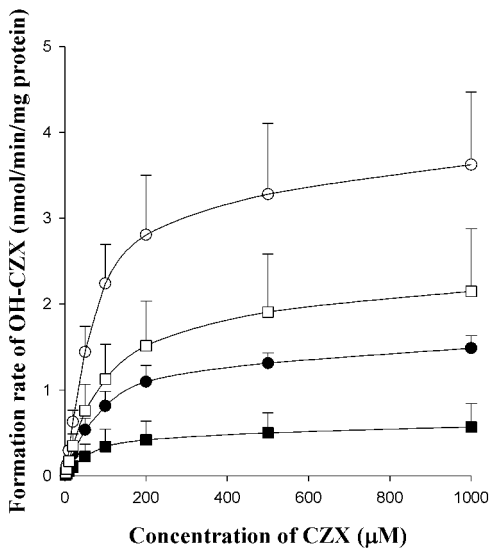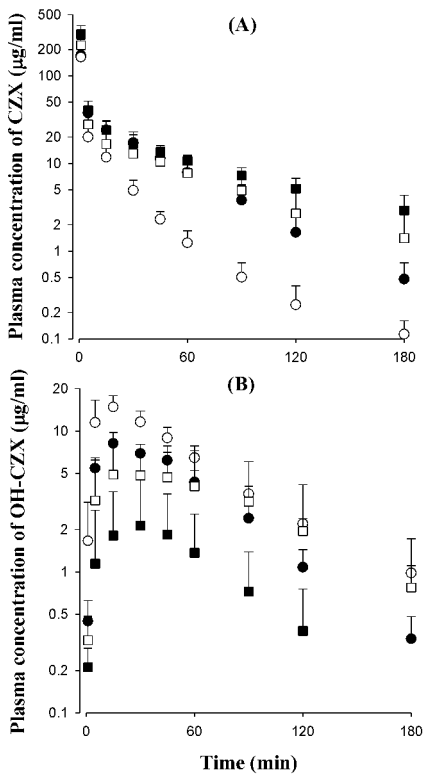
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FIG. 1.

FIG. 2.

FIG. 3.



(A)

(B)

Time (min)

FIG. 4.