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, South Korea (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, South Korea (C.Y.A.); Department of Clinical Pharmacology, Busan Paik Hospital, Inje University, Busan, South Korea (S.K.B.); and Department of Diagnostic Pathology, College of Medicine, University of Ulsan, Asan Foundation, Asan Medical Center, Seoul, South Korea (I.L.)

Received June 26, 2007; accepted March 27, 2008

ABSTRACT:
Protein expression of the hepatic CYP2E1 has been reported to be increased in diabetic rats. This enzyme is the primary metabolizer of chlorzoxazone (CZX) to 6-hydroxychlorzoxazone (OH-CZX). Although patients with liver cirrhosis have a higher prevalence of diabetes mellitus, there have been no reported studies on the protein expression of CYP2E1 in rats induced to have liver cirrhosis and diabetes mellitus by injection of N-dimethylaminosamine followed by streptozotocin [liver cirrhosis with diabetes mellitus (LCD)]. 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 (Clint; formation of OH-CZX per milligram protein) was comparable in both groups of rats. As a result, the relative Clint was also comparable for the two groups. Thus, OH-CZX formation in LCD and control rats was expected to be similar. As expected, after i.v. (20 mg/kg) and p.o. (50 mg/kg) administration of CZX, the area under the curve (AUC) of OH-CZX was comparable in control and LCD rats (i.v., 571 ± 85.8 and 578 ± 413 µg · min/ml, respectively; p.o., 1540 ± 338 and 2170 ± 1070 µg · min/ml, respectively). In LCD rats, the AUCOH-CZX/AUCCZX ratio was similar to the value in control rats after i.v. and p.o. administration. These results indicate that OH-CZX can be used as a chemical probe to assess the activity of CYP2E1 in LCD rats.

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 (P450) enzyme 2E1 in humans (Conney and Burns, 1960; Desiraju et al., 1983). 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 (Clint; formation of OH-CZX per milligram protein) was comparable in both groups of rats. As a result, the relative Clint was also comparable for the two groups. Thus, OH-CZX formation in LCD and control rats was expected to be similar. As expected, after i.v. (20 mg/kg) and p.o. (50 mg/kg) administration of CZX, the area under the curve (AUC) of OH-CZX was comparable in control and LCD rats (i.v., 571 ± 85.8 and 578 ± 413 µg · min/ml, respectively; p.o., 1540 ± 338 and 2170 ± 1070 µg · min/ml, respectively). In LCD rats, the AUCOH-CZX/AUCCZX ratio was similar to the value in control rats after i.v. and p.o. administration. These results indicate that OH-CZX can be used as a chemical probe to assess the activity of CYP2E1 in LCD rats.

ABBRVIATIONS: CZX, chlorzoxazone; OH-CZX, 6-hydroxychlorzoxazone; P450, cytochrome P450; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; PBS-T, phosphate-buffered saline/Tween 20; Clint, intrinsic clearance; Cl, time-averaged total body clearance; Clr, time-averaged renal clearance; Cln, time-averaged non-renal clearance; Clr, creatinine clearance; MRT, mean residence time; Vss, apparent volume of distribution at steady state; F, extent of absolute oral bioavailability; AEx, percentage of the dose excreted in the 24-h urine; GI24 h, percentage of the dose recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h.
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 high-performance liquid chromatography (HPLC) analysis of CZX and OH-CZX], monoclonal anti-β-actin antibody, NADPH (tetrasodium salt), Tris buffer, EDTA (disodium salt), β-glucuronidase (type H-1, from Helix pomatia), streptozotocin, and Kodak X-OMAT film were purchased from Sigma-Aldrich (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 fromDetroit R&D (Detroit, MI), and horseradish peroxidase-conjugated goat anti-rabbit antibody was from Bio-Rad (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 (Varese, Italy) under filtered, pathogen-free air, with food (Sam Yang Company, Pyungtaek, South Korea) and water available ad libitum.

Induction of 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 i.p. at a dose of 0.01 mg/kg on three consecutive days per week for 4 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 LC have clinical features similar to those of humans with LC, such as increased mortality, hepatic parenchymal cell destruction, formation of connective tissue, and nodular regeneration (Kang et al., 2002). LC in the LC rats was evident by histological examination of the whole liver was estimated by the protein expression below and expressed as a percentage of the controls (100%): total liver protein (mg) ×
protein expression of CYP2E1 (% relative to controls) × Clint (ml/min/mg protein).

**Pretreatment of Rats for i.v. or p.o. Study.** Early in the morning on day 36, each rat was anesthetized by i.m. injection of ketamine hydrochloride at a dose of 100 mg/kg. The jugular vein (for drug administration in the i.v. 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. Each rat then was housed individually in a rat metabolic cage (Daejung Scientific Company, Seoul, South Korea) and allowed to recover from anesthesia for 4 h before beginning the experiment. Thus, the rats were not restrained in the present study. Ketamine was used instead of ether to minimize the effect on CYP2E1 because Liu et al. (1993) reported that ether anesthesia alone increased the protein expression of CYP2E1 (% relative to controls) at a dose of 20 mg/kg.

**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 i.v. 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 Stiff, 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-μl 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. The abdomen then 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 i.v. study) at a dose of 50 mg/kg was administered p.o. (total p.o. volume of 3 ml/kg) using a feeding tube to rats in each group (n = 8, 7, 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 p.o. administration of CZX. Other procedures were similar to those described above for the i.v. 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 Jasko, 1984) in a 1-ml dialysis cell (Fisher Scientific, Fair Lawn, NJ) fitted with a Spectra/Por 4 membrane (molecular mass 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 oscillations/min) for 2 h at 37°C. A 50-μl aliquot of methanol containing 10 mg/ml of 3-amino-phenyl 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 (16,000g, 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 nitrate, 10 mM phosphate buffer (pH 7.0)].
acetate/acetonitrile/tetrahydrofuran (72:22.5:5.5, v/v/v)], and a 50-μl aliquot was directly injected onto a reversed-phase (C18) 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 β-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 g/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 noncompartmental analysis (WinNonlin; Pharsight Corporation): the time-averaged total body, renal, and nonrenal clearances (Cl, Clr, and Clnr, respectively), the terminal half-life (t1/2), the first moment of AUC, the mean residence time (MRT), the apparent volume of distribution at steady state (Vss), and the extent of absolute oral bioavailability (F). The peak plasma concentration (Cmax) and the time to reach Cmax (Tmax) 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 Inc., Chicago, IL) posteriori analysis of variance among the four means for the unpaired data. All the data are expressed as the mean ± S.D., with the exception of Tmax, 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, Clcr, 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
albamin; 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 Clint 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 (precirrhotic 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).

**TABLE 2**

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Control (n = 5)</th>
<th>LC (n = 5)</th>
<th>DM (n = 5)</th>
<th>LCD (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>384 ± 18.5</td>
<td>292 ± 25.6</td>
<td>309 ± 37.3</td>
<td>253 ± 18.2</td>
</tr>
<tr>
<td>Liver weight (% of body weight)</td>
<td>3.25 ± 0.418</td>
<td>2.55 ± 0.568</td>
<td>3.17 ± 0.246</td>
<td>3.52 ± 0.263</td>
</tr>
<tr>
<td>Vmax (mmol/min/mg protein)</td>
<td>1.63 ± 0.139</td>
<td>0.612 ± 0.270</td>
<td>3.95 ± 0.995</td>
<td>2.38 ± 0.785</td>
</tr>
<tr>
<td>Km (μM)</td>
<td>110 ± 48.3</td>
<td>108 ± 45.2</td>
<td>86.8 ± 20.3</td>
<td>119 ± 31.8</td>
</tr>
<tr>
<td>Clint (ml/min/mg protein)</td>
<td>0.0165 ± 0.00520</td>
<td>0.00720 ± 0.00509</td>
<td>0.0461 ± 0.00970</td>
<td>0.0214 ± 0.00866</td>
</tr>
<tr>
<td>Total protein (mg/whole liver)</td>
<td>373 ± 113</td>
<td>121 ± 24.7</td>
<td>239 ± 13.6</td>
<td>143 ± 35.7</td>
</tr>
<tr>
<td>Protein expression of CYP2E1 (%)</td>
<td>100 ± 15.0</td>
<td>22 ± 10</td>
<td>358 ± 22.0</td>
<td>224 ± 36</td>
</tr>
<tr>
<td>Relative Clint (%)</td>
<td>100</td>
<td>3.11</td>
<td>64.1</td>
<td>113</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.D.

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 Clint 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 the 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 with that in control rats and that the $V_{\text{max}}$, $\text{Cl}_{\text{int}}$ per milligram protein, and relative $\text{Cl}_{\text{int}}$, based on the whole liver, were similar to values observed in control rats.

Mizuno et al. (2000) reported a $K_{m}$ of $73 \pm 3.1$ $\mu$M and a $V_{\text{max}}$ of 1.09 ± 0.38 mmol/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 versus 0.2 mg) and substrate (0.01–1 versus 2.5–1000 $\mu$M) used, or in the incubation time (15 versus 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 values 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 $\mu$g/ml, which yielded values of 68.3 ± 3.51, 69.6 ± 1.91, and 67.3 ± 5.44%, respectively. Thus, a CZX concentration of 10 $\mu$g/ml was arbitrarily chosen for the plasma protein binding studies.

**Pharmacokinetics of CZX and OH-CZX after i.v. Administration of CZX.** The mean arterial plasma CZX concentration–time profiles for i.v. 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 $t_{\frac{1}{2}}$ (max) and MRT were significantly longer; and the $\text{Cl}$, $\text{Cl}_{\text{r}}$, and $\text{Cl}_{\text{int}}$ were significantly lower than those in control rats. In DM rats, the AUC was significantly smaller; the terminal $t_{\frac{1}{2}}$ (max) was more significant than that in control rats. Interestingly, the AUC, MRT, $\text{Cl}$, $\text{Cl}_{\text{r}}$, and $\text{Cl}_{\text{int}}$ of CZX were similar between LCD and control rats. The contribution of the Cl to the Cl of CZX was almost negligible; the values were less than 3.63% in all the rats studied. However, the $V_{\text{max}}$ of CZX and the percentage of the i.v. dose of CZX excreted in the 24-h urine as unchanged drug ($A_{\text{E0-24 h}}$) were not significantly different among the four groups. CZX was undetectable (under the detection limit) in the gastrointestinal tract at 24 h (GI24 h) in all the rats. Thus, the contribution of changes in the Cl of CZX to other pharmacokinetic changes of CZX may also be almost negligible.

For the i.v. 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 $T_{\text{max}}$ within 5 to 60 min. In LC rats, the AUC of OH-CZX was significantly smaller, the $C_{\text{max}}$ was significantly lower, the $T_{\text{max}}$ was significantly longer, the $A_{\text{E0-24 h}}$ of total OH-CZX was significantly smaller, and the $\text{AUC}_{\text{OH-CZX}} = \text{AUC}_{\text{OH-CZX}}$ ratio was significantly smaller than those in controls. In DM rats, the AUC of OH-CZX was significantly greater, the terminal $t_{\frac{1}{2}}$ was significantly longer, the $C_{\text{max}}$ was significantly higher, the $A_{\text{E0-24 h}}$ of total OH-CZX was significantly larger, and the $\text{AUC}_{\text{OH-CZX}} = \text{AUC}_{\text{OH-CZX}}$ ratio was significantly greater than those in controls. Interestingly, in LCD rats, the AUC, $C_{\text{max}}$, $A_{\text{E0-24 h}}$, of total OH-CZX, and $\text{AUC}_{\text{OH-CZX}} = \text{AUC}_{\text{OH-CZX}}$ ratio were similar to those in control rats. OH-CZX was also undetectable in GI24 h for all the rats studied.

The ratios of $A_{\text{E0-24 h}}$ to total OH-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 de-
Discussion

The presence of LC and DM 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 under Results.

Baek et al. (2006) reported that the Clint of CZX could represent the metabolic clearance of the drug in rats. Additionally, the Clint of CZX listed in Table 3 could represent the hepatic metabolic clearance of CZX. Thus, changes in the Clint 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 may 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 mM 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 Clint of CZX was comparable for the two groups (Table 2), which may be because of the accumulation of extracellular matrix in combination with a decrease in liver parenchymal cells.

After i.v. 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 Clint of CZX (37.5% decrease) in the LC rats because the two groups had comparable Cl 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 caused by a significantly slower (96.9% decrease) relative Clint 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 AUCOH-CZX/AUCCLX ratio (82.0% decrease) (Table 3).
In contrast to LC rats, DM rats exhibited a significant (57%) decrease in AUC of CZX after i.v. 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%) Clint in CZX than in the controls (Table 3). Although Cl in CZX was significantly faster (by 146%) in DM rats than in the control rats, the contribution of the Cl in the CZX almost approached, 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 Clint 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 attributed by the significantly greater (by 282%) AUCOH-CZX/AUCCZX ratio compared with that in the control rats (Table 3).

Similar results for the pharmacokinetics of CZX and OH-CZX and for Clint per milligram protein have been reported for other rat studies (Baek et al., 2006). Wang et al. (2003) also reported that following p.o. administration of 500 mg of CZX to patients with type 1 diabetes or obese, type 2 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 among the three groups, Wang et al. (2003) also reported increased mRNA levels for CYP2E1 in peripheral blood mononuclear cells in both types of diabetes.

Protein 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 Clint 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, Clp, and Clint of CZX, and the AUC and Clmax of OH-CZX, the AUCOH-CZX/AUCCZX ratio did not differ significantly between control and LCD rats (Table 3). After p.o. 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 the result of an increase or decrease in gastrointestinal absorption of CZX found in LC and DM rats, respectively, compared with the control and LCD rats because the Glmax values were undetectable for both groups after i.v. and p.o. 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 of the groups of rats. Similar results were obtained from our i.v. 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 pri-
marily 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 \( V_{\text{max}} \), \( K_{\text{m}} \), and \( C_{\text{int}} \) for the formation of OH-CZX were not significantly different for the DDT-treated versus 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 CYP1A1 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 i.v. (Table 3) and p.o. (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 \( C_{\text{int}} \) for the formation of OH-CZX, based on total liver, was comparable with 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.

References